VNIVERSITAT
D VALÈNCIA

Facultat de Química

Departamento de Química Analítica

Nuevos desarrollos de la espectroscopia para el análisis mineral de alimentos

Memoria presentada para la obtención
del título de Doctor por

Alba Mir Marqués

Mayo 2017

Programa de Doctorado
“Técnicas Experimentales en Química”

Directores:
M. Luisa Cervera Sanz
Miguel de la Guardia Cirugeda

Vniversitat de València Facultat de Química

El Prof. Dr. Miguel de la Guardia Cirugeda, Catedrático de Universidad, y el Prof. Dr. M. Luisa Cervera Sanz, Catedrático de Universidad del Departamento de Química Analítica de la Universidad de Valencia (Estudi General)

CERTIFICAN

QUE D. Alba Mir Marqués ha realizado la presente Tesis Doctoral titulada **"Nuevos desarrollos de la espectroscopia para el análisis mineral de alimentos"** bajo su dirección en el Departamento de Química Analítica de la Universidad de Valencia, y autorizan su presentación para optar al Grado de Doctor.

Y para que así conste, firman la presente en Burjassot a 22 de mayo de 2017.

Prof. Dr. M. Luisa Cervera Sanz

Prof. Dr. Miguel de la Guardia Cirugeda

A mi familia

AGRAÏMENTS

M’agradaria donar les gràcies a totes les persones que durant aquests anys de treball m’han ajudat a dur a terme aquest projecte que ara està arribant a la fi.

En primer lloc, voldria agrair als meus directors de Tesi, Marisa i Miguel, tot el recolzament que m’han donat durant tots aquests anys, especialment en aquests últims mesos per tota la dedicació i ajuda amb els tràmits finals de la Tesi. Voldria estendre l’agraïment a tots els membres del grup Solinquiana, tant professors, com becaris i estudiants, per tot allò que m’han pogut aportar durant els anys de treball al laboratori; a Gianni per tot allò que m’ha ensenyat i tots els consells que m’ha donat durant aquests anys; i a Manuela pels moments compartits al laboratori.

A la meua companya Merce, per que sense ella açò haguera sigut molt més difícil. Moltes gràcies per estar ahí, tant als bons moments com als moments més difícils, per ser la persona qui ha escoltat les meues penes i alegries durant els últims 5 anys de dilluns a divendres, i qui ha tingut que aguantar-me més hores al laboratori.

Als meus “quimiquillos” (Alaina, Asun, Anna, Carla, Rosa, Marc i Javi), amb qui vaig començar aquesta aventura de la química fa 12 anys, amb els que cada moment que passem junts es especial, i els que encara que tingues un mal dia, amb ells es converteix en un dia divertit, on les penes desapareixen. A Enrique, Isuha i Juanlu, per compartir grans moments amb ells, i per tota l’ajuda prestada quan ho he necessitat.

Als meus pares vull agrair-los tot allò que han fet per mi durant tota la seua vida, per que sense ells no seria la persona que sóc en aquests moments. Gràcies per fer possible complir tots els meus somnis, per aguantar-me als dies difícils i per estar ahí sempre que ho necessite. També a la meua germana Mireia, per estar sempre ahí, i per tot el que hem compartit juntes.

Finalment, a la meua meitat, per que sense ell no haguera pogut acabar la Tesi. Es qui m’ha alçat cada vegada que volia abandonar, qui m’ha ajudat als moments més difícils, qui ha confiat en que jo podia, qui dia a dia suporta el meu mal humor, les males contestacions, els dies tristos i qui fa que cada dia siga millor.

GRÀCIES A TOTS!

FACTOR DE IMPACTO DE LAS PUBLICACIONES

Tesis doctoral presentada por **Alba Mir Marqués** en la modalidad de "compendio de publicaciones". Esta tesis doctoral se presenta como compendio de los siguientes 9 artículos científicos y un capítulo de libro:

Artículo I: Mir-Marqués, A., González-Masó, A., Cervera, M.L., de la Guardia, M. Mineral profile of Spanish commercial baby food. *Food Chemistry*, 172 (2015) 238-244.

Factor de impacto en Journal Citation Reports (JCR), 2015: 4.052
Categoría y posición: Food, Science & Technology, 7/125 (Q1)
Número de citas: 4

Capítulo libro I: Mir-Marqués, A., Cervera, M.L., de la Guardia, M. Baby foods, published in: Handbook of Mineral Elements in Food, de la Guardia, M. and Garrigues, S. (Eds.). Chichester, John Wiley & Sons, 2015, pp. 709-723.

Artículo II: Mir-Marqués, A., Cervera, M.L., de la Guardia, M. A preliminary approach to the mineral composition of Spanish diet established from university canteen menus. *Journal of Food Composition and Analysis*, 27 (2012) 160-168.

Factor de impacto en Journal Citation Reports (JCR), 2015: 2.780
Categoría y posición: Food, Science & Technology, 22/125 (Q1)
Número de citas: 11

Artículo III: Mir-Marqués, A., Cervera, M.L., de la Guardia, M. Mineral analysis of human diets by spectrometry methods. *Trends in Analytical Chemistry*, 82 (2016) 457-467.

Factor de impacto en Journal Citation Reports (JCR), 2015: 7.487
Categoría y posición: Analytical Chemistry, 2/75 (Q1)

Artículo IV: Mir-Marqués, A., Garrigues, S., Cervera, M.L., de la Guardia, M. Direct determination of minerals in human diets by infrared spectroscopy and X-ray fluorescence. *Microchemical Journal*, 117 (2014) 156-163.

Factor de impacto en Journal Citation Reports (JCR), 2015: 2.893
Categoría y posición: Analytical Chemistry, 17/75 (Q1)
Número de citas: 3

Artículo V: Mir-Marqués, A., Domingo, A., Cervera, M.L., de la Guardia, M. Mineral profile of kaki fruits (*Diospyros kaki L.). Food Chemistry*, 172 (2015) 291-297.

Factor de impacto en Journal Citation Reports (JCR), 2015: 4.052
Categoría y posición: Food, Science & Technology, 7/125 (Q1)
Número de citas: 9

Artículo VI: Mir-Marqués, A., Cervera, M.L., de la Guardia, M. Authentication of the protected designation of origin “Kaki Ribera del Xúquer” from its mineral profile. *Ciencia*, 24 (2016) 153-164.

Revista no indexada en JCR.

Artículo VII: Khanmohammadi, M., Karami, F., Mir-Marqués, A., Bagheri Garmarudi, A., Garrigues, S., de la Guardia, M. Classification of persimmon fruit origin by near infrared spectrometry and least squares-support vector machines. *Journal of Food Engineering*, 142 (2014) 17-22.

Factor de impacto en Journal Citation Reports (JCR), 2015: 3.199
Categoría y posición: Food, Science & Technology, 17/125 (Q1)
Número de citas: 14

Artículo VIII: Mir-Marqués, A., Martínez-García, M., Garrigues, S., Cervera, M.L., de la Guardia, M. Green direct determination of mineral elements in artichokes by infrared spectroscopy and X-ray fluorescence. *Food Chemistry*, 196 (2016) 1023-1030.

Factor de impacto en Journal Citation Reports (JCR), 2015: 4.052
Categoría y posición: Food, Science & Technology, 7/125 (Q1)
Número de citas: 5

Artículo IX: Mir-Marqués, A., Elvira-Sáez, C., Cervera, M.L., Garrigues, S., de la Guardia, M. Authentication of protected designation of origin artichokes by spectroscopy methods. *Food Control*, 59 (2016) 74-81.

Factor de impacto en Journal Citation Reports (JCR), 2015: 3.388
Categoría y posición: Food, Science & Technology, 15/125 (Q1)
Número de citas: 1

Tabla de contenidos

4. "Mineral analysis of human diets by spectrometry methods"

Capítulo 5. Determinación directa de minerales en dietas mediante espectroscopia infrarroja y fluorescencia de rayos X ………………… 175

5. “Direct determination of minerals in human diets by infrared spectroscopy and X-ray fluorescence”

Capítulo 6. Perfil mineral de caquis (*Diospyros kaki L.)* ……………… 207

6. “Mineral profile of kaki fruits (*Dyospiros kaki L.*)

Capítulo 7. Autentificación de la denominación de origen “Kaki Ribera del Xúquer” a partir de su perfil mineral ……………………………… 231

7. “Authentication of the protected designation of origin “Kaki Ribera del Xúquer” from its mineral profile”

Sección I

Resumen

Los elementos minerales son indispensables para la vida. La exposición a estos elementos esenciales se produce por distintas vías, una de las más importantes es a través de la dieta. En los alimentos hay una gran cantidad de elementos minerales esenciales que pasan al organismo después de su ingesta, aunque también pueden introducirse a través de los alimentos elementos que pueden ser perjudiciales para el ser humano, es decir, tóxicos. Los elementos tóxicos, generalmente, se encuentran en los alimentos en muy baja concentración, y su presencia suele deberse a contaminación de las materias primas o durante el proceso de producción. Incluso los elementos minerales considerados como esenciales para el organismo, pueden encontrarse en concentraciones elevadas pudiendo llegar a ser tóxicos [1].

En algunos casos, se añaden elementos minerales a los alimentos procesados para aumentar la funcionalidad del producto final o para proporcionarle cualidades específicas. Las cantidades añadidas de estos minerales a los alimentos están reguladas, generalmente, como niveles máximos permitidos, definidas en la legislación de cada grupo de alimentos y cada país, para garantizar la seguridad del consumidor [2]. Debido a esto, es realmente importante controlar todos los elementos ingeridos a través de la dieta, principalmente con dos fines; uno es controlar que no se ingieren cantidades de elementos tóxicos que pudieran acarrear un problema de salud, y otro es controlar que se ingiere la cantidad necesaria de aquellos elementos que se necesitan para un correcto desarrollo y funcionamiento del organismo humano. Una escasa ingesta de elementos esenciales puede traer asociada deficiencias estructurales o fisiológicas en el cuerpo humano. Generalmente, los estudios sobre elementos minerales en dietas se habían realizado como estudios de dieta total (Total Diet Study, TDS) analizando alimentos por separado y, a partir de encuestas de consumo, realizando una estimación de las concentraciones de elementos minerales ingeridos [3–5]. Nos pareció de mayor interés, controlar dietas reales que ingieren las personas de nuestro alrededor. En el caso de los niños, a partir del análisis de tarritos de comida infantil comerciales, y en el caso de adultos empleando el estudio de menús completos de cafeterías universitarias.

El primer año de vida de los humanos es un periodo crítico en el desarrollo del niño. Es un periodo de crecimiento rápido, con un gran número de cambios en las funciones motoras y cognitivas. El análisis de tarritos infantiles es muy importante; ya que, a partir de los 6 meses de edad, los bebés necesitan algunos nutrientes que solamente con la ingesta de leche materna no se pueden aportar [6, 7], por lo que es necesario introducir otro tipo de alimentación; la ausencia o inapropiada composición de estos alimentos puede producir un problema en el crecimiento o acarrear problemas de salud en la etapa adulta [8]. En los últimos años, los tarritos de comida infantil se han convertido en una importante parte de la alimentación de los bebés, debido a los cambios que se están produciendo en la sociedad, principalmente por que las familias disponen de poco tiempo para poder preparar comidas caseras alternativas [9, 10]. Para proporcionar nuevos datos de composición mineral sobre alimentos infantiles, se analizaron muestras de tarritos infantiles de diferentes marcas comerciales de carne, pescado, verduras y fruta (capítulo 1).

En el capítulo 2 de esta tesis doctoral se revisan las principales estrategias utilizadas en los últimos años para determinar el perfil mineral de alimentos infantiles comúnmente consumidos en distintos países, incluyendo el muestreo, la preparación de muestra y las técnicas analíticas de medida. Además, se han actualizado los datos bibliográficos sobre el contenido mineral en alimentos infantiles para niños entre 6 y 12 meses de edad. En el estudio bibliográfico sobre el estado actual del análisis mineral en alimentos infantiles se puso de manifiesto la escasez de dichos estudios.

Otra preocupación es la alimentación en las cafeterías universitarias, puesto que en ellas come diariamente una gran cantidad de personas, principalmente adolescentes que tienen un gran desgaste mental y necesitan una correcta alimentación, que incluya principalmente todos aquellos elementos necesarios para un correcto funcionamiento de su cuerpo y su mente. También es muy importante controlar el nivel de sodio que se ingiere; ya que en las cafeterías se suele tender a cocinar con más sal de la debida, y esto puede crear un problema para las personas que comen todos los días en estos establecimientos. Por lo que, se decidió estudiar los menús de las cafeterías del campus de Burjassot de la Universidad de Valencia. Hasta la fecha la mayoría de estudios de dieta que se habían hecho o estaban en proceso eran estudios de dieta total, generalmente a cargo de instituciones involucradas en los temas de la alimentación o por los gobiernos estatales de todo el mundo [11–28]. Los estudios de menús son más escasos[29–34], pero proporcionan una visión mucho más real de la ingesta que se produce en el lugar de estudio.

Las técnicas analíticas más utilizadas para la determinación del perfil mineral de alimentos infantiles y dietas se han recopilado en los capítulos 2 y 4, en ellos se puede concluir que éstas son la espectroscopia de absorción y emisión atómica, y la espectrometría de masas. Para elementos minoritario como el Fe, Zn o Ca se suele utilizar espectroscopia de absorción atómica en llama (Flame Atomic Absorption Spectroscopy, FAAS), mientras que para el análisis de trazas suele utilizarse una técnica más sensible como es la espectroscopia de absorción atómica con cámara de grafito (Graphite Furnace Atomic Absorption Spectroscopy, GFAAS), aunque estas dos técnicas son útiles para al análisis de uno o pocos elementos. Si se necesita analizar una gran cantidad de elementos se utiliza la espectroscopia de emisión óptica con plasma de acoplamiento inductivo (Inductively Coupled Plasma-Optical Emission Spectroscopy, ICP-OES) o la espectrometría de masas con plasma de acoplamiento inductivo (Inductively Coupled Plasma-Mass Spectrometry ICP-MS). Para realizar la medida de los analitos de interés utilizando dichas técnicas instrumentales, es necesario un tratamiento previo de las muestras. Los elementos minerales en los alimentos se pueden disolver simplemente usando reactivos a temperatura ambiente, proporcionando así diluciones o "*slurries*" de las muestras. Es cierto que muchos de los elementos a analizar son fácilmente solubles en agua o en ácidos diluidos, pero estas diluciones pueden causar problemas relacionados con los efectos de la matriz durante el análisis y obligan al uso de técnicas altamente sensibles para la determinación de elementos a niveles de traza y ultra-traza. La digestión por vía seca o vía húmeda son las estrategias mayormente utilizadas para la puesta en disolución de las muestras de alimentos y dietas. La vía seca utiliza básicamente la temperatura y la descomposición total de la matriz, proporcionando así una alta pre-concentración de las muestras a analizar. Sin embargo, está claro que el simple uso de la temperatura y el tiempo puede provocar la pérdida de elementos volátiles y compuestos, por lo tanto, se han propuesto alternativas para reducir estos problemas. De hecho, el uso combinado de temperatura y ácidos inorgánicos es la técnica más útil para la disolución completa de los alimentos. Además, si se añade el uso de la presión es probablemente la mejor manera de acelerar la digestión de muestras de alimentos.

Para el análisis de tarritos infantiles y menús se decidió utilizar la digestión de las muestras asistida por microondas, ya que se trata de una metodología rápida y en la que se utiliza poca cantidad de reactivos para realizar una correcta digestión, y nuestro grupo de investigación tiene una gran experiencia en el uso de microondas para la puesta en disolución de alimentos [35, 36]. El método de digestión se adaptó para este tipo de muestras utilizando como reactivos ácido nítrico y peróxido de hidrógeno, en proporción 4:1.

Dado que los alimentos son perecederos, previo a la digestión, para conservar mejor la muestra es muy recomendable congelarla o secarla para que no se produzcan procesos de descomposición de la misma que puedan afectar al resultado final. La liofilización es una buena opción para realizar el secado de las muestras de dietas antes de su análisis porque durante este proceso la estructura original de los productos permanece inalterada y sólo se elimina el agua a baja temperatura [26, 29, 37–41]. Esto hace mucho más fácil su posterior manipulación para realizar los ensayos y análisis pertinentes, al tiempo que supone una pre-concentración de la muestra.

Para lograr una caracterización completa del perfil mineral de alimentos y dietas, se requieren métodos analíticos que proporcionen una gran cantidad de información y que sean altamente sensibles. Las técnicas analíticas más utilizadas para el análisis mineral de alimentos, como se ha comentado, son la espectroscopia atómica y la espectrometría de masas. La espectroscopia de emisión con plasma de acoplamiento inductivo (ICP-OES) es una técnica que permite la detección multi-elemental de manera rápida y robusta en muestras de alimentos. Para diseñar un método adecuado para el análisis mineral en distintos alimentos, es necesario establecer algunos parámetros instrumentales como la potencia de radiofrecuencia, los flujos de gas (argón) y el caudal de aspiración de la muestra, además hay que seleccionar la longitud de onda de medida para cada elemento, donde se recomienda seleccionar aquella que sea más sensible, siempre y cuando esté libre de interferencias espectrales o interferencias producidas por la matriz. En nuestro caso, se eligieron las dos longitudes de onda más sensibles para cada elemento, con el fin de garantizar que alguna de ellas no presentara interferencias de ningún tipo en las muestras analizadas. Para asegurar la calidad del método de análisis, se prepararon blancos de reactivos y muestras certificadas que pasaron por todo el proceso de medida, desde la digestión de la muestra hasta el análisis final mediante ICP-OES. Además, cada muestra fue digerida y medida por triplicado. Se evaluó la metodología empleada calculando los límites de detección (LOD) y cuantificación (LOQ), así como la reproducibilidad del método en términos de desviación estándar relativa (RSD). La medida de las muestras certificadas permitió la determinación de la exactitud del método.

El método desarrollado para determinar el perfil mineral de tarritos infantiles es un método exacto, preciso y reproducible, ya que los valores de RSD en todos los casos fueron inferiores al 4% y, se obtuvieron unos límites de detección muy bajos, comparados con los indicados en la bibliografía siendo de destacar que solo se observan LODs inferiores a los obtenidos cuando se utiliza ICP-MS para el análisis mineral de alimentos infantiles.

El método desarrollado para determinar el perfil mineral en dietas es exacto, preciso y reproducible, con valores de RSD inferiores al 2.5% excepto para el As, Pb, Se y Tl, elementos que presentan una baja repetitividad debido a sus bajas concentraciones y a la baja sensibilidad de estos elementos en ICP-OES. Los LODs obtenidos fueron bajos. Se utilizó un método de regresión para comparar los datos certificados de materiales de referencia de harina de arroz, hepatopáncreas de langosta e hígado bovino con los datos obtenidos mediante el método propuesto, encontrando coeficientes de regresión para las distintas muestras certificadas analizadas superiores a 0.997 en todos los casos.

Los datos obtenidos de composición mineral en tarritos infantiles y menús se compararon con los datos encontrados en la bibliografía de distintos países, y se observó que, en general, son similares, pero en las dietas se encontraron diferencias con respecto al contenido en sodio.

Los datos de composición mineral obtenidos se utilizaron para determinar la ingesta media diaria de cada elemento, ya que cada vez hay una mayor preocupación por conocer la ingesta diaria para cada individuo. Las cantidades de elementos minerales recomendadas vienen determinadas por distintos parámetros fijados por organizaciones internacionales como la Organización Mundial de la Salud (World Health Organization, WHO) o la Organización de las Naciones Unidas para la Alimentación y la Agricultura (Food and Agriculture Organization of the United Nations, FAO) [42–45]. Los parámetros más utilizados a nivel mundial son el ***requerimiento medio estimado*** (Estimated average requirement, EAR), ***tolerancia diaria recomendada o ingesta de nutrientes recomendada*** (Recommended daily allowance, RDA o Recommended nutrient intake, RNI), ***ingesta adecuada*** (Adequate Intake, AI) o ***nivel superior de ingesta tolerable*** (Tolerable upper intake level, UL). Estos parámetros se utilizan para comparar los datos obtenidos en distintos estudios de alimentos, y así comprobar si existe riesgo alimentario por ingesta de elementos tóxicos

[46–48], o si hay alguna deficiencia de elementos esenciales para el organismo humano, que pudiese producir algún trastorno o anomalía en el funcionamiento correcto del cuerpo humano [49–51].

En la Tabla 1 se indica el contenido mínimo y máximo de elementos esenciales que aporta una dieta a base de tarritos infantiles, y se puede apreciar que el contenido en hierro y en zinc es mucho menor que el necesario para una ingesta adecuada de dichos elementos, en el caso del hierro inferior al 20 %. Así pues, se pone de manifiesto la necesidad de enriquecer los tarritos de comida infantil comerciales para la correcta alimentación del niño. Por otra parte, la cantidad de sodio, magnesio, cobre, manganeso y potasio encontrada en los tarritos de comida infantil varía entre un 30 % y un 160 % de la ingesta recomendada; por lo que se puede concluir en este estudio, que la alimentación a base de tarritos infantiles cumple con los requisitos necesarios de ingesta de elementos esenciales excepto en el caso del hierro y zinc, que es muy escasa. De los datos sobre composición mineral en alimentos infantiles encontrados en la bibliografía y recopilados en el capítulo 2 se concluye que las cantidades de K, Ca y Na en este tipo de alimentos se encuentran a niveles de porcentaje, los niveles de Mg, Fe, Zn, Cu, Mn y Al a niveles de ppm, mientras que el Ni, Cr y Se se encuentran a niveles de ppb, con ligeras variaciones dependiendo del país de origen de las muestras. Por otra parte, es muy importante remarcar la ausencia o las bajas concentraciones encontradas para el As, Pb, Cd y Hg en alimentos infantiles comerciales.

Tabla 1. Contribución mínima y máxima (en porcentaje, %) a la ingesta adecuada (AI) y al requerimiento medio estimado (EAR) para niños de 6 a 8 meses, y de 9 a 12 meses

	6-8 meses		**9-12 meses**	
	min	max	min	max
Ca	4.8	58.5	7.9	66.6
Cu	16.7	111.5	31.1	137.9
Fe	1.9	15.3	3.4	19.6
K	26.8	119.8	53.0	157.3
Mg	17.9	75.9	32.6	99.2
Mn	10.4	69.6	16.5	90.7
Na	4.5	144.1	17.4	147.5
Zn	8.2	50.0	14.8	54.9

El aporte mineral de cada menú de cafetería universitaria a la ingesta diaria recomendada (RDI), se presenta en la Figura 1, calculado como miligramos de cada elemento que aporta cada menú dividido por el valor de RDI y multiplicado por 100. Los valores de RDI utilizados son 800, 0.7, 6, 4700, 350, 2, 2400 y 9.4 mg dia^{-1} para Ca, Cu, Fe, K, Mg, Mn, Na y Zn, respectivamente. Se puede observar que los menús estudiados son adecuados en cuanto a ingesta diaria recomendada para todos los elementos analizados, excepto para el sodio, que en la mayoría de los casos es bastante elevado, superando en diversos casos el 150% de la cantidad diaria recomendada. La contribución a la RDI es de 33-66% para el Ca, 34-152% para el Cu, 24-133% para el Fe, 20-81% para el K, 28-109% para el Mg, 41-93% para el Mn, 61-202% para el Na y 33-72% para el Zn. Por otra parte, la concentración de elementos no esenciales encontradas en las muestras de menús analizadas no supero la cantidad de 3 mg para el Al, 0.8 mg para el As, 0.5 mg de Ba, 0.05 mg de Li, 2 mg de Sr y 1 mg de Ti. Estos resultados demuestran una buena calidad de los menús de las cafeterías universitarias en cuanto a la ausencia de elementos tóxicos y a la adecuación de los elementos esenciales presentes, exceptuando la cantidad de sodio que aportan y que debería reducirse.

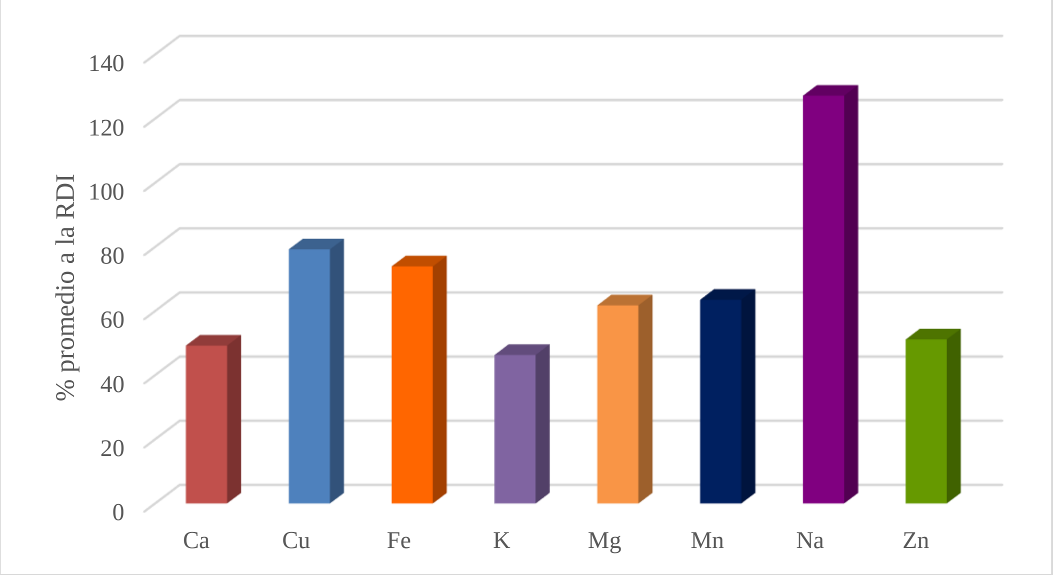

Figura 1. Contribución promedio de las muestras de menús estudiadas a la ingesta diaria recomendada de elementos esenciales estudiados

Las técnicas de ICP-OES e ICP-MS son muy útiles para el análisis multi-elemental, ya que son técnicas rápidas, sensibles y muy robustas. A pesar de ello, presentan ciertas desventajas como son el elevado coste de la instrumentación, el que los instrumentos no sean portátiles, que se requiera la puesta en disolución de la muestra, lo que lleva asociado procedimientos largos con un consumo de ácidos fuertes y oxidantes, y la generación de residuos.

Por todo lo antedicho, surge la necesidad de buscar alternativas más respetuosas con el medio ambiente. La química analítica verde implica esfuerzos para evitar el uso de sustancias tóxicas o peligrosas, junto con la reducción en el consumo de energía, reactivos y disolventes que implican la generación de residuos tóxicos [52]. Para atender los objetivos que plantea la química analítica verde, durante los últimos años se han buscado métodos de análisis no invasivos, o al menos que eviten el tratamiento químico de las muestras para el análisis mineral de alimentos. Se ha producido un aumento considerable en el desarrollo de métodos espectroscópicos que cumplieran con estos propósitos de la química analítica verde [53].

Los procedimientos analíticos basados en el espectro infrarrojo permiten un análisis directo y rápido de las muestras sin ningún proceso de preparación de las mismas, evitando así el uso de reactivos y la consiguiente generación de residuos, además de reducir el tiempo de análisis debido a la supresión del paso de digestión de la muestra, y el abaratamiento del coste de análisis [54]. La espectroscopia de fluorescencia de rayos X también es una alternativa a la espectroscopia atómica e iónica comúnmente utilizada en al análisis mineral de alimentos, especialmente las técnicas de fluorescencia de rayos X basadas en la dispersión de la energía (Energy Dispersion X-Ray Fluorescence, ED-XRF), ya que permite la determinación mineral con la mínima manipulación de muestra, y sin generar residuos [55]. Con esto se puede decir que se trata de técnicas que cumplen con los propósitos de la química analítica verde.

Los estudios de minerales en alimentos mediante espectroscopia infrarroja han aumentado drásticamente desde hace unos años [56–65], ya que el uso de los espectros de infrarrojo junto con herramientas quimiométricas puede proporcionar métodos alternativos muy satisfactorios. Aunque las correlaciones entre el espectro de infrarrojo y los elementos minerales no parecen creíbles, la predicción de algunos elementos minerales mayoritarios mediante NIR puede llevarse a cabo debido a su relación con la matriz orgánica. Estos estudios se limitan, en muchos casos, al análisis de elementos mayoritarios y minoritarios como el potasio, sodio, calcio, magnesio, hierro y zinc, y a unos pocos alimentos [66–68].

No todas las zonas del espectro electromagnético correspondientes a la luz infrarroja se utilizan para el análisis mineral en alimentos, sino que se suele usar solo la zona del infrarrojo cercano NIR (14000 a 4000 cm^{-1}) e infrarrojo medio MIR (4000 a 400 cm^{-1}). A pesar de que en la zona del infrarrojo medio se producen las vibraciones de los estados fundamentales y la zona del infrarrojo cercano corresponde con sobretonos de las bandas fundamentales, es la zona del infrarrojo cercano la más usada debida a su versatilidad y facilidad de manejo de sus equipos, así como a la repetitividad de las medidas [69].

Las técnicas IR presentan algunas desventajas, como la baja sensibilidad y un elevado efecto matriz. Utilizando espectros de infrarrojo adquiridos directamente o después de la etapa de pre-tratamiento, se construyen modelos quimiométricos para establecer una relación entre la información espectral y los datos de referencia obtenidos con otra técnica., En nuestro caso los datos de referencia fueron los datos obtenidos mediante el análisis mineral a partir de ICP-OES y/o ICP-MS. Para construir estos modelos se utilizó una parte de las muestras como conjunto de calibración, y la otra parte para verificar el modelo creado, con el fin de comprobar si la concentración predicha corresponde con la que se había medido previamente en ese conjunto de muestras denominado conjunto de validación.

La espectroscopia de fluorescencia de rayos X (XRF) consiste en la emisión de rayos X secundarios o fluorescentes característicos de un analito que ha sido excitado al ser bombardeado con rayos X de alta energía. Es una técnica muy utilizada en química analítica para la determinación mineral, generalmente de muestras sólidas. La radiación de rayos X cubre una parte de la radiación electromagnética de longitud de onda corta y abarca desde 100 hasta 0.5 Å, implicando unas energías en el intervalo de 0.1 a 25 KeV [55]. Cuando un fotón de rayos X de energía suficiente golpea un átomo, desprende un electrón de uno de los orbitales internos, de la capa K y/o L. Para recuperar la estabilidad, un electrón de uno de los orbitales externos llena la vacante y en este proceso, el exceso de energía se libera en forma de un fotón de rayos X. Debido a que los estados cuánticos de los electrones de cada átomo son únicos, la energía de los fotones emitidos es característica de los elementos presentes en la muestra excitada y el número de fotones detectados a una energía específica es proporcional a la concentración de ese elemento en la muestra [70].

Hay tres tipos básicos de equipos de XRF, los dispersivos de longitud de onda, los dispersivos de energía y los no dispersivos. Tradicionalmente, el gran tamaño y el elevado coste de los equipos de dispersión de longitud de onda ha hecho que se utilice en el campo de la geología, la arqueología y la ciencia forense pero en los últimos años, debido al desarrollo instrumental y tecnológico, especialmente en los equipos portátiles de dispersión de la energía (ED-XRF), se ha hecho posible el uso de esta técnica para análisis de rutina, permitiendo evaluar el perfil mineral de distintas muestras, incluidas las muestras de alimentos[70–75]. Los equipos portátiles de XRF dispersivos de energía sólo permiten analizar elementos de la tabla periódica a partir del magnesio, además presentan una baja sensibilidad comparada con las técnicas comúnmente utilizadas para el análisis mineral de alimentos, reduciendo su aplicabilidad a la determinación de elementos mayoritarios y minoritarios. La principal ventaja que presenta el uso de XRF en el análisis mineral de alimentos es que permite la determinación del perfil mineral de muestras sólidas sin tratamiento previo, o simplemente secando la muestra, evitando así el uso de reactivos y por consiguiente la generación de residuos [76–83]. En muchos casos en necesario compactar la muestra y hay que preparar pastillas [84]. Estas pastillas se pueden preparar sobre un soporte de aluminio con una cantidad aproximada de 3-4 g de muestra seca, generalmente liofilizada, que se prensa posteriormente y se recubre con una capa fina de polipropileno (Figura 2(a)). Otra opción cuando se dispone de poca cantidad de muestra, es preparar pastillas sin soporte, simplemente, se introduce una cantidad aproximada de 0.2 g de muestra en un molde y posteriormente se prensa (Figura 2(b)).

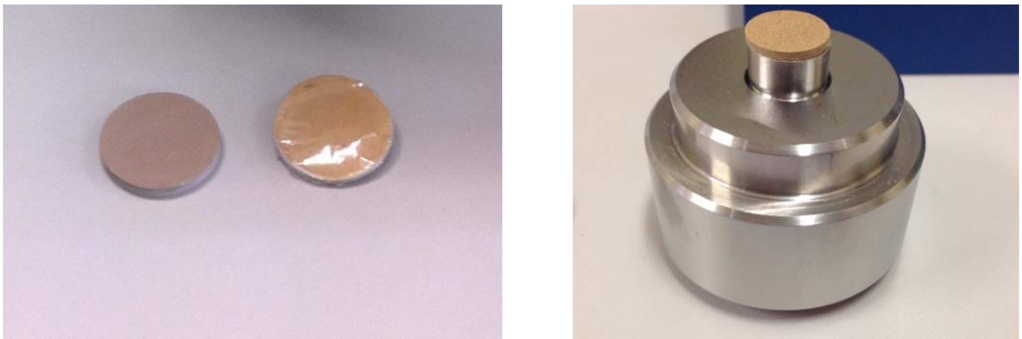
(a) (b)

Figura 2. Tipos de pastillas de muestra para la medida en XRF portátil

La matriz de alimentos influye fuertemente en la intensidad de la radiación de rayos X emitida y esto afecta la capacidad de la técnica para obtener datos cuantitativos exactos [55]. Además, la calibración interna de los equipos suele referirse al análisis de rocas, minerales o sedimentos, no para el análisis de alimentos, es por eso que los datos de concentración que proporciona no son fiables, y por ello no se utilizaron, empleándose los espectros obtenidos con esta técnica para calibrar externamente. Realizar una calibración adecuada es una cuestión complicada, pero mediante el uso de herramientas quimiométricas se pueden modelizar las señales de fluorescencia de rayos X de las muestras para predecir su perfil mineral, operando del mismo modo que con la espectroscopia infrarroja.

Para crear modelos quimiométricos a partir de los espectros de IR o XRF junto con los datos de referencia obtenidos mediante técnicas de espectroscopia atómica o de masas, uno de los algoritmos utilizado comúnmente es el análisis por mínimos cuadrados parciales (PLS) [57], [58], [60]–[62], [67], [85], [86]. Las referencias bibliográficas encontradas en este campo, son únicamente de unos pocos alimentos y no se había realizado hasta el momento el análisis mineral de dietas completas por espectroscopia infrarroja o con un equipo de fluorescencia de rayos X portátil; por lo que se decidió aplicar estas técnicas al análisis mineral en dietas (Capítulo 5).

Cuando se usa PLS como herramienta quimiométrica hay que tener en cuenta algunos parámetros como son el pretratamiento de las señales, el intervalo espectral utilizado, así como el número de variables latentes. Para una estimación fiable de la capacidad de predicción de los modelos quimiométricos construidos a partir de los espectros de IR y/o XRF, los datos espectrales (matriz X) y los datos de concentración de referencia (matriz Y), deben dividirse en dos conjuntos: uno de calibración y otro de validación. Los datos de calibración se utilizaron durante la optimización de los parámetros del modelo (selección de variables, pre-procesado de la señal o intervalo espectral idóneo). Para ello se realizó una validación cruzada (CV) en la que el conjunto de modelos calculados usando diferentes parámetros se evalúa mediante el error cuadrático medio de validación cruzada (RMSECV) [87].

En esta tesis, para seleccionar el conjunto de calibración más apropiado se utilizó el algoritmo de Kennard-Stone (KS). Este algoritmo selecciona un subconjunto representativo de las muestras basándose únicamente en las señales instrumentales; el subgrupo seleccionado debe cubrir homogéneamente toda la región experimental eligiendo objetos que están uniformemente distribuidos en el espacio experimental [88].

El algoritmo intervalo PLS (iPLS) se utilizó para desarrollar modelos locales PLS equidistantes de sub-intervalos de todo el espectro con el fin de proporcionar una imagen global de la información pertinente en diferentes subdivisiones espectrales y, por tanto, reducir las importantes variables espectrales [89].

Para aumentar la señal-ruido y por consiguiente la señal de los analitos de interés se puede realizar un pre-tratamiento de las señales. Los pre-tratamientos estudiados durante la tesis fueron los siguientes:

- *Centrado de los datos* (MC): se calcula el valor medio de cada variable de la matriz de datos y se resta éste en cada punto de la columna de datos. El valor medio corresponde al centro del modelo, y los valores de todas las variables se refieren a ese centro.
- *Suavizado* (SMO): es un filtro utilizado para eliminar el ruido de alta frecuencia de las muestras. Se realiza por separado en cada fila de la matriz de datos y actúa sobre variables adyacentes. El suavizado supone que las variables que están próximas entre sí en la matriz de datos están relacionadas entre sí y contienen información similar que se puede promediar conjuntamente para reducir el ruido sin pérdida significativa de la señal de interés.
- *Primera derivada de Savintzky-Golay* (FD): se basa en el uso de señales derivadas de primer orden en lugar de espectros de orden cero. Permite eliminar señales idénticas entre dos variables y deja sólo la parte de señal que es diferente. Además, elimina los desplazamientos de línea base. El algoritmo de Savintzky-Golay también suaviza los datos espectrales.
- *Variación estándar normal* (SNV): es una normalización ponderada, con el fin de reflejar que no todos los puntos contribuyen a la normalización por igual. Calcula la desviación estándar de todas las variables agrupadas para la muestra dada, y se normaliza la muestra para ese valor. Se utiliza principalmente para corregir el efecto de dispersión (scattering).
- *Corrección de dispersión multiplicativa* (MSC): se realiza la regresión de un espectro medido contra un espectro de referencia y luego la corrección del espectro medido usando la pendiente de este ajuste. Tiene la misma utilidad del SNV, pero depende del conjunto de muestras al que se le aplica.

- *Corrección ortogonal de la señal* (OSC): permite eliminar toda la información de los espectros que no está correlacionada con la propiedad que se quiere determinar. Permite la obtención de modelos de calibración más simples.

Después de la selección de los parámetros óptimos, el modelo se debe evaluar utilizando el conjunto de datos de validación, no empleados en la construcción y optimización del modelo, evitando así figuras de mérito demasiado optimistas. Las muestras del conjunto de validación se analizan en las condiciones seleccionadas. La potencia predictiva de los modelos ensayados se puede evaluar mediante el error cuadrático medio de la predicción (RMSEP) y/o la relación del rendimiento de la desviación (RPD). Éste último parámetro se calcula como la desviación estándar de las muestras dividido por el error cuadrático medio para cada modelo. Según Bellon-Maurel et al. (2010) [90] si RDP es mayor que 2 el modelo puede ser considerado “excelente”, si los valores de RPD están entre 1.4 y 2, el modelo es “justo”, pero si RPD es menor que 1.4 el modelo debe ser considerado como “no fiable”.

En la Tabla 2 se presenta un resumen de los modelos creados para la determinación mineral de distintos elementos en dietas mediante MIR, NIR y XRF, mostrando únicamente los valores de validación del método, y se puede concluir que el método es bueno para la determinación de potasio y zinc por las tres técnicas empleadas. Además, se pone de manifiesto que los datos obtenidos mediante XRF son mejores que mediante MIR o NIR para todos los elementos estudiados exceptuando para el Fe (mediante MIR), y el Na dado que el equipo de XRF portátil no permite la determinación de este elemento.

Tabla 2. Resumen de los datos de los modelos de validación creados para la determinación de elementos minerales en dietas mediante espectroscopia MIR, NIR y XRF.

	MIR		**NIR**		**XRF**	
Elemento	**R^2_{val}**	**RRMSEP (%)**	**R^2_{val}**	**RRMSEP (%)**	**R^2_{val}**	**RRMSEP (%)**
Ca	0.63	29	0.42	51	0.86	19
K	0.86	11	0.9	11	0.92	10
Fe	0.76	19	0.41	37	0.6	34
Mg	0.57	16	0.405	19	0.63	18
Na	0.95	30	0.8	30	----	---
Zn	0.82	19	0.69	25	0.84	3

La importancia del análisis mineral de alimentos también radica en la detección de adulteraciones o fraudes. El fraude alimentario relacionado con los productos con Denominación de Origen Protegida (DOP) o con Indicación Geográfica Protegida (IGP), producen una gran pérdida económica en España, valorada en 2013 en 90 millones de euros anuales. Se ha constatado que entre un 10 y un 15 % de las ocasiones, el producto referenciado no se corresponde con el protegido por una Denominación de Origen o Indicación Geográfica, si no que se trata de un producto diferente. En la Comunidad Valenciana hay un total de 22 productos con DOP o IGP, en la Figura 3 se muestran algunos de los sellos distintivos con los que cuentan las principales denominaciones de origen de la Comunidad Valenciana. En nuestro grupo de investigación tenemos experiencia en el desarrollo de estrategias contra el fraude alimentario de las denominaciones de origen, ya que se ha trabajado con “Vinos de Valencia”, “Herbero de la Sierra de Mariola” “Arroz de Valencia”, “Horchata de Valencia” y “Cerezas de la Montaña de Alicante”. Para poder realizar la autentificación de estos productos se ha usado el perfil mineral de dichos alimentos, proporcionando resultados satisfactorios en cuanto a la determinación del origen [91]–[93]. De los pocos alimentos con denominación de origen protegida que no habían sido analizados en nuestro laboratorio se decidió escoger “Kaki Ribera del Xúquer” y “Alcachofa de Benicarló”. Ambos productos son muy característicos y presentan diferencias gustativas con sus homólogos procedentes de otras zonas.

Figura 3. Sellos distintivos de algunas de las Denominaciones de Origen Protegida de la Comunidad Valenciana

El caqui es una fruta originaria de China, pero que se cultiva en zonas cálidas en muchas partes del mundo. La variedad Rojo Brillante se cultiva en la Ribera del Xúquer (Valencia). Este cultivo en la zona cuenta con el distintivo de Denominación de Origen Protegida "Kaki Ribera del Xúquer". Las características del clima en esta zona de producción influyen en gran parte en la calidad y características de esta fruta. Además, la composición del suelo afecta a la composición mineral del caqui, por lo que puede ser una manera de identificar la procedencia de los caquis según su composición mineral.

En el capítulo 6 se aporta el estudio de la determinación mineral de más de 150 muestras de caqui de distintas regiones de España mediante ICP-OES e ICP-MS. Siguiendo con la tradición del grupo de investigación en el análisis de alimentos con denominación de origen y aplicando los conocimientos aprendidos anteriormente en los estudios de dietas, se desarrolló una metodología basada en la digestión por microondas de las muestras de caqui, seguida de la determinación mediante espectroscopia de la composición mineral. Con el fin de obtener la máxima sensibilidad se optó por utilizar un nebulizador ultrasónico en las medidas de ICP-OES, y además analizar las muestras mediante ICP-MS para tener la máxima información mineral posible de las muestras, y así establecer el aporte mineral de este alimento a la ingesta diaria recomendada y poder caracterizar las muestras bajo el amparo de la denominación de origen protegida "Kaki Ribera del Xúquer".

Se adaptó la digestión en microondas para este tipo de muestra, reduciendo la masa de muestra utilizada y la potencia necesaria para llevar a cabo la digestión. Los parámetros instrumentales del ICP-OES también se adecuaron al análisis de muestras de caquis, se probaron distintos nebulizadores y finalmente se escogió el nebulizador ultrasónico ya que daba buenos resultados con una gran sensibilidad en la medida. Para cada elemento se escogió la línea de emisión, de las dos estudiadas, que presentaba una mayor sensibilidad y estaba libre de interferencias espectrales. Además, se realizaron pruebas con distintas diluciones de las muestras digeridas (1:2, 1:5, 1:10 y 1:100) para realizar las medidas, escogiendo al final una dilución 1:2 para analizar todas las muestras mediante el ICP-OES. En cuanto a la medida en ICP-MS se evaluaron todos los parámetros instrumentales con una disolución conteniendo 12 elementos. Se realizaron calibrados con concentraciones inferiores a las utilizadas en ICP-OES, y se estudiaron diferentes diluciones para medir las muestras de caquis, escogiendo la dilución 1:5, que fue la que dio mejores resultados.

En cuanto al control de calidad de las medidas, se analizaron blancos en cada tanda de digestión en microondas, pasando por todas las etapas del proceso. Todas las muestras fueron analizadas por triplicado, incluyendo los blancos y las muestras certificadas. En este caso la muestra certificada analizada fue hojas de tomate (NIST 1573a), y se compararon los datos certificados con los obtenidos mediante la metodología propuesta encontrando una recta de regresión con coeficiente de 0.9997. También se realizaron ensayos de recuperación a dos niveles de concentración 0.1 y 0.5 mg L^{-1}, obteniéndose valores de recuperación entre 91 y 111 %.

El análisis mineral de las muestras de caqui mediante ICP-OES e ICP-MS proporcionó datos semejantes a los escasos datos indicados en la bibliografía sobre dicha fruta (tres trabajos únicamente). El caqui es una fruta que aporta una gran cantidad de potasio y cobre a la ingesta diaria recomendada, valores adecuados de calcio, hierro y magnesio, y un bajo aporte en sodio y zinc. Se puede concluir que tomar una pieza de caqui puede ser una buena opción para la gente con problemas de corazón o hipertensión. Los datos de concentración de elementos minerales en caquis, se utilizaron para realizar un primer análisis estadístico que mostró algunas diferencias entre las distintas procedencias de las muestras.

Siguiendo con la autentificación de caquis, se realizaron análisis quimométricos univariantes y multivariantes. Se realizó un test ANOVA de una vía para comprobar si existían diferencias significativas entre las distintas zonas de producción a partir del perfil mineral de las muestras analizadas mediante ICP-OES e ICP-MS. Para todos los elementos se encontraron diferencias significativas con un nivel de probabilidad del 95%, además se realizó un ANOVA de dos vías añadiendo como variable el año de la cosecha de las muestras. En este caso solo se observaron diferencias significativas en la concentración de cinco de los elementos analizados. Posteriormente, se realizaron análisis multivariantes que incluían distintas herramientas quimiométricas como análisis de componentes principales (PCA), análisis jerárquico (HCA), análisis lineal discriminante (LDA) y análisis de árboles de regresión (CART).

Con todas las herramientas quimiométricas utilizadas se observó una diferencia entre las distintas zonas de producción, pero no se puedo autentificar al 100% la procedencia de cada muestra, y sólo en un 67%, se clasificaron correctamente las muestras usando análisis discriminante lineal. Por ello se optó por intentar clasificar las muestras de caqui a partir de su espectro de infrarrojo cercano.

Se obtuvieron los espectros de muestras liofilizadas por espectroscopia NIR con transformada de Fourier (FT-NIR) y se utilizó como algoritmo matemático para clasificar las muestras máquinas de soporte vectorial por mínimos cuadrados (Least Squares-Support Vector Machines, LS-SVM) con OSC como pre-tratamiento previo de la señal. LS-SVM es un algoritmo novedoso [94] que presenta una buena alternativa quimiométrica al análisis discriminante por mínimos cuadrados parciales (PLS-DA) más utilizado hasta el momento para la autentificación de muestras.

Las muestras, previamente liofilizadas, se introdujeron en viales de vidrio y se midieron en el equipo de FT-NIR. La adquisición de los espectros se realizó usando medidas de reflectancia difusa por acumulación de 50 barridos, en el intervalo de 740 a 2700 nm. Los espectros de las muestras se dividieron en dos conjuntos aleatoriamente, uno para calibración con 116 muestras y otro para validación externa con 50 muestras no incluidas en el conjunto de calibración. Los estudios quimiométricos se realizaron con el programa MATLAB y el complemento Toolbox. Se realizaron análisis no supervisados como PCA y HCA con y sin pre-tratamiento espectral previo. Se observó, que cuando no se usaba tratamiento espectral no había grupos de muestra diferenciados, en cambio cuando se aplicaba el pre-tratamiento de OSC se observaban grupos naturales de muestras, lo que también ocurría en análisis de clústeres. Lo mismo ocurrió al realizar el análisis mediante LS-SVM con y sin pre-tratamiento OSC, los datos obtenidos fueron realmente buenos, obteniendo un error de predicción de sólo el 2% cuando se utilizaba OSC para el conjunto de validación externa, y un error del 0% para el conjunto de calibración, en cambio sin pre-tratamiento espectral el error de calibración y validación externa fue del 28 y 24%, respectivamente. Estos datos evidenciaron que la espectroscopia de infrarrojo junto con el análisis quimiométrico es una alternativa para la autentificación de productos con denominación de origen, y permite un mayor control de las muestras y evita en gran medida el fraude.

La alcachofa “Blanca de Tudela” es una variedad que se cultiva en España, y que, cuando se produce en el área de Benicarló está bajo el amparo de la denominación de origen protegida “Alcachofa de Benicarló”. Se cultiva en la provincia de Castellón, en concreto es una zona muy restringida que abarca únicamente 4 pueblos (Benicarló, Vinaròs, Càlig y Penyíscola). La alcachofa es un alimento que favorece el metabolismo de las grasas y de la urea, además contribuye a la regulación de las funciones del hígado y del riñón.

Las muestras de alcachofa previamente liofilizadas y trituradas, se midieron por espectroscopia de infrarrojo cercano para obtener su espectro. Parte de estas muestras se utilizó para preparar unas pastillas compactas para obtener su espectro de XRF. Estos espectros junto con los datos de composición mineral, obtenidos a partir de ICP-OES, se utilizaron para crear modelos quimiométricos que relacionasen los espectros con la composición de distintos elementos. Para ello, las muestras se separaron en dos conjuntos, uno de calibración y otro de validación, utilizando la herramienta de Kennard-Stone, del mismo modo que para el análisis de dietas. Se estudiaron diferentes pre-tratamientos espectrales y se eligieron los que mejores resultados proporcionaron. En la Tabla 3 se indica un resumen de los principales datos obtenidos en la validación tanto de los modelos creados a partir de los espectros de NIR como de los obtenidos a partir de los espectros de XRF.

Tabla 3. Resumen de los datos de los modelos de validación creados para la determinación de elementos minerales en alcachofas mediante espectroscopia NIR y XRF.

Elemento	**NIR**			**XRF**		
	R^2_{val}	**RRMSEP (%)**	**RPD_{val}**	**R^2_{val}**	**RRMSEP (%)**	**RPD_{val}**
Ca	0.61	19.2	1.8	0.96	9	3.7
K	0.79	4.5	2.2	0.93	3	4.8
Mg	0.53	9.4	1.2	0.8	5.2	2.7
Fe	0.77	11.7	3.7	0.79	14.3	2.0
Mn	0.54	14.3	1.5	0.76	17.7	1.9
Zn	0.60	21.1	1.5	0.90	14.5	3.0

Nota: R^2_{val} coeficiente de correlación para el conjunto de validación. RRMSEP error cuadrático medio relativo de predicción. RPD_{val} relación del rendimiento de la desviación.

Con el fin de determinar su composición mineral, se escogieron muestras de alcachofas procedentes de la PDO “Alcachofa de Benicarló”, y muestras procedentes de la región de Valencia y Murcia. Las muestras se analizaron mediante ICP-OES tras la digestión en horno microondas en presencia de ácido nítrico concentrado. Los datos obtenidos se utilizaron con tres objetivos: 1) controlar la cantidad de minerales que aporta el consumo de alcachofa a la ingesta diaria recomendada; 2) como datos de referencia para la creación de modelos quimiométricos a partir de los espectros NIR y

XRF de las alcachofas para crear herramientas de determinación directa de elementos minerales en alcachofas; y 3) para autentificar la procedencia de las muestras de alcachofa a partir de los espectros de NIR y XRF.

Considerando un consumo de tres piezas de alcachofa, el aporte de éstas a la RDA es bueno respecto al potasio, manganeso y magnesio ya que aportan un 15-20% de estos minerales a la RDA, mientras que podría considerarse bajo o muy bajo el aporte de calcio, hierro y zinc, que es inferior al 10% de la RDA. Estos valores se consideran adecuados, teniendo en cuenta que la alcachofa no es una de las principales fuentes de minerales que ingieren los humanos en su dieta diaria.

Los modelos de PLS-NIR para la predicción del contenido mineral de las alcachofas no fueron demasiado buenos y se obtuvieron coeficientes de regresión bajos como se puede observar en la Tabla 3, a pesar de que la precisión de los modelos fue alta con valores de RSD inferiores al 10%. La fiabilidad de los modelos de calibración PLS-NIR es alta siguiendo el criterio de Bellon et al., 2010 [90], ya que en todos los casos los RPDs son mayores de 2, en cuanto a los modelos de validación no ocurre lo mismo; se consideran modelos “excelentes” para el K y el Fe, “justos” para el Ca, Mn y Zn, y “no fiables” para el Mg. Por el contrario, los modelos PLS-XRF fueron muy buenos, con coeficientes de regresión bastante buenos, principalmente para el Ca, K y Zn, con unos valores de RSD inferiores al 9%; y como se puede ver en la Tabla 3 unos modelos de validación en todos los casos “excelentes”, excepto para el Mn para el que el valor de RPD indica que es un modelo “justo”. Se puede concluir que la capacidad predictiva de los modelos PLS-XRF desarrollados es muy buena para la concentración de calcio, potasio, hierro, magnesio, manganeso y zinc. Estos resultados se corresponden con el hecho de que las líneas de los espectros de XRF están relacionadas con la presencia de elementos minerales, mientras que las bandas que se obtiene en los espectros de NIR están relacionadas con los sobretonos de “stretching” molecular.

Los datos de composición mineral de alcachofa medidos mediante ICP-OES, así como los espectros NIR y XRF se utilizaron para la autentificación de la “Alcachofa de Benicarló”. Se intentó clasificar las muestras como procedentes de la PDO o no procedentes de la PDO, y en un segundo estudio se intentaron clasificar según sus tres orígenes (Benicarló, Valencia y Murcia). En ambos estudios, se realizaron análisis estadísticos tanto supervisados (análisis discriminante por mínimos cuadrados parciales, PLSDA) como no supervisados (PCA y HCA). PCA y HCA se utilizaron para observar la presencia de grupos de muestras naturales, mientras que en el análisis supervisado PLSDA se utilizaron dos conjuntos de muestras, para calibración y validación, usando la herramienta de Kennard-Stone.

La clasificación de las muestras de alcachofa en función de su origen a partir de sus espectros NIR, no fue satisfactoria. A priori, el análisis mediante PCA y HCA no mostró diferencias claras entre los distintos orígenes de las muestras. En cuanto al análisis quimométrico por PLSDA se obtuvieron porcentajes de clasificación correctos para la validación de los modelos creados del 88% en el caso muestras procedentes de la PDO y no procedentes de la PDO, y de un 80% cuando se intentaron autentificar los tres orígenes de las muestras de alcachofa (ver Tabla 4).

Tabla 4. Resumen de los datos obtenidos mediante PLSDA para la autentificación de muestras de alcachofa según su procedencia

Técnica	**Nº clases**	**% correcto de calibración**	**% correcto de validación**
NIR	2	100	88
	3	98	80
XRF	2	100	100
	3	100	93
ICP-OES	2	100	100
	3	100	100

Los datos de autentificación del origen de las alcachofas a partir de su perfil mineral medido mediante ICP-OES, fueron mucho mejores que a partir de su espectro de infrarrojo, ya que se obtuvieron porcentajes de clasificación correctos del 100% en todos los casos. Para el análisis quimómetrico a partir del perfil mineral de alcachofa, se analizaron 42 elementos de los cuales finalmente solo se utilizaron 6 elementos para el análisis quimiométrico, ya que eran los que presentaban diferencias significativas entre las distintas procedencias y, además, presentaron mayor peso en el estudio mediante PCA. Las variables utilizadas fueron la concentración de Al, Fe, K, Na, Sr y V en mg kg^{-1}.

Con respecto a la autentificación del origen de muestras de alcachofa a partir de su espectro de XRF, los datos obtenidos fueron satisfactorios. A simple vista, el análisis por PCA muestra una clara diferencia entre las muestras procedentes de la PDO "Alcachofa de Benicarló" y el resto de las procedencias. En cuanto al análisis supervisado mediante PLSDA se obtuvieron porcentajes correctos de clasificación del 100% en todos los casos, excepto cuando se intentaron autentificar los tres orígenes diferentes, y aquí una muestra de Valencia se clasificó erróneamente como si fuese de Murcia (ver Tabla 4).

Podemos concluir que la autentificación de muestras de alcachofa a partir de su perfil mineral medido mediante ICP-OES, da muy buenos resultados y es una manera eficaz de poder evitar el fraude que se produce con los productos amparados bajo la denominación de origen protegida. Del mismo modo, podemos afirmar lo mismo para la autentificación de muestras de alcachofa a partir de su espectros de XRF, además con esta última metodología contribuimos un poco más a la química verde, al aplicar un estudio propuesto por Armenta et al., 2015 [95] del consumo energético, junto con los residuos producidos, la metodología propuesta utilizando XRF obtendría un certificado energético A, mientras que con el ICP-OES se obtendría un certificado B, un poco menos respetuoso con el medio ambiente.

Bibliografía

[1] C. G. Fraga, 'Relevance, essentiality and toxicity of trace elements in human health', *Mol. Aspects Med.*, vol. 26, no. 4–5, pp. 235–244, Aug. 2005.

[2] World Health Organization, 'Codex alimentario'. 2015.

[3] European Food Safety Authority and Food and Agriculture Organization of the United Nations (FAO), 'Towards a harmonised Total Diet Study approach: a guidance document: Guidance for the use of the total diet study approach', *EFSA J.*, vol. 9, no. 11, p. 2450, Nov. 2011.

[4] Food Standards Australia New Zealand, *The 22nd Australian total diet study*. Canberra: Food Standards Australia New Zealand (FSANZ), 2008.

[5] U.S. Food and Drug Administration, 'Total Diet Study Statistics on Element Results. Market Baskets 2006-1 through 2008-4'. 2010.

[6] B. de Benoist, World Health Organization, and Centers for Disease Control and Prevention (U.S.), *Worldwide prevalence of anaemia 1993-2005 of WHO Global Database of anaemia*. Geneva: World Health Organization, 2008.

[7] C. Agostoni *et al.*, ‘Complementary Feeding: A Commentary by the ESPGHAN Committee on Nutrition’:, *J. Pediatr. Gastroenterol. Nutr.*, vol. 46, no. 1, pp. 99–110, Jan. 2008.

[8] World Health Organization, *Infant and young child feeding: Model Chapter for textbooks for medical students and allied health professionals.* Geneva: World Health Organization, 2009.

[9] R. Melo, K. Gellein, L. Evje, and T. Syversen, ‘Minerals and trace elements in commercial infant food’, *Food Chem. Toxicol.*, vol. 46, no. 10, pp. 3339–3342, Oct. 2008.

[10] V. Okesli, C. A. González-Bermúdez, M. L. Vidal-Guevara, J. Dalmau, and G. Ros, ‘Alimentación complementaria: ¿es igual el perfil nutricional de un pure casero que el de un tarrito comercial?’, *Acta Pediátrica Esp.*, vol. 69, pp. 440–448, 2011.

[11] J. C. Leblanc, T. Guérin, L. Noël, G. Calamassi-Tran, J. L. volatier, and P. Verger, ‘Dietary exposure estimates of 18 elements from the 1st French Total Diet Study’, *Food Addit. Contam.*, vol. 22, no. 7, pp. 624–641, Jul. 2005.

[12] O. Muñoz *et al.*, ‘Estimation of the dietary intake of cadmium, lead, mercury, and arsenic by the population of Santiago (Chile) using a Total Diet Study’, *Food Chem. Toxicol.*, vol. 43, no. 11, pp. 1647–1655, Nov. 2005.

[13] Bureau of Chemical Safety and Health Products and Food Branch, ‘Canadian Total Diet Study’. 2007.

[14] B. M. Thomson, R. W. Vannoort, and R. M. Haslemore, ‘Dietary exposure and trends of exposure to nutrient elements iodine, iron, selenium and sodium from the 2003–4 New Zealand Total Diet Survey’, *Br. J. Nutr.*, vol. 99, no. 03, Mar. 2008.

[15] Ministry of Agriculture and Forestry, *2009 NEW ZEALAND TOTAL DIET STUDY. Agricultural compound residues, selected contaminant and nutrient elements*. New Zealand: Crown, 2011.

[16] L. Nasreddine *et al.*, ‘Dietary exposure to essential and toxic trace elements from a Total diet study in an adult Lebanese urban population’, *Food Chem. Toxicol.*, vol. 48, no. 5, pp. 1262–1269, May 2010.

[17] M. Rose, M. Baxter, N. Brereton, and C. Baskaran, ‘Dietary exposure to metals and other elements in the 2006 UK Total Diet Study and some trends over the last 30 years’, *Food Addit. Contam. Part A*, vol. 27, no. 10, pp. 1380–1404, Oct. 2010.

[18] R. P. Avegliano, V. A. Maihara, and F. F. da Silva, ‘A Brazilian Total Diet Study: Evaluation of essential elements’, *J. Food Compos. Anal.*, vol. 24, no. 7, pp. 1009–1016, Nov. 2011.

[19] S. Millour *et al.*, ‘Pb, Hg, Cd, As, Sb and Al levels in foodstuffs from the 2nd French total diet study’, *Food Chem.*, vol. 126, no. 4, pp. 1787–1799, Jun. 2011.

[20] C. M. Tanase, P. Griffin, K. G. Koski, M. J. Cooper, and K. A. Cockell, ‘Sodium and potassium in composite food samples from the Canadian Total Diet Study’, *J. Food Compos. Anal.*, vol. 24, no. 2, pp. 237–243, Mar. 2011.

[21] N. Arnich *et al.*, ‘Dietary exposure to trace elements and health risk assessment in the 2nd French Total Diet Study’, *Food Chem. Toxicol.*, vol. 50, no. 7, pp. 2432–2449, Jul. 2012.

[22] L. Noël *et al.*, ‘Li, Cr, Mn, Co, Ni, Cu, Zn, Se and Mo levels in foodstuffs from the Second French TDS’, *Food Chem.*, vol. 132, no. 3, pp. 1502–1513, Jun. 2012.

[23] China National Center For Food Safety Risk Assessment, ‘China Total Diet Study’, *China Food Safety*, 2013. [Online]. Available: http://www.chinafoodsafety.net/detail.aspx?id=2F8F16B8AB6EB2D2D514FFBE794B3FB98C5610E779E432B5.

[24] M. M. Gimou, U. R. Charrondière, J. C. Leblanc, L. Noël, T. Guérin, and R. Pouillot, ‘Dietary exposure and health risk assessment for 11 minerals and trace elements in Yaoundé: the Cameroonian Total Diet Study’, *Food Addit. Contam. Part A*, vol. 30, no. 9, pp. 1556–1572, Sep. 2013.

[25] US Food and Drug Administration, ‘Total Diet Study Statistics on Element Results - 2006-2011’. US Food and Drug Administration, 2014.

[26] Food Standards Australia New Zealand, *24th Australian Total Diet Study*. Food Standards Australia New Zealand, 2014.

[27] M. M. Gimou, U. R. Charrondière, J. C. Leblanc, R. Pouillot, L. Noël, and T. Guérin, ‘Concentration data for 25 elements in foodstuffs in Yaoundé: The Cameroonian Total Diet Study’, *J. Food Compos. Anal.*, vol. 34, no. 1, pp. 39–55, May 2014.

[28] M. M. Gimou, R. Pouillot, U. R. Charrondiere, L. Noël, T. Guérin, and J.-C. Leblanc, ‘Dietary exposure and health risk assessment for 14 toxic and essential trace elements in Yaoundé: the Cameroonian total diet study’, *Food Addit. Contam. Part A*, vol. 31, no. 6, pp. 1064–1080, Jun. 2014.

[29] N. N. Aung, J. Yoshinaga, and J. I. Takahashi, ‘Dietary intake of toxic and essential trace elements by the children and parents living in Tokyo Metropolitan Area, Japan’, *Food Addit. Contam.*, vol. 23, no. 9, pp. 883–894, Sep. 2006.

[30] R. Raghunath, R. Tripathi, B. Suseela, S. Bhalke, V. Shukla, and V. Puranik, ‘Dietary intake of metals by Mumbai adult population’, *Sci. Total Environ.*, vol. 356, no. 1–3, pp. 62–68, Mar. 2006.

[31] J. M. Bastias *et al.*, ‘Determination of Dietary Intake of Total Arsenic, Inorganic Arsenic and Total Mercury in the Chilean School Meal Program’, *Food Sci. Technol. Int.*, vol. 16, no. 5, pp. 443–450, Oct. 2010.

[32] P. Liu, C. N. Wang, X. Y. Song, and Y. N. Wu, ‘Dietary intake of lead and cadmium by children and adults – Result calculated from dietary recall and available lead/cadmium level in food in comparison to result from food duplicate diet method’, *Int. J. Hyg. Environ. Health*, vol. 213, no. 6, pp. 450–457, Nov. 2010.

[33] J. L. Domingo, G. Perelló, and J. Giné Bordonaba, ‘Dietary Intake of Metals by the Population of Tarragona County (Catalonia, Spain): Results from a Duplicate Diet Study’, *Biol. Trace Elem. Res.*, vol. 146, no. 3, pp. 420–425, Jun. 2012.

[34] C. Cabrera-Vique and M. Mesías, ‘Content and Bioaccessibility of Aluminium in Duplicate Diets from Southern Spain: Aluminium in duplicate diets from Spain’, *J. Food Sci.*, vol. 78, no. 8, pp. T1307–T1312, Aug. 2013.

[35] M. N. Matos-Reyes, M. L. Cervera, R. C. Campos, and M. de la Guardia, ‘Total content of As, Sb, Se, Te and Bi in Spanish vegetables, cereals and pulses and

estimation of the contribution of these foods to the Mediterranean daily intake of trace elements', *Food Chem.*, vol. 122, no. 1, pp. 188–194, Sep. 2010.

[36] H. Sousa-Ferreira, M. N. Matos-Reyes, M. L. Cervera, S. L. Costa-Ferreira, and M. de la Guardia, 'Screening of Toxic Inorganic Arsenic Species in Garlic (Allium sativum L.)', *Food Anal. Methods*, vol. 4, no. 4, pp. 447–452, Dec. 2011.

[37] S. W. Cheung Chung, K. P. Kwong, J. Yau, and W. Wong, 'Dietary exposure to antimony, lead and mercury of secondary school students in Hong Kong', *Food Addit. Contam. Part A*, vol. 25, no. 7, pp. 831–840, Jul. 2008.

[38] C. Velasco-Reynold, M. Navarro-Alarcon, H. Lopez-Ga de la Serrana, V. Perez-Valero, A. Agil, and M. C. Lopez-Martinez, 'Dialysability of Magnesium and Calcium from Hospital Duplicate Meals: Influence Exerted by Other Elements', *Biol. Trace Elem. Res.*, vol. 133, no. 3, pp. 313–324, Mar. 2010.

[39] D. K. Adotey, V. Stibilj, Y. Serfor-Armah, B. J. B. Nyarko, and R. Jaćimović, 'Dietary supply of selenium for adolescents in three residential care orphanages in Southern Ghana', *Sci. Total Environ.*, vol. 410, no. 411, pp. 72–79, Dec. 2011.

[40] M. das G. Vaz-Tostes, T. A. Verediano, E. G. de Mejia, and N. M. Brunoro Costa, 'Evaluation of iron and zinc bioavailability of beans targeted for biofortification using *in vitro* and *in vivo* models and their effect on the nutritional status of preschool children: Iron and zinc bioavailability of beans targeted for biofortification', *J. Sci. Food Agric.*, vol. 96, no. 4, pp. 1326–1332, Mar. 2016.

[41] World Health Organization, *Guidelines for the study of dietary intakes of chemiscal contaminants*. Geneva, 1985.

[42] Joint FAO/WHO Expert Committee on Food Additives, Food and Agriculture Organization of the United Nations, and World Health Organization, Eds., *Evaluation of certain contaminants in food: seventy-second report of the Joint FAO/WHO Expert Committe on Food Additives*. Geneva, Switzerland: World Health Organization, 2011.

[43] FAO/WHO, 'Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation (2n edition)'. 2004.

[44] World Health Organization, FAO, and Internationale Atomenergie-Organisation, Eds., *Trace elements in human nutrition and health*. Geneva, 1996.

[45] Food and Nutrition Board, ‘Dietary Reference Intake: the essential guide to nutrient requirements’. National Academies Press, 2006.

[46] J. M. Llobet, G. Falcó, C. Casas, A. Teixidó, and J. L. Domingo, ‘Concentrations of Arsenic, Cadmium, Mercury, and Lead in Common Foods and Estimated Daily Intake by Children, Adolescents, Adults, and Seniors of Catalonia, Spain’, *J. Agric. Food Chem.*, vol. 51, no. 3, pp. 838–842, Jan. 2003.

[47] L. Noël, J. C. Leblanc, and T. Guérin, ‘Determination of several elements in duplicate meals from catering establishments using closed vessel microwave digestion with inductively coupled plasma mass spectrometry detection: estimation of daily dietary intake’, *Food Addit. Contam.*, vol. 20, no. 1, pp. 44–56, Jan. 2003.

[48] I. Martorell, G. Perelló, R. Martí-Cid, J. M. Llobet, V. Castell, and J. L. Domingo, ‘Human Exposure to Arsenic, Cadmium, Mercury, and Lead from Foods in Catalonia, Spain: Temporal Trend’, *Biol. Trace Elem. Res.*, vol. 142, no. 3, pp. 309–322, Sep. 2011.

[49] S. K. Egan, S. S.-H. Tao, J. A. T. Pennington, and P. M. Bolger, ‘US Food and Drug Administration’s Total Diet Study: intake of nutritional and toxic elements, 1991–96’, *Food Addit. Contam.*, vol. 19, no. 2, pp. 103–125, Feb. 2002.

[50] B. Deutch, J. Dyerberg, H. S. Pedersen, E. Aschlund, and J. C. Hansen, ‘Traditional and modern Greenlandic food — Dietary composition, nutrients and contaminants’, *Sci. Total Environ.*, vol. 384, no. 1–3, pp. 106–119, Oct. 2007.

[51] W. Becker, L. Jorhem, B. Sundström, and K. P. Grawé, ‘Contents of mineral elements in Swedish market basket diets’, *J. Food Compos. Anal.*, vol. 24, no. 2, pp. 279–287, Mar. 2011.

[52] S. Armenta, S. Garrigues, and M. de la Guardia, ‘Green Analytical Chemistry’, *TrAC Trends Anal. Chem.*, vol. 27, no. 6, pp. 497–511, Jun. 2008.

[53] S. Garrigues and M. de la Guardia, ‘Publishing in Green Analytical Chemistry’, in *Handbook of Green Analytical Chemistry*, M. de la Guardia and S. Garrigues, Eds. Chichester, UK: John Wiley & Sons, Ltd, 2012, pp. 55–66.

[54] S. Garrigues and M. de la Guardia, ‘Vibrational spectroscopy’, in *Handbook of Mineral Elements in Food*, M. de la Guardia and S. Garrigues, Eds. Chichester, UK: John Wiley & Sons, Ltd, 2015, pp. 301–312.

[55] M. de la Guardia and S. Garrigues, ‘X-ray’, in *Handbook of Mineral Elements in Food*, M. de la Guardia and S. Garrigues, Eds. Chichester, UK: John Wiley & Sons, Ltd, 2015, pp. 285–300.

[56] J. Moros, I. Llorca, M. L. Cervera, A. Pastor, S. Garrigues, and M. de la Guardia, ‘Chemometric determination of arsenic and lead in untreated powdered red paprika by diffuse reflectance near-infrared spectroscopy’, *Anal. Chim. Acta*, vol. 613, no. 2, pp. 196–206, Apr. 2008.

[57] H. Soyeurt *et al.*, ‘Potential estimation of major mineral contents in cow milk using mid-infrared spectrometry’, *J. Dairy Sci.*, vol. 92, no. 6, pp. 2444–2454, Jun. 2009.

[58] D. Wu, Y. He, J. Shi, and S. Feng, ‘Exploring Near and Midinfrared Spectroscopy to Predict Trace Iron and Zinc Contents in Powdered Milk’, *J. Agric. Food Chem.*, vol. 57, no. 5, pp. 1697–1704, Mar. 2009.

[59] V. Lebot, A. Champagne, R. Malapa, and D. Shiley, ‘NIR Determination of Major Constituents in Tropical Root and Tuber Crop Flours’, *J. Agric. Food Chem.*, vol. 57, no. 22, pp. 10539–10547, Nov. 2009.

[60] C. Collell, P. Gou, P. Picouet, J. Arnau, and J. Comaposada, ‘Feasibility of near-infrared spectroscopy to predict aw and moisture and NaCl contents of fermented pork sausages’, *Meat Sci.*, vol. 85, no. 2, pp. 325–330, Jun. 2010.

[61] D. Cozzolino, W. Cynkar, N. Shah, and P. Smith, ‘Quantitative analysis of minerals and electric conductivity of red grape homogenates by near infrared reflectance spectroscopy’, *Comput. Electron. Agric.*, vol. 77, no. 1, pp. 81–85, Jun. 2011.

[62] M. Plans, J. Simó, F. Casañas, and J. Sabaté, ‘Near-Infrared Spectroscopy Analysis of Seed Coats of Common Beans (Phaseolus vulgaris L.): A Potential Tool for Breeding and Quality Evaluation’, *J. Agric. Food Chem.*, vol. 60, no. 3, pp. 706–712, Jan. 2012.

[63] D. Martínez-Valdivieso, R. Font, P. Gómez, T. Blanco-Díaz, and M. Del Río-Celestino, ‘Determining the mineral composition in *Cucurbita pepo* fruit using near infrared reflectance spectroscopy: Predicting mineral content in summer

squash fruit by NIRS', *J. Sci. Food Agric.*, vol. 94, no. 15, pp. 3171–3180, Dec. 2014.

[64] X. Zhu, G. Li, and Y. Shan, 'Prediction of Cadmium content in brown rice using near-infrared spectroscopy and regression modelling techniques', *Int. J. Food Sci. Technol.*, vol. 50, no. 5, pp. 1123–1129, May 2015.

[65] S. Obregón-Cano, R. Moreno-Rojas, A. M. Jurado-Millán, and A. De Haro-Bailón, 'Análisis del contenido en minerales en nabizas y grelos (*Brassica rapa* L. var. *rapa*) mediante reflectancia en el infrarrojo cercano', *CyTA - J. Food*, vol. 14, no. 2, pp. 359–367, Apr. 2016.

[66] I. Gonzalez-Martin, C. Gonzalez-Perez, J. Hernandez-Mendez, and N. Alvarez-Garcia, 'Mineral analysis (Fe, Zn, Ca, Na, K) of fresh Iberian pork loin by near infrared reflectance spectrometry - Determination of Fe, Na and K with a remote fibre-optic reflectance probe', *Anal. Chim. Acta*, vol. 468, no. 2, pp. 293–301, Sep. 2002.

[67] D. Cozzolino *et al.*, 'Analysis of elements in wine using near infrared spectroscopy and partial least squares regression', *Talanta*, vol. 74, no. 4, pp. 711–716, Jan. 2008.

[68] D. Cozzolino and A. Moron, 'Exploring the use of near infrared reflectance spectroscopy (NIRS) to predict trace minerals in legumes', *Anim. Feed Sci. Technol.*, vol. 111, no. 1–4, pp. 161–173, Jan. 2004.

[69] S. Schmitt, S. Garrigues, and M. de la Guardia, 'Determination of the Mineral Composition of Foods by Infrared Spectroscopy: A Review of a Green Alternative', *Crit. Rev. Anal. Chem.*, vol. 44, no. 2, pp. 186–197, Apr. 2014.

[70] P. T. Palmer, R. Jacobs, P. E. Baker, K. Ferguson, and S. Webber, 'Use of fiel-portable XRF analyzers for rapid screening of toxic elements in FDA-regulated products', *J. Agric. Food Chem.*, vol. 57, pp. 2605–2613, 2009.

[71] H. Ida and J. Kawai, 'Analysis of wrapped or cased object by a hand-held X-ray fluorescence spectrometer', *Forensic Sci. Int.*, vol. 151, no. 2–3, pp. 267–272, Jul. 2005.

[72] D. L. Anderson, ‘Screening of foods and related products for toxic elements with a portable X-ray tube analyzer’, *J. Radioanal. Nucl. Chem.*, vol. 282, no. 2, pp. 415–418, Nov. 2009.

[73] R. Xiu-qin, Y. Yi-bin, H. Hai-bo, S. Zhou, and Z. Lian-qing, ‘Identification of Tea from Different Regions Using X-Ray Fluorescence’, *Spectrosc. Spectr. Anal.*, vol. 29, pp. 837–839, 2009.

[74] A. Agostino, and R. Falcone, ‘Rapid quantitative analysis of lead and other trace metals in glass containers by handheld ED-XRF’, *Glass Technol. - Eur. J. Glass Sci. Technol. Part A*, vol. 53, pp. 60–64, 2012.

[75] G. Reames and V. Charlton, ‘Lead detection in food, medicinal, and ceremonial items using a portable X-ray fluorescence (XRF) instrument’, *J. Environ. Health*, vol. 75, no. 6, pp. 16–20, Feb. 2013.

[76] L. Perring, D. Andrey, M. Basic-Dvorzak, and D. Hammer, ‘Rapid quantification of iron, copper and zinc in food premixes using energy dispersive X-ray fluorescence’, *J. Food Compos. Anal.*, vol. 18, no. 7, pp. 655–663, Nov. 2005.

[77] U. Çevik *et al.*, ‘Assessment of metal element concentrations in mussel (M. Galloprovincialis) in Eastern Black Sea, Turkey’, *J. Hazard. Mater.*, vol. 160, no. 2–3, pp. 396–401, Dec. 2008.

[78] P. Anacleto *et al.*, ‘Total Arsenic Content in Seafood Consumed in Portugal’, *J. Aquat. Food Prod. Technol.*, vol. 18, no. 1–2, pp. 32–45, Mar. 2009.

[79] A. Mohapatra, T. R. Rautray, A. K. Patra, V. Vijayan, and R. K. Mohanty, ‘Trace element-based food value evaluation in soft and hard shelled mud crabs’, *Food Chem. Toxicol.*, vol. 47, no. 11, pp. 2730–2734, Nov. 2009.

[80] L. Perring and F. Monard, ‘Improvement of Energy Dispersive X-Ray Fluorescence Throughput: Influence of Measuring Times and Number of Replicates on Validation Performance Characteristics’, *Food Anal. Methods*, vol. 3, no. 2, pp. 104–115, Jun. 2010.

[81] Z. Matasin, M. Ivanusic, V. Orescanin, S. Nejedli, and I. Tlak Gajge, ‘Heavy Metal Concentrations in Predator Fish’, *J. Anim. Vet. Adv.*, vol. 10, no. 9, pp. 1214–1218, Sep. 2011.

[82] N. G. Paltridge, L. J. Palmer, P. J. Milham, G. E. Guild, and J. C. R. Stangoulis, ‘Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain’, *Plant Soil*, vol. 361, no. 1–2, pp. 251–260, Dec. 2012.

[83] N. G. Paltridge *et al.*, ‘Energy-dispersive X-ray fluorescence spectrometry as a tool for zinc, iron and selenium analysis in whole grain wheat’, *Plant Soil*, vol. 361, no. 1–2, pp. 261–269, Dec. 2012.

[84] M. West *et al.*, ‘Atomic spectrometry update-X-ray fluorescence spectrometry’, *J. Anal. At. Spectrom.*, vol. 25, no. 10, pp. 1503–1545, Oct. 2010.

[85] J. Moros, F. A. Iñón, M. Khanmohammadi, S. Garrigues, and M. de la Guardia, ‘Evaluation of the application of attenuated total reflectance–Fourier transform infrared spectrometry (ATR–FTIR) and chemometrics to the determination of nutritional parameters of yogurt samples’, *Anal. Bioanal. Chem.*, vol. 385, no. 4, pp. 708–715, Jun. 2006.

[86] R. Font, M. Del Río-Celestino, D. Vélez, A. De Haro-Bailón, and R. Montoro, ‘Visible and Near-Infrared Spectroscopy as a Technique for Screening the Inorganic Arsenic Content in the Red Crayfish (*Procambarus c larkii* Girard)’, *Anal. Chem.*, vol. 76, no. 14, pp. 3893–3898, Jul. 2004.

[87] B. M. Wise, J. M. Shaver, N. B. Gallagher, W. Windig, R. Bro, and R. S. Koch, ‘Chemometrics Tutorial for PLS_Toolbox and Solo Eigenvector Research Inc’. 2006.

[88] R. W. Kennard and L. A. Stone, ‘Computer Aided Design of Experiments’, *Technometrics*, vol. 11, no. 1, pp. 137–148, Feb. 1969.

[89] L. Nørgaard, A. Saudland, J. Wagner, J. P. Nielsen, L. Munck, and S. B. Engelsen, ‘Interval Partial Least Squares Regression (iPLS): A Comparative Chemometric Study with an Example from Near-Infrared Spectroscopy’, *Appl. Spectrosc.*, vol. 54, pp. 413–419, 2000.

[90] V. Bellon-Maurel, E. Fernandez-Ahumada, B. Palagos, J. M. Roger, and A. McBratney, ‘Critical review of chemometric indicators commonly used for assessing the quality of the prediction of soil attributes by NIR spectroscopy’, *TrAC Trends Anal. Chem.*, vol. 29, no. 9, pp. 1073–1081, Oct. 2010.

[91] A. Gonzálvez, A. Llorens, M. L. Cervera, S. Armenta, and M. de la Guardia, ‘Elemental fingerprint of wines from the protected designation of origin Valencia’, *Food Chem.*, vol. 112, no. 1, pp. 26–34, Jan. 2009.

[92] K. Boeting, Z. Aguilera de Benzo, M. L. Cervera, and M. de la Guardia, ‘Authentication of the protected designation of origin horchata de Valencia through the chemometric treatment of mineral content’, *Anal. Methods*, vol. 2, no. 11, p. 1723, 2010.

[93] A. Gonzálvez, S. Armenta, and M. de la Guardia, ‘Geographical traceability of “Arròs de Valencia” rice grain based on mineral element composition’, *Food Chem.*, vol. 126, no. 3, pp. 1254–1260, Jun. 2011.

[94] V. N. Vapnik, *The Nature of Statistical Learning Theory*. New York, NY: Springer New York: Imprint: Springer, 2000.

[95] S. Armenta, M. de la Guardia, and J. Namiesnik, ‘Green Microextraction’, in *Analytical Microextraction Techniques*, Bentham Science Publishers, 2017, pp. 3–27.

Sección II

Capítulo 1

Perfil mineral de alimentos infantiles en España

Mineral profile of Spanish commercial baby food

Alba Mir-Marqués, Anna González-Masó, M. Luisa Cervera, Miguel de la Guardia

Department of Analytical Chemistry, University of Valencia, Jerónimo Muñoz Building,
50^{th} Dr. Moliner St., 46100 Burjassot (Valencia), Spain

Received 26 February 2013,

Received in revised form 9 September 2014,

Accepted 13 September 2014,

Available online 20 September 2014

Food Chemistry 172 (2015) 238-244

DOI 10.1016/j.foodchem.2014.09.074

Resumen

Los alimentos infantiles comerciales suponen una importante parte de la ingesta diaria de niños de 6 a 12 meses de edad. Se determinó el perfil mineral de los alimentos infantiles comerciales para establecer los niveles de elementos esenciales y no esenciales, y su contribución a la ingesta adecuada (AI) y al requerimiento medio estimado (EAR). Se obtuvieron treinta y cinco potitos comerciales, hechos a base de carne, pescados, vegetales y fruta, en distintos mercados españoles y se determinó posteriormente la composición de 14 elementos minerales. En general, los alimentos infantiles analizados mostraron una composición adecuada a la ingesta mineral, pero con unas contribuciones a la AI y el EAR para el hierro, zinc y calcio demasiado bajas (5-20%, 10-60% y 10-70%, respectivamente). Esta deficiencia podría asociarse con problemas de crecimiento o enfermedades cuando sean adultos, por lo que sería recomendable una fortificación con estos elementos.

Abstract

Commercial baby foods are an important part of the daily intake of babies from 6-12 month. The mineral profile of commercial baby foods in Spain was determined to establish levels of essential and non-essential elements, and their contribution to adequate intake (AI) and estimated average requirement (EAR). Thirty-five jars of commercial foods containing meat, fish, vegetables and fruit were obtained from the Spanish market and the mineral composition determined for 14 elements. In general, the baby foods analysed were sufficient for an adequate mineral intake, but contributions to AI and EAR for iron, zinc and calcium were very low (5-20%, 10-60% and 10-70%, respectively). This deficiency could be associated with growth problems or diseases in adulthood, and fortification of commercial products is recommended.

1.1. Introduction

Adequate nutrition during infancy and early childhood is essential to ensure the growth, health, and development of children to their full potential. Poor nutrition increases the risk of illness in adulthood. Early nutritional deficits are also linked to long-term impairment in growth and health [1]. From the age of 6 months, infants' needs for energy and nutrients start to exceed that provided by milk alone and thus, complementary feeding becomes necessary to fill the energy and nutrient gap [2]. Complementary foods must be introduced at this age and their absence or inappropriate composition can affect growth and development. Complementary foods need to be safe and nutritionally adequate meet energy and nutrient needs [1].

In recent years, commercial baby jars have become an important part of baby food due changes in lifestyle, and because many families do not have time to prepare homemade alternatives. These foods are sometimes the main source of food for babies between 6 and 12 month; therefore, detailed analysis of the mineral content is of particular interest to ensure infants have a balanced diet to develop properly [3,4]. In the aforementioned context, there are different parameters to be determined in baby food. In the case of essential elements, the recommended daily allowance (RDA), the adequate intakes (AI) and the estimated average requirement (EAR) must be considered. RDA is 'the amount of a nutrient that a healthy person should eat each day on average through diet to maintain good health', defined by the Food and Nutrition Board of the US National Academy of Sciences [5]. AI are the recommended average daily intakes based on observed or experimentally determined nutrient intake in a group of apparently healthy people that are assumed to be adequate; used when a RDA cannot be determined. EAR is the average daily nutrient intake level that meets the needs of 50% of healthy individuals in a particular age and gender group. For iron and zinc, EAR can be used as an estimation of the risk (%) for low intakes [6]. In the case of toxic elements, the tolerable upper intake level (UL) is the most common parameter for non-essential elements used to evaluate safety limits for babies. It is an estimate of the highest level of intake that carries no appreciable risk of adverse health effects [7].

In the literature, there are a large number of studies on breast and formula feeding, but there are few studies of complementary feeding (as baby foods) between 6 and 12 months, which according to European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) is the period where major changes occur in both macronutrients and micronutrients intakes [8]. Thus, an exhaustive analysis of baby food mineral composition for this age group is essential. Table 1.1 shows currently available literature describing the analysis of essential and non-essential elements in complementary baby foods, and it can be seen there are only a few references, which are often based on a limited number of elements (1-3) or the analyses of only a reduced number of samples. Considering the analytical approaches, it is clear the analysis of baby food mineral content requires accurate and sensitive techniques that allow multi-element determination. The use of optical emission spectroscopy with inductively coupled plasma (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) can be useful for determining the mineral profile of infant foods, in particular pureed and hermetically packaged ready meal (jars).

The main purpose of this study was to determine the level of essential and non-essential elements in commercially available baby foods in Spain and evaluate intake of essential elements as well as assessing the risk of exposure to toxic minerals.

1.2. Materials and methods

1.2.1. Sample collection

Thirty-five baby food samples (four brands, 1-4) available across the Spanish market sector were purchased. The samples were classified based on basic composition; meat (17), fish (7), vegetables only (5) and fruits only (6). The composition of each baby food sample analysed in this study is detailed in Table 1.2. Baby foods collected were recommended for children aged 6 to 12 months.

Table 1.1. Published literature on the mineral composition of baby foods

Sample	Country	n	Elements	Technique	Reference
Commercial baby food of vegetables (16) and fruits (16)	Belgium	32	Ca, Fe, Zn	FAAS	[9]
Commercial baby food of chicken based (10) and hake based (4)	Spain, UK, China, USA	14	Ca, Na, Fe, Cu, Zn, Mn, Se, Cr, Co, Ni, As, Pb, Cd, Hg	ICP-MS, AAS	[10]
Commercial baby food of chicken based (11) and fish based (8)	Spain, UK, China, USA	19	As	ICP-MS, HPLC-ICP-MS	[11]
Commercial baby food of vegetable based	Nigeria	4	Ca, Cu, Fe, K, Mg, Mn, P, Zn	ICP-OES	[12]
Commercial baby food of meat, fish, vegetables and fruit	Norway	72	Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mo, Na, Ni, Zn	HR-ICP-MS	[3]
Commercial and homemade baby food of lamb (4), beef (4) and chicken (4)	Spain	12	Na	FAAS	[4]
Commercial baby food	Italy, Spain, Slovakia, Sweden	112	Cd, Zn	ICP-OES, ICP-sf-MS	[13]
Total Diet Survey	New Zealand		Fe, Na, Se	ICP-OES	[14]
Commercial baby food of meat (12) and vegetables (12)	UK	24	Ca, Cu, Mg, Fe, Zn, K, Na, Se	ICP-OES, ICP-MS	[15]
Commercial baby food of chicken based (4) and fish based (4)	UK	8	Ca, Fe, Mg, K, Na, Zn, Se, Mo, Co, Cu, Cr, Mn, As, Ba, Ni, Cd, Sb, Pb, Hg, Al	ICP-OES, ICP-MS	[16]

NOTE: parentheses indicate the number of samples of each type of baby food analysed in each study. FAAS: Flame atomic absorption spectroscopy. AAS: atomic absorption spectroscopy. ICP-MS: inductively coupled plasma mass spectrometry. HPLC-ICP-MS: high performance liquid chromatography-inductively coupled plasma mass spectrometry. HR-ICP-MS: High resolution- inductively coupled plasma mass spectrometry. ICP-sf-MS: inductively coupled plasma sector field mass spectrometry.

Table 1.2. Composition of baby foods analysed through this study

	Sample	Composition	Recommended age	Brand
MEAT	M1	Ham, beef and vegetables	from 6 month	1
	M2	Turkey with vegetables	from 6 month	1
	M3	Chicken with rice	from 6 month	1
	M4	Lamb stew	from 6 month	1
	M5	Lamb stew	from 6 month	2
	M6	Beef with vegetables	from 6 month	2
	M7	Rice and chicken	from 6 month	2
	M8	Mashed vegetables with chicken and beef	from 6 month	2
	M9	Mashed vegetables and turkey	from 6 month	2
	M10	Rice and beef	from 6 month	2
	M11	Chicken, beef and vegetables	from 6 month	1
	M12	Beef with carrots	from 6 month	1
	M13	Beef with vegetables	from 6 month	1
	M14	Chicken with vegetables	from 6 month	1
	M15	Vegetables and rice with turkey	from 8 month	3
	M16	Mashed zucchini with beef	from 8 month	4
	M17	Vegetables with beef	from 8 month	4
FISH	M18	Sole with white sauce	from 8 month	1
	M19	Rice with hake	from 8 month	1
	M20	Selected vegetables and sea bass	from 8 month	2
	M21	Selected vegetables and monkfish	from 8 month	2
	M22	Mashed peas and rice with hake	from 8 month	2
	M23	White sauce and hake	from 8 month	2
	M24	Whiting vegetables in cream	from 8 month	1
VEGETABLES	M25	Carrots with rice in chicken broth	from 4 month	1
	M26	Mixed vegetables	from 4 month	1
	M27	Cream of green beans with potatoes	from 6 month	1
	M28	Soft cream carrots and potatoes	from 8 month	4
	M29	Soft cream of peas with potatoes	from 8 month	4
FRUIT	M30	Peach and banana	from 4 month	1
	M31	3 Fruit (pear, peach and banana)	from 4 month	1
	M32	Banana, tangerine and pear	from 4 month	1
	M33	Varied fruit (peach, apple, banana, apricot, orange)	from 4 month	1
	M34	Fruit salad and cereals	from 6 month	2
	M35	Tangerine, apple and biscuits	from 6 month	1

1.2.2. Apparatus

ICP-OES measurements were performed using a Perkin Elmer, Model Optima 5300 DV spectrometer (Norwalk, CT, USA) equipped with an auto sampler AS 93-plus and a cross flow nebulizer. Argon C-45 (purity higher than 99.995%), supplied by Carburos Metálicos (Barcelona, Spain), was employed as plasmogen and carrier gas.

A microwave laboratory system, Ethos SEL from Milestone (Sorisole, Italy), equipped with a thermocouple probe for automatic temperature control and an automatic gas leak detector, was employed for samples digestion using high pressure Teflon vessels of 100 mL inner volume. Teflon vessels were cleaned with vapours of nitric acid (69%), using the Trace CLEAN from Milestone (Sorisole, Italy) to avoid cross-contamination.

Other equipment used for sample pre-treatment included a Cryodos 50 lyophiliser Telstar (Barcelona, Spain) and an ultrasound water bath from Selecta (Barcelona, Spain, 9 L, 50 W, 50 Hz).

1.2.3. Reagents

A multi-element standard solution (100 mg L^{-1}) containing 26 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Se, Na, Sr, Tl, Ti, V and Zn) dissolved in 5% HNO_3 from Scharlau (Barcelona, Spain) was used to prepare the calibration standards. Additionally, a 1 g L^{-1} sodium standard solution for atomic absorption and 1 g L^{-1} potassium standard solution for atomic absorption, both from Scharlau, were employed for calibration.

A 1 g L^{-1} scandium atomic spectroscopy standard solution was purchased from Fluka (Buchs, Switzerland) and used an internal standard.

Nitric acid (HNO_3 69%) for trace analysis and hydrogen peroxide (H_2O_2 35%) reagent grade, both from Scharlau, were used for sample digestion.

All solutions were prepared with analytical reagent grade chemicals and ultrapure water (18 MΩ cm) obtained by purifying distilled water with the Milli-Q Millipore system (Molsheim, France).

1.2.4. Sample pre-treatment

Baby food purée samples were poured into different containers and frozen at -20ºC in a freezer before lyophilisation. They were lyophilised for a minimum of 48h at a chamber pressure of 50 Pa. Powdered samples were homogenised in a domestic Braun mixer (Kronberg, Germany) and stored in polyethylene bottles until analysis.

The water content of samples varied between 76.6 to 87.6 %, and moisture was independent of brand and composition of baby food.

Freeze-dried samples (1 g) were accurately weighed in to the Teflon digestion vessels and 8 mL of nitric acid were added, before being sonicated in an ultrasound water bath for 30 minutes at room temperature. Then 2 mL of hydrogen peroxide were added and the mixtures sonicated again for further 40 minutes. This approach was used to avoid foam formation and gas leaks during digestion. After that, the reactors were closed and placed inside the microwave oven. The microwave digestion programme was optimised previously for digestion of adult complete diets [17]. The following program was run: 3 min to reach 85°C; 85-145°C for 12 min; 145-180 °C for 10 min; 15 min at 180°C; and finally cooling down for 25 minutes. After digestion, the reactors were opened and sonicated. The digested samples were diluted in 20 mL polyethylene tubes with nanopure water before analysis using ICP-OES. Each sample, reagent blank and certified reference material (CRM) were digested in triplicate.

1.2.5. ICP-OES determination

The operating conditions of the ICP-OES equipment were as follow: 1200 W RF power, 15 L min^{-1} plasma gas flow rate, 0.5 L min^{-1} auxiliary gas flow rate, 0.7 L min^{-1}

nebuliser gas flow rate, 1.10 mL min^{-1} sample flow rate. All elements were detected in axial mode except calcium, potassium, magnesium and sodium, which were detected in radial mode. For the background correction, 2 points were used. Multi-elemental standard solutions were used for external calibration. Two sets of standards were used for minor and trace elements (0, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2 and 5 mg L^{-1}) and for potassium and sodium (0, 2, 4, 6, 8 and 10 mg L^{-1}). Digested samples were measured with a final dilution 1:2, and 1:100 for calcium, potassium, magnesium and sodium. Scandium (0.5 mg L^{-1}) was used as the internal standard and added to all samples, reagents blanks and standards.

1.2.6. Quality control

To establish precision and accuracy, CRMs were analysed under the same conditions as samples: NIST 1568a Rice Flour (National Institute of Standards and Technology, Gaithensburg, MD, USA); TORT-2 Lobster Hepatopancreas (National Research Council, Ottawa, Canada) and IAEA-359 Cabbage (International Atomic Energy Agency, Vienna, Austria).

Two reagent blanks were prepared and measured together with each batch of samples, and CRMs were digested and measured together with sample batches. Additional, control standards were measured for every series of 10 independent sample measurements.

Detection (LOD) and quantification limits (LOQ) were calculated as three- and ten- times the standard deviation of ten reagent blanks, respectively, taking into account the analytical sensitivity and sample mass. Reproducibility was calculated as the relative standard deviation of method (RSDm, %) for three independent analyses of three independent portions of the same sample. Instrument repeatability was calculated as the relative standard deviation (RSDi, %) of 10 independent measurements of a 100 µg L^{-1} multi-elemental standard solution (not including calcium, potassium, magnesium and sodium for which a standard solution of 5 mg L^{-1} was used).

1.3. Results and discussion

1.3.1. Analytical characteristics of the method

The analytical figures of merit for the method are shown in supplementary data (Table S1.1). RSDi values obtained were less than 4%. For most elements, the LOD was lower than those reported in the literature (see Table 1.3), the only exceptions being those analysed using ICP-MS.

No infant food certified materials were available and, thus, three CRM of different types of foods, similar to the analysed samples, were chosen. Table 1.4 shows the results obtained. It can be seen that they are in good agreement with the reference data. A statistical evaluation based on the regression between average obtained values and certified values indicated regression lines $y = (0.996 \pm 0.005)x - (7 \pm 20)$ for IAEA-359, $y = (0.998 \pm 0.012)x + (0.12 \pm 0.14)$ for NIST 1568a and $y = (1.007 \pm 0.017)x + (0.096 \pm 1.433)$ for TORT-2, suggesting the intercept and slope correspond in all cases to 0 and 1, and regression coefficients of 0.9999, 0.998 and 0.998, respectively.

1.3.2. Mineral content of commercial baby foods

The concentration of mineral elements in meat, fish, vegetables and fruit based baby foods, expressed in mg kg^{-1} of dry weight, are reported in supplementary data (Tables S1.2 to S1.5).

Table 1.5 shows the percentage of AI provided by each baby food group with respect to calcium, copper, potassium, magnesium, manganese and sodium. In the case of iron and zinc EAR was considered. AI values for infants of 6 to 12 months are 260, 0.22, 700, 75, 0.6 and 370 mg day^{-1} for calcium, copper, potassium, magnesium, manganese and sodium, respectively [5], and the EAR are 6.5 and 2.5 mg day^{-1} for iron and zinc, respectively [18].

Table 1.3. Detection limit values reported for the determination of mineral elements in baby foods

Element	LOD (mg/L) [9]	LOD (mg/kg) [14]	LOD (mg/kg) [10]	LOD (mg/kg) [13]	LOQ (mg/kg) [16]	LOD (mg/kg) This study
Al					0.332 (ICP-MS)	0.15 (ICP-OES)
As			0.006 (ICP-MS)		0.01 (ICP-MS)	0.3 (ICP-OES)
Ba					0.053 (ICP-MS)	0.009 (ICP-OES)
Ca			10 (AAS)	2 (ICP-sf-MS)	5.2 (ICP-OES)	0.10 (ICP-OES)
Cd			0.007 (ICP-MS)	0.0002 (ICP-sf-MS)	0.02 (ICP-MS)	0.02 (ICP-OES)
Co			0.037 (ICP-MS)		0.005 (ICP-MS)	0.05 (ICP-OES)
Cr			0.005 (ICP-MS)		0.026 (ICP-MS)	0.04 (ICP-OES)
Cu	0.0005 (GFAAS)		0.11 (ICP-MS)	0.2 (ICP-sf-MS)	0.12 (ICP-MS)	0.03 (ICP-OES)
Fe	0.06 (FAAS)		7 (AAS)	0.2 (ICP-sf-MS)	0.5 (ICP-OES)	0.05 (ICP-OES)
K					0.1 (ICP-OES)	3.0 (ICP-OES)
Mg					1.2 (ICP-OES)	0.03 (ICP-OES)
Mn			0.02 (ICP-MS)	0.04 (ICP-sf-MS)	0.048 (ICP-MS)	0.003 (ICP-OES)
Mo					0.023 (ICP-MS)	0.03 (ICP-OES)
Na		50 (ICP-OES)	10 (AAS)		1.1 (ICP-OES)	0.5 (ICP-OES)
Ni			0.038 (ICP-MS)	0.2 (ICP-sf-MS)	0.08 (ICP-MS)	0.07 (ICP-OES)
Pb			0.039 (ICP-MS)	0.0002 (ICP-sf-MS)	0.014 (ICP-MS)	0.3 (ICP-OES)
Se		0.001 (ICP-OES)	0.013 (ICP-MS)	0.2 (ICP-sf-MS)	0.022 (ICP-MS)	1.3 (ICP-OES)
Zn	0.015 (FAAS)		0.16 (ICP-MS)	0.2 (ICP-sf-MS)	0.5 (ICP-OES)	0.2 (ICP-OES)

Table 1.4. Evaluation of the accuracy of the method employed for ICP-OES determination of the mineral profile of baby foods through the comparison between values found and those certified in a series of food reference materials.

Elements	**IAEA-359**		**NIST 1568a**		**TORT-2**	
	Found values	**Certified range**	**Found values**	**Certified range**	**Found values**	**Certified range**
Al	---	---	4.8 ± 0.3	3.4 - 5.4	---	---
Ba	10.75 ± 0.5	10.5 - 11.5	---	---	---	---
Ca	18200 ± 100	17990 -19010	0.012 ± 0.003a	0.0112 - 0.0124a	---	---
Cd	0.12 ± 0.06	0.115 - 0.125	ND	0.020 - 0.024	26.2 ± 0.5	26.1 – 27.3
Cr	1.27 ± 0.04	1.24 - 1.36	---	---	ND	0.62 – 0.92
Cu	5.6 ± 0.2	5.49 - 5.85	2.65 ± 0.09	2.1 - 2.7	113 ± 5	96 – 116
Fe	146 ± 1	144.1 - 151.9	7.1 ± 0.9	6.5 - 8.3	104 ± 10	92 – 118
K	32500 ± 200	31810 - 33190	0.127 ± 0.008a	0.1272 - 0.1288a	---	---
Mg	2160 ± 10	2110 - 2210	0.056 ± 0.03a	0.054 - 0.058a	---	---
Mn	32.2 ± 0.5	31.3 - 32.5	19.8 ± 0.5	18.4 - 21.6	12.5 ± 0.5	12.4 – 14.8
Mo	---	---	1.4 ± 0.1	1.38 - 1.54	0.91 ± 0.05	0.85 – 1.05
Na	591.5 ± 0.9	567 - 601	7.12 ± 0.08	5.8 - 7.4	---	---
Ni	1.09 ± 0.1	1.00 - 1.10	---	---	2.6 ± 0.3	2.31 – 2.69
Sr	50.2 ± 0.4	47.8 - 50.6	---	47.8 - 50.6	46 ± 4	43.3 – 47.1
Zn	38.9 ± 0.5	37.9 – 39.3	18.95 ± 0.06	18.9 - 19.9	179 ± 7	174 - 186

NOTE: Analytical values are based on the “dry-weight” of reference material. Concentration expressed in mg kg^{-1}.a: concentration expressed in %.

Table 1.5. Baby foods contribution (%) to the Adequate Intake of essential elements for babies 6 to 12 moths (percentage that provides jars to the AI)

	Meat jars				**Fish jars**			
	Min.	Max.	Average	Median	Min.	Max.	Average	Median
Ca	4	25	12	10	6	38	20	22
Cu	18	73	42	41	15	53	30	28
Fe	3	11	7	6	1	10	4	3
K	25	78	53	52	29	78	47	41
Mg	14	52	34	34	22	47	37	43
Mn	9	47	26	24	11	42	26	27
Na	26	135	64	67	8	76	41	36
Zn	16	61	35	33	10	18	12	12
	Vegetable jars				**Fruit jars**			
	Min.	Max.	Average	Median	Min.	Max.	Average	Median
Ca	12	54	28	24	6	16	9	8
Cu	29	72	46	45	38	78	64	67
Fe	3	10	6	6	3	9	5	4
K	54	75	63	58	52	83	72	75
Mg	29	53	41	43	33	47	40	41
Mn	12	42	33	38	33	50	39	37
Na	48	77	63	64	1	7	4	3
Zn	13	21	17	16	6	10	7	7

Following the recommendations of Dewey and Brown [19], regarding the amount of energy to be provided by complementary foods, and with the proviso that jars are consumed at least once a day, we can estimate the contribution of available nutrients from the baby foods analysed. More specifically, at 6-8 months' complementary foods should provide 200 kcal day^{-1}, and infants should have 2-3 meals per day consisting of half jar at each meal, and at 9 to 12 months 300 kcal day^{-1} from 3-4 meals per day. Minimum and maximum values of mineral elements provided for each range are shown in Figure 1.1.

Considering the contribution to the daily intake of commercially available baby foods in Spain, it can be seen that the content of aluminium in the studied samples is higher in vegetable jars, between 4.8 to 11.5 mg kg$^{-1,}$ while the aluminium content of baby food meats, fish and fruit are very similar to each other with an average value of 3.5 mg kg^{-1}, and in the same order of magnitude as Norwegian baby foods reported by

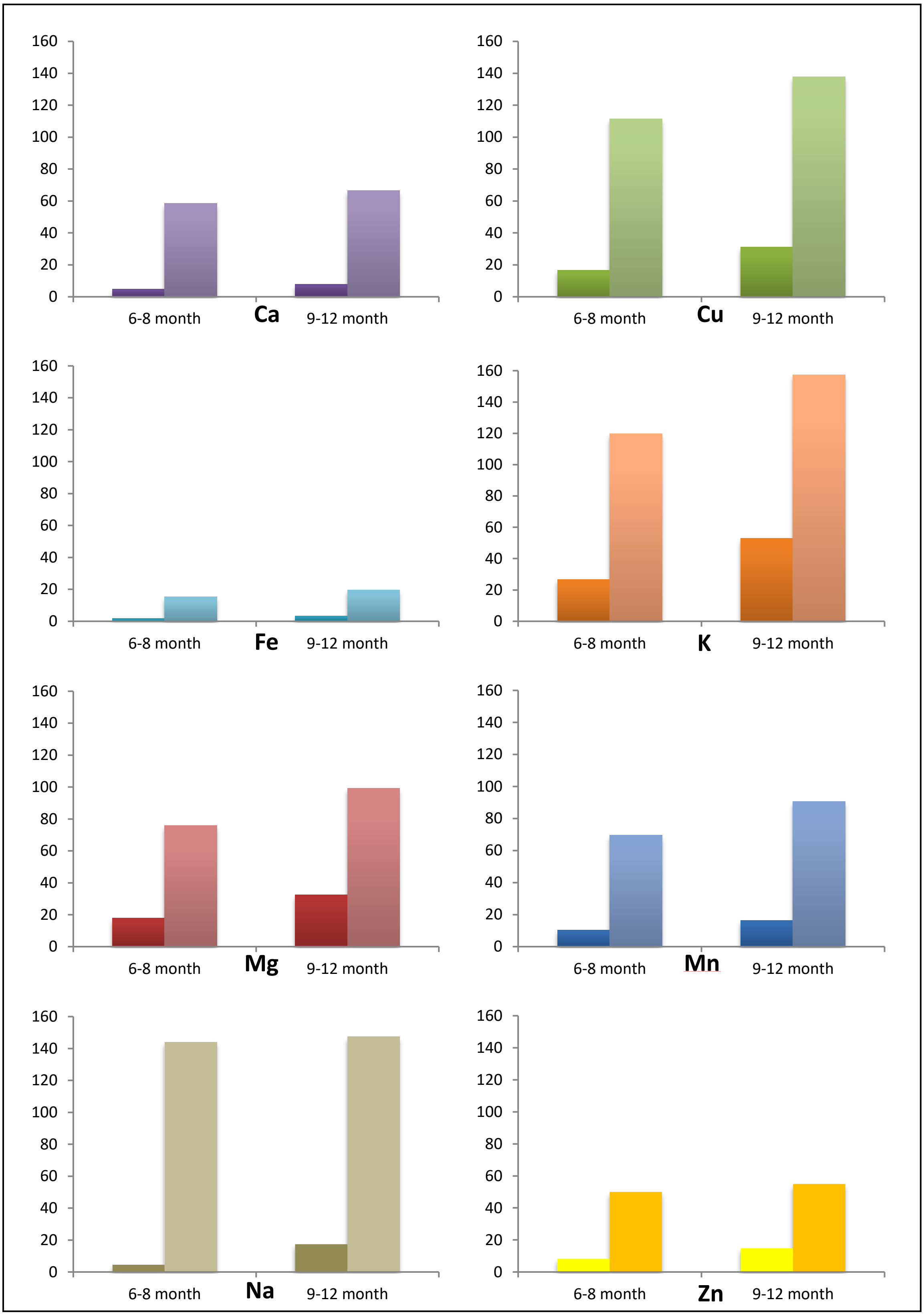

Figure 1.1. Minimum and maximum contribution (in percentage %) of the studied samples to the adequate intake (AI) and estimated average requirement (EAR) for babies 6 to 8 month and 9 to 12 month regarding the recommendations values

Melo et al. (2008) [3]. Aluminium compounds are probably added during processing, from substances such as flour or colouring agents, because fresh foods such as fruits, vegetables, and meat contain very little naturally. In this sense, it must be taken into consideration that high intakes of aluminium can cause bone diseases in infants [20].

Barium is present in all foods, above all in fish and certain plants. Usually levels of barium in food are in the range of 0.05-1.0 mg kg^{-1} [21, 22]. Barium content was highest in vegetable (average amount).

Calcium is an essential nutrient that plays a vital role in neuromuscular function, blood clotting and providing rigidity to the skeleton. In the first two years of life, infants need to absorb 120 mg of calcium daily to allow for normal growth. In the case ofbreast-feeding, a mean intake of 240 mg is required for absorption of 120 mg of calcium. Thus, FAO and WHO recommended an intake of 300 mg [23]. The amount of calcium in meat and fish baby foods was higher than vegetable and fruit jars, in some cases double the amount. The values of calcium were similar to data reported in the past for Spanish meat and fish jars [10], similar to baby food data from Norway [3], and also to meat, fish and vegetables baby foods from UK [15, 16].

Chromium is important in the metabolism of fats and carbohydrates. Chromium stimulates fatty acid and cholesterol synthesis, which are important for brain function and other body processes. Chromium is also important in the breakdown of insulin. Chromium deficiency may be seen as impaired glucose tolerance and has been reported in infants with protein-calorie malnutrition. Supplementation of chromium helps with management of these conditions. Because of the low absorption and high excretion rates of chromium, toxicity of this element is not common [24]. The values of chromium found in baby foods were lower than those previously reported in commercial baby food from Spain, Norway and UK [3, 10, 16].

It is well established that copper is essential for life, being required for infant growth, host defence mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol and glucose metabolism [25]. The highest amount of copper was found in fruit jars, followed by vegetable, meat and fish jars. The values of copper were similar to those reported previously in Spain for meat and fish jars [10] and meat baby food from UK [15], but lower than that reported for vegetable and fruit jars from Belgium [9] and higher than that found for vegetable jars from UK [15].

In the first year of life, body weight typically triples and body-wide iron content should double. After four months, iron requirements increase due to rapid growth and iron reserves diminish to pathological levels if the needs are not covered with dietary iron [26]. Commercial products are regularly fortified with iron and ascorbic acid, and usually given together with fruit juices and solid foods containing meat, fish, and vegetables. The fortification of products with iron is important in meeting the high dietary needs, especially considering the importance of optimal iron nutrition during this phase of brain development [23]. The amount of iron in analysed samples was very small; this is an indication that the jars analysed were not fortified with iron and, therefore, do not meet the needs of children between 6 and 12 months. The iron content found in samples was very similar to that reported in vegetable and fruit jars from Belgium [9], but lower than those found in baby food from Norway [3] and UK [15, 16].

Potassium is an essential mineral that works to maintain the body’s water and acid balance. It plays a role in transmitting nerve impulses to muscles, in muscle contractions and in the maintenance of normal blood pressure [27]. The greatest amounts of potassium were found in fruit baby foods following by vegetable, meat and fish jars. The content of potassium was very similar to that found in vegetable and fruit jars from Belgium [9], baby food from Norway [3] and baby food from UK [15, 16].

Lithium is present in all food, albeit at low levels. The lowest content of lithium was found in fish-based foods.

Magnesium is needed for more than 300 biochemical reactions in the body. It helps to maintain normal muscle and nerve function, keeps heart rhythm steady, supports a healthy immune system, and keeps bones strong. Magnesium also helps to regulate blood sugar levels, promotes normal blood pressure, and is known to be involved in energy metabolism and protein synthesis [28]. The content of magnesium found was very similar to that reported in vegetable jars from Belgium [9], baby food from Norway [3] and meat and vegetable baby food from UK [16], especially those based on chicken and fish baby food from UK [15].

Manganese is an essential element for many living organisms, including humans. Adverse health effects can be caused by inadequate intake or over-exposure. Manganese deficiency in humans appears to be rare, because manganese is present in many common foods [29]. Babies are exposed to manganese through foods. The human diet typically provides the necessary amount of manganese for normal body function [30]. The values of manganese found through this study were similar to data reported in the Spain for meat and fish jars [10], similar to those found in fruit jars from Belgium [9] but less than that reported from UK [16].

Sodium is an essential nutrient that is involved in numerous physiological functions, such as the maintenance of the liquid extracellular osmolarity, the control of the volume of body water compartments and the maintenance of normal blood pressure. It also plays a role in the acid-base balance. But, to meet these requirements, only a small amount of sodium is required from the diet [26]. The sodium content of fruit jars was found to be very low, but high in meat, vegetables and fish jars. The concentrations of sodium found in this study were similar to data reported in Spain for meat and fish jars [4, 10] and fruit jars from Belgium [9], but higher than baby food from UK [15, 16]. In any case, the sodium content exceeded the maximum permitted level of 200mg/100g food [31].

Nickel is not currently considered an essential nutrient for humans. Nickel is conjectured to play a role in processes related to the vitamin B12 and folic acid dependent pathway in methionine metabolism [18]. It is likely that the health effects seen in babies exposed to nickel will be similar to the effects seen in adults. We do not know whether infants differ from adults in their susceptibility to nickel. The major dietary source of nickel is plant foods. Animal foods are low in nickel [32]. The major content of nickel, as expected, was found in vegetable jars, and the least in meat and fish jars. The content of nickel was similar to chicken and fish baby food from UK [16].

Babies are exposed to stable strontium in the same manner as adults, usually in small amounts in drinking water and food. Young children who have more hand-to-mouth activity or who eat soil may accidentally eat more strontium. Infants and children with active bone growth absorb more strontium from the gut than adults. Excess stable strontium causes problems with growing bone. For this reason, babies are more susceptible to the effects of stable strontium than adults who have mature bone. Babies who unusually eat high levels of strontium may have problems with bone growth, but only if the diet is low in calcium and protein [33]. Strontium was highest in meat and vegetable jars, and the least in fruit jars.

Zinc is essential for proper growth and development of babies. Little is known about whether infants who eat too much zinc will react differently from adults who have ingested large amounts of zinc [34]. The best dietary sources of zinc are meat, particularly viscera, and shellfish, while eggs and dairy products are also relatively good sources of zinc. The bioavailability of zinc is inhibited by phytate, which is present in large amounts in cereals and legumes. The clearest indication of zinc deficiency is stunted baby growth; there is evidence that zinc supplementation can improve the growth of stunted baby [35]. Zinc was highest in meat jars, and levels were very similar to baby food from Norway [3] and baby food from UK [15, 16].

1.4. Conclusions

The levels of essential and trace elements in commercial baby foods were inadequate, in some cases (iron, zinc and calcium), to meet the AI of babies of 6 to 12-month age. Iron intake provided by the commercial baby food was between 5 and 20% of the EAR; at this age, it should be around 97% because breast milk provides only 3% of essential iron. This shows the lack of fortification of commercial baby foods analysed and the need for supplementary mineral for appropriate growth. The same is true for zinc and calcium, although these were not deficient: zinc intake provided was between 10 to 60% (should be 86%) and calcium was between 10 to 70% (should be 72%). The amounts of sodium, magnesium, copper, manganese and potassium varied between 30 and 140%; this means if receiving a varied diet, the infant would be consuming a good supply of these minerals. The concentration of toxic elements, such as cadmium and lead, was in all cases below LOD, confirming good practices in production.

Acknowledgments

Authors acknowledge the financial support of the Generalitat Valenciana (project PROMETEO 2010-055) and the Ministerio de Economia y Competitividad-Feder (project CTQ2011-25743 and project CTQ2012-38635). Alba Mir-Marqués acknowledges a grant 'Atracció de Talent' provided by the University of Valencia.

References

[1] WHO (World Health Organization), *Infant and Young Child Feeding. Model Chapter for Textbooks for Medical Students and Allied Health Professionals*, WHO, Geneva, Switzerland, 2009.

[2] WHO (World Health Organization). *Strengthening action to improve feeding of infants and Young children 6-23 months of age in nutrition and child health programmes.* WHO, Geneva, October 6-9, 2008.

[3] Melo, R., Gellein, K., Evje, L., & Syversen, T. (2008). Minerals and trace elements in commercial infant food. *Food and Chemical Toxicology, 46*, 3339-3342.

[4] Okesli, V., González-Bermúdez, C. A., Vidal-Guevara, M. L., Dalmau, J., & Ros, G. (2011). Alimentación complementaria: ¿es igual el perfil nutricional de un pure casero que el de un tarrito commercial?. *Acta Pediátrica España, 69*, 235-240.

[5] Food and Nutrition Board, Institute of Medicine, 2004. Dietary Reference Intake (DRIs): Recommended Intakes for Individuals Elements. National Academies Press.

[6] Butte, N. F., Fox, M. K., Briefel, R. R., Siega-Riz, A. M., Dwyer, J. T., Deming, D. M., & Reidy, K.C. (2010). Nutrient intakes of US infants, toddlers, and preschoolers meet or exceed dietary reference intakes. *Journal of the American Dietetic Association, 110*, S27-S37.

[7] EFSA (European Food Safety Authority), 2006. Tolerable upper intake levels for vitamins ans minerals. Scientific Committee on Food, Scientific Panel of Dietetic

Products, Nutrition and Allergies, from: www.efsa.europa.eu/en/ndatopics/docs/ndatolerableuil.pdf

[8] ESPGHAN Committee on Nutrition. (2008). Complementary Feeding: a commentary by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, *46*, 99-110.

[9] Bosscher, D., Van Cauwenbergh, R., Van der Auwera, J., Robberecht, H., & Deelstra, H. (2002). Calcium, iron and zinc availability from weaning meals. *Acta Paediatrica, 91*, 761-768.

[10] Carbonell-Barrachina, A. A., Ramirez-Gandolfo, A., Wu, X., Norton, G. J., Burlo, F., Deacon, C., & Meharg, A. (2012). Essential and toxic elements in infant foods from Spain, UK, China and USA. *Journal of Environmental Monitoring, 14*, 2447-2455.

[11] Carbonell-Barrachina, A. A., Wu, X., Ramirez-Gandolfo, A., Norton, G. J., Burlo, F., Deacon, C., & Meharg, A. (2012). Inorganic arsenic contents in rice based infant food from Spain, UK, China and USA. *Environmental Pollution*, *163*, 77-83.

[12] Fernandez, D. R., Vanderjagt, D.J., Williams, M., Huang, Y.S., Chuang, L., Millson, M. (2002). Fatty amino acid and trace mineral analyses of five weaning foods from Jos, Nigeria. *Plants Foods for Human Nutrition, 57*, 257-271.

[13] Pandelova, M., Lopez, W. L., Michalke, B., Schramm, K. (2012). Ca, Cd, CU, Fe, Hg, Mn, Ni, Pb, Se, and Zn contents in baby foods from the EU market: Comparison of assessed infant intakes with the present safety limits for minerals and trace elements. *Journal of Food Composition and Analysis, 27*, 120-127.

[14] Thomson, B. M., Vannoort, R. W., Haslemore, R. M. (2008). Dietary exposure and trends of exposure to nutrient elements iodine, iron, selenium and sodium from the 2003-4 New Zealand Total Diet Survey. *British Journal of Nutrition, 99*, 614-625.

[15] Zand, N., Chowdhry, B. Z., Zotor, F. B., Wray, D. S., Amuna, P., & Pullen, F. S. (2011). Essential and trace elements content of commercial infant foods in the UK. *Food Chemistry*, *128*, 123-128.

[16] Zand, N., Chowdhry, B. Z., Wray, D. S., Pullen, F. S., & Snowden, M. J. (2012). Elemental content of commercial 'ready to-feed' poultry and fish based infant foods in the UK. *Food Chemistry, 135*, 2796-2801.

[17] Mir-Marqués, A., Cervera, M. L., & de la Guardia, M. (2012). A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus. *Journal of Food Composition and Analysis, 27*, 160-168.

[18] NCR (National Research Council), *Dietary References Intakes for Vitamin A, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*, National Academy of Sciences, Washington, D.C., 2002, pp. 197-223.

[19] Dewey, K., & Brown, K. (2003). Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food and Nutrition Bulletin, 24*, 5–28.

[20] ATSDR (Agency of Toxic Substances & Disease Registry), *Public Health Statement for Al (2008), Ba (2007), Mn (2000a), Ni (2005), Sr (2004), Zn (2000b)*, from: www.atsdr.cdc.gov

[21] Reilly, C. (2002). *Metal Contamination of Food*, (3rd ed). Oxford Bookes University, Oxford, UK.

[22] ATSDR (Agency of Toxic Substances & Disease Registry), *Public Health Statement for Ba (2007)*, from: www.atsdr.cdc.gov

[23] FAO/WHO Human Vitamin and Mineral Requirements, 2002. Report of a Joint FAO/WHO Expert Consultation Bangkok, Thailand.

[24] Food and Nutrition Board, Institute of Medicine, 2011. Chromium in diet: MedlinePlus Medical Encyclopedia. U.S. National Library of Medicine.

[25] SCF (Scientific Committe on Food), *Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Copper*. SCF/CS/NUT/UPPLEV/57 Final, European Commission, 2003.

[26] Gil Hernández, A., Uauy Dagach, R., & Dalmau Serra, J., Comité de Nutrición de la AEP. (2006). Bases for adequate complementary feeding in infants and young children. *Anales de Padiatría (Barc), 65*, 481-495.

[27] FAO (Food, Nutrition and Agriculture), *Alimentation, nutrition et agriculture. Alimentación, nutrición y agricultura*, 2009, from: www.fao.org/docrep/x2650T/x2650T00.htm

[28] Office of Dietary Supplements, 2009. *Strengthening knowledge and understanding of dietary supplements: Magnesium*. National Institutes of Health, US.

[29] WHO (World Health Organization), *Guidelines for Drinking-water Quality: Manganese in Drinking-water*. WHO/SDE/WSH/03.04/104/Rev/1, 2011, from: www.who.int/water_sanitation_health/dwq/chemicals/manganese.pdf

[30] ATSDR (Agency of Toxic Substances & Disease Registry), *Public Health Statement for Mn (2000a),* from: www.atsdr.cdc.gov

[31] Directiva 2006/125/CE de la Comisión de 5 de diciembre de 2006 relativa a los alimentos elaborados a base de cereals y alimentos infantiles para lactantes y niños de corta edad. DOCE nº.L339/16

[32] ATSDR (Agency of Toxic Substances & Disease Registry), *Public Health Statement for Ni (2005),* from: www.atsdr.cdc.gov

[33] ATSDR (Agency of Toxic Substances & Disease Registry), *Public Health Statement for Sr (2004)*, from: www.atsdr.cdc.gov

[34] ATSDR (Agency of Toxic Substances & Disease Registry), *Public Health Statement for Zn (2000b)*, from: www.atsdr.cdc.gov

[35] Brown, K. H., Wuehler, S. E., & Peerson, J. M. (2001). The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. *Food and Nutrition Bulletin, 22*, 113-125.

Supplementary material

Table S1.1. Analytical figures of merit of ICP-OES determination of the mineral composition of baby food samples

Elements	LODi	LODm	LOQi	LOQm	RSDi
	(mg L^{-1})	(mg kg^{-1})	(mg L^{-1})	(mg kg^{-1})	(%)
Al	0.004	0.15	0.012	0.5	0.1
As	0.008	0.3	0.03	1.0	2.0
Ba	0.0002	0.009	0.0007	0.03	0.3
Be	0.002	0.06	0.005	0.2	1.3
Bi	0.010	0.4	0.03	1.4	2.0
Ca	0.003	0.10	0.009	0.3	3.0
Cd	0.0006	0.02	0.002	0.08	0.6
Co	0.0012	0.05	0.004	0.16	0.4
Cr	0.0009	0.04	0.003	0.12	0.3
Cu	0.0004	0.03	0.0015	0.06	0.2
Fe	0.0011	0.05	0.004	0.15	0.3
K	0.08	3.0	0.3	10	1.0
Li	0.00004	0.001	0.0001	0.003	1.1
Mg	0.0008	0.03	0.003	0.11	3.0
Mn	0.00007	0.003	0.0002	0.010	0.3
Mo	0.0008	0.03	0.003	0.11	2.0
Na	0.013	0.5	0.04	2.0	2.0
Ni	0.002	0.07	0.006	0.2	0.2
Pb	0.008	0.3	0.03	1.0	1.3
Se	0.03	1.3	0.10	4.0	4.0
Sr	0.00006	0.002	0.0002	0.007	4.0
Ti	0.0003	0.013	0.0011	0.04	0.2
Tl	0.003	0.11	0.009	0.4	3.0
V	0.004	0.2	0.013	0.5	0.4
Zn	0.004	0.2	0.013	0.5	0.9

NOTE: LODi instrumental detection limit. LODm detection limit of method. LOQi instrumental quantification limit. LOQm quantification limit of method. RSDi instrumental relative standard deviation.

Table S1.2. Mineral content of baby food meat jars (mg kg^{-1} dry weight)

	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**
Al	2.3 ± 0.3	2.5 ± 0.3	3.5 ± 0.4	4.6 ± 0.6	5.3 ± 0.5	6.0 ± 0.3	2.06 ± 0.17	5.5 ± 0.3	2.2 ± 0.4	3.1 ± 0.5
Ba	0.655 ± 0.014	0.477 ± 0.017	1.92 ± 0.02	1.86 ± 0.07	1.58 ± 0.08	2.17 ± 0.03	0.613 ± 0.006	2.04 ± 0.06	1.48 ± 0.07	1.361 ± 0.019
Cr	0.080 ± 0.007	0.08 ± 0.04	0.056 ± 0.011	0.14 ± 0.05	0.124 ± 0.011	0.151 ± 0.007	<LOD	0.146 ± 0.009	0.10 ± 0.03	0.157 ± 0.005
Cu	1.86 ± 0.03	2.30 ± 0.05	1.22 ± 0.03	2.96 ± 0.13	2.43 ± 0.17	3.857 ± 0.013	1.31 ± 0.03	2.59 ± 0.07	2.50 ± 0.10	3.4 ± 0.6
Fe	15.2 ± 0.4	16.9 ± 0.4	8.00 ± 0.15	25.4 ± 0.8	30 ± 2	25.7 ± 0.8	6.916 ± 0.014	30.1 ± 0.9	14.7 ± 0.3	18.60 ± 0.16
Li	<LOD	0.4 ± 0.3	<LOD	<LOD	0.80 ± 0.06	0.839 ± 0.013	0.64 ± 0.02	0.736 ± 0.009	0.219 ± 0.004	0.1497 ± 0.0015
Mn	3.36 ± 0.06	4.26 ± 0.11	3.12 ± 0.04	3.82 ± 0.14	6.9 ± 0.4	5.11 ± 0.09	2.736 ± 0.006	5.28 ± 0.13	3.25 ± 0.04	4.33 ± 0.03
Ni	<LOD	<LOD	<LOD	0.0912 ± 0.0017	0.17 ± 0.05	0.17 ± 0.09	<LOD	0.19 ± 0.09	<LOD	<LOD
Sr	3.21 ± 0.09	6.646 ± 0.008	5.65 ± 0.11	11.1 ± 0.5	10.5 ± 0.7	5.70 ± 0.07	2.92 ± 0.03	5.15 ± 0.15	5.21 ± 0.19	3.379 ± 0.006
Zn	29.3 ± 0.5	30.6 ± 0.4	18.8 ± 0.5	27.9 ± 0.9	24.4 ±1.2	21.7 ± 0.3	18.0 ± 0.2	39.0	28.9 ± 0.8	28.69 ± 0.03
Ca	288 ± 15	365 ± 12	351 ± 12	690 ± 20	1460 ± 80	1019 ± 16	1635 ± 18	1300 ± 30	1510 ± 30	637 ± 15
K	8900 ± 200	7280 ± 30	5930 ± 120	11400 ± 500	12000 ± 700	11500 ± 100	8430 ± 160	12900 ± 400	10000 ± 300	9640 ± 60
Mg	611 ± 13	639 ± 3	494 ± 10	833 ± 40	800 ± 50	693 ± 5	548 ± 7	750 ± 30	580 ± 10	518 ± 3
Na	6560 ± 150	6130 ± 30	7420 ± 180	6300 ± 300	4600 ± 300	2980 ± 50	2450 ± 40	3700 ± 100	3000 ± 100	2720 ± 8

Table S1.2. (continued) Mineral content of baby food meat jars (mg kg^{-1} dry weight)

	M11	**M12**	**M13**	**M14**	**M15**	**M16**	**M17**	**Minimum**	**Maximum**	**Mean**	**Median**
Al	4.0 ± 0.5	4.5 ± 0.5	3.5 ± 0.4	3.9 ± 0.4	2.05 ± 0.31	4.3 ± 1.6	6.4 ± 0.5	2.1	6.4	3.9	3.9
Ba	1.68 ± 0.15	1.49 ± 0.07	0.98 ± 0.05	2.48 ± 0.10	1.89 ± 0.05	0.22 ± 0.08	1.87 ± 0.04	0.22	2.48	1.46	1.58
Cr	0.24 ± 0.04	0.062 ± 0.013	0.20 ± 0.06	0.19 ± 0.07	0.055 ± 0.006	0.46 ± 0.09	0.336 ± 0.017	<LOD (1/17)	0.46	0.153	0.140
Cu	3.04 ± 0.19	1.85 ± 0.10	3.34 ± 0.18	2.89 ± 0.13	2.3 ± 0.3	1.6 ± 0.3	2.99 ± 0.05	1.22	3.857	2.50	2.50
Fe	25.5 ± 1.8	23.3 ± 1.3	31.4 ± 1.5	19.5 ± 0.5	11.0 ± 1.0	18 ± 3	25.6 ± 0.5	6.916	31.4	20.3	19.5
Li	0.106 ± 0.006	0.094 ± 0.005	0.102 ± 0.006	0.1046 ± 0.0018	0.124 ± 0.005	0.071 ± 0.017	0.0874 ± 0.0015	<LOD (3/17)	0.839	0.262	0.106
Mn	5.2 ± 0.4	3.23 ± 0.18	4.9 ± 0.3	4.35 ± 0.11	4.83 ± 0.14	2.3 ± 0.4	4.54 ± 0.07	2.3	6.9	4.2	4.3
Ni	0.32 ± 0.03	<LOD	0.207 ± 0.019	0.187 ± 0.019	<LOD	1.58 ± 0.05	1.79 ± 0.06	<LOD (8/17)	1.79	0.31	0.07
Sr	12.5 ± 1.0	8.1 ± 0.7	11.5 ± 0.7	8.48 ± 0.08	7.06 ± 0.27	2.2 ± 0.4	7.6 ± 0.3	2.2	12.5	6.9	6.6
Zn	28 ± 2	46.0 ± 1.5	47 ± 2	21.6 ± 0.7	20.8 ± 0.9	19 ±4	27.1 ± 0.8	17.99	47	28.1	27.9
Ca	890 ± 70	430 ± 30	870 ± 50	730 ± 18	1110 ± 40	600 ± 100	970 ± 30	288	1635	873	865
K	11700 ± 800	10200 ± 900	14100 ± 900	15190 ± 130	6300 ± 200	7100 ± 1100	9600 ± 400	5930	15190	10118	10031
Mg	920 ± 70	640 ± 50	1000 ± 60	930 ± 13	584 ± 18	420 ± 70	760 ± 30	420	1000	689	639
Na	7500± 500	8700 ± 700	8000 ± 500	8700 ± 100	14400 ± 600	9820 ± 1600	10100 ± 300	2450	14400	6654	6557

Table S1.3. Mineral content of baby food fish jars (mg kg^{-1} dry weight)

	M18	M19	M20	M21	M22	M23	M24	Minimum	Maximum	Mean	Median
Al	4.1 ± 0.4	1.6 ± 0.3	5.2 ± 0.5	2.8 ± 0.3	3.4 ± 0.7	2.26 ± 0.10	5.85 ± 0.09	1.6	5.85	3.59	3.35
Ba	0.326 ± 0.010	0.161 ± 0.008	2.191 ± 0.018	1.589 ± 0.007	0.50 ± 0.02	<LOD	0.076 ± 0.012	<LOD (1/7)	2.191	0.693	0.326
Cr	0.112 ± 0.015	<LOD	0.16 ± 0.03	0.236 ± 0.006	0.156 ± 0.013	0.15 ± 0.06	0.30 ± 0.06	0.057	0.30	0.169	0.156
Cu	1.65 ± 0.04	1.38 ± 0.04	2.94 ± 0.08	1.870 ± 0.018	2.64 ± 0.02	1.007 ± 0.015	1.164 ± 0.010	1.007	2.94	1.81	1.65
Fe	8.9 ± 1.2	5.8 ± 0.7	18.32 ± 0.09	18.4 ± 0.3	24.9 ± 0.4	4.5 ± 0.6	8 ± 3	4,5	24.9	12.6	8.9
Li	<LOD	<LOD	<LOD	0.80 ± 0.03	0.0875 ± 0.0008	0.0361 ± 0.0013	0.0582 ± 0.0013	<LOD (3/7)	0.80	0.155	0.036
Mn	2.23 ± 0.07	4.05 ± 0.12	5.34 ± 0.07	5.53 ± 0.05	5.78 ± 0.10	2.064 ± 0.007	5.480 ± 0.005	2.064	5.78	4.35	5.34
Ni	<LOD	<LOD	0.21 ± 0.05	0.044 ± 0.011	0.216 ± 0.015	<LOD	1.53 ± 0.02	<LOD (3/7)	1.53	0.286	0.013
Sr	5.60 ± 0.10	5.97 ± 0.17	7.81 ± 0.05	8.52 ± 0.10	4.98 ± 0.11	1.432 ± 0.018	4.163 ± 0.008	1.432	8.52	5.50	5.60
Zn	11.4 ± 0.3	8.8 ± 0.3	12.6 ± 0.2	9.5 ± 1.8	12.3 ± 0.3	10.53 ± 0.15	10.82 ± 0.04	8.8	12.6	10.85	10.82
Ca	2600 ± 50	400 ± 20	1510 ± 30	1630 ± 10	508 ± 6	1760 ± 20	1970 ± 15	400	2600	1482	1628
K	11300 ± 300	8100 ± 200	9410 ± 70	13650 ± 170	6190 ± 140	6050 ± 130	8670 ± 70	6050	13650	9048	8670
Mg	870 ± 20	810 ± 20	714 ± 6	875 ± 8	746 ± 18	497 ± 7	802 ± 1	497	875	758	802
Na	6570 ± 150	7170 ± 180	1830 ± 30	2240 ± 20	696 ± 15	4010 ± 70	7980 ± 70	696	7980	4355	4011

Table S1.4. Mineral content of baby foods vegetable jars (mg kg^{-1} dry weight)

	M25	M26	M27	M28	M29	Minimum	Maximum	Mean	Median
Al	4.8 ± 0.3	5.1 ± 0.9	6.7 ± 0.5	6.4 ± 0.3	11.5 ± 0.5	4.8	11.5	6.9	6.4
Ba	5.86 ± 0.05	1.69 ± 0.15	1.24 ± 0.09	3.13 ± 0.14	1.36 ± 0.03	1.24	5.86	2.66	1.69
Cr	0.15 ± 0.02	0.38 ± 0.06	0.30 ± 0.05	0.242 ± 0.010	0.27 ± 0.03	0.15	0.38	0.27	0.27
Cu	2.60 ± 0.02	3.1 ± 0.2	1.80 ± 0.11	3.5 ± 0.5	4.83 ± 0.07	1.80	4.83	3.2	3.1
Fe	10.9 ± 0.4	25 ± 2	18.8 ± 1.6	12.7 ± 0.6	32.2 ± 0.5	10.9	32.2	20.0	18.8
Li	<LOD	0.121 ± 0.008	0.114 ± 0.005	0.087 ± 0.003	0.095 ± 0.002	<LOD (1/5)	0.121	0.090	0.095
Mn	5.47 ± 0.07	7.8 ± 0.6	6.5 ± 0.4	2.62 ± 0.12	7.27 ± 0.15	2.62	7.8	5.9	6.5
Ni	0.15 ± 0.07	0.41 ± 0.04	1.69 ± 0.12	1.57 ± 0.06	2.24 ± 0.03	0.15	2.24	1.21	1.57
Sr	12.30 ± 0.10	15.9 ± 1.7	13.1 ± 0.9	6.0 ± 0.4	2.80 ± 0.08	2.8	15.9	10.0	12.3
Zn	16.52 ± 0.18	12.1 ± 0.8	13.7 ± 1.0	14.9 ± 0.6	19.0 ± 0.3	12.1	19.0	15.2	14.9
Ca	912 ± 10	1200 ± 120	4000 ± 300	3400 ± 200	1930 ± 50	912	4000	2283	1932
K	11000 ± 100	15000 ± 2000	14800 ± 1100	14000 ± 1000	12000 ± 400	11000	15000	13533	14441
Mg	655 ± 5	1110 ± 120	1120 ± 80	790 ± 50	980 ± 30	655	1120	930	981
Na	6840 ± 50	5400 ± 600	7300 ± 400	7300 ± 500	8700 ± 200	5400	8700	7100	7270

Table S1.5. Mineral content of baby foods fruit jars (mg kg^{-1} dry weight)

	M30	M31	M32	M33	M34	M35	Minimum	Maximum	Mean	Median
Al	1.87 ± 0.13	3.6 ± 0.6	3.13 ± 0.05	3.78 ± 0.10	3.3 ± 0.3	3.44 ± 0.14	1.87	3.78	3.18	3.37
Ba	0.553 ± 0.013	0.389 ± 0.005	1.29 ± 0.03	0.59 ± 0.02	0.688 ± 0.009	1.012 ± 0.015	0.389	1.29	0.753	0.637
Cu	2.99 ± 0.03	3.14 ± 0.04	2.652 ± 0.002	2.96 ± 0.14	2.945 ± 0.018	1.85 ± 0.03	1.85	3.14	2.757	2.953
Fe	8.8 ± 0.3	6.5 ± 0.6	9.9 ± 0.5	11.22 ± 0.09	7.68 ± 0.08	21 ± 3	6.5	21	10.8	9.3
Li	0.1006 ± 0.0002	0.191 ± 0.003	0.1392 ± 0.0003	0.107 ± 0.002	0.0415 ± 0.0004	0.087 ± 0.003	0.0415	0.191	0.1111	0.1040
Mn	6.05 ± 0.06	3.79 ± 0.03	4.20 ± 0.09	4.13 ± 0.07	4.32 ± 0.04	5.32 ± 0.08	3.79	6.05	4.64	4.26
Ni	0.56 ± 0.12	0.47 ± 0.19	0.30 ± 0.19	<LOD	0.33 ± 0.02	<LOD	<LOD (2/6)	0.56	0.30	0.31
Sr	2.695 ± 0.019	4.46 ± 0.08	4.94 ± 0.12	4.20 ± 0.07	3.15 ± 0.02	3.51 ± 0.11	2.695	4.94	3.826	3.857
Ti	<LOD	0.031 ± 0.002	0.040 ± 0.004	0.10 ± 0.05	0.064 ± 0.016	0.065 ± 0.008	<LOD (1/6)	0.10	0.052	0.052
Zn	4.79 ± 0.10	4.25 ± 0.07	4.23 ± 0.10	3.73 ± 0.06	5.033 ± 0.012	3.99 ± 0.07	3.73	5.033	4.34	4.24
Ca	288 ± 3	408 ± 3	559 ± 10	396 ± 7	299 ± 3	940 ± 20	288	940	481	402
K	10900 ± 90	9610 ± 160	10200 ± 200	11810 ± 160	9210 ± 160	8200 ± 200	8200	11810	9995	9928
Mg	670 ± 7	527 ± 10	621 ± 11	605 ± 9	599 ± 7	551 ± 12	527	670	595	602
Na	197.1 ± 1.1	233 ± 3	485 ± 11	179 ± 4	45.0 ± 0.3	555 ± 15	45	555	282	215

Capítulo 2

Análisis mineral de alimentos infantiles

Baby foods

Alba Mir-Marqués, M. Luisa Cervera and Miguel de la Guardia

Department of Analytical Chemistry. University of Valencia. Jeronimo Muñoz research building, 50th Dr. Moliner str. 46100 Burjassot. Valencia. Spain.

Chapter 32 Baby foods, published in:

Handbook of Mineral Elements in Food

ISBN: 978-1-118-65436-1

May 2015, Wiley-Blackwell

Resumen

El perfil mineral en alimentos infantiles es muy importante con el fin de establecer los niveles de elementos esenciales y no esenciales y, su contribución a la ingesta adecuada (AI) y al requerimiento medio estimado (EAR) para proveer de una nutrición adecuada durante la infancia. Esto es esencial para asegurar el crecimiento, salud y desarrollo del niño en todo su potencial, ya que una pobre nutrición aumenta el riesgo de enfermedades en la edad adulta. Este capítulo discute las diferentes estrategias para determinar el perfil mineral en los alimentos infantiles más comunes, con respecto a la preparación de muestra y método analítico usado para este fin. También se han actualizado los resultados de la bibliografía sobre contenido mineral en alimentos infantiles para niños entre 6 y 12 meses de edad.

Abstract

The mineral profile of baby food is very important in order to establish the levels of essential and non-essential elements and their contribution to adequate intake (AI) and estimated average requirement (EAR) for providing adequate nutrition during infancy. This is essential to ensure the growth, health and development of children to their full potential, because poor nutrition increases the risk of illness in adulthood. This chapter discusses different strategies for determining the mineral profile in most common childhood foods, with regard to both sample preparation and analytical method employed for determination. The results in the literature on the mineral content of baby foods used from 6 to 12 months of age are also updated.

2.1. Introduction

The first year of human life is a critical period in the development of a child. It is a period of rapid growth, with major changes in motor and cognitive function. Adequate nutrition during infancy and childhood is essential to ensure the growth, health and development of children. Malnutrition during the first 2 years of life increases the risk of illness in adulthood, principally chronic malnutrition and not attaining full growth potential. High intake of food can also have negative consequences, such as childhood obesity. Thus, it is vital to thoroughly survey the nutrient intake of infants, since nutrition at this age marks the future quality of life.

Some authorities encourage the timely introduction of complementary foods during infancy, which may be necessary for both nutritional and development reasons, and to enable the transition from breast milk to infant foods, because the ability of breast milk to meet requirements for macronutrients and micronutrients becomes limited with increasing age of the baby [1]. Early diet has an immediate effect on the health of infants in economically developing countries because of factors such as the lack of availability of suitable alternatives to breast milk, microbial contamination of foods and beverages, displacement of breast milk by less nutritious alternatives, and the physiological maturity of gastrointestinal and renal function [2].

Agencies such as the World Health Organization (WHO) and the United Nations Children’s Fund (UNICEF) have produced recommendations that encourage exclusive breastfeeding for 6 months (180 days) and nutritionally adequate and safe complementary feeding starting from the age of 6 months with continued breastfeeding up to 2 years of age or beyond [3,4].

From the age of 6 months, infant needs for energy and nutrients start to exceed that provided by breast milk and thus complementary feeding becomes necessary to fill the energy and nutrient gap [5]. The stage between 6 and 24 months of age is the most critical in terms of malnutrition, since children cease taking all the nutrients from breast milk and the nutrient composition of other foods becomes the key problem. This introduces many factors that affect infant nutrition, such as the amount of supplementary feed and its composition, consistency and bioavailability of nutrients.

In general, up to 6 months of age babies fed only on breast milk are supplied with necessary micronutrients, including Fe and Zn, which are present in low concentration but with high bioavailability and absorption. After 6 months, the child can eat solid or semi-solid food in the form of porridge or mashed foods. These foods are usually milk mixed with cereals or baby rice or mixed vegetables with meat or fruit porridge. After 9 months of age, baby food includes many types of meat and the child is introduced to fish and eggs, which are not included in the diet until 1 year of age. Additionally, the consistency of food must be such that babies can pick up the food. After 12 months, the child can start eating family foods, but chopped or mashed [2,3].

Various national and international health agencies recommend the levels of mineral elements needed for appropriate baby nutrition. Table 2.1 compares the levels recommended by different agencies for each essential element. Recommended values for 12 essential elements (Ca, Cr, Cu, Fe, Mg, Mn, Mo, P, Se, Na and Zn), together with the daily intake recommendations according to infant nutrition at three different ages (0–5, 6–11 and 12–23 months), were taken from different organizations in the USA and Canada [6], Australia and New Zealand [7], Europe [8] and the WHO [9]. From the data summarised in Table 2.1 it can be concluded that the recommended values are very similar among the different organizations. Additionally, it can be seen that essential elements can be classified according to their required amounts: K and Na at levels of around 1 g and 0.4 g day^{-1}, respectively; Ca and P at levels in the order of hundreds of milligrams per day; Mg, Fe, Zn and Mn also required at milligram per day levels; Cu at the hundreds of micrograms per day level; and Se, Mo and Cr in the low micrograms per day level. Because of this, all these elements must be carefully controlled in baby foods.

The main parameters are reported as follows:

- Estimated average requirement (EAR): the average daily nutrient intake that meets the needs of 50% of healthy individuals in a particular age and gender group.
- Recommended daily allowance (RDA), equivalent to recommended nutrient intake (RNI): the average daily nutrient intake sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a particular life stage and gender group.

- Adequate intake (AI): the recommended average daily intake based on observed or experimentally determined approximations or estimations of nutrient intake by a group of apparently healthy people that are assumed to be adequate.
- Tolerable upper intake level (UL): when an RDA cannot be determined. This is the highest average daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population [9,10].

2.2 Methods employed for determination of the mineral composition of baby foods

The main analytes to be determined in baby foods are those that can cause problems for the child's health, either by excessive or insufficient intake. For example, iron and zinc are the most problematic elements for baby's health, because of their relatively low bioavailability in infant food. Sodium content can also be a problem for infants, because despite its essential character an excess of this element may cause hypertension. The most common baby food samples are purées of different foods, as mixtures of meat or fish with vegetables or mixtures of fruits. The nutritional content of these purées is usually about 80–85% water, 10% carbohydrate, 3.2% protein, 2% fat, 1% fibre and 0.1% sodium.

Baby food samples have high water content, and samples can be analysed either wet or after being dried. The drying process allows pre-concentration of the elements to be determined and ensures better preservation of samples than that achieved for wet foods. The drying process can be accomplished by application of temperature in an oven (normally about 100°C), by applied vacuum or by lyophilisation. For oven drying, Carbonell-Barrachina et al. [12] suggest placing the samples in an oven for 78 hours at 80°C. Lyophilisation involves very low temperatures and pressures of around –50°C and 0.05 mbar, respectively [13], and these conditions are highly recommended in order to avoid the loss of highly volatile elements and chemical forms of minerals like Hg and chloride compounds.

Table 2.1. Recommended values of essential element intake for infants obtained from different national and international agencies

ELEMENT	AGE	Food and Nutrition Board (USA and Canada) [6]				Australia and New Zealand [7]				WHO [9]		Europe [8]
	months	**EAR**	**RDA**	**AI**	**UL**	**EAR**	**RDI**	**AI**	**UL**	**RDI**	**UL**	**RDA**
Ca (mg/day)	**0-5**			200	1000			210		300		
	6-11			260	1500			270		400		450
	12-23	500	700		2500	360	500		2500	500	3000	550
Cr (µg/day)	**0-5**			0.2				0.2				
	6-11			5.5				5.5				10
	12-23			11				11				20
Cu (µg/day)	**0-5**			200				200				
	6-11			220				220				400
	12-23	260	340		1000			700	1000	500[c]	1500[c]	500
Fe (mg/day)	**0-5**			0.27	40			0.2	20			
	6-11	6.9	11		40	7	11		20	7.7[a]		8
	12-23	3.0	7		40	4	9		20	4.8[a]		8
K (g/day)	**0-5**			0.4				0.4				
	6-11			0.7				0.7				0.7
	12-23			3.0				2				1.0
Mg (mg/day)	**0-5**			30				30		26		
	6-11			75				75		54		75
	12-23	65	80			65	80			60	65	80
Mn (mg/day)	**0-5**			0.003				0.003				
	6-11			0.6				0.600				0.7
	12-23			1.2	2			2.0				1.2
Mo (µg/day)	**0-5**			2				2				
	6-11			3				3				15
	12-23	13	17		300	13	17		300			25

NOTE: EAR: estimated average requirement. RDA: recommended daily allowance. RDI: recommended daily intake. AI: adequate intake. UL: tolerable upper intake level. [a] assuming 12% bioavailability; [b] assuming a moderate bioavailability; [c] [11].

Table 2.1. (continued) Recommended values of essential element intake for infants obtained from different national and international agencies

ELEMENT	AGE	Food and Nutrition Board (USA and Canada) [6]				Australia and New Zealand [7]				WHO [9]		Europe [8]
	months	**EAR**	**RDA**	**AI**	**UL**	**EAR**	**RDI**	**AI**	**UL**	**RDI**	**UL**	**RDA**
P (mg/day)	**0-5**			100				100				
	6-11			275				275				350
	12-23	380	460		3000	380	460		3000			550
Se (µg/day)	**0-5**			15	45			12	45	6		
	6-11			20	60			15	60	10		15
	12-23	17	20		90	20	25		90	17		20
Na (g/day)	**0-5**			0.12				0.12				
	6-11			0.37				0.17				0.3
	12-23			1.0	1.5			0.2-0.4	1			0.4
Zn (mg/day)	**0-5**			2	4			2.0	4	2.8[b]		
	6-11	2.5	3		5	2.5	3.0		5	4[b]		4
	12-23	2.5	3		7	2.5	3.0		7	4[b]		5

NOTE: EAR: estimated average requirement. RDA: recommended daily allowance. RDI: recommended daily intake. AI: adequate intake. UL: tolerable upper intake level. [a] assuming 12% bioavailability; [b] assuming a moderate bioavailability; [c] [11].

The most common measurement techniques for the analysis of mineral composition in baby foods are atomic absorption and emission spectrometry. However, to carry out the mineral analysis using these methodologies requires liquid samples, so that baby food samples need previous dissolution or digestion. Figure 2.1 shows the methodology used to determine mineral elements in infant food, and also shows how mineral speciation could be accomplished using prior separation of the different mineral compounds of the same element by chromatographic or non-chromatographic techniques.

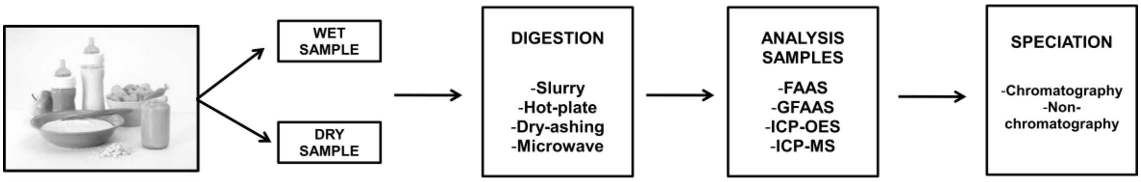

Figure 2.1. Scheme of general methodology used for mineral element analysis in baby food

2.2.1 Mineralisation and dissolution of samples

The organic nature of baby foods makes samples insoluble in water and therefore digestion of baby food samples with acid is mandatory in order to obtain a solution of the mineral compounds. Figure 2.2 shows the parameters (temperature, pressure, reagents) required for complete digestion of baby foods in order to destroy the matrix without loss of minerals.

Mineral elements can be dissolved by simply using reagents at room temperature, thus providing solutions or slurries of samples. It is true that many of the elements to be analysed are easily soluble in water or dilute acids, but such dilution of baby food samples with aqueous extraction solutions causes problems related to matrix effects during measurement and compels the use of highly sensitive techniques for determination of trace and ultratrace elements.

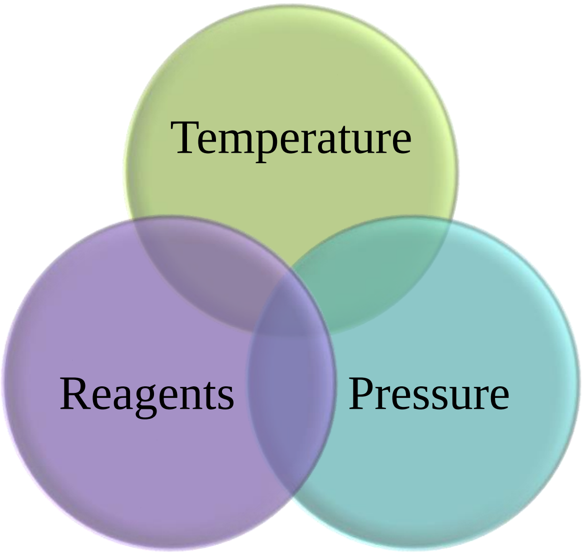

Figure 2.2. Key parameters used for the digestion of baby food samples

Dry ashing or wet ashing are the main strategies for digestion of baby food samples. Dry ashing uses basically temperature and total decomposition of the matrix, thus providing a high pre-concentration of samples to be analysed. However, it is clear that the simple use of temperature and time causes the loss of volatile elements and compounds and so ashing aids have been proposed to reduce these problems. Indeed, the combined use of temperature and acids is the most useful technique for complete dissolution of the food ash. The use of pressure is probably the best way to accelerate food sample digestion. Nowadays, pressurised microwave-assisted digestion is the most commonly used digestion process.

In some cases, direct analysis of solid samples can be performed by using simple laser ablation of samples or by direct atomisation of sample slurry in a graphite furnace. Methods employed for mineralisation of baby food samples are summarised in Table 2.2 and it can be seen that sample slurries, digestion with HNO_3 and HCl on a hot plate, dry ashing and microwave-assisted digestion are the most commonly used procedures.

Slurry sampling

The simplest sample preparation is direct analysis of samples without applying temperature or pressure or the addition of any reagent, despite there are no similar studies in the literature in recent years. However, it is quite common to prepare sample slurries in water when analysing infant food, in which case it is only necessary to add aqueous solutions of acids or surfactants and then homogenise the sample. Slurry sampling has many advantages, such as reduced sample preparation time, decreased analyte losses, reduced risk of contamination, and elimination of hazards associated with the use of acids at high temperatures. Slurries can then be nebulised into a flame (FAAS) or plasma (ICP-OES/MS) or deposited in a graphite tube for GFAAS.

Table 2.2. Methods employed in the analytical literature for the mineralization of baby food samples

Sample	Digestion process	Reference
Baby food reference (TUGAP 1201 R)	Slurry (Triton X-114/HNO_3) MW (HNO_3/H_2O_2)	[14]
Commercial baby food of chicken, hake, vegetables and fruit based	Slurry (Triton X-100/HNO_3/H_2O_2) MW (HNO_3/H_2O_2)	[15]
Commercial baby food of fish based	Slurry (Triton X-100/HNO_3/H_2O_2) MW (HNO_3/H_2O_2)	[16]
Commercial infant rice, pureed infant foods (hake and chicken based)	Dry-ashing	[19]
Commercial and home-made baby food of lamb, beef and chicken	Dry-ashing	[21]
Home-made baby food meat based (chicken and veal)	Dry-ashing	[23]
Commercial baby food of vegetable based	Hot-plate (HNO_3/HCl)	[17]
Total diet survey	HNO3/HCl	[18]
Commercial infant cereals, baby food of chicken based, hake based and infant rice	MW(HNO_3/H_2O_2)	[12]
Foods consumed by children	MW (HNO_3/H_2O_2)	[25]
Commercial baby food of vegetables and fruits	MW (HNO_3/H_2O_2)	[26]
Commercial baby food of meat and vegetables	MW (HNO_3/H_2O_2)	[27]
Commercial baby food of meat, fish, vegetables and fruit	MW (HNO_3)	[28]
Commercial baby food	MW (HNO_3)	[29]
Infant food	MW (HNO_3)	[30]
Commercial baby food of chicken and fish	MW (HNO_3/HCl/H_2O_2)	[31]

NOTE: MW: Microwave assisted digestion

Baby food slurry is usually composed of a small amount of sample plus Triton X-100, nitric acid and an antifoam agent. The addition of nitric acid facilitates extraction of the analyte or analytes into the liquid phase of the slurry, while Triton X-100 serves as a dispersing agent of solid particles. Ozbekand Akman [14] reported a method for slurry analysis of baby food for Fe and Zn determination using FAAS. To prepare the slurry, 0.5 g of sample were weighed and suspended in 20 mL of a diluent containing 0.05% Triton X-114 and 0.1 M nitric acid. The slurries were homogenized using an Ultra-Turrax at 15,000 rpm for 5 min. This method was compared with microwave-assisted digestion, using GBW-08503 Wheat flour and IRMM-804 Rice flour, obtaining recovery percentages of 95% and 98% for Fe and 103–104% and 101–102% for Zn, respectively for slurry and microwave digestion. The total time for slurry preparation was 20 min, while the time required for microwave digestion was 135 min. For the determination of Cu, Co, Ni, Mn, Se, Pb and Cd in samples of meat, fish, vegetable and fruit baby foods and fish baby foods, Viñas et al. [15,16] weighed the samples and prepared suspensions by diluting with 5 mL of a solution containing 0.1% w/v Triton X-100, 1% v/v nitric acid and 30% w/v hydrogen peroxide (1% w/v ammonium dihydrogen phosphate only for Cu), with measurement by GFAAS. Slurries were homogenized by movements of the plunger, which took about 5 min. The suspensions were sonicated for a few minutes and stirred with a magnetic stirrer before measurement by GFAAS. The accuracy of the method was verified by using the reference materials HDP Total diet, DORM-2 Dogfish muscle, SRM 1566a Oyster tissue, SRM 1577b Bovine liver, SRM 1568a Rice flour and SRM 1572 Citrus leaves. This sample preparation method was compared with microwave-assisted digestion; Student’s t-test revealed no difference between the results obtained using either of the assayed procedures at a level of significance of 0.05.

Wet digestion

Wet digestion of baby foods uses mixtures of acids, generally nitric acid mixed with other acids or with hydrogen peroxide, which are heated on a hot plate. In a study involving the determination of eight essential elements (Ca, Cu, Fe, K, Mg, Mn, P and

Zn) by ICP-OES [17], the decomposition of different weaning foods was made on a hot plate, with 0.1 g of sample being digested in 125 mL beakers with 20 mL concentrated nitric acid and 1 mL concentrated perchloric acid. Samples were covered with glass and set on a hot-plate at 120°C for 1 hour. After that, the temperature was increased to 150°C and samples refluxed overnight. Then, samples were taken to almost dryness in order to reduce the volume to approximately 1 mL, at the same temperature. Dried samples were treated with 2.5 mL of nitric/perchloric acid (4:1). After cooling, solutions were transferred to tubes and diluted to a 50 mL final volume with deionised water.

Thomson *et al*. [18] determined Na and Fe in the New Zealand Total Diet Survey by ICP-OES by also using wet digestion. In this study, 0.5–2.5 g of the homogenised food were digested with nitric and hydrochloric acids. The accuracy of the method was verified by measurement of NIST 1549 Non-fat milk powder, SRM 1577b Bovine liver, RM 8414 Bovine muscle, SRM 1568a Rice flour, SRM 1515 Apple leaves and SRM 1566b Oyster tissue. The accuracy of the determinations, based on recovery from certified reference materials, was better than 85%. These results indicate the suitability of wet digestion at atmospheric pressure for the determination of trace elements in baby foods. However, the atmospheric pressure wet digestion is time-consuming, analyte contamination and losses occur, and often this kind of digestion does not allow complete decomposition of the sample matrix.

Dry ashing

Dry ashing has been commonly used in baby food analysis. This method allows ash dissolution in small amounts of reagents, whereas wet digestion uses large amounts of reagents. On the other hand, dry digestion has the drawback of time (1 day or more) and requires attention from the analyst.

Burló *et al*. [19] determined As in baby food using dry ashing at 450°C for 12 hours and HGAAS. In this method, 1 g of dry sample was weighed, adding 1 mL ashing aid and 5 mL nitric acid 50% v/v, then the mixture was evaporated on a sand bath total dryness. Samples were mineralized as described [20]. Ashes from the digested samples were dissolved in 5 mL HCl 50% (v/v) and 5 mL KI-ascorbic acid. After 30 min, solutions were diluted to the volume with HCl 50% (v/v) and filtered. Okesli *et al*. [21] used an official method [22] for the determination of sodium by flame atomic emission spectrometry and previous incineration of the samples in a muffle furnace at 525°C, while Van den Boom *et al*. [23] used the dry digestion official method [24] for the determination of Na, K, Ca, Mg, Fe and Zn by atomic spectrometry.

Microwave-assisted digestion

Wet sample digestion can be performed on a heating plate at atmospheric pressure or inside closed vessels by microwave heating at high pressure. The use of microwave-assisted sample digestion has increased considerably in the last few decades. This technique is rapid and allows total decomposition of sample with minor risks of contamination and minor analyte losses. In the majority of published papers nitric acid and hydrogen peroxide were used for the digestion of baby food samples. The volume of nitric acid varies from 2 to 8 mL and hydrogen peroxide from 0.5 to 2 mL, depending on the study. For example, Bosscher *et al*. [25,26] used 2 mL of nitric acid and 0.5 mL of H_2O_2 for the digestion of 0.4 g of baby food to be analysed by FAAS for determination of Ca, Fe and Zn. The accuracy of the method was verified by measurement of NIST 1573a Tomato leaves, NBS 1549 Non-fat milk powder and NBS 1571 Orchard leaves, obtaining recovery of 94, 114 and 107% for Ca, Fe and Zn, respectively. Carbonell-Barrachina *et al*. [12] used 2 mL of HNO_3 and 2 mL of H_2O_2 to digest meat and fish baby food samples, with digestion of 30 min at 95°C.The method was validated using NIST SRM 1568a Rice Flour, obtaining recoveries of 64.2–99.5% for 13 analytes studied by ICP-MS. Ozbekand Akman [14] proposed the use of 7 mL of HNO_3 and 1 mL of H_2O_2, with a digestion step at 190°C for 15 min. IRMM-804 Rice Flour and GBW-08503 Wheat flour were used as certified reference materials for evaluation of the effectiveness of the digestion. The recovery percentages obtained were 98% for Fe and 101–102% for Zn. Zand *et al*. [27] used 5 mL of HNO_3 and 0.5 mL of H_2O_2 to digest 0.5 g of wet baby food sample. The recoveries obtained for Ca, Cu, Mg, Fe, Zn, K, Na and Se were 90–112%. Other authors [28–30] used only HNO_3 for sample digestion, with acid volumes of 3–8 mL.

As can be seen, microwave digestion is very versatile, provides a digestion time lower than that required for digestion with acids at atmospheric pressure or for dry ashing, and reagent consumption is not very high, especially when working with sealed reactors. It allows fast and complete dissolution of both dry and wet baby food samples.

2.2.2 Analytical methods

Various factors should be considered when choosing an appropriate measurement technique for determination of the mineral profile in baby foods. Such factors include detection capability and sensitivity, availability of instrumentation, speed of analysis, cost and application, as well as classical features like accuracy, precision and selectivity. Table 2.3 shows a comparison of the characteristics of the main measurement techniques used to determine mineral elements in baby food. The analytical methods employed in the literature to determine minerals in baby food are indicated in Table 2.4, from which it can be seen that minor elements like Fe, Zn and Ca have been frequently determined by FAAS. Trace elements like Cu, Co, Ni, Mn, Se, Cd and Pb were analysed using GFAAS and a more or less complete mineral profile of baby foods was established using ICP-OES or ICP-MS.

Table 2.3. Comparison of the main characteristics of measurement techniques commonly employed for the determination of the mineral profile of baby foods

Technique	**Multielement**	**LOD (µg/kg)**	**Precision (%)**	**Cost**
FAAS	No	1-100	0.1-1	Low
GFAAS	No	0.01-1	1-5	Moderate
ICP-OES	Yes	1-100	0.1-1	High
ICP-MS	Yes	0.001-0.1	0.2-2	High

Flame atomic absorption spectrometry

FAAS is a well-known technique for the analysis of trace elements in baby food samples. However, despite being a simple and robust tool for the analysis of a wide range of elements, it is a single-elemental technique.

Fe and Zn have been determined by FAAS in vegetable and fruit baby foods [25,26]. Samples were digested with HNO_3 and H_2O_2 in a microwave oven and analysed using the method of standard addition from 0 to1 mg L^{-1} for Fe, and from 0 to 0.4 mg L^{-1} for Zn, with a dilution factor of 4 for Fe and 5 for Zn. This method shows linearity between 0 and 10 mg L^{-1} and between 0 and 0.5 mg L^{-1} for Fe and Zn, respectively, with good detection limits of 0.06 mg L^{-1} and 0.015 mg L^{-1} (Table 2.5). The reproducibility of the method for 10 measurements of vegetable jars was 2.5–15% for Fe and 8–9.4% for Zn, and for fruit jars was 4.7–14.4% and 8.9–15.8% for Fe and Zn, respectively.

Table 2.4. Analytical methods employed in the recent literature to determine mineral elements in baby food

Sample	**Elements**	**Analytical technique**	**Reference**
Baby food reference (TUGAP 1201 R)	Fe, Zn	FAAS	[14]
Foods consumed by children	Fe, Zn	FAAS	[25]
Commercial baby food of vegetables and fruits	Ca, Fe, Zn	FAAS	[26]
Commercial baby food of chicken, hake, vegetables and fruit based	Co, Cu, Ni, Mn	GFAAS	[15]
Commercial baby food of fish based	Cd, Se, Pb	GFAAS	[16]
Foods consumed by children	Cu	GFAAS	[25]
Commercial infant cereals, baby food of chicken based, hake based and infant rice	Ca, Co, Cr, Cu, Fe, Mn, Na, Ni, Se, Zn	ICP-MS	[12]
Commercial baby food of meat and vegetables	Ca, Cu, Fe, K, Mg, Na, Se, Zn	ICP-OES/ICP-MS	[27]
Commercial baby food of meat, fish, vegetables and fruit	Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mo, Na, Ni, Zn	ICP-MS	[28]
Commercial baby food	Ca, Cu, Fe, Mn, Ni, Se, Zn	ICP-OES	[29]
Commercial baby food of chicken and fish	Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Se, Zn	ICP-OES/ICP-MS	[31]

Ozbekand Akman [14] used FAAS for Fe and Zn determination in baby food samples. In this case, slurry sampling with Triton X-114 and nitric acid was used for the direct analysis of these elements. For the determination of Fe a wavelength of 256.8 nm was used, with a limit of detection (LOD) of 5.46 mg kg^{-1}. In the case of Zn, a wavelength of 213.9 nm was used, with an LOD of 3.37 mg kg^{-1} (Table 2.5). These LODs are quite high, but bearing in mind that samples were not digested and were directly determined in sample slurry, it must be accepted that the sensitivity is sufficient for screening purposes.

Table 2.5. Limit of detection values reported in the literature of recent years for the determination of mineral elements in baby foods

Element	LOD (mg/kg) [12]	LOD (mg/kg) [13]	LOD (mg/kg) [14]	LOD (mg/L) [25]	LOD (mg/kg) [29]	LOD (mg/kg) [31]
Al		0.15 (ICP-OES)				0.1 (ICP-MS)
As	0.006 (ICP-MS)	0.3 (ICP-OES)				0.003 (ICP-MS)
Ba		0.009 (ICP-OES)				0.02 (ICP-MS)
Ca	10 (AAS)	0.10 (ICP-OES)			2 (ICP-OES)	1.56 (ICP-OES)
Cd	0.007 (ICP-MS)	0.02 (ICP-OES)				0.006 (ICP-MS)
Co	0.037 (ICP-MS)	0.05 (ICP-OES)				0.0015 (ICP-MS)
Cr	0.005 (ICP-MS)	0.04 (ICP-OES)				0.0078 (ICP-MS)
Cu	0.11 (ICP-MS)	0.03 (ICP-OES)		0.0005 (GFAAS)	0.2 (ICP- OES)	0.036 (ICP-MS)
Fe	7 (AAS)	0.05 (ICP-OES)	5.46 (FAAS)	0.06 (FAAS)	0.2 (ICP- OES)	0.15 (ICP-OES)
K		3.0 (ICP-OES)				0.03 (ICP-OES)
Mg		0.03 (ICP-OES)				0.36 (ICP-OES)
Mn	0.02 (ICP-MS)	0.003 (ICP-OES)			0.04 (ICP- OES)	0.014 (ICP-MS)
Mo		0.03 (ICP-OES)				0.0069 (ICP-MS)
Na	10 (AAS)	0.5 (ICP-OES)				0.33 (ICP-OES)
Ni	0.038 (ICP-MS)	0.07 (ICP-OES)			0.2 (ICP- OES)	0.024 (ICP-MS)
Pb	0.039 (ICP-MS)	0.3 (ICP-OES)				0.0042 (ICP-MS)
Se	0.013 (ICP-MS)	1.3 (ICP-OES)			0.2 (ICP- OES)	0.0066 (ICP-MS)
Zn	0.16 (ICP-MS)	0.2 (ICP-OES)	3.37 (FAAS)	0.015 (FAAS)	0.2 (ICP- OES)	0.15 (ICP-OES)

In infant food analysis, Fe and Zn are the two most important elements, and their lack in infant nutrition often creates greater deficiency problems in childhood. Thus, it can be concluded that FAAS is a good technique if only Fe and Zn are to be measured, as it has good sensitivity and accuracy and low cost, which makes this an adequate methodology for small laboratories.

Graphite furnace atomic absorption spectrometry

GFAAS provides a part per billion detection capability using only a few microlitre samples deposited inside a graphite tube. This technique is a versatile and highly sensitive tool for food analysis. Viñas *et al*. [15] reported a method for the analysis of meat, fish, vegetable and fruit baby food slurries with regard to the determination of Cu, Co, Ni and Mn. Optimum atomization temperatures for the analytes were 1800°C for Cu, 2600°C for Co, 2600°C for Ni and 2000°C for Mn. The wavelength used to determine Cu, Co, Ni and Mn in baby food were 324.8, 240.7, 232.0 and 279.5 nm, respectively. To evaluate the precision of the method, the relative standard deviation (RSD) values were 3.7–11.2% for Cu, 0.7–1.7% for Mn, 2.4–9.9% for Ni and 6.2–9.6% for Co. Lower RSD values were found for jars of vegetables and fruits, and the highest RSD values were obtained for meat and fish jars.

Viñas *et al*. [16] also employed baby food slurries to determine toxic elements (Cd and Pb) by GFAAS. They used wavelengths of 283.3 and 228.8 nm for Pb and Cd analysis, respectively. LODs obtained by this method were 0.4 μg kg^{-1} for Cd and μg kg^{-1} for Pb, and the corresponding RSD values were 2.5 and 2.7%.

Determination of Cu in samples of food for children aged 2–3 years was made by GFAAS, after a previous microwave-assisted digestion of samples with HNO_3 and H_2O_2 [25]. The applied dilution factor was 10, with LOD of 0.5 μg L^{-1}and RSD of 2% for 10 independent determinations in the same sample.

Inductively coupled plasma optical emission spectrometry

ICP-OES is a powerful multi-element technique for the determination of trace elements in foods and offers a very wide linear dynamic range of about five orders of magnitude. Usually, the LODs obtained by ICP-OES are comparable with those obtained by FAAS and higher than those found by GFAAS by about one or two orders of magnitude.

In a study of the mineral profile of infant food, Ca, Cu, Fe, Mn, Ni, Se and Zn were determined by ICP-OES [29]. The wavelengths selected were 393.366, 324.752, 259.939, 257.61, 231.604, 196.026 and 213.857 nm for Ca, Cu, Fe, Mn, Ni, Se and Zn, respectively. LODs obtained are shown in Table 2.5. The accuracy of the method was calculated by RSD, and the values obtained were less than 2% for Mn and Zn, less than 3% for Ca and Fe, less than 5% for Cu, less than 7% for Ni and less than 12% for Se.

In other multi-element studies of baby food samples, ICP-OES was used to determine Ca, Cu, Mg, Fe, Zn, K and Na in meat and vegetable jars [27] or to determine Ca, Fe, Mg, Mn, Na, K and Zn in meat and fish jars [31]. In both cases, microwave digestion with nitric acid and hydrogen peroxide for sample preparation were used. The limits of quantification (LOQ) data were 5.2, 0.5, 0.1, 0.048, 1.2, 1.1 and 0.5 mg kg^{-1} for Ca, Fe, Mg, Mn, K, Na and Zn, respectively.

In short, it can be concluded that ICP-OES is a very good technique for evaluating the mineral profile of baby foods, because it allows a large number of elements to be determined simultaneously in a short period of time. It is a robust and sensitive technique with good LODs and the cost of analysis is not excessive despite the high consumption of gas required for plasma stabilization and analyte measurement. ICP-OES is a good choice when the aim is to determine elements at mg L^{-1} levels in baby foods. Conversely, if the objective is to measure elements at ultratrace levels, the sensitivity of this technique is not sufficiently high and a pre-concentration step will be mandatory.

Inductively coupled plasma mass spectrometry

ICP-MS involves the detection of ions extracted from the sample nebulised inside a high-temperature argon plasma. ICP-MS provides multi-element data with LODs lower than those of FAAS and ICP-OES. Some of the specific advantages of ICP-MS include extremely good sensitivity and selectivity, wide linear dynamic range and very fast acquisition of quantitative information about the elements present in the dissolved samples.

In a multi-element study of infant cereal and puréed infant food, Cu, Mn, Zn, Se, Cr, Ni and Co were determined by ICP-MS [12]. LODs obtained with this method can be seen in Table 2.5. The recovery percentages obtained varied were between 80 and 120%. In this study samples from different countries (Spain, UK, China and USA) were compared.

ICP-MS was also used to determine Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mn, Mo, Na, Ni, Pb and Zn in 74 infant food products for babies aged 4–18 months [28]. The accuracy of the method was verified by analysis of a certified material NIST 1577b Bovine Liver. The concentrations found were within 95–105% of the certified values. To assess possible contamination during sample preparation, blanks were prepared using the same procedure employed for samples.

2.3 Mineral composition of baby foods

The daily mineral intake of children can be evaluated by direct determination of the mineral profile of baby foods (Figure 2.3) or by indirect strategies based on both the estimation of food consumed by babies and the mineral composition of basic foods consumed in a country. One of the most common strategies employed to evaluate the mineral intake of baby foods is based on the Feeding Infants and Toddlers Study (FITS). This provides an estimate of the elemental composition of baby foods by surveying parents about the food intake of their children and by accessing data collected in different food data bases. Devaney *et al*. [32] and Heird *et al*. [33] reported data for

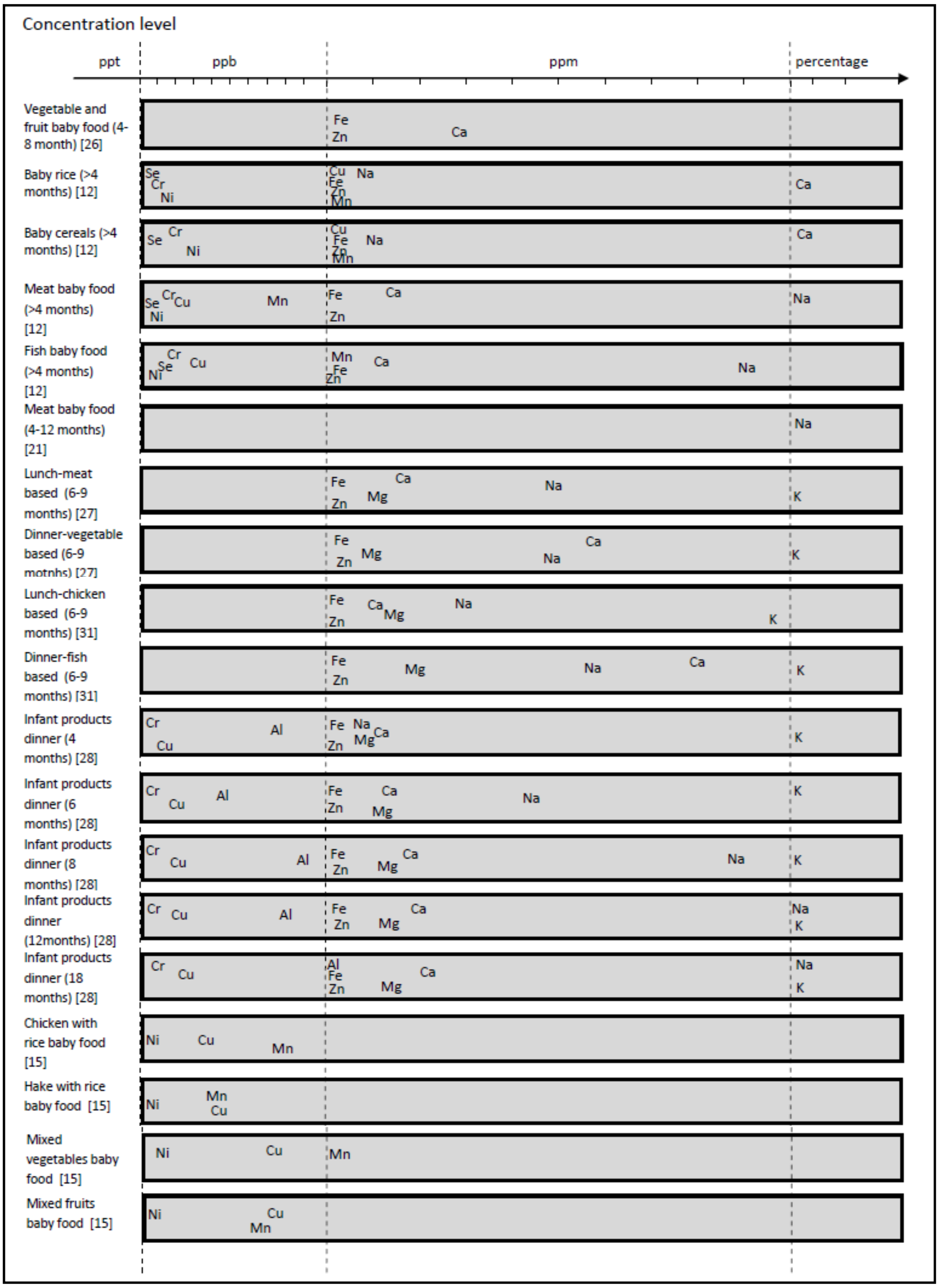

Figure 2.3. Concentration values of mineral elements in baby food samples

Ca, P, Mg, Fe, Zn, Na and K for infants and toddlers aged 4–24 months. These studies consisted of three separate telephone interviews: a household interview, a 24-hour dietary recall and a second-day 24-hour dietary recall for a random subsample of respondents with supplementary questions on growth, development and feeding patterns and a second dietary recall for a random subset of samples. Data obtained with the survey were entered in the Software of Intake Distribution Estimation (Iowa State University). This program provides estimates of the percentiles of usual nutrient intake distributions, as well as estimates of the proportion above or below defined cut-off values. Six years later, Butte *et al*. [34] followed the same procedure to estimate the intake of Ca, P, Mg, Fe, Zn, Na and K in children aged 0–4 years.

2.3.1 Essential element content in baby foods

As indicated in the introduction, mineral elements play an important role in the normal development of the child. Essential elements are involved in numerous biochemical reactions in the body, affecting physiological, neuromuscular and cardiac functions. They also participate in the development and maintenance of bones and muscles. In this section, we will comment in detail on the influence of each of the commonly analysed elements in baby foods.

Potassium is the major cation of intracellular fluid and an almost constant component of lean body tissues, and maintains body water and acid balance. It plays a role in transmission of nerve impulses to muscles, in muscle contraction and in the maintenance of normal blood pressure [35]. About 85% of potassium is absorbed by the human organism. Potassium is a non-problematic element in infant feeding. It is usually present in baby food in adequate quantities at recommended intake values.

Sodium is an essential nutrient that is involved in numerous physiological functions, such as maintenance of extracellular osmolarity, controlling the volume of body water compartments and the maintenance of normal blood pressure [36]. The intake of calcium and potassium can affect sodium needs. The major adverse effect of increased sodium chloride intake in babies concerns the increase in blood pressure; a risk factor for cardiovascular and renal diseases [37]. Sodium is one of the most important elements to be controlled in baby diets, because abuse of this element can cause various health problems. Because of this, it is very important to control sodium in commercially available baby foods, and it is recommended to not add salt to baby food. In the literature, values of sodium agree well with the recommended values, but in two studies of meat-based infant food the content of sodium was higher than the recommended values [21,23].

Calcium is required for the correct development and maintenance of the skeleton. It plays a vital role in neuromuscular and cardiac functions. It is stored in the teeth and bones where it provides structure and strength. In the first 2 years of life, infants need 120 mg of calcium daily to allow for normal growth. With human milk, absorption of 120 mg of calcium requires a mean intake of 240 mg and the recommended intake is 300 mg [38]. Sodium intake can affect calcium requirements; 2.3 g of sodium removes about 40 mg of calcium. The average concentration of calcium in breast milk for infants aged 7–12 months is 210 mg L^{-1}. This provides a contribution of 126 mg day^{-1} from breast milk that is added to the 140 mg day^{-1} from complementary foods, giving an AI of 270 mg day^{-1} [9]. Following these recommendations, several studies in the literature indicate that the amount of calcium is lower than recommended, for an average consumption of 400 mg of baby food [12,28]. On the other hand, Zand *et al*. [27,31] found levels of calcium in vegetable- and fish-based infant food to be higher than those in foods based on meat.

Magnesium is involved in over 300 biochemical reactions in the body. It helps to maintain normal muscle and nerve function, maintains a regular heart rhythm, supports a healthy immune system, and keeps bones strong. Magnesium also helps to regulate blood sugar levels, promotes normal blood pressure, and is known to be involved in energy metabolism and protein synthesis [39]. Magnesium in breast milk or infant formula is absorbed at levels of 50–90% [40,41]. As for potassium, there is no problem with magnesium because infants can access the recommended amounts in available foods.

Iron requirements are higher during the periods of rapid growth in early childhood and adolescence. During the first year of life, children increase their weight threefold and should bend body iron content. At 4 months of life there is an increased iron requirement of 0.78 mg day^{-1} due to rapid growth and the iron reserves could have diminished to pathological levels if needs are not covered with dietary iron [36]. The presence of other nutrients, such as vitamin C and citric, lactic or malic acids, can increase the absorption from vegetables consumed at the same time. In contrast, calcium and zinc can inhibit the absorption of iron [9]. Commercial baby food products are regularly fortified with iron and ascorbic acid. These fortifications must be carefully controlled because a high intake of iron can affect the absorption of other nutrients, such as calcium or zinc. Iron is the most problematic element in terms of bioavailability. In all cases reported in the literature, the amount of Fe in baby food is lower than the recommended intake [12,23,25–28,31].

Zinc is essential for growth and development of young children. Dietary intake of iron in some supplements can decrease zinc absorption, which is of particular concern during lactation. The bioavailability of zinc is also inhibited by phytate, which is present in large amounts in cereals and legumes [42]. Absorption of zinc is higher from breast milk than from infant formula based on cow milk or soy; these formulas have a much higher content of zinc that breast milk, which compensates for the low absorption efficiency. Zinc is also problematic in terms of bioavailability, so it is necessary to control the amount of zinc in infant foods.

Manganese is an essential element involved in the formation of bone and the metabolism of carbohydrates, cholesterol and amino acids. Manganese is present in many common foods. Therefore, it is extremely rare to have a deficiency of this element in humans [43]. In infant foods based on meat, fish or fruit, manganese levels are lower than the AI or RDA, but in vegetable-based infant food Mn values agree with the recommended values [12,15].

Copper is essential for life. It is required for infant growth, bone strength, iron transport, and cholesterol and glucose metabolism [44]. Very high levels of zinc and iron, generally taken as supplements, can affect the absorption of Cu in infants. Copper deficiency can affect the central nervous system and the immune and cardiovascular systems, notably in infants [45]. In general, the values of Cu in complementary foods are adequate to meet the recommended intake, although in some cases values of Cu are lower than the recommended values, for example in dinner products for babies aged 12–18 months analysed by Melø *et al.* [28], meat- and fish-based baby foods from Spain [12] or commercial baby foods containing chicken with rice and hake with rice from Spain [15]. However, it must be taken into account that milk also provides small amounts of Cu.

Selenium has been implicated in the protection of body tissues against oxidative stress, the maintenance of defences against infection, and the modulation of growth and development. Cow milk-based formula provides less than one-third of the selenium content of human milk. Estimates of selenium intake by 2-month-old infants were 7.8 μg day^{-1} from cow-milk compared with 22.4 μg day^{-1} from human milk [7]. Normal content of selenium in baby foods is 50–120 μg kg^{-1} depending on type of baby food. High levels of Se have been reposted in fish-based foods [12].

Molybdenum is absorbed very efficiently by passive transport. Legumes are major contributors of molybdenum in the diet, as are grain products and nuts. Animal foods, fruits and vegetables are low in molybdenum [7]. Molybdenum intakes in Asian countries are higher due to the predominantly vegetarian diet in these countries. Between weaning and 3 years of age, molybdenum intakes appear to be higher but decline thereafter [11].

Chromium is important in the metabolism of fats and carbohydrates. Several studies have indicated the beneficial effects of chromium on circulating glucose, insulin and lipids in humans. Chromium deficiency may be seen as impaired glucose tolerance and can be found in infants with protein-calorie malnutrition. Supplementation of chromium could be recommended but Cr absorption is low and its excretion rate is high. Additionally, toxicity of this element is not common [46]. A study on the mineral and trace elements in commercial infant food [28] for infants aged 4–18 months found high values of Cr compared with the AI and RDA. The same results were found in a study of essential elements in infant foods from Spain, the UK, China and the USA [12].

From literature data summarised in Figure 2.3 it can be seen that K followed by Ca and Na are present at percentage levels in baby foods analysed worldwide, with Mg, Fe, Zn, Cu, Mn and Al at ppm levels and Ni, Cr and Se at ppb levels. However, depending on the type of food analysed and the country of origin, the concentrations of the mentioned elements vary. On the other hand, it is important to note that highly toxic elements like As, Pb, Cd or Hg have not been reported in baby foods.

References

[1] Committee on Nutrition. Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2008; 46:99–110.

[2] Foote KD, Marriott LD. Weaning of infants. *Arch Dis Child* 2003; 88:488–492.

[3] World Health Organization. Infant and Young Child Feeding. Model Chapter for Textbooks for Medical Students and Allied Health Professionals. WHO, Geneva, 2009.

[4] World Health Organization. Guiding Principles for Complementary Feeding of the Breastfed Child. WHO, Geneva, 2003.

[5] World Health Organization. Strengthening Action to Improve Feeding of Infants and Young Children 6–23 Months of Age in Nutrition and Child Health Programmes. WHO, Geneva, 2008.

[6] Food and Nutrition Board, Institute of Medicine. *Dietary Reference Intake (DRIs): Recommended Intakes for Individuals Elements*. National Academies Press, Washington, DC, 2004.

[7] National Health and Medical Research Council. Nutrient reference values for Australia and New Zealand. National Health and Medical Reserch Council, Canberra, Australia, 2005.

[8] Scientific Committe on Food. Opinion of the Scientific Committee on Food on the revision of reference values for nutrition labelling. SCF/CS/NUT/GEN/18 Final, European Commission, 2003.

[9] FAO/WHO. Vitamin and Mineral Requirements in Human Nutrition: Report of a Joint FAO/WHO Expert Consultation, 2nd edn. Bangkok, Thailand, 2004.

[10] Food and Nutrition Board. *Dietary Reference Intake: The Essential Guide to Nutrient Requirements*. National Academies Press, Washington, DC, 2006.

[11] World Health Organization. *Trace-Elements in Human Nutrition and Health*. WHO, Geneva, 1996.

[12] Carbonell-Barrachina AA, Ramirez-Gandolfo A, Wu X*et al*. Essential and toxic

elements in infant foods from Spain, UK, China and USA. *J Environ Monit* 2012; 14:2447–2455.

[13] Mir-Marqués A, González-Masó A, Cervera ML, de la Guardia M. Mineral profile of Spanish commercial baby food. *Food Chem* 2015; 172:238-244.

[14] Ozbek N, Akman S.A slurry sampling method for the determination of iron and zinc in baby food by flame atomic absorption spectrometry. *Food Addit Contam* 2012; 29:208–216.

[15] Viñas P, Pardo-Martínez M, Hernández-Córdoba M. Determination of copper, cobalt, nickel, and manganese in baby food slurries using electrothermal atomic absorption spectrometry. *J Agric Food Chem* 2000; 48:5789–5794.

[16] Viñas P, Pardo-Martínez M, Hernández-Córdoba M. Rapid determination of selenium, lead and cadmium in baby food samples using electrothermal atomic absorption spectrometry and slurry atomization. *Anal Chim Acta* 2000; 412:121–130.

[17] Fernandez DR, Vanderjagt DJ, Williams M*et al*.Fatty acid, amino acid, and trace mineral analyses of five weaning foods from Jos, Nigeria. *Plant Food Hum Nutr* 2002; 57:257–274.

[18] Thomson BM,Vannoort RW, Haslemore RM. Dietary exposure and trends of exposure to nutrient elements iodine, iron, selenium and sodium from the 2003–4 New Zealand Total Diet Survey. *Br J Nutr* 2008; 99:614–625.

[19] Burló F, Ramírez-Gandolfo A, Signes-Pastor A, Haris P, Carbonell-Barrachina A. Arsenic contents in Spanish infant rice, pureed, infant foods and rice. *J Food Sci* 2012; 71: T15–T19.

[20] Ybañez N, Cervera ML, Montoro R, de la Guardia M. Comparison of dry mineralization and microwave-oven digestion for the determination of arsenic in mussel products by platform in furnace Zeeman-effect atomic-absorption spectrometry. *J Anal Atom Spectrom* 1991; 6:379–384.

[21] Okesli V, González-Bermúdez CA, Vidal-Guevara ML, Dalmau J, Ros G. Alimentación complementaria: ¿es igual el perfil nutricional de un pure casero que el de un tarrito commercial? *Acta Pediátr España* 2011; 69:235–240.

[22] Association of Official Analytical Chemists. *Official Methods of Analysis*, 15th edn. AOAC, Rockville, MD, 1990, pp. 931–945.

[23] Van den Boom S, Kimber AC, Morgan JB. Nutritional composition of home-prepared baby meals in Madrid. Comparison with comercial products in Spain and home-made meals in England. *Acta Paediatr* 1997; 86:57–62.

[24] Association of Official Analytical Chemists. *Official Methods of Analysis*, 15th edn. AOAC, Rockville, MD, 1990, pp. 806–807.

[25] Bosscher D, Van Cauwenbergh R, Robberecht H, Van Caillie-Bertrand M, Deelstra H. Daily dietary iron, zinc and copper intake of infants in Belgium. *Eur Food Res Technol* 2002; 215:275–278.

[26] Bosscher D, Van Cauwenbergh R, Van der Auwera J, Robberecht H, Deelstra H.Calcium, iron and zinc availability from weaning meals. *Acta Paediatr* 2002; 91:761–768.

[27] Zand N, Chowdhry BZ, Zotor FB, Wray DS, Amuna P, Pullen FS.Essential and trace elements content of commercial infant foods in the UK. *Food Chem* 2011; 128:123–128.

[28] Melø R, Gellein K, Evje L, Syversen T. Minerals and trace elements in commercial infant food. *Food Chem Toxicol* 2008; 46:3339–3342.

[29] Pandelova M, Lopez WL, Michalke B, Schramm K. Ca, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn contents in baby foods from the EU market: comparison of assessed infant intakes with the present safety limits for minerals and trace elements. *J Food Compos Anal* 2012; 27:120–127.

[30] Plessi M, Bertelli D, Monzani A. Determination of aluminum and zinc in infants formulas and infant foods. *J Food Compos Anal* 1997; 10:36–42.

[31] Zand N, Chowdhry BZ, Wray DS, Pullen FS, Snowden MJ. Elemental content of commercial ‘ready to-feed’ poultry and fish based infant foods in the UK. *Food Chem* 2012; 135:2796–2801.

[32] Devaney B, Ziegler P, Psc S, Karwe V, Barr S. Nutrient intake of infants and toddlers. *J Am Diet Assoc* 2004; 104: S14–S21.

[33] Heird W, Ziegler P, Reidy K, Briefel R. Current electrolyte intakes of infants and toddlers. *J Am Diet Assoc* 2006;106: S43–S51.

[34] Butte NF, Fox MK, Briefel RR *et al*. Nutrient intakes of US infants, toddlers, and preschoolers meet or exceed dietary reference intakes. *J Am Diet Assoc* 2010;110:S27–S37.

[35] Food and Agriculture Organization. Alimentation, nutrition et agricultura, Alimentación, nutrición y agricultura. Available at: www.fao.org/docrep/x2650T/x2650T00.htm

[36] Gil Hernández A, Uauy Dagach R, Dalmau Serra J.Bases for adequate complementary feeding in infants and young children [in Spanish]. *An Pediatr (Barc)* 2006; 65: 481–495.

[37] Graudal NA, Galloe AM, Garred P. Effects of sodium restriction on blood pressure, renin, aldosterone, catecholamines, cholesterols and triglyceride: a meta-analysis. *JAMA* 1998; 279: 1383–1391.

[38] FAO/WHO. Human Vitamin and Mineral Requirements. Report of a Joint FAO/WHO Expert Consultation. Bangkok, Thailand, 2002.

[39] Office of Dietary Supplements. Strengthening knowledge and understanding of dietary supplements: Magnesium. Available at: http://ods.od.nih.gov/factsheets/Magnesium-Consumer/

[40] Lonnerdal B. Magnesium nutrition of infants. *Magnesium* 1995; 8: 99–105.

[41] Lonnerdal B. Effects of milk and milk components on calcium, magnesium and trace element absorption during infancy. *Physiol Rev* 1997; 77: 643–669.

[42] Brown KH, Wuehler SE, Peerson JM. The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. *Food Nutr Bull* 2001; 22: 113–125.

[43] World Health Organization. *Guidelines for Drinking-water Quality: Manganese in Drinking-water*. Available at: www.who.int/water_sanitation_health/dwq/chemicals/manganese.pdf

[44] Scientific Committe on Food. Opinion of the Scientific Committee on Food on the

Tolerable Upper Intake Level of Copper. SCF/CS/NUT/UPPLEV/57 Final, European Commission, 2003.

[45] Turnlund JR. Copper. In: Shils ME, Olson JA, Shike M, Ross AC (eds) *Modern Nutrition in Health and Disease*, 9th edn. Williams & Williams, Baltimore, 1999, pp. 241–252.

[46] Food and Nutrition Board, Institute of Medicine. Chromium in diet: Medline Plus Medical Encyclopedia, US National Library of Medicine, 2011.

Capítulo 3

Estudio de la ingesta diaria de minerales en España a partir del análisis de menús de cafeterías universitarias

A preliminary approach to the mineral composition of Spanish diet established from university canteen menus

Alba Mir-Marqués, M. Luisa Cervera, Miguel de la Guardia

Department of Analytical Chemistry, University of Valencia, Jeronimo Muñoz Building,
50th Dr. Moliner St., 46100 Burjassot (Valencia), Spain

Received 20 December 2011,

Received in revised form 16 May 2012,

Accepted 17 June 2012,

Journal of Food Composition and Analysis 27 (2012) 160-168

DOI 10.1016/j.jfca.2012.06.006

Resumen

La concentración de elementos minerales en menús completos en España ha sido determinada con el objetivo de establecer los niveles de elementos esenciales y tóxicos y, su contribución a la ingesta máxima recomendada. Trece menús procedentes de las cafeterías universitarias del campus de Burjassot, en Valencia, se analizaron para establecer una primera aproximación de la composición mineral de la dieta española. Los menús estaban compuestos por una mezcla de cereales, carne, pescado, vegetales, frutas y productos lácteos. Las muestras se trituraron, homogenizaron y liofilizaron, determinando 25 elementos mediante espectroscopia de emisión óptica con plasma de acoplamiento inductivo (ICP-OES) tras su digestión en horno microondas. Los menús analizados son, en general, adecuados para una adecuada ingesta mineral, proporcionando del orden de 33-66 % de la ingesta diaria recomendad (RDI) para el Ca, 34-152 % para el Cu, 24-133 % para el Fe, 20-81 % para el K, 28-109 % para el Mg, 41-93 % para el Mn y 33-72 % para el Zn, no obstante, se encontró un excesivo uso de cloruro de sodio que se tradujo en un aporte del 61-202 % de sodio a la RDI. Por otra parte, las cantidades de elementos no esenciales que aportan los menús analizados variaron desde no detectados hasta 3 mg de Al, 0.8 mg de As, 0.5 mg de Ba, 0.05 mg de Li, 2 mg de Sr y 1 mg de Ti.

Abstract

The concentration of mineral elements in complete menus in Spain has been determined in order to establish the levels of essential and toxic elements and their contribution to the maximum recommended intake. Thirteen university canteen menus obtained from the Burjassot campus, in Valencia, were analysed to establish a first approach on the mineral composition of Spanish diet. The menus were composed of a mixture of cereals, meat, fish, vegetables, fruits and dairy products. Samples were crushed, homogenised and lyophilised, and 25 elements were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) after digestion of samples in a microwave oven. Menu samples analysed are in general adequate for an appropriate mineral intake providing from 33-66% of the recommended daily intake (RDI) for Ca, 34-152% for Cu, 24-133% for Fe, 20-81% for K, 28-109% for Mg, 41-93% for Mn and 33-72% for Zn, with however excessive use of sodium chloride, 61-202% of the RDI. On the other hand, the absolute amount of non-essential elements provided by the menus studied varied from non-detectable amounts to 3 mg Al, 0.8 mg As, 0.5 mg Ba, 0.05 mg Li, 2 mg Sr or 1 mg Ti.

3.1. Introduction

Human exposure to mineral elements is an important problem from the point of view of both nutritional and environmental toxicology, because some mineral elements are essentials for life but many heavy metals are regarded as toxic to living organisms. Therefore, efforts should be focused on the estimation of daily intakes of mineral elements by consumers. To accomplish this, the most informative and sensitive analytical methods are required, in order to monitor human ingestion and evaluate the risks associated with the use of foods produced in polluted areas or subjected to bad processing practices.

There are two main alternative ways to estimate the daily mineral intake in a country, one corresponds to Total Diet Study (TDS) market basket studies, and consists of representative key foods (raw and processed using commonly used methods) [1-8]. The other way is based on the duplicate portion studies analysis of duplicate portions of foods in representative meals and analysis of individual food items [9-12]. With this in mind, in the present study we determined the mineral composition of university canteen menu samples because many people eat in these places every day, and thus the data obtained could be of great value for an estimation of the total diet in a country to be compared with data from other sources.

To date, many total diet studies have been carried out to determine the quantity of mineral elements ingested with the foods [2,7,13-17], along with studies of specific meals to determine the number of essential elements ingested with the foods [18-22] and duplicated portion studies [12,23-27]. There are also studies in the literature on exposure to toxic elements such as As, Cd, Pb or Hg in diets [10,13,18,19,21,23,28]. Other studies have focused on a specific essential element, for example Se [24,29]. Most mineral components to be determined in diet samples are present at trace and ultra-trace levels; hence analytical techniques with adequate sensitivity are required for their accurate determination. Additionally, it is important to remember that the accuracy of the quantitative analysis procedures is strongly dependent on the sampling and sample preparation steps. The techniques most employed for trace element analysis in foods are those based on atomic spectrometry and inorganic mass spectrometry. To analyse a few elements, it is very common the use electrothermal atomic absorption or hydride generation atomic absorption or fluorescence spectrometry. However, when there is a general interested on the mineral composition of foods and diets, multi-elemental techniques based on inductively coupled plasma optical emission (ICP-OES) [10,22,30-32] or inductively coupled plasma mass spectrometry (ICP-MS) [5,12,19] are the best options.

The main purpose of this study was to evaluate the nutrient profile and exposure to chemical contaminants of students at the University of Valencia. Data obtained have been used for establishing an approximation of the daily intake of essential and toxic elements by people living in the same area.

3.2. Materials and methods

3.2.1. Instruments and reagents

ICP-OES measurements were carried out using a Perkin Elmer Model Optima 5300 DV spectrometer (Norwalk, CT, USA) equipped with an auto sampler AS 93-plus, and a cross flow nebuliser. Argon C-45 (purity higher than 99.995%), supplied by Carburos Metálicos (Barcelona, Spain), was employed as plasmogen and carrier gas. The operating conditions of the ICP-OES equipment are summarised in Table 3.1.

A microwave laboratory system, Ethos SEL from Millestone (Sorisole, Italy), equipped with a thermocouple, for automatic temperature control, and an automatic gas leaks detector has been employed for samples digestion. The system operated at a maximum exit power of 1000 W, was employed for microwave-assisted digestion of menu samples using high pressure Teflon vessels of 100 mL inner volume. For cleaning the vessels an automatic cleaning device traceCLEAN from Millestone was used.

Other equipment included a Cryodos 50 lyophiliser Telstar (Barcelona, Spain) employed for sample preservation, and an ultrasound water bath from Selecta (Barcelona, Spain), of 9 L volume with 50 W power and 50 Hz frequency, which was used for sample sonication.

Table 3.1. Instrumental parameters employed for ICP-OES determination of the mineral composition of menu samples

Parameter	Value
RF power	1200 W
Gas	Argon
Plasma gas	15 L min^{-1}
Auxiliary gas	0.5 L min^{-1}
Nebuliser gas	0.7 L min^{-1}
Sample aspiration rate	1.10 mL min^{-1}
View	Axial and Radial for Ca, K, Mg and Na
Background correction	2 points
Number of replicates	3
Nebuliser	Cross-flow

All reagents used were of analytical grade and all solutions were prepared in ultrapure water with a minimum resistivity of 18.0 MΩ cm obtained from a Milli-Q Millipore system (Bedford, MA, USA).

Stock solutions of a multi-element standard solution (100 mg L^{-1}) containing 26 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Se, Na, Sr, Tl, Ti, V and Zn) dissolved in 5% HNO_3 from Scharlau (Barcelona, Spain), and a multi-element solution containing 20 mg L^{-1} Dy, Er, Eu, Gd, Ho, Lu, Sm, Sc, Tb, Tm, Yb and Y and 100 mg L^{-1} Ce, La, Nd and Pr dissolved in 5% HNO_3, also from Scharlau, were employed to prepare the calibration standards.

HNO_3 69% for trace analysis and H_2O_2 35% reagent grade, both from Scharlau, were used for sample treatment.

A 1000 mg L^{-1} Ruthenium Atomic Spectroscopy Standard Solution prepared from $RuCl_3$ in 1M HCl Fluka (Buchs, Switzerland) and 1000 mg L^{-1} Scandium Atomic Spectroscopy Standard Solution prepared with Sc_2O_3 in 0.5 M HNO_3 Fluka were used as internal standards. A 1000 mg L^{-1} Sodium Standard Solution for AA prepared with $NaNO_3$ in 0.5 M HNO_3 Scharlau, a 1000 mg L^{-1} Potassium Standard Solution for Atomic Absorption prepared with KNO_3 in 0.5 M HNO_3 Scharlau, were also employed.

3.2.2. Samples and sampling

Samples of different menus were purchased in 2010 at two canteens of the campus of Burjassot of the University of Valencia. Menus were composed by entries, a main dish and dessert and also include a piece of 50-70g of bread. The foods corresponding to the starter, main course, bread and dessert were separated in different plastic containers, and transported to the laboratory.

The composition of each sample menu analysed is detailed in Table 3.2. The samples were chosen to include all the foods offered to the students in the canteen menus. Different starters and main courses were selected randomly between those offered each day in order to have a representative description of the diet followed in the area of study.

3.2.3. Sample storage and handling

Samples were handled immediately if possible or stored in refrigerator up to handling for a period of less than 24 hours. The edible part of the foods was weight and mixed, and immediately after the samples were crushed and homogenised with a domestic Braun mixer (Kronberg, Germany), and finally frozen at -20°C in a freezer. Afterwards, they were lyophilised for a minimum of 48 h at a chamber pressure of 0.05 mbar. The dried complete menu samples were pulverised with a domestic mixer, and the powdered samples were stored in polyethylene bottles until their analysis.

3.2.4. Sample digestion

A 1 g freeze-dried menu sample was accurately weighed inside the Teflon digestion vessel and 8 mL of concentrate nitric acid were added, allowing the samples to be predigested by standing open in an ultrasound water bath for a minimum of 15 min. Then 2 mL of H_2O_2 were added and the mixture was sonicated for a further 45 min. After that, the Teflon reactors were closed and placed inside the microwave oven. The following program was run: step 1, 3 min to reach 85°C; step 2, 12 min to reach 145°C; step 3, 10 min to reach 180°C; step 4, 15 min at 180°C; and step 5, cooling down. The reactors were opened and sonicated. The resulting solution was quantitatively transferred to a plastic flask and made up to 20 mL with nanopure water.

Table 3.2. Food composition of the menu samples analysed through this study

Menu	Starters	Main courses	Dessert
1	Seafood paella (rice, mussels, grouper, squid rings)	Salmon with chips	Pear
2	Vegetable pie with tomato sauce (carrots, tomatoes, peppers, beets, zucchini, ...)	Tuna omelette with smashed potatoes	Home-made crème caramel
3	Lentil (350 g)	Ham croquettes (170 g) with chips (100 g)	Skimmed yogurt (120 g)
4	Spaghetti with carbonara sauce (380 g)	Sausages (140 g) with chips (80 g) and vegetables (peppers, cabbage and garlic) (25 g)	Fried milk with sugar and cinnamon (90 g)
5	Salad (lettuce, tomato, corn, carrot, eggs, cucumber, pepper, soy and olives) (300 g)	Mixed-up of spinach and mushrooms (190 g) with steamed potatoes (90 g)	Orange (180 g)
6	Soup with bread, garlic, egg, onion... (330 g)	Grilled tuna (120 g) with steamed potatoes (150 g)	Lemon yogurt (125 g)
7	Macaroon with tomato (390 g)	Ham (30 g), bacon (20 g) and sausage (50 g) grilled with potatoes (85 g), cabbage and steamed carrot (120 g)	Apple (120 g)
8	Beans (360 g)	Meatballs with sauce (peas, carrots and potatoes) (200 g) with chips (80 g)	Orange gelatine (75 g)
9	Vegetable cream (260 g)	Roast chicken (110 g) with green pepper (55 g) and chips (130 g)	Orange (80 g)
10	Spaghetti with carbonara sauce (310 g)	Ham schnitzel (85 g) with chips (70 g)	Vanilla custard (120 g)
11	Salad (lettuce, carrots, ham, soy, cheese and mayonnaise) (225 g)	Cod (130 g) with carrots, parsley and other vegetables (60 g)	Orange (200 g)
12	Beans with ham (250 g)	Zucchini gratin (180 g) with béchamel sauce, cheese and bacon (80 g)	Strawberry and orange juice (110 g)
13	Rice with squid, cuttlefish and prawns (260 g)	Pork loin (80 g) with carrots (15 g), peas (18 g) and chips (85 g)	Lemon yogurt (125 g)

Note: all menus include approximately 70-75 g (M1-M9) and 50g (M10-M13) of bread.

Samples were pre-digested inside the reactors vessels with HNO_3 and after that with H_2O_2, by sonication in an ultrasound water bath for 1 h. This step (60 min) is time-consuming because the amount of sample employed (1 g) is higher compared with the conventional procedures for microwave- assisted digestion, but it is necessary in order to avoid foam formation and excess of gases inside the reactors during the digestion step which could procedure too much pressure. This process removes the main part of the gases formed during the decomposition of samples, and prevents leaks. Finally, reactors were introduced in the microwave oven. After digestion, the solutions were sonicated to eliminate nitrous vapours. A complete digestion for all the samples assayed was carried out, obtaining a clear and precipitate free solutions for all the samples assayed.

3.2.5. ICP-OES determination

The calibration range for the 24 trace elements studied was established from 0.05 to 5 mg L^{-1}, and for K and Na it was evaluated from 2 to 10 mg L^{-1}. Scandium (0.5 mg L^{-1}) was used as internal standard and added to all samples and standards. Digested samples were measured with a final dilution 1:2 and making 1:100 dilution to determine the concentration of Ca, K, Mg and Na. Determinations were made using the instrument parameters indicated in Table 3.1.

3.2.6. Quality control

Samples were prepared and measured in triplicate. Reagent blank were made regularly together with each batch of sample digestion and certified reference materials were digested and measurement together with the different sample batches. Additionally, control standards were measured each series of 10 independent sample measurements.

Three different certified reference materials were analysed: NIST 1568a (Rice Flour) obtained from the NIST, National Institute of Standards and Technology (Gaithensburg, MD, USA); TORT-2 (Lobster Hepatopancreas), purchased from the NRC, National Research Council (Ottawa, Canada) and BCR-185R (Bovine liver) obtained from the IRMM, Institute for Reference Materials and Measurements (Geel, Belgium).

The limit of detection of instrument (LODi) values were calculated as the concentrations corresponding to signals equal to 3 times the standard deviation of 10 reagents blank solutions. Additionally, limits of detection of the method values (LODm) were referred to the original samples (mg kg^{-1}), taken into consideration the amount of sample digested and the final dilution employed in the recommended procedure. The limits of quantification of instrument (LOQi) were determined in the same way for a factor of 10 times the deviation of blank measurements, and the limits of quantification of the method (LOQm) were calculated in terms of concentration in the original sample.

The repeatability of the instrumental measurements was calculated as the relative standard deviation (RSD %) of 10 independent measurements of a 100 µg L^{-1} multi-elemental standard solution, except for Ca, K, Mg and Na for which a standard solution of 5 mg L^{-1} was used. The repeatability of the whole method was calculated as the relative standard deviation (RSD %), for 3 independent analysis of 3 independent portions of the same sample.

3.3. Results and discussion

3.3.1. Sample measurements

Wavelengths recommended by the manufacturer and additional wavelengths were employed for the determination of each element in order to verify possible interferences and to establish appropriate baseline correction at different spectral regions. In general, those lines recommended by the instrument manufacturer were chosen, as they are the most sensitive ones. Table 3.3 shows the selected wavelengths employed to analyse the menu samples.

Table 3.3. Analytical figures of merit of ICP-OES determination of the mineral composition of menu samples

	Spectral line (nm)	**LODi (mg L^{-1})**	**LODm (mg kg^{-1})**	**LOQi (mg L^{-1})**	**LOQm (mg kg^{-1})**	**RSDi (%)**
Al	396.153	0.06	2.4	0.20	8	2.1
As	188.979	0.014	0.54	0.047	1.8	8.7
Ba	233.527	0.0025	0.10	0.0083	0.33	1.6
Be	313.042	0.002	0.08	0.0067	0.27	0.2
Bi	223.061	0.01	0.4	0.033	1.3	2.2
Ca	317.933	0,25	10	0.83	33	0.8
Cd	228.802	0.0007	0.027	0.0023	0.09	1.7
Co	228.616	0.0013	0.05	0.0043	0.17	1.5
Cr	267.716	0.002	0.08	0.0067	0.27	1.7
Cu	327.393	0.001	0.04	0.0033	0.13	0.5
Fe	238.204	0.006	0.23	0.020	0.77	1.5
K	766.490	0.2	7.5	0.67	25	1.5
Li	670.784	0.00006	0.0025	0.0002	0.008	0.9
Mg	285.213	0.01	0.4	0.033	1.3	0.7
Mn	257.610	0.0012	0.05	0.004	0.17	0.4
Mo	202.031	0.0024	0.1	0.008	0.33	1.6
Na	589.592	0.26	10.6	0.87	35	0.7
Ni	231.604	0.0025	0.1	0.008	0.33	1.3
Pb	220.353	0.0032	0.13	0.011	0.43	3.5
Se	196.026	0.027	1.1	0.090	3.7	7.5
Sr	407.771	0.0006	0.026	0.002	0.09	2.2
Ti	334.940	0.0004	0.016	0.001	0.05	0.3
Tl	190.801	0.007	0.3	0.023	1.0	17
V	290.880	0.005	0.21	0.017	0.7	1.1
Zn	206.200	0.005	0.21	0.017	0.7	1.5

Note: LODi: limit of detection of instrument, LODm: limit of detection of the method, LOQi: limit of quantification of instrument, LOQm: limit of quantification of the method, RDSi: relative standard deviation of instrumental measures.

In ICP-OES measurements one of the main problems is due to the high emission intensity of alkaline and alkaline-earth elements. These elements can modify excitation and ionization plasma conditions. Additionally, the presence of major elements can affect the introduction rate of the aforementioned samples, through changes in the sample viscosity and surface tension, thus creating physical and matrix interferences in the trace element determinations. One way to solve problems produced by the matrix consists of diluting the samples. A study of different dilution factors: 1:10^4, 1:10^3, 1:10^2, 1:10 and 1:2 was carried out for all considered elements and data found were compared with the direct measurement of undiluted digested samples. It was chosen the dilution 1:100 to measure major elements (Ca, Na, K, Mg). For the rest of the elements, studies of standard addition on the sample digested undiluted and with dilution 1:2 and 1:10 were carried out. Table 3.4 shows the comparison of the slopes for the standard addition and multi-element external calibration obtained, in order to evaluate the matrix effect and any transports and nebulisation interferences.

For undiluted digested samples, relative error higher than 10 % were found for half of the elements; however, for the dilution 1:2, relative errors were lower than 10 % for all element except Al, thus this dilution (1:2) was chosen for sample measurements.

Table 3.4. Study of standard addition: comparison of the slope for standard addition in front of external multi-element calibration for the undiluted digested sample and for the 1:2 dilution factors

		Dilution 1:2		**Undiluted**	
Analyte	**Slope multielemental calibration**	**Slope standard addition**	**Relative error**	**Slope standard addition**	**Relative error**
Al	47232	57440	22	58741	24
As	846	829	-2	757	-10
Ba	39620	38372	-3	35738	-10
Be	586373	556369	-5	496472	-15
Bi	1200	1219	2	1156	-4
Cd	18031	17972	-0.3	16538	-8
Co	10122	9776	-3	8914	-12
Cr	26613	26411	-1	24823	-7
Cu	92702	101363	9	100994	9
Fe	21441	20643	-4	18915	-12
Mn	151774	145985	-4	136142	-10
Mo	6340	6291	-1	5898	-7
Ni	8593	8553	-0.5	7895	-8
Pb	1844	1820	-1	1665	-10
Se	447	449	0.5	417	-7
Sr	1445955	1453792	1	1375736	-5
Ti	130737	130239	-0.4	123854	-5
Tl	887	870	-2	780	-12
V	29640	29557	-0.3	27767	-6
Zn	7850	7853	0.04	6868	-13

NOTE: Relative error was calculated (((slope standard addition - slope multi-elemental calibration)/ slope multi-elemental calibration)*100)

3.3.2. The use of internal standard

The choice of a suitable internal standard and the use of relative measurements of the emission intensities between the analyte and the internal standard can compensate some matrix induced signal variations as well as the background drift, thus improving of accuracy and repeatability. Ru and Sc were tested as internal standard for the determination of mineral elements in menu samples. Results obtained from the analysis of samples showed higher sensitivity for Sc as compared with Ru (Sc provides 490000-550000 emission units while Ru provides 7500-10000 emission units). So, the use of Sc is recommended because of the greater number of emission units obtained, resulting in the most effective the internal standard correction. This internal standard was added to all samples, calibration standards and blanks at a fixed concentration of 0.5 mg L^{-1}.

3.3.3. Analytical characteristics of the method

The mineral contents provided in the present study were submitted to a quality control of the measurements through certified referential materials, reagent blanks and standards controls in every batch sample. Table 3.3 shows the main analytical figures of merit; the procedures carried out were defined in section 3.2.6 concerning quality control.

All RSD instrumental values were equal to or lower than 2.5%, except for the As, Pb, Se and Tl which provided a poorer repeatability, due to the low sensitivity for these elements (see slope of calibration in Table 3.4). The RSD of the method (for triplicate analysis of samples) was lower than 10% for all elements in all samples except for Al, As, Cr, Mo and Ti for which it was around 20% in some cases. This may be due to the low content of the aforementioned elements in samples analysed which were close the limit of detection of the method.

The accuracy of the procedure was tested based on the analysis of 3 reference materials, and Table 3.5 shows the results obtained; it can be seen that they are in good agreement with the reference data. A statistical evaluation of the comparison between the two data population was made on the regression between average obtained values and certified ones provided a regression line y = (0.997 ± 0.017)x + (0.17 ± 1.28), y = (0.952 ± 0.027)x - (0.058 ± 0.329), y = (1.02 ± 0.02)x - (1.59 ± 3.37) respectively for TORT-2, NIST 1568a and BCR-185R, which demonstrates that the intercept and the slope correspond statistically to 0 and 1, respectively, with a regression coefficient (r^2) of 0.998, 0.997 and 0.9991 respectively.

Table 3.5. Evaluation of the accuracy of the method employed through the comparison between values found and those certified in a series of food reference materials.

	TORT-2		**NIST 1568a**		**BCR-185R**	
Element	**Obtained (mg kg^{-1})**	**Certified (mg kg^{-1})**	**Obtained (mg kg^{-1})**	**Certified (mg kg^{-1})**	**Obtained (mg kg^{-1})**	**Certified (mg kg^{-1})**
Al	----	----	3.5 ± 0.5	4.4 ± 1.0	----	----
As	24 ± 1	21.6 ± 1.8	<LOD(0.54)	0.29 ± 0.03	<LOD(0.54)	0.033 ± 0.002
Cd	25.9 ± 0.4	26.7 ± 0.6	<LOD(0.027)	0.022 ± 0.002	<LOD(0.027)	0.544 ± 0.017
Cr	0.64 ± 0.03	0.77 ± 0.15	----	----	----	----
Cu	109.0 ± 0.5	106 ± 10	2.55 ± 0.08	2.4 ± 0.3	284 ± 3	277 ± 5
Fe	98 ± 3	105 ± 13	7.3 ± 0.2	7.4 ± 0.9	----	----
Mn	12.7 ± 0.2	13.6 ± 1.2	18.5 ± 0.3	20.0 ± 1.6	10.7 ± 0.2	11.07 ± 0.29
Mo	0.79 ± 0.04	0.95 ± 0.1	1.33 ± 0.07	1.46 ± 0.08	----	----
Na	----	----	<LOD(10.6)	6.6 ± 0.8	----	----
Se	6.8 ± 0.6	5.63 ± 0.67	<LOD(1.1)	0.38 ± 0.04	2.1 ± 0.3	1.68 ± 0.14
Sr	47 ± 3	45.2 ± 1.9	----	----	----	----
Zn	182 ± 1	180 ± 6	18.9 ± 0.2	19.4 ± 0.5	134 ± 2	138.6 ± 2.1

NOTE: Samples were analysed in triplicate

3.3.4. Mineral content of menu samples

Table 3.6 shows the concentration values of Al, As, Ba, Ca, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Sr, Ti and Zn in the different menu samples analysed, expressed in mg kg^{-1} of dry sample. As it can be seen, from the initial 25 elements investigated, only 16 are present in detectable concentrations in the edible part of considered samples.

Aluminium (Al). Aluminium is a well-known neurotoxicant, but its possible connection with Alzheimer’s disease has been subjected to considerable controversy in recent years. In spite of aluminium is ubiquitous in the environment, as well as it is present in all diets, little is normally absorbed from foods or beverages [33]. Fresh foods like fruits, vegetables, and meat contain very little aluminium. The major source of aluminium in the diet in many countries is processed food. Aluminium compounds (permitted additives) may be added during processing of foods, such as flour, colouring agents and anti-caking agents [34]. The content of aluminium in the studied samples is high in those menus which include pre-processed foods (M2, M6, M7 and M8). The values of the mineral element concentrations found in this study were compared with values found in the literature and it can be concluded that Al concentrations are similar to those found in studies made in France [12] and lower than those obtained in Italy [35] or US [14].

Arsenic (As). It occurs in the environment in both organic and inorganic compounds in its trivalent or pentavalent state. The toxicity of the arsenic is highly dependent of their chemical form, in contrast to inorganic compounds, organic arsenicals are less or non-toxic. Inorganic arsenic induces skin lesions and disturbances on heart, circulatory and nervous systems [36]. Certain fish and crustaceans contain very high levels of organic arsenic, often as arsenobetaine. In most other foodstuffs, levels of arsenic are low, but the form is not known, being their toxicological risk unknown. The predominant dietary source of arsenic is seafood, followed by rice, mushrooms, and poultry. The total daily intake is depending of seafood consumed. The highest levels of As correspond to samples with fish (M6 and M11). The values obtained for As are similar to those obtained in studies related to Spanish foods [15,19,37], but higher than values found in other countries [12,13,23,28].

Barium (Ba). Barium is found in all foods, usually at levels of about 0.05-1.0 mg kg^{-1}. There is no particularly good food source of barium with exception of Brazil nuts; the highest levels are also found in pecan nuts and dry cocoa [33]. Some foods, such as seaweed, fish, and certain plants, may also contain high amounts of barium. The amount of barium found in food and water usually is not high enough to be a health concern [38]. When the menu contains a lot of vegetables Ba content is high as for example M3, M5, M8 and M11. The values obtained for Ba are similar to those obtained in studies related to UK [16].

Table 3.6. Mineral composition of menu samples

	Sample concentration (mg kg^{-1}dry weight)						
Analyte	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**
Al	7.5 ± 0.3	11.4 ± 0.2	7.9 ± 0.2	5.2 ± 0.2	5.3 ± 0.4	9.1 ± 0.2	12 ± 1
As	1.0 ± 0.3	0.70 ± 0.08	< 0.54	< 0.54	< 0.54	2.72 ± 0.07	< 0.54
Ba	0.25 ± 0.01	1.25 ± 0.04	1.778 ± 0.006	0.63 ± 0.02	1.46 ± 0.03	0.53 ± 0.01	0.69 ± 0.02
Ca	850 ± 50	2370 ± 30	1460 ± 30	1460 ± 50	2090 ± 70	1550 ± 10	970 ± 40
Cr	<0.08	0.23 ± 0.06	<0.08	<0.08	<0.08	0.164 ± 0.007	0.19 ± 0.03
Cu	1.94 ± 0.08	1.41 ± 0.02	3.55 ± 0.01	2.12 ± 0.09	2.55 ± 0.08	1.129 ± 0.007	2.19 ± 0.01
Fe	33 ± 1	23.2 ± 0.4	24.3 ± 0.5	15.6 ± 0.3	31.7 ± 0.5	22 ± 2	18.1 ± 0.6
K	5600 ± 300	6360 ± 70	8500 ± 100	5400 ± 200	10400 ± 400	7690 ± 10	5290 ± 20
Li	0.092 ± 0.004	0.120 ± 0.003	0.111 ± 0.001	0.0691 ± 0.0001	0.302 ± 0.005	0.130 ± 0.001	0.120 ± 0.002
Mg	560 ± 20	539 ± 2	710 ± 20	500 ± 10	780 ± 30	600 ± 2	477 ± 4
Mn	4.9 ± 0.2	4.8 ± 0.1	6.83 ± 0.03	5.06 ± 0.08	6.71 ± 0.05	4.388 ± 0.002	5.19 ± 0.09
Mo	<0.1	0.11 ± 0.01	0.58 ± 0.05	<0.1	0.12 ± 0.02	<0.1	<0.1
Na	9500 ± 400	11700 ± 200	11000 ± 100	10300 ± 400	7900 ± 300	11410 ± 30	13200 ± 100
Sr	5.8 ± 0.4	4.15 ± 0.02	3.67 ± 0.04	4.2 ± 0.4	10.3 ± 0.4	2.54 ± 0.04	4.13 ± 0.07
Ti	0.28 ± 0.03	0.120 ± 0.008	0.11 ± 0.03	0.51 ± 0.06	0.10 ± 0.03	0.237 ± 0.008	0.35 ± 0.08
Zn	13.7 ± 0.4	16.5 ± 0.3	16.5 ± 0.2	15.8 ± 0.2	19.6 ± 0.2	16.83 ± 0.07	12.3 ± 0.3

NOTE: Three independent samples of each item were analysed. For details about each menu sample composition see Table 3.2

Table 3.6. (continued) Mineral composition of menu samples

	Sample concentration (mg kg^{-1}dry weight)					
Analyte	**M8**	**M9**	**M10**	**M11**	**M12**	**M13**
Al	11 ± 1	4.4 ± 0.8	3.2 ± 0.3	3.4 ± 0.4	4.0 ± 0.1	3.5 ± 0.5
As	< 0.54	< 0.54	< 0.54	5.0 ± 0.1	1.157 ± 0.003	1.6 ± 0.1
Ba	1.28 ± 0.05	0.91 ± 0.03	0.56 ± 0.02	1.62 ± 0.03	0.76 ± 0.01	0.26 ± 0.03
Ca	885 ± 8	970 ± 30	1450 ± 20	3130 ± 50	2620 ± 50	1300 ± 100
Cr	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
Cu	3.69 ± 0.02	2.92 ± 0.02	2.00 ± 0.04	1.74 ± 0.06	2.65 ± 0.06	2.01 ± 0.03
Fe	29.4 ± 0.2	22.9 ± 0.2	12.8 ± 0.2	10.7 ± 0.3	24.1 ± 0.9	17.4 ± 0.6
K	10700 ± 100	10800 ± 300	5640 ± 40	8800 ± 200	14100 ± 200	6100 ± 200
Li	0.1370 ± 0.0004	0.105 ± 0.001	0.048 ± 0.001	0.143 ± 0.002	0.052 ± 0.003	0.078 ± 0.002
Mg	870 ± 7	750 ± 20	497 ± 2	720 ± 10	960 ± 10	520 ± 20
Mn	6.44 ± 0.03	5.3 ± 0.2	4.46 ± 0.06	3.96 ± 0.06	9.5 ± 0.2	3.79 ± 0.08
Mo	1.28 ± 0.04	0.481 ± 0.005	<0.1	<0.1	0.27 ± 0.03	<0.1
Na	11500 ± 100	12200 ± 300	6560 ± 40	12800 ± 300	9500 ± 100	9200 ± 300
Sr	4.08 ± 0.07	4.39 ± 0.08	3.35 ± 0.03	14.1 ± 0.2	8.9 ± 0.6	3.7 ± 0.3
Ti	0.19 ± 0.02	0.10 ± 0.03	<0.016	< 0.016	<0.016	0.07 ±0.02
Zn	16.8 ± 0.3	21.6 ± 0.3	19.83 ± 0.08	18.0 ± 0.2	20.1 ± 0.3	21.5 ± 0.8

NOTE: Three independent samples of each item were analysed. For details about each menu sample composition see Table 3.2

Calcium (Ca). Calcium is an essential nutrient that plays a vital role in neuromuscular function, many enzyme-mediated processes, blood clotting, and providing rigidity to the skeleton by its phosphate salts. Its non-structural roles require the strict maintenance of ionised calcium concentration in tissue fluids at the expense of the skeleton if necessary and it is therefore the skeleton which is at risk if the supply of calcium falls short of requirement [39]. The richest sources of calcium are dairy products. In countries where milk is scarce, calcium may be obtained from certain cereals (notably millets). Small saltwater and freshwater fish such as sardines and sprats supply good quantities of calcium since they are usually eaten whole, bones and all. Vegetables and pulses provide some calcium [40]. The values of Ca are similar to data reported in US [41], higher than in France [12], Greenlandic [42], Brazil [43] or UK [16] and less than in Swedish [44].

Chromium (Cr). Trivalent chromium is an essential nutrient for humans, which is involve in the glucose tolerance. Adequate chromium nutrition may reduce risk factors associated with cardiovascular disease as well as diabetes mellitus. Hexavalent chromium is, in contrast, extremely toxic [33]. The major source of trivalent chromium is meat and vegetables. But this level could be increased by migration into the food from stainless steel cooking equipments and cans. The amount of Cr is higher in menus that include tune or canned food (M2, M6 and M7). The values of Cr average values obtained through this study are similar to those found in the literature [12,17] but higher than Swedish [44] or Brazil [43].

Copper (Cu). It is an essential trace element for humans, copper is a vital component of several enzymes. Its absorption depends on the amount ingested, its chemical form, and the composition of other dietary components such as zinc. Drinking water has a significant contribution to dairy intake due to the use of domestic copper pipes. Offal, such as liver and kidney, contain high copper levels. Fish, fruits, cereals, nuts, and green vegetables are good sources of copper, whereas meat and dairy products contain lower levels [36]. The amount of Cu is higher in menus that include a lot of vegetables (for example M3, M8, M9, M12). The values of Cu average values obtained through this study are similar to those found in the literature [12,16,17,44].

Iron (Fe). Iron is needed for the synthesis of blood pigments, as well as for many other essential activities of cells. The best food source of iron is animal offal, especially liver. Other animal products, like red meat are also rich in iron. The levels in cereals and vegetables are generally low, with the exception of dark green vegetables, such as spinach, which are rich in iron [33]. The amount of Fe in each menu depends on the type of foods, and in some cases, is quite low and in other cases very high. So, as much as possible variability of the foods in the diet must be recommended for an equilibrated daily mineral intake. Values obtained through this study are similar to those found in the literature [12,16,41,44], but in Greenlandic iron values are higher than in this study [42].

Potassium (K). Potassium is an essential mineral that works to maintain the body's water and acid balance. It plays a role in transmitting nerve impulses to muscles, in muscle contraction and in the maintenance of normal blood pressure [45]. Potassium can be found in vegetables, fruits, potatoes, meat, bread, milk and nuts. The highest amounts have been found in menus that include several vegetables and orange fruit (M5, M8, M9, M11 and M12). The potassium average values obtained through this study are similar to those found in France [12], Belgium [16] and Swedish [44] but less than in the US [41] and higher than Brazil [43].

Lithium (Li). The essential role of lithium has not been established, but its use in psychiatric medicine to treat manic-depressive psychosis is well known. Lithium is present in all food, though at low levels [33]. The concentrations obtained in this study are much higher than those found in France [12] or UK [16].

Magnesium (Mg). In spite of the structural function of magnesium in the skeleton, it has many functions in muscles and soft tissues, such as a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes. Dietary deficiency of magnesium of a severity sufficient to provoke pathologic changes is rare [39]. Magnesium is widely distributed in plant and animal foods. Most green vegetables, legume seeds, peas, beans, and nuts are rich in magnesium, as are also some shellfish, spices, and soya flour. The values of Mg are similar in all menus, between 91 and 225 mg day^{-1}. The Mg concentrations are comparable to those found in Spain [19] and Swedish [44] but higher than values reported in France [12].

Manganese (Mn). This is an essential element for humans and animals. Manganese is present in all plant and animal tissues, with food the major source in human intake. The highest manganese concentrations are found in some foods of plant origin, especially wheat and rice. High concentrations are also found in nuts, tea, legumes, pineapples, and lower levels in milk products, meats, fish and eggs [36]. The Mn concentrations are similar to those found in France [12], UK [16] and Swedish [44].

Molybdenum (Mo). This is an essential constituent of several enzymes, and dietary recommendations have been established. The foods contributing the most molybdenum to the diet are grains, milk, dairy products, and legumes. In contrast, animal products generally have lower level. The Mo data are of the same order as those reported in other countries [12,16].

Sodium (Na). This element is necessary for humans in order to maintain balance in physical fluids systems. Sodium is also required for the operation of nerves and muscles, but an excess of sodium can damage the kidneys and increase the possibilities of hypertension. In general, the sodium content was very high in all samples and must be attributed to the addition of sodium chloride while cooking the foods. Figure 3.1 shows the frequency of the values of the sodium concentration in the samples in descending order of magnitude. The values found are also higher than the values reported in the literature of France [12], Belgium [26], US [41], Brazil [43] and Swedish [44].

Strontium (Sr). This is a non-essential trace element, which can substitute for calcium in bone. Food and drinking water are the highest sources of exposure to strontium. Grains, leafy vegetables, and dairy products contribute the greatest percentage of dietary strontium to humans. Problems with bone growth may occur in children eating or drinking unusually high levels of strontium, especially if the diet is low in calcium and protein [46]. The amount of Sr is higher in menus that include several vegetables (M5, M11 and M12). No data were found in the literature.

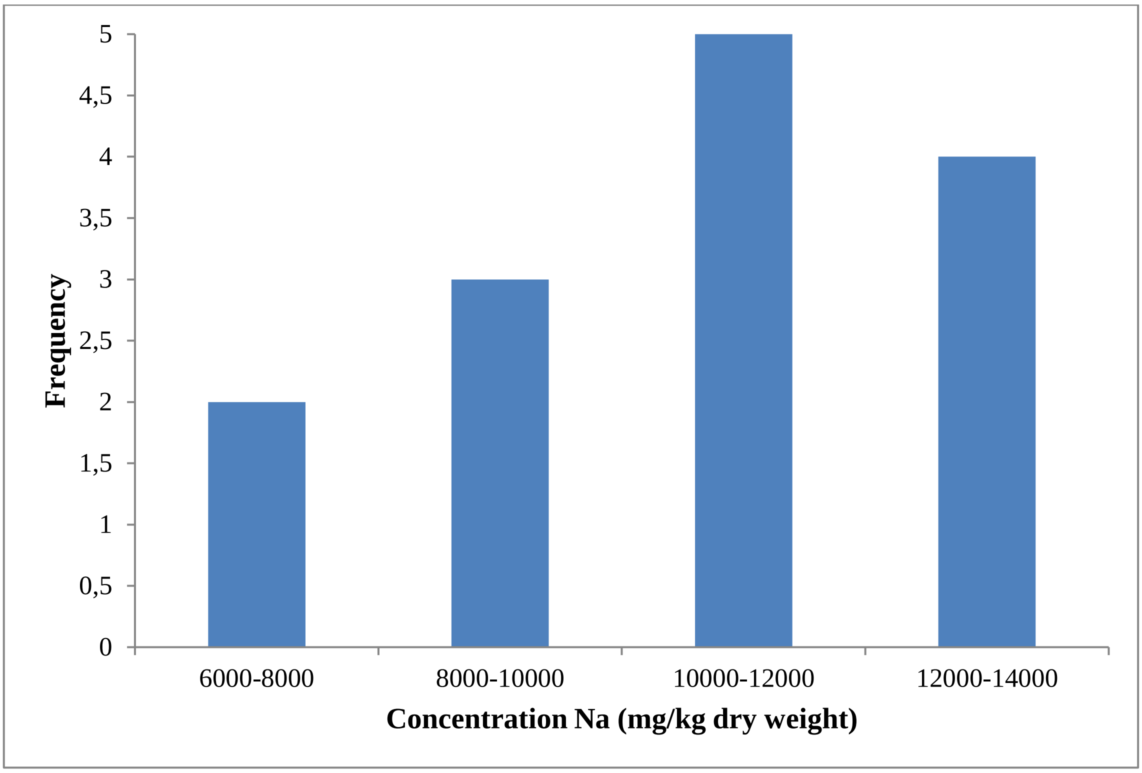

Figure 3.1. Histogram with the sodium concentration in dry weight (mg kg^{-1})

Titanium (Ti). There is no evidence that titanium is an essential element for humans. It is poorly absorbed by plants and animals. High levels of titanium could be found for vegetables from contaminated soils and in certain types of cheese from the addition of titanium dioxide as whitener and an aging accelerator [36]. The values of Ti found are between 0.02 and 1.66 mg day^{-1}. These values agree with data reported (about 2 mg kg^{-1}) in cereals, vegetables, dairy products and some other foods [33].

Zinc (Zn). Zinc is an essential element in human nutrition; it is present in many important enzymes essential for metabolism. Zinc is present in most foods both of vegetable and of animal origin, but the richest sources tend to be protein-rich foods such as meat, seafoods and eggs [40]. The amount of Zn is higher in menus that include vegetables and meat than in those based on pasta and fish. The values of Zn average values obtained through this study are similar to those found in the literature [15-17,44] and higher than Brazil [43].

Table 3.7 shows the percentage of the recommended daily intake (RDI) that provides each menu analysed for Ca, Cu, Fe, K, Mg, Mn, Na and Zn. The percentage of

RFI was calculated as milligrams of every element that gives each menu divided by the values of RDI reported in the literature and multiplied by 100. The RDI values were 800, 0.7, 6, 4700, 350, 2, 2400 and 9.4 mg day^{-1} for Ca, Cu, Fe, K, Mg, Mn, Na and Zn respectively [39,47]. From comparing data found with the recommended RDI values it can be seen that the contribution of Ca, K, Mg, Mn and Zn to the daily intake is between 30 and 70% of the RDI, thus indicating that their concentration level in analysed menus is appropriate to meet the daily needs of the aforementioned minerals. On the other hand, the contribution of sodium is very high in many cases, reaching 200% of the RDI in some sample. This level of Na can create problems for people with hypertension. The amount of Cu and Fe found in each menu depends on the type of foods and in some cases, is quite low and in other cases very high. Therefore, it is recommended to vary as much as possible the foods in the diet to arrive at a balance daily mineral intake.

Table 3.7. Contribution of the studied samples to the daily intake of essential elements

	Percentage that provides each menu to the RDI (%)							
Sample	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Zn**
M1	41	78	133	38	63	69	137	48
M2	41	34	57	20	28	41	79	35
M3	62	143	98	60	84	96	162	58
M4	66	97	71	47	70	81	180	63
M5	43	64	80	44	54	59	68	43
M6	45	34	65	43	54	46	128	44
M7	43	91	75	41	59	75	202	44
M8	41	152	121	81	109	93	177	61
M9	33	97	76	62	70	62	140	63
M10	59	74	48	41	56	58	91	65
M11	65	40	24	36	47	32	103	36
M12	47	50	46	46	52	63	61	33
M13	54	76	67	43	57	51	125	72

NOTE: RDI (Recommended Daily Intake) as indicated in (FAO/WHO, 2002; Food and Nutrition Board, 2004)

3.4. Conclusions

The data results of this study demonstrate the general good quality of university canteen menus with respect to daily intake of mineral elements, as well as low levels of potentially toxic elements. On considering the average concentration of trace essential elements found in present study, it can be concluded that they are adequate as compared with the recommended daily intake by the international organisms, providing from 46 to 79% of the RDI for the essential elements, except for Na (127 %). Only in the case of Na, action must be taken to reduce the excessively high levels, namely using less sodium chloride during cooking. In general, the contents of Cu and Fe are relatively high but it should be noted that Spanish menus provide high concentrations for Ca, K and Mg as well.

The mineral contents provided in the present study have been submitted to a quality control of the measurements being employed certified referential materials, reagent blanks and standards controls in each batch samples.

Finally, in order to collect data on the mineral composition of typical menu samples is a given country can be provide an alternative to studies on diet composition based on questionnaires and reference data.

Acknowledgements

Authors acknowledge the financial support of the Generalitat Valenciana Project PROMETEO 2010-055 and University of Valencia Project UVINV-AE11-42834.

References

[1] European Food Safety Authority (EFSA). (2011). Towards a harmonised Total Diet Study approach: a guidance document, from: www.efsa.europa.eu/en/efsajournal/doc/2450.pdf

[2] Food Standards Australia New Zealand (2008). The 22th Australian Total Diet Survey, a total diet study of five trace elements: I, Se, Cr, Mo, Ni, from: http://www.foodstandards.gov.au/newsroom/publications/22tdaustraliantotaldietstudy/index.cfm

[3] French Total Diet Study 2. Tome 1: Contaminants inorganiques, minéraux, pollutants organiques persistants, mycotoxines, phyto-estrogènes. (2011). Agence nationale de sécurité sanitaire alimentation, environnement, travall, from: http://www.anses.fr/Documents/PASER2006sa0361Ra1.pdf

[4] Millour, S., Nöel, L., Chekri, R., Vastel, C., Kadar, A., Guérin, T. (2010). Internal quality controls applied in inductively coupled plasma mass spectrometry multi-elemental analysis in the second French Total Diet Study. *Accreditation and Quality Assurence*, 15, 503-513.

[5] Millour, S., Nöel, L., Kadar, A., Chekri, R., Vastel, C., Guérin, T. (2011a). Simultaneous analysis of 21 elements in foodstuffs by ICP-MS after closed-vessel microwave digestion: Method validation. *Journal of Food Composition and Analysis*, 24, 111-120.

[6] Millour, S., Nöel, L., Kadar, A., Chekri, R., Vastel, C., Sirot, V., Leblanc, J. C., Guérin, T. (2011b). Pb, Hg, Cd, As, Sb and Al levels in foodstuffs from the 2nd French total diet study. *Food Chemistry*, 126, 1787-1799.

[7] U.S. Food and Drug Administration. Centre for Food Safety and Applied Nutrition, College Park, MD. (2010). Total Diet Study Statistics on Element Results. Market Baskets 2006-1 through 2008-4, from: http://www.fda.gov/downloads/food... totaldietstudy/ucm184301.pdf

[8] Tanase, C.M., Griffin, P., Koski, K.G., Cooper, M.J., Cockell, K.A. (2011). Sodium ans potassium in composite food samples from Canadian Total Diet Study. *Journal of Food Composition and Analysis*, 24, 237-243.

[9] Bastías, J. M., Bermúdez, M., Carrasco, J., Espinoza, O., Muñoz, M., Galotto, M. J., Muñoz, O. (2010). Determination of Dietary Intake of Total Arsenic, Inorganic Arsenic and Total Mercury in the Chilean School Meal Program. *Food Science and Technology International*, 00, 0001-0008.

[10] Lee, H. S., Cho, Y. H., Park, S. O., Kye, S. H., Kim, B. H., Hahm, T. S., Kim, M., Lee, J. O., Kim, C. (2006). Dietary exposure of the Korean population to arsenic, cadmium, lead and mercury. *Journal of Food Composition and Analysis*, 19, S31-S37.

[11] Marzec, Z., Schlegel-Zawadzka, M. (2004). Exposure to cadmium, lead and mercury in the adult population from Eastern Poland, 1990-2002. *Food Additives and Contaminants*, 21, 963-970.

[12] Nöel, L., Leblanc, J. C., Guérin, T. (2003). Determination of several elements in duplicate meals from catering establishments using closed vessels microwave digestion with inductively coupled plasma mass spectrometry detection: estimation of daily dietary intake. *Food Additives and Contaminants,* 20, 44-56.

[13] Muñoz, O., Bastias, J. M., Araya, M., Morales, A., Orellana, C., Rebolledo, R., Velez, D. (2005). Estimation of the dietary intake of cadmium, lead, mercury, and arsenic by the population of Santiago (Chile) using a Total Diet Study. *Food and Chemical Toxicology*, 43, 1647-1655.

[14] Pennington, J., Schoen, S. A. (1995). Estimates of dietary exposure to aluminium. *Food additives and contaminants,* 12, 119-128.

[15] Urieta, I., Jalon, M., Eguileor, I. (1996). Food surveillance in the Basque Country (Spain). 2. Estimation of the dietary intake of organochlorine pesticides, heavy metals, arsenic, aflatoxin M(1), iron and zinc through the total diet study, 1990/91. *Food additives and contaminants*, 13, 29-56.

[16] Ysart, G., Miller, P., Crews, H., Robb, P., Baxter, M., de L'argy, C., Lofthouse, S., Sargent, C., Harrison, N. (1999). Dietary exposure estimates of 30 elements from the UK Total Diet Stuy. *Food additives and contaminants*, 16, 391-403.

[17] Ysart, G., Miller, P., Croasdale, M., Crews, H., Robb, P., Baxter, M., de L'Argy, C., Harrison, N. (2000). 1997 UK Total Diet Study- dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. *Food and additives and contaminants*, 17, 775-786.

[18] Cheung, S. W., Ping, K., Yau, J., Wong, W. (2008). Dietary exposure to antimony, lead and mercury of secondary school students in Hong Kong. *Food Additives and Contaminants*, 25, 831-840.

[19] Llobet, J. M., Falcó, G., Casas, C., Teixidó, A., Domingo, J. L. (2003). Concentrations of Arsenic, Cadmium, Mercury, and Lead in Common Foods and

Estimated Daily Intake by Children, Adolescents, Adults, and Seniors of Catalonia, Spain. *Journal of Agricultural and Food Chemistry*, 51, 838-842.

[20] Rubio, C., González-Iglesias, T., Revert, C., Reguera, J. I., Gutiérrez, A., Hardisson, A. (2005). Lead dietary intake in the Spanish Population (Canary Islands). *Food Additives and Contaminants*, 53, 6543-6549.

[21] Rubio, C., Hardisson, A., Reguera, J. I., Revert, C., Lafuente, M. A., González-Iglesias, T. (2006). Cadmium dietary intake in the Canary Islands, Spain. *Environmental Research*, 100, 123-129.

[22] Sola-Larrañaga, C., Navarro-Blasco, I. (2009). Chemometric analysis of mineral and trace elements in raw cow milk from the community of Navarra, Spain. *Food Chemistry*, 112, 189-196.

[23] Maihara, V. A., Vasconcellos, M. B. A., Cordeiro, M. B., Cozzolino, S. M. F. (1998). Estimates of toxic element intake in diets of pre-school children and elderly collected by duplicate portion sampling. *Food additives and contaminants*, 15, 782-788.

[24] Mainhara, V. A., Gonzaga, I. B., Silva, V. L., Fávaro, D. I. T., Vasconcellos, M. B. A., Cozzolino, S. M. F. (2004). Daily dietary selenium intake of selected Brazilian population groups. *Journal of Radioanalytical and Nuclear Chemistry*, 259, 465-468.

[25] Robberecht, H., Van Cauwenbergh, R., Bosscher, D., Cornelis, R.,Deelstra, H. (2002). Daily dietary total arsenic intake in Belgium using duplicate portion sampling and elemental content of various foodstuffs. *European Food and Research Technology*, 214, 27-32.

[26] Van Cauwenbergh, R., Hendrix, P., Robberecht, H. J., Deelstra, H. A. (1999). Daily dietary sodium and potassium intake in Belgium, using duplicate portion sampling. *Eur Food Res Technol*, 209, 63-67.

[27] Van Cauwenbergh, R., Bosscher, D., Robberecht, H., Deelstra, H. (2000). Daily dietary cadmium intake in Belgium using duplicate portion sampling. *European Food and Research Technology*, 212, 13-16.

[28] Maclntosh, D. L., Spengler, J. D., Özkaynak, H., Tsai, L., Ryan, P. B. (1996). Dietary exposures to selected metals and pesticides. *Environmental health perspectives*, 104, 202-209.

[29] Pedrero, Z., Madrid, Y. (2009). Novel approaches for selenium speciation in foodstuffs and biological specimens: A review. *Analytical Chimica Acta*, 634, 135-152.

[30] Boeting, K., Aguilera de Benzo, Z., Cervera, M. L., de la Guardia, M. (2010). Authentication of the protected designation of origin horchata de Valencia through the chemometric treatment of mineral content. *Analytical Methods*, 2, 1723-1728.

[31] Gonzálvez, A., Llorens, A., Cervera, M. L., Armenta, S., de la Guardia, M. (2009). Elemental fingerprints of wines from the protected designation of origin Valencia. *Food Chemistry*, 112, 26-34.

[32] Gonzálvez, A., Armenta, S., de la Guardia, M. (2011). Geographical traceability of "Arròs de Valencia" rice grain based on mineral element composition. *Food Chemistry*, 126, 1254-1260.

[33] Reilly, C. (2002). Metal contamination of food (Third edition). *Oxford Brookes University*, Oxford, UK.

[34] Agency of Toxic Substances & Disease Registry (ATSDR), Public health statement for Al (2008), from: www.atsdr.cdc.gov

[35] Gramiccioni, L., Ingrao, G., Milarna, M. R., Santaroni, P., Tomassi, G. (1996). Aluminium levels in Italian diets and in selected foods from aluminium utensils. *Food additives and contaminants*, 13, 767-774.

[36] Nordberg, G. F., Fowler, B. A., Nordberg, M., Friberg, L.T. (2007). *Handbook on toxicology of metals* (Third edition). *Elsevier*.

[37] Martorell, I., Perelló, G., Martí-Cid, R., Llobet, J.M., Castell, V., Domingo, J.L. (2011). Human exposure to arsenic, cadmium, mercury, and lead from foods in Catalonia, Spain: Temporal Trend. *Biological Trace Element Research*, 142, 309-322.

[38] Agency of Toxic Substances & Disease Registry (ATSDR), Public health statement for a Ba (2007), from: www.atsdr.cdc.gov

[39] FAO/WHO Human Vitamin and Mineral Requirements. (2002). Report of a joint FAO/WHO expert consultation Bangkok, Thailand.

[40] FAO Human nutrition in the developing world. (1997). Food and nutrition series nº 29 by Michael C. Latham, from www.feo.org/docrep/W0073E/w0007300.htm

[41] Egan, S.K., Tao, S.S-H., Pennington, J.A.T., Bolger, P.M. (2002). US Food and Drug Administration’s Total Diet Study: intake of nutrional and toxic elements 1991-96. *Food Additives and Contaminants*, 19, 103-125.

[42] Deutch, B., Byerberg, J., Pedersen, H.S., Aschlund, E., Hansen, J.C. (2007). Traditional and modern Greenlandic food – Dietary composition, nutrients and contaminants. *Science of the Total Environment*, 384, 106-119.

[43] Avegliano, R.P., Maihara, V.A., da Silva, F.F. (2011). A Brazilian Total Diet Study: Evaluation of essential elements. *Journal of Food Composition and Analysis*, 24, 1009-1016.

[44] Becker, W., Jorhem, L., Sundström, B., Petersson Grawé, K. (2011). Contents of mineral elements in Swedish market basket diets. *Journal of Food Composition and Analysis*, 24, 279-287.

[45] FAO Food, nutrition and agriculture. Alimentation, nutrition et agriculture. Alimentación, nutrición y agricultura. (1999)., from www.fao.org/docrep/x2650T/x2650T00.htm

[46] Agency of Toxic Substances & Disease Registry (ATSDR), Public health statement for Sr (2004), from: www.atsdr.cdc.gov

[47] Food and Nutrition Board, Institute of Medicine, National Academies Press. (2004). Dietary Reference Intake (DRIs): Recommended Intakes for Individuals Elements.

Capítulo 4

Análisis mineral en dietas mediante métodos espectroscópicos

Mineral analysis of human diets by spectrometry methods

Alba Mir-Marqués, M.Luisa Cervera, Miguel de la Guardia

Department of Analytical Chemistry. University of Valencia. Jeronimo Muñoz research building, 50th Dr. Moliner str. 46100 Burjassot. Valencia. Spain.

Received 11 February 2016,

Received in revised form 20 July 2016,

Accepted 21 July 2016,

Available online 25 July 2016

Trends in Analytical Chemistry 82 (2016) 457-467

DOI 10.1016/j.trac.2016.07.007

Resumen

La determinación de elementos minerales en dietas es muy importante para la salud del ser humano, debido a la presencia de elementos esenciales y elementos tóxicos en los alimentos o su incorporación durante la manipulación y el proceso de cocinado. Se han utilizado diferentes técnicas instrumentales para la determinación de elementos minerales en dietas humanas, pero las técnicas basadas en la espectroscopia atómica y la espectrometría de masas son las más comúnmente empleadas. La etapa más crítica en el análisis mineral en dietas es el procedimiento de muestreo, por lo que se suelen usar protocolos estandarizados. Este capítulo pretende resumir críticamente las últimas tendencias en el análisis mineral en dietas humanas, considerando el muestreo, la preparación de la muestra y el procedimiento de determinación de los elementos. Los datos encontrados en la bibliográfica procedentes de diferentes países se han comparado entre ellos y, con los valores de ingesta diaria recomendados establecidos por las agencias internacionales para la salud humana.

Abstract

Mineral element determination in human diets is very important for human health, due to the presence in foods of essential and toxic elements or their incorporation in the manipulation and cooking food process. Different instrumental techniques have been used to determine mineral elements in human diets, but atomic spectroscopy and mass spectrometry based ones are the most commonly employed. Sampling procedures for diet analysis are the main critically step for mineral element determination, being employed different standardised protocols. This review summarised critically the state-of-the-art of mineral analysis in human diets, considering sampling, sample preparation and determination procedures. Results obtained from different countries are compared between them and with recommended daily intake values established by international agencies for the preservation of human health.

4.1. Introduction

The general population is exposed to a wide range of mineral elements through food, water, air and other environmental sources. The diet together with tap water is the main route of exposure to toxic elements, but also the way to obtain essential and non-essential elements [1, 2]. Food, as consumed, contains a lot of different compounds which some are necessary and other unnecessary for a correct human health. Nutrients are a natural part of food and are the basis for human nutrition. In some cases, especially in processed foods, they may also be added to ensure the optimal functionality of the final product and to provide specific qualities [3]. The amounts that can be added to processed foods are regulated by maximum permitted levels defined in food legislation for each food category and each country to ensure the consumer safety [4]. Essential elements are very important for the correct functioning of human body, because of their interactions in genetic and physiological processes. A deficiency of essential elements could bring on structural and physiological abnormalities, while a higher concentration than recommended ones may be harmful.

Potentially toxic elements may be also found in foods but, fortunately, in lower concentrations than essential ones. The presence of toxic and non-essential elements in foods is attributed to natural sources; such as raw materials or contamination during their production and processing. Arsenic, cadmium, mercury, lead, aluminium, barium, antimony and tin can accumulate in foods creating potentially dangerous situation for the consumers. Based on the aforementioned considerations, it is very important to control the concentration of mineral elements in foods.

Daily intake for essential elements is recommended by international organisms and the maximum permitted levels of toxic ones are regulated by the legislation concerning each food category to avoid hazards for human health [5]. To accomplish a complete characterisation of the mineral profile of foods and human diets, highly informative and sensitive analytical methods are required. To monitor human ingestion and to evaluate the risks associated with the use of foods produced or subjected to bad processing practices. An evaluation element by element and food by food of the human mineral intake is a very complex task and cannot provide a realistic picture about the lack of essential elements and the risks associated to the toxic ones in the different communities all around the world. Because of that, the control of diets seems the most appropriate approach for screening the human consumption of mineral elements.

The main components of mineral to be determined in diet samples are present at trace levels; hence analytical techniques with adequate sensitivity are required for their accurate determination. Additionally, it is important to remember that the accuracy of the quantitative analysis procedures is strongly dependent on the sampling and sample preparation steps, and thus, these two aspects must be controlled in food analysis together with the most employed techniques for trace element analysis in foods.

Another point to consider is the bioavailability of minerals in food, as not all the minerals that human ingest are available for the human organism, only a fraction of them present in the food matrix is bioavailable and can be absorbed and utilised by the human body. The fraction which can be bioaccessible to the human body depends on the speciation of the elements, on the behaviour of organometallic species and complexes in the gastrointestinal tract, and on interactions with the food matrix [6]. Because of that, in recent years some diet studies are focusing on study the minerals bioavailability in the human diet, not only in total mineral content of food.

On the other hand, the new approaches in analytical chemistry are focused on finding more environmental friendly techniques including in food chemistry. In the literature, it can find enough scientific articles regarding green techniques for analysis of minerals in foods, while they are not often applied to the analysis of complete diets.

The main objective of this review has been to provide an update of methods proposed in the literature to determine essential, non-essential and toxic elements in human diets, being that the most direct way to evaluate mineral element intake of humans.

4.2. Mineral composition of diet samples and their contribution to the daily intake

The control of elements ingested through the diet is very important to avoid levels of toxic elements that can cause health damages and moreover to ensure the daily recommended intake of essential elements, since their deficiency might also cause health troubles.

4.2.1. Toxic and non-essential elements

Toxic elements are those can cause hazardous effects in human health. It is not always possible to clearly differentiate between toxic elements, elements that play essential roles in human body functions and ones with unknown effects. However, it must be taken into account that at high concentration levels practically all the mineral element present deleterious effects on the health. The most relevant toxic elements in the diet are As, Cd, Pb and Hg, and similarly exposure to minor toxic metals as Al, Ba, Be, Cr (VI), Sb, Sn, Tl and U must be taken into consideration and, in some cases, are considered in the legislation about maximum tolerable levels in food. Figure 4.1 summarised the concentration levels found for toxic and non-essential elements in studies concerning human diet analysis all around the world.

Some international agencies as the World Health Organization (WHO), the European Food Safety Authority (EFSA) and the Agency for Toxic Substances and Disease Registry (ATSDR) are evaluating the adverse health effects of heavy metals and other elements. The most frequently parameters used are the acceptable daily intake (ADI), tolerable daily intake (TDI), provisional tolerable daily intake (PTDI) or provisional tolerable weekly intake (PTWI) and the tolerable upper level intake (UL). PTWI for As, Cd, Hg and Pb are 15, 7, 5 and 25 µg per kg body weight per week, respectively [7, 8]. PTWI values represent permissible human weekly exposure to contaminants unavoidably associated with the consumption of foods. The term ‘provisional’ is related to the lack of a deep knowledge on the consequences of human exposure at levels approaching those established by the Joint FAO/WHO Expert Committee of Food Additives (JECFA) [9].

The contribution of arsenic, cadmium, mercury and lead to the PTWI found in the literature are lower than 100% in all cases. The highest values of PTWI (>80%), found in the literature, for Cd and Hg were to a duplicate diet study in a restaurant from Tarragona (Spain) [10]; and for Pb were to a total diet study from Chilean population [11].

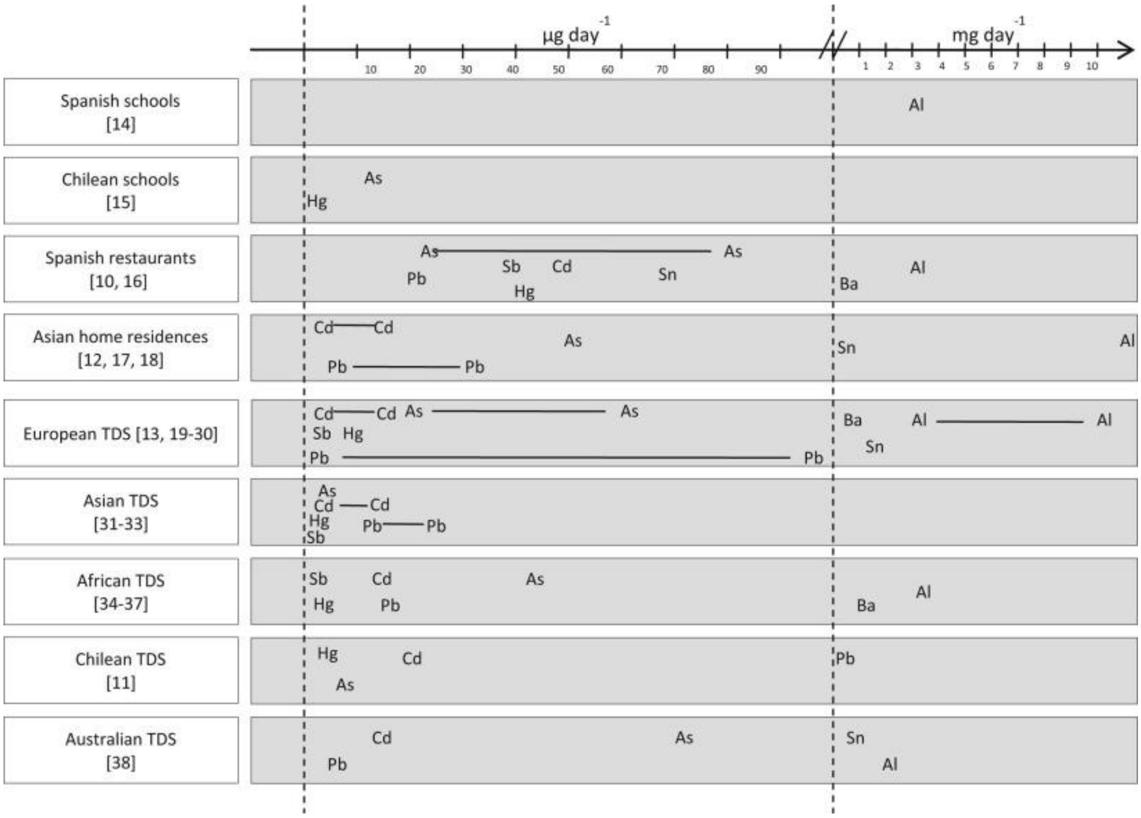

Figure 4.1. Daily intake of toxic and non-essential elements in human diets obtained from published data during the last ten years

From the data reported for non-essential elements in diets, it can be say that the content of elements like aluminium, barium, antinomy and tin, in different kind of menus and total diet studies, is very low and therefore, being not a problem for the population. The weekly intake values of Ba, Sn and Sb found in the literature are lower than 20% of the established PTWI. In the case of Al, the weekly intake are lower than 40% of the PTWI in all cases, except to diet analysis made in Tokio (Japan) [12] and Canary Island (Spain) [13], but no way are these values dangerous from human health.

4.2.2. Essential elements

Calcium, iron, potassium, magnesium, sodium and zinc are considered essential elements and their intake are required at milligrams per kilogram level to maintain the human organisms free from deficiency effects. Additionally, copper, cobalt, chromium, manganese, molybdenum, nickel and selenium are needed at trace levels in the human diets. Figure 4.2 shows the range of concentration of essential elements found in the recent literature regarding diet analysis.

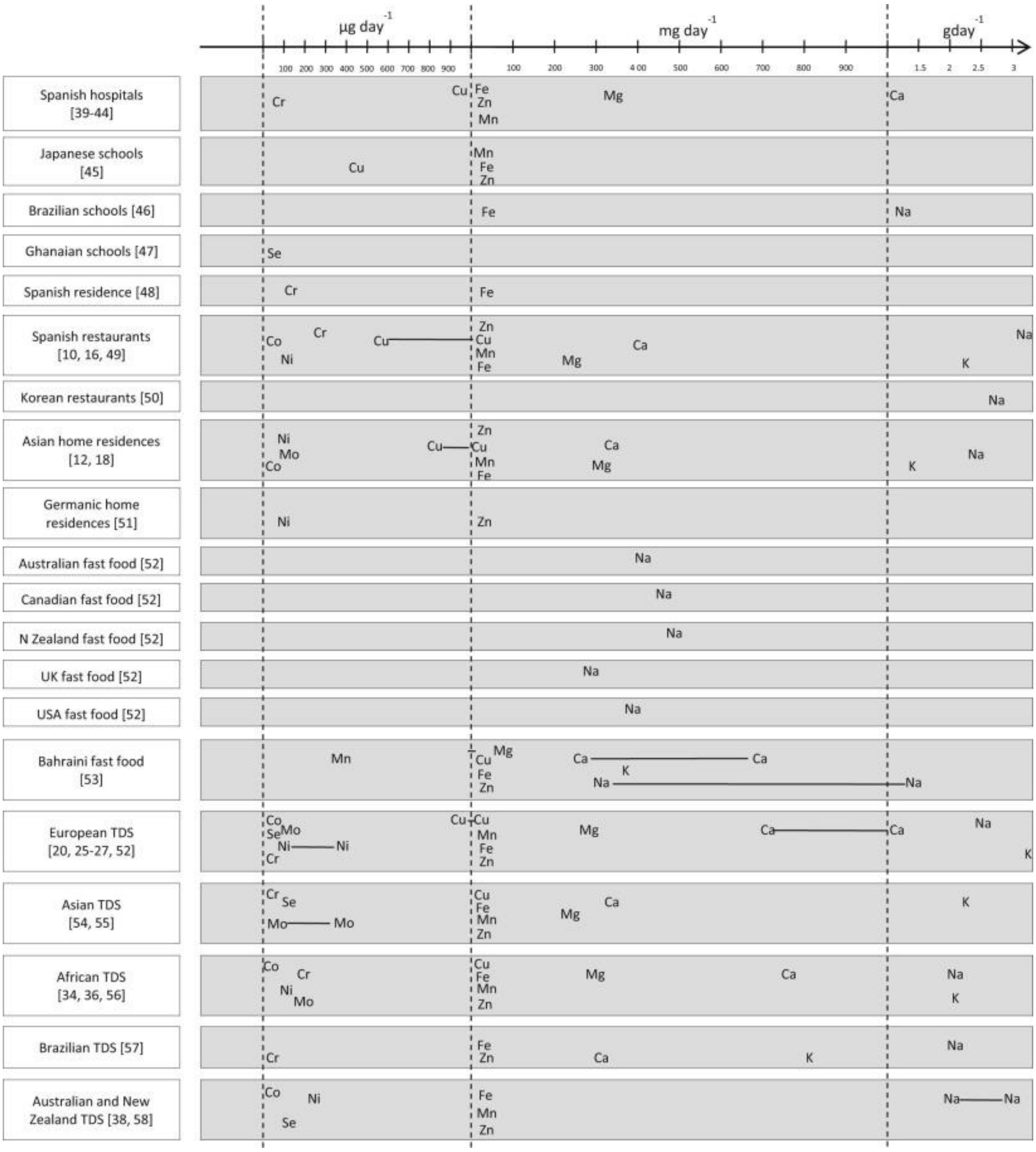

Figure 4.2. Daily intake of essential elements inhuman diets obtained from published data during the last ten years

Unfortunately, sodium is an essential element present in diets at g day^{-1} level followed by potassium, calcium and magnesium. High sodium intake is associated with high risk of incident stroke, fatal stroke and fatal coronary heart disease. WHO recommends a reduction to less than 2 g day^{-1} of sodium (5 g day^{-1} salt) in adults [59]. In the 40% of the data found in the literature, sodium content exceeds this value, especially in human diets obtained from restaurants. On the other hand, WHO recommends an increase in potassium intake from food in order to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart disease in adults, mitigating the negative effects of elevated sodium consumption [60, 61]. WHO suggests a potassium intake of at least 3510 mg day^{-1} for adults. Calcium is essential for the correct development and maintenance of the skeleton, muscular function, nerve conduction, and it is involved in multiple intracellular pathways and enzymatic reactions. The recommended daily intake for calcium or recommended daily allowance is 1000 mg day^{-1} for adults older than 19 years [62]. Fortunately, levels of calcium reported in diet studies are appropriated, because most of the diets provide between 75 to 110% of the recommended daily intake. The main role of magnesium is the potassium fluxes regulation and its involvement in the metabolism of calcium. Magnesium content is abundant in most green vegetables, legume seeds, beans, and nuts, some shellfish, spices, and soya flour, which usually contain more than 500 mg kg^{-1} in fresh weight. The recommended daily intake of magnesium is between 220 and 260 mg day^{-1} for adult females and males, respectively [62].

The concentration found in the literature for Fe, Zn, Mn and Cu are lower than 100 mg day^{-1}. Iron has several vital functions in the body, being calcium the only dietary factor that negatively influences in their mechanism of absorption. The recommended daily intake of iron depends on its bioavailability and varies between 19.6 to 58.5 mg day^{-1} for females and 9.1 until 27.4 mg day^{-1} for males [62]. Taking into account the bioavailability of iron from ingested food, the iron content in diets is very low for women and can cause anaemia, WHO reported that 30% of non-pregnant women suffer from anaemia [63]. Zinc is an essential component of more than 300 enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. The recommended daily intake of zinc depends on its bioavailability, for a low bioavailability (15%) the RDI value is 9.8 and 14 mg day^{-1}, for a medium bioavailability (30%) is 4.9 and 7 mg day^{-1} and for high bioavailability (50%) is 3 and 4.2 mg day^{-1} for females and males, respectively [62]. In the case of zinc, the intake in reported studies is generally higher than the recommended values, especially when the bioavailability of zinc in foods is medium or high. Manganese is present in all plant and animal tissues, being food the major source of manganese for humans [64]. The RDI value is 1.8 and 2.3 mg day^{-1} for females and males, respectively. These values are lower than most data found in the literature for diet analysis. Copper is an essential trace element as a vital part of several enzymes. Fish, fruits, cereals, nuts, and green vegetables are good sources of copper [64]. WHO suggested a daily intake of copper of 1.2 and 1.3 mg day^{-1} for females and males, respectively [65]. Most data found in the literature about copper intake in diets reach the recommended values by the WHO.

In almost studies found in the literature Cr, Se, Mo, Co and Ni are present at levels below 400 µg day^{-1}. For Cr, the 50% of the data reported in the recent literature concerning the contribution to the recommended daily intake values exceeded this limit [25, 42, 48, 56]. In most cases, levels of molybdenum and selenium reported in diet studies are higher than recommended values, and for Co and Ni values found in the literature agree with the values of recommended daily intake.

4.2.3. Mineral bioavailability in diets

To evaluate the real intake of essential micronutrients from the diet, the determination of the potentially bioaccessible fraction from food must be taken into account. There are factors which enhancing or inhibiting the bioaccessibility of minerals, like interactions between different foods and physiological ones (sex, age, and nutritional status). Scarce studies of bioavailability of minerals in complete human diets are found in the literature. Lazarte et al. [66] analysed the content of zinc, iron, calcium and phytate in the 16 most consumed foods from five villages in a tropical rural area of Bolivia, and they evaluated the mineral bioavailability by calculation the molar ratio of phytate and each mineral. They concluded that phytate may be one of the main factors cause zinc and iron deficiencies.

Singh et al. [67] reported the total content of Fe and Zn and their bioavailability in the food samples which are commonly consumed by Fiji population. This study showed that there are many factors which affect minerals bioavailability like mineral content of soil, climatic conditions, storage, ripping system and cooking methods of foods. The absorption of Fe and Zn mineral from foods depends on their contents and other factors (phytate, tannin, etc.) available in the food samples.

Moreover, it can be found several references in the literature on mineral bioavailability, generally in vegetables, which have a high content of certain minerals but low bioavailability [68, 69].

4.3. Sample treatment for determination of the mineral composition of diets

The most common methods employed for the analysis of mineral composition of human diets are those based on atomic and mass spectrometry. However, to carry out the mineral analysis using these methodologies samples must be previously dissolved or digested in order to obtain appropriate solutions, and to do it, sampling and sample preparation procedures must be critically discussed.

4.3.1. Diet sampling methods

Sampling is the most important step in the analytical process in order to obtain reliable analytical results. It is essential that samples are taken from the lot in a representative way [70]. Sampling methods for diet analyses are very complex, because diet samples include many ingredients from liquids to solid with different textures. Because of that, there are detailed sampling protocols for diets in the literature as it can be seen in Figure 4.3.

Sampling methods employed in diet analysis can be separated in two groups, direct and indirect approaches. Direct sampling allows the incorporation of a large number of personal exposure monitoring and biological markers of exposure. These methods are simple and accurate.

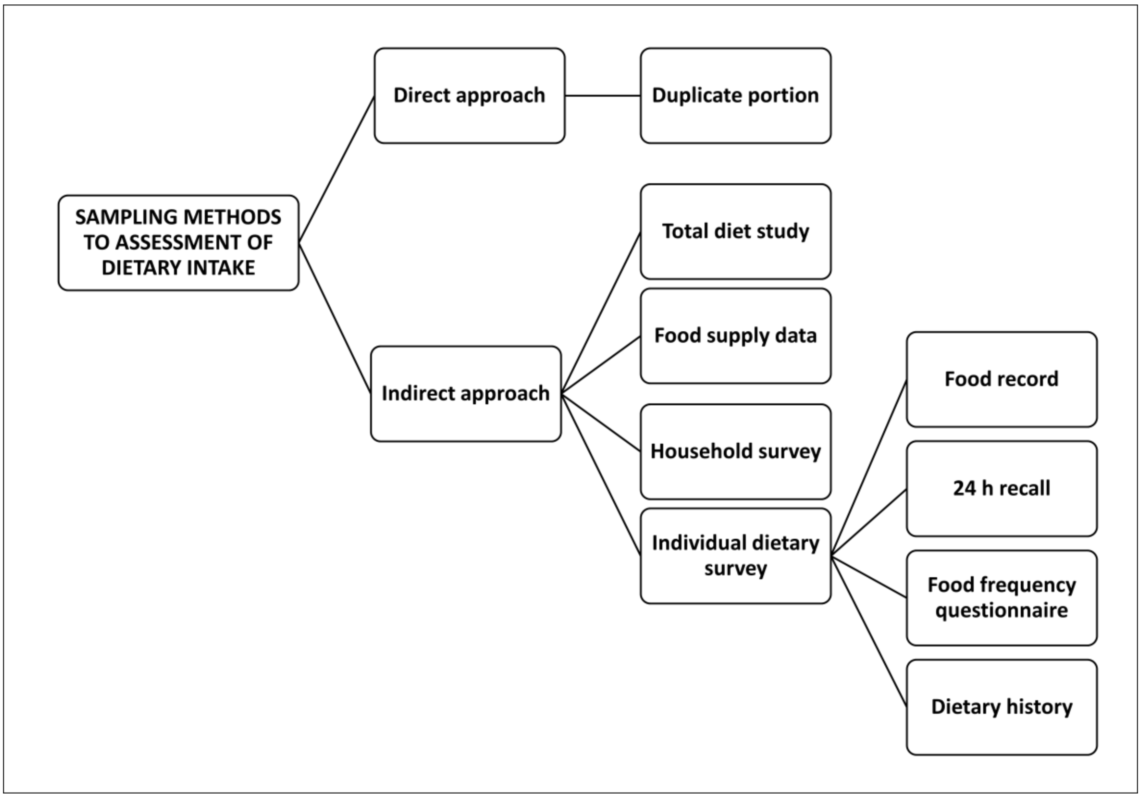

Figure 4.3. Scheme about different kind of sampling methods in human diet analysis

Duplicate portion method provides an useful methodology that supplies the most accurate information on the ingested dose of mineral elements in a diet [71]. This approach is based on the preparation of twice the usual portions of food that would be consumed by an individual within the home or institution. Residential homes [12, 17, 18, 51, 72, 73], schools [14, 15, 45–48, 74], hospitals [39–44], cafeterias [10, 16, 18, 49, 50] and fast food restaurants [52, 53, 75, 76] have been reported as the basis through with duplicate portion studies were organised. Aung et al. [12] did their study during seven consecutive days with 25 pairs of Japanese children and their parents in the city centre and in the suburb regions of Tokyo; Wittsiepe et al. [51] also carried out their study during seven consecutive days in young children living in urban and rural areas of Germany, both in residential homes. Cabrera-Vique et al. [14, 75] and Adotey et al. [47] also did their sampling during seven consecutive days in female university residences from Spain and adolescent orphanages from Ghana, respectively. Liu et al. [17, 72] and Sun et al. [73] did their study with 30 children and 30 adults from south-east of China during three consecutive days. Raghunat et al. [18] did the sampling only during one day but extended to 250 volunteers belonged to the middle class from Mumbai (India). Although some authors prefer to do the sampling during many days, especially when they have few places for do it. Weber & Morais [46] performed their sampling during four weeks in 15 canteens from public and private primary schools in Brazil. In a hospital from Motril (Spain) it was made a sampling during 36 consecutive days by Velasco-Reynold et al. [39–44]. Duplicate portion is a suitable method for institutional groups and for small surveys but, because of the cost and time involved, it is not appropriate for large-scale food consumption studies [77].

Total diet study, food supply data, household data, and individual dietary surveys are included within indirect methods for assessment of dietary intake. Indirect methods make predictions of personal exposure to mineral elements using exposure factor information, modelling and questionnaires combined with the analysis of selected foods or database mineral content of foods. **Total diet study** (TDS) also known as a market basket survey (MBS) is the most common approach to evaluate daily intake of mineral elements from the analysis of individual food items. A list which includes foods that constitutes the largest part of the total diet of the studied population is compiled, and then they are purchased and prepared as usually consumed by the population using the most common local practices and combining them into composite samples before analysis for contaminants. Food consumption and content data must be combined to evaluate the intakes within the population. This distribution is compared with international reference recommendation for essential elements and toxicological values in order to assess the safety of food consumed by the population [1]. It is very common that health agencies from different countries are responsible to carry out or supervise the total diet studies like Australia, US, Canada, New Zealand and China [78–82].

Food supply data approach, also known as food balance sheets (FBS), has been developed because of the need for assessing the amount of food and nutrients available for human consumption. Results are an estimation of the average value per head of the population classified for age or gender. However, food supply data do not indicate any difference in food consumption levels between different regions, occupations or income levels. Becker et al. [20] based their study on food supply data of 1998 from Sweden. They did a shopping list where separated categories into food items using data on their market shares. Food items consumed on average less than 0.5 kg per person in one year were excluded from the list. The shopping list finally consisted of 116 foods and beverages and covered approximately 90% of the total annual consumption expressed in kg per person.

In contrast to food supply data, **household surveys** provide information about food consumption levels in different regions, occupations or income levels, although not provide information on how food is handled within the household, or members' consumption. Data on the quantity of consumed food may be collected by record keeping, by interviews or by both methods. Gimou et al. [34, 56, 83] carried out their diet sampling based on the 2nd Cameroonian Household Budget Survey from 2001. They purchased a total of 1773 foods representing 203 food items that were prepared as consumed in Cameroon. Avegliano et al. [57] did the sampling survey of 2017 household, based on the food consumption data base of the National Household Food Budget Survey from 2003. They purchased 5440 food items and prepared them at the university restaurant of University of Sao Paulo (Brazil).

To obtain information on average food and nutrition intake and their distribution over groups of individuals it has been used **individual dietary surveys**. Methods to collect dietary intake data at an individual level can be divided into two categories, record and recall methods. Record methods collect information on intake over one or more days. Recall methods reflect the past consumption, varying from intake of previous days (24-hour recall) to usual food intake (food frequency or dietary history). So, the following methodologies have been applied in the literature: i) *Food record*, which is kept for a specific time period, usually 1-7 days. The food records include all foods and beverages consumed at meals and in quantified amounts, as well as the inedible waste, the total cooked weight of meals, the cooked weight of the individual portion and dish waste, ii) In *recall method* for which the subject does a list of foods and beverages consumed the previous day or the 24 hours prior to the recall interview, and describes the kind and amounts of all foods and beverages ingested during this period of time. Food quantities are usually assessed by using household measures, food models or photographs, iii) *Food frequency questionnaire*, a method used as a tool in assessing the frequency of individual foods or food group's intake over extended periods of time (weeks, months, or years). The questionnaire, in this case, may include questions regarding the amount of food consumed, usual food preparation methods, including the use of dietary supplements and iv) in *dietary history method*, the main purpose is obtaining a picture of dietary consumption habits of the studied population. A trained interviewer assesses an individual's total usual food intake and meal pattern.

The main part of studies use 24 h recall to obtain daily intake information, because this method provides detailed quantitative assessment of intake and thus, these data can be used to estimate acute or chronic exposure, offer a possibility of calculating average and distribution of exposure and estimates that can be produced for specific population groups [11, 21-24, 31, 54, 71].

In order to collect data on mineral intake from the diet in small scale we consider that the best solution is the duplicate portion approach which is faster and easier than total diet studies. The determination of mineral composition of typical menu samples in a given country can provide an alternative to the studies on diet composition based on questionnaires and reference data.

4.3.2. Diet sample pre-treatment

Sample pre-treatment should involve as few steps as possible, reducing the probability of sample and analyte losses, or alteration of species. Sample pre-treatment procedures used in diet analysis by atomic spectrometry and mass spectrometry methods include digestion of target samples by dry-ashing and wet-digestion procedures. Other available techniques; such as infrared or X-ray fluorescence, only require drying of samples for mineral determination [49].

Diet samples required preservation, avoiding microbiological degradation by drying or freezing. Due to high degree of heterogeneity in diet samples is recommended pulverise and homogenise the sample previously.

In general, results of mineral analysis in diet are expressed on a dry-weight basis, because drying is the way to preserve samples during extended period of time and allows pre-concentration of the elements to be determined. Samples usually were dried inside the oven until constant weight at different temperatures, according to the literature, as 40 ºC [23, 32, 34, 35, 83–85], 50 ºC [11], 65 ºC [15], 60-80 ºC [13, 41–43], 100 ºC [22], 102 ºC [46] or 105 ºC [36, 37].

On the other hand, freeze-drying is also commonly used to dry diet samples before their analysis because it permits that the original structure of the products remain unaltered and only water is removed during this process [12, 16, 33, 47, 51, 54, 57, 86]. To start the freeze-drying process, it is necessary to freeze the raw material at -18°C approximately. The second step of the process is the extraction of the water from the raw material, for that, the food is placed in a vacuum chamber. Under very low pressure, the frozen water contained in its structure is removed in the form of vapour. Some authors use different methods like microwave [14, 48] or infrared lamp [21] to dry the samples. It has been reported that during food drying steps some compounds could be lost and, because of that, other authors prefer to treat directly wet samples for their mineral analysis in diet. In these cases, samples are just frozen to improve its preservation until their analysis [10, 19, 20, 25-27, 31, 38, 45, 73, 74, 87].

Sample matrix treatment can be carried out by dry-ashing or wet digestion procedures that permit the decomposition of organic compounds and produce a residue of inorganic compounds followed for the elemental analysis by atomic spectroscopy methods.

Dry-ashing of diets

Ashing methods ensure the complete decomposition of organic matter by heating the sample at high temperatures inside a muffle furnace. Samples are heated between 400 ºC [15] to 550 ºC [31], being the most common temperature used 450 ºC [11, 13, 22, 24, 74]. Samples heating at high temperature can involve losses of some elements by volatilisation, like Hg, As, Se, Te, Sb or halogen compound. So, this problem may be avoided by addition of ashing aids, as magnesium nitrate or magnesium oxide [11, 15].

Ashes are dissolved with nitric acid or hydrochloric acid, in variable concentrations depending of authors (see Table 4.1) and, in some cases, solutions are filtered before their analysis [11].

Dry-ashing methodology uses few millilitres of reagents for completely dissolve the ashes, but requires long mineralisation time.

Table 4.1. Methods reported in the literature for the mineralisation of human diet samples

Digestion process	**References**
Dry-ashing followed by dissolution of ashes with HNO_3	[13, 18, 20-22]
Dry-ashing followed by dissolution of ashes with HNO_3-HCl	[15, 24, 71, 88]
Dry-ashing followed by dissolution of ashes with HNO_3-H_2O_2	[11, 45, 89]
Dry-ashing followed by dissolution of ashes with HCl	[31]
Wet digestion in hot-plate with HNO_3	[10, 12, 28, 29, 38]
Wet digestion in hot-plate with HNO_3-HCl	[58]
Wet digestion in hot-plate with HNO_3-H_2O_2-HCl	[33]
Wet digestion in MW with HNO_3-H_2O_2	[16, 17, 30, 32, 36, 37, 51, 54, 73, 85]
Wet digestion in MW with HNO_3-H_2O	[26, 34, 35, 56, 83-84]
Wet digestion in MW with HNO_3-$HClO_4$	[14, 28, 55, 76]
Wet digestion in MW with HNO_3-H_2SO_4	[23]
Wet digestion in MW with HNO_3-HCl	[27]
Wet digestion in MW with HNO_3-HCl-HF	[19]

NOTE: MW microwave-assisted digestion

Wet digestion of diets

Wet digestion requires lower temperatures than those applied for dry-ashing methods, being carried out wet digestion until clear solutions, at temperatures in the range 80-120 ºC in hot plate [10, 28, 29, 33] or between 180 and 210 ºC in microwave oven [16, 17, 30, 73], until to clear solutions. A pre-digestion step at the beginning, can be employed for accelerate sample dissolution during a time of 15 min to 8 h. Ultra-sonication can be also applied directly to the samples or indirectly through the walls of the sample container using a water bath for pre-digestion [90].

Sample amounts and reagents used in microwave digestion methodologies are similar to those employed in hotplate digestion. In the case of wet sample treatment, the amount of sample varies between 0.2 to 3 g and, in the case of dry samples 0.1 to 1 g of sample diet were treated. Generally, the most widely used combination reagents in microwave-assisted digestion treatment is nitric acid with hydrogen peroxide in varying proportions (see Table 4.1).

The aforementioned wet digestion methodologies at atmospheric pressure are time-consuming, and analyte contamination or losses can occur. So, the use of microwave-assisted digestion (MW) could be a solution to provide a rapid total decomposition of diet sample with reduced risks of contamination and analyte losses [91].

The most employed methods reported in the literature for the mineralisation of human diets are summarised in Table 4.1.

4.4. Analytical methods for mineral analysis of diets

Total concentration of elements in complete diet samples are mainly determined by atomic spectrometry and mass methods, including inductively coupled plasma mass spectrometry (ICPMS), inductively coupled plasma optical emission spectroscopy (ICPOES), graphite furnace atomic absorption spectroscopy (GFAAS) and flame atomic absorption spectroscopy (FAAS). One of the complexities in food analysis is that the essential elements like K, Mg, Ca, Na, Fe and Zn are present at relatively high concentration levels, while toxic elements as Pb, Cd, Hg and As should be present at trace or ultra-trace levels. So, for toxic elements and trace minerals determination in diets ICPMS and GFAAS are often used due the capability to work at ppb level with limit of detection (LOD) values very low, being the working range for ICPOES and FAAS at ppm level (see Figure 4.4).

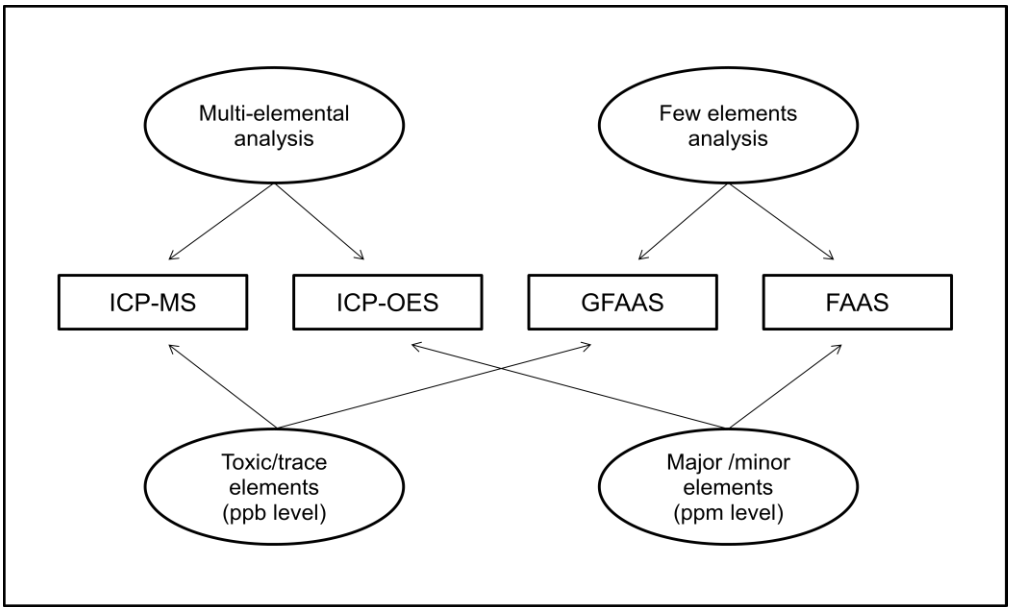

Figure 4.4. Principal uses of atomic and mass spectrometry techniques

Looking at the recently published works (see Table 4.2) it can be appreciated that in 36% of cases ICPMS was employed as analysis technique, 24% used GFAAS, 24% used FAAS and 16% used ICPOES. To choose an appropriate measurement technique for determination of the mineral profile of diet samples various factors, such as detection capability, sensitivity, speed of analysis, cost and applicability, must be taken into account. ICP-based techniques are particularly effective in the multi-elemental analysis of diet samples within wide concentration ranges. ICPMS is considered the most suitable technique for elemental food analysis, because it allows quantify a big number of contaminants, minerals and nutrients whose concentration ranges varied widely. GFAAS and FAAS are more time-consuming than ICP-based techniques due to their mono-elemental detection, but still quite often applied when studies are focused on a few number of elements, because of their relatively low cost. Table 4.3 shows LODs found in the literature to determine elements in human diets.

In order to reduce time and cost of analysis, spectrometric methods that combine fast analysis together with a simple sample preparation have a great interest for the analysis of food samples. Energy dispersive X-ray fluorescence (EDXRF) and infrared spectroscopy (IR) eliminate the need of sample pre-treatment because they can be applied to solid samples and providing rapid measurements. The combination of infrared spectroscopy or XRF measurements with chemometric data treatment could offer increasing possibilities for the direct determination of mineral components in food samples. In the literature, partial least squares (PLS) or modified-partial lest squares (MPLS) have been employed for developing models for quantitative determination of mineral elements in foods [92–96] and diet analysis [49]. Unfortunately, XRF and IR suffer from relatively low sensitivity and strong inter-element and matrix effects.

Essential and toxic elements in diet samples can be also determined using instrumental neutron activation analysis (INAA) which is a really effective technique to measure major, minor and trace elements in foods [47, 57]. However, this technique has been rarely used, probably due to its expensive costs, the use of big facilities, additionally than the long time required. Generally, the potential toxic elements as cadmium and lead are analysed by GFAAS [11, 20–22, 32, 72], while arsenic and mercury are analysed by HGAFS [11, 15, 17, 23, 32, 73]. However, some authors prefer to determine the last four elements by ICPMS [10, 26–30, 38, 83, 85].

4.5. Conclusions

The analysis of the mineral composition of complete diets offers the best way to obtain true values on the daily intake of minerals by a target population. It must be preferred in front of those approaches based on food surveys and average data on the concentration of mineral elements in each kind of foods.

Table 4.2. Common analytical techniques reported in the literature for the elemental analysis of human diets

	ANALYTICAL TECHNIQUES				
Element	**ICPMS**	**ICPOES**	**GFAAS**	**FAAS**	**Other technique**
Al	[12, 26, 27, 34, 35, 38, 83, 84]	[13, 16, 19]	[14]		
As	[10, 26-30, 35, 38, 56, 83, 84]	[31]			HGAFS [11, 15, 17, 32]
Ba	[19, 27, 34, 54, 84]	[16, 25]			
Ca	[26, 54]	[16, 20, 86]		[18, 40, 56, 74, 83, 88]	INAA [57], MIR, NIR and XRF [49]
Cd	[10, 12, 19, 26-30, 35-37, 56, 83, 84]	[31]	[11, 20, 22, 32, 72]	[18, 24]	
Co	[10, 19, 26, 35, 36, 38, 54, 56, 83, 85]		[20]	[18]	
Cr	[10, 12, 26, 27, 29, 54, 56, 83, 85]	[41]	[20, 25]	[42, 48]	INAA [57]
Cu	[10, 12, 19, 26, 27, 34, 36, 38, 54, 83, 85]	[16, 55]	[20, 43]	[18, 45, 74]	
Fe	[34, 36, 56, 83]	[16, 19, 58]	[20, 48]	[18, 39, 45, 46, 74, 88, 89]	INAA [57], MIR, NIR and XRF [49]
Hg	[10, 26-28, 30, 33, 35, 37, 38, 56, 83, 84]				CVAFS [11, 23], HGAFS [15, 32, 73]
K	[26, 54]	[16, 20, 86]		[18, 56, 83, 87]	INAA [57], MIR, NIR and XRF [49]
Li	[19, 26, 34, 83, 85]	[16, 25]			
Mg	[20, 26, 54]	[16, 86]		[18, 40, 56, 83]	MIR, NIR and XRF [49]
Mn	[10, 12, 26, 27, 29, 34, 36, 38, 54, 83, 85]	[16, 19]		[18, 20, 41, 45]	
Mo	[12, 19, 26, 27, 34, 54, 55, 83, 85]	[16]			
Na	[26, 50]	[16, 20, 58]		[18, 46, 56, 83, 87]	INAA [57], MIR and NIR [49]
Ni	[10, 12, 19, 24, 26, 29, 34-36, 38, 54, 83, 84]		[20, 51]	[18, 24]	
Pb	[10, 12, 19, 26-30, 34-38, 54, 74, 83, 84]	[31]	[11, 20, 21, 32, 72]	[18, 24]	
Sb	[10, 26, 27, 33-35, 54, 74, 83, 85]				
Se	[12, 26, 27, 38, 55, 56, 83, 85]	[20, 58]			INAA [47]
Sn	[10, 12, 19, 27, 29]				
Sr	[27, 34, 54, 83, 86]	[16, 25]			
Zn	[10, 12, 19, 26, 27, 36, 38, 54, 56, 83, 85]	[16]	[44, 51]	[18, 20, 45, 74, 88, 89]	INAA [57], MIR,NIR and XRF[49]

NOTE: inductively coupled plasma mass spectrometry ICPMS; inductively coupled plasma optical emission spectroscopy ICPOES; graphite furnace atomic absorption spectroscopy GFAAS; flame atomic absorption spectroscopy FAAS; hydrogen generation atomic fluorescence spectroscopy HGAFS; cold vapour atomic fluorescence spectroscopy CVAFS; middle infrared spectroscopy MIR; near infrared spectroscopy NIR; x-ray fluorescence XRF; instrumental neutron activation analysis INAA.

Table 4.3. Detection limits reported in the literature for the elemental analysis of human diets

LOD in µg kg^{-1} except for elements Al, Ca, Fe, K, Mg and Na in mg kg^{-1}					
Element	**ICPMS**	**ICPOES**	**GFAAS**	**FAAS**	**HGAFS**
Al	0.01-0.29 [8, 44, 45, 55, 56, 66, 67]	2.4 [16]			
As	1-200 [27, 43, 44, 52, 55, 56, 66, 71–73]	10-540 [16, 31]			2.2-10 [15, 17, 32]
Ba	7-45 [44, 45, 52, 67]	100 [16]			
Ca	8-160 [52, 66]	10 [16]		12.5 [56, 83]	
Cd	0.13-25 [8, 44, 45, 52, 55, 56, 60, 61, 66, 67, 71–73]	5-27 [16, 31]	1-22 [20, 32]	2 [24]	
Co	1.5-16 [43, 44, 52, 55, 57, 60, 66]	50 [16]	3.5 [20]		
Cr	3-140 [43, 44, 52, 55, 57, 66]	80 [16]	5 [20]		
Cu	0.5-40 [44, 52, 57, 60, 66, 67]	40 [16]	84 [20]		
Fe	0.043[a]-1.1 [43, 44, 52, 60]	0.23 [16]	0.14 [20]		
Hg	0.5-100 [43, 44, 55, 56, 61, 66, 67, 71, 73]				0.02-0.12 [15, 32, 73]
K	83 [66]	7.5 [16]		1.5-4.5 [56, 83, 84, 87]	
Mg	0.246-1.6 [43, 44, 52, 56, 66]	0.4 [16]		2.5 [56, 83, 84]	
Mn	0.5-28 [8], [44, 45, 52, 57, 60, 67, 72]	50 [16]		20 [20]	
Mo	0.02-61 [8, 44, 45, 52, 57, 67, 77]	100 [16]			
Na	2.9 [43, 44, 56, 66]	10.6 [16]		2.4-4.15 [83, 84, 86, 86]	
Ni	0.5-110 [8, 44, 45, 52, 53, 55, 56, 67, 72]	100 [16]	18 [20]	0.8 [24]	
Pb	0.5-25 [8, 44, 45, 52, 55, 60, 61, 66, 67, 71–73]	10-130 [16, 31]	3.5-5 [20, 32]	0.8 [24]	
Sb	0.1-9.9 [44, 45, 52, 55, 56, 66, 67]				
Se	0.6-50 [8, 43, 44, 57, 67, 77]	110 [16]			
Sn	0.19-50 [8, 67, 72]				
Sr	2.9-20 [44, 45, 52, 67]	26 [16]			
Zn	0.5-110 [8, 43, 44, 52, 57, 60, 66, 67]	210 [16]			

[a] with cell collision

Regarding the sample treatment microwave assisted digestion employing nitric acid hydrogen peroxide is the most used procedure, being also employed dry-ashing and hot-plate digestion.

In spite of the new developments in analytical chemistry, we have confirmed that for mineral analysis of diet samples atomic spectroscopy and mass spectrometry based techniques are the most employed to obtain the available data for human diets (36% for ICPMS, 24% for GFAAS, 24% for FAAS and 16% for ICPOES).

Future directions in mineral analysis of diets are likely to focus on the development of improved sample preparation, mineral bioavailability, direct analytical techniques and the implementation of green direct analytical tools that could provide screening information about the presence of essential, non-essential and toxic elements in both, the main foods which compose the human diets and the complete diets.

Acknowledgement

Authors acknowledge the financial support the Ministerio de Economia y Competitividad-Feder (project CTQ2012-38635 and project CTQ2014-52841) and Generalitat Valenciana (project PROMETEO-II 2014-077). Alba Mir-Marqués acknowledges the grant ‘Atracció de Talent’ (PREDOC13-110514) provided by the University of Valencia.

References

[1] Food and Agriculture Organization of the United Nations FAO and World Health Organization WHO, Principles and methods for the risk assessment of chemicals in food. Geneva, 2009. Available: http://www.who.int/foodsafety/publications/chemical-food/en/

[2] World Health Organization WHO and Food and Agriculture Organization of the United Nations FAO, Food safety risk analysis: a guide for national food safety authorities. Rome, 2007. Available: http://www.fao.org/docrep/012/a0822e/a0822e00.htm

[3] R. Kroes, D. Müller, J. Lambe, M.R. Löwik, J. van Klaveren, J. Kleiner, R. Massey, S. Mayer, I. Urieta, P. Verger, A. Visconti, Assessment of intake from the diet, Food Chem. Toxicol. 40 (2002) 327–385.

[4] World Health Organization WHO and Food and Agriculture Organization of the United Nations FAO, Codex Alimentarius, 2015. Available: http://www.codexalimentarius.org/

[5] Joint FAO/WHO Expert Committee on Food Additives, World Health Organization WHO, Evaluation of certain food additives and contaminants: seventy-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 2011. Available:http://apps.who.int/iris/bitstream/10665/44788/1/WHO_TRS_966 eng.pdf

[6] R.B. Khouzam, P. Pohl, R. Lobinski, Bioaccessibility of essential elements from white cheese, bread, fruit and vegetables, Talanta 86 (2011) 425-428.

[7] Joint FAO/WHO Expert Committee on Food Additives, Food and Agriculture Organization of the United Nations, and World Health Organization, Evaluation of certain contaminants in food: seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland, 2011. Available: http://apps.who.int/iris/bitstream/10665/44514/1/WHO_TRS_959_eng.pdf

[8] Joint FAO/WHO Expert Committee on Food Additives and World Health Organization, Evaluation of certain food additives and contaminants: seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland, 2011. Available: http://apps.who.int/iris/bitstream/10665/44515/1/WHO_TRS_960_eng.pdf

[9] Food and Agriculture Organization of the United Nations, and World Health Organization, Working document for information and use in discussions related to contaminants and toxins in the GSCTFF. Geneva, 2011. Available: ftp://ftp.fao.org/codex/meetings/ CCCF/cccf5/cf05_INF.pdf

[10] J.L. Domingo, G. Perelló, J. Giné Bordonaba, Dietary Intake of Metals by the Population of Tarragona County (Catalonia, Spain): Results from a Duplicate Diet Study, Biol. Trace Elem. Res. 146 (2012) 420–425.

[11] O. Muñoz, J.M. Bastias, M. Araya, A. Morales, C. Orellana, R. Rebolledo, D. Velez, Estimation of the dietary intake of cadmium, lead, mercury, and arsenic by the population of Santiago (Chile) using a Total Diet Study, Food Chem. Toxicol. 43(2005) 1647–1655.

[12] N.N. Aung, J. Yoshinaga, J.I. Takahashi, Dietary intake of toxic and essential trace elements by the children and parents living in Tokyo Metropolitan Area, Japan, Food Addit. Contam. 23 (2006) 883–894.

[13] D. González-Weller, A.J. Gutiérrez, C. Rubio, C. Revert, A. Hardisson, Dietary Intake of Aluminum in a Spanish Population (Canary Islands), J. Agric. Food Chem. 58 (2010) 10452–10457.

[14] C. Cabrera-Vique, M. Mesías, Content and Bioaccessibility of Aluminium in Duplicate Diets from Southern Spain: Aluminium in duplicate diets from Spain, J. Food Sci. 78 (2013) T1307–T1312.

[15] J.M. Bastias, M. Bermudez, J. Carrasco, O. Espinoza, M. Munoz, M.J. Galotto, O. Munoz, Determination of Dietary Intake of Total Arsenic, Inorganic Arsenic and Total Mercury in the Chilean School Meal Program, Food Sci. Technol. Int. 16 (2010) 443–450.

[16] A. Mir-Marqués, M.L. Cervera, M. de la Guardia, A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus, J. Food Compos. Anal. 27 (2012) 160–168.

[17] P. Liu, C.N. Wang, X.Y. Song, Y.F. Yu, Y.N. Wu, Dietary intake of arsenic by children and adults from Jinhu area of China, Food Addit. Contam. Part A 27 (2010) 1128–1135.

[18] R. Raghunath, R. Tripathi, B. Suseela, S. Bhalke, V. Shukla, V. Puranik, Dietary intake of metals by Mumbai adult population, Sci. Total Environ. 356 (2006) 62–68.

[19] G. Turconi, C. Minoia, A. Ronchi, C. Roggi, Dietary exposure estimates of twenty-one trace elements from a Total Diet Study carried out in Pavia, Northern Italy, Br. J. Nutr. 101 (2009) 1200.

[20] W. Becker, L. Jorhem, B. Sundström, K.P. Grawé, Contents of mineral elements in Swedish market basket diets, J. Food Compos. Anal. 24 (2011) 279–287.

[21] C. Rubio, T. González-Iglesias, C. Revert, J.I. Reguera, A.J. Gutiérrez, A. Hardisson, Lead Dietary Intake in a Spanish Population (Canary Islands), J. Agric. Food Chem. 53 (2005) 6543–6549.

[22] C. Rubio, A. Hardisson, J. Reguera, C. Revert, M. Lafuente, T. Gonzaleziglesias, Cadmium dietary intake in the Canary Islands, Spain, Environ. Res. 100 (2006) 123–129.

[23] C. Rubio, A.J. Gutiérrez, A. Burgos, A. Hardisson, Total dietary intake of mercury in the Canary Islands, Spain, Food Addit. Contam. Part A 25, (2008) 946–952.

[24] Z. Marzec, W. Koch, A. Marzec, W. Żukiewicz-Sobczak, Dietary exposure to cadmium, lead and nickel among students from the south-east region of Poland, Ann. Agric. Environ. Med. 21 (2014) 825–828.

[25] D. González-Weller, C. Rubio, A.J. Gutiérrez, G.L. González, J.M.C. Mesa, C.R. Gironés, A. B. Ojeda, A. Hardisson, Dietary intake of barium, bismuth, chromium, lithium, and strontium in a Spanish population (Canary Islands, Spain), Food Chem. Toxicol. 62 (2013) 856–868.

[26] J.C. Leblanc, T. Guérin, L. Noël, G. Calamassi-Tran, J.L. Volatier, P. Verger, Dietary exposure estimates of 18 elements from the 1st French Total Diet Study, Food Addit. Contam. 22 (2005) 624–641.

[27] M. Rose, M. Baxter, N. Brereton, C. Baskaran, Dietary exposure to metals and other elements in the 2006 UK Total Diet Study and some trends over the last 30 years, Food Addit. Contam. Part A 27 (2010) 1380–1404.

[28] R. Martí-Cid, J.M. Llobet, V. Castell, J.L. Domingo, Dietary intake of arsenic, cadmium, mercury, and lead by the population of Catalonia, Spain, Biol. Trace Elem. Res. 125, (2008) 120–132.

[29] A. Bocio, M. Nadal, J.L. Domingo, Human exposure to metals through the diet in Tarragona, Spain: Temporal Trend, Biol. Trace Elem. Res. 104 (2005) 193–202.

[30] I. Martorell, G. Perelló, R. Martí-Cid, J.M. Llobet, V. Castell, J.L. Domingo, Human exposure to Arsenic, Cadmium, Mercury, and Lead from foods in Catalonia, Spain: Temporal Trend, Biol. Trace Elem. Res. 142 (2011) 309–322.

[31] H.S. Lee, Y.H. Cho, S.O. Park, S.H. Kye, B.H. Kim, T.S. Hahm, M. Kim, J. Ok Lee, C. Kim, Dietary exposure of the Korean population to arsenic, cadmium, lead and mercury, J. Food Compos. Anal. 19 (2006) S31–S37.

[32] C. Chen, Y. Qian, Q. Chen, C. Li, Assessment of Daily Intake of Toxic Elements Due to Consumption of Vegetables, Fruits, Meat, and Seafood by Inhabitants of Xiamen, China, J. Food Sci. 76 (2011) T181–T188.

[33] S.W. Cheung Chung, K.P. Kwong, J. Yau, W. Wong, Dietary exposure to antimony, lead and mercury of secondary school students in Hong Kong, Food Addit. Contam. Part A 25 (2008) 831–840.

[34] M.M. Gimou, R. Pouillot, U.R. Charrondiere, L. Noël, T. Guérin, J.C. Leblanc, Dietary exposure and health risk assessment for 14 toxic and essential trace elements in Yaoundé: the Cameroonian total diet study, Food Addit. Contam. Part A 31 (2014) 1064–1080.

[35] N. Arnich, V. Sirot, G. Rivière, J. Jean, L. Noël, T. Guérin, J.C. Leblanc, Dietary exposure to trace elements and health risk assessment in the 2nd French Total Diet Study, Food Chem. Toxicol. 50 (2012) 2432–2449.

[36] L. Nasreddine, O. Nashalian, F. Naja, L. Itani, D. Parent-Massin, M. Nabhani-Zeidan, N. Hwalla, Dietary exposure to essential and toxic trace elements from a Total diet study in an adult Lebanese urban population, Food Chem. Toxicol. 48 (2010) 1262–1269.

[37] L. Nasreddine, N. Hwalla, O. El Samad, J.C. Leblanc, M. Hamzé, Y. Sibiril, D. Parent-Massin, Dietary exposure to lead, cadmium, mercury and radionuclides of an adult urban population in Lebanon: A total diet study approach, Food Addit. Contam. 23 (2006) 579–590.

[38] A. Callan, A. Hinwood, A. Devine, Metals in commonly eaten groceries in Western Australia: a market basket survey and dietary assessment, Food Addit. Contam. Part A 31 (2014) 1968–1981.

[39] C. Velasco-Reynold, M. Navarro-Alarcon, H. Lopez-Ga de la Serrana, V. Perez-Valero, M.C. Lopez-Martinez, Iron Dialyzability from Hospital Duplicate Meals: Daily Intake, Biol. Trace Elem. Res. 130 (2009) 241–248.

[40] C. Velasco-Reynold, M. Navarro-Alarcon, H. Lopez-Ga de la Serrana, V. Perez-Valero, A. Agil, M.C. Lopez-Martinez, Dialysability of Magnesium and Calcium from Hospital Duplicate Meals: Influence Exerted by Other Elements, Biol. Trace Elem. Res. 133 (2010) 313–324.

[41] C. Velasco-Ryenold, M. Navarro-Alarcón, H. Lopez-Ga de la Serrana, V. Perez-Valero, M.C. Lopez-Martinez, Total and dialyzable levels of manganese from duplicate meals and influence of other nutrients: Estimation of daily dietary intake, Food Chem. 109 (2008) 113–121.

[42] C. Velasco-Reynold, M. Navarro-Alarcón, H. Lopez-Ga de la Serrana, V. Perez-Valero, M.C. Lopez-Martinez, Determination of daily dietary intake of chromium by duplicate diet sampling: *In vitro* availability study, Food Addit. Contam. Part A 25 (2008) 604–610.

[43] C. Velasco-Reynold, M. Navarro-Alarcon, H. López-Ga de la Serrana, V. Perez-Valero, M.C. Lopez-Martinez, Analysis of total and dialyzable copper levels in duplicate meals by ETAAS: daily intake, Eur. Food Res. Technol. 227 (2008) 367–373.

[44] C. Velasco-Reynold, M. Navarro-Alarcon, H. López-Ga de la Serrana, V. Perez-Valero, M.C. Lopez-Martinez, In vitro determination of zinc dialyzability from duplicate hospital meals: influence of other nutrients, Nutrition 24 (2008) 84–93.

[45] M. Goshima, T. Murakami, H. Nakagaki, T. Shibata, T. Sugiyama, K. Kato, N. Narita, M. Nishimuta, Iron, zinc, manganese and copper intakes in Japanese children aged 3 to 5 years, J. Nutr. Sci. Vitaminol. 54 (2008) 475–482.

[46] M.L. Weber, T.B. Morais, Nutritional composition, assessed by chemical analyses, of prepared foods available for primary-school children: a comparison of public and private schools, Public Health Nutr. 13 (2010) 1855–1862.

[47] D.K. Adotey, V. Stibilj, Y. Serfor-Armah, B.J.B. Nyarko, R. Jaćimović, Dietary supply of selenium for adolescents in three residential care orphanages in Southern Ghana, Sci. Total Environ. 410 (2011) 72–79.

[48] C. Cabrera-Vique, M. Mesías, Chromium and iron content in duplicate meals at a university residence: daily intake and dialysability, Br. J. Nutr. 105 (2011) 1546–1552.

[49] A. Mir-Marqués, S. Garrigues, M.L. Cervera, M. de la Guardia, Direct determination of minerals in human diets by infrared spectroscopy and X-ray fluorescence, Microchem. J. 117 (2014) 156–163.

[50] H.R. Park, G.O. Jeong, S.L. Lee, J.Y. Kim, S.A. Kang, K.Y. Park, H.J. Ryou, Workers intake too much salt from dishes of eating out and food service cafeterias; direct chemical analysis of sodium content, Nutr. Res. Pract. 3 (2009) 328.

[51] J. Wittsiepe, K. Schnell, A. Hilbig, P. Schrey, M. Kersting, M. Wilhelm, Dietary intake of nickel and zinc by young children – Results from food duplicate portion measurements in comparison to data calculated from dietary records and available data on levels in food groups, J. Trace Elem. Med. Biol. 23 (2009) 183–194.

[52] E. Hobin, C. White, Y. Li, M. Chiu, M.F. O'Brien, D. Hammond, Nutritional quality of food items on fast-food "kids" menus': comparisons across countries and companies, Public Health Nutr. 17 (2014) 2263–2269.

[53] A.O. Musaiger, J.H. Al-Jedah, R. D'souza, Proximate, mineral and fatty acid composition of fast foods consumed in Bahrain, Br. Food J. 110 (2008) 1006–1018.

[54] H. Marcussen, B.H. Jensen, A. Petersen, P.E. Holm, Dietary Exposure to Essential and Potentially Toxic Elements for the Population of Hanoi, Vietnam, Asia Pac. J. Clin. Nutr. 22 (2013) 300–311.

[55] M.K. Choi, M.H. Kang, M.H. Kim, The Analysis of Copper, Selenium, and Molybdenum Contents in Frequently Consumed Foods and an Estimation of Their Daily Intake in Korean Adults, Biol. Trace Elem. Res. 128 (2009) 104–117.

[56] M.M. Gimou, U.R. Charrondière, J.C. Leblanc, L. Noël, T. Guérin, R. Pouillot, Dietary exposure and health risk assessment for 11 minerals and trace elements in Yaoundé: the Cameroonian Total Diet Study, Food Addit. Contam. Part A 30 (2013) 1556–1572.

[57] R.P. Avegliano, V.A. Maihara, F.F. da Silva, A Brazilian Total Diet Study: Evaluation of essential elements, J. Food Compos. Anal. 24 (2011) 1009–1016.

[58] B.M. Thomson, R.W. Vannoort, R.M. Haslemore, Dietary exposure and trends of exposure to nutrient elements iodine, iron, selenium and sodium from the 2003–4 New Zealand Total Diet Survey, Br. J. Nutr. 99 (2008) 614-625.

[59] World Health Organization, Guideline: Sodium intake for adults and children. Geneva, 2012. Available: http://www.who.int/nutrition/publications/guidelines/sodium_intake_printversion.pdf

[60] D.B. Young, Role of potassium in preventive cardiovascular medicine, 6th ed., Kluwer Academic Inc., Boston, 2001.

[61] World Health Organization, Guideline: Potassium intake for adults and children. Geneva, 2012. Available: http://www.who.int/nutrition/publications/guidelines/potassium_intake_printversion.pdf

[62] World Health Organization and Food and Agriculture Organization of the United Nations, Vitamin and mineral requirements in human nutrition, 2nd ed. Rome, 2004. Available: http://apps.who.int/iris/bitstream/10665/42716/1/9241546123.pdf

[63] B. De Benoist, World Health Organization, and Centers for Disease Control and Prevention (U.S.), Worldwide prevalence of anaemia 1993-2005 of WHO Global Database of anaemia. Geneva, 2008. Available: http://apps.who.int/iris/bitstream/10665/43894/1/9789241596657_eng.pdf

[64] G.F. Nordberg, Handbook on the toxicology of metals, 3th ed., Elsevier, Amsterdam, 2007.

[65] Scientific Committee on Food European Commission, Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Copper. Brussels, 2003. Available: http://ec.europa.eu/food/fs/sc/scf/out176_en.pdf

[66] C.E: Lazarte, N.G. Carlsson, A. Almgren, A.S. Sandberg, Y. Granfeldt, Phytate, zinc, iron and calcium content of common Bolivian food, and implications for mineral bioavailability, J. Food Comp. Anal. 39 (2015) 111-119.

[67] P. Singh, S. Prasad, W. Aalbersbe, Bioavailability of Fe and Zn in selected legumes, cereals, meat and milk products consumed in Fiji, Food CHem. 207 (2016) 125-131.

[68] R.L. Bertin, H.F. Maltez, J.S. Gois, D.L.G. Borges, G. Borges, L. Gonzaga, R. Fett, Mineral composition and bioacessibility in Sacocornia ambigua using ICP-OES, J. Food Comp. Anal. 47 (2016) 45-51.

[69] M. Vaz-Tostes, T. Verediano, E.G. de Mejia, N. Brunoro, Evaluation of iron and zinc bioavailability of beans targeted for biofortification using in-vitro and in-vivo models and their effect on the nutrition status of preschool children, J. Sci. Food Agri. 96 (2016) 1326-1332.

[70] L.D. Martínez, R.A. Gil, P. H. Pacheco, S. Cerutti, Elemental composition analysis of food by FAES and ICP-OES, in: M. de la Guardia, S. Garrigues (Eds.), Handbook of mineral elements in food, John Wiley & Sons Inc., Chichester, UK, 2015, pp. 219–238.

[71] J. Zukowska, M. Bizuk, Methodological evaluation of method for dietary heavy metal intake, J. Food Sci. 73 (2008) R21-R29.

[72] P. Liu, C.N. Wang, X.Y. Song, Y.N. Wu, Dietary intake of lead and cadmium by children and adults – Result calculated from dietary recall and available lead/cadmium level in food in comparison to result from food duplicate diet method, Int. J. Hyg. Environ. Health 213 (2010) 450–457.

[73] J. Sun, C. Wang, X. Song, Y. Wu, B. Yuan, P. Liu, Dietary intake of mercury by children and adults in Jinhu area of China, Int. J. Hyg. Environ. Health 214 (2011) 246–250.

[74] F. Cámara, M.A. Amaro, R.Barberá, G. Clemente, Bioaccessibility of minerals in school meals: Comparison between dialysis and solubility methods, Food Chem. 92 (2005) 481–489.

[75] C. Cabrera-Vique, M. Mesías, P.R. Bouzas, Nickel levels in convenience and fast foods: In vitro study of the dialyzable fraction, Sci. Total Environ. 409 (2011) 1584–1588.

[76] C. Cabrera-Vique, P.R. Bouzas, Chromium and manganese levels in convenience and fast foods: In vitro study of the dialyzable fraction, Food Chem. 117 (2009) 757–763.

[77] World Health Organization, Guidelines for the study of dietary intakes of chemical contaminants. Geneva, 1985. Available: http://apps.who.int/iris/handle/10665/ 39255

[78] Food Standards Australia New Zealand, 24th Australian Total Diet Study. Kingston, 2014. Available: http://www.foodstandards.gov.au/publications/Pages/ 24th-Australian-Total-Diet-Study.aspx

[79] US Food and Drug Administration, Total Diet Study Statistics on Element Results - 2006-2011. College Park, Maryland, 2014. Available: http://www.fda.gov/ downloads/Food/FoodScienceResearch/TotalDietStudy/UCM184301.pdf

[80] Bureau of Chemical Safety and Health Products and Food Branch, Canadian Total Diet Study. Ottawa, Ontario, 2007. Available: http://www.hc-sc.gc.ca/fn-an/surveill/total-diet/index-eng.php.

[81] Ministry of Agriculture and Forestry, 2009 New Zealand Total Diet Study. Agricultural compound residues, selected contaminant and nutrient elements. Crown, 2011. Available: http://www.foodsafety.govt.nz/science-risk/programmes/ total-diet-survey. htm

[82] China National Center for Food Safety Risk Assessment, China Total Diet Study. Beijing, 2013. Available: http://www.chinafoodsafety.net/detail.aspx?id=2F8F16B8AB6EB2D2D514FFBE79 4B3FB98C5610E779E432B5.

[83] M.M. Gimou, U.R. Charrondière, J.C. Leblanc, R. Pouillot, L. Noël, T. Guérin, Concentration data for 25 elements in foodstuffs in Yaoundé: The Cameroonian Total Diet Study, J. Food Compos. Anal. 34 (2014) 39–55.

[84] S. Millour, L. Noël, A. Kadar, R. Chekri, C. Vastel, V. Sirot, J.C. Leblanc, T. Guérin, Pb, Hg, Cd, As, Sb and Al levels in foodstuffs from the 2nd French total diet study, Food Chem. 126 (2011) 1787–1799.

[85] L. Noël, R. Chekri, S. Millour, C. Vastel, A. Kadar, V. Sirot, J.C. Leblanc, T. Guérin, Li, Cr, Mn, Co, Ni, Cu, Zn, Se and Mo levels in foodstuffs from the Second French TDS, Food Chem. 132 (2012) 1502–1513.

[86] A.G. Gharib, M.G. Gharib, Intakes of radiologically important trace and minor elements from Iranian daily diets, Iran, J. Sci. Technol. Trans. Sci. 34 (2010) 227–236.

[87] C.M. Tanase, P. Griffin, K.G. Koski, M.J. Cooper, K.A. Cockell, Sodium and potassium in composite food samples from the Canadian Total Diet Study, J. Food Compos. Anal. 24 (2011) 237–243.

[88] M. Umeta, C.E. West, H. Fufa, Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia, J. Food Compos. Anal. 18 (2005) 803–817.

[89] A.M. Karunaratne, P.H. Amerasinghe, V.M. Sadagopa Ramanujam, H.H. Sandstead, P.A.J. Perera, Zinc, iron and phytic acid levels of some popular foods consumed by rural children in Sri Lanka, J. Food Compos. Anal. 21 (2008) 481–488, Sep. 2008.

[90] M. das G. Andrade Korn, E.S. da Boa Morte, D.C.M. Batista dos Santos, J.T. Castro, J.T.P. Barbosa, A.P. Teixeira, A.P. Fernandes, B. Welz, W.P.C. dos Santos, E.B.G. Nunes dos Santos, M. Korn, Sample preparation for the determination of metals in food samples using spectroanalytical methods—A review, Appl. Spectrosc. Rev. 43 (2008) 67–92.

[91] A. Mir-Marques, M.L. Cervera, M. de la Guardia, Baby foods, in: M. de la Guardia, S. Garrigues (Eds.) Handbook of mineral elements in food, John Wiley & Sons Inc., Chichester, UK, 2015, pp. 709–723.

[92] C. Collell, P. Gou, P. Picouet, J. Arnau, J. Comaposada, Feasibility of near-infrared spectroscopy to predict aw and moisture and NaCl contents of fermented pork sausages, Meat Sci. 85 (2010) 325–330.

[93] D. Cozzolino, W. Cynkar, N. Shah, P. Smith, Quantitative analysis of minerals and electric conductivity of red grape homogenates by near infrared reflectance spectroscopy, Comput. Electron. Agric. 77 (2011) 81–85.

[94] A. Lucas, D. Andueza, E. Rock, B. Martin, Prediction of dry matter, fat, pH, vitamins, minerals, carotenoids, total antioxidant capacity, and color in fresh and freeze-dried cheeses by visible-near-infrared reflectance spectroscopy, J. Agric. Food Chem. 56 (2008) 6801–6808.

[95] J. Moros, I. Llorca, M. L. Cervera, A. Pastor, S. Garrigues, M. de la Guardia, Chemometric determination of arsenic and lead in untreated powdered red paprika by diffuse reflectance near-infrared spectroscopy, Anal. Chim. Acta 613 (2008) 196–206.

[96] A. Mir-Marqués, M. Martínez-García, S. Garrigues, M.L. Cervera, M. de la Guardia, Green direct determination of mineral elements in artichokes by infrared spectroscopy and X-ray fluorescence, Food Chem. 196 (2016) 1023-1030.

Capítulo 5

Determinación directa de minerales en dietas mediante espectroscopia infrarroja y fluorescencia de rayos X

Direct determination of minerals in human diets by infrared spectroscopy and X-ray fluorescence

Alba Mir-Marqués, Salvador Garrigues, M.Luisa Cervera and Miguel de la Guardia

Department of Analytical Chemistry. University of Valencia. Jeronimo Muñoz research building, 50th Dr. Moliner str. 46100 Burjassot. Valencia. Spain.

Received 19 June 2014,

Accepted 27 June 2014,

Available online 4 July 2014

Microchemical Journal 117 (2014) 156-163

DOI 10.1016/j.microc.2014.06.028

Resumen

En este trabajo se investigó el empleo de la espectroscopia de infrarrojo cercano (NIR) y medio (MIR) junto con la fluorescencia de rayos X (XRF) para determinar la concentración de elementos minerales en dietas españolas para humanos. Se analizaron treinta y cinco alimentos infantiles comerciales, seis menús infantiles de comida rápida y trece menús de cafeterías universitarias, mediante espectroscopia infrarroja y de fluorescencia de rayos X, y sus espectros se evaluaron usando como datos de referencia los obtenidos mediante espectroscopia de emisión óptica con plasma de acoplamiento inductivo (ICP-OES). Se crearon y validaron modelos quimiométricos para la determinación de calcio, potasio, hierro, magnesio, sodio y zinc. Los espectros fueron tratados usando diferentes algoritmos de pre-procesado (corrección multiplicativa de dispersión, variación estándar normal, primera derivada, corrección ortogonal de señal, suavizado y centrado en la media) antes del desarrollo de los modelos de calibración usando mínimos cuadrados parciales, y se evaluó también la validación cruzada y validación externa. Los coeficientes de correlación más altos para la validación externa y los menores errores relativos de predicción se obtuvieron para el potasio en el caso de MIR (0.86 y 11%), NIR (0.9 y 11%) y XRF (0.9 y 10%).

Abstract

The use of near and mid infrared spectroscopy and X-ray fluorescence (XRF) to determine the concentration of mineral elements in Spanish human diets was investigated. Thirty-five commercial baby foods, 6 children fast food menus and 13 university canteen menu samples were analysed by infrared and XRF spectroscopy and spectra evaluated by using reference data obtained by inductively coupled plasma optical emission spectroscopy (ICP-OES). Models for calcium, potassium, iron, magnesium, sodium and zinc determination were built and validated. Spectra were pre-treated by using different pre-processing algorithms (multiplicative scatter correction, standard normal variation, first derivate, orthogonal signal correction, smoothing and mean centre) prior to developing calibration models using partial least squares and were evaluated by cross-validation and external validation. The highest coefficients of determination in validation (R^2_{val}) and the lowest relative root-mean-square error of prediction were obtained for potassium determination by MIR (0.86 and 11%), NIR (0.9 and 11%) and XRF (0.9 and 10%).

5.1. Introduction

In the last years, significant changes in the lifestyle have improved the consume of baby jars in the period from 6 till 12 months, and modified the nutrition habits, moving from home meals to the frequent use of fast food and canteen menus. Because of that, analytical control of this kind of diets has an increased importance. Additionally, the mineral profile of foods is very important in order to assure the recommended daily intake of essential elements and to control excessive consume of salt, frequently added to cooked foods [1–3].

The determination of the mineral profile of foods and diets is commonly made by using atomic spectroscopy and mass spectrometry methods, being the most common used techniques for this purpose inductively coupled plasma optical emission (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) [4–8]. These techniques are very useful for the multi-elemental analysis of samples in a few minutes. However, to do it, additionally than the use of high cost and non-portable instrumentation it is required a previous sample digestion and dissolution, thus involving the use of long and tedious sample pre-treatment with the use of strong acids and oxidants and a lot of energy, also providing non-degradable wastes.

Green alternatives to the aforementioned techniques could be based on direct measurements by infrared spectroscopy [9,10] and X-ray fluorescence (XRF) [11]. These techniques permit a direct and fast analysis of solid samples without any chemical sample pre-processing, thus avoiding reagent consumption and toxic waste generation making these analyses very easy and cheap. Another advantage is the reduced cost of the new IR and XRF portable instrumentation as compared with the atomic and mass spectrometry ones. However, the main disadvantage of both infrared and XRF is their low sensitivity and high matrix effects. Therefore, it is essential to use chemometric tools to build appropriate calibration models from the spectra of well characterised samples in order to increase the prediction capability of measurements made [12]. Being those reasons for which IR and XRF methods have been mainly considered as semi-quantitative tools.

The combination of middle infrared (MIR), near infrared (NIR) and XRF measurements with chemometric data treatment could offer increasing possibilities for the direct determination of mineral components in food samples [13] and to do it, partial least squares (PLS) or modified-partial lest squares (MPLS) have been employed for building models proposed in the literature to quantitative determine mineral elements in foods [14–19]. So, the main purpose of this study was to develop chemometric based methods to extract information about as much as possible mineral elements in different human diets from the MIR, NIR and XRF spectra of solid freeze dried samples.

5.2. Materials and methods

5.2.1. Apparatus and instruments

Reference values on the mineral composition of samples were determined by ICP-OES after microwave-assisted digestion of samples. It was used an optima 5300 DV ICP-OES Perkin Elmer (Norwalk, CT, USA) equipped with an auto sampler AS 93-plus and a cross flow nebuliser. Samples were previously digested using a Milestone Ethos SEL microwave laboratory system (Sorisole, Italy), equipped with a thermocouple probe for automatic temperature control, and an automatic gas leak detector using high pressure Teflon vessels of 100 mL inner volume. Teflon vessels were cleaned with vapours of nitric acid (69%), using the Trace CLEAN from Milestone (Sorisole, Italy) to avoid cross-contamination.

To obtain the MIR spectra it was employed a model Tensor 27 Fourier transform mid-infrared (FT-MIR) spectrometer from Bruker (Bremen, Germany) equipped with a DLaTGS detector and a dry-air purge in-compartment DuraSampleIR accessory for attenuated total reflectance measurements (ATR) from Smiths Detection Inc. (Warrington, UK) with a three reflection diamond/ZnSe DuraDisk plate. For instrument control and data acquisition, OPUS software 6.5 from Bruker was used.

For NIR measurements it was employed a MPA model Multipurpose Analyser Fourier transform near-infrared (FT-NIR) spectrometer from Bruker equipped with an integrating sphere, used to diffuse reflectance spectra acquisition. For instrument control and data acquisition, the OPUS software 6.5 from Bruker was used.

X-ray fluorescence spectra were obtained using a portable model S1 Titan energy dispersive X-ray fluorescence (ED-XRF) spectrometer from Bruker equipped with a Rhodium X-ray tube and X-Flash® SDD detector. For instrument control S1RemoteCtrl and S1Sync software from Bruker were employed; and for spectra treatment the ARTAX software from Bruker was used.

5.2.2. Reagents

For the reference procedure argon C-45 (purity higher than 99.995%) supplied by Carburos Metálicos (Barcelona, Spain) was used as plasmogen, and stock solution of a multi-element standard solution (100 mg L^{-1}) containing 26 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Se, Na, Sr, Tl, Ti, V and Zn) dissolved in 5% HNO_3 obtained from Scharlau (Barcelona, Spain), a 1000 mg L^{-1} Scandium Standard Solution for ICP, HNO_3 69% for trace analysis and H_2O_2 35% reagent grade, all from Scharlau were used. Ultra-pure water with a minimum resistivity of 18.2 MΩ cm, obtained using a Milli-Q plus Millipore system (Molsheim, France) was employed to prepare the standard and sample solution.

5.2.3. Sample collection

A total of 54 samples of different types were purchased in Spain from different markets, restaurants and university canteens, including 35 samples of commercial baby food jars, 6 samples of fast food for children and 13 samples of university canteen menus.

Jar samples were composed by meat with vegetables, fish with vegetables, only vegetables or only fruit. Baby foods were recommended for children aged of 6 and 12 months. Fast foods for children were composed by meat, cheese, chips, bread, ketchup and mustard, dairy products and beverage. University canteen menus were composed by entries, a main dish and dessert and also included a piece of 50-70 g of bread. Detailed composition of all food samples used in this study is indicated in Supplementary material (Table S5.1.).

In the case of menus, the edible part of the foods was weight and mixed. After that, samples were crushed and homogenised with a domestic Braun mixer (Kronberg, Germany), and finally frozen at -20°C in a freezer. Afterwards, they were lyophilised in a Cryodos 50 lyophiliser Telstar (Barcelona, Spain) for a minimum of 48 h at a chamber pressure of 50 Pa. The dried complete samples were pulverised and homogenised with a domestic mixer, and the powdered samples were stored in polyethylene bottles until their analysis.

5.2.4. Reference procedure

Reference data were obtained by ICP-OES after microwave-assisted digestion. For samples digestion, 1 g portions of freeze-dried sample were accurately weight inside Teflon reactors and 8 mL of concentrated nitric acid and 2 mL of H_2O_2 were added. Reactors were closed and placed inside the microwave oven with a digestion step of 15 min at 180º C (700 W). The resulting solution was quantitatively transferred to a plastic flask and made up to 20 mL with nanopure water and preserved at 4º C until to be analysed.

Digested samples were measured with a final dilution to 40 mL. Additional, control standards were measured for every series of 10 independent sample measurements. Scandium (0.5 mg L^{-1}) was used as internal standard and added to all samples, reagent blanks and standards. The average concentration of blanks was subtracted from the analytical signals of digested samples after interpolation on the calibration graphs. Results were expressed as a basis of dry sample weight.

To establish precision and accuracy of the reference method, CRMs were analysed under the same conditions than samples: NIST 1568a Rice Flour (National Institute of Standards and Technology, Gaithensburg, MD, USA); TORT-2 Lobster

Hepatopancreas (National Research Council, Ottawa, Canada) and IAEA-359 Cabbage (International Atomic Energy Agency, Vienna, Austria). A statistical evaluation based on the regression between average obtained values and certified values provided a regression lines of $y = (0.996 \pm 0.005)x - (7 \pm 20)$ for IAEA-359, $y = (0.998 \pm 0.012)x + (0.12 \pm 0.14)$ for NIST 1568a and $y = (1.007 \pm 0.017)x + (0.096 \pm 1.433)$ for TORT-2, evidencing that the intercept and the slope values correspond, in all cases, statistically to 0 and 1, with regression coefficients (r^2) of 0.9999, 0.998 and 0.998, respectively. Detection limits (LOD) were calculated as three times the standard deviation of ten reagent blanks, taking into account the analytical sensitivity and sample mass. LODs for calcium, potassium, iron, magnesium, sodium and zinc were 0.25, 0.2, 0.06, 0.01, 0.26 and 0.005 mg kg^{-1} respectively. Reproducibility was calculated as the relative standard deviation of method (RSD), for three independent analyses of three independent portions of the same sample. RSD for all elements was lower than 4%.

5.2.5. General procedures

MIR measurements

Direct ATR-FTIR measurements were performed by placing approximately 100 mg of dry sample directly onto the diamond ATR crystal and pressed with the corresponding accessory. Spectra were recorded in the range between 4000 and 600 cm^{-1} by accumulating 50 scans per spectrum, at a resolution of 4 cm^{-1}. The background spectrum was acquired from a spectrum of the clean ATR crystal, obtained immediately before acquiring the spectrum of each sample. After measurement, samples were aspirated and the ATR crystal cleaned with cellulose. ATR correction was applied to all spectra.

NIR measurements

Approximately, 0.5-1 g of dry samples were placed inside glass vials of 20 mL (22 mm of internal diameter and 44 mm high) to obtain their NIR spectra by diffuse reflectance. The vials were rotated to obtain triplicate measurements for each sample. Diffuse reflectance near spectra (DRIFT) were recorded in Kubelka-Munk units, in the 14000-4000 cm^{-1} spectral region, using a resolution of 4 cm^{-1} and cumulating 50 scans per spectrum. The background spectrum was acquired from the closed integrating sphere using the same instrumental conditions than those employed for samples.

XRF measurements

Approximately three grams of dry samples were compacted in a special holder of stainless steel and were pressed to obtain pellets of a diameter of 40 mm using a hydraulic press for 1 min at a pressure of 120 psi. Pellets were covered with a thin window of Mylar® to protect and to avoid hydration from air humidity during manipulation, and spectra obtained by triplicate using 50 kV voltage, 11µA current and 60 s as acquisition time (30 s for high atomic number elements, and 30 s for low atomic number elements).

5.2.6. Chemometric data treatment

Models for the prediction of the mineral elements content from spectral measurements of samples were performed using partial least squares regression (PLS) using Matlab 2013a software from Mathworks (Natick, MA, USA) and the PLS Toolbox 6.2 from Eigenvector Research Inc. (Wenatchee, WA, USA). Kennard-Stone (KS) algorithm was assayed to select the most appropriate sample calibration set. The number of samples in the calibration set was fixed as 40 and the validation set was composed by 14 additional samples, being used the same number of samples in sets for each one of the three considered methodologies (MIR, NIR and XRF). KS is a well-known method for the selection of a representative subset of samples based only on the instrumental signals. The selected subset should cover the experimental region homogeneously by choosing objects which are uniformly distributed in the experimental space.

For building the best PLS models, different spectral regions were tested. Interval PLS (iPLS) was used to develop local PLS models on equidistant subintervals of the whole spectrum in order to provide an overall picture of the relevant information in different spectral subdivisions and thereby narrowing the important spectral variables [20]. Local PLS models were developed on spectral subintervals of equal width, and the prediction performance of these local models and that based on the whole spectrum were compared, based on the use of root mean square error of cross-validation (RMSECV). Other parameters such as squared correlation coefficient (R^2), slope and offset were also consulted to ensure a comprehensive model overview.

Prior to PLS model calculation, several pre-treatments of spectra were tested for normalisation, filtering, scaling and centring. Pre-treatment models assayed were multiplicative scatter correction (MSC), standard normal variation (SNV), first derivate (FD), orthogonal signal correction (OSC), smoothing (SMO) and mean centre (MC) also combination of them. The best one was selected for each element on the basis of the lowest root mean square error of calibration (RMSEC) and cross-validation (RMSECV). The optimum number of PLS factors was determined by cross-validation using venetian blinds with (m/5) splits in order to obtain the minimum value of RMSECV [21]. Samples from the validation set were then analysed in the selected conditions. Predictive power of assayed models was evaluated through the use of the root mean square error of prediction (RMSEP). PLS models were evaluated for all elements with available reference data but only the most significant information about mineral content of diet samples is presented through this paper.

5.3. Results and discussion

5.3.1. MIR spectra of diet samples

Figure 5.1 shows the typical ATR-FTIR spectra between 4000-600 cm^{-1} for baby foods (B), children fast food (F) and adult menu samples (M). The broad and strong peak from 3600-3200 cm^{-1} was due to O-H and N-H stretching vibrations. Peaks at 2925 and 2855 cm^{-1} correspond to CH_2 asymmetric and symmetric stretching vibration of the fatty acids, respectively. The band at 1745 cm^{-1} can be associated with the C=O stretching, in this region dominate protein and peptide bands. The C=C stretching vibration provided showed a peak at 1650 cm^{-1}. The region between 1500 and 1200 cm^{-1} is a mixed region containing vibrational bands of fatty acids, proteins and polysaccharides. The region at 1200-900 cm^{-1} is related to polysaccharides and sucrose. Finally, the absorbance bands obtained under 900 cm^{-1} correspond to the fingerprint [22,23].

5.3.2. PLS models for determination of minerals by ATR-FTIR

For the selection of the two sets of samples to be employed for ATR-FTIR determination of minerals as calibration and validation ones, Kennard-Stone algorithm was used; remaining samples were considered for validation purpose. For calibration set, 25 baby foods, 5 infant fast food and 10 menus were selected. The mean, concentration range and standard deviation for the concentration of assayed elements in diet samples for both, the calibration and validation, sets employed for ATR-FTIR analysis are shown in Table 5.1.

Statistics of the PLS calibration models developed for calcium, potassium, iron, magnesium, sodium and zinc in diet samples using ATR-FTIR and pre-processing models were built and compared in order to evaluate their prediction capability. The parameters chosen for the best model for each mineral element are also reported in

Table 5.1. For every element, all MIR spectra (4000-600 cm^{-1}) were used to build PLS models.

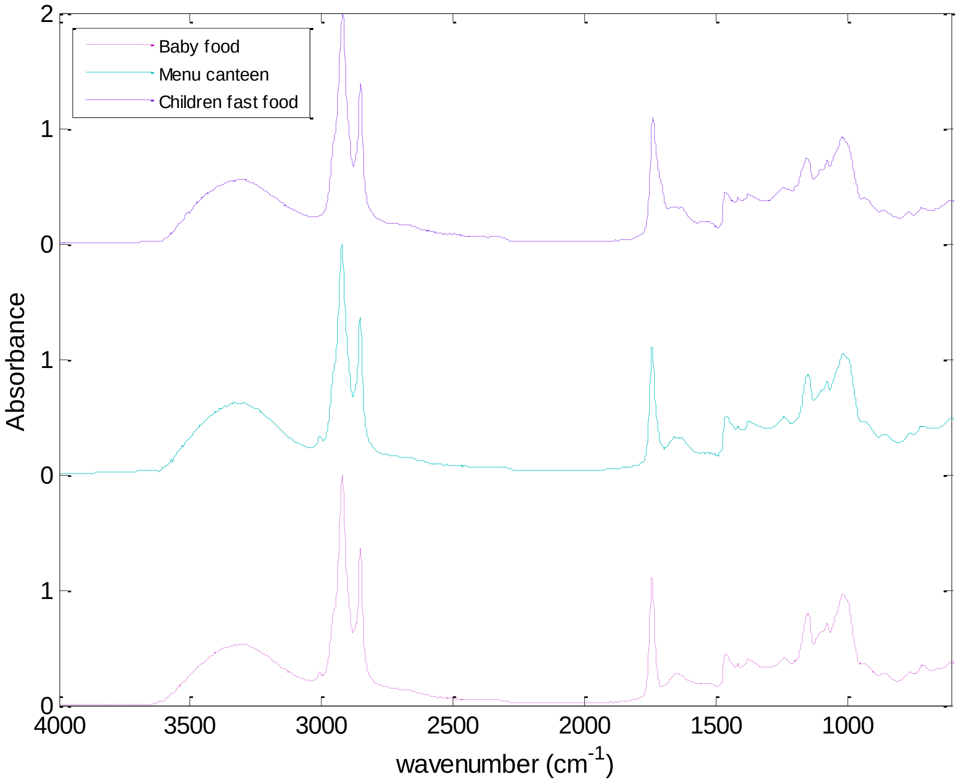

Figure 5.1. Mean ATR-FTIR spectra of baby foods, children fast food and adult menus

NOTE: spectra were shift in the Y axe to improve their presentation

Coefficients of determination in calibration (R^2_{cal}) and cross-validation (R^2_{cv}) were fairly good; for calibration R^2 varied between 0.72 and 0.994, and for cross-validation were lower than previous ones (0.22-0.73). Regarding the number of required latent variables (LV) only two were employed for sodium PLS model and the cumulative variance percentage explained was 99.4% in contrast to potassium and iron PLS models which needed eight LV to explain 86 and 87% of cumulative variance, respectively (see Table 5.1). The highest coefficients of determination in cross-validation (R^2_{cv}) and the lowest errors of cross-validation (RSECV) were obtained for potassium (0.52 and 2219 mg kg^{-1}), magnesium (0.4 and 155 mg kg^{-1}) and sodium (0.73 and 1887 mg kg^{-1}).

Table 5.1. Statistics of PLS- ATR-FTIR models employed for the determination of mineral elements in human diets, also including the reference data of sample sets used for calibration and validation

Element	Set	Min	Max	Mean	SD	Pre-process	LV	R^2_{cal}	R^2_{CV}	R^2_{val}	RMSEC	RMSECV	RMSEP	RRMSEP
Ca	CAL	288	4000	1426	872	OSC+MC	4	0.97	0.22	0.63	146	1147	263	29
	VAL	365	1635	891	426									
K	CAL	3860	15190	9329	3204	MSC+FD	8	0.86	0.52	0.86	1189	2219	1010	11
	VAL	5290	12000	9069	2489									
Fe	CAL	2.1	33	17	8	SNV+FD	8	0.87	0.46	0.76	2.98	6.48	3.4	19
	VAL	6.9	30	19	7									
Mg	CAL	402	1120	692	192	OSC	6	0.92	0.4	0.57	52	155	102	16
	VAL	461	920	637	134									
Na	CAL	45	14400	7111	3558	OSC+MC	2	0.994	0.73	0.95	266	1887	1606	30
	VAL	179	13200	5293	4115									
Zn	CAL	3.99	47	18	9	SNV	6	0.72	0.41	0.82	4.68	7.22	3.63	19
	VAL	3.73	46	21	11									

NOTE: minimum (min), maximum (max), mean and standard deviation (SD) in mg kg^{-1} dry weight for calibration (CAL) and validation (VAL) sets. RMSEC root-mean-square error of calibration in mg kg^{-1}. RMSECV root-mean-square error of cross-validation in mg kg^{-1}. RMSEP root-mean-square error of prediction in mg kg^{-1}. RRMSEP relative root-mean-square error of prediction in percentage (%).

5.3.3. Prediction capability of PLS-ATR-FTIR for mineral elements determination in diets

Cross-validation does not inform about the capability of the developed models to be used for the prediction of unknown samples, different than those included in the calibration. Because of that, the prediction capability of PLS-ATR-FTIR methods has been evaluated by using an independent external validation set of diet samples, not employed during the calibration stage. Coefficients of determination in validation (R^2_{val}) were quite good; being between 0.57 for Mg and 0.95 for Na (see Table 5.1). The highest coefficients of determination in validation (R^2_{val}) and the lowest relative root-mean-square error of prediction (RRMSEP) were obtained for potassium (0.86 and 11%), iron (0.76 and 19%) and zinc (0.82 and 19%). The minimum RRMSEP was obtained for potassium.

The prediction capability of the PLS-ATR-FTIR method was good for almost all considered elements, RRMSEP being lower than 20% for potassium, magnesium, iron and zinc. However, for calcium and sodium this error was higher than 25%, and Figure 5.2 shows the regression between predicted values for potassium concentration and those obtained by reference method.

5.3.4. NIR spectra of diet samples

Figure 5.3 shows the typical near DRIFT spectra obtained between 14000 and 4000 cm^{-1} for baby foods (B), children fast food (F) and adult menu samples (M).

The broad and strong peak from 8300 cm^{-1} was due to second overtone C-H stretching vibrations. At 7000-6400 cm^{-1} it can be seen a broad peak which corresponds to the first overtone of N-H and the O-H stretching of water. Peaks at 5700 and 5800 cm^{-1} correspond to first overtone C-H stretching. O-H first and second overtone can be seen at 5155 cm^{-1}. A strong peak at 4732 cm^{-1} was due to N-H and O-H stretching. Double peak at 4330 and 4260 cm^{-1} was due to combination of C-H stretching [14, 23].

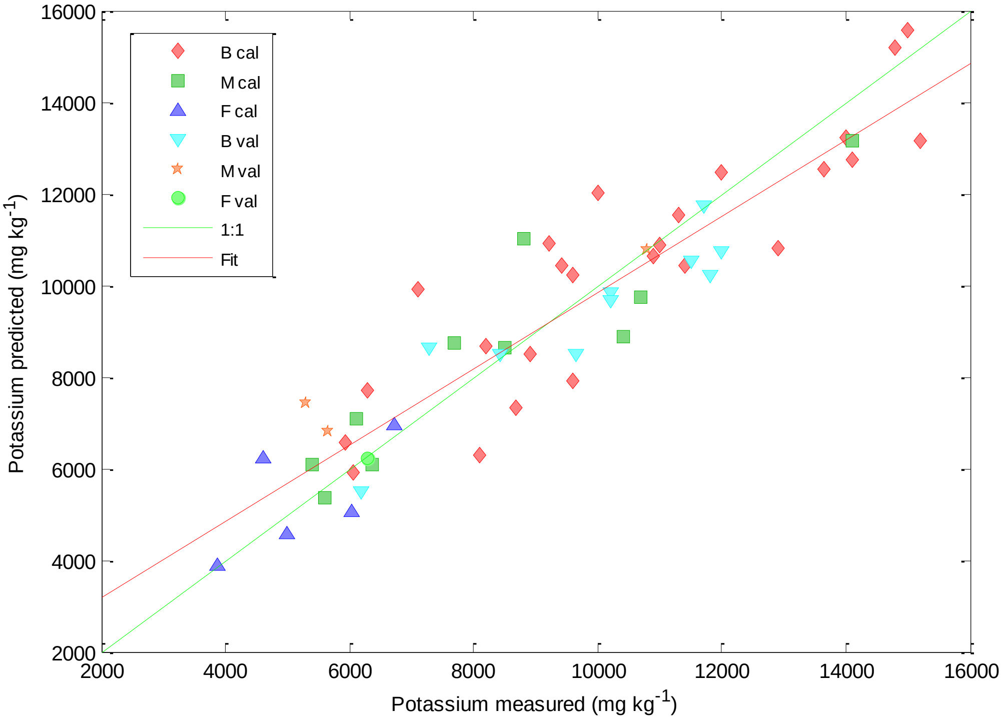

Figure 5.2. Predicted vs. measured potassium values for the PLS-ATR-FTIR model employed for the analysis of baby foods and diet samples

5.3.5. PLS models for the determination of minerals by NIR

For the selection of the two sets of samples, to be used for calibration and validation, the same methodology than that employed for MIR was used. For calibration, 24 baby foods, 5 infant fast foods and 11 adult menus were selected; remaining samples constitute the validation set. The mean, concentration range and standard deviation for the elements measured in diet samples in both sets employed for NIR analysis are shown in Table 5.2.

Several spectral regions and different pre-processing strategies were assayed to build the best models and those compared in order to evaluate their prediction capability. Statistics for the PLS-NIR determination of calcium, iron, magnesium and zinc, using the NIR wavenumber region (14000-4000 cm^{-1}) are shown in Table 5.2. For potassium and sodium, the best spectral range used was 9000-4000 cm^{-1}. The most common pre-treatment used was OSC with mean centring.

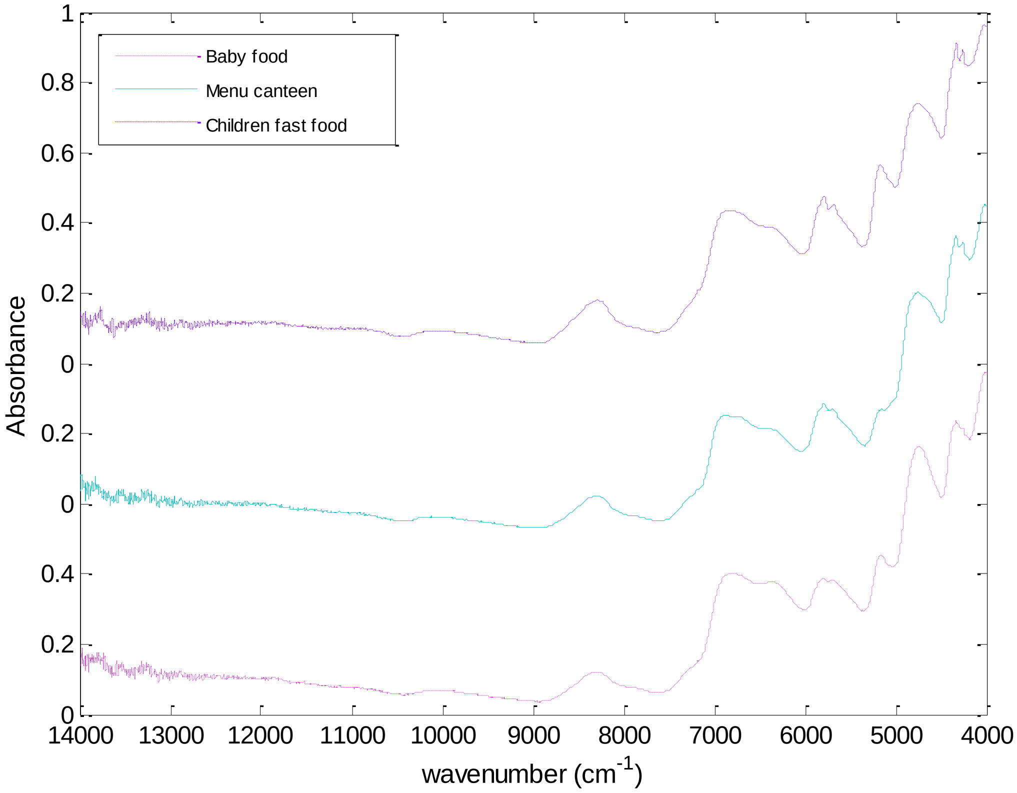

Figure 5.3. Mean DRIFT spectra of baby foods, children fast food and adult menus

NOTE: spectra were shift in the Y axe for clarity

The highest coefficients of determination in cross-validation (R^2_{cv}) and the lowest RMSECV were obtained for potassium (0.7 and 18%) and magnesium (0.71 and 15%). Coefficients of determination in calibration varied between 0.9 and 0.997, and coefficients of cross-validation were lower than previous ones. Regarding the number of LV selected, with only three LVs for calcium PLS model the cumulative variance percentage explained was 99.7% but RMSECV was high; being the same for iron. For zinc and sodium PLS models needed eight or nine LV to explain 99 and 90% of cumulative variance, respectively (see Table 5.2).

Table 5.2. Statistics of PLS- NIR models employed for the determination of mineral elements in human diets, also including the reference data of sample sets used for calibration and validation

Element	Set	Min	Max	Mean	SD	Pre-process	LV	R^2_{cal}	R^2_{CV}	R^2_{val}	RMSEC	RMSECV	RMSEP	RRMSEP
Ca	CAL	288	4000	1275	790	OSC+MC	3	0.997	0.22	0.42	44	797	668	51
	VAL	351	3400	1324	905									
K	CAL	4600	15190	9293	3085	OSC+MC	8	0.9	0.7	0.9	961	1711	997	11
	VAL	3860	14000	9172	2910									
Fe	CAL	2.1	33	18	8	OSC	7	0.991	0.355	0.41	0.76	6.75	6.39	37
	VAL	6.9	29.4	16	8									
Mg	CAL	402	1120	675	190	MSC+MC	9	0.988	0.689	0.405	19.8	104	119	19
	VAL	477	920	685	150									
Na	CAL	45	14400	6874	3774	OSC+MC	9	0.9	0.64	0.8	1173	2413	1711	30
	VAL	485	13200	5967	3762									
Zn	CAL	3.7	47	20	10	OSC+MC	8	0.985	0.62	0.69	1.22	6.29	4.41	25
	VAL	4.23	28.69	17	7									

NOTE: minimum (min), maximum (max), mean and standard deviation (SD) in mg kg^{-1} dry weight for calibration (CAL) and validation (VAL) sets. RMSEC root-mean-square error of calibration in mg kg^{-1}. RMSECV root-mean-square error of cross-validation in mg kg^{-1}. RMSEP root-mean-square error of prediction in mg kg^{-1}. RRMSEP relative root-mean-square error of prediction in percentage (%).

5.3.6. Prediction capability of PLS-NIR for mineral elements determination in diets

External validation was used to evaluate the PLS-NIR methodology, using an independent set of diet samples, not employed during the calibration step. Coefficients of determination in validation were good and varied between 0.41 for Mg and 0.9 for K (see Table 5.2). The minimum RRMSEP obtained was for potassium, only 11%, the same as that obtained by MIR procedure (see Figure 5.4). However, the prediction capability of PLS-NIR was lower than that of PLS-ATR-FTIR and only for potassium and magnesium RRMSEP was lower than 20%; for calcium, iron, sodium and zinc this value was higher than 20%. These results demonstrate the good predictive capability of the PLS-NIR models developed for potassium and magnesium determination, while a poor prediction was found for the other considered elements in diet samples.

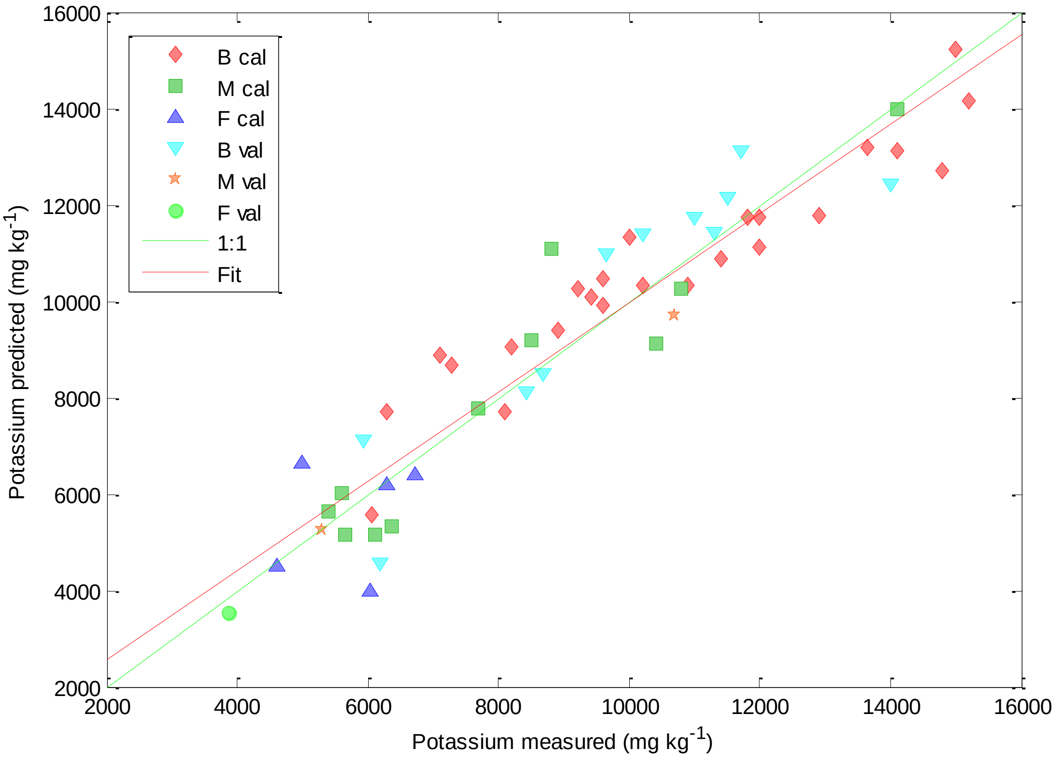

Figure 5.4. Predicted vs. measured potassium values for PLS-NIR model employed for the analysis of baby foods and diet samples

5.3.7. XRF spectra of diet samples

Figure 5.5 shows the typical EDXRF spectra between 0 and 30 keV for baby foods (B), children fast food (F) and adult menu (M) samples. All elements studied in this paper provided bands at energies lower than 10 keV, except sodium, which cannot be determined using this portable instrument. The Kα lines of magnesium, potassium, calcium and zinc are at 1.25, 3.31, 3.69 and 8.64 keV, respectively. Peaks higher than 15 keV correspond to the radiation due to the XR tube.

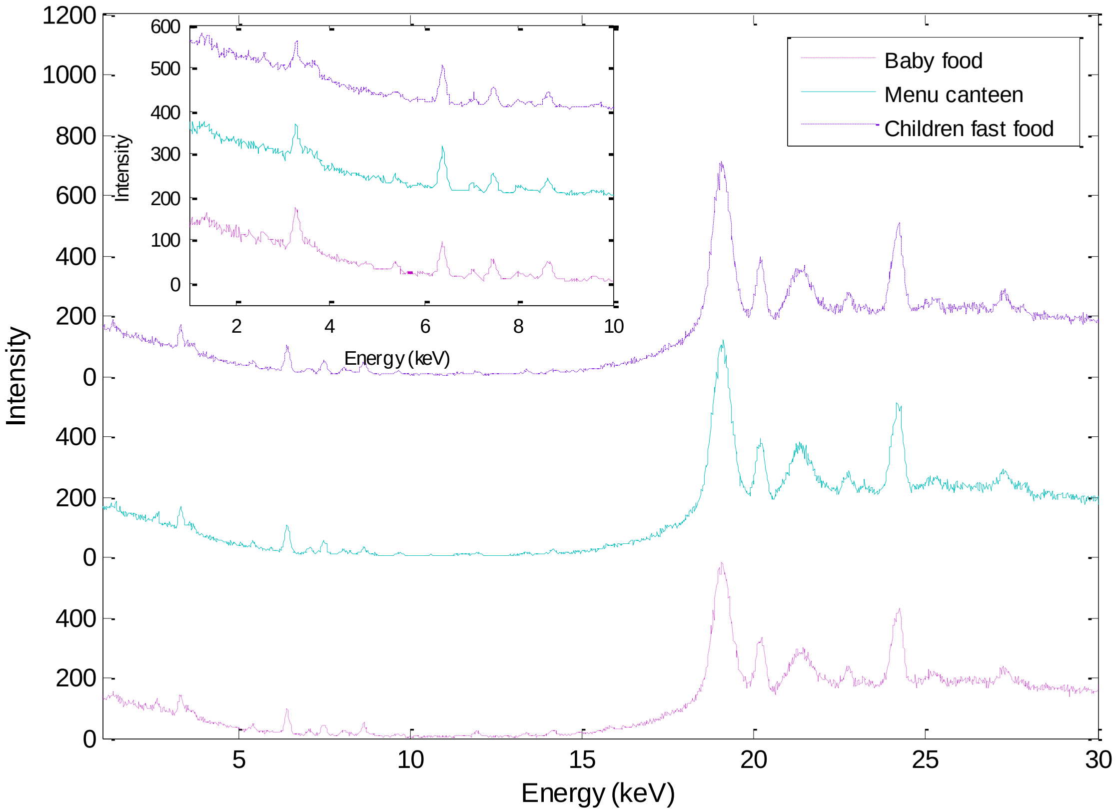

Figure 5.5. XRF spectra of baby foods, children fast food and adult menus. Inset of the region between 1 and 10 keV.

NOTE: spectra shift in the Y axe for clarity

5.3.8. PLS models for the determination of mineral elements in diets by XRF

For the selection of the two sample sets used for calibration and validation, the Kennard-Stone method was used. For calibration set, 22 baby foods, 5 infant fast food and 13 adult menus were selected, remaining 14 samples were used for validation. The mean, concentration range and standard deviation for the elements determined in diet samples in both, calibration and validation, sets employed for XRF are shown in Table 5.3. Several spectral regions and different pre-processing strategies were assayed to build the models and compared in order to evaluate their prediction capability. iPLS was used to select the spectral range for each considered element. Spectral regions selected included in all cases the Kα energy corresponding to each element. In general, XRF spectra were smoothed, being also employed OSC or MSC as pre-processing of signals (see Table 5.3).

Coefficients of determination in calibration varied between 0.7 and 0.999, and coefficients of cross-validation between 0.22 and 0.9. Regarding the number of LV, few factors, two for magnesium, three for potassium, four for calcium, five for zinc and six for iron, were necessary to obtain good models, with cumulative variance percentage explained around 77-99%. EDXRF provided RMSECV values lower than those found by IR spectroscopy, except for iron (see Tables 5.1-5.3). The highest R^2_{CV} and the lowest RSECV were obtained for zinc being 0.9 and 2.4 mg kg^{-1}, which evidenced an excellent coherence of the developed model.

5.3.9. Prediction capability of PLS-XRF for the determination of mineral elements in diets

An external sample set was used for the validation of the PLS-XRF developed methodology, using an independent set of samples, not employed during the calibration step. Coefficients of determination in validation were quite good, between 0.6 for Fe and 0.92 for K (see Table 5.3). The minimum RRMSEP obtained was 3% for zinc, better than that found for this element by MIR and NIR (see Figure 5.6). The prediction capability of PLS-XRF was higher than PLS-ATR-FTIR and PLS-NIR, for every element considered RRMSEP was lower than 20% in all cases except for iron. These results demonstrate a good predictive capability of the PLS-XRF models developed to predict the concentration of calcium, potassium, magnesium and zinc in different diets. PLS-XRF models for sodium were not created because the EDXRF portable instrument only measured elements with atomic number higher than 12.

5.3.10. Comparison of MIR, NIR and XRF for mineral analysis of diet samples

PLS models were established to determine mineral elements in human diets using MIR, NIR and XRF spectra. Tables 5.1-5.3 show prediction results for calcium, potassium, iron, magnesium, sodium and zinc in human diets based on MIR, NIR and XRF spectra. In general, XRF provided much better results than MIR and NIR spectra with lower LVs, and potassium was the best element prediction except in the case of sodium determination for which it must be used IR spectroscopy.

For calcium determination PLS-XRF (R^2_{val}=0.86, RMSEP=226 mg kg^{-1}) model provided better results than PLS-ATR-MIR (R^2_{val}=0.63, RMSEP=263 mg kg^{-1}) and PLS-NIR (R^2_{val}=0.42, RMSEP=668 mg kg^{-1}). For potassium results found by the three techniques were similar (R^2_{val}=0.92, RMSEP=1037 mg kg^{-1} for PLS-XRF, R^2_{val}=0.86, RMSEP=1010 mg kg^{-1} for ATR-MIR-PLS and R^2_{val}=0.9, RMSEP=997 mg kg^{-1} for NIR-PLS). However, PLS-XRF model was built with three LVs while MIR and NIR models needed eight LVs. For prediction of iron content PLS-ATR-MIR was the best alternative (R^2_{val}=0.76, RMSEP=3.4 mg kg^{-1}) with eight LVs. This element is very important for infants, because after 4 months of life the iron requirements increase due to the rapid growth of baby and iron reserves diminished till pathological levels if the needs are not covered with dietary iron [24]. For determination of magnesium, PLS-XRF only with two LVs provided R^2_{val}=0.63 and RMSEP=130 mg kg^{-1}, thus, better results than MIR or NIR models. For sodium prediction, PLS-XRF model was not able but, PLS-ATR-MIR and PLS-NIR models were very similar. However, for MIR two LVs were used while in NIR nine LVs were used. For zinc determination, the best method was PLS-XRF which provided very good values (R^2_{val}=0.84 and RMSEP=4.2 mg kg^{-1}).

Table 5.3. Statistics of PLS- XRF models employed for the determination of mineral elements in human diets, also including the reference data of sample sets used for calibration and validation

Element	Set	Min	Max	Mean	SD	Pre-process	LV	R^2_{cal}	R^2_{CV}	R^2_{val}	RMSEC	RMSECV	RMSEP	RRMSEP
Ca	CAL	288	4000	1438	887	SMO+MSC	4	0.77	0.62	0.86	417	535	226	19
	VAL	351	2600	1268	561									
K	CAL	3860	15190	8866	3225	OSC+FD	3	0.94	0.77	0.92	795	1530	1037	10
	VAL	4600	15000	9911	2983									
Fe	CAL	2.1	33	20	7	SMO+OSC	6	0.94	0.22	0.6	1.5	7.3	6.1	34
	VAL	4.5	31.4	18	9									
Mg	CAL	402	1120	680	180	SMO+OSC	2	0.999	0.76	0.63	2	89	130	18
	VAL	460	1110	709	208									
Zn	CAL	8.8	46	20	8	SMO+MSC	5	0.97	0.9	0.84	1.3	2.4	4.2	3
	VAL	10.53	47	22	10									

NOTE: minimum (min), maximum (max), mean and standard deviation (SD) in mg kg^{-1} dry weight for calibration (CAL) and validation (VAL) sets. RMSEC root-mean-square error of calibration in mg kg^{-1}. RMSECV root-mean-square error of cross-validation in mg kg^{-1}. RMSEP root-mean-square error of prediction in mg kg^{-1}. RRMSEP relative root-mean-square error of prediction in percentage (%).

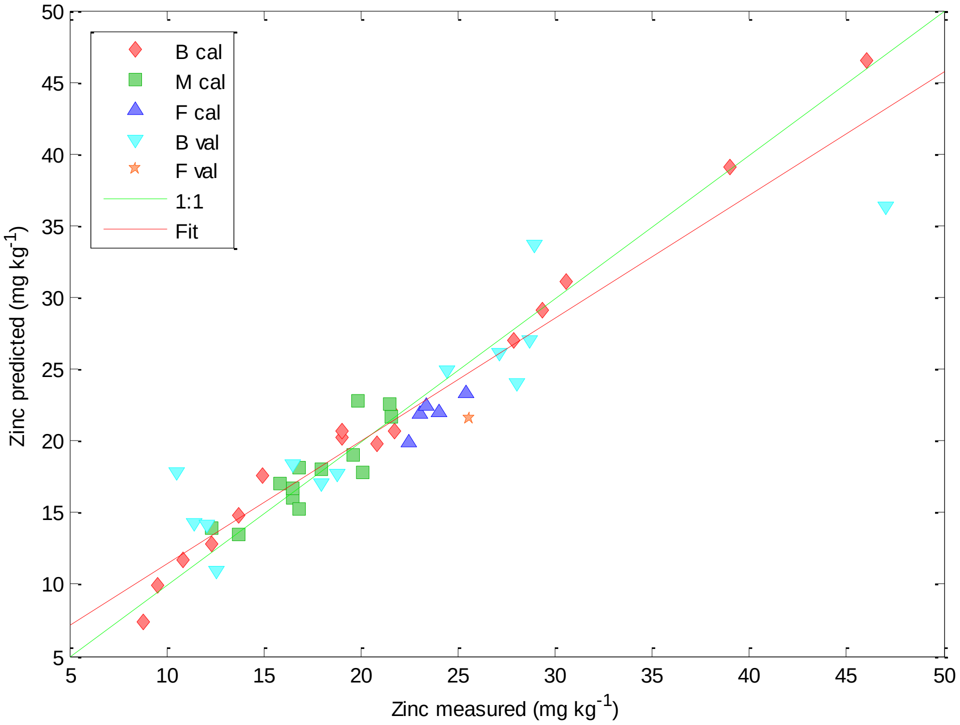

Figure 5.6. Predicted vs. measured zinc values for PLS-XRF model employed in baby foods and diet sample analysis

5.4. Conclusions

MIR, NIR spectroscopy and X-ray fluorescence techniques provided fast and green alternatives to determine calcium, potassium, iron, magnesium and zinc in human diets, being XRF the best choice in the main part of cases. Potassium and zinc content were easily predicted with all the techniques, obtaining good results. For iron, MIR was the best technique to do it and for calcium XRF was the best. Results found through this study indicate that XRF, MIR and NIR spectroscopy, combined with chemometrics could be applied as a rapid and green method for the determination of the main minerals in human diets. For sodium, XRF cannot be used and for magnesium content prediction using XRF, NIR and MIR must be further investigated. In short, these studies evidenced the strengths and weakness of direct spectroscopy methods for the determination of the major minerals presents in diets and offer fast procedures for the valuation of these samples.

Acknowledgements

The authors acknowledge the financial support of the Ministerio de Economia y Competitividad-Feder (project CTQ2011-25743 and project CTQ2012-38635). Alba Mir-Marqués acknowledges a grant ‘Atracció de Talent’ provided by the University of Valencia.

References

[1] FAO/WHO, “Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation (2n edition).” 2004.

[2] Food and Nutrition Board, “Dietary Reference Intake: the essential guide to nutrient requirements.” National Academies Press, 2006.

[3] WHO, “Trace-elements in human nutrition and health.” 1996.

[4] A. A. Carbonell-Barrachina, A. Ramirez-Gandolfo, X. Wu, G. J. Norton, F. Burlo, C. Deacon, and A. A. Meharg, “Essential and toxic elements in infant foods from Spain, UK, China and USA,” *J. Environ. Monit.*, 14 (2012) 2447–2455.

[5] R. Melo, K. Gellein, L. Evje, and T. Syversen, “Minerals and trace elements in commercial infant food,” *Food Chem. Toxicol.*, 46 (2008) 3339–3342.

[6] A. Mir-Marques, M. L. Cervera, and M. de la Guardia, “A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus,” *J. Food Compos. Anal.*, 27 (2012) 160–168.

[7] L. Noël, J.-C. Leblanc, and T. Guérin, “Determination of several elements in duplicate meals from catering establishments using closed vessel microwave

digestion with inductively coupled plasma mass spectrometry detection: estimation of daily dietary intake," *Food Addit. Contam.*, 20 (2003) 44–56.

[8] N. Zand, B. Z. Chowdhry, F. B. Zotor, D. S. Wray, P. Amuna, and F. S. Pullen, "Essential and trace elements content of commercial infant foods in the UK," *Food Chem.*, 128 (2011) 123–128.

[9] M. de la Guardia and S. Garrigues (eds.), *Handbook of green analytical chemistry*. Chichester: John Wiley & Sons, 2012.

[10] M. de la Guardia and S. Garrigues (eds.), *Challenges in green analytical chemistry*. Cambridge: RSC Publishing, 2011.

[11] P. T. Palmer, R. Jacobs, P. E. Baker, K. Ferguson, and S. Webber, "Use of fiel-portable XRF analyzers for rapid screening of toxic elements in FDA-regulated products," *J. Agric. Food Chem.*, 57 (2009) 2605–2613.

[12] M. de la Guardia and S. Garrigues (eds.), *Handbook of mineral elements in food*. Chichester: John Wiley & Sons, (in press).

[13] S. Schmitt, S. Garrigues, and M. de la Guardia, "Determination of the Mineral Composition of Foods by Infrared Spectroscopy: A Review of a Green Alternative," *Crit. Rev. Anal. Chem.*, 44 (2014) 186–197.

[14] C. Collell, P. Gou, P. Picouet, J. Arnau, and J. Comaposada, "Feasibility of near-infrared spectroscopy to predict aw and moisture and NaCl contents of fermented pork sausages," *Meat Sci.*, 85 (2010) 325–330.

[15] D. Cozzolino and A. Moron, "Exploring the use of near infrared reflectance spectroscopy (NIRS) to predict trace minerals in legumes," *Anim. Feed Sci. Technol.*, 111 (2004) 161–173.

[16] D. Cozzolino, W. Cynkar, N. Shah, and P. Smith, "Quantitative analysis of minerals and electric conductivity of red grape homogenates by near infrared reflectance spectroscopy," *Comput. Electron. Agric.*, 77 (2011) 81–85.

[17] I. Gonzalez-Martin, C. Gonzalez-Perez, J. Hernandez-Mendez, and N. Alvarez-Garcia, "Mineral analysis (Fe, Zn, Ca, Na, K) of fresh Iberian pork loin by near

infrared reflectance spectrometry - Determination of Fe, Na and K with a remote fibre-optic reflectance probe,” *Anal. Chim. Acta*, 468 (2002) 293–301.

[18] A. Lucas, D. Andueza, E. Rock, and B. Martin, “Prediction of dry matter, fat, pH, vitamins, minerals, carotenoids, total antioxidant capacity, and color in fresh and freeze-dried cheeses by visible-near-infrared reflectance spectroscopy,” *J. Agric. Food Chem.*, 56 (2008) 6801–6808.

[19] J. Moros, I. Llorca, M. L. Cervera, A. Pastor, S. Garrigues, and M. de la Guardia, “Chemometric determination of arsenic and lead in untreated powdered red paprika by diffuse reflectance near-infrared spectroscopy,” *Anal. Chim. Acta*, 613 (2008) 196–206.

[20] L. Nørgaard, A. Saudland, J. Wagner, J. P. Nielsen, L. Munck, and S. B. Engelsen, “Interval Partial Least Squares Regression (iPLS): A Comparative Chemometric Study with an Example from Near-Infrared Spectroscopy,” *Appl. Spectrosc.*, 54 (2000) 413–419.

[21] D. A. Burns and E. W. Ciurczak, *Handbook of Near-Infrared Analysis*. New York: Marcel Dekker, 1992.

[22] J. Moros, F. A. Iñón, M. Khanmohammadi, S. Garrigues, and M. de la Guardia, “Evaluation of the application of attenuated total reflectance–Fourier transform infrared spectrometry (ATR–FTIR) and chemometrics to the determination of nutritional parameters of yogurt samples,” *Anal. Bioanal. Chem.*, 385 (2006) 708–715.

[23] D. Wu, Y. He, J. Shi, and S. Feng, “Exploring Near and Midinfrared Spectroscopy to Predict Trace Iron and Zinc Contents in Powdered Milk,” *J. Agric. Food Chem.*, 57 (2009) 1697–1704.

[24] A. Gil Hernández, R. Uauy Dagach, and J. Dalmau Serra, “Bases para una alimentación complementaria adecuada de los lactantes y los niños de corta edad,” *An. Pediatría*, 65 (2006) 481–495.

Supplementary material

Table S5.1. Composition of baby foods and human diets employed in this study

Sample	Composition	Brand
B1	Ham, beef and vegetables	1
B2	Turkey with vegetables	1
B3	Chicken with rice	1
B4	Lamb stew	1
B5	Lamb stew	2
B6	Beef with vegetables	2
B7	Rice and chicken	2
B8	Mashed vegetables with chicken and beef	2
B9	Mashed vegetables and turkey	2
B10	Rice and beef	2
B11	Chicken, beef and vegetables	1
B12	Beef with carrots	1
B13	Beef with vegetables	1
B14	Chicken with vegetables	1
B15	Vegetables and rice with turkey	3
B16	Mashed zucchini with beef	4
B17	Vegetables with beef	4
B18	Sole with white sauce	1
B19	Rice with hake	1
B20	Selected vegetables and sea bass	2
B21	Selected vegetables and monkfish	2
B22	Mashed peas and rice with hake	2
B23	White sauce and hake	2
B24	Whiting vegetables in cream	1
B25	Carrots with rice in chicken broth	1
B26	Mixed vegetables	1
B27	Cream of green beans with potatoes	1
B28	Soft cream carrots and potatoes	4
B29	Soft cream of peas with potatoes	4
B30	Peach and banana	1
B31	3 Fruit (pear, peach and banana)	1
B32	Banana, tangerine and pear	1
B33	Varied fruit (peach, apple, banana, apricot, orange)	1
B34	Fruit salad and cereals	2
B35	Tangerine, apple and biscuits	1
M1	Seafood paella. Salmon with chips. Pear. Bread.	1
M2	Vegetable pie with tomato sauce. Tuna omelette with smashed potatoes. Crème caramel. Bread	1
M3	Lentil. Ham croquettes with chips. Skimmed yogurt. Bread.	1
M4	Spaghetti with carbonara sauce. Sausages with chips and vegetables. Fried milk. Bread.	1
M5	Salad. Mixed-up of spinach and mushrooms with potatoes. Orange. Bread.	1
M6	Soup with bread and garlic. Grilled tuna with potatoes. Lemon yogurt. Bread.	1
M7	Macaroon with tomato. Ham, bacon and sausage grilled with potatoes. Apple. Bread.	1
M8	Beans. Meatballs with sauce with chips. Orange gelatine. Bread.	1
M9	Vegetable cream. Roast chicken with chips. Orange. Bread.	1
M10	Spaghetti with carbonara sauce. Ham schnitzel with chips. Vanilla custard. Bread.	2

NOTE: B corresponds to baby food jars, M corresponds to adult menus and F corresponds to children fast food menus.

Table S5.1. (continued) Composition of baby foods and human diets employed in this study

Sample	Composition	Brand
M11	Salad. Cod with vegetables. Orange. Bread.	2
M12	Beans with ham. Zucchini gratin with béchamel sauce, cheese and bacon. Strawberry and orange juice. Bread.	2
M13	Rice with fish. Pork loin with vegetables and chips. Lemon yogurt. Bread.	2
F1	Bread, hamburger, cheese, chips, ketchup, mustard, yogurt and cola refresh.	1
F2	Bread, hamburger, cheese, chips, ketchup, mustard, yogurt and orange refresh.	1
F3	Bread, hamburger, cheese, chips, ketchup, mustard, yogurt and lemon refresh.	2
F4	Bread, hamburger, cheese, chips, ketchup, mustard, yogurt and cola refresh.	2
F5	Bread, hamburger, cheese, chips, ketchup, mustard, yogurt and tea with lemon.	1
F6	Bread, hamburger, cheese, chips, ketchup, mustard, yogurt and cola refresh.	2

NOTE: B corresponds to baby food jars, M corresponds to adult menus and F corresponds to children fast food menus.

Capítulo 6

Perfil mineral de caquis (Diospyros kaki L.)

Mineral profile of kaki fruits (*Diospyros kaki L.*)

Alba Mir-Marqués, Ana Domingo, M. Luisa Cervera*, Miguel de la Guardia

Department of Analytical Chemistry, University of Valencia, Jerónimo Muñoz Building, 50th Dr. Moliner St., 46100 Burjassot (Valencia), Spain

Received 19 April 2013,

Received in revised form 8 September 2014,

Accepted 13 September 2014,

Available online 20 September 2014

Food Chemistry 172 (2015) 291-297

DOI 10.1016/j.foodchem.2014.09.076

Resumen

El principal objetivo de este estudio fue determinar el perfil mineral de 167 muestras de fruta caqui (*Diospyros kaki L.*) producidas en diferentes regiones de España, incluyendo muestras bajo la denominación de origen protegida “Kaki Ribera del Xúquer” Valencia (España). Las muestras fueron analizadas mediante espectroscopia de emisión óptica con plasma de acoplamiento inductivo (ICP-OES) y espectrometría de masas con plasma de acoplamiento inductivo (ICP-MS). El consumo de una pieza de caqui (200-400 g) puede aportar una ingesta entre el 1-10 % de la cantidad diaria recomendada (RDA) para el calcio, entre 1-30 % para el cobre y el potasio, entre 1-15 % para el hierro y magnesio, menos de 1 % para el sodio, y menos de 4 % para el zinc. El análisis ANOVA indica que existen diferencias entre muestras procedentes de diferentes regiones de España, lo que nos indica una posible manera para autentificar el origen de muestras con DOP.

Abstract

The main objective of this study was the determination of the mineral profile of 167 kaki fruit (*Diospyros kaki L.*) samples produced from different regions of Spain, including samples with the protected designation of origin (PDO) ‘Kaki Ribera del Xúquer’ Valencia (Spain). Samples were analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Consumption of one piece of kaki fruit (200 – 400 g) would give a mineral intake providing 1-10% of the recommended daily allowance (RDA) for calcium, 1-30% for copper and potassium, 1-15% from iron and magnesium, nearly to 1% of sodium, and nearly to 4% of zinc. ANOVA analysis indicates differences between samples from different Spanish region, thus offering a way for authentication of PDO sample origin.

6.1. Introduction

Persimmon (*Diospyros kaki* L.) is a fruit native to China, but cultivated in warm regions all around the world. Rojo Brillante is an astringent persimmon and this cultivar originates in la Ribera del Xúquer (Valencia) region of Spain. It has the protected designation of origin (PDO) 'Kaki Ribera del Xúquer', which includes more than 95% of persimmon grown in the region, production being around 25 million kg per year [1-3].

Lots of the kaki farmlands are locate in the low plain of the rivers Xúquer and Magro, and has compact and rich soil. Moreover, in the lowlands of the valley, there are soils with the capacity to sustain intensive exploitation. The region enjoys a mild climate favourable for the cultivation of persimmon with an average temperature of 17 °C, low cloud cover, and average annual rainfall of 400-500 mm. Furthermore, the surrounding mountains protect crops against frost especially in the valleys. These characteristics determine the quality and character of the 'Kaki Ribera del Xúquer' fruits. The shape of the kaki fruit not only depends on the variety, but also the climate. The ratio height to diameter is larger and more pointed in hot-dry climates, which provides a point of differentiation from fruits not protected by PDO status (see Figure 6.1) [1].

The cultivation of persimmon is very important in the 'Ribera del Xúquer' for economic and traditional reasons. Rojo Brillante is highly sought after because of its excellent nutritional and sensorial qualities. It is important to know the chemical composition of this fruit, particularly the content of minerals and trace elements, since this reflects the soil type and the growing environment, and may offer a route for authenticating fruit produced inside the PDO. In addition, the mineral composition of foods has a vital role in human health and adequate intakes of many elements are the key to a healthy diet.

Essential elements are those needed in the human diet to maintain normal physiological functions. Thus, risk assessment in terms of dietary intake concerns toxic substances, which must be absent and essential elements, high intakes of which could result in toxic effects and low intakes in nutritional inadequacy [4]. It is important a thorough analysis of mineral profiles in foods, including essential and toxic elements, is carried out to know the contribution of foods to the recommended daily amount (RDA). The RDA is the 'amount of a nutrient a healthy person should eat each day on average through diet to maintain a good health' [5]. To achieve this, analytical techniques with sufficient sensitivity are required.

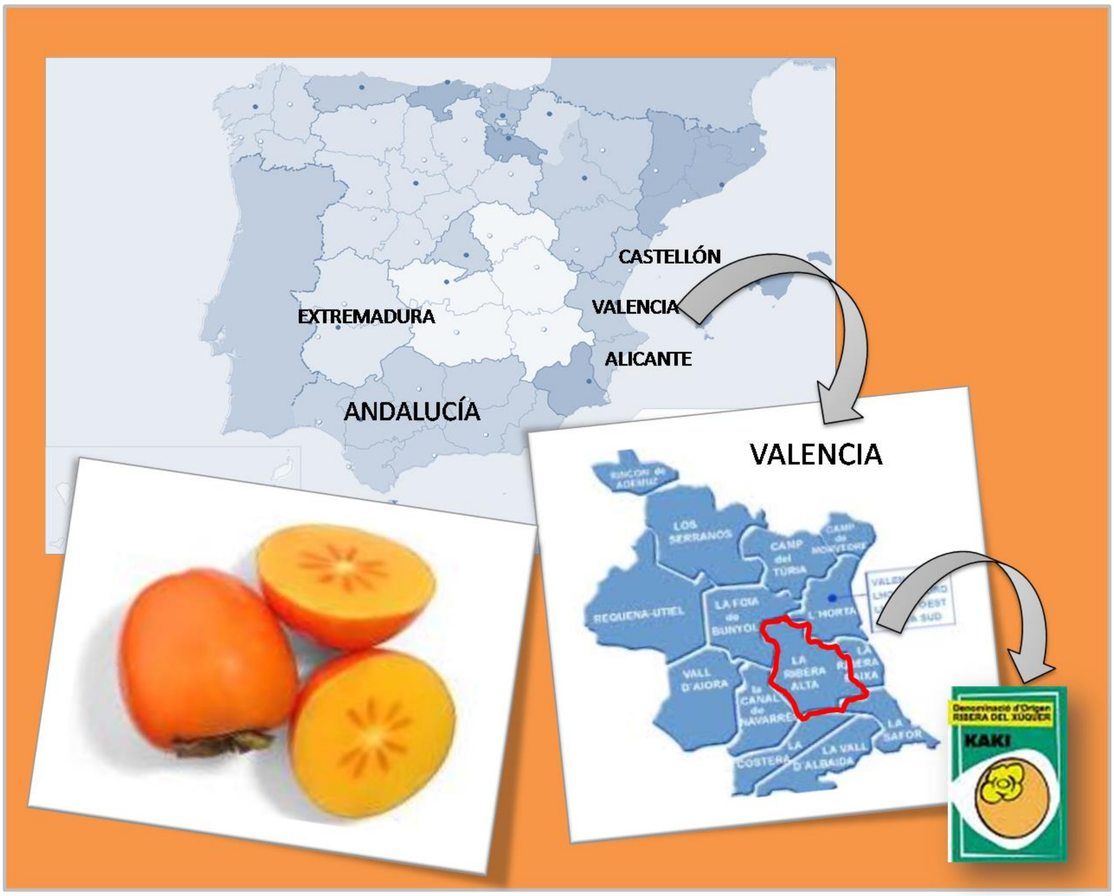

Figure 6.1. Map of Spain indicating the different regions where the kaki fruit samples employed through this study were produced, the area covered by PDO 'Kaki Ribera del Xúquer' from Valencia and the PDO label.

Different techniques, such as flame atomic absorption spectroscopy (FAAS) or graphite furnace atomic absorption spectroscopy (GFAAS) [6, 7], have been employed for the determination of mineral elements in fruits. However, inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are useful techniques for complete mineral profile determination of foods [8-11] and have been used as a basis for authentication of PDO products [12-16]. These techniques provide a wide linear dynamic range and permit the determination of several elements in a same sample.

The main purpose of this study was to determine the mineral profile of persimmon from different areas in Spain. Data were used to categorise (adequate, insufficient or toxic) daily intake of minerals following regular consumption of kaki fruit, and characterisation of samples produced under de PDO ‘Kaki Ribera del Xúquer’.

6.2. Materials and methods

6.2.1. Apparatus

An ICP-OES Optima 5300 DV inductively coupled plasma optical emission spectrometer Perkin Elmer (Norwalk, CT, USA) equipped with an auto sampler AS 93-plus, and a ultrasonic nebuliser U6000AT$^+$ Cetac (Nebraska, EEUU) and ICP-MS Perkin-Elmer SCIEX Elan 6100 DRCII apparatus, equipped with a Meinhard nebuliser and an autosampler Perkin-Elmer AS-91, were used for mineral determination.

The apparatus employed for sample pre-treatment was a microwave laboratory system, Ethos SEL from Millestone (Sorisole, Italy), equipped with a thermocouple for automatic temperature control, an automatic gas leaks detector and high pressure Teflon vessels of 100 mL inner volume. For cleaning the vessels, an automatic cleaning device, traceCLEAN from Millestone (Sorisole, Italy), was used.

A LyoAlfa Plus 10-55 lyophiliser Telstar (Barcelona, Spain) employed for sample drying and preservation, and an ultrasound water bath from Selecta (Barcelona, Spain, 9 L, 50 W, 50 Hz) was used for sample sonication.

6.2.2. Reagents

Stock solutions of a multi-element standard solution (100 mg L^{-1}) containing 26 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Se, Na, Sr, Tl, Ti, V and Zn) dissolved in 5% nitric acid, a multi-element solution containing 20 mg L^{-1} Dy, Er, Eu, Gd, Ho, Lu, Sm, Sc, Tb, Tm, Yb and Y and 100 mg L^{-1} Ce, La, Nd and Pr dissolved in 5% nitric acid, a 1 g L^{-1} potassium standard solution for atomic absorption in 0.5 mol L^{-1} nitric acid, a 1 g L^{-1} calcium standard solution for atomic absorption in 0.5 mol L^{-1} nitric acid, a 1 g L^{-1} magnesium standard solution for atomic absorption in 0.5 mol L^{-1} nitric acid all from Scharlau (Barcelona, Spain), were employed to prepare the calibration standards.

Nitric acid (69% for trace analysis) and hydrogen peroxide (35% reagent grade), both from Scharlau, and ultra-pure water with a minimum resistivity of 18.2 MΩ cm, obtained using a Milli-Q plus Millipore system (Molsheim, France), were used for sample digestion and dilution.

A 1 g L^{-1} ruthenium standard solution for ICP in hydrochloric acid 5%, and a 1 g L^{-1} rhodium standard solution for ICP in hydrochloric acid 5%, both from Scharlau (Barcelona, Spain) were used as internal standards. Argon C-45 (purity higher than 99.995%), supplied by Carburos Metálicos (Barcelona, Spain), was employed as plasmogen and carrier gas.

6.2.3. Sampling, sample storage and pre-treatment

167 kaki fruit samples were collected from different agricultural cooperatives and producers in Spain during 2010 and 2011. Samples (113) were produced under the PDO ‘Kaki Ribera del Xúquer’, four samples were from Alicante, 11 from Andalucía, 10 from Castellón, three from Extremadura, 13 from the Ribera area (outside the PDO), and 13 from Valencia. Regions where kaki fruit samples were obtained are shown in Figure 6.1. The edible parts of the fruit were cut into pieces and frozen at -20°C for a minimum of 24 h. Subsequently, they were lyophilised for a minimum of 48 h under 50

Pa. Dried samples were pulverised with a domestic Braun mixer (Kronberg, Germany), and the powdered samples stored in polyethylene bottles until analysis. In order to have a fixed reference for mineral elements concentration, the water content of samples was determined, and found to be 81 ± 2 % for all the samples analysed.

6.2.4. Sample digestion

The microwave-assisted treatment was adapted from that employed by us for the determination of the mineral profile of diets [17] according to the microwave laboratory system manufacturer recommendations and our own experience.

A mass of 0.5 g freeze-dried sample was weighed accurately in the Teflon digestion vessel and 8 mL of nitric acid (69 %) added. Samples were allowed to pre-digest in an ultrasound water bath for 30 min at room temperature. Then, 2 mL of hydrogen peroxide (35 %) were added and the mixture was sonicated for further 45 min. After that, the Teflon reactors were closed and placed inside the microwave oven. The following program was run: step 1) 3 min to reach 85°C (500 W), step 2) 12 min to reach 145°C (500 W), step 3) 10 min to reach 180°C (700 W), step 4) 15 min at 180°C (700 W), and step 5) cooling down. After cooling to ambient temperature, the reactors were opened and sonicated to eliminate the nitrous vapours. The resulting solution was quantitatively transferred to a plastic flask and made up to 20 mL with ultra-pure water. Teflon digestion vessels were previously cleaned in nitric acid solution to avoid cross-contamination. All samples were analysed in triplicate to eliminate any specific error, and to verify the homogeneity of samples and to evaluate the repeatability of the procedure. Blanks were prepared in the same way, but the sample omitted, for each batch of samples, and the certified reference material was also digested using the same method.

6.2.5. ICP-OES determination

The operating conditions of the ICP-OES equipment were as follow 15 L min^{-1} of argon plasma gas flow rate, 0.2 L min^{-1} of auxiliary gas flow rate, 0.8 L min^{-1} nebuliser gas flow rate, 1300 W of radio frequency (RF) power, 1.10 mL min^{-1} of sample flow rate. All elements were detected in axial mode except calcium, magnesium, lithium, potassium, sodium and strontium, which were detected in radial mode. For the signal integration four points per peak were used while for the background correction two points were employed. All analytes were measured in two different emission lines. In order to check for matrix effects on the sensitivity and selectivity, a scanning of the emission lines was obtained for a standard, a digested sample and a digested spiked sample. The emission line was selected, taking into account non-spectral interferences and the best signal to background ratio for all elements, and background corrected manually for all emission lines selected (see Table 6.1).

The calibration standards were prepared in 0.5% nitric acid. The calibration range for all elements was evaluated from 0.05 to 2 mg L^{-1} except calcium and magnesium for which calibration curves were prepared from 2 to 10 mg L^{-1} and potassium for which the calibration was from 20 to 100 mg L^{-1}. Ruthenium (1 mg L^{-1}) was used as internal standard and added to all samples, reagent blanks and standards. Digested samples were diluted 1:2, 1:5, 1:10 and 1:100, 1:10 and, additionally, undiluted samples were analysed by using the standard addition method in order to evaluate the presence of matrix effect and its correction. From the results, a final dilution of 1:2 was selected. The average value for blank samples was subtracted from the analytical signals of digested samples after interpolation on the corresponding calibration graphs.

Table 6.1. Analytical figures of merit of ICP-OES and ICP-MS determination of the mineral composition of kaki fruit samples

Elements	**ICP-OES**			**ICP-MS**		
	Wavelength (nm)	**LODm (mg kg^{-1})**	**LOQm (mg kg^{-1})**	**m/z**	**LODm (µg kg^{-1})**	**LOQm (µg kg^{-1})**
Al	396.153	0.12	0.4	-	-	-
As	188.979	0.3	0.8	-	-	-
Ba	233.527	0.07	0.2	138	3	9
Be	313.042	0.05	0.16	-	-	-
Bi	223.061	0.3	0.9	209	0.5	2
Ca	317.933	3	9	-	-	-
Cd	228.802	0.011	0.04	114	2	7
Co	228.616	0.016	0.05	59	1.5	5
Cr	267.716	0.07	0.2	52	71	237
Cu	327.393	0.05	0.17	65	25	82
Fe	238.204	0.3	0.9	-	-	-
K	766.490	2	7	-	-	-
Li	670.784	0.005	0.017	7	8	26
Mg	285.213	0.09	0.3	-	-	-
Mn	257.610	0.011	0.04	55	34	113
Mo	202.031	0.04	0.13	95	10	34
Na	589.592	1.2	4	-	-	-
Ni	231.604	0.10	0.3	60	58	194
Pb	220.353	0.05	0.15	206	7	22
Se	196.026	0.4	0.3	-	-	-
Sr	407.771	0.02	0.07	88	5	16
Ti	334.940	0.04	0.13	47	66	219
Tl	190.801	0.09	0.3	205	0.2	0.8
V	290.880	0.05	0.16	51	3	9
Zn	206.200	0.14	0.5	66	93	310

NOTE: LOD and LOQ data are referred to dry sample taking into account the sample mass 0.5 g and dilution carried out for the measurement.

6.2.6. ICP-MS determination

For the analysis by ICP-MS, a solution (prepared daily) containing 10 µg L^{-1} of barium and 1 µg L^{-1} of magnesium, aluminium, chromium, manganese, copper, rhodium, cadmium, indium, cerium, lead, and thorium was used to optimise the operating conditions. A radio frequency (RF) power of 1125 W, argon plasma gas of 15 L min^{-1}, auxiliary gas of 1.2 L min^{-1}, nebuliser gas flow rate of 0.95 L min^{-1}, and lens voltage of 7 V were automatically selected by the instrument during the optimisation step. The dwell time was 50 ms. The selected mass numbers employed to analyse the kaki fruit samples are shown in Table 6.1.

The calibration standards were prepared in 0.5% nitric acid. The calibration range for all studied elements was from 2 to 400 µg L^{-1}. Rhodium (1 µg L^{-1}) was used as internal standard and added to all samples and standards. Digested samples were measured with a final dilution 1:5. The average of blank ICP-MS signals was subtracted from analytical signals of digested samples after interpolation on the corresponding calibration graphs.

6.2.7. Quality control

Kaki fruit samples were prepared and measured in triplicate. Reagent blanks were made regularly together with each batch of sample digestion, and certified reference materials were digested and measured together with the different sample batches in order to control the trueness of data found. Additionally, control standards were measured for every series of ten independent sample measurements.

For the precision and trueness control of the method, a kaki fruit sample was spiked with two concentration levels (0.1 and 0.5 mg L^{-1}), and a certified reference material of NIST 1573a (Tomato leaves), obtained from the National Institute of Standards and Technology (Gaithensburg, MD, USA), was analysed under the same conditions as the samples.

Detection limits of instrument (LODi) were calculated as the concentrations corresponding to signals equal to three times the standard deviation of ten reagents blank measurements. Additionally, detection limits of the method (LODm) were calculated based on the original samples (mg kg^{-1} in dry weight), taking into consideration the amount of sample digested and the final dilution employed in the recommended procedure. Quantification limits of instrument (LOQi) were determined in the same way for a factor of ten times the deviation of blank measurements, and the quantification limits of the method (LOQm) were calculated in terms of concentration in the original sample in dry weight.

6.3. Results and discussion

6.3.1. Method quality assurance

Table 6.1 shows the main analytical figures of merit, LODm and LOQm for ICP-OES and ICP-MS measurements. In general, ICP-OES LODm and LOQm were higher than those obtained for ICP-MS. Thus, we used ICP-MS for lithium, nickel, titanium and zinc because it is not easy to determine these by ICP-OES.

The results found for certified reference material (NIST 1573a) were statistically compared with certified values. The regression line of average content versus certified values for each element was $y = (1.013 \pm 0.016)x - (2.917 \pm 3.25)$, which indicates the intercept and slope correspond to 0 and 1, and the regression coefficient was 0.9997. The percentage of recoveries evaluated by comparison between concentration data found and the certified values of the reference material provided recovery values from 97% for Na to 114% for Mg. It can be seen in Table 6.2 that the recovery percentages for samples spiked at two concentrations (0.1 mg L^{-1} and 0.5 mg L^{-1}) were 91 to 111%.

6.3.2. Mineral content of samples

Minimum, maximum, mean and median concentration of elements Al, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Sr, Ti and Zn found in kaki fruit samples are shown in Table 6.3. Data were classified as a function of the production regions (PDO Ribera del Xúquer, Alicante, Andalucía, Castellón, Extremadura, Ribera outside the PDO and Valencia) and expressed in mg kg^{-1} of dry weight. Data found for each assayed sample are included in the supplementary material. As indicated by the initial investigation of 42 elements, only 14 were present at detectable concentrations in the edible portion of the samples analysed.

The concentration of potassium in kaki samples (between 6500 and 15500 mg kg^{-1} dry weight) provided values comparable with those obtained in bananas [18, 19].

Thus, kaki fruits may be a good source of potassium, which is essential to maintain body water content and acid balance. Levels of calcium and magnesium were 400 mg kg^{-1}. Calcium allows the correct formation of the skeleton during childhood and has an essential role in preventing osteoporosis in later life. The values of calcium found through this study were lower than data reported for kakis by Farran et al. [20] and Souza et al. [21], and very similar to values reported by Gorinstein et al. [6] with exception of samples from Alicante. Magnesium is also essential for humans; it is needed for more than 300 biochemical reactions in the body, helps to regulate blood sugar levels and is involved in energy metabolism and protein synthesis. The values of magnesium found through this study are higher than ones reported from Brazil [21]. Sodium concentrations found in this study were lower than those reported by Gorinstein et al. [6]. Sodium is also an essential element, but is only necessary in small amounts. High intakes of this element are associated with increased blood pressure and risk of cardiovascular disease [22]. Therefore, the kaki fruits could be beneficial because its consumption is not associated with hypertension.

Table 6.2. Evaluation of the trueness of the method employed for ICP-OES and ICP-MS determination of the mineral profile of kaki fruit

Element	Certified (mg kg^{-1})	Obtained (mg kg^{-1})	Instrument	Recovery[b](%)	Recovery[c] (%)
Al	598 ± 12	587 ± 6	ICP-OES	91	103
Ba	63	60 ± 3	ICP-MS	107	107
Ca	5.05 ± 0.09[a]	5 ± 0.5[a]	ICP-OES	---	---
Cu	4.70 ± 0.14	4.7 ± 0.4	ICP-MS	---	---
Fe	368 ± 7	363 ± 2	ICP-OES	103	104
K	2.70 ± 0.05[a]	3.02 ± 0.08[a]	ICP-OES	---	---
Mg	1.2[a]	1.23 ± 0.04[a]	ICP-OES	---	---
Mn	246 ± 8	248 ± 10	ICP-MS	106	111
Na	136 ± 4	155 ± 5	ICP-OES	---	---
Ni	1.59 ± 0.07	1.66 ± 0.12	ICP-MS	105	105
Sr	85	87 ± 3	ICP-MS	96	101
Zn	30.9 ± 0.7	30 ± 3	ICP-MS	107	111

NOTE: data obtained were found as the average of 15 independent analysis made in different sessions by the proposed method. [a] Data reported in percentage (%). [b] Spiked level (0.1 mg/L). [c] Spiked level (0.5 mg/L).

Table 6.3. Mineral composition of kaki fruit samples from different Spain production areas

		Al	**Ba**	**Ca**	**Cu**	**Fe**	**K**	**Li**
PDO “Kaki Ribera del Xúquer”	**min**	< 0.12	0.4	210	0.1	< 0.3	6800	< 0.005
	max	8.6	4.8	540	2.2	11.0	12600	1.0
	mean	2.6 ± 1.9	1.7 ± 0.8^{b}	380 ± 70^{b,c}	1.1 ± 0.5^{a,b}	2.6 ± 2.3	9600 ± 1400^{b}	0.16 ± 0.22^{b}
	median	2.4	1.6	380	1.0	2.4	9700	0.09
Alicante	**min**	1.2	0.14	500	1.32	0.57	9400	< 0.005
	max	2.7	7.8	1000	2.10	3.68	11600	0.06036
	mean	1.9 ± 0.8	3.2 ± 3.4^{a,b}	800 ± 200^{a}	1.60 ± 0.34^{a}	2.00 ± 1.60	10300 ± 900^{a,b}	0.015 ± 0.030^{a,b}
	median	1.9	2.4	800	1.49	1.87	10100	0
Andalucía	**min**	0.5	< 0.07	230	0.3	0.6	9200	< 0.005
	max	9.3	4.0	550	2.0	8.7	15517	0.56
	mean	3.4 ± 3.1	2.2 ± 1.2^{a,b}	400 ± 80^{b,c}	1.1 ± 0.5^{a,b}	4.1 ± 2.2	11200 ± 1800^{a}	0.16 ± 0.20^{a,b}
	median	2.6	2.3	400	1.3	4.2	10600	0.09
Castellón	**min**	< 0.12	0.46	240	0.19	0.33	6500	< 0.005
	max	2.60	2.51	430	1.04	4.05	11200	0.194
	mean	1.08 ± 1.02	1.45 ± 0.68^{b}	350 ± 60^{b,c}	0.70 ± 0.26^{b}	2.17 ± 1.38	9000 ± 1300^{b}	0.070 ± 0.081^{b}
	median	0.69	1.51	340	0.72	2.10	8900	0.041
Extremadura	**min**	0.3	2.59	230	0.25	4.0	9300	< 0.005
	max	0.7	4.08	270	1.45	8.2	9900	0.08
	mean	0.5 ± 0.2	3.53 ± 0.82^{a}	250 ± 20^{c}	0.72 ± 0.64^{a,b}	6.0 ± 2.1	9600 ± 300^{a,b}	0.03 ± 0.05^{a,b}
	median	0.7	3.91	250	0.45	5.6	9700	0
Ribera ouside PDO “Kaki Ribera del Xúquer”	**min**	< 0.12	< 0.07	210	0.7	< 0.3	7600	< 0.005
	max	10	3	520	2.1	13	11100	1.0
	mean	3 ± 4	2 ± 0.8^{a,b}	410 ± 90^{b,c}	1.2 ± 0.4^{a,b}	4 ± 3.5	9000 ± 1100^{b}	0.38 ± 0.26^{a}
	median	2	2	400	1.2	3	9100	0.43
Valencia	**min**	< 0.12	0.5	270	0.6	< 0.3	8000	< 0.005
	max	14.3	5.1	660	3.3	6.3	12300	0.77
	mean	3.2 ± 4.1	2.0 ± 1.2^{a,b}	450 ± 130^{b}	1.4 ± 0.7^{a}	2.0 ± 1.8	10600 ± 1400^{a,b}	0.18 ± 0.24^{a,b}
	median	2.1	1.7	400	1.3	1.7	11100	0.03

NOTE: concentration in mg/kg dry weight. Mean values in the same column with different superscripts (a-c) are significantly different ($p<0.05$) with respect of the origin of kaki fruits by Tukey’s test.

Table 6.3. (continued) Mineral composition of kaki fruit samples from different Spain production areas

		Mg	Mn	Na	Ni	Sr	Ti	Zn
PDO “Kaki Ribera del Xúquer”	**min**	240	0.4	< 1.2	< 0.10	0.3	< 0.04	0.4
	max	500	14.2	200	2.3	9.8	3.6	4.5
	mean	$400 \pm 50^{a,b}$	5.4 ± 3.3^{a}	50 ± 30^{a}	0.7 ± 0.4^{b}	4.6 ± 1.7^{a}	2.3 ± 0.7	$1.7 \pm 0.6^{a,b}$
	median	400	4.4	40	0.7	4.5	2.5	1.6
Alicante	**min**	420	4.3	40	0.85	1.8	2.6	1.70
	max	540	11.1	110	2.55	3.0	3.3	2.77
	mean	480 ± 50^{a}	$7.6 \pm 2.8^{a,b}$	$70 \pm 30^{a,b}$	1.66 ± 0.69^{a}	$2.3 \pm 0.5^{a,b}$	3.0 ± 0.3	2.37 ± 0.48^{a}
	median	480	7.5	70	1.63	2.3	3.0	2.50
Andalucía	**min**	380	2	30	0.3	0.2	1.4	1.1
	max	610	35	90	2.5	5.1	3.7	3.0
	mean	440 ± 70^{a}	$9 \pm 10^{a,b}$	$60 \pm 20^{a,b}$	$0.9 \pm 0.6^{a,b}$	2.6 ± 1.6^{b}	2.6 ± 0.6	$1.8 \pm 0.5^{a,b}$
	median	410	6	50	0.8	3.0	2.6	1.7
Castellón	**min**	330	3.1	10	< 0.10	2.0	1.0	< 0.14
	max	420	11.6	40	0.75	4.1	3.2	1.44
	mean	360 ± 30^{b}	5.6 ± 2.4^{b}	$20 \pm 10^{a,b}$	0.35 ± 0.27^{b}	$3.1 \pm 0.7^{a,b}$	2.3 ± 0.6	0.76 ± 0.54^{c}
	median	360	5.0	20	0.39	3.2	2.3	0.95
Extremadura	**min**	290	7.0	4.7	< 0.10	1.07	1.28	0.83
	max	350	19.7	12.4	0.44	1.22	1.58	1.12
	mean	320 ± 30^{b}	15.4 ± 7.2^{a}	$9.7 \pm 4.4^{a,b}$	0.20 ± 0.22^{b}	1.16 ± 0.08^{b}	1.40 ± 0.16	$0.97 \pm 0.15^{b,c}$
	median	300	19.4	12.1	0.17	1.19	1.33	0.97
Ribera ouside PDO “Kaki Ribera del Xúquer”	**min**	330	3	20	< 0.10	1.2	1.5	1.0
	max	500	10	130	1.2	7	3	3
	mean	$410 \pm 60^{a,b}$	7 ± 2^{b}	$50 \pm 30^{a,b}$	$0.8 \pm 0.3^{a,b}$	$4 \pm 1.6^{a,b}$	3 ± 0.7	$2 \pm 0.5^{a,b,c}$
	median	400	7	50	0.8	3	3	1.4
Valencia	**min**	340	0.7	3	0.4	1.0	1.7	0.7
	max	630	22	60	5.1	9.3	4.2	2.9
	mean	440 ± 90^{a}	$7.7 \pm 6.5^{a,b}$	22 ± 20^{b}	1.3 ± 1.2^{a}	$3.2 \pm 2.4^{a,b}$	2.5 ± 0.7	$1.9 \pm 0.6^{a,b}$
	median	400	5.1	20	1.0	2.4	2.4	1.8

NOTE: concentration in mg/kg dry weight. Mean values in the same column with different superscripts (a-c) are significantly different ($p<0.05$) with respect of the origin of kaki fruits by Tukey’s test.

Manganese, strontium, iron, aluminium and titanium concentration levels in kaki samples were less than 5 mg kg^{-1} but, in general, greater than 2 mg kg^{-1}. Manganese content was very similar to that reported by Gorinstein et al. [6], except in the case of kaki fruit from Extremadura. The values for iron found in this study were the same order as those reported by Gorinstein et al. [6], but lower than those reported by Farran et al. [20]. Manganese and iron are essential nutrients for humans. An adequate supply of iron is very important for females between 14 to 50 years old and for babies in the first months of life. Strontium, aluminium and titanium are not essential for humans, but it is important to monitor their levels in foods, since excess could cause health problems. Barium, zinc, copper and nickel concentrations were around 1 mg kg^{-1} in the kaki fruits analysed. Lithium was determined between 0.005 and 1 mg kg^{-1}. Copper and zinc content were, in general, higher than levels found by Gorisntein et al. [6]. In all cases, the content of lead and cadmium were lower than the LOD values obtained by ICP-MS, using the proposed method, at 2 and 7 µg kg^{-1}, respectively. Therefore, lead and cadmium content do not exceed the maximum levels permitted in fruits (0.10 and 0.05 mg kg^{-1} respectively) according to the European Commission [23].

The contribution of calcium, copper, iron, potassium, magnesium, sodium and zinc to the recommended daily intake provided by one piece of kaki fruit (200-400 g fresh weight) are shown in Table 6.4. The percentage of RDA was calculated as milligrams of essential elements provided by each sample divided by the recommended values of RDA [5, 24] and multiplied by 100. The contribution of calcium, iron and magnesium is between 1 and 15 % of the RDA. The percentage of copper and potassium is 5-30% whereas the contribution of sodium and zinc is negligible (1 and 4 %, respectively).

We performed a one-way ANOVA for each analyte to see whether there were significant differences between mineral contents from different regions. This analysis indicates the differences were statistically significant at a probability level of 95% for all the elements determined, except aluminium. Additionally, Tukey’s test was applied with $p<0.05$ significance level and samples classified in groups with respect to their mean values (see Table 6.3). This information could for the basis of kaki fruit PDO authentication.

Table 6.4. Contribution of one kaki fruit per day to the recommended daily allowance of essential elements

Element	RDA	RDA percentage provided by one piece of kaki fruit						
		PDO Ribera	Alicante	Andalucía	Castellón	Ribera	Extremadura	Valencia
Ca	800	1 - 6	3 - 10	1 - 6	1 - 5	1 - 6	1 - 4	1 - 7
Cu	0.7	1 - 29	7 - 21	2 - 22	1 - 13	5 - 20	1 - 20	3 - 35
Fe	6	0 - 14	0 - 4	0 - 13	0 - 7	0 - 15	3 - 10	0 - 8
K	4700	5 - 23	6 - 22	7 - 26	7 - 20	6 - 20	9 - 20	6 - 25
Mg	350	3 - 12	4 - 11	4 - 14	4 - 10	4 - 13	4 - 10	4 - 15
Na	2400	0 - 1	0 - 0.3	0 - 0.3	0 - 0.2	0 - 0.4	0 - 0.05	0 - 0.2
Zn	9.4	0 - 4	1 - 2	0 - 3	0 - 1	0 - 3	0 - 1	0 - 3

NOTE: RDA values in mg, obtained from FAO/WHO, 2002 [24] and Food and Nutrition Board, 2004 [5].

6.4. Conclusions

The mineral contents indicated in the present study were submitted to quality control using certified referential materials, reagent blanks and standards controls in each batch samples. Data obtained in this study, demonstrate the nutritional quality of kaki fruits produced in Spain, and their contribution to daily intake of essential mineral elements as well as the potential intake of low levels of toxic elements. It can be concluded that the intake of calcium, iron and magnesium are adequate compared with the RDA. Kaki fruits provide high levels of potassium and copper, and very low levels of zinc and sodium. Kaki fruits could be a healthy option for people with heart diseases and hypertension as well as the winder population. Also, our data suggests the mineral profile of kaki fruits could be used for the authentication of fruit covered by PDO.

Acknowledgements

Authors acknowledge the financial support of the Generalitat Valenciana (project PROMETEO 2010-055) and the Ministerio de Economia y Competitividad-Feder (project CTQ2011-25743 and project CTQ2012-38635). Also acknowledge for “Coagri (Alginet)”, “Agrícola de Catadau”, “Coop. Agrícola del Marquesat (Llombai)”, “Canso (L’Alcúdia)”, “Coop. Agrícola San Felipe Benicio (Benimodo)”, “Copal

(Algemesí)", "Coop. Carlet", "Coalfa (Alfarp)", "Coagal (Almussafes)", "Alzicoop (Alzira)", "Cohoca (Benifaió)", "Frutas Teresí Alegre (Alzira)", "Hnos Gimenez (Alzira)", "Cofrudeca (Belgida)", "Coop. Agrícola Cristo de la Salud (Navarrés)", "Coop. San Isidro Labrador (Segorb)", "Vale Marc (Pego)", "Naturcrex (Cáceres)", "Ecotiendadelavera (Cáceres)", "Grupo Medina (Huelva)", "Agrícola Antoluz (Huelva)" and "Supra Cañamas (Sevilla)" to provide kaki fruit samples. AMM acknowledge the 'Atracció de Talent' grant provided by the University of Valencia'.

References

[1] CRDO (2011). Consejo Regulador de la Denominación de Origen "Kaki Ribera del Xúquer". Available from: http://www.kakifruit.com

[2] Orihuel-Iranzo, B., Miranda, M., Zacarías, L., Lafuente, M. T. (2010). Temperature and ultra low oxygen effects and involment of ethylene in chilling injury of "Rojo Brillante" Persimmon fruit. *Food Science and Technology International*, 16, 159-167.

[3] Plaza, L., Colina, C., de Ancos, B., Sanche-Moreno, C., Cano, M. P. (2012). Influence of ripening and astringency on carotenoid content of high-pressure treated persimmon fruit (*Diospyros kaki L.*). *Food Chemistry*, 130, 591-597.

[4] Goldhaber, S. (2003). Trace element risk assessment: Essentiality vs. toxicity. *Regulatory Toxicology and Pharmacology, 38*, 232-242.

[5] Food and Nutrition Board, Institute of Medicine, National Academies Press. (2004). Dietary Reference Intake (DRIs): Recommended Intakes for Individuals Elements.

[6] Gorinstein, S., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., Zemser, M., Weisz, M., Trakhtenberg, S., Martín-Belloso, O. (2001). Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples. *Journal of Agricultural and Food Chemistry, 49*, 952-957.

[7] Radwan, M. A., Salama, A. K. (2006). Market basket survey for some heavy metals in egyptian fruits and vegetables. *Food and Chemical Toxicology, 44*, 1273-1278.

[8] Moreno-Rojas, R., Sanchez-Segarra, P. J., Camara-Martos, F., Amaro-Lopez, M. A. (2010). Multivariate analysis techniques as tools for categorization of southern spanish cheeses: Nutritional composition and mineral content. *European Food Research and Technology, 231*, 841-851.

[9] Terrab, A., Hernanz, D., Heredia, F. (2004). Inductively coupled plasma optical emission spectrometric determination of minerals in thyme honeys and their contribution to geographical discrimination. *Journal of Agricultural and Food Chemistry, 52*, 3441-3445.

[10] Wall, M. (2006). Ascorbic acid, vitamin A, and mineral composition of banana (musa sp.) and papaya (carica papaya) cultivars grown in hawaii. *Journal of Food Composition and Analysis, 19*, 434-445.

[11] Zeiner, M., Steffan, I., Cindric, I. (2005). Determination of trace elements in olive oil by ICP-AES and ETA-AAS: A pilot study on the geographical characterization. *Microchemical Journal, 81*, 171-176.

[12] Ariyama, K., Aoyama, Y., Mochizuki, A., Homura, Y., Kadokura, M., Yasui, A. (2007). Determination of the geographic origin of onions between three main production areas in japan and other countries by mineral composition. *Journal of Agricultural and Food Chemistry, 55*, 347-354.

[13] Boeting, K., Aguilera de Benzo, Z., Luisa Cervera, M., de la Guardia, M. (2010). Authentication of the protected designation of origin horchata de valencia through the chemometric treatment of mineral content. *Analytical Methods, 2*, 1723-1728.

[14] Brescia, M., Monfreda, M., Buccolieri, A., Carrino, C. (2005). Characterisation of the geographical origin of buffalo milk and mozzarella cheese by means of analytical and spectroscopic determinations. *Food Chemistry, 89*, 139-147.

[15] Gonzalvez, A., Llorens, A., Cervera, M. L., Armenta, S., de la Guardia, M. (2009). Elemental fingerprint of wines from the protected designation of origin Valencia. *Food Chemistry, 112*, 26-34.

[16] Matos-Reyes, M. N., Simonot, J., Lopez-Salazar, O., Cervera, M. L., de la Guardia, M. (2013). *Food Chemistry, 141*, 2191-2197.

[17] Mir-Marqués, A., Cervera, M. L., de la Guardia, M. (2012). A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus. *Journal of Food Composition and Analysis*, 27, 160-168.

[18] Hardisson, A., Rubio, C., Baez, A., Martin, M., Alvarez, R., Diaz, E. (2001). Mineral composition of the banana (*Musa acuminata*) from the island of Tenerife. *Food Chemistry, 73,* 153-161.

[19] Paul, M., Rodríguez, E., Darias, J., Díaz, C. (2002). Statistical differentiation of bananas according to their mineral composition. *Journal of Agricultural and Food Chemistry*, 50, 6130-6135.

[20] Farran, A., Zamora, R., Cervera, P. (2004). Tabla de composición de alimentos del CESNID, from: Base de Datos Española de Composición de Alimentos www.bedca.net

[21] Souza, E. L., Argenta, L. C., Rombaldi, C. V., Souza, A. L. K. & Almarante, C. V. T. (2012). Diagnosis of fruit quality and mineral contents of 'Fuyu' persimmon produced in southern Brazil. *Acta Horticulturae*, 934, 775-781.

[22] WHO *Guideline: Sodium intake for adults and children* (2012). World Health Organization (WHO), Geneva.

[23] EC (2006). Commission of the European Communities. Commission Regulation (EC) No. 1881/2006 Regulation of setting maximum levels for certain contaminants in foodstuffs. Official Journal European Union L364-5/L364-24.

[24] FAO/WHO Human Vitamin and Mineral Requirements (2002). Report of a joint FAO/WHO expert consultation Bangkok, Thailand.

Capítulo 7

Autentificación de la denominación de origen “Kaki Ribera del Xúquer” a partir de su perfil mineral

Authentication of the protected designation of origin “Kaki Ribera del Xúquer” from its mineral profile

Alba Mir-Marqués, M. Luisa Cervera*, Miguel de la Guardia

Department of Analytical Chemistry, University of Valencia, Jerónimo Muñoz Building, 50th Dr. Moliner St., 46100 Burjassot (Valencia), Spain.

Received 24 November 2015,

Accepted 29 September 2016,

Ciencia 24 (2016) 153-164

Resumen

Muestras de caqui con denominación de origen protegida (DOP) “Kaki Ribera del Xúquer” fueron discriminadas de otras con distinta procedencia española: de ciudades cerca de Valencia, Alicante, Andalucía, Castellón y la Ribera producidas fuera de la DOP. Se usaron herramientas de análisis multivariante como el análisis de componentes principales (PCA), análisis jerárquico de clústers (HCA), análisis discriminante lineal (LDA) y árboles de clasificación y regresión (CARTs) para evaluar la correcta clasificación de los seis grupos de origen. Para el análisis quimiométrico se utilizaron 14 variables (contenido mineral en mg kg^{-1}, determinado por ICP-OES e ICP-MS). HCA y CARTS no permiten la total autentificación de caquis; CART proporciona una separación del 79 % para las muestras de DOP, mientras que PCA sólo permite discriminar bien las muestras a partir de su año de cosecha. LDA proporciona unos resultados bastante satisfactorios ya que se obtiene una clasificación correcta del 100 % para el set de calibración y de 67 % para el set de validación.

Abstract

Protected designation of origin (PDO) “Kaki Ribera del Xúquer” samples were discriminated from those produced in other five origins concerning: towns surrounding Valencia, Alicante, Andalucía, Castellón and also Ribera, produced outside the PDO frame. Multivariate analysis tools; such as principal components analysis (PCA), hierarchical cluster analysis (HCA), linear discriminate analysis (LDA) and classification and regression trees (CARTs) were evaluated in order to achieve a correct sample classification of the six sample origin groups considered. Chemometric analysis was conducted with 14 variables (content of different elements in mg kg^{-1}, determined by ICP-OES and ICP-MS). HCA and CART cannot authenticate the origin of kaki fruits at 100 %; CART provided a separation of 79 % for the PDO samples. PCA only discriminated well the samples as a function of their different harvest but LDA provided quite satisfactory results obtaining a classification rate of 100 % for samples of the calibration set and 67 % for those included in the validation set.

7.1. Introduction

Kaki is a seasonal fruit whose origin is China and Japan, and it was brought to Spain during the 19th century. One of the main geographical locations of kaki fruit production is the river Xúquer region (Valencia, Spain). The local variety “Rojo Brillante” has the protected designation of origin (PDO) “Kaki Ribera del Xúquer” from the European Union.

Authenticity is a quality criterion for food which importance increases within Europe as a result of legislative protection of regional foods, based on the approval of different labels [1,2]. The PDO label is used to describe foodstuffs, with regional identity, that are produced, processed and prepared in a specific geographical area [3,4]. From the economic point of view, food authenticity has great importance, for the sectors involved in food production and also for the consumer. This is a very important factor because the authenticity helps to guarantee the characteristics and quality of food products and to prevent overpayment [5,6].

The use of analytical techniques is the best way to authenticate foods without any doubt in order to determine their geographical origin. There are many papers that try to discriminate foods between PDO areas to those produced outside the PDO, using different analytical methods. The most commonly analytical techniques used are GC-MS [7-11] and HPLC [12-14] for the determination of organic compounds, but organic compounds vary easily due to a lot of factors: fertilization, climatic conditions… So, it is sometimes very difficult to have definitive authentication. The mineral profile of foods is characteristic due to the soil type and the climatic conditions. Therefore, the determination of the mineral profile is a good technique to assure the geographical origin of food samples [5]. The most commonly techniques employed to determine mineral elements in food in order to do their authentication are flame atomic absorption spectrometry FAAS [15,16], inductively coupled plasma optical emission spectrometry ICP-OES [17-20] and inductively coupled plasma mass spectrometry ICP-MS [21,22].

The main purpose of this study is the authentication of kaki fruit samples produced from PDO “Kaki Ribera del Xúquer” using their mineral profile information together with multivariate analysis.

7.2. Materials and methods

7.2.1. Apparatus

An ICP-OES Optima 5300 DV inductively coupled plasma optical emission spectrometer Perkin Elmer (Norwalk, CT, USA) equipped with an auto sampler AS 93-plus, and an ultrasonic nebuliser U6000AT$^+$ Cetac (Nebraska, EEUU) was used for mineral determinations. ICP-MS measurements were carried out with a Perkin-Elmer SCIEX Elan 6100 DRCII apparatus, equipped with a Meinhard nebuliser and an autosampler Perkin-Elmer AS-91 was used for sample introduction.

The apparatus employed for sample pre-treatment was a microwave laboratory system, Ethos SEL from Milestone (Sorisole, Italy), equipped with a thermocouple for automatic temperature control, and an automatic gas leaks detector.

7.2.2. Reagents

Argon C-45 (purity higher than 99.995%) supplied by Carburos Metálicos (Barcelona, Spain), was employed as plasmogen and carrier gas for ICP-OES and ICP-MS.

Stock solutions of a multi-element standard solution (100 mg L^{-1}) containing 26 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Se, Na, Sr, Tl, Ti, V and Zn) dissolved in 5% HNO_3 obtained from Scharlau (Barcelona, Spain), and a multi-element solution containing 20 mg L^{-1} Dy, Er, Eu, Gd, Ho, Lu, Sm, Sc, Tb, Tm, Yb and Y and 100 mg L^{-1} Ce, La, Nd and Pr dissolved in 5% HNO_3, also from Scharlau, were employed to prepare the calibration standards.

For sample digestion, it was employed HNO_3 69 % for trace analysis and H_2O_2 35 % reagent grade, both from Scharlau, and nanopure water with a minimum resistivity of 18.0 MΩ cm, obtained from a Milli-Q Millipore system (Bedford, MA, USA).

A 1000 mg L^{-1} ruthenium standard solution for ICP and a 1000 mg L^{-1} rhodium standard solution for ICP both from Scharlau, were used as internal standards.

7.2.3. Samples

162 kaki fruit samples from different Spanish agricultural cooperatives were collected during 2010 and 2011 harvests. Each sample corresponds to a mixture of 2-4 pieces of kaki fruit obtained from the same producer but from different trees. 114 samples were produced under the PDO "Kaki Ribera del Xúquer", four samples from Alicante, 12 from Andalucía, ten from Castellón, nine from Ribera (outside the PDO) and 13 from Valencia (see Figure 7.1). The samples were taken from October to December, for each harvest. The edible parts were lyophilised, pulverised and stored in polyethylene bottles until analysis.

Figure 7.1. Map of Spain indicating the different regions where the kaki fruit samples employed through this study were produced

The microwave-assisted treatment was adapted from that provided by Mir-Marqués et al., 2012 [23] according to the microwave laboratory system manufacturer recommendations and our experience.

Freeze-dried samples (0.5 g) were digested with 8 mL of concentrated nitric acid and 2 mL of H_2O_2 into the Teflon vessel inside the microwave oven. The digestion step of 15 min at 180 °C (700 W) was employed. The resulting solution was quantitatively transferred to a plastic flask and made up to 20 mL with ultrapure water. All samples were analysed in triplicate to eliminate any specific error, to verify the homogeneity of samples and to evaluate the repeatability of the procedure. Blanks and certified material (NIST 1573a Tomato Leaves from the National Institute of Standards and Technology (Gaithensburg, MD, USA)) were prepared in the same way than the samples.

7.2.4. Analytical procedures

The calibration range for all the 42 elements evaluated was established from 0.05 to 2 mg L^{-1} and ruthenium (1 mg L^{-1}) was used as internal standard and added to all samples, blanks and standards for ICP-OES analysis. Digested samples were measured with a final dilution of 40 mL. For ICP-MS analysis, the calibration range for all the 42 elements evaluated was from 2 to 400 µg L^{-1} and rhodium (10 µg L^{-1}) was used as internal standard and added to samples, blanks and standards. In this case, digested samples were measured with a final dilution of 100 mL. Additional, control standards were measured for every series of 10 independent sample measurements and a reference material every series.

The average measurement of blanks was subtracted from the analytical signals of digested samples after interpolation on the calibration graphs. Results were expressed as a basis of dry weight.

7.2.5. Statistical analysis

Both, univariate and simple multivariate methodologies were employed through this study for the authentication of the origin of samples from their mineral profile.

Univariate analysis

Analysis of variance was applied to all variables studied considering only those elements present in the samples over the detection limit of the method employed. In cases where the concentration was below the detection limit, this value was taken as data for the chemometric studies. The mean values obtained for samples with different origin were compared by using one-way ANOVA and two-way ANOVA including the production year.

Multivariate analysis

Statistical multivariate analysis was performed by using the software XLSTAT2008 from Addinsoft (Barcelona, Spain). Principal component analysis (PCA), hierarchical cluster analysis (HCA), linear discriminate analysis (LDA) and classification and regression trees (CART) were performed on the mineral elements concentration of the studied samples.

Although 42 elements were evaluated in the 162 kaki fruit samples analysed, calculations were performed using only 14 variables considering the content of Al, Ba, Ca, Cu, Fe, Li, K, Mg, Mn, Na, Ni, Sr, Ti and Zn, in mg kg^{-1}, determined by ICP-OES and ICP-MS. The concentrations of other elements were lower than the detection limit (LOD) in almost samples analysed in this study and thus they were not used in data treatment.

7.3. Results and discussion

7.3.1. Mineral content of kaki fruit

Table 7.1 shows the maximum, minimum, mean and median concentration of elements (Al, Ba, Ca, Cu, Fe, Li, K, Mg, Mn, Na, Ni, Sr, Ti and Zn) present in all samples at concentration levels higher than the LOD as a function of their production region (Alicante, Andalucía, Castellón, PDO Ribera del Xúquer, Ribera outside PDO, Valencia) expressed in mg kg^{-1} of dry weight.

7.3.2. Statistical analysis

7.3.2.1. Univariate analysis

ANOVA was used to compare elemental profiles of food in different origin [6]. We performed a one-way ANOVA for each one of the 14 analytes present in all samples over the LOD of the employed method: Al, Ba, Ca, Cu, Fe, Li, K, Mg, Mn, Na, Ni, Sr, Ti and Zn, to find whether there were significant differences between samples produced in different regions. ANOVA analysis shows that the differences were statistically significant at a probability level of 95 % ($p<0.05$) since F calculated is greater than F critic (F critic 2.16) for all elements except for aluminium. So, this information evidences that the mineral profile could be important as a basis of the kaki fruit PDO authentication. We also performed a two-way ANOVA including the production year, in this case samples from Alicante and Castellon had not considered because we did not harvested samples in 2010 (see Table 7.2). As can be seen in Table 7.2 from the two-way ANOVA there were differences between harvest areas for all the considered elements. On the other hand, Cu, Li, K, Mg, Mn, Na, Ni, Sr, Ti and Zn did not present differences among production year.

Table 7.1. Mineral composition of kaki fruit samples from different Spain production areas

		Al	**Ba**	**Ca**	**Cu**	**Fe**	**K**	**Li**	**Mg**	**Mn**	**Na**	**Ni**	**Sr**	**Ti**	**Zn**
PDO “Kaki Ribera del Xúquer” n= 114	**min**	< 0.12	0.4	210	0.1	< 0.3	6800	< 0.005	240	0.4	< 1.2	< 0.10	0.3	< 0.04	0.4
	max	8.6	4.8	540	2.2	11.0	12600	1.0	500	14.2	200	2.3	9.8	3.6	4.5
	mean	3 ± 2	1.7 ± 0.8	380 ± 70	1.1 ± 0.5	3 ± 2	9600 ± 1400	0.2 ± 0.2	400 ± 50	5 ± 3	50 ± 30	0.7 ± 0.4	4.6 ± 1.7	2.3 ± 0.7	1.7 ± 0.6
	median	2.4	1.6	380	1.0	2.4	9700	0.09	400	4.4	40	0.7	4.5	2.5	1.6
Alicante n= 4	**min**	1.2	0.14	500	1.32	0.57	9400	< 0.005	420	4.3	40	0.85	1.8	2.6	1.70
	max	2.7	7.8	1000	2.10	3.68	11600	0.06036	540	11.1	110	2.55	3.0	3.3	2.77
	mean	1.9 ± 0.8	3 ± 3	800 ± 200	1.6 ± 0.3	2.0 ± 1.6	10300 ± 900	0.02 ± 0.03	480 ± 50	8 ± 3	70 ± 30	1.7 ± 0.7	2.3 ± 0.5	3.0 ± 0.3	2.4 ± 0.5
	median	1.9	2.4	800	1.49	1.87	10100	0	480	7.5	70	1.63	2.3	3.0	2.50
Andalucía n= 12	**min**	0.5	< 0.07	230	0.3	0.6	9200	< 0.005	380	2	30	0.3	0.2	1.4	1.1
	max	9.3	4.0	550	2.0	8.7	15517	0.56	610	35	90	2.5	5.1	3.7	3.0
	mean	3 ± 3	2.2 ± 1.2	400 ± 80	1.1 ± 0.5	4 ± 2	11200 ± 1800	0.2 ± 0.2	440 ± 70	9 ± 10	60 ± 20	0.9 ± 0.6	2.6 ± 1.6	2.6 ± 0.6	1.8 ± 0.5
	median	2.6	2.3	400	1.3	4.2	10600	0.09	410	6	50	0.8	3.0	2.6	1.7
Castellón n= 10	**min**	< 0.12	0.46	240	0.19	0.33	6500	< 0.005	330	3.1	10	< 0.10	2.0	1.0	< 0.14
	max	2.60	2.51	430	1.04	4.05	11200	0.194	420	11.6	40	0.75	4.1	3.2	1.44
	mean	1.1 ± 1.0	1.5 ± 0.7	350 ± 60	0.7 ± 0.3	2.2 ± 1.4	9000 ± 1300	0.07 ± 0.08	360 ± 30	6 ± 3	20 ± 10	0.4 ± 0.3	3.1 ± 0.7	2.3 ± 0.6	0.8 ± 0.5
	median	0.69	1.51	340	0.72	2.10	8900	0.041	360	5.0	20	0.39	3.2	2.3	0.95
Ribera outside PDO n= 9	**min**	< 0.12	< 0.07	210	0.7	< 0.3	7600	< 0.005	330	3	20	< 0.10	1.2	1.5	1.0
	max	10	3	520	2.1	13	11100	1.0	500	10	130	1.2	7	3	3
	mean	3 ± 4	2.0 ± 0.8	410 ± 90	1.2 ± 0.4	4 ± 4	9000 ± 1100	0.4 ± 0.3	410 ± 60	7 ± 2	50 ± 30	0.8 ± 0.3	4.0 ± 1.6	3.0 ± 0.7	2.0 ± 0.5
	median	2	2	400	1.2	3	9100	0.43	400	7	50	0.8	3	3	1.4
Valencia n= 13	**min**	< 0.12	0.5	270	0.6	< 0.3	8000	< 0.005	340	0.7	3	0.4	1.0	1.7	0.7
	max	14.3	5.1	660	3.3	6.3	12300	0.77	630	22	60	5.1	9.3	4.2	2.9
	mean	3 ± 4	2.0 ± 1.2	450 ± 130	1.4 ± 0.7	2.0 ± 1.8	10600 ± 1400	0.2 ± 0.2	440 ± 90	8 ± 7	22 ± 20	1.3 ± 1.2	3 ± 2	2.5 ± 0.7	1.9 ± 0.6
	median	2.1	1.7	400	1.3	1.7	11100	0.03	400	5.1	20	1.0	2.4	2.4	1.8

NOTE: concentration in mg kg^{-1} dry weight. n= number of samples

Table 7.2. Two-way ANOVA evaluation of the comparability of the mineral profile of kaki samples produced inside and outside the PDO and the production year

Element	F_A	F_B	Element	F_A	F_B
Al	47.16	9.78	**Mg**	14.47	0.86
Ba	55.2	11.4	**Mn**	19.02	2.74
Ca	28.01	4.98	**Na**	15.26	1.75
Cu	11.94	0.86	**Ni**	5.35	0.67
Fe	26.38	4.25	**Sr**	16.86	2.21
Li	19.04	2.47	**Ti**	10.81	0.53
K	10.60	0.42	**Zn**	47.16	9.78

NOTE: F_A is calculated Fisher's F to different origin, F value critic is 2.14. F_B is calculated Fisher's F to different production year, F value critic is 2.75.

7.3.2.2. Multivariate analysis

In this study, the concentration of 14 elements was used to evaluate the fruits coming from PDO "Kaki Ribera del Xúquer" produced in a restricted area near Valencia and to discriminate between different regions of kaki fruit production close to the zone covered by the PDO label. In this sense, mineral content of the edible part of the samples was used as chemical descriptor for building the statistical methods, in order to establish differences between PDO kaki fruit and the other ones. As it has been indicated, PCA, HCA, LDA and CART models were employed for chemometric treatment. Results obtained from this chemometric models were compared between them, in order to look for the best discrimination processing tool.

Principal component analysis

Principal component analysis is an unsupervised technique that allows visualising the information of the data set in a few principal components retaining the maximum possible variability within that set. From the loadings of original variables in the two first considered principal components (see Table 7.3). Principal component 1 (PC1) represents 24.80 % of the total variance. Cu, Ni, Mg and Sr were identified as the dominant variables in the PC1 while Fe and Na show the lowest values. PC2 explains 15.54 % of the total variance. Revising loadings, K, Mg and Ba result as the most dominant variables in PC2, while Li and Mn show the lowest dominant values. PC1 and PC2 account for 40.34 % of the total variance, and it allows differentiating two groups by harvest years, 2010 and 2011 (see Figure 7.2). However, it is really difficult to discriminate between samples produced in different areas during the two evaluated years, neither within each of the considered harvest years. The values of total variance explained are very similar than to values reported in bananas from Tenerife and Ecuador [24] and different banana flours [25].

Table 7.3. Loadings of the variables for the two first Principal Components established from the mineral profile of kaki samples produced in different parts of Spain during the period 2010-2011

Variable	PC1	PC2	Variable	PC1	PC2
Al	-0.131	-0.312	**Mg**	0.620	0.579
Ba	0.543	-0.531	**Mn**	0.277	-0.011
Ca	0.590	-0.128	**Na**	0.095	0.444
Cu	0.731	-0.288	**Ni**	0.692	-0.104
Fe	-0.020	-0.284	**Sr**	0.609	-0.342
K	0.284	0.856	**Ti**	0.480	0.455
Li	0.390	0.061	**Zn**	0.703	-0.108

Hierarchical cluster analysis

Hierarchical cluster analysis is an unsupervised technique that uses the information obtained from the measured mineral elements, to determine if there are cluster association between investigated samples with the same origin. Based on the previous results obtained by PCA analysis, HCA was applied to the whole sample population and was separately used in order to evaluate samples produced in 2010 and 2011. As it can be seen in Figure 7.3, seven clusters were obtained from the 162 samples considered. However, clusters A1 and A2 concerns both the Alicante samples and because of their vicinity they were cumulated in a single cluster A. Cluster B contains two samples from PDO, one sample from Ribera and another sample from Valencia. Cluster C includes samples of four different origins (PDO (8), Castellón (1), Ribera (4) and Valencia (2)). Cluster D contains a single sample from PDO. Cluster E contains the main part of samples produced from the PDO (65 samples) which are accompanied by 25 samples from different origins (Alicante (1), Andalucía (7), Castellón (7), Ribera (2) and Valencia (8), thus providing also a bad classification of kaki fruit from their origin. Cluster F contains 49 samples (PDO (36), Andalucía (4), Castellón (2), Ribera (2) and Valencia (5)). So, it can be concluded that the mineral composition of kaki fruits produced in different parts of Spain during the period 2010-2011 cannot be described correctly by HCA in order to authenticate their origin.

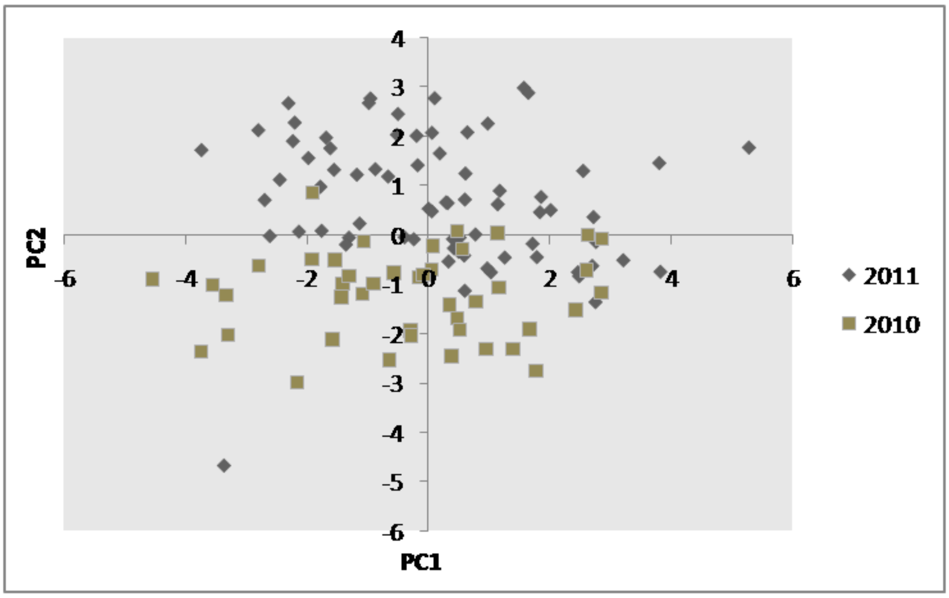

Figure 7.2. Principal components analysis (PC1 vs. PC2) of the mineral profile of kaki samples produced in different parts of Spain

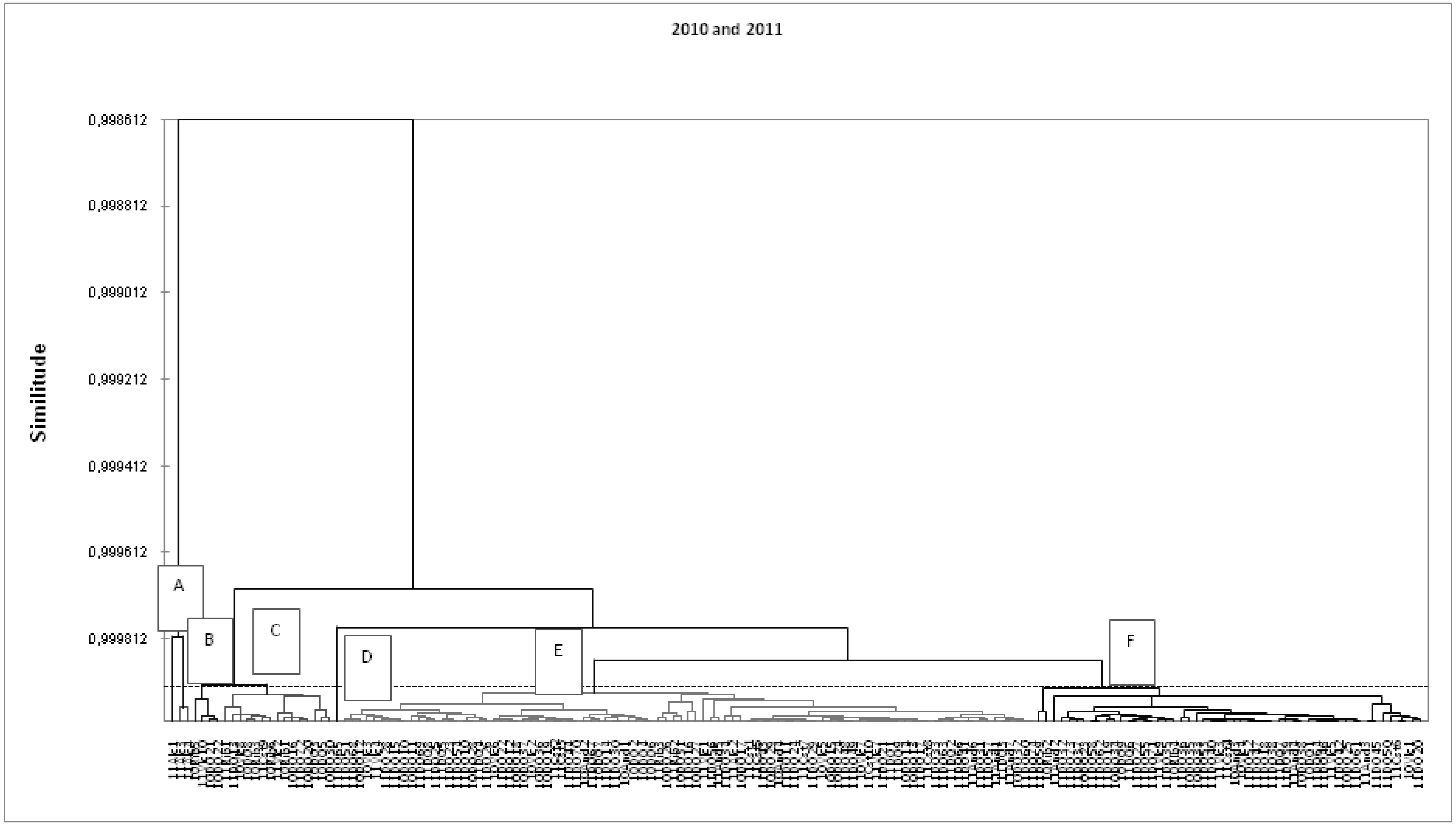

Figure 7.3. Cluster classification of kaki fruit samples obtained from their mineral profile

The HCA of the mineral profile of samples corresponding to 2010 and 2011 harvest provided five and seven clusters respectively. However, the distribution of the 56 samples collected in 2010 between the five clusters was arbitrary, and as an example 38 samples of the PDO were classified in all clusters. Similar results were found applying HCA to samples collected in 2011. Only one of the clusters correctly classified Alicante samples. The remaining clusters contained samples of the other origins. Therefore, it can be concluded that HCA is an unsuitable tool to do the authentication of kaki fruits from their mineral profile. Compared with other authors, Foster et al., 2002 [24] differentiated between Tenerife and Ecuador bananas by HCA, this may be due to the large distance between the two origins, because they belong to different continents with very different characteristics. According to the investigated kaki fruits, all samples belong to the same country and all regions analyzed show very similar characteristics in both climatic and soil conditions.

Linear discriminate analysis

Linear discriminate analysis is a supervised technique for classifying a set of observations into predefined classes. LDA maximises the variance between categories, minimises the variance within categories, and suppresses the correlation among variables.

LDA was performed by separating the samples in 6 classes: Alicante, Andalucía, Castellón, PDO Kaki Ribera del Xúquer, Ribera (outside the PDO) and Valencia. The kaki samples were divided in two groups: a training set consisting in 108 samples (2 from Alicante, 8 from Andalucía, 6 from Castellón, 78 from PDO, 6 from Ribera outside PDO and 8 from Valencia) and another set for validation containing 54 samples (2 from Alicante, 4 from Andalucía, 4 from Castellón, 36 from PDO, 3 from Ribera outside the PDO and 5 from Valencia). The division into two groups was made randomly. Results were satisfactory, obtaining a classification rate for calibration of 100 % and 67 % for validation (see Table 7.4) for the validation set.

In Table 7.4 it is also included the confusion matrix for the validation samples. For the 2010 harvest, a total of 56 samples were divided into 2 sets; one for calibration, containing 36 samples (3 from Andalucía, 23 from PDO, 7 from Ribera and 3 from Valencia), and another set for validation, containing 20 samples (1 from Andalucía, 16 from PDO, 3 from Ribera and 0 from Valencia). In this case, a correct classification rate of 85 % was obtained for validation.

In the case of the 2011 harvest, a total of 106 samples were divided into a set of calibration, containing 66 samples (2 from Alicante, 4 from Andalucía, 5 from Castellón, 47 from PDO, 2 from Ribera and 6 from Valencia), and a set of validation with 40 samples (2 from Alicante, 3 from Andalucía, 5 from Castellón, 25 from PDO, one from Ribera and 4 from Valencia). As it can be seen in Table 3 a correct classification rate of 68 % was obtained.

So, as it can be seen, the capability of LDA for a correct classification of kaki fruit samples from their mineral profile is quite good and does not depends dramatically on the harvest. So, it is possible authentication of Kaki Ribera del Xúquer at a 69 % correct level; being those samples produced in the same area, but outside the protected label, the most critical group to obtain a correct differentiation. Other authors obtained a correct classification of 100 % in bananas and cherries [20,25] but they do not explain if it corresponds to classification set or validation set, in this case we could think that they have not performed external validation sets, so their data are comparable with ours.

Classification analysis regression trees

CART technique was applied to the samples collected during the two harvests (2010 and 2011) in order to check the capability of classification of determined concentration of mineral elements in kaki fruits.

This technique divides the data into sub-groups (nodes) that are more homogeneous with respect to the response of the initial data set. When performing the CART data with both harvests, we obtain a separation of 79 % for the PDO, with 4 nodes (see Figure 7.4), been characterised these samples by low concentrations for calcium and nickel and high concentrations for sodium and strontium. This separation is not pure, and does not let us to separate completely the PDO samples from other ones produced in the Ribera (7), Valencia (2), Andalucía (5) and Castellón (2).

Table 7.4. LDA confusion matrix of validation set for the classification of kaki fruit samples from their mineral profile

	Assigned group **Original group**	**Alic**	**And**	**Cast**	**PDO**	**Rib**	**Val**	**Total**	**% Correct**
All samples (harvest 2010 and 2011)	Alic	2	0	0	0	0	0	2	100%
	And	0	3	0	1	0	0	4	75%
	Cast	0	0	2	1	0	1	4	50%
	PDO	0	1	2	25	5	3	36	**69%**
	Rib	0	0	0	2	1	0	3	33%
	Val	0	0	1	1	0	3	5	60%
	Total	2	4	5	30	6	7	54	67%

	Assigned group **Original group**	**And**	**PDO**	**Rib**	**Val**	**Total**	**% Correct**
Samples of the 2010 harvest	And	1	0	0	0	1	100%
	PDO	1	14	1	0	16	**87%**
	Rib	0	1	2	0	3	67%
	Val	0	0	0	0	0	0%
	Total	2	15	3	0	20	85%

	Assigned group **Original group**	**Alic**	**And**	**Cast**	**PDO**	**Rib**	**Val**	**Total**	**% Correct**
Samples of the 2011 harvest	Alic	2	0	0	0	0	0	2	100%
	And	0	2	0	1	0	0	3	67%
	Cast	0	0	3	1	0	1	5	60%
	PDO	0	2	5	17	1	0	25	**65%**
	Rib	0	0	0	0	1	0	1	100%
	Val	0	0	0	1	0	3	4	75%
	Total	2	4	8	20	2	4	40	68%

On the other hand, CART analysis of samples collected in the 2010 harvest provided a correct classification of 84 % of samples produced inside the PDO with two nodes corresponding to low concentrations of Ca and Li. However once again, the separation was not complete. It was the same for samples produced in 2011, for which the separation was 85 % with 5 nodes corresponding to low concentrations of Ca and Ni, and high concentrations of Zn and Sr.

So, it can be concluded that CART provided coherent results with those suggested by LDA.

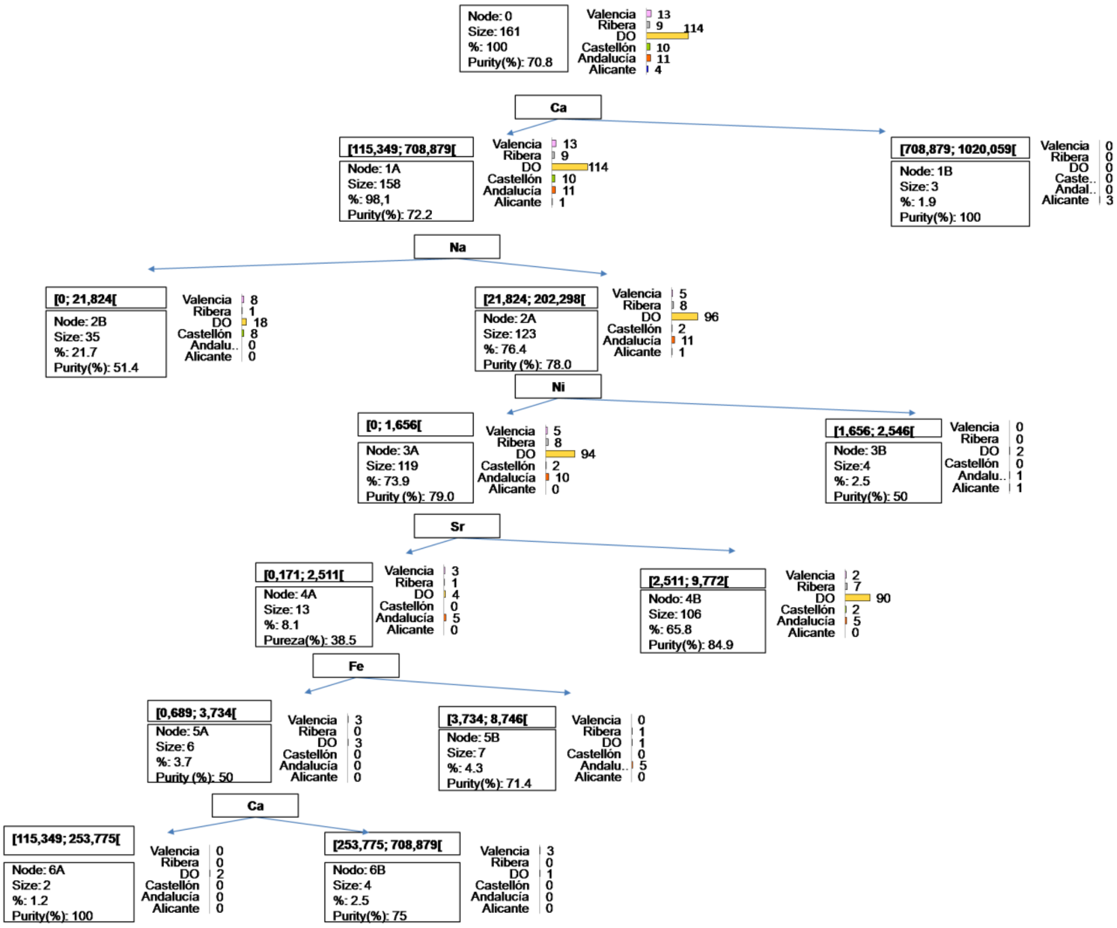

Figure 7.4. Classification analysis regression trees for kaki fruit samples collected in 2010 and 2011 harvests

7.4. Conclusions

Mineral profile of kaki fruit samples from different origin produced in Spain cannot be clearly discriminate by their production area. Because of that, kaki fruits produced inside the PDO “Kaki Ribera del Xúquer” cannot be totally authenticate, being obtained uncorrected classification of other samples produced in Alicante, Andalucía, Castellón, Ribera outside PDO and Valencia by using HCA, PCA and CART chemometric treatment. LDA provided satisfactory results to the authentication of PDO "Kaki Ribera del Xúquer", obtaining a classification rate of 100 % for samples of the calibration set and 67 % for validation set. Although, additional data must be required for a complete description of the fruit PDO and new efforts are in due course to use improved chemometric tools for data treatment.

Acknowledgements

Authors acknowledge the financial support the Ministerio de Economia y Competitividad-Feder (project **CTQ2012-38635**) and Generalitat Valenciana (project **PROMETEO II 2014-077**). "Coagri (Alginet)", "Agrícola de Catadau", "Coop. Agrícola del Marquesat (Llombai)", "Canso (L'Alcúdia)", "Coop. Agrícola San Felipe Benicio (Benimodo)", "Copal (Algemesí)", "Coop. Carlet", "Coalfa (Alfarp)", "Coagal (Almussafes)", "Alzicoop (Alzira)", "Cohoca (Benifaió)", "Frutas Teresí Alegre (Alzira)", "Hnos Gimenez (Alzira)", "Cofrudeca (Belgida)", "Coop. Agrícola Cristo de la Salud (Navarrés)", "Coop. San Isidro Labrador (Segorb)", "Vale Marc (Pego)", "Naturcrex (Cáceres)", "Ecotiendadelavera (Cáceres)", "Grupo Medina (Huelva)", "Agrícola Antoluz (Huelva)" and "Supra Cañamas (Sevilla)" to provide kaki fruit samples. Alba Mir-Marqués acknowledges a grant "**Atracció de Talent**" provided by the University of Valencia.

References

1. EU Regulation. European Union Regulation (EEU) 2081/1992
2. EU Regulation. European Union Regulation (EEU) 1151/2012
3. KELLY S., HEATON K., HOOGEWERFF J. ***Trends Food Sci Tech*** 16: 555-567. 2005.
4. DE LA GUARDIA M., GONZÁLVEZ A. (Eds). Food ***Protected Designation of Origin: methodologies and applications***. Elsevier. Amsterdam (Netherland). 2013.

5. GONZÁLVEZ A., ARMENTA S., DE LA GUARDIA M. ***Trac-trend Anal Chem*** 28: 1295-1311. 2009.

6. DRIVELOS S.A., GEORGIOU C.A. ***Trac-trend Anal Chem*** 40: 38-51. 2012.

7. BIANCHI F., CARERI M., CHIAVARO E., MUSCI M., VITTADINI E. ***Food Chem*** 110: 787-793. 2008.

8. AQUILANTI L., SANTARELLI S., BABINI V., OSIMANI A., CLEMENTI F. ***Int Dairy J*** 29: 42-52. 2013.

9. INNOCENTE N., MUNARI M., BIASUTTI M. ***J Dairy Sci*** 96: 26-32. 2013.

10. KARALI F., GEORGALA A., MASSOURAS T., KAMINARIDES S. (2013). ***J Sci Food Agr*** 93: 1845-1851. 2013.

11. MONTERO-PRADO P., BENTAYEB K., NERIN C. ***Food Chem*** 138: 724-731. 2013.

12. GALAUP P., FLAMIN C., CARLET E., DUFOSSÉ L. ***Food Res Int*** 28: 855-860. 2005.

13. BARREIRA J.C.M., CASAL S., FERREIRA I.C.F.R., PERES A.M., PEREIRA J.A., OLIVEIRA M.B.P.P. ***J Agr Food Chem*** 60: 9697-9704. (2012).

14. RUSSO R., SEVERINO V., MENDEZ A., LLIBERIA J., PARENTE A., CHAMBERY A. ***J Mass Spectrom*** 47: 1407-1414. 2012.

15. ARIYAMA K., HORITA H., YASUI A. ***Anal Sci*** 20: 871-877. 2004.

16. MORENO-ROJAS R., SANCHEZ-SEGARRA P.J., CAMARA-MARTOS F., AMARO-LOPEZ M.A. ***Eur Food Res Tech*** 231: 841-851. 2010.

17. ANDERSON K., SMITH B. ***J Agr Food Chem*** 53: 410-418. 2005.

18. ARIYAMA K., AOYAMA Y., MOCHIZUKI A., HOMURA Y., KADOKURA M., YASUI A. ***J Agr Food Chem*** 55: 347-354. 2007.

19. GONZÁLVEZ A., ARMENTA S., DE LA GUARDIA M. ***Food Chem*** 126: 1254-1260. 2011.

20. MATOS-REYES M.N., SIMONOT J., LÓPEZ-SALAZAR O., CERVERA M.L., DE LA GUARDIA M. ***Food Chem*** 141: 2191-2197. 2013.

21. DI GIACOMO F., DEL SIGNORE A., GIACCIO M. ***J Agr Food Chem*** 55: 860-866. 2007.

22. COSTAS-RODRIGUEZ M., LAVILLA I., BENDICHO C. ***Anal Chim Acta*** 664: 121-128. 2010.

23. MIR-MARQUÉS A., CERVERA M.L., DE LA GUARDIA M. ***J Food Compos Anal*** 27: 160-168. 2012.
24. FOSTER M., RODRIGUEZ E., MARTIN J., ROMERO C. ***J Agr Food Chem*** 50: 6130-6135. 2002.
25. ALKARKHI A.F.M., BIN RAMLI S., EASA A.M. ***Int J Food Sci Nutr*** 60: 116-125. 2009.

Capítulo 8

Clasificación de caquis según su origen mediante infrarrojo cercano (NIR)

Classification of persimmon fruit origin by near infrared spectrometry and least squares-support vector machines

M. Khanmohammadi[a], F. Karami[a], A. Mir-Marqués[b], A. Bagheri Garmarudi[a],
S. Garrigues[b], M. de la Guardia[b]

[a] *Chemistry Department, Faculty of Science, IKIU, Qazvin, Iran*

[b] *Department of Analytical Chemistry. University of Valencia. Jeronimo Muñoz research building, 50th Dr. Moliner str. 46100 Burjassot. Valencia. Spain*

Received 1 February 2014,

Received in revised form 30 May 2014,

Accepted 2 June 2014,

Available online 11 June 2014

Journal of Food Engineering 142 (2014) 17-22

DOI 10.1016/j.jfoodeng.2014.06.003

Resumen

El principal objetivo de este trabajo ha sido la autentificación del origen de caquis cultivados en diferentes regiones de España mediante espectroscopia de infrarrojo cercano con transformada de Fourier (FT-NIR). Para conseguir este objetivo, 166 muestras de caquis de 7 regiones diferentes de España fueron analizadas mediante espectroscopia FT-NIR. Los sets de calibración y validación se construyeron usando técnicas quimiométricas, en concreto máquinas de soporte vectorial por mínimos cuadrados (LS-SVM). Previamente a la clasificación se llevó a cabo el uso de corrección ortogonal de la señal y análisis por componentes principales. Se validó el método empleando 50 muestras del conjunto de validación con un error de predicción del 2%.

Abstract

The main objective of this work has been the authentication by Fourier transform near infrared (FT-NIR) spectrometry of the origin of persimmon fruits cultivated in different regions of Spain. In order to achieve this goal, 166 persimmon samples from 7 different regions of Spain were analyzed by FT-NIR spectrometry. By splitting the spectral data in training and independent test sets, a classification model was built using least squares support vector machines chemometric technique. Orthogonal signal correction and principal component analysis were performed prior to conduct the classification strategy. The verified model was applied for the prediction of the origin of 50 samples from the independent test set being the prediction error 2%.

8.1. Introduction

Quality control in horticultural products is a serious concern for food industry. It is an important issue for every producer and buyer, whether dealing with manufactured commodities to farmed fruits and products. Even local farmers must organise their activities on high quality reputation to sustain development, fruitfulness, profitability and competitiveness.

Experimental inspection regulations lay down stringent requirements which would guarantee the quality of products based on declared standards. In addition, there are some quality schemes which help in identification of specifications of products and foodstuffs farmed and produced. Quality control in this field consists of different subtitles. Considering the probable contamination of fruit products with chemical pollutants e.g. pesticides, there are several analytical approaches for evaluation of the quality of fruits prior to be consumed in the market. On the other hand, there are several important nutritional parameters, affecting the quality of fruits. Mineral profile of the fruits together with other food components e.g. vitamins are the main quality parameters influencing the product.

The characteristics of foods are highly depending on the geographical region and farming practices in which the fruit garden has been located. Thus, the origin of a fruit product is an important factor, affecting its quality. The protected designation of origin (PDO) is a status given by the name of a specific geographical area, used as a designation for an agricultural product or a foodstuff which has been produced, prepared or processed there. Thus, the final product acquires unique properties and sometimes its quality is exclusively determined via its origin geographical environment i.e. natural and human factors [1].

Persimmon is a native Chinese fruit, cultivated in warm regions worldwide. There are several different persimmon species farmed in Spain; such as Rojo Brillante astringent type. Mild climate is a favourable requirement for cultivation of persimmon. The main land areas in which this type of fruit is grown in Spain are La Ribera del Xúquer with European PDO register label, Alicante, Andalucía, Castellón, Extremadura, Ribera without PDO and Valencia.

Climate characteristics of the production area are important factors influencing the quality and features of the fruits. The variety of persimmon fruit and climate conditions also determine the shape of it [2]. In addition, the soil chemical composition is an important parameter in the nutritional profile of persimmon. All of these effective parameters are mainly depending on the geographical location and for this reason it is favourable to determine the origin of the fruit and also to discriminate the fruit samples from different origins while some may be PDO stated.

Generally, there are few reliable methods available for origin recognition of fruits. The main technique for this aim is to use 26s rDNA and 28s rDNA analysis [3] and free amino acid distribution [4] as the features to do the differentiation of samples. However, there are numerous analytical approaches for quality control of fruits, introduced to enable the authorities in reliable evaluation of unknown origin samples. Quantitative determination of phenolic compounds in fruit is a common tool for discrimination of fruit species [5]. Isorhamnetin glycosides and phloridsin are typical contents of pear and apple have been also employed for their authentication [6], food authenticity control has been also performed by NMR spectroscopy [7] together with fractionation and characterisation of cell wall polysaccharides [8].

Spectroscopy techniques have been widely used for qualitative and quantitative determination of different components in fruit samples. They have been utilised for the quantitative determination of several compounds. Also, the output data from these techniques are useful tools to access reliable conclusions on their origin. One of the spectroscopy techniques used in food chemistry is Fourier transform infrared (FTIR) spectroscopy especially in the near infrared (NIR) spectral region (800–2500 nm) [9-15].

Obtained data from NIR spectroscopy measurements are very advantageous when well-defined data processing is performed. Nowadays, chemometrics data processing methods are important tools to obtain information and data from NIR spectra. NIR spectroscopy has been a beneficial technique in determination of the quality of persimmon fruit [16].

Main chemometrics methods used in analytical chemistry concern calibration, both supervised and unsupervised, classification and experimental design. Versatile combination of chemometrics and IR spectroscopy has been applied in food industry, agriculture and horticulture; such as partial least squares discrimination analysis (PLS-DA) which is a reliable classification technique in chemometrics and mid infrared (mid-IR) diffuse reflectance spectroscopy (3–5 μm) [17-21]. Among several chemometric techniques, support vector machine (SVM) is a classification approach, found to be very useful for different aims [22, 23]. Considering the current interest in development of SVM based classification models in chemometrics and also the verified capabilities of NIR spectroscopy, in this work we have used multi class least squares SVM for treatment of NIR spectral data of persimmon samples as a tool for discriminating the origin of fruits cultivated in different regions of Spain.

8.2. Materials and methods

8.2.1. Sampling and analysis

166 Samples produced in Spain were collected from different agricultural cooperatives and producers during the harvests of 2010 and 2011. Among these samples 122 were produced under the PDO status as “Ribera del Xúquer”, 4 samples from Alicante, 11 samples from Andalucía, 10 samples from Castellón, 3 samples from Extremadura, 13 samples from the Ribera area but produced without PDO status, and 13 samples from Valencia. Geographical distribution of the regions from which persimmon samples used in this study were provided is shown in Figure 8.1. The edible parts of fruits were frozen and lyophilised during 72 h and after that samples were pulverised and stored until their analysis. The NIR spectra were acquired by using a Bruker® MPA (Bremen, Germany) FT-NIR analyser equipped with an air cooled NIR source, TE-InGaAs detector and a quartz beam splitter. The scanner of the interferometer was operated at a He–Ne laser modulation frequency of 10 kHz and total reflectance spectra of samples were obtained in glass vials of 20 mL with an internal diameter of 23 mm. The spectral range considered covered between 740 and 2700 nm spectral region and spectra were obtained with a resolution of 4 cm^{-1} and cumulating 50 scans.

Figure 8.1. Map of Spain indicating the different regions where the persimmon fruit samples employed through this study were collected.

8.2.2. Data set and treatment

NIR spectra of 166 persimmon samples from 7 regions of Spain (see Figure 8.2) were splitted in 2 subsets. In this regard, 116 samples were randomly selected from the data set to form the training set and the remaining 50 samples were used as independent test set for the external evaluation of the different models built. Chemometric data processing was performed by using MATLAB 8.0 and appropriate toolboxes. The first step in chemometric data treatment was to evaluate the data set by principal component analysis (PCA). Then the capabilities of cluster analysis (CA) in data classification were evaluated. Next step was to conduct the Orthogonal Signal Correction (OSC) pre-treatment and to track the origin pattern recognition of persimmon samples by least squares-support vector machine (LS-SVM). In order to classify the NIR spectra, samples from Kaki Ribera del Xúquer were labelled as class 1, the Ribera area samples produced outside the PDO were class 2, Andalucía samples were class 3, while

Valencia, Alicante, Castellón and Extremadura samples were class 4, 5, 6 and 7 respectively.

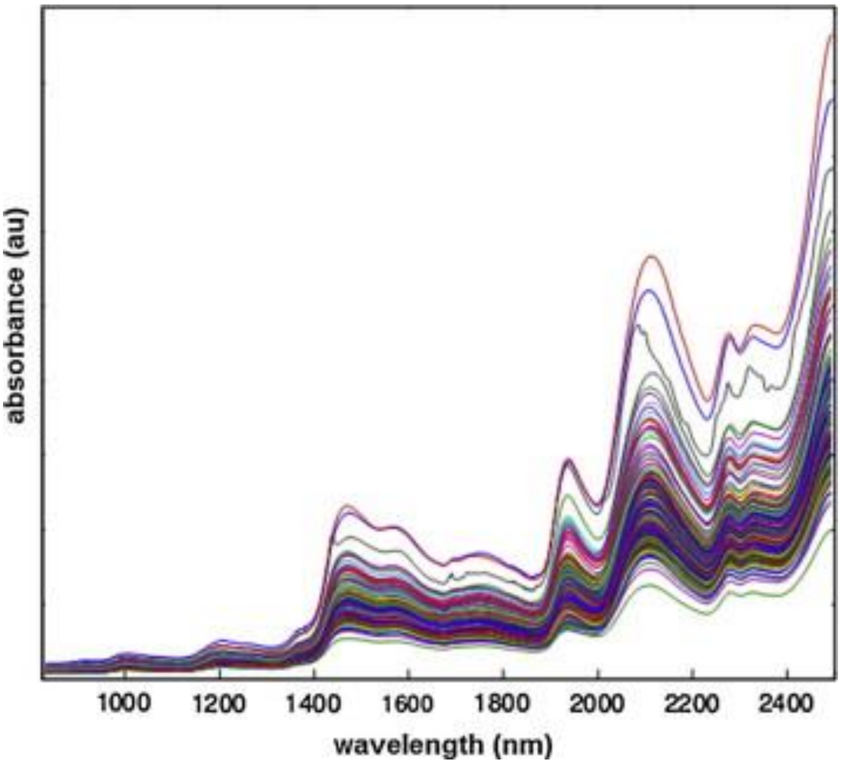

Figure 8.2. Raw FT-NIR spectra of persimmon samples employed through this study.

8.3. Results and discussion

8.3.1. Initial NIR spectra evaluation

As observed in Figure 8.2 absorbance bands obtained from total reflectance of lyophilised kaki samples measured inside glass vials are due to overtones and combination vibrations. There is a weak signal around 1400 nm due to first stretching vibration overtone of O–H. This peak is related to phenolic compounds and organic acids that they are main ingredients in persimmon [24]. Also, the amine based structure provided signals around 2100 nm. In data processing, all of the wavelengths were used because the results showed that application of the domain completely, decreases the error of prediction. In order to study the general possibility for locating the persimmon samples in different sub-spaces, all the spectra were projected in a 2 dimension space based on the use of 2 different wavelengths randomly selected (see Figure 8.3). The allocating plot of data by using the aforementioned two variables, 2010.6 and 2497.7 nm, confirmed that there is no adequate separation between classes based only on the crude spectra.

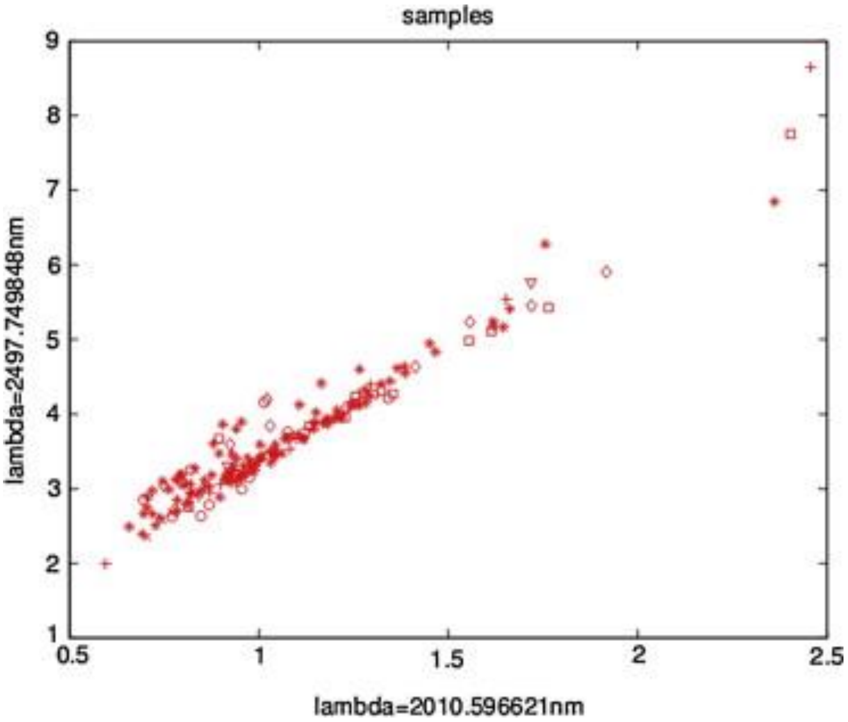

Figure 8.3. NIR spectra data distribution plot obtained by using the two wavelengths 2010.6 and 2497.7 nm.

Note: identification of sample classes: class 1 (✱), class 2 (□), class 3 (+), class 4 (○), class 5 (×), class 6 (◇) and class 7 (∇).

8.3.2. Orthogonal Signal Correction (OSC)

Multivariate analysis based on NIR spectra often needs pre-processing as an essential step. Systematic variation with NIR spectra unrelated to responses may disturb the multivariate modelling and cause prediction errors. Systematic variation in the data can be removed by differentiation and signal correction methods. The only concern dealing with these methods may be the probable removal of some useful information from original data set. Orthogonal Signal Correction (OSC) [25] is a method that performs uncorrelated signal removing by considering the response. It also removes information which is uncorrelated to the response. The spectra after OSC are shown in Figure 8.4 in which it can be also seen the presence of one outlier.

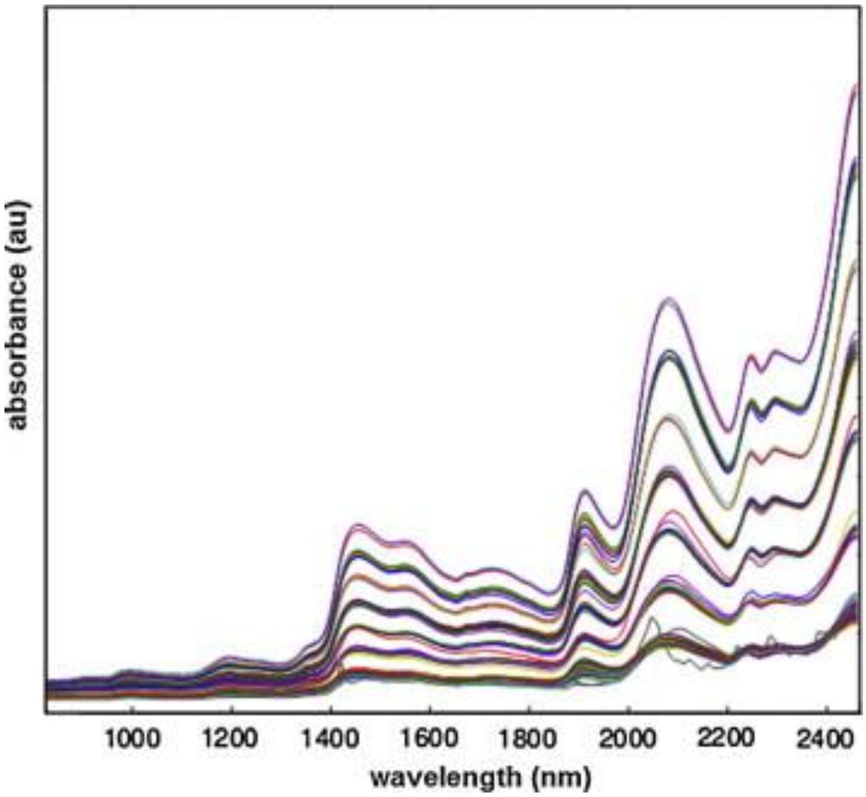

Figure 8.4. FT-NIR spectra of persimmon samples obtained after OSC.

8.3.3. Cluster analysis (CA) and principal component analysis (PCA)

The CA discrimination is an unsupervised classification technique [26, 27] in chemometrics and it is successful after OSC pre-processing in discrimination among classes. The CA discrimination was performed utilising Mahalanobis distance, the parameter which determines the similarity between samples (see Figure 8.5). Regions B and D are those parts of the data set in which the CA classification had been successful while in A and C regions, there are several misclassified samples. In the A region, samples from classes 2 and 3 are complicatedly present while the B region consists of only class 1 samples. Samples from class 4 are in the C region while classed 5, 6 and 7 are disordered in the D region.

As it can be observed in Figure 8.5 the overall scheme of classification after OSC treatment of persimmon NIR spectra is a binary system for distances higher than 0.6 and the absolute distance in which the performance of discrimination is visually realised would be seriously doubtful. It is obvious from the dendrogram that the quality of classification is not reliable enough to be evaluated as a practical way for origin based authentication of persimmon samples.

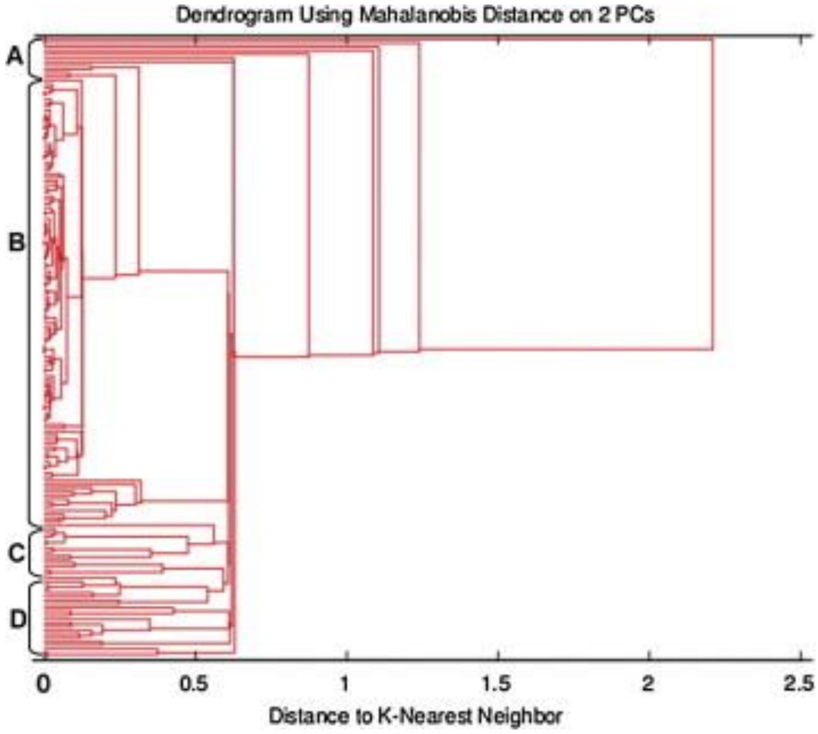

Figure 8.5. Dendrogram of CA on persimmon NIR spectra after OSC.

PCA is a well-known chemometric technique [28] for size reduction in multivariate data analysis. It is commonly used for constructing new dimensions of data set by reducing the total number of variables into a small one displaying most of the original variability of data in a reduced space. The elements of the new space are linear combinations of the original variables, orthogonal and contain the maximum variance within them. The PCA output consists of a spectrum like patterns, called loading matrix, representing the principal components (PC's) and a score matrix, which provides the coordinates of the original spectra on the new axes determined by the corresponding PC's.

However, constructing the PCA model based on the OSC treated data; an initial separation between the 7 classes would be clearly obtained (see Figure 8.6). Score plot for OSC treated data shows a justifiable separation within classes in a well-defined scheme that correlates well with geographical distances between them. As an example, samples from Alicante, Castellón and Valencia, which are close regions, are also close in the PCA graph. Scores of samples in class 1, class 2 and class 3 are less distributed across the 2nd PC. On the other hand, both PDO and non-PDO samples from Ribera region are also close located. The only deviation is related to Extremadura and Andalucía samples that in spite of the proximity of their geographical origin would far PC location.

8.3.4. Least squares-SVM (LS-SVM)

Support vector machines (SVM) is a powerful method for classification and regression aims [29]. Training process in SVM is a quadratic programming problem [30] and the technique is originally developed for binary classification. It maps the original data points from original data space to high or infinite dimensional feature space. Then, a hyper plane is constructed to discriminate the classes [31, 32]. Data set is $\{x_i, y_i\}^N_{i=1}$ with $x_i \in R^d$ that they are input vectors and $y_i \in \{-1, +1\}$ that they are the class labels. Mapping of data from real space to feature space is performed by using kernel function which could be a linear or non-linear function or radial basis function (RBF). The most popular kernel function used in SVM is RBF. Each kernel function consists of a set of tuneable parameters. In the case of RBF there is only one parameter (σ) which is radial width of kernel function. In the SVM classification approaches

C and σ are two mathematical parameters that they should be optimised, where C is positive regularization constant and σ radial width of kernel function.

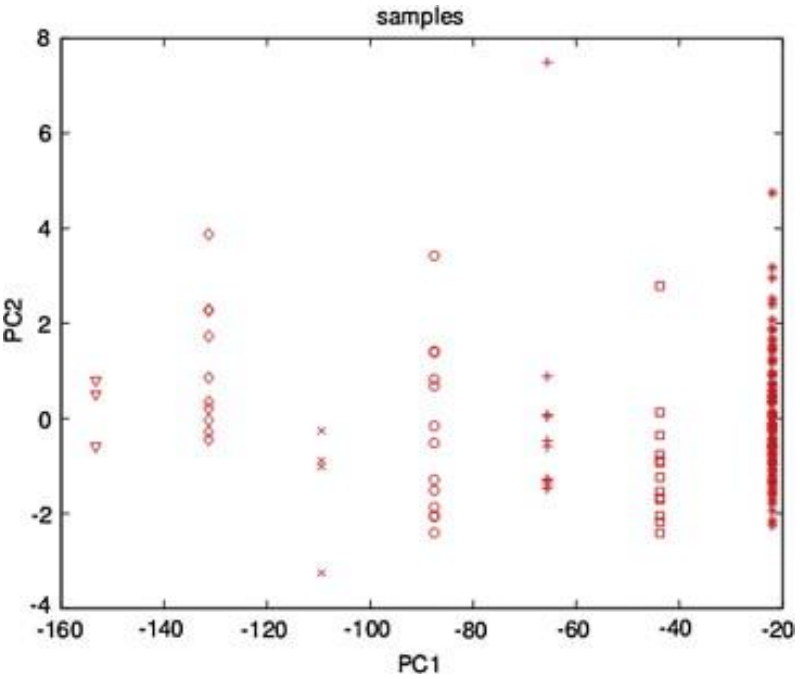

Figure 8.6. PCA score pot obtained for persimmon sample NIR spectra after performing OSC.

Note: identification of sample classes: class 1 (✱), class 2 (□), class 3 (+), class 4 (○), class 5 (×), class 6 (◇) and class 7 (∇).

SVM is originally a binary classification method but by utilising some strategies it can be used for discriminating more than two classes. In one of main strategies the multiclass problem is divided into several binary class problems and the results of all binary classifications are combined. In every stage discrimination of one class is performed from other classes. This strategy is named one against all. In another strategy, k classes of multiclass problem are converted to $k(k-1)/2$ binary classifiers and each classifier separates two classes of the total k classes. This strategy is named one against one. In least-squares SVM (LS-SVM) solution of problem is obtained by determining a set of linear equations instead of quadratic programming [22]. Utilising this method, the complexity is reduced significantly. In LS-SVM positive regularisation constant is shown by γ.

8.3.5. Recognition of the origin of persimmon samples

LS-SVM was performed for predicting the geographical origin of persimmon samples based on their NIR spectra. Training set was used for model construction with and without OSC pre-processing while PCA was previously conducted. First two PCs were used and LS-SVM parameters (γ and σ) were tuned by simplex and leave one out cross validation methods. The interval used for tuning of each of the parameters was [exp(−10) exp(10)]. Different combination or binary set of the aforementioned parameters were used to make LS-SVM model to get minimum error of cross validation. Considering these routes, the LS-SVM optimized parameters were 0.2 for γ and 2.8 for σ in raw LS-SVM while they were 2.6 for γ and 1.3 σ OSC supported LS-SVM. One against one strategy was utilized for this multiclass recognition case, trying to locate each spectrum in a unique position in the LS-SVM space. The statistical results for this classification issue are shown in Table 8.1, demonstrating the dramatic effect of OSC on SVM output. Comparing the schematic output of SVM (Figure 8.7A) with that of OSC-SVM (Figure 8.7B), it is obviously concluded that role of OSC is highly important in achieving a robust classification mode. One of the main properties of the developed method is that we can easily and exactly report the error of the work in comparison with other reported methods which determine the origin qualitatively. Also, the results of proposed method are not influenced by some parameters such as pollution of the skin of fruits during the transportation.

Table 8.1. Statistical output of SVM different models for classification of persimmon samples.

	SVM	**OSC-SVM**
Training error	28%	0%
Prediction error	24%	2%
Training R^2	0.33	1.00
Prediction R^2	0.15	0.99

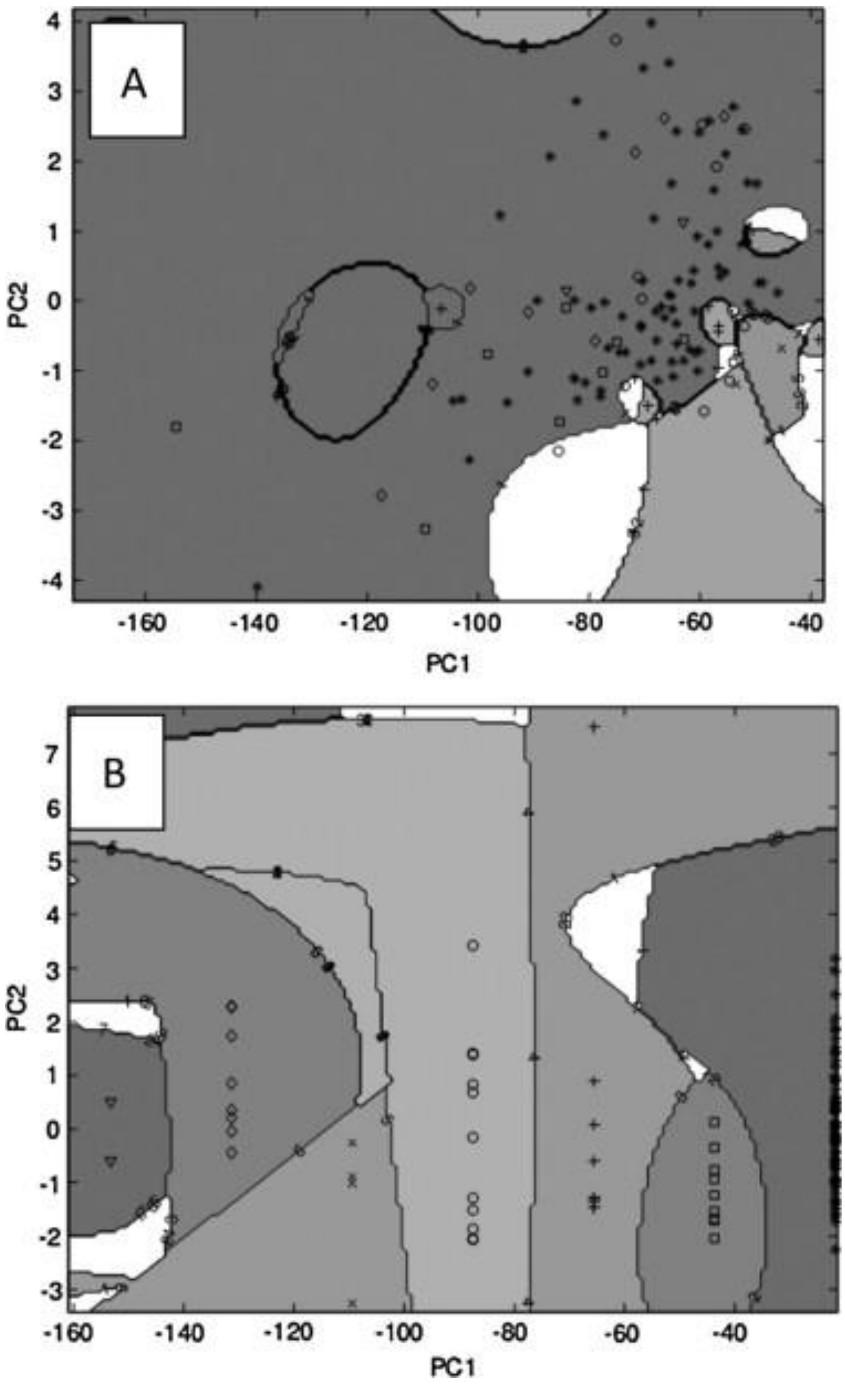

Figure 8.7. Classification of NIR persimmon data by LS-SVM without performing OSC (A) and after performing OSC (B).

Note: identification sample classes: class 1 (✱), class 2 (□), class 3 (+), class 4 (○), class 5 (×), class 6 (♢) and class 7 (∇).

8.4. Conclusion

Authentication of the origin, nutritional characteristics and performing quality control of fruits is one of the most important needs in food industry. In order to achieve this goal, it could be utilised different methods. Most of these techniques employ complicated chemical analysis. Consequently, they are time consuming and expensive. Also, they use chemicals, such as solvents for separating ingredients, causing environmental concerns and for this reason they are not environmental green methods. Thus, developing new methods for persimmon evaluating is an important task. This study evidenced that LS-SVM classification method, after OSC treatment of NIR spectra for noise removing from signals, can be used as an easy and fast approach with high reliability for the authentication of the geographical origin of persimmon fruits cultivated in various regions of Spain, thus providing a green alternative to other authentication procedures based on the determination of chemical parameters of samples after their digestion or dissolution. Additionally, it is clear that the developed methodology could be also suitable for the authentication of other foods produced in different areas and covered by protected designation of origin (PDO) or protected geographical indications (PGI).

References

[1] M. De la Guardia, A. Gonzálvez (Eds.), Food Protected Designation of Origen: Methodologies and Applications, Elsevier, Amsterdam (2013)

[2] CRDO Consejo Regulador de la Denominación de Origen “Kaki Ribera del Xúquer”. Available (2011) from: http://www.kakifruit.com

[3] A.F. El Sheikha, A. Condur, I. Métayer, D.D. Nguyen, G. Loiseau, D. Montet. Determination of fruit origin by using 26S rDNA fingerprinting of yeast communities by PCR-DGGE: preliminary application to Physalis fruits from Egypt

[4] Licciardello, F., Muratore, G., Avola, C., Tomaselli, F., Maccarone, E., 2011. Geographical origin assessment of orange juices by comparison of free aminoacids

distribution. In: ISHS Acta Horticulture 892: II International Symposium on Citrus Biotechnology. Available from: http://www.actahort.org/books/892/892_48.htm

[5] B.M. Silva, P.B. Andrade, P. Valentao, G.C. Mendes, R.M. Seabra, M.A. Ferreira. Phenolic profile in the evaluation of commercial quince jellies authenticity. Food Chem., 71 (2) (2000), pp. 281–285

[6] E. Hvattum. Determination of phenolic compounds in rose hip (Rosa canina) using liquid chromatography coupled to electrospray ionisation tandem mass spectrometry and diode-array detection. Rapid Commun. Mass Spectrom., 16 (7) (2002), pp. 655–662

[7] I.J. Colquhoun. High resolution NMR spectroscopy in food analysis and authentication. Spectrosc. Europe, 10 (1) (1998), pp. 8–18

[8] R. Feugel, R. Carle, A. Schieber. A novel approach to quality and authenticity control of fruit products using fractionation and characterisation of cell wall polysaccharides. Food Chem., 87 (1) (2004), pp. 141–150

[9] K.H. Norris. Design and development of a new moisture meter. Agric. Eng., 45 (7) (1964), pp. 370–372

[10] G.S. Birth, G.G. Dull, W.T. Renfroe, S.J. Kays. Nondestructive spectrophotometric determination of dry matter in onions. J. Am. Soc. Horticultural Sci., 110 (2) (1985), pp. 297–303

[11] A.M. Davies, A. Grant. Review: near infra-red analysis of food. Int. J. Food Sci. Technol., 22 (3) (1987), pp. 191–207

[12] S. Roy, R. Anantheswaran, J. Shenk, M.O. Westerhaus, R. Beelman. Determination of moisture content of mushrooms by Vis—NIR spectroscopy. J. Sci. Food Agric., 63 (3) (1993), pp. 355–360

[13] B.M. Nicolaï, K. Beullens, E. Bobelyn, A. Peirs, W. Saeys, K.I. Theron, J. Lammertyn. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: a review. Postharvest Biol. Technol., 46 (2) (2007), pp. 99–118

[14] G. Fan, J. Zha, R. Du, L. Gao. Determination of soluble solids and firmness of apples by Vis/NIR transmittance. J. Food Eng., 93 (4) (2009), pp. 416–420

[15] A. Terdwongworakul, N. Nakawajana, S. Teerachaichayut, A. Janhiran. Determination of translucent content in mangosteen by means of near infrared transmittance

[16] A.D. Mowat, Philip R. Poole. Non-destructive discrimination of persimmon fruit quality using visible-near infrared reflectance spectrophotometry. Acta Hort. (ISHS), 436 (1997), pp. 159–164

[17] L. Wang, B. Mizaikoff. Application of multivariate data-analysis techniques to biomedical diagnostics based on mid-infrared spectroscopy. Anal. Bioanal. Chem., 391 (5) (2008), pp. 1641–1654

[18] J.S. Ribeiro, T.J. Salva, M. Ferreira. Chemometric studies for quality control of processed Brazilian coffee using DRIFTS. J. Food Quality, 33 (2) (2010), pp. 212–227

[19] Y. Ni, M. Mei, S. Kokot. Analysis of complex, processed substances with the use of NIR spectroscopy and chemometrics: classification and prediction of properties – the potato crisps example. Chemom. Intelligent Lab. Syst., 105 (2) (2011), pp. 147–156

[20] C. Sârbu, R.D. Nascu-Briciu, A. Kot-Wasik, S. Gorinstein, A. Wasik, J. Namiesnik Classification and fingerprinting of kiwi and pomelo fruits by multivariate analysis of chromatographic and spectroscopic data. Food Chem., 130 (4) (2012), pp. 994–1002

[21] A.M. Gómez-Caravaca, R.M. Maggio, V. Verardo, A. Cichelli, L. Cerretani. Fourier transform infrared spectroscopy–Partial Least Squares (FTIR–PLS) coupled procedure application for the evaluation of fly attack on olive oil quality. Lebensmittel-Wissenschaft & Technologie, 50 (1) (2013), pp. 153–159

[22] V.N. Vapnik. The Nature of Statistical Learning Theory (second ed.) Springer, New York (2000)

[23] Y. Xu, S. Zomer, R.G. Brereton. Support vector machines: a recent method for classification in chemometrics. Critical Rev. Anal. Chem., 36 (3–4) (2006), pp. 177–188

[24] Y. Park, S. Jung, S. Kang, E. Delgado-Licon, A.L.M. Ayala, M.S. Tapia, O.

Martin-Belloso, S. Trakhtenberg, S. Gorinstein. Drying of persimmons (*Diospyros kaki* L.) and the following changes in the studied bioactive compounds and the total radical scavenging activities. LWT, 39 (7) (2006), pp. 748–755

[25] S. Wold, H. Antti, F. Lindgren, J. Ohman. Orthogonal signal correction of near-infrared spectra. Chemom. Intelligent Lab. Syst., 44 (1–2) (1998), pp. 175–185

[26] S. Sharma. Applied Multivariate Techniques. Wiley, New York (1996)

[27] J.A.S. Almeida, L.M.S. Barbosa, A.A.C.C. Pais, S.J. Formosinho. Improving hierarchical cluster analysis: a new method with outlier detection and automatic clustering. Chemom. Intelligent Lab. Syst., 87 (2) (2007), pp. 208–217

[28] B. Flury, H. Riedwyl. Multivariate Statistics—A Practical Approach. Chapman & Hall, London (1988)

[29] V.N. Vapnik. The Nature of Statistical Learning Theory (second ed.) Springer-Verlag, New York (1995)

[30] S. Tao, D. Chen, W. Zhao. Fast pruning algorithm for multi-output LS-SVM and its application in chemical pattern classification. Chemom. Intelligent Lab. Syst., 96 (1) (2009), pp. 63–69

[31] J.A.K. Suykens, J. Vandewalle. Least squares support vector machine classifiers. Neural Process. Lett., 9 (3) (1999), pp. 293–300

[32] J. Lutsa, F. Ojeda, R. Van de Plas, B. De Moor, S. Van Huffel, J.A.K. Suykens. A tutorial on support vector machine-based methods for classification problems in chemometrics. Anal. Chim. Acta, 665 (2) (2010), pp. 129–145

Capítulo 9

Determinación directa de elementos minerales en alcachofa mediante espectroscopia infrarroja y fluorescencia de rayos X

Green direct determination of mineral elements in artichokes by infrared spectroscopy and X-ray fluorescence

Alba Mir-Marqués, Maria Martínez-García, Salvador Garrigues, M.Luisa Cervera and Miguel de la Guardia

Department of Analytical Chemistry. University of Valencia. Jeronimo Muñoz research building, 50th Dr. Moliner str. 46100 Burjassot. Valencia. Spain.

Received 29 May 2015,

Received in revised form 2 October 2015,

Accepted 11 October 2015,

Available online 22 October 2015

Food Chemistry 196 (2016) 1023-1030

DOI 10.1016/j.foodchem.2015.10.048

Resumen

Espectroscopia de infrarrojo cercano (NIR) y fluorescencia de rayos X (XRF) se utilizaron para la predicción de la concentración de calcio, potasio, hierro, magnesio, manganeso y zinc en muestras de alcachofa. Se compraron sesenta muestras de alcachofa procedentes de diferentes regiones de España (Benicarló, Valencia y Murcia). Para desarrollar modelos quimiométricos de predicción de la concentración de elementos minerales se usaron los espectros de NIR y XRF de las muestras combinados con el tratamiento de datos por mínimos cuadrados parciales (PLS). Con el fin de obtener los datos de referencia, las muestras se digirieron y se analizaron mediante espectroscopia de emisión óptica con plasma de acoplamiento inductivo (ICP-OES). Los coeficientes de determinación obtenidos para la regresión entre valores predichos y valores de referencia usando los espectros NIR fueron 0.61, 0.79, 0.53, 0.77, 0.54 y 0.60 para el calcio, potasio, magnesio, hierro, manganeso y zinc respectivamente; y usando los espectros de XRF fueron 0.96, 0.93, 0.80, 0.79, 0.76 y 0.90. Ambas metodologías ensayadas, ofrecen una alternativa “verde” al clásico análisis mineral, aunque XRF proporciona mejores resultados que NIR como método cuantitativo de “screening”.

Abstract

Near infrared (NIR) and X-ray fluorescence (XRF) spectroscopy were investigated to predict the concentration of calcium, potassium, iron, magnesium, manganese and zinc in artichoke samples. Sixty artichokes were purchased from different Spanish areas (Benicarló, Valencia and Murcia). NIR and XRF spectra, combined with partial least squares (PLS) data treatment, were used to develop chemometric models for the prediction of mineral concentration. To obtain reference data, samples were mineralised and analysed by inductively coupled plasma optical emission spectrometry (ICP-OES). Coefficients of determination obtained for the regression between predicted values and reference ones for calcium, potassium, magnesium, iron, manganese and zinc were 0.61, 0.79, 0.53, 0.77, 0.54 and 0.60 for NIR and 0.96, 0.93, 0.80, 0.79, 0.76 and 0.90 for XRF, respectively. Both assayed methodologies, offer green alternatives to classical mineral analysis, but XRF provided the best results in order to be used as a quantitative screening method.

9.1. Introduction

The determination of the mineral profile in foods is commonly made by using atomic spectroscopy and mass spectrometry methods, being the most common used techniques for this purpose inductively coupled plasma optical emission (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) [1-5]. Mineral analysis in artichokes has been done commonly by the aforementioned techniques [6-8]. These techniques are very useful for the multi-elemental analysis of samples in a few minutes. However, to do it, a costly instrumentation is required and liquid samples that involves the use of strong acids and other reagents, apparatus, energy and time-consuming, also generating a lot of wastes. A green alternative for the evaluation of the presence of mineral elements in solid foods could be a methodology that combined fast sample preparation, or no sample pre-treatment, easy detection of the target analysis and minimal generation of residues. Near infrared (NIR) spectroscopy has been employed as an alternative to wet chemistry-based atomic spectroscopy procedures for food analysis [9]. Although correlations between spectra and mineral element contents seem unreliable, prediction of some major mineral by NIR may be possible through their association with the organic matrix and based on the use of chemometrics to model the NIR spectra from a series of samples well characterised in order to determine their mineral profile in a series of samples, including some foods [10]. Other green alternative for the analysis of minerals in foods could be based on X-ray fluorescence (XRF). This technique is very commonly employed in geological materials, archaeology objects, and environmental samples, but it is not very often used to food and beverage analysis [11]. XRF, specially the techniques based on energy dispersion (EDXRF), bring great advantages to the analysis of mineral elements by ICP-OES or ICP-MS, as: the elimination of sample chemical pre-treatment, the lack of use of reagents and acids, the direct and rapid measurement of spectra and the fact that XRF provides a non-destructive sample technique thus deleting the waste generation and reducing drastically the environmental side effects and costs [10].

However, the main disadvantage of both, NIR and XRF, is their low sensitivity and high matrix effects. Therefore, it is essential to use chemometric tools to build appropriate calibration models from the spectra of well characterised samples in order to increase the prediction capability of measurements made [9,10].

The combination of NIR and XRF measurements with chemometric data treatment could offer exciting possibilities for the direct determination of mineral components in food samples. Partial least squares (PLS) or modified-partial lest squares (MPLS) have been employed for building models proposed in the literature to do the quantitative determine mineral elements in vegetables [12-15].

Artichoke (*Cynara Scolymus L.*) is one of the oldest cultivated plants, and it is used as a food since the 15th century. There are different types of artichokes and Spain is one of the most important producers in the European Union. The ‘Blanca de Tudela’ variety is labelled as protected designation of origin (PDO) 'Alcachofa de Benicarló' (Castellón, Spain) and artichokes are also produced in different areas of the País Vasco, Aragon, Valencia and Murcia. Artichoke is a very good food for diabetics and also favours the fat and urea metabolism, contributing to the regulation of liver and kidney functions [16]. Artichokes contain a lot of minerals such as calcium, potassium, magnesium, iron, manganese and zinc. Controlling minerals in artichoke is important to maintain and verify the quality control of these products before reaching the consumer and to generate a correct daily intake of essential elements.

The main purpose of this study was the development of a green analytical methodology for mineral element determinations in artichokes, based on NIR and XRF spectra with PLS treatment in order to provide a green methodology useful for quality control of essential minerals in solid foods.

9.2. Materials and methods

9.2.1. Samples

60 artichoke samples from three different Spanish origins were analysed, 38 samples were produced under the PDO ‘Alcachofa de Benicarló’ (Castellón, Spain), 12 samples from Valencia (Spain) and 10 from Murcia (Spain). Each sample was a

combination of 2-4 artichokes of the same field. Samples were washed with ultrapure water with a resistivity of 18.2 MΩ cm, obtained using a Milli-Q plus Millipore system (Molsheim, France), and the edible parts were lyophilised in a Cryodos 50 lyophiliser Telstar (Barcelona, Spain) for a minimum of 48 h at a chamber pressure of 50 Pa. Then, samples were pulverised and homogenised with a domestic Braun mixer (Kronberg, Germany). Finally, samples were sieved through a particle size of 250 µm and stored in polyethylene bottles until their analysis.

9.2.2. Reference procedure

Reference data were obtained by ICP-OES using an Optima 5300 DV Perkin Elmer (Norwalk, CT, USA), equipped with an auto sampler AS 93-plus and a GemCone nebuliser, after microwave-assisted digestion using a Milestone Ethos SEL microwave laboratory system (Sorisole, Italy), equipped with a thermocouple probe for automatic temperature control, and an automatic gas leak detector, using high pressure Teflon vessels of 100 mL inner volume. For samples digestion, 0.5 g of each freeze-dried sample were accurately weight inside Teflon reactors and 10 mL of concentrated HNO_3 (69% for trace analysis, Scharlau, Barcelona, Spain) were added. Reactors were closed and placed inside the microwave oven using a digestion step of 15 min at 180º C (700 W) [17]. Digested samples were measured with a final dilution volume of 50 mL. The calibration range for iron, manganese and zinc was established from 0.01 to 2 mg L^{-1}, for calcium and magnesium it was made from 10 to 100 mg L^{-1} and for potassium was made from 20 to 900 mg L^{-1}. Rhenium (1 mg L^{-1}) was used as internal standard and added to all samples, reagent blanks and standards. The average concentration of blanks was subtracted from the analytical signals of digested samples after interpolation on the calibration graphs. Results were expressed as a basis of dry sample weight. All samples were analysed in duplicate together with blanks and certified reference materials, Tomato Leaves (NIST 1573a, National Institute of Standards and Technology, Gaithersburg, MD, USA) and Cabbage (IAEA-359, International Atomic Energy Agency, Vienna, Austria), were measured in each session in order to control possible contaminations and to verify the accuracy of the methods. Limits of detection (LOD) were calculated as three times the standard deviation of reagent blanks. LODs for calcium, potassium, magnesium, iron, manganese and zinc were 0.22, 0.24, 0.07, 0.06, 0.08 and 0.017 mg kg^{-1} respectively, taking into account the amount of samples assayed and the final dilution employed for determinations.

9.2.3. NIR procedure

0.5-1 g of dry samples were placed inside glass vials. The vials were rotated to obtain triplicate measurements for each sample. For acquisition data diffuse reflectance infrared Fourier transform (DRIFT) were recorded in Kubelka-Munk units, in the range of 12500-3500 cm^{-1}, with spectral resolution of 4 cm^{-1} and cumulating 50 scans per spectrum using a MPA model Multipurpose Analyser Fourier transforms near-infrared (FT-NIR) spectrometer, from Bruker (Bremen, Germany), equipped with an integrating sphere. The background spectrum was acquired from the closed integrating sphere using the same instrumental conditions than those employed for samples. For instrument control and data acquisition, the OPUS software 6.5 from Bruker was used.

9.2.4. XRF procedure

0.3 g of dry samples were placed inside a pellet box and were compacted using a hydraulic press for 1 min at a pressure of $5.1 \cdot 10^3$ kg. Pellets were stored inside desiccators to avoid hydration from air humidity. XRF spectra were obtained by triplicate at 50 kV, 11 µA and 60 s acquisition time (30 s for high atomic number elements, and 30 s for low atomic number elements) using a portable model S1 Titan energy dispersive X-ray fluorescence (ED-XRF) spectrometer from Bruker equipped with a Rhodium X-ray tube and X-Flash® SDD detector. For instrument control S1RemoteCtrl and S1Sync software from Bruker were employed; and for spectra treatment the ARTAX software from Bruker was used.

9.2.5. Chemometrics data treatment

Models for the prediction of mineral element content of artichoke samples from their spectral measurements were performed using partial least squares regression (PLS) by Matlab 2014a software from Mathworks (Natick, MA, USA) and the PLS Toolbox 6.2 from Eigenvector Research Inc. (Wenatchee, WA, USA). PLS regression is a technique used to evaluate data that contain correlated variables. This technique creates new predictor variables, known as latent variables, as linear combinations of the original variables, while considering the observed response values, leading to a model with reliable predictive power. So, PLS analysis is based on the use of combinations of the predictors that have a large covariance with the response values [18]. In this study, PLS was used to build the relationship between the spectral data matrix (X) and reference concentration of elements matrix (Y) for predicting the concentration of a separate set of samples with the smallest number of latent variables (LV) [19]. For a reliable estimation of the prediction capability of chemometric models built, the preliminary data set, including the spectra matrix (X) and the reference concentration data (Y), must be divided into separate calibration and validation sets. Cross-validation was used to validate the quality of the models and to prevent over-fitting of the calibration. For a given set of data, cross-validation involves a series of steps called sub-validation ones in which a subset of objects is removed from the set of data, to build a model using the remaining objects as the set of data, and then applying the resulting model to the removed objects [18]. In all cases, leave-one-out cross validation (CV) was employed for the selection of the optimum number of latent variables of the models according to the root mean square error of cross validation (RMSECV) values. After selection of the best parameters as variable and data pre-processing, the model must be evaluated using an independent set of measurements obtained from samples included in the validation set, which were not employed in building and optimizing the model. So, it permits to obtain a non-overoptimistic value of the prediction error.

Kennard-Stone (KS) algorithm was assayed to select the most appropriate sample calibration set. KS is a method for the selection of a representative subset of samples based only on the instrumental signals. The selected subset should cover the experimental region homogeneously by choosing objects which are uniformly distributed in the experimental space [20]. Different data pre-treatment, such as, mean centring (MC), standard normal variate (SNV), Savitzky-Golay first derivate (FD) and also combinations of them, were evaluated for NIR and XRF measurements. Samples from the validation set were analysed in the selected conditions. Mean-centring calculates the mean of each variable and subtract this from the obtained values. Another way of interpreting mean-centred data is that, after mean-centring, each row of the mean-centred matrix data includes only how that row differs from the average sample in the original data matrix. The SNV normalization method is a weighted normalization, in order to reflect that not all points contribute to the normalization equally. SNV calculates the standard deviation of all the pooled variables for the given sample. The entire sample is then normalized by this value. First derivative is based on the use of first order derivative signals instead of zero order ones. It permits to remove the signal which is the same between the two variables and leaves only the part of the signal which is different. Derivatives de-emphasize low frequencies and emphasize high frequencies, they tend to accentuate noise and for this reason, the Savitzky-Golay algorithm is often used to simultaneously smooth the data as greatly improving the utility of derivative signals [18].

9.3. Results and discussion

9.3.1. NIR spectra of artichokes

Figure 9.1 shows the typical near DRIFT spectra obtained between 9000 and 4000 cm^{-1} for artichoke samples from different Spanish origin. The first spectrum is the average of those found for Benicarló samples, the second that is corresponds to Murcia samples and the last one is that obtained for Valencia samples. The spectra regions between 12500-9000 cm^{-1} and 4000-3500 cm^{-1} were removed for data processing. The broad and strong peak around 8300 cm^{-1} was due to the second overtone C-H stretching vibrations. At 7000–6800 cm^{-1} it can be seen a broad peak, which corresponds to the combination of C-H and the N-H stretch first overtone. Peak at 5800 cm^{-1} corresponds to the C-H stretch first overtone. O-H first and second overtone can be seen at 5155 cm^{-1}. A strong peak at 4732 cm^{-1} was due to C-O stretch combination and O-H bend, characteristics of carbohydrates. Double peak at 4350 and 4250 cm^{-1} were due to C-H and CH_2 bend second overtone, corresponding to proteins [21].

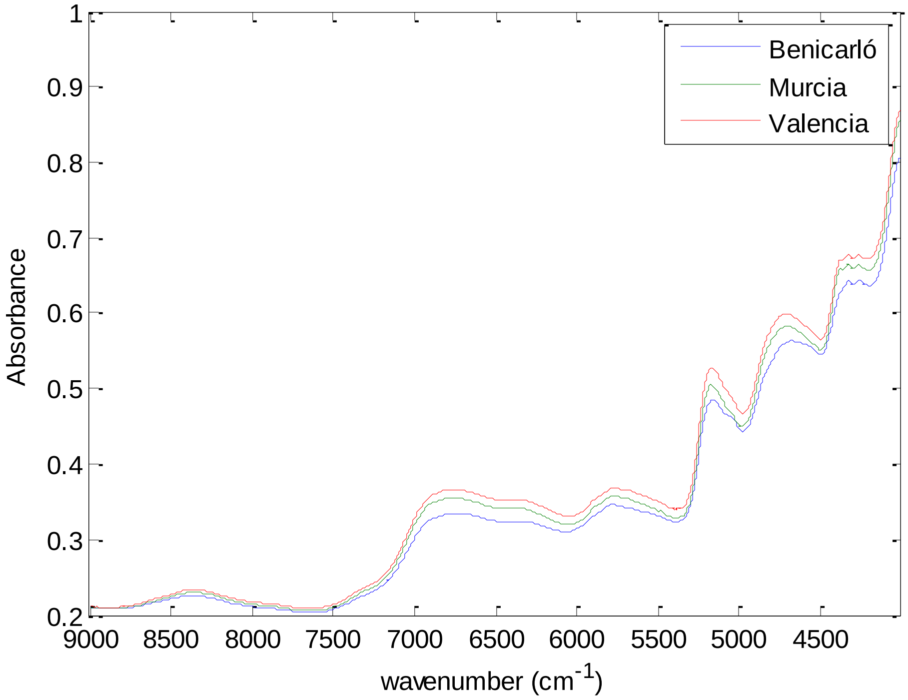

Figure 9.1. Mean DRIFT spectra of artichoke samples

9.3.2. PLS-NIR models for determination of mineral elements in artichokes

The complete data set of spectra was divided in two independent sets, calibration and validation, with 43 and 17 samples, respectively, using KS algorithm. In order that the calibration set could be representative of all the samples considered, it was composed by 24 Benicarló samples, 10 Valencia samples and 9 Murcia samples. The concentration range, mean and standard deviation for the elements determined in artichokes for both, calibration and validation sets, are shown in Table 9.1. In all PLS-NIR models, from the total spectral region (4667 variables) only 2796 variables (4000-

9000 cm^{-1}) were used to build the models. These variables were removed because they contain useless or irrelevant information for the mineral element determinations, such as noise and background, which can worse the predictive ability of PLS-NIR models. For the selection of the optimum number of LV, several parameters; such as the predictive residual error sum of squares (PRESS), root mean square error for cross-validation (RMSECV) and X and Y explained variances were evaluated (see Table 9.1). For the micronutrients, iron, manganese and zinc, 2, 3 and 10 LV, respectively, were necessary.

The RMSECV values were 7.2 (iron), 3.1 (manganese) and 8.2 mg kg^{-1} (zinc), and the explained variances for X and Y were 97.4 and 83.4% for iron, 96.3 and 79.6% for manganese and 99.9 and 95.3% for zinc, respectively. For the macronutrients, calcium, potassium and magnesium, 13, 4 and 10 LV were needed to build the corresponding PLS models. Explained variances for X and Y were 99.7 and 97.1% for calcium, 97.4 and 78.0% for potassium and 97.4 and 97.8% for magnesium.

Ratio of performance to deviation (RPD) values was calculated for all models built (see Table 9.1). RPD was calculated as standard deviation of the samples divided by root mean squared error for each model. According to Bellon-Maurel et al. (2010) [22] if RDP is higher than 2 the model can be considered as excellent, if RPD values is between 1.4 to 2 the model is fair but if RPD is lower than 1.4 the model must be considered as non-reliable. For calibration models built from NIR spectra RPD values were greater than 2, in all cases. On the other hand, for calcium, potassium and magnesium, a Savitzky-Golay first derivate, with a window of 11 points and a second order polynomial, and mean centring treatment were chosen as signal pre-processing. For iron, manganese and zinc mean centring was chosen. Additionally, concentration data were mean centred and auto scaled in all cases.

9.3.3. Prediction capability of PLS-NIR for mineral element determination in artichokes

External validation was used to evaluate the PLS-NIR methodology, using an independent set of artichoke samples, not employed during the calibration step. Predicted values of analytes versus those obtained by the reference method are shown in Figure 9.2. In general, it can be seen that the best models are not good enough,

Table 9.1. Reference data and statistics of mineral content of artichokes used for calibration and validation sets employed for PLS-NIR determination

Element	Set	Samples	Range	Mean	SD	Pre-process	LV	R^2_{cal}	RMSEC	R^2_{CV}	RMSECV	R^2_{val}	RMSEP	RRMSEP
Ca	CAL	43	900-3384	1715	594	MC+FD	13	0.97	94	0.52	393	0.61	355	19.2
	VAL	17	970-3368	1850	623									
K	CAL	43	25882-50541	39935	6814	MC+FD	4	0.77	3116	0.61	4070	0.79	1900	4.5
	VAL	17	32923-47329	42267	4148									
Mg	CAL	43	1816-3523	2834	447	MC+FD	10	0.98	59	0.70	226	0.53	271	9.4
	VAL	17	2325-3533	2874	325									
Fe	CAL	43	17.7-70.5	47.9	15.1	MC	2	0.83	6.2	0.77	7.2	0.77	6.3	11.7
	VAL	17	27.0-78.7	53.9	13.3									
Mn	CAL	43	6.7-28.9	18.1	6.3	MC	3	0.80	2.6	0.72	3.1	0.54	2.8	14.3
	VAL	17	10.5-24.7	19.6	4.1									
Zn	CAL	43	11.7-61.6	32.9	12.8	MC	10	0.90	3.9	0.61	8.2	0.60	8.0	21.1
	VAL	17	19.1-60.0	38.0	12.1									

NOTE: Range, mean and standard deviation (SD) in mg kg^{-1} dry weight for calibration (CAL) and validation (VAL) data sets. RMSEC, root-mean-square error of calibration in mg kg^{-1}; RMSECV, root-mean-square error of cross-validation in mg kg^{-1}; RMSEP, root-mean-square error of prediction in mg kg^{-1}; RRMSEP, relative root-mean square error of prediction in percentage (%).

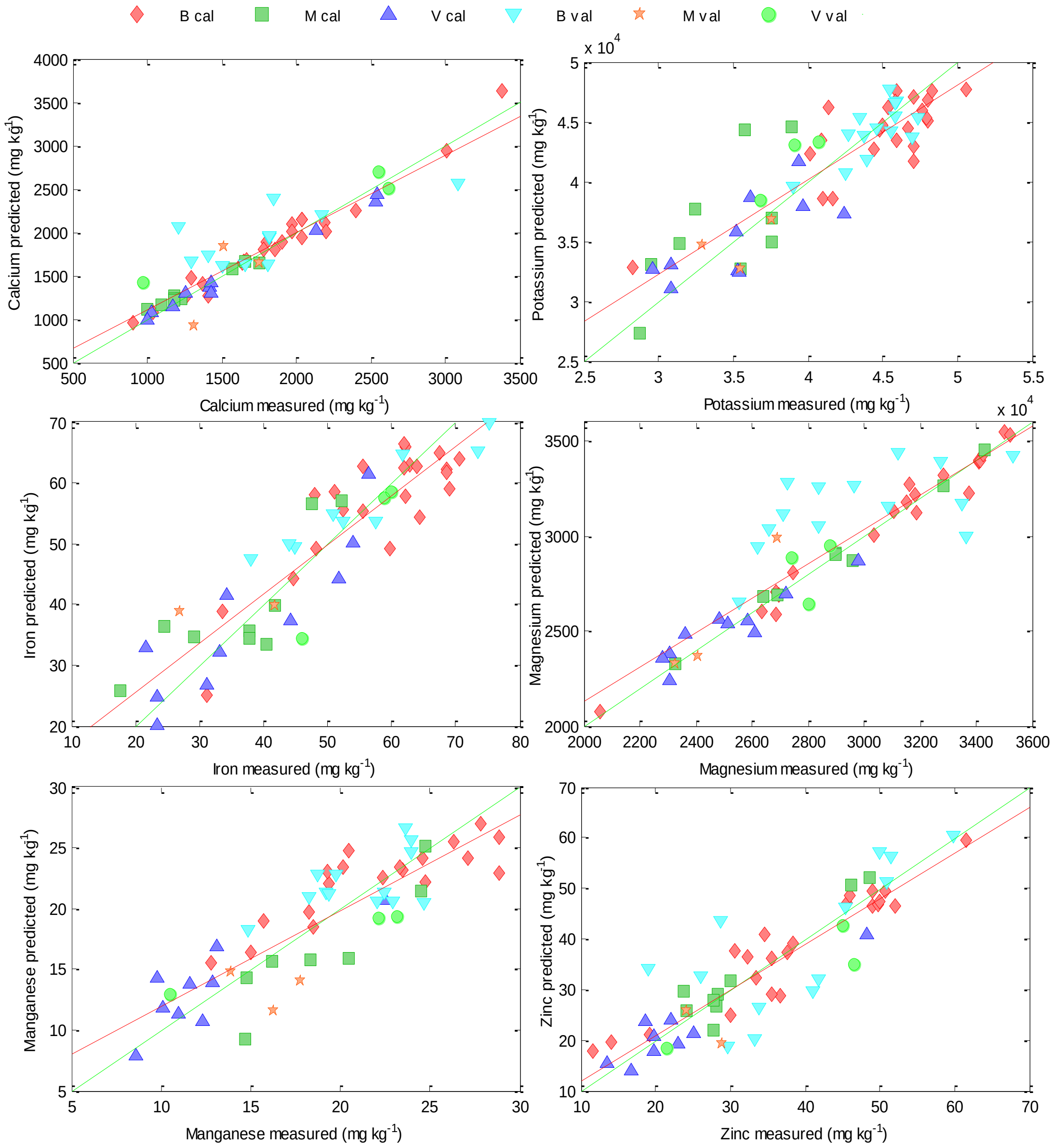

Figure 9.2. Predicted values by PLS-NIR vs. reference values of mineral elements in artichoke samples

NOTE: B samples from Benicarló, M samples from Murcia, V samples from Valencia, cal samples from calibration set, val samples from validation set

since the calibration and validation samples do not fit the regression line with zero intercept and one slope. Good accuracy was obtained in the validation step for potassium and magnesium and acceptable accuracy calcium, iron, manganese and zinc.

Average relative prediction errors of models were 18.6% for calcium, 3.9% for potassium, 8.0% for magnesium, 10.4% for iron, 14.0% for manganese and 18.5% for zinc, with coefficients of determination between 0.53 for magnesium and 0.79 for potassium. The precision of the models built was very high. For potassium, iron and manganese, relative standard deviation (RSD) values were lower than 2.5 %, and in all cases RSD was lower than 10 %. The minimum relative root mean square error of prediction (RRMSEP) obtained was 4.5% for potassium and in all the cases it was lower than 21% (see Table 9.1). These results, together with RPD values, demonstrate a good predictive capability of the PLS-NIR models developed to predict the concentration of potassium and iron in artichoke samples together with an acceptable prediction for calcium, manganese and zinc concentration, but a non-acceptable prediction of magnesium concentration. Thus, is reduced the capability of PLS-NIR as a green alternative for the direct determination of the aforementioned elements in dried samples without a previous sample digestion.

9.3.4. XRF spectra of artichokes

Figure 9.3 shows the typical EDXRF spectra between 0 and 14 keV of artichoke samples from different Spanish origin. The first spectrum corresponds to the average spectrum of Benicarló samples, the second spectrum corresponds to Valencia samples and the last one is that of Murcia samples. All elements studied provided specific Kα and Kβ bands at lower energies than 10 keV. The Kα lines of magnesium, potassium, calcium, manganese, iron and zinc are at 1.25, 3.31, 3.69, 5.90, 6.40 and 8.64 keV, respectively.

To build PLS models, 43 samples were used for calibration set (24 Benicarló samples, 10 Valencia samples and 9 Murcia samples) and remaining 17 samples were used for validation set (14 Benicarló samples, two Valencia samples and one Murcia sample). The concentration range, mean and standard deviation for each one of elements determined in artichokes for both, calibration and validation, sets are shown in Table 9.2. In all PLS-XRF models, from the total spectral region (2047 variables) only 708 variables those corresponding to the energy range (0.9-14 keV) were used for built the models. For the selection of the optimum number of LV, several parameters such as PRESS, RMSECV and X and Y explained variances were evaluated, the same as those used for the NIR methodology.

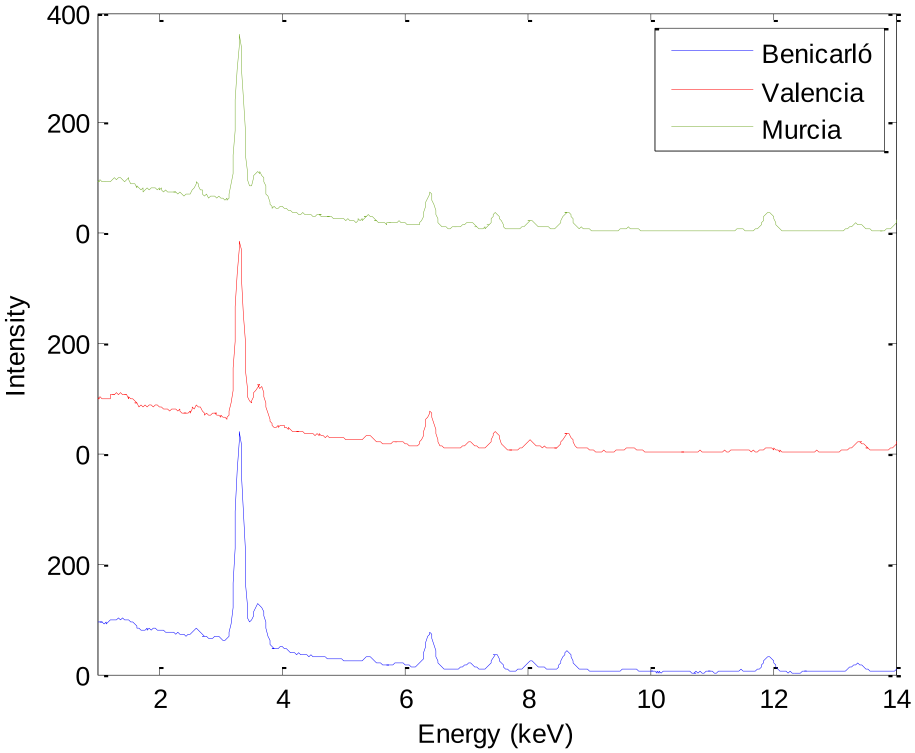

Figure 9.3. Mean XRF spectra of artichoke samples produced in different areas

NOTE: spectra were shift in the Y axe for clarity

9.3.5. PLS-XRF models for determination of minerals in artichokes

For iron, manganese and zinc, six, four and five LV, respectively, were necessary to build good models. Explained variances for X and Y were 96.9 and 95.5% for iron, 96.3 and 90.8% for manganese and 90.1 and 95.9% for zinc, respectively. For calcium, potassium and magnesium, it was needed five, two and five LV, respectively, to build the best models. Explained variances for X and Y were 95.0 and 96.7% for calcium, 97.2 and 96.1% for potassium and 92.9 and 91.2% for magnesium. For calibration models built from XRF spectra, RPD values were greater than 2, in all cases. Therefore, these calibration models could be considered as excellent. For potassium,

Table 9.2. Reference data and statistics of mineral content of artichokes used for calibration and validation sets employed for PLS-XRF determination

Element	Set	Range	Mean	SD	Pre-process	LV	R^2 cal	RMSEC	R^2 CV	RMSECV	R^2 val	RMSEP	RRMSEP
Ca	CAL	998-3384	1805	630	MC	5	0.97	113	0.91	200	0.96	150	9.0
	VAL	900-3087	1668	556									
K	CAL	28268-50541	40460	6013	MC+FD	2	0.96	1175	0.95	1350	0.93	1280	3.1
	VAL	25882-48021	41767	6122									
Mg	CAL	2058-3533	2851	418	MC	5	0.91	122	0.55	280	0.80	149	5.2
	VAL	1816-3503	2855	404									
Fe	CAL	17.7-78.7	50.5	14.6	MC+FD	6	0.96	2.93	0.76	6.74	0.79	6.96	14.3
	VAL	21.5-68.6	48.6	14.1									
Mn	CAL	6.7-28.8	18.4	5.6	MC+FD	4	0.91	1.69	0.71	3.0	0.76	3.25	17.7
	VAL	9.8-28.9	18.4	6.0									
Zn	CAL	16.7-60.0	34.0	12.0	MC+FD	5	0.96	2.42	0.84	4.82	0.90	5.10	14.5
	VAL	11.7-61.6	35.1	15.5									

NOTE: Range, mean and standard deviation (SD) in mg kg^{-1} dry weight for calibration (CAL) and validation (VAL) data sets. RMSEC, root-mean-square error of calibration in mg kg^{-1}; RMSECV, root-mean-square error of cross-validation in mg kg^{-1}; RMSEP, root-mean-square error of prediction in mg kg^{-1}; RRMSEP, relative root-meansquare error of prediction in percentage (%).

iron, manganese and zinc first derivate and mean centring were used for spectra pre-processing, and for calcium and magnesium only mean centred was chosen. Concentration data were mean centred and auto scaled in all cases. The most important calibration parameters of the PLS-XRF models are summarised in Table 9.2.

9.3.6. Prediction capability of PLS-XRF for mineral element determination in artichokes

External validation was used to evaluate the PLS-XRF methodology, using 17 artichoke samples not chose to create the corresponding models. Predicted values of analytes versus those obtained by the reference method are shown in Figure 9.4. As it can be seen, calibration and validation sample points were distributed near the optimum regression line between predicted and measured values with zero intercept and one slope. Coefficients of determination in the validation set were good and varied between 0.76 for manganese and 0.96 for calcium (see Table 9.2). The differences found in the external validation set between predicted concentrations and reference values vary from an average value between 8.4% for calcium, 2.5% for potassium, 4.4% for magnesium, 14.7% for iron, 14.1% for manganese and 13.2% for zinc. So, it can be concluded that the accuracy of the validation is good for calcium, potassium and magnesium, but it is only acceptable for iron, manganese and zinc, since the latter prediction errors are greater than 10%. The precision of the models built was very high, for potassium and manganese with RSD values lower than 4 %, and in all cases this parameter was lower than 9 %. The minimum RRMSEP obtained was 3.1% for potassium and in all cases, it was lower than 18% (See Table 9.2). These results, together with the obtained RPD values, demonstrate the excellent predictive capability of the PLS-XRF models developed to predict the concentration of calcium, potassium, iron, magnesium and zinc in artichokes, and a good predictive capability for manganese concentration.

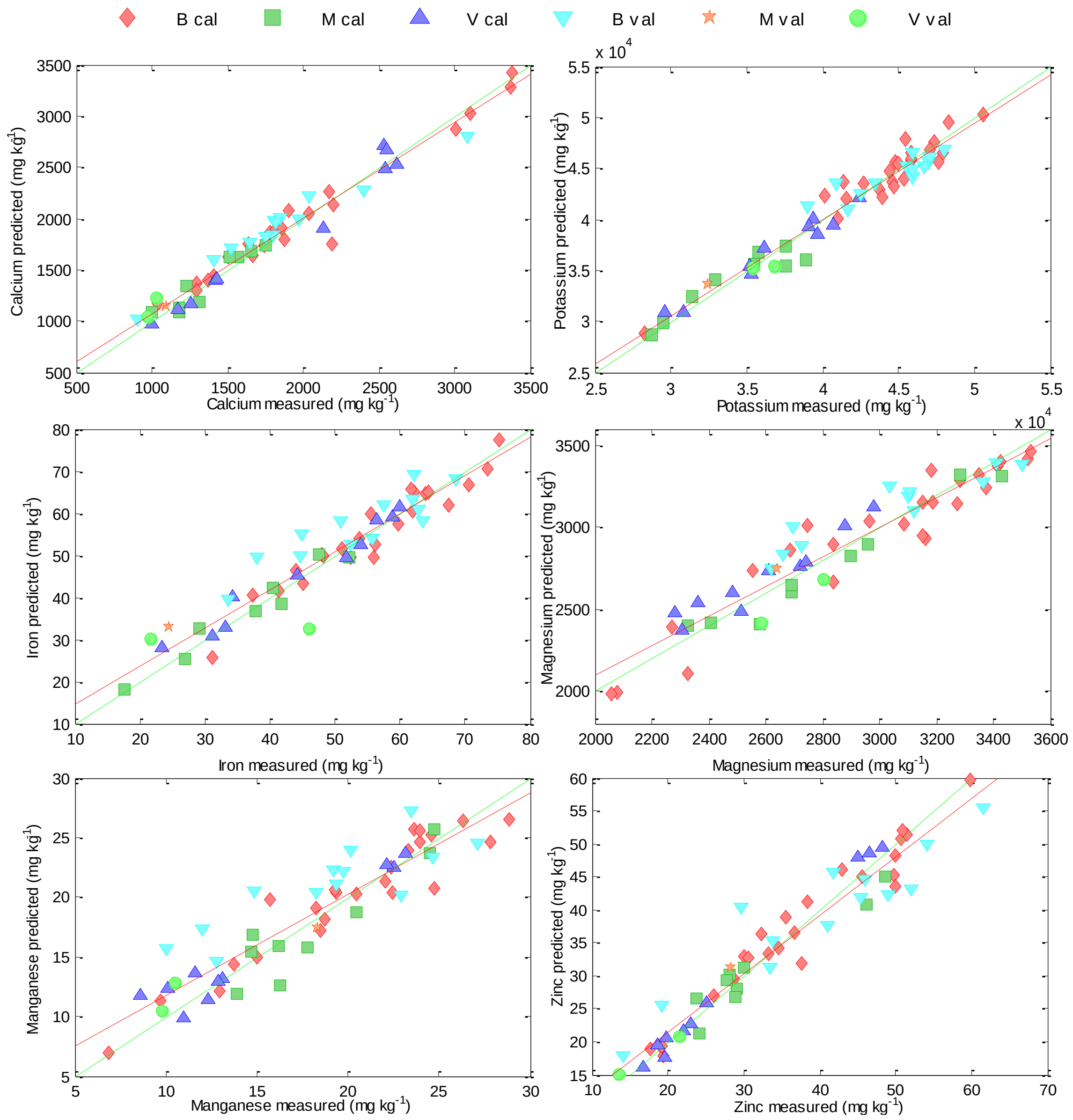

Figure 9.4. Predicted values by PLS-XRF vs. reference values of mineral elements in artichoke samples

NOTE: B samples from Benicarló, M samples from Murcia, V samples from Valencia, cal samples from calibration set, val samples from validation set.

9.3.7. Comparison of NIR and XRF for mineral analysis of artichokes

PLS models were established to determine mineral elements in artichokes using NIR and XRF spectra. Prediction results for calcium, potassium, iron, magnesium, manganese and zinc in artichokes are shown in Table 9.1 and 9.2. For calcium determination PLS-XRF (R^2_{val}=0.96, RMSEP=150 mg kg^{-1}) model provided better results than PLS-NIR (R^2_{val}=0.61, RMSEP=355 mg kg^{-1}). Additionally, PLS-XRF model was built with five LVs while NIR model needed thirteen LVs. For prediction of potassium content PLS-XRF was the best alternative (R^2_{val}=0.93, RMSEP=1280 mg kg^{-1}) with two LVs. For determination of magnesium, PLS-XRF with five LVs provided R^2_{val}=0.80 and RMSEP=149 mg kg^{-1}, thus, better results than the NIR model. For iron prediction, PLS-XRF and PLS-NIR models were very similar. However, for NIR two LVs were used while in XRF six LVs were needed. For manganese results found by XRF were R^2_{val}=0.76 and RMSEP=3.25 mg kg^{-1}, while PLS-NIR prediction results were R^2_{val}=0.54 and RMSEP=2.8 mg kg^{-1}. For zinc determination, the best method was PLS-XRF which provided very good values (R^2_{val}=0.90 and RMSEP=5.10 mg kg^{-1}).

From the results obtained, it can be concluded that XRF presents lower calibration, cross validation, and prediction errors than NIR, with better coefficients of prediction for the determination of all assayed mineral elements which, in fact is quite correct taking into account that XRF bands are close related to the presence of mineral elements being NIR bands related to the overtones of the main molecular stretching.

9.3.8. Contribution of artichokes to the daily mineral intake of essential elements

The contribution of artichoke consumption to the calcium, iron, potassium, magnesium, manganese and zinc to daily intake was evaluated from the recommended daily allowance (RDA) and adequate intake (AI), considering the consume of three pieces of artichoke of approximately 43 g of fresh weight each one. The percentage of RDA or AI was calculated as milligrams of essential elements provided a sample taking into account that the RDA values for men between 19 and 50 years are 1000, 410, 8 and 11 mg day^{-1} for calcium, magnesium, iron and zinc; and for women, in the same period of age, are 1000, 320, 18 and 8 mg day^{-1}, respectively. The adequate intake values for men are 4700 and 2.3 mg day^{-1} for potassium and manganese; and for women 4700 and 1.8 mg day^{-1} [23]. So, the contribution of calcium and zinc can be between 4% and 9% of the RDA, and for iron 6% regarding women consumption, being the aforementioned values a little bit low. The percentage of iron for men, potassium, magnesium and manganese are 14, 19, 15-19% and 17-22%, respectively. These values could be considered as adequate. However, it is clear that artichoke consumption is not the main source of studied minerals for humans.

9.3.9. Greenness of the methods employed for mineral elements determination in artichokes

The greenness of the methodologies employed were evaluated by using the eco-scale proposed by Van Aken et al. (2006) [24], developed by Galuszka et al. (2012) [25] and modified by Armenta et al. (in press) [26] to establish the green certificate of analytical methods. The modified system includes a new criterion to quantify the penalty point values as a function of the volume of consumed reagents and generated wastes through the use of mathematical expressions and to associate the eco-scale value to a category class, from A to G, in the so called Green Certificate. The general form of employed equations is as following

$$(y = a \times x^{b}) \qquad (Equation\ 9.1)$$

being a=0.61±0.05 and b=0.31±0.02 for reagents consumption and a=1.50±0.08 and b=0.40±0.02 for waste generation. So, the penalty points obtained for reagents consumption should be multiplied by coefficients of hazards: the absence of pictograms in the Globally Hazardous System Classification and Labelling of Chemicals concerning 0 penalty points, less severe hazard, described by warning pictogram, concerns 1 penalty point and more severe hazard indicated by danger pictogram involves 2 penalty points.

The energy consumes, together with the amount of reagents employed and their toxicity, were evaluated, also considering the amount of wastes generated and the occupational hazard of method application. From the initial scale of 100 points, NIR methodology and XRF methodology only involve 1 penalty point for the consume of energy, due to the use of sample lyophilisation. So, it can be concluded that these are ideal green methods, with a category class of green certificate A. ICP-OES methodology, from an initial scale of 100 points, involves 3 penalty points due to the use of HNO_3 for the treatment of samples, the use of standard solutions for calibration and argon in plasma generation; the energy consumes by the lyophiliser, microwave oven and ICP-OES provided additional penalty of 3 points. Extra points from occupational hazard and wastes involve 8.5 penalty points. So, the final score of the method was 85.5, obtaining a B class in green certificate. So, it can be concluded that NIR and XRF methods evaluated through this study are clearly green alternatives to the determination of mineral elements in artichoke samples.

9.4. Conclusions

NIR and XRF spectroscopy techniques were evaluated to determine calcium, potassium, magnesium, iron, manganese and zinc in artichoke samples. PLS models built from NIR and XRF spectra allowed the determination of mineral concentrations with quite good results in a green way. Artichoke samples can be analysed without any chemical sample preparation except physical lyophilisation. Proposed methodologies are greener than conventional ICP-OES methodology from the reagents, time-consuming and analysis cost.

Therefore, from the obtained results, it can be seen that NIR spectroscopy and X-ray fluorescence techniques offer fast and green alternatives to determine calcium, potassium, magnesium, iron, manganese and zinc in artichokes, being XRF the best choice in the main part of cases.

Acknowledgements

Authors acknowledge the financial support the Ministerio de Economia y Competitividad-Feder (project CTQ2011-25743 and project CTQ2012-38635) and Generalitat Valenciana (project PROMETEO-II 2014-077). Alba Mir-Marqués acknowledges the grant ‘Atracció de Talent’ (PREDOC13-110514) provided by the University of Valencia.

References

[1] dos Santos, A.M.P., Lima, J.S., Anunciacao, D.S., Souza, A.S., dos Santos, D.C.M.B., & Matos, G.D. (2013). Determination and evaluation employing multivariate analysis of the mineral composition of broccoli (*Brassica oleracea* L. var. Italica). *Food Analytical Methods*, 6, 745–752.

[2] Kelly, S.D., & Bateman, A.S. (2010). Comparison of mineral concentrations in commercially grown organic and conventional crops - Tomatoes (*Lycopersicon esculentum*) and lettuces (*Lactuca sativa*). *Food Chemistry*, 119, 738–745.

[3] Mahmood, T., Anwar, F., Iqbal, T., Bhatti, I.A., & Ashraf, M. (2012) Mineral composition of strawberry, mulberry and cherry fruits at different ripening stages as analyzed by Inductively Coupled Plasma-Optical Emission Spectroscopy. *Journal of Plant Nutrition*, 35, 111–122.

[4] Mir-Marqués, A., Domingo, A., Cervera, M.L., & de la Guardia, M. (2015). Mineral profile of kaki fruits (*Diospyros kaki* L.). *Food Chem*istry, 172, 291-297.

[5] Mitic, S.S., Obradovic, M.V., Mitic, M.N., Kostic, D.A., Pavlovic, A.N., Tosic, S.B., & Stojkovic, M.D. (2012). Elemental composition of various sour cherry and table grape cultivars using Inductively Coupled Plasma Atomic Emission Spectrometry method (ICP-OES). *Food Analytical Methods*, 5, 279–286.

[6] Harmankaya, M., Al Juhaimi, F., & Musa Özcan, M. (2012). Mineral contents of Jerusalem artichoke (*Helianthus tuberosus* L.) growing wild in Turkey. *Analytical Letters*, 45, 2269-2275.

[7] Paudino, G., Lombardo, S., Mauromicale, G. (2011). Mineral profile in globre artichoke as affected by genotype, head part and environment. *Journal of Science and Food Agricultural*, 91, 302-308.

[8] Terzic, S., Atlagic, J., Maksimovic, I., Zeremski, T., Zoric, M., Miklic, V., Balalic, I. (2012). Genetic variability for concentrations of essential elements in tubers and leaves of Jerusalem artichoke (*Helianthus tuberosus* L.). *Scientia Horticulturae*, 136, 135-144.

[9] Schmitt, S., Garrigues, S., & de la Guardia, M. (2014). Determination of the mineral composition of foods by Infrared Spectroscopy: a review of a green alternative. *Critical Reviews in Analytical Chemistry*, 44, 186–197.

[10] de la Guardia, M. & Garrigues, S. (2015). *Handbook of mineral elements in food*. John Wiley & Sons, Chichester.

[11] West, M., Ellis, A.T., Potts, P.J., Streli, C., Vanhoof, C., Wegrzynek, D., & Wobrauschek, P. (2010). Atomic spectrometry update-X-ray fluorescence spectrometry. *Journal of Analytical Atomic Spectrometry*, 25, 1503–1545.

[12] Cozzolino, D., & Moron, A. (2004). Exploring the use of near infrared reflectance spectroscopy (NIRS) to predict trace minerals in legumes. *Animal Feed Science and Technology*, 111, 161–173.

[13] Cozzolino, D., Cynkar, W., Shah, N., & Smith, P. (2011). Quantitative analysis of minerals and electric conductivity of red grape homogenates by near infrared reflectance spectroscopy. *Computers and Electronics in Agriculture*, 77, 81–85.

[14] Manhas Verbi Pereira, F., Rodrigues Pereira-Filho, E., & Maretti Silveira Bueno, M.I. (2006). Development of a methodology for calcium, iron, potassium, magnesium, manganese, and zinc quantification in teas using X-ray spectroscopy and multivariate calibration. *Journal of Agricultural and Food Chemistry*, 54, 5723–5730.

[15] Moros, J., Llorca, I., Cervera, M.L., Pastor, A., Garrigues, S., & de la Guardia, M. (2008). Chemometric determination of arsenic and lead in untreated powdered red paprika by diffuse reflectance near-infrared spectroscopy. *Analytica Chimica Acta*, 613, 196–206.

[16] CRDO. (2014). Consejo Regulador de la Denominación de Origen "Alcachofa de Benicarló". Available from: www.alcachofabenicarlo.com

[17] Mir-Marques, A., Cervera, M. L., & de la Guardia, M. (2012). A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus. *Journal of Food Composition and Analysis*, 27, 160–168.

[18] Wise, B.M., Shaver, J.M., Gallagher, N.B., Windig, W., Bro, R., & Koch, R.S. (2006) Chemometrics Tutorial for PLS_Toolbox and Solo Eigenvector Research Inc, Wenatchee, USA.

[19] Wold, S., Sjöström, M., & Eriksson, L. (2001). PLS-regression: a basic tool of chemometrics. *Chemometrics and Intelligent Laboratory Systems*, 58,109-130.

[20] Kennard, R.W., & Stone, L. A. (1989). Computer aided design of experiments. *Technometrics*, 11, 137-148.

[21] Burns, D.A., & Ciurczack, E.W. (2007). Handbook of near-infrared analysis. CRC press, New York.

[22] Bellon-Maurel, V., Fernandez-Ahumada, E., Palagos, B., Roger, J.M., & McBratney, A. (2010). Critical review of chemometric indicators commonly used for assessing the quality of the prediction of soil attributes by NIR spectroscopy. *Trac-Trend in Analytical Chemistry*, 29, 1073-1081.

[23] Food and Nutrition Board, Institute of Medicine. (2011). *Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements*. National Academies Press.

[24] Van Aken, K., Strekowski, L., & Patiny, L. (2006). EcoScale, a semi-quantitative tool to select an organic preparation based on economical and ecological parameters, *Beilstein Journal of Organic Chemistry*, 2, 3.

[25] Galuszka, A., Konieczka, P., Migaszewski, Z.M., & Namiésnik, J. (2012). Analytical Eco-Scale for assessing the greenness of analytical procedures. *Trac-Trend in Analytical Chemistry*, 37, 61-72.

[26] Armenta, S., de la Guardia, M., & Namiesnik, J. (in press). Green Microextraction. In M. Valcarcel (Ed.), *Analytical Microextraction Techniques*. Bentham Science.

Capítulo 10

Autentificación de alcachofas con denominación de origen protegida mediante métodos espectroscópicos

Authentication of protected designation of origin artichokes by spectroscopy methods

Alba Mir-Marqués, Cristina Elvira-Sáez, M. Luisa Cervera, Salvador Garrigues and Miguel de la Guardia

Department of Analytical Chemistry, University of Valencia, Jerónimo Muñoz Building,
50th Dr. Moliner St., 46100 Burjassot (Valencia), Spain.

Received 4 February 2015,

Received in revised form 15 April 2015,

Accepted 4 May 2015,

Available online 9 May 2015

Food Control 59 (2016) 74-81

DOI 10.1016/j.foodcont.2015.05.004

Resumen

Muestras de alcachofa con denominación de origen protegida (DOP) “Alcachofa de Benicarló” fueron discriminadas de las producidas cerca de Valencia y Murcia, fuera del indicativo de la DOP. Plasma de acoplamiento inductivo (ICP-OES), infrarrojo cercano (NIR) y fluorescencia de rayos X (XRF) se ensayaron con el fin de lograr una correcta clasificación de muestras de alcachofa procedentes de tres regiones diferentes (Benicarló, Valencia y Murcia). Se usaron estudios quimiométricos para comparar la capacidad de clasificación de las alcachofas a partir de su origen usando los datos de concentración de elementos minerales determinados mediante ICP-OES, espectros NIR y espectros XRF. Para el tratamiento de datos se emplearon herramientas de análisis multivariante, como análisis de componentes principales (PCA), análisis jerárquico de clústers (HCA) y análisis discriminantes por mínimos cuadrados parciales (PLSDA). Este estudio evidenció que el perfil mineral de alcachofas es una buena manera para realizar una correcta identificación del origen de muestras y que el uso directo de espectros XRF de la parte comestible de alcachofas liofilizadas puede usarse como una alternativa barata y respetuosa con el medio ambiente para la autentificación de muestras producidas bajo la DOP “Alcachofa de Benicarló” y para diferenciarlas de las producidas en áreas cercanas.

Abstract

Artichoke samples with the protected designation of origin (PDO) ‘Alcachofa de Benicarló’ were discriminated from those produced in other towns surrounding Valencia and Murcia, outside the PDO frame. Inductively coupled plasma (ICP-OES), near infrared (NIR) and X-ray fluorescence (XRF) were assayed in order to achieve a correct sample classification of the three different origin sample groups (Benicarló, Valencia and Murcia). Chemometrics was used to compare the ability of classification of artichoke from their origin by using concentration data of mineral elements, determined by ICP-OES, NIR spectra and XRF spectra. Multivariate analysis tools, as principal component analysis (PCA), hierarchical cluster analysis (HCA) and partial least square discriminant analysis (PLSDA), were used for chemometric data treatment. This study evidenced that the mineral profile of artichokes is a good way for the correct identification of sample origin and that the direct use of XRF spectra of the lyophilised edible part of artichoke can be used as a cheap and green alternative for the authentication of samples produced in the PDO ‘Alcachofa de Benicarló’ and to differentiate from those produced in near areas.

10.1. Introduction

Authenticity is a quality criterion for food which has an increasing interest in Europe as a result of legislative protection of regional foods, based on the approval of different labels [1]. The PDO label identifies foodstuffs, with regional identity, that are produced, processed and prepared in a specific geographical area and protects regional foods in front of the concurrency of a cheapest production of the same food from other areas or countries [2,3]. From the economic point of view, food authenticity has a great importance for the sectors involved in food production and also for consumers. This is important because the food authentication helps to guarantee the characteristics and quality of food products and to prevent overpayment [4,5].

Artichoke (*Cynara Scolymus L.*) is one of the oldest cultivated plants, and it is used as a food since the 15th century. There are different types of artichokes but only ‘Blanca de Tudela’ variety is labelled as PDO 'Alcachofa de Benicarló'. The area of cultivation of the ‘Alcachofa de Benicarló’ includes the areas of Benicarló, Vinaròs, Càlig and Penyíscola (Castellón, Spain). The cultivation land is very flat with average slopes lower than 5%, and is practically at the sea level. This leads to a special micro clime that makes the production of the ‘Blanca de Tudela’ in the extra and first categories and permits the compaction of the fruit with a consequent increase of product quality, which translates into good quotes of the market. It must be also highlighted its unique dimple shape and its duration without sacrificing quality offered [6].

The use of analytical techniques is the best way to authenticate foods without any doubt in order to determine their geographical origin. There are many papers that discriminate foods produced inside PDO areas to those produced outside the covered region, using different analytical methods. The most commonly analytical techniques used are gas chromatography mass spectrometry (GC-MS) [7-9] and high performance liquid chromatography (HPLC) [10,11] generally employed for the determination of organic compounds. However, the concentration of organic compounds in foods varies easily due to many factors including weather conditions additionally than land composition. So, it is sometimes very difficult to have a definitive authentication based on the organic profile of foods. Because of that, isotope relation [12,13] and the mineral profile of foods [5] have been used to characterise foods from the soil type and the climatic conditions. The most commonly techniques employed to determine mineral elements in foods are flame atomic absorption spectrometry (FAAS) [14,15], inductively coupled plasma optical emission spectrometry (ICP-OES) [16-18] and inductively coupled plasma mass spectrometry (ICP-MS) [19,20].

On the other hand, in recent years, vibrational spectroscopy has been explored as a successfully alternative to classify and authenticate foods with advantages based on the direct acquisition of signals from untreated samples, or with a minimum preparation, which provides green analytical tools. Complementary, vibrational spectroscopy offers information about major and minor components [21]. Additionally, direct fluorescence signals obtained by energy dispersion X-ray (ED-XRF) provides also a potential way for the characterisation of foods [22].

The correct characterisation of PDO food generally involves the determination of many analytical features or the consideration of several variables included in the sample spectral fingerprinting and therefore requires the deployment of complex mathematical processes in order to obtain the relevant information. This is the main reason of the extended use of multivariate pattern recognition approaches to build successful models to verify PDO claims. Chemometric tools generally used for PDO authentication are principal component analysis (PCA), as unsupervised method [23,24], and linear discriminant analysis (LDA), partial least squares discriminant analysis (PLSDA) and soft independent modelling of class analogy (SIMCA) as supervised methods [17,18,25,26].

The main purpose of this study has been the authentication of artichoke samples produced from PDO ‘Alcachofa de Benicarló’ using their mineral profile information, and also the XRF and NIR spectra to differentiate between samples produced inside the PDO and those coming from near regions.

10.2. Materials and methods

10.2.1. Apparatus

An ICP-OES Optima 5300 DV inductively coupled plasma optical emission spectrometer Perkin Elmer (Norwalk, CT, USA), equipped with an auto sampler AS 93-plus and a GemCone nebuliser, was used for mineral profile determination. Samples were digested inside Teflon vessels of 100 mL inner volume using a microwave laboratory system, Ethos SEL from Millestone (Sorisole, Italy), equipped with a thermocouple for automatic temperature control and an automatic gas leaks detector.

For NIR measurements, it was employed a Multipurpose Analyser Fourier transform near-infrared (FT-NIR) spectrometer MPA model from Bruker (Bremen, Germany) equipped with an integrating sphere, used for the acquisition of diffuse reflectance spectra. For instrument control and data acquisition, the OPUS software 6.5 from Bruker was used.

X-ray fluorescence spectra were obtained using a portable model S1 Titan energy dispersive X-ray fluorescence (ED-XRF) spectrometer from Bruker equipped with a Rhodium X-ray tube and X-Flash® SDD detector. For instrument control, the S1RemoteCtrl and S1Sync software, from Bruker, were employed, and spectra were treated by the ARTAX software also from Bruker.

10.2.2. Reagents

Nitric acid 69% for trace analysis from Scharlau (Barcelona, Spain) was employed for sample digestion and ultrapure water, with a minimum resistivity of 18.2 MΩ cm, obtained using a Milli-Q plus Millipore system (Molsheim, France), was used for dilution of samples and standards.

Argon C-45 (purity higher than 99.995%), supplied by Carburos Metálicos (Barcelona, Spain), was employed as plasmogen and carrier gas for ICP-OES.

Stock solutions of a multi-element standard solution (100 mg L^{-1}) containing 26 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Se, Na, Sr, Tl, Ti, V and Zn) dissolved in 5% HNO_3 and a multi-element solution containing 20 mg L^{-1} Dy, Er, Eu, Gd, Ho, Lu, Sm, Sc, Tb, Tm, Yb and Y and 100 mg L^{-1} Ce, La, Nd and Pr dissolved in 5% HNO_3, both from Scharlau, were employed to prepare the calibration standards. Additionally, a 1 g L^{-1} calcium standard solution for atomic absorption, a 1 g L^{-1} magnesium standard solution for atomic absorption, a 1 g L^{-1} sodium standard solution for atomic absorption and a 1 g L^{-1} potassium standard solution for atomic absorption, all from Scharlau, were employed for calibration. A 1 g L^{-1} rhenium standard solution for ICP, from Scharlau, was used as internal standard.

Certified references materials as NIST 1573a (Tomato Leaves) and IAEA-359 (Cabbage) obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA) and from the International Atomic Energy Agency (Vienna, Austria) respectively, were used to control the accuracy of the method employed by determinations of the artichoke mineral profile by ICP-OES.

10.2.3. Samples

60 artichoke samples from different Spanish agriculture cooperatives were collected during the same period of time. Each sample corresponds to a mixture of 2-4 pieces of artichoke. 38 samples were produced under the PDO ‘Alcachofa de Benicarló’ (Castellón, Spain), 10 samples from Valencia (Spain) and 12 from Murcia (Spain). Samples were washed with ultrapure water and the edible parts were lyophilised, pulverised, sieved through a particle size of 250 µm and stored in polyethylene bottles until their analysis.

10.2.4. Analytical procedures

ICP-OES measurements

A 0.5 g of freeze-dried samples were digested according to a previous protocol developed for food analysis by Mir-Marqués et al. [27] using 10 mL of concentrate nitric acid and a digestion step of 15 min at 180ºC (700 W) inside a microwave oven. All samples were analysed in duplicate and blanks and certified reference materials were measured in each session in order to control possible contaminations and to assure the accuracy of the method. Digested samples were measured with a final dilution volume of 50 mL.

The calibration range for all the 42 elements evaluated was established from 0.01 to 2 mg L^{-1} for all elements except calcium, magnesium, sodium (10 to 100 mg L^{-1}) and potassium (20 to 900 mg L^{-1}). Rhenium (1 mg L^{-1}) was used as internal standard and added to all samples, blanks and standards for ICP-OES analysis. Additionally, control standards were measured for every series of 10 independent sample measurements and a reference material was analysed in each series.

The average measurement of blanks was subtracted from the analytical signals of digested samples after interpolation on the calibration graphs. Results were expressed as a basis of dry weight.

NIR measurements

Approximately, 0.5-1 g of dry samples were placed inside glass vials of 20 mL (22 mm of internal diameter and 44 mm high) to obtain their NIR spectra by diffuse reflectance. The vials were rotated to obtain triplicate measurements for each sample. Diffuse reflectance near spectra (DRIFT) were recorded in Kubelka-Munk units, in the range of 12500-3500 cm^{-1}, using a spectral resolution of 4 cm^{-1} and cumulating 50 scans per spectrum. The background spectrum was acquired from the closed integrating sphere using the same instrumental conditions than those employed for samples. The signals obtained were employed for artichoke authentication.

XRF measurements

A 0.12 g of dry samples were placed inside a pellet box and were compacted using a hydraulic press for 1 min at a pressure of $5.1 \cdot 10^3$ kg. Pellets were stored inside desiccators to avoid hydration from air humidity. Spectra were obtained by triplicate using 50 kV voltage, 11 µA current and 60 s acquisition time (30 s for high atomic number elements, and 30 s for low atomic number elements). Signals obtained, in the aforementioned conditions, were used for sample authentication.

10.2.5. Statistical analysis

Statistical multivariate analysis was performed with the Matlab software 2013a from Mathworks (Natick, MA, USA) and the PLS Toolbox 6.2 from Eigenvector Research Inc. (Wenatchee, WA, USA). Principal component analysis (PCA), hierarchical cluster analysis (HCA) and partial least squares discriminant analysis (PLSDA) were used to process the data of concentration of the mineral elements determined by ICP-OES and also to process NIR and XRF spectra of the studied samples.

Leave-one-out cross validation and external validation were assayed to validate the prediction quality of employed models and to prevent overfitting of the calibration. Additional samples, not included in the calibration set, were used for external validation. Kennard-Stone (KS) algorithm was used to select the most appropriate calibration and validation sample sets. Different data pre-treatments, such as, mean centred, autoscale, standard normal variate, first derivate and also combinations of them, were evaluated for NIR, ICP-OES and XRF measurements.

10.3. Results and discussion

10.3.1. NIR analysis of artichoke samples

The diffuse reflectance spectra of artichokes were employed as descriptor of samples collected from three different production areas and evaluated in order to be used for authentication purpose.

10.3.1.1. NIR spectra of artichoke samples

Figure 10.1 shows the typical near DRIFT spectra obtained between 9000 and 4000 cm^{-1} for Benicarló (B), Valencia (V) and Murcia (M) samples. Spectra regions between 12500-9000 cm^{-1} and 4000-3500 cm^{-1} were removed for data processing. The broad and strong peak around 8300 cm^{-1} was due to the 2nd overtone C-H stretching vibrations. At 7000–6800 cm^{-1} it can be seen a broad peak, which corresponds to the combination of C-H and the N-H stretch 1st overtone. Peak at 5800 cm^{-1} corresponds to the C-H stretch 1st overtone. O-H 1st and 2nd overtone can be seen at 5155 cm^{-1}. A strong peak at 4732 cm^{-1} was due to C-O stretch combination and O-H bend, characteristics of carbohydrates. Double peak at 4350 and 4250 cm^{-1} were due to C-H and CH_2 bend 2nd overtone, corresponding to proteins [28].

10.3.1.2. Chemometric treatment of NIR spectra

NIR spectra of artichoke samples were pre-processed using first derivate and mean-centring before the application of modelling procedures.

An exploratory analysis was initially performed in order to observe the existence of natural groups. For this purpose, PCA and HCA were applied to the spectral data set. PCA and HCA analysis did not show clear differences between the origin groups of samples considered.

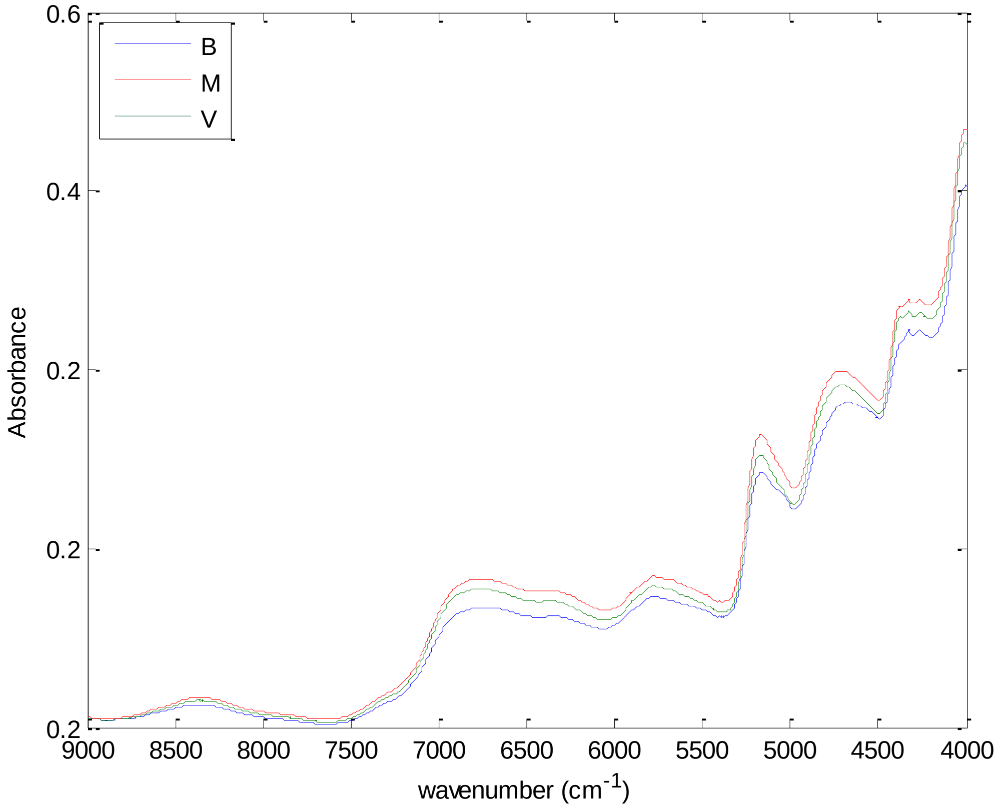

Figure 10.1. Mean DRIFT spectra of artichoke samples

The authentication of PDO ‘Alcachofa de Benicarló’ samples was performed by PLSDA. 44 artichokes (28 samples from PDO and 16 from not PDO) and 16 artichoke samples (10 samples from PDO and 6 from not PDO) were selected for calibration and validation sets by using the Kennard-Stone algorithm. Results found provided classification rates of 100% for calibration, 93% for cross validation and 88% for external validation in order to discriminate samples produced inside and outside the PDO (see Table 10.1), with five latent variables (LV), 95 and 87 % of explained variance of X and Y blocks, and regression coefficients of 0.87, 0.57 and 0.59 for calibration, cross validation and external validation, respectively. It must be noticed that two samples not produced inside the PDO were confused as coming from Benicarló.

Additionally, a study to identify the three origins of samples (Benicarló, Murcia and Valencia) was made. In this case, results were worse those obtained in the study of two classes. These results provided a correct classification of 98% for calibration, 82%

for cross validation and 80% for external validation (see Table 10.1). To build this model, six LV were chosen, with 95 and 74% of explained variance of X and Y blocks, respectively. One sample from Murcia was confused as coming from Benicarló and two samples from Valencia were identified as produced in Murcia and Benicarló. So, for a complete classification of artichoke samples origin from their NIR spectra, it can be seen the restricted capability of PLSDA.

Table 10.1. PLSDA external validation confusion matrix for the classification of artichoke samples from their NIR spectra

Assigned group **Original group**	**PDO**	**Not PDO**	**Total**	**% Correct**
PDO	10	0	10	100%
Not PDO	2	4	6	67%
Total	12	4	16	88%

Assigned group **Original group**	**Benicarló**	**Murcia**	**Valencia**	**Total**	**% Correct**
Benicarló	10	0	0	10	100%
Murcia	1	1	0	2	50%
Valencia	1	1	1	3	33%
Total	12	2	1	15	80%

10.3.2. Mineral profile of artichoke samples obtained by ICP-OES

The mineral profile of artichoke samples was used as a descriptor of their relationship with the production area, based on the interaction of plant-soil-weather conditions and from these data it was evaluated the capability of trace elements for the discrimination between samples produced in Murcia, Valencia and Benicarló.

10.3.2.1. Mineral content of artichoke samples by ICP-OES

Table 10.2 shows the minimum, maximum and mean concentration of 15 elements (Al, B, Ba, Cu, Fe, Mn, Mo, Ni, Sr, Ti, V, Zn, Ca, K, Mg and Na) which were present in almost samples at concentration levels higher than the limit of detection

(LOD) of the methodology classified, as a function of their production region. Although 42 elements were evaluated in the 60 artichoke samples analysed, chemometric calculations were performed using only 6 variables considering the content of Al, Fe, K, Na, Sr and V, in mg kg^{-1}, determined by ICP-OES which were identified as the most relevant variables in the PCA study.

10.3.2.2. Chemometric treatment of mineral composition of artichoke samples

PCA and HCA were applied to the data set of the concentration of the 6 selected elements to observe the existence of natural groups. For building the PLSDA method it was used the mineral content of the edible part of artichoke as variables. All data were auto-scaled and mean-centred before modelling procedures.

Hierarchical cluster analysis

As it can be seen in Figure 10.2, three clusters were obtained from the 60 samples considered for a variance weighted distance of 10. Cluster 1 concerns only the Murcia samples. Cluster 2 includes 14 samples from Benicarló PDO, one sample from Murcia and 12 samples from Valencia thus providing a difficult differentiation between artichoke production areas. Cluster 3 includes only samples from the PDO area. So, it can be seen that HCA allows a clear discrimination of samples from Murcia to those produced in Valencia and Benicarló, but does not differentiate between Valencia samples and Benicarló ones, thus being unable for PDO artichoke authentication from their mineral profile.

Table 10.2. Minimum, maximum and mean concentration of mineral elements determined in artichoke samples from different Spain production areas (expressed in mg kg^{-1} dry weight)

	Benicarló			**Valencia**			**Murcia**		
	MIN	**MAX**	**MEAN**	**MIN**	**MAX**	**MEAN**	**MIN**	**MAX**	**MEAN**
Al	< 1.1	38	7 ± 10	< 1.1	4.4	2.1 ± 1.0	<1.1	3.5	1.5 ± 0.7
B	< 4	25	5 ± 4	< 4	6.5	4.3 ± 0.8	< 4	6.6	4.2 ± 0.8
Ba	< 0.1	4	1.0 ± 0.8	< 0.1	2.0	0.7 ± 0.6	< 0.1	2.5	0.7 ± 0.8
Cu	1	12	7 ± 3	2.7	6.9	5.0 ± 1.5	3	11	5 ± 3
Fe	31	75	55 ± 11	22	60	43 ± 14	18	79	40 ± 17
Mn	7	29	20 ± 5	9	23	14 ± 6	14	25	18 ± 4
Mo	0.14	0.64	0.36 ± 0.11	0.22	0.41	0.31 ± 0.05	0.28	0.48	0.36 ± 0.06
Ni	< 0.3	1.0	0.4 ± 0.2	< 0.3	0.52	0.34 ± 0.09	< 0.3	< 0.3	< 0.3
Sr	2	13	6 ± 3	11	41	25 ± 12	19	78	42 ± 20
V	< 2	4.2	2.2 ± 0.3	2.2	4.6	3.2 ± 0.9	4	12	8 ± 3
Zn	12	62	37 ± 13	13	48	27 ± 13	24	49	31 ± 9
Ca	900	3400	1900 ± 600	1000	2600	1700 ± 700	1000	1800	1400 ± 300
K	26000	51000	44000 ± 5000	30000	42000	37000 ± 4000	29000	39000	34000 ± 4000
Mg	1800	3500	3000 ± 400	2300	3000	2600 ± 200	2300	3400	2800 ± 400
Na	400	2200	1000 ± 400	300	2000	1200 ± 600	1000	6000	4000 ± 2000

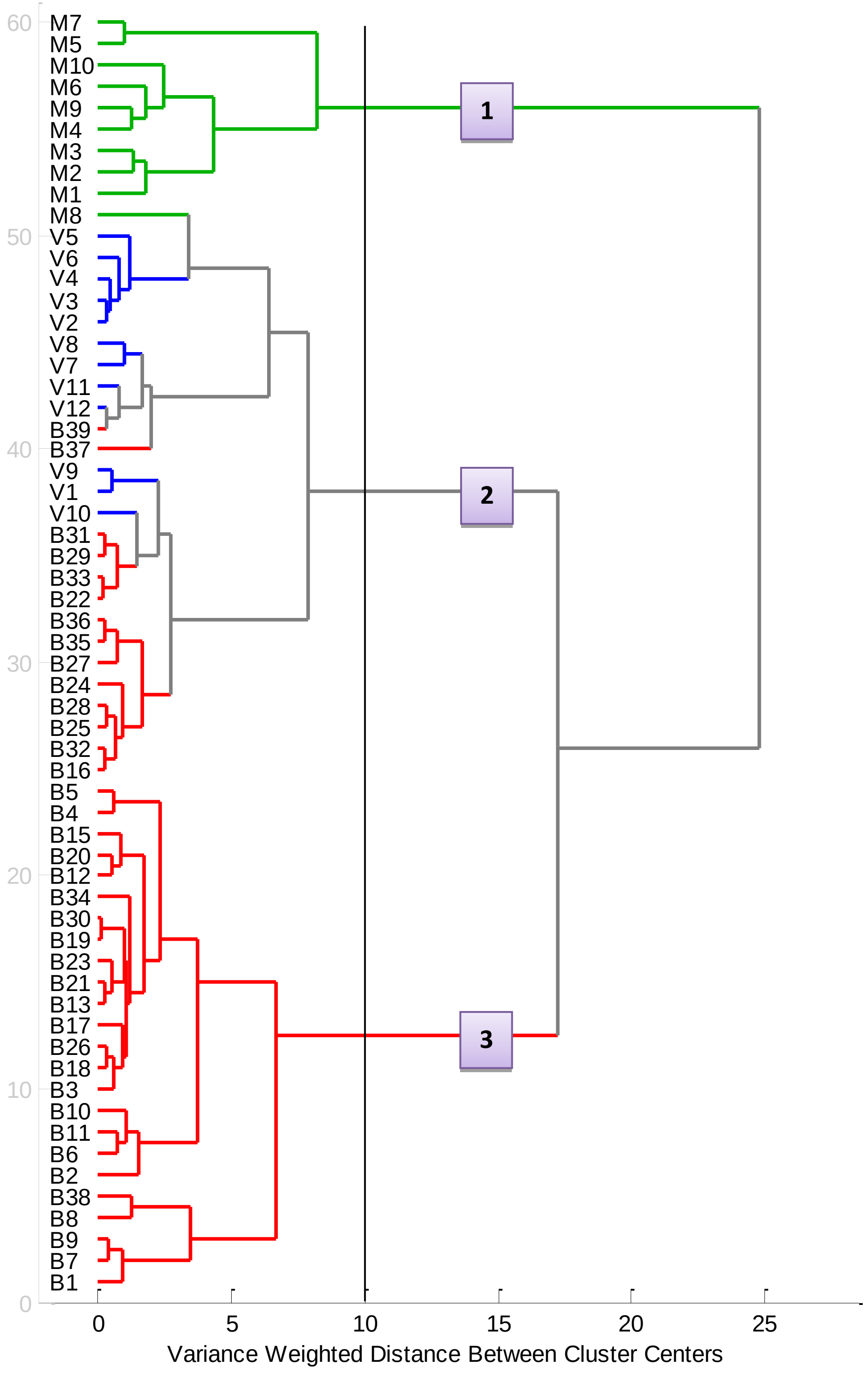

Figure 10.2. Cluster classification of artichoke samples obtained from their mineral profile

NOTE: B, V and M indicate samples from Benicarló, Valencia and Murcia origin, respectively.

Principal component analysis

The loadings of original mineral profile variables in the two first considered principal components were used to discriminate artichoke samples (see Table 10.3). Principal component 1 (PC1) represents 52.30% of the total variance, including strontium, vanadium, sodium and potassium, which were identified as the dominant variables in the PC1, while iron and aluminium show the lowest values. PC2 explains 20.04% of the total variance. Evaluating the loadings, potassium, iron and sodium resulted as the most dominant variables in PC2. The two first principal components take into account 72.34% of the total variance of the mineral profile of artichokes, and it allows differentiating three groups by origin almost 100%, except two samples of Benicarló and one sample of Murcia which were grouped from Valencia.

Table 10.3. Loadings of the variables considered for the two first Principal Components established from the mineral profile of artichoke samples produced in different parts of Spain

Variable	PC1	PC2	Variable	PC1	PC2
Al	-0.21491	0.07135	**Na**	0.47213	0.36608
Fe	-0.28216	0.70526	**Sr**	0.50000	0.28655
K	-0.41374	0.48772	**V**	0.48010	0.20702

Partial least squares discriminant analysis

Authentication of artichokes with protected designation of origin from its mineral profile was carried out by building PLSDA models. Two groups of samples were selected by using the Kennard-Stone algorithm, a first group to calibrate the model and another group to validate it. The calibration set consisted of 44 samples, of which 28 belonged to the PDO and 16 samples from Valencia and Murcia. For the validation set 16 samples were selected, 10 from the PDO and 6 samples outside the PDO. Results found for this model, with five latent variables and an explained variance of 95 and 78%

for X and Y blocks, respectively, were 100, 93.4 and 100% of correct classification rate for calibration, cross validation and validation external (see Table 10.4).

Table 10.4. PLSDA external validation confusion matrix for the classification of artichoke samples from their mineral profile

Assigned group Original group	**PDO**	**Not PDO**	**Total**	**% Correct**
PDO	10	0	10	100%
Not PDO	0	6	6	100%
Total	10	6	16	100%

Assigned group Original group	**Benicarló**	**Murcia**	**Valencia**	**Total**	**% Correct**
Benicarló	10	0	0	10	100%
Murcia	0	2	0	2	100%
Valencia	0	0	3	3	100%
Total	10	2	3	15	100%

To differentiate between the three geographical areas assayed (Benicarló, Murcia and Valencia), following the above methodology, with 45 samples used for calibration (28 samples from Benicarló, 8 from Murcia and 9 from Valencia) and 15 samples for external validation (10 samples from Benicarló, 2 from Murcia and 3 from Valencia), results were similar to those mentioned above, getting classification coefficients of 100, 93.4 and 100% for calibration, cross-validation and external validation, respectively (see Table 4). In this case, the explained variances for X and Y blocks were 97 and 71%, with five LV. So, it can be concluded that the capability of PLSDA for a correct authentication of artichoke samples from their mineral profile is very good, being thus possible to discriminate correctly the artichokes produced inside the PDO at a 100% level, in front of those produced in close areas.

10.3.3. XRF analysis of artichoke samples

The energy dispersive X-ray fluorescence spectra are directly related to the mineral composition of foods and were employed, in this study, as a fingerprint of artichoke samples in order to do their origin authentication.

10.3.3.1. XRF spectra of artichoke samples

The XRF spectra of artichoke samples of different origin are similar. However, as it can be seen in Figure 10.3, spectra present some differences that allow their discrimination. Peak at 3.3 KeV, corresponding to Kα of potassium, in Benicarló samples is higher than in the other origin samples. In addition, Murcia and Benicarló samples have a peak at 12 KeV corresponding to Kα of bromine, while in Valencia samples this peak does not appear. So, it indicates that bromine could be a discriminating element. On the other hand, samples from Valencia and Murcia have a fairly strong peak at 14.3 KeV that belongs to Kα of strontium and another less intense at 2.7 KeV belonging to Kα of chlorine which could make differentiation from Benicarló samples. Other peaks present in the spectra of artichoke samples correspond to Kα of calcium (3.7 KeV), chromium (5.45 KeV), copper (8 KeV), nickel (7.6 KeV) and zinc (8.6 KeV) with intensities very similar between samples from different sources with the exception of calcium, which has a peak height a little bit higher in artichokes coming from Benicarló than that found in samples from Murcia and Valencia.

10.3.3.2. Chemometric treatment of XRF spectra of artichoke samples

The XRF spectra were used directly to discriminate between PDO ‘Alcachofa de Benicarló’ and other ones. In this sense, XRF spectra of the edible part of the samples were used as chemical descriptor for building the PLSDA method. PCA and HCA were also performed, based just on the XRF spectra, in order to observe the existence of natural groups. All data were auto-scaled and mean-centred before to make the modelling procedures.

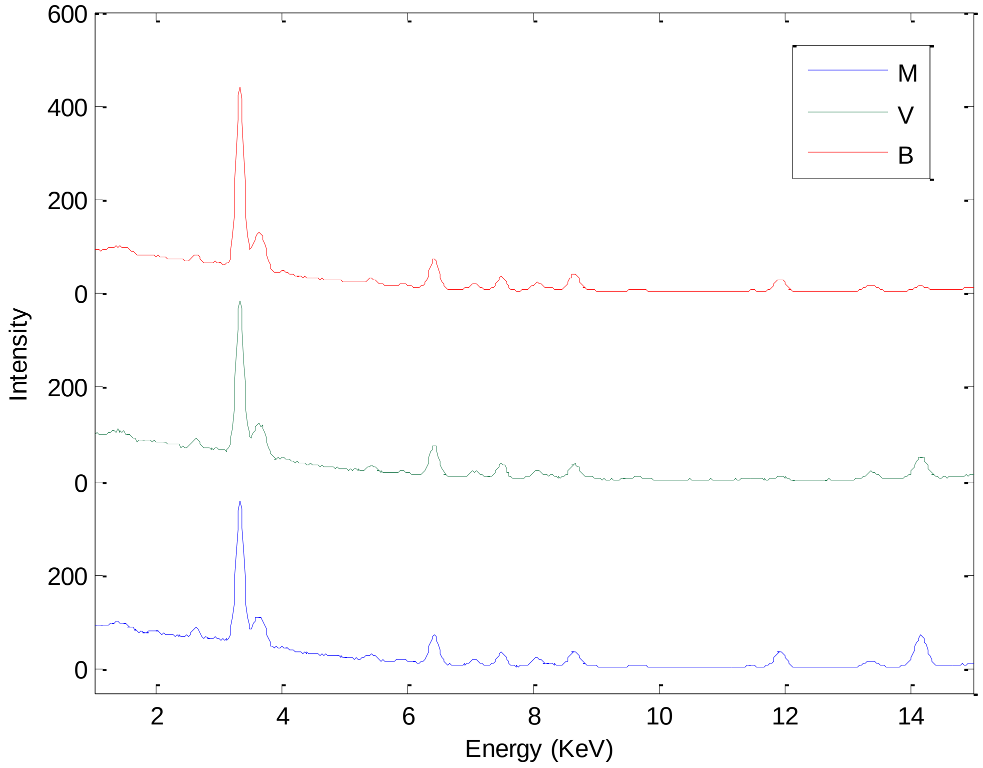

Figure10. 3. Mean XRF spectra of artichoke samples produced in different areas

NOTE: spectra were shift in the Y axe for clarity

Hierarchical cluster analysis

Four clusters were obtained, for a variance distance of 100, for the 60 samples considered. Cluster 1 concerns 23 samples of Benicarló, cluster 2 contains six samples from Murcia and five samples from Valencia, cluster 3 includes 14 samples from the PDO and one from Valencia and cluster 4 contains samples from Benicarló (1), Valencia (6) and Murcia (4). So, it can be concluded that HCA of the XRF spectra of artichokes allows the discrimination of the main part of samples produced from the Benicarló PDO to those produced in Valencia and Murcia samples. However, the distribution of the Benicarló samples in cluster 2 and 4 and the difficulty for a correct differentiation between Valencia and Murcia artichoke samples, evidences that it is not a good chemometric tool for the treatment of XRF spectra and other tools must be assayed for the treatment of these signals.

Principal component analysis

From the loadings of original variables corresponding to the two first considered principal components obtained for the evaluation of the XRF spectra of artichoke samples, it was observed that PC1 represents 86.36% of the total variance, being identified signals of strontium, bromine, iron and potassium, in the XRF spectra, as the dominant variables, while chromium and nickel peaks show the lowest values. PC2 explains 2.50% of the total variance. Potassium and strontium fluorescence areas result as the most dominant variables in PC2. PC1 and PC2 take account 88.86% of the total variance, and allow differentiating between samples produced in Benicarló and those coming from Murcia and Valencia, but it does not discriminate the samples between Murcia and Valencia origin as it can be seen in Figure 10.4.

Partial least squares discriminant analysis

To discriminate samples from PDO ‘Alcachofa de Benicarló’ and samples from other Spanish origin, PLSDA models were built. Two sets of samples were used, one for calibration and another for external validation of the model; for which 44 (28 samples from PDO and 16 from not PDO) and 16 artichoke samples (10 samples from PDO and 6 samples from not PDO) were chosen, respectively. This model was built with two LV and 68 and 87% explained variance in the X and Y blocks, obtaining a correct classification ratio of 100, 98 and 100 % for calibration, cross-validation and external validation, and coefficients of regression between 0.73 and 0.90 (see Table 10.5).

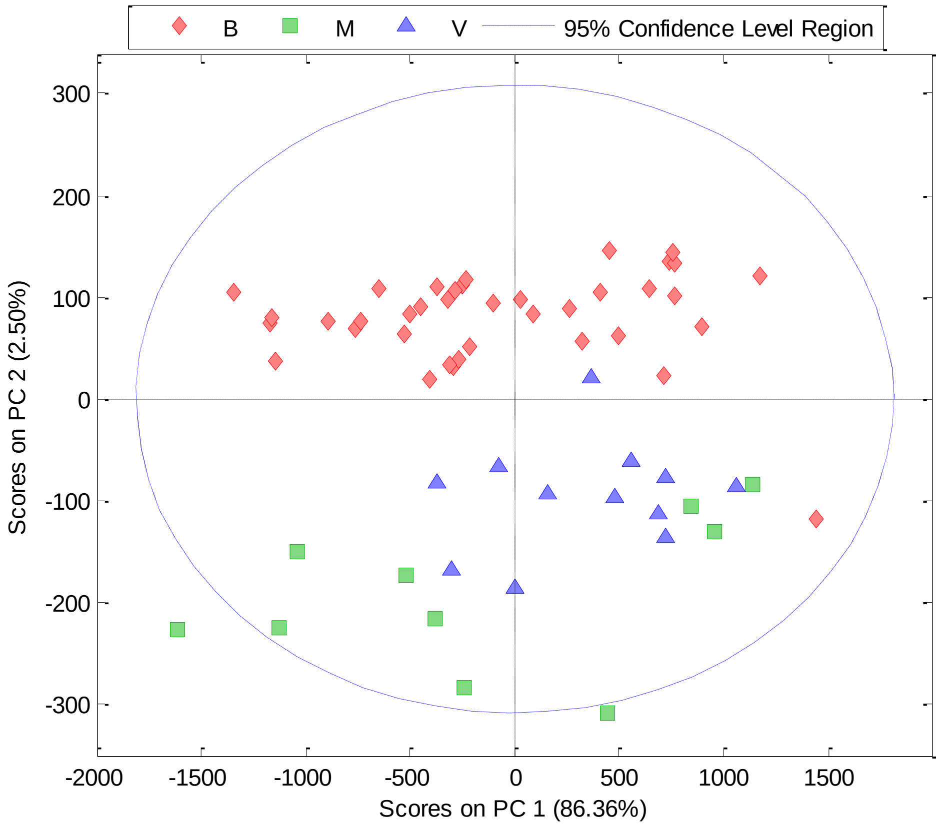

Figure 10.4. Principal components analysis of the XRF spectra of artichoke samples produced in different parts of Spain

Furthermore, PLSDA was performed by separating the samples in the three classes corresponding to Benicarló, Murcia, and Valencia. Results obtained after PLSDA treatment of XRF spectra were satisfactory, obtaining a classification rate of 100, 89 and 93% for calibration, cross validation and external validation, respectively (see Table 10.5). Three latent variables were chosen with explained variances of 79 and 88% for X and Y blocks.

As it can be seen, the capability of PLSDA for a correct classification of artichoke samples from their XRF spectra is quite good and comparable to that found based on the mineral profile obtained by ICP-OES with the advantage of the lack of sample treatment no signal calibration. This methodology allows the authentication of PDO ‘Alcachofa de Benicarló’ at 100% correct level in spite that one samples from Murcia was incorrectly identified as produced in Valencia.

Table 10.5. PLSDA external validation confusion matrix for the classification of artichoke samples from their XRF spectra

Assigned group **Original group**	**PDO**	**Not PDO**		**Total**	**% Correct**
PDO	10	0		10	100%
Not PDO	0	6		6	100%
Total	10	6		16	100%
Assigned group **Original group**	**Benicarló**	**Murcia**	**Valencia**	**Total**	**% Correct**
Benicarló	10	0	0	10	100%
Murcia	0	2	0	2	100%
Valencia	0	1	2	3	66.6%
Total	10	3	2	15	93.4%

10.3.4. Evaluation of green parameters of the assayed methods

The greenness of the methodologies employed were evaluated by using the eco-scale proposed by Van Aken et al. [29] and developed by Galuszka et al. [30] for evaluation of analytical methods. The energy consumes, together with the amount of reagents employed and their toxicity, were evaluated, also considering the amount of wastes generated and the occupational hazard of method application. From the initial scale of 100 points, NIR methodology and XRF methodology only involve 1 penalty point for the consume of energy, due to the sample lyophilisation. So, it can be concluded that these are ideal green methods. ICP-OES methodology, from an initial scale of 100 points, involves 11 penalty points due to the use of HNO_3 for the treatment of samples, the use of standard solutions for calibration and argon in plasma generation; the energy consumes by the lyophiliser, microwave oven and ICP-OES provided additional penalty of 3 points. Extra points from occupational hazard and wastes involve 8 penalty points. So, the final score of the method was 78 which mean an excellent green analysis although clearly worse than that based on NIR and XRF spectra.

10.3.5. Comparison of NIR, ICP-OES and XRF for origin authentication of artichokes

In view of the obtained results in this study, which are summarized in Table 10.6, if it wants to authenticate the samples belonging to the PDO ‘Alcachofa de Benicarló’, both, ICP-OES and XRF provide satisfactory results at 100% level. So, XRF technique can be used as an alternative to the ICP-OES, as it is a much greener technology, as it can be seen in the 10.3.4 section, much faster, as it requires little sample preparation and it is cheaper than ICP-OES.

However, if we want to discriminate perfectly the production origin of each sample, XRF provides satisfactory results to 93% of the samples included in the external validation set; which is quite good results, but if we want a perfect differentiation between the three origins studied, we would need to use ICP-OES.

Additionally, XRF and NIR spectra were used together to build PLSDA models and the results obtained are shown in Table 10.6. Results found were similar to those obtained only by XRF spectra. So, it could conclude that the joint use of NIR and XRF is not necessary since XRF provided satisfactory enough results.

10.4. Conclusions

Studies carried out evidenced the possibility to authenticate artichoke samples produced from the PDO ‘Alcachofa de Benicarló’ based on its mineral profile, established by ICP-OES, and sample XRF spectra after the appropriate chemometric treatment. On comparing these two analytical approaches it can be seen that the direct use of XRF spectra of the lyophilised edible part of artichokes provides a cheap direct and green way for the characterisation of the sample origin and the clear discrimination of samples produced inside the PDO to other samples coming from close areas. On the other hand, the use of NIR spectra is not powerful enough to do a correct identification of artichoke sample origin.

Table 10.6. Summary of obtained results by PLSDA

Technique	N classes	LV	% Variance X block	% Variance Y block	R^2_{cal}	R^2_{CV}	R^2_{pred}	% Correct of calibration	% Correct of cross validation	% Correct of external validation
NIR	2	5	95	87	0.87	0.57	0.59	100	93	88
	3	6	95	74	0-62-0.88	0.12-0.55	0.10-0.62	98	82	80
ICP-OES	2	5	95	78	0.78	0.67	0.79	100	96	100
	3	5	97	71	0.55-0.83	0.4-0.7	0.67-0.87	100	93	100
XRF	2	2	68	87	0.90	0.73	0.83	100	98	100
	3	3	70	88	0.84-0.91	0.3-0.75	0.44-0.82	100	89	93
NIR+XRF	2	4	85	92	0.92	0.58	0.89	100	96	100
	3	6	87	94	0.91-0.96	0.25-0.66	0.37-0.85	100	91	93

Acknowledgements

Authors acknowledge the financial support the Ministerio de Economia y Competitividad-Feder (project **CTQ2011-25743** and project **CTQ2012-38635**) and Generalitat Valenciana (project **PROMETEO-II 2014-077**). Alba Mir-Marqués acknowledges the grant '**Atracció de Talent**' provided by the University of Valencia.

References

[1] EU Regulation (2012). European Union Regulation (EEU) 1151/2012

[2] M. de la Guardia, A. Gonzálvez (Eds.), Food Protected Designation of Origin: methodologies and applications. Elsevier, Amsterdam, 2013.

[3] S. Kelly, K. Heaton, J. Hoogewerff. Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis. Trends Food Sci. Tech. 16 (2005) 555-567.

[4] S.A. Drivelos, C.A. Georgiou. Multi-element and multi-isotope-ratio analysis to determine the geographical origin of foods in the european union. Trac-Trend. Anal. Chem. 40 (2012) 38-51.

[5] A. Gonzálvez, S. Armenta, M. de la Guardia. Trace-element composition and stable-isotope ratio for discrimination of foods with Protected Designation of Origin. Trac-Trend. Anal. Chem. 28 (2009) 1295-1311.

[6] CRDO (2014). Consejo Regulador de la Denominación de Origen "Alcachofa de Benicarló". Available from: www.alcachofabenicarlo.com

[7] L. Aquilanti, S. Santarelli, V. Babini, A. Osimani, F. Clementi. Quality evaluation and discrimination of semi-hard and hard cheeses from the marche region (central italy) using chemometric tools. Int. Dairy J. 29 (2013) 42-52.

[8] F. Karali, A. Georgala, T. Massouras, S. Kaminarides. Volatile compounds and lipolysis levels of kopanisti, a traditional greek raw milk cheese. J. Sci. Food Agri. 93 (2013) 1845-1851.

[9] P. Montero-Prado, K. Bentayeb, C. Nerin. Pattern recognition of peach cultivars (Prunus persica L.) from their volatile components. Food Chem. 138 (2013) 724-731.

[10] J.C.M. Barreira, S. Casal, I.C.F.R. Ferreira, A.M. Peres, J.A. Pereira, M.B.P.P. Oliveira. Supervised chemical pattern recognition in almond (prunus dulcis) portuguese PDO cultivars: PCA- and LDA-based triennial study. J. Agri. Food Chem. 60 (2012) 9697-9704.

[11] R. Russo, V. Severino, A. Mendez, J. Lliberia, A. Parente, A. Chambery. Detection of buffalo mozzarella adulteration by an ultra-high performance liquid chromatography tandem mass spectrometry methodology. J. Mass Spectrom. 47 (2012) 1407-1414.

[12] S.D. Kelly, A.S. Bateman. Comparison of mineral concentrations in commercially grown organic and conventional crops - Tomatoes (Lycopersicon esculentum) and lettuces (Lactuca sativa). Food Chem. 119 (2010) 738-745.

[13] S. Swoboda, M. Brunner, S.F. Boulyga, P. Galler, M. Horacek, U. Prohaska. Identification of Marchfeld asparagus using Sr isotope ratio measurements by MC-ICP-MS. Anal. Bioanal. Chem. 390 (2008) 487-494.

[14] K. Ariyama, H. Horita, A. Yasui. Chemometric techniques on inorganic elements composition for the determination of the geographic origin of welsh onions. Anal. Sci. 20 (2004) 871-877.

[15] R. Moreno-Rojas, P.J. Sanchez-Segarra, F. Camara-Martos, M.A. Amaro-Lopez. Multivariate analysis techniques as tools for categorization of southern Spanish cheeses: Nutritional composition and mineral content. Eur. Food Res. Technol. 231 (2010) 841-851.

[16] K. Ariyama, Y. Aoyama, A. Mochizuki, Y. Homura, M. Kadokura, A. Yasui. Determination of the geographic origin of onions between three main production areas in japan and other countries by mineral composition. J. Agr. Food Chem. 55 (2007) 347-354.

[17] K. Boeting, Z.A. de Benzo, M.L. Cervera, M. de la Guardia. Authentication of the protected designation of origin horchata de Valencia through the chemometric treatment of mineral content. Anal. Method 2 (2010) 1723-1728.

[18] M.N. Matos-Reyes, J. Simonot, O. López-Salazar, M.L. Cervera, M. de la Guardia. Authentication of Alicante's Mountain cherries protected designation of origin by their mineral profile. Food Chem.141 (2013) 2191-2197.

[19] M. Costas-Rodriguez, I. Lavilla, C. Bendicho. Classification of cultivated mussels from galicia (northwest spain) with european protected designation of origin using trace element fingerprint and chemometric analysis. Anal. Chim. Acta 664 (2010) 121-128.

[20] F. Di Giacomo, A. Del Signore, M. Giaccio. Determining the Geographic Origin of Potatoes Using Mineral and Trace Element Content. J. Agri. Food Chem. 55 (2007) 860-866.

[21] S. Garrigues, M. de la Guardia, Vibrational Spectroscopy, in: M. de la Guardia, A. Gonzálvez (Eds.), Food protected designation of origin: methodologies and applications, Elsevier, Amsterdam, 2013, pp 101-122.

[22] S. Garrigues, M. de la Guardia (Eds.), Handbook of mineral elements in food, John Wiley & Sons, Chichester, 2015 (in press).

[23] A. Gori, R.M. Maggio, L. Cerretani, M. Nocetti, M.F. Caboni. Discrimination of grated cheeses by Fourier transform infrared spectroscopy coupled with chemometric techniques. Int. Dairy J. 23 (2012) 115-120.

[24] Z.X. Zhang, P. Liu, H.J. Kang, C.C. Liao, Z.L. Chen, G.D. Xu. A study of the diversity of different geographical populations of Emmenopterys henryi using FTIR based on principal component analysis and cluster analysis. Spectrosc. Spect. Anal. 28 (2008) 2081-2086.

[25] M. Bevilacqua, R. Bucci, A.D. Magri, A.L. Magri, F. Marini. Tracing the origin of extra virgin olive olis by infrared spectroscopy and chemometrics: a case study. Anal. Chim. Acta 717 (2012) 39-51.

[26] R. Korifi, Y. Le Dreau, J. Molinet, J. Artaud, N. Dupuy. Composition and authentication of virgin olive oil from French PDO regions by chemometric treatment of Raman spectra. J. Raman Spectrosc. 42 (2011) 1540-1547.

[27] A. Mir-Marqués, M.L. Cervera, M. de la Guardia. A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus. J. Food Comp. Anal. 27 (2012)160-168.

[28] D.A. Burns, E.W. Ciurczack (Eds.). Handbook of near-infrared analysis, 3rd edition. CRC press, New York, 2007.

[29] K. Van Aken, L. Strekowski, L. Patiny. EcoScale, a semi-quantitative tool to select and organic preparation based on economical and ecological parameters. Beilstein J. Org. Chem. (2006) 2:3.

[30] A. Galuszka, P. Konieczka, Z.M. Migaszewski, J. Namiésnik. Analytical eco-scale for assessing the greenness of analytical procedures. Trac-Trend. Anal. Chem. 37 (2012) 61-72.

Anexo

Food Chemistry 172 (2015) 238–244

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Mineral profile of Spanish commercial baby food

Alba Mir-Marqués, Anna González-Masó, M. Luisa Cervera *, Miguel de la Guardia

Department of Analytical Chemistry, University of Valencia, Jerónimo Muñoz Building, 50th Dr. Moliner St., 46100 Burjassot (Valencia), Spain

ARTICLE INFO

Article history:
Received 26 February 2013
Received in revised form 9 September 2014
Accepted 13 September 2014
Available online 20 September 2014

Keywords:
Baby food
Infant food
Essential elements
Toxic elements
Adequate intake (AI)
Estimated average requirement (EAR)
Inductively coupled plasma optical emission spectroscopy (ICP-OES)

ABSTRACT

Commercial baby foods are an important part of the daily intake of babies from 6 to 12 months. The mineral profile of commercial baby foods in Spain was determined to establish levels of essential and non-essential elements, and their contribution to adequate intake (AI) and estimated average requirement (EAR). Thirty-five jars of commercial foods containing meat, fish, vegetables and fruit were obtained from the Spanish market and the mineral composition determined for 14 elements. In general, the baby foods analysed were sufficient for an adequate mineral intake, but contributions to AI and EAR for iron, zinc and calcium were very low (5–20%, 10–60% and 10–70%, respectively). This deficiency could be associated with growth problems or diseases in adulthood, and fortification of commercial products is recommended.

1. Introduction

Adequate nutrition during infancy and early childhood is essential to ensure the growth, health, and development of children to their full potential. Poor nutrition increases the risk of illness in adulthood. Early nutritional deficits are also linked to long-term impairment in growth and health (WHO, 2009). From the age of 6 months, infants' needs for energy and nutrients start to exceed that provided by milk alone and thus, complementary feeding becomes necessary to fill the energy and nutrient gap (WHO, 2008). Complementary foods must be introduced at this age and their absence or inappropriate composition can affect growth and development. Complementary foods need to be safe and nutritionally adequate meet energy and nutrient needs (WHO, 2009).

In recent years, commercial baby jars have become an important part of baby food due changes in lifestyle, and because many families do not have time to prepare homemade alternatives. These foods are sometimes the main source of food for babies between 6 and 12 months; therefore, detailed analysis of the mineral content is of particular interest to ensure infants have a balanced diet to develop properly (Melo, Gellein, Evje & Syversen, 2008; Okesli, González-Bermúdez, Vidal-Guevara, Dalmau & Ros, 2011). In the aforementioned context, there are different parameters to be determined in baby food. In the case of essential elements, the recommended daily allowance (RDA), the adequate intakes (AI) and the estimated average requirement (EAR) must be considered. RDA is 'the amount of a nutrient that a healthy person should eat each day on average through diet to maintain good health', defined by the Food and Nutrition Board of the US National Academy of Sciences (Food and Nutrition Board, 2004). AI are the recommended average daily intakes based on observed or experimentally determined nutrient intake in a group of apparently healthy people that are assumed to be adequate; used when a RDA cannot be determined. EAR is the average daily nutrient intake level that meets the needs of 50% of healthy individuals in a particular age and gender group. For iron and zinc, EAR can be used as an estimation of the risk (%) for low intakes (Butte et al., 2010). In the case of toxic elements, the tolerable upper intake level (UL) is the most common parameter for non-essential elements used to evaluate safety limits for babies. It is an estimate of the highest level of intake that carries no appreciable risk of adverse health effects (EFSA, 2006).

In the literature, there are a large number of studies on breast and formula feeding, but there are few studies of complementary feeding (as baby foods) between 6 and 12 months, which according to European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) is the period where major changes occur in both macronutrients and micronutrients intakes (ESPGHAN, 2008). Thus, an exhaustive analysis of baby food mineral composition for this age group is essential. Table 1 shows currently available literature describing the analysis of essential and

---

* Corresponding author. Tel.: +34 96 354 3486; fax: +34 96 354 4845.
E-mail address: m.luisa.cervera@uv.es (M.L. Cervera).

http://dx.doi.org/10.1016/j.foodchem.2014.09.074
0308-8146/

Table 1
Published literature on the mineral composition of baby foods.

Sample	Country	*n*	Elements	Technique	References
Commercial baby food of vegetables (16) and fruits (16)	Belgium	32	Ca, Fe, Zn	FAAS	Bosscher et al. (2002)
Commercial baby food of chicken based (10) and hake based (4)	Spain, UK, China, USA	14	Ca, Na, Fe, Cu, Zn, Mn, Se, Cr, Co, Ni, As, Pb, Cd, Hg	ICP-MS, AAS	Carbonell-Barrachina, Ramirez-Gandolfo et al. (2012)
Commercial baby food of chicken based (11) and fish based (8)	Spain, UK, China, USA	19	As	ICP-MS, HPLC-ICP-MS	Carbonell-Barrachina, Wu et al. (2012)
Commercial baby food of vegetable based	Nigeria	4	Ca, Cu, Fe, K, Mg, Mn, P, Zn	ICP-OES	Fernandez et al. (2002)
Commercial baby food of meat, fish, vegetables and fruit	Norway	72	Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mo, Na, Ni, Zn	HR-ICP-MS	Melo et al. (2008)
Commercial and homemade baby food of lamb (4), beef (4) and chicken (4)	Spain	12	Na	FAAS	Okesli et al. (2011)
Commercial baby food	Italy, Spain, Slovakia, Sweden	112	Cd, Zn	ICP-OES, ICP-sf-MS	Pandelova et al. (2012)
Total Diet Survey	New Zealand		Fe, Na, Se	ICP-OES	Thomson et al. (2008)
Commercial baby food of meat (12) and vegetables (12)	UK	24	Ca, Cu, Mg, Fe, Zn, K, Na, Se	ICP-OES, ICP-MS	Zand et al. (2011)
Commercial baby food of chicken based (4) and fish based (4)	UK	8	Ca, Fe, Mg, K, Na, Zn, Se, Mo, Co, Cu, Cr, Mn, As, Ba, Ni, Cd, Sb, Pb, Hg, Al	ICP-OES, ICP-MS	Zand et al. (2012)

Note: Parentheses indicate the number of samples of each type of baby food analysed in each study. FAAS: Flame atomic absorption spectroscopy. AAS: atomic absorption spectroscopy. ICP-MS: inductively coupled plasma mass spectrometry. HPLC–ICP-MS: high performance liquid chromatography–inductively coupled plasma mass spectrometry. HR-ICP-MS: High resolution-inductively coupled plasma mass spectrometry. ICP-sf-MS: inductively coupled plasma sector field mass spectrometry.

non-essential elements in complementary baby foods, and it can be seen there are only a few references, which are often based on a limited number of elements (1–3) or the analyses of only a reduced number of samples. Considering the analytical approaches, it is clear the analysis of baby food mineral content requires accurate and sensitive techniques that allow multi-element determination. The use of optical emission spectroscopy with inductively coupled plasma (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) can be useful for determining the mineral profile of infant foods, in particular pureed and hermetically packaged ready meal (jars).

The main purpose of this study was to determine the level of essential and non-essential elements in commercially available baby foods in Spain and evaluate intake of essential elements as well as assessing the risk of exposure to toxic minerals.

2. Materials and methods

2.1. Sample collection

Thirty-five baby food samples (four brands, 1–4) available across the Spanish market sector were purchased. The samples were classified based on basic composition; meat (17), fish (7), vegetables only (5) and fruits only (6). The composition of each baby food sample analysed in this study is detailed in Table 2. Baby foods collected were recommended for children aged 6–12 months.

2.2. Apparatus

ICP-OES measurements were performed using a Perkin Elmer, Model Optima 5300 DV spectrometer (Norwalk, CT, USA) equipped with an auto sampler AS 93-plus and a cross flow nebulizer. Argon C-45 (purity higher than 99.995%), supplied by Carburos Metálicos (Barcelona, Spain), was employed as plasmogen and carrier gas.

A microwave laboratory system, Ethos SEL from Milestone (Sorisole, Italy), equipped with a thermocouple probe for automatic temperature control and an automatic gas leak detector, was employed for samples digestion using high pressure Teflon vessels of 100 mL inner volume. Teflon vessels were cleaned with vapours of nitric acid (69%), using the Trace CLEAN from Milestone (Sorisole, Italy) to avoid cross-contamination.

Other equipment used for sample pre-treatment included a Cryodos 50 lyophiliser Telstar (Barcelona, Spain) and an ultrasound water bath from Selecta (Barcelona, Spain, 9 L, 50 W, 50 Hz).

2.3. Reagents

A multi-element standard solution (100 mg L^{-1}) containing 26 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Se, Na, Sr, Tl, Ti, V and Zn) dissolved in 5% HNO_3 from Scharlau (Barcelona, Spain) was used to prepare the calibration standards. Additionally, a 1 g L^{-1} sodium standard solution for atomic absorption and 1 g L^{-1} potassium standard solution for atomic absorption, both from Scharlau, were employed for calibration.

A 1 g L^{-1} scandium atomic spectroscopy standard solution was purchased from Fluka (Buchs, Switzerland) and used an internal standard.

Nitric acid (HNO_3 69%) for trace analysis and hydrogen peroxide (H_2O_2 35%) reagent grade, both from Scharlau, were used for sample digestion.

All solutions were prepared with analytical reagent grade chemicals and ultrapure water (18 MΩ·cm) obtained by purifying distilled water with the Milli-Q Millipore system (Molsheim, France).

2.4. Sample pre-treatment

Baby food purée samples were poured into different containers and frozen at −20 °C in a freezer before lyophilisation. They were lyophilised for a minimum of 48 h at a chamber pressure of 50 Pa. Powdered samples were homogenised in a domestic Braun mixer (Kronberg, Germany) and stored in polyethylene bottles until analysis.

The water content of samples varied between 76.6% and 87.6%, and moisture was independent of brand and composition of baby food.

Freeze-dried samples (1 g) were accurately weighed into the Teflon digestion vessels and 8 mL of nitric acid were added, before being sonicated in an ultrasound water bath for 30 min at room temperature. Then 2 mL of hydrogen peroxide were added and the mixtures sonicated again for further 40 min. This approach was used to avoid foam formation and gas leaks during digestion. After that, the reactors were closed and placed inside the microwave oven. The

Table 2
Composition of baby foods analysed through this study.

	Sample	Composition	Recommended age	Brand
Meat	M1	Ham, beef and vegetables	From 6 month	1
	M2	Turkey with vegetables	From 6 month	1
	M3	Chicken with rice	From 6 month	1
	M4	Lamb stew	From 6 month	1
	M5	Lamb stew	From 6 month	2
	M6	Beef with vegetables	From 6 month	2
	M7	Rice and chicken	From 6 month	2
	M8	Mashed vegetables with chicken and beef	From 6 month	2
	M9	Mashed vegetables and turkey	From 6 month	2
	M10	Rice and beef	From 6 month	2
	M11	Chicken, beef and vegetables	From 6 month	1
	M12	Beef with carrots	From 6 month	1
	M13	Beef with vegetables	From 6 month	1
	M14	Chicken with vegetables	From 6 month	1
	M15	Vegetables and rice with turkey	From 8 month	3
	M16	Mashed zucchini with beef	From 8 month	4
	M17	Vegetables with beef	From 8 month	4
Fish	M18	Sole with white sauce	From 8 month	1
	M19	Rice with hake	From 8 month	1
	M20	Selected vegetables and sea bass	From 8 month	2
	M21	Selected vegetables and monkfish	From 8 month	2
	M22	Mashed peas and rice with hake	From 8 month	2
	M23	White sauce and hake	From 8 month	2
	M24	Whiting vegetables in cream	From 8 month	1
Vegetables	M25	Carrots with rice in chicken broth	From 4 month	1
	M26	Mixed vegetables	From 4 month	1
	M27	Cream of green beans with potatoes	From 6 month	1
	M28	Soft cream carrots and potatoes	From 8 month	4
	M29	Soft cream of peas with potatoes	From 8 month	4
Fruit	M30	Peach and banana	From 4 month	1
	M31	3 Fruit (pear, peach and banana)	From 4 month	1
	M32	Banana, tangerine and pear	From 4 month	1
	M33	Varied fruit (peach, apple, banana, apricot, orange)	From 4 month	1
	M34	Fruit salad and cereals	From 6 month	2
	M35	Tangerine, apple and biscuits	From 6 month	1

microwave digestion programme was optimised previously for digestion of adult complete diets (Mir-Marqués, Cervera, de la Guardia, 2012). The following program was run: 3 min to reach 85 °C; 85–145 °C for 12 min; 145–180 °C for 10 min; 15 min at 180 °C; and finally cooling down for 25 min. After digestion, the reactors were opened and sonicated. The digested samples were diluted in 20 mL polyethylene tubes with nanopure water before analysis using ICP-OES. Each sample, reagent blank and certified reference material (CRM) were digested in triplicate.

2.5. ICP-OES determination

The operating conditions of the ICP-OES equipment were as follow: 1200 W RF power, 15 L min^{-1} plasma gas flow rate, 0.5 L min^{-1} auxiliary gas flow rate, 0.7 L min^{-1} nebuliser gas flow rate, 1.10 mL min^{-1} sample flow rate. All elements were detected in axial mode except calcium, potassium, magnesium and sodium, which were detected in radial mode. For the background correction, 2 points were used. Multi-elemental standard solutions were used for external calibration. Two sets of standards were used for minor and trace elements (0, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2 and 5 mg L^{-1}) and for potassium and sodium (0, 2, 4, 6, 8 and 10 mg L^{-1}). Digested samples were measured with a final dilution 1:2, and 1:100 for calcium, potassium, magnesium and sodium. Scandium (0.5 mg L^{-1}) was used as the internal standard and added to all samples, reagents blanks and standards.

2.6. Quality control

To establish precision and accuracy, CRMs were analysed under the same conditions as samples: NIST 1568a Rice Flour (National Institute of Standards and Technology, Gaithersburg, MD, USA); TORT-2 Lobster Hepatopancreas (National Research Council, Ottawa, Canada) and IAEA-359 Cabbage (International Atomic Energy Agency, Vienna, Austria).

Two reagent blanks were prepared and measured together with each batch of samples, and CRMs were digested and measured together with sample batches. Additional, control standards were measured for every series of 10 independent sample measurements.

Detection (LOD) an quantification limits (LOQ) were calculated as three- and ten- times the standard deviation of ten reagent blanks, respectively, taking into account the analytical sensitivity and sample mass. Reproducibility was calculated as the relative standard deviation of method (RSDm,%) for three independent analysis of three independent portions of the same sample. Instrument repeatability was calculated as the relative standard deviation (RSDi,%) of 10 independent measurements of a 100 μg L^{-1} multi-elemental standard solution (not including calcium, potassium, magnesium and sodium for which a standard solution of 5 mg L^{-1} was used).

3. Results and discussion

3.1. Analytical characteristics of the method

The analytical figures of merit for the method are shown in Supplementary data (Table S1). RSDi values obtained were less than 4%. For most elements, the LOD was lower than those reported in the literature (see Table 3), the only exceptions being those analysed using ICP-MS.

Table 3
Detection limit values reported for the determination of mineral elements in baby foods.

Element	Bosscher et al. (2002) LOD (mg L^{-1})	Thomson et al. (2008) LOD (mg kg^{-1})	Carbonell-Barrachina, Ramirez-Gandolfo et al. (2012) LOD (mg kg^{-1})	Pandelova et al. (2012) LOD (mg kg^{-1})	Zand et al. (2012) LOQ (mg kg^{-1})	This study LOD (mg kg^{-1})
Al					0.332 (ICP-MS)	0.15 (ICP-OES)
As			0.006 (ICP-MS)		0.01 (ICP-MS)	0.3 (ICP-OES)
Ba					0.053 (ICP-MS)	0.009 (ICP-OES)
Ca			10 (AAS)	2 (ICP-sf-MS)	5.2 (ICP-OES)	0.10 (ICP-OES)
Cd			0.007 (ICP-MS)	0.0002 (ICP-sf-MS)	0.02 (ICP-MS)	0.02 (ICP-OES)
Co			0.037 (ICP-MS)		0.005 (ICP-MS)	0.05 (ICP-OES)
Cr			0.005 (ICP-MS)		0.026 (ICP-MS)	0.04 (ICP-OES)
Cu	0.0005 (GFAAS)		0.11 (ICP-MS)	0.2 (ICP-sf-MS)	0.12 (ICP-MS)	0.03 (ICP-OES)
Fe	0.06 (FAAS)		7 (AAS)	0.2 (ICP-sf-MS)	0.5 (ICP-OES)	0.05 (ICP-OES)
K					0.1 (ICP-OES)	3.0 (ICP-OES)
Mg					1.2 (ICP-OES)	0.03 (ICP-OES)
Mn			0.02 (ICP-MS)	0.04 (ICP-sf-MS)	0.048 (ICP-MS)	0.003 (ICP-OES)
Mo					0.023 (ICP-MS)	0.03 (ICP-OES)
Na		50 (ICP-OES)	10 (AAS)		1.1 (ICP-OES)	0.5 (ICP-OES)
Ni			0.038 (ICP-MS)	0.2 (ICP-sf-MS)	0.08 (ICP-MS)	0.07 (ICP-OES)
Pb			0.039 (ICP-MS)	0.0002 (ICP-sf-MS)	0.014 (ICP-MS)	0.3 (ICP-OES)
Se		0.001 (ICP-OES)	0.013 (ICP-MS)	0.2 (ICP-sf-MS)	0.022 (ICP-MS)	1.3 (ICP-OES)
Zn	0.015 (FAAS)		0.16 (ICP-MS)	0.2 (ICP-sf-MS)	0.5(ICP-OES)	0.2 (ICP-OES)

Table 4
Evaluation of the accuracy of the method employed for ICP-OES determination of the mineral profile of baby foods through the comparison between values found and those certified in a series of food reference materials.

Elements	IAEA-359		NIST 1568a		TORT-2	
	Found values	Certified range	Found values	Certified range	Found values	Certified range
Al	–	–	4.8 ± 0.3	3.4–5.4	–	–
Ba	10.75 ± 0.5	10.5–11.5	–	–	–	–
Ca	18,200 ± 100	17,990–19,010	0.012 ± 0.003[a]	0.0112–0.0124[a]	–	–
Cd	0.12 ± 0.06	0.115–0.125	ND	0.020–0.024	26.2 ± 0.5	26.1–27.3
Cr	1.27 ± 0.04	1.24–1.36	–	–	ND	0.62–0.92
Cu	5.6 ± 0.2	5.49–5.85	2.65 ± 0.09	2.1–2.7	113 ± 5	96–116
Fe	146 ± 1	144.1–151.9	7.1 ± 0.9	6.5–8.3	104 ± 10	92–118
K	32,500 ± 200	31,810–33,190	0.127 ± 0.008[a]	0.1272–0.1288[a]	–	–
Mg	2160 ± 10	2110–2210	0.056 ± 0.03[a]	0.054–0.058[a]	–	–
Mn	32.2 ± 0.5	31.3–32.5	19.8 ± 0.5	18.4–21.6	12.5 ± 0.5	12.4–14.8
Mo	–	–	1.4 ± 0.1	1.38–1.54	0.91 ± 0.05	0.85–1.05
Na	591.5 ± 0.9	567–601	7.12 ± 0.08	5.820137.4	–	–
Ni	1.09 ± 0.1	1.00–1.10	–	–	2.6 ± 0.3	2.31–2.69
Sr	50.2 ± 0.4	47.8–50.6	–	47.8–50.6	46 ± 4	43.3–47.1
Zn	38.9 ± 0.5	37.9 – 39.3	18.95 ± 0.06	18.9–19.9	179 ± 7	174–186

Note: Analytical values are based on the "dry-weight" of reference material. Concentration expressed in mg kg^{-1}.

[a] Concentration expressed in %.

No infant food certified materials were available and, thus, three CRM of different types of foods, similar to the analysed samples, were chosen. Table 4 shows the results obtained. It can be seen that they are in good agreement with the reference data. A statistical evaluation based on the regression between average obtained values and certified values indicated regression lines $y = (0.996 \pm 0.005)x - (7 \pm 20)$ for IAEA-359, $y = (0.998 \pm 0.012)x + (0.12 \pm 0.14)$ for NIST 1568a and $y = (1.007 \pm 0.017)x + (0.096 \pm 1.433)$ for TORT-2, suggesting the intercept and slope correspond in all cases to 0 and 1, and regression coefficients of 0.9999, 0.998 and 0.998, respectively.

3.2. Mineral content of commercial baby foods

The concentration of mineral elements in meat, fish, vegetables and fruit based baby foods, expressed in mg kg^{-1} of dry weight, are reported in Supplementary data (Tables S2–S5).

Table 5 shows the percentage of AI provided by each baby food group with respect to calcium, copper, potassium, magnesium, manganese and sodium. In the case of iron and zinc EAR was considered. AI values for infants of 6–12 months are 260, 0.22, 700, 75, 0.6 and 370 mg day^{-1} for calcium, copper, potassium, magnesium, manganese and sodium, respectively (Food and Nutrition Board, 2004), and the EAR are 6.5 and 2.5 mg day^{-1} for iron and zinc, respectively (NCR, 2002).

Following the recommendations of Dewey and Brown, 2003, regarding the amount of energy to be provided by complementary foods, and with the proviso that jars are consumed at least once a day, we can estimate the contribution of available nutrients from the baby foods analysed. More specifically, at 6–8 months complementary foods should provide 200 kcal day^{-1}, and infants should have 2–3 meals per day consisting of half jar at each meal, and at 9–12 months 300 kcal day^{-1} from 3 to 4 meals per day. Minimum and maximum values of mineral elements provided for each range are shown in Fig. 1.

Considering the contribution to the daily intake of commercially available baby foods in Spain, it can be seen that the content of aluminium in the studied samples is higher in vegetable jars, between 4.8 and 11.5 mg kg^{-1}, while the aluminium content of baby food meats, fish and fruit are very similar to each other with an average value of 3.5 mg kg^{-1}, and in the same order of magnitude as Norwegian baby foods reported by Melo et al. (2008). Aluminium compounds are probably added during processing, from substances such as flour or colouring agents, because fresh foods such as fruits,

Table 5
Baby foods contribution (%) to the Adequate Intake of essential elements for babies 6–12 months (percentage that provides jars to the AI).

	Meat jars				Fish jars				Vegetable jars				Fruit jars			
	Min.	Max.	Average	Median	Min.	Max.	Average	Median	Min.	Max.	Average	Median	Min.	Max.	Average	Median
Ca	4	25	12	10	6	38	20	22	12	54	28	24	6	16	9	8
Cu	18	73	42	41	15	53	30	28	29	72	46	45	38	78	64	67
Fe	3	11	7	6	1	10	4	3	3	10	6	6	3	9	5	4
K	25	78	53	52	29	78	47	41	54	75	63	58	52	83	72	75
Mg	14	52	34	34	22	47	37	43	29	53	41	43	33	47	40	41
Mn	9	47	26	24	11	42	26	27	12	42	33	38	33	50	39	37
Na	26	135	64	67	8	76	41	36	48	77	63	64	1	7	4	3
Zn	16	61	35	33	10	18	12	12	13	21	17	16	6	10	7	7

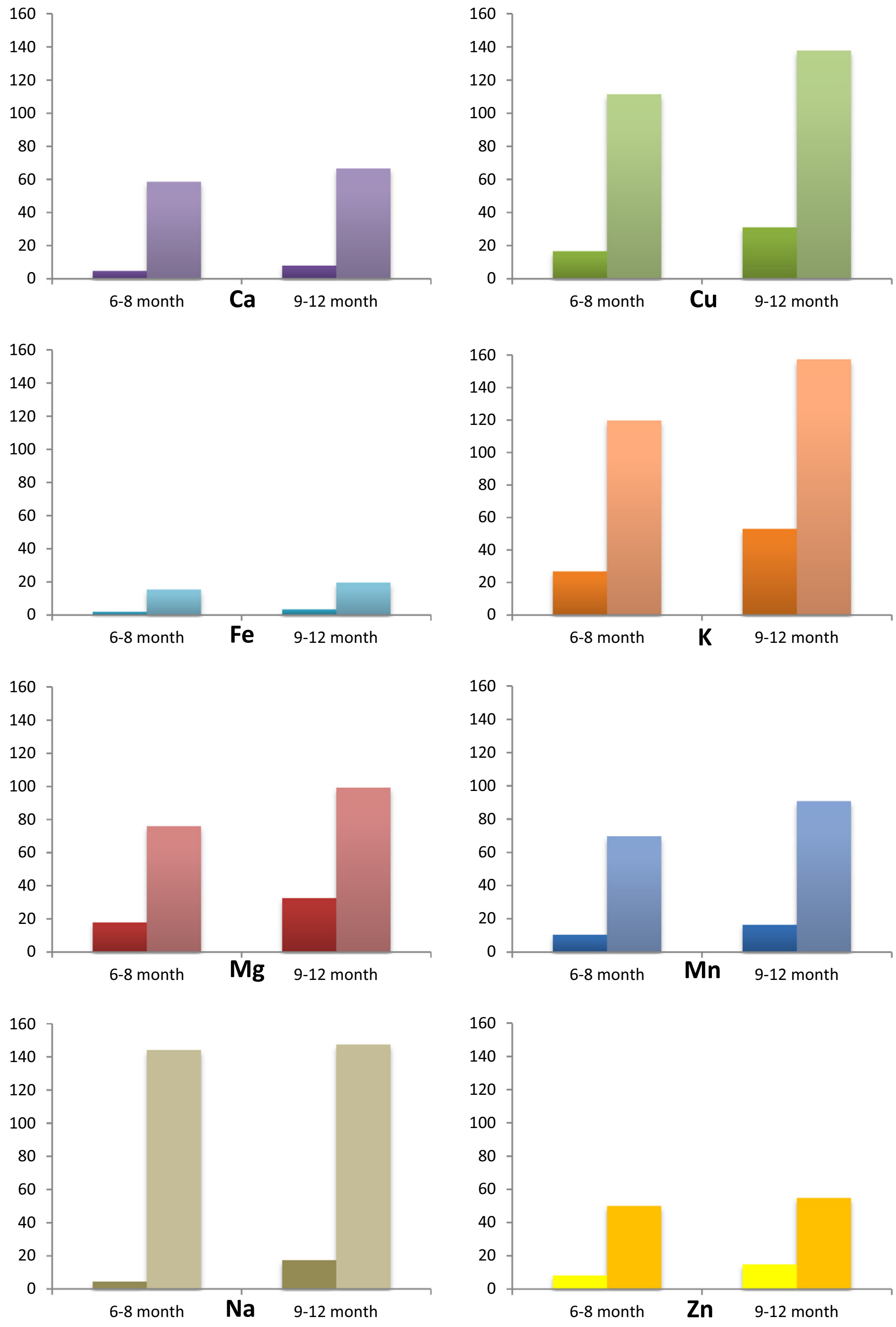

Fig. 1. Minimum and maximum contribution (in percentage %) of the studied samples to the adequate intake (AI) and estimated average requirement (EAR) for babies 6–8 months and 9–12 months regarding the recommendations values by Dewey and Brown, 2003; WHO, 2003.

vegetables, and meat contain very little naturally. In this sense, it must be taken into consideration that high intakes of aluminium can cause bone diseases in infants (ATSDR, 2008).

Barium is present in all foods, above all in fish and certain plants. Usually levels of barium in food are in the range of 0.05–1.0 mg kg^{-1} (ATSDR, 2007; Reilly, 2002). Barium content was highest in vegetable (average amount).

Calcium is an essential nutrient that plays a vital role in neuromuscular function, blood clotting and providing rigidity to the skeleton. In the first two years of life, infants need to absorb 120 mg of calcium daily to allow for normal growth. In the case of breast-feeding, a mean intake of 240 mg is required for absorption of 120 mg of calcium. Thus, FAO and WHO recommended an intake of 300 mg (FAO/WHO, 2002). The amount of calcium in meat and fish baby foods was higher than vegetable and fruit jars, in some cases double the amount. The values of calcium were similar to data reported in the past for Spanish meat and fish jars (Carbonell-Barrachina, Ramirez-Gandolfo, et al., 2012), similar to baby food data from Norway (Melo et al. 2008), and also to meat, fish and vegetables baby foods from UK (Zand, Chowdhy, Wray, Pullen & Snowden, 2012; Zand et al., 2011).

Chromium is important in the metabolism of fats and carbohydrates. Chromium stimulates fatty acid and cholesterol synthesis, which are important for brain function and other body processes. Chromium is also important in the breakdown of insulin. Chromium deficiency may be seen as impaired glucose tolerance and has been reported in infants with protein-calorie malnutrition. Supplementation of chromium helps with management of these conditions. Because of the low absorption and high excretion rates of chromium, toxicity of this element is not common (Food and Nutrition Board, 2011). The values of chromium found in baby foods were lower than those previously reported in commercial baby food from Spain, Norway and UK (Carbonell-Barrachina, Ramirez-Gandolfo, et al., 2012; Melo et al., 2008; Zand et al., 2012).

It is well established that copper is essential for life, being required for infant growth, host defence mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol and glucose metabolism (SCF, 2003). The highest amount of copper was found in fruit jars, followed by vegetable, meat and fish jars. The values of copper were similar to those reported previously in Spain for meat and fish jars (Carbonell-Barrachina, Ramirez-Gandolfo, et al., 2012) and meat baby food from UK (Zand et al., 2011), but lower than that reported for vegetable and fruit jars from Belgium (Bosscher, Van Cauwenbergh, Van der Auwera, Robberecht & Deelstra, 2002) and higher than that found for vegetable jars from UK (Zand et al., 2011).

In the first year of life, body weight typically triples and body-wide iron content should double. After 4 months, iron requirements increase due to rapid growth and iron reserves diminish to pathological levels if the needs are not covered with dietary iron (Gil Hernández, Uauy Dagach & Dalmau Serra, 2006). Commercial products are regularly fortified with iron and ascorbic acid, and usually given together with fruit juices and solid foods containing meat, fish, and vegetables. The fortification of products with iron is important in meeting the high dietary needs, especially considering the importance of optimal iron nutrition during this phase of brain development (FAO/WHO, 2002). The amount of iron in analysed samples was very small; this is an indication that the jars analysed were not fortified with iron and, therefore, do not meet the needs of children between 6 and 12 months. The iron content found in samples was very similar to that reported in vegetable and fruit jars from Belgium (Bosscher et al., 2002), but lower than those found in baby food from Norway (Melo et al., 2008) and UK (Zand et al., 2011, 2012).

Potassium is an essential mineral that works to maintain the body's water and acid balance. It plays a role in transmitting nerve impulses to muscles, in muscle contractions and in the maintenance of normal blood pressure (FAO, 2009). The greatest amounts of potassium were found in fruit baby foods following by vegetable, meat and fish jars. The content of potassium was very similar to that found in vegetable and fruit jars from Belgium (Bosscher et al., 2002), baby food from Norway (Melo et al., 2008) and baby food from UK (Zand et al., 2011, 2012).

Lithium is present in all food, albeit at low levels. The lowest content of lithium was found in fish-based foods.

Magnesium is needed for more than 300 biochemical reactions in the body. It helps to maintain normal muscle and nerve function, keeps heart rhythm steady, supports a healthy immune system, and keeps bones strong. Magnesium also helps to regulate blood sugar levels, promotes normal blood pressure, and is known to be involved in energy metabolism and protein synthesis (Office Dietary Supplements, 2009). The content of magnesium found was very similar to that reported in vegetable jars from Belgium (Bosscher et al., 2002), baby food from Norway (Melo et al., 2008) and meat and vegetable baby food from UK (Zand et al., 2012), especially those based on chicken and fish baby food from UK (Zand et al., 2011).

Manganese is an essential element for many living organisms, including humans. Adverse health effects can be caused by inadequate intake or over-exposure. Manganese deficiency in humans appears to be rare, because manganese is present in many common foods (WHO, 2011). Babies are exposed to manganese through foods. The human diet typically provides the necessary amount of manganese for normal body function (ATSDR, 2000a). The values of manganese found through this study were similar to data reported in the Spain for meat and fish jars (Carbonell-Barrachina, Ramirez-Gandolfo, et al., 2012), similar to those found in fruit jars from Belgium (Bosscher et al., 2002) but less than that reported from UK (Zand et al., 2012).

Sodium is an essential nutrient that is involved in numerous physiological functions, such as the maintenance of the liquid extracellular osmolarity, the control of the volume of body water compartments and the maintenance of normal blood pressure. It also plays a role in the acid-base balance. But, to meet these requirements, only a small amount of sodium is required from the diet (Gil Hernández et al., 2006). The sodium content of fruit jars was found to be very low, but high in meat, vegetables and fish jars. The concentrations of sodium found in this study were similar to data reported in Spain for meat and fish jars (Carbonell-Barrachina, Ramirez-Gandolfo, et al., 2012; Okesli et al., 2011) and fruit jars from Belgium (Bosscher et al., 2002), but higher than baby food from UK (Zand et al., 2011, 2012). In any case, the sodium content exceeded the maximum permitted level of 200 mg/100 g food (DOCE, 2006).

Nickel is not currently considered an essential nutrient for humans. Nickel is conjectured to play a role in processes related to the vitamin B12 and folic acid dependent pathway in methionine metabolism (NCR, 2002). It is likely that the health effects seen in babies exposed to nickel will be similar to the effects seen in adults. We do not know whether infants differ from adults in their susceptibility to nickel. The major dietary source of nickel is plant foods. Animal foods are low in nickel (ATSDR, 2005). The major content of nickel, as expected, was found in vegetable jars, and the least in meat and fish jars. The content of nickel was similar to chicken and fish baby food from UK (Zand et al., 2012).

Babies are exposed to stable strontium in the same manner as adults, usually in small amounts in drinking water and food. Young children who have more hand-to-mouth activity or who eat soil may accidentally eat more strontium. Infants and children with active bone growth absorb more strontium from the gut than adults. Excess stable strontium causes problems with growing bone. For this reason, babies are more susceptible to the effects of stable strontium than adults who have mature bone. Babies who unusually eat high levels of strontium may have problems with bone growth, but only if the diet is low in calcium and protein (ATSDR, 2004). Strontium was highest in meat and vegetable jars, and the least in fruit jars.

Zinc is essential for proper growth and development of babies. Little is known about whether infants who eat too much zinc will react differently from adults who have ingested large amounts of zinc (ATSDR, 2000b). The best dietary sources of zinc are meat, particularly viscera, and shellfish, while eggs and dairy products are also relatively good sources of zinc. The bioavailability of zinc is inhibited by phytate, which is present in large amounts in cereals and legumes. The clearest indication of zinc deficiency is stunted baby growth; there is evidence that zinc supplementation can improve the growth of stunted baby (Brown, Wuehler & Peerson, 2001). Zinc was highest in meat jars, and levels were very similar to baby food from Norway (Melo et al., 2008) and baby food from UK (Zand et al. 2011, 2012).

4. Conclusions

The levels of essential and trace elements in commercial baby foods were inadequate, in some cases (iron, zinc and calcium), to meet the AI of babies of 6–12 months age. Iron intake provided by the commercial baby food was between 5 and 20% of the EAR; at this age, it should be around 97% because breast milk provides only 3% of essential iron. This shows the lack of fortification of commercial baby foods analysed and the need for supplementary mineral for appropriate growth. The same is true for zinc and calcium, although these were not deficient: zinc intake provided was between 10% and 60% (should be 86%) and calcium was between 10% and 70% (should be 72%). The amounts of sodium, magnesium, copper, manganese and potassium varied between 30% and 140%; this means if receiving a varied diet, the infant would be consuming a good supply of these minerals. The concentration of toxic elements, such as cadmium and lead, was in all cases below LOD, confirming good practices in production.

Acknowledgments

Authors acknowledge the financial support of the Generalitat Valenciana (Spain) (project PROMETEO 2010-055) and the Ministerio de Economia y Competitividad (Spain)-Feder (project CTQ2011-25743 and project CTQ2012-38635). Alba Mir-Marqués acknowledges a grant ‘Atracció de Talent’ provided by the University of Valencia (Spain).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.09.074.

References

ATSDR (Agency of Toxic Substances & Disease Registry), *Public Health Statement for Al (2008)*, from: <www.atsdr.cdc.gov>.

ATSDR (Agency of Toxic Substances & Disease Registry), *Public Health Statement for Ba (2007)*, from: <www.atsdr.cdc.gov>.

ATSDR (Agency of Toxic Substances & Disease Registry), *Public Health Statement for Mn (2000a)*, from: <www.atsdr.cdc.gov>.

ATSDR (Agency of Toxic Substances & Disease Registry), *Public Health Statement for Ni (2005)*, from: <www.atsdr.cdc.gov>.

ATSDR (Agency of Toxic Substances & Disease Registry), *Public Health Statement for Sr (2004)*, from: <www.atsdr.cdc.gov>.

ATSDR (Agency of Toxic Substances & Disease Registry), *Public Health Statement for Zn (2000b)*, from: <www.atsdr.cdc.gov>.

Bosscher, D., Van Cauwenbergh, R., Van der Auwera, J., Robberecht, H., & Deelstra, H. (2002). Calcium, iron and zinc availability from weaning meals. *Acta Paediatrica, 91*, 761–768.

Brown, K. H., Wuehler, S. E., & Peerson, J. M. (2001). The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. *Food and Nutrition Bulletin, 22*, 113–125.

Butte, N. F., Fox, M. K., Briefel, R. R., Siega-Riz, A. M., Dwyer, J. T., Deming, D. M., et al. (2010). Nutrient intakes of US infants, toddlers, and preschoolers meet or exceed dietary reference intakes. *Journal of the American Dietetic Association, 110*, S27–S37.

Carbonell-Barrachina, A. A., Ramirez-Gandolfo, A., Wu, X., Norton, G. J., Burlo, F., Deacon, C., et al. (2012). Essential and toxic elements in infant foods from spain, UK, china and USA. *Journal of Environmental Monitoring, 14*, 2447–2455.

Carbonell-Barrachina, A. A., Wu, X., Ramírez-Gandolfo, A., Norton, G. J., Burló, F., Deacon, C., et al. (2012). Inorganic arsenic contents in rice-based infant food from Spain, UK, China and USA. *Environmental Pollution, 163*, 77–83.

Dewey, K., & Brown, K. (2003). Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food and Nutrition Bulletin, 24*, 5–28.

Directiva 2006/125/CE de la Comisión de 5 de diciembre de 2006 relativa a los alimentos elaborados a base de cereals y alimentos infantiles para lactantes y niños de corta edad. DOCE no. L339/16.

EFSA (European Food Safety Authority), 2006. Tolerable upper intake levels for vitamins ans minerals. Scientific Committee on Food, Scientific Panel of Dietetic Products, Nutrition and Allergies, from: <www.efsa.europa.eu/en/ndatopics/docs/ndatolerableuil.pdf>.

ESPGHAN Committee on Nutrition (2008). Complementary feeding: A commentary by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology and Nutrition, 46*, 99–110.

FAO (Food, Nutrition and Agriculture), *Alimentation, nutrition et agriculture. Alimentación, nutrición y agricultura*, 2009, from: <www.fao.org/docrep/x2650T/x2650T00.htm>.

FAO/WHO Human Vitamin and Mineral Requirements, 2002. Report of a Joint FAO/WHO Expert Consultation Bangkok, Thailand.

Fernandez, D. R., Vanderjagt, D. J., Williams, M., Huang, Y. S., Chuang, L., Millson, M., et al. (2002). Fatty acid, amino acid, and trace mineral analyses of five weaning foods from Jos, Nigeria. *Plants Foods for Human Nutrition, 57*, 257–274.

Food and Nutrition Board, Institute of Medicine. (2004). *Dietary reference intake (DRIs): Recommended intakes for individuals elements*. National Academies Press.

Food and Nutrition Board, Institute of Medicine. (2011). *Chromium in diet: MedlinePlus medical encyclopedia*. U.S. National Library of Medicine.

Gil Hernández, A., Uauy Dagach, R., Dalmau Serra, J., & Comité de Nutrición de la AEP (2006). Bases for adequate complementary feeding in infants and young children. *Anales de Padiatría (Barc), 65*, 481–495.

Melo, R., Gellein, K., Evje, L., & Syversen, T. (2008). Minerals and trace elements in commercial infant food. *Food and Chemical Toxicology, 46*, 3339–3342.

Mir-Marqués, A., Cervera, M. L., & de la Guardia, M. (2012). A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus. *Journal of Food Composition and Analysis, 27*, 160–168.

NCR (National Research Council). (2002). *Dietary references intakes for vitamin A, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*. Washington, D.C.: National Academy of Sciences [pp. 197–223].

Office of Dietary Supplements (2009). *Strengthening knowledge and understanding of dietary supplements: Magnesium*. US: National Institutes of Health.

Okesli, V., González-Bermúdez, C. A., Vidal-Guevara, M. L., Dalmau, J., & Ros, G. (2011). Alimentación complementaria: ¿es igual el perfil nutricional de un pure casero que el de un tarrito commercial? *Acta Pediátrica España, 69*, 235–240.

Pandelova, M., Lopez, W. L., Michalke, B., & Schramm, K. (2012). Ca, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn contents in baby foods from the EU market: Comparison of assessed infant intakes with the present safety limits for minerals and trace elements. *Journal of Food Composition and Analysis, 27*, 120–127.

Reilly, C. (2002). *Metal contamination of food* (3rd ed). Oxford, UK: Oxford Bookes University.

SCF (Scientific Committee on Food), *Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Copper*. SCF/CS/NUT/UPPLEV/57 Final, European Commission, 2003.

Thomson, B. M., Vannoort, R. W., & Haslemore, R. M. (2008). Dietary exposure and trends of exposure to nutrient elements iodine, iron, selenium and sodium from the 2003–4 New Zealand Total Diet Survey. *British Journal of Nutrition, 99*, 614–625.

WHO (World Health Organization). (2003). *Guiding principles for complementary feeding of the breastfed child*. Washington: WHO.

WHO (World Health Organization). (2008). *Strengthening action to improve feeding of infants and Young children 6–23 months of age in nutrition and child health programmes*. Geneva: WHO [October 6–9].

WHO (World Health Organization). (2009). *Infant and young child feeding. Model chapter for textbooks for medical students and allied health professionals*. Geneva, Switzerland: WHO.

WHO (World Health Organization), *Guidelines for Drinking-water Quality: Manganese in Drinking-water*. WHO/SDE/WSH/03.04/104/Rev/1, 2011, from: <www.who.int/water_sanitation_health/dwq/chemicals/manganese.pdf>.

Zand, N., Chowdhry, B. Z., Wray, D. S., Pullen, F. S., & Snowden, M. J. (2012). Elemental content of commercial ‘ready to-feed’ poultry and fish based infant foods in the UK. *Food Chemistry, 135*, 2796–2801.

Zand, N., Chowdhry, B. Z., Zotor, F. B., Wray, D. S., Amuna, P., & Pullen, F. S. (2011). Essential and trace elements content of commercial infant foods in the UK. *Food Chemistry, 128*, 123–128.

CHAPTER 32

Baby foods

Alba Mir-Marqués, Maria Luisa Cervera & Miguel de la Guardia
Department of Analytical Chemistry, University of Valencia, Burjassot, Valencia, Spain

Abstract: The mineral profile of baby food is very important in order to establish the levels of essential and non-essential elements and their contribution to adequate intake (AI) and estimated average requirement (EAR) for providing adequate nutrition during infancy. This is essential to ensure the growth, health and development of children to their full potential, because poor nutrition increases the risk of illness in adulthood. This chapter discusses different strategies for determining the mineral profile in most common childhood foods, with regard to both sample preparation and analytical method employed for determination. The results in the literature on the mineral content of baby foods used from 6 to 12 months of age are also updated.

Keywords: baby foods, infant foods, mineral composition, essential elements

32.1 Baby food and essential element requirements

The first year of human life is a critical period in the development of a child. It is a period of rapid growth, with major changes in motor and cognitive function. Adequate nutrition during infancy and childhood is essential to ensure the growth, health and development of children. Malnutrition during the first 2 years of life increases the risk of illness in adulthood, principally chronic malnutrition and not attaining full growth potential. High intake of food can also have negative consequences, such as childhood obesity. Thus it is vital to thoroughly survey the nutrient intake of infants, since nutrition at this age marks the future quality of life.

Some authorities encourage the timely introduction of complementary foods during infancy, which may be necessary for both nutritional and development reasons, and to enable the transition from breast milk to infant foods, because the ability of breast milk to meet requirements for macronutrients and micronutrients becomes limited with increasing age of the baby [1]. Early diet has an immediate effect on the health of infants in economically developing countries because of factors such as the lack of availability of suitable alternatives to breast milk, microbial contamination of foods and beverages, displacement of breast milk by less nutritious alternatives, and the physiological maturity of gastrointestinal and renal function [2].

Agencies such as the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) have produced recommendations that encourage exclusive breastfeeding for 6 months (180 days) and nutritionally adequate and safe complementary feeding starting from the age of 6 months with continued breastfeeding up to 2 years of age or beyond [3, 4].

From the age of 6 months, infant needs for energy and nutrients start to exceed that provided by breast milk and thus complementary feeding becomes necessary to fill the energy and nutrient gap [5]. The stage between 6 and 24 months of age is the most critical in terms of malnutrition, since children cease taking all the nutrients from breast milk and the nutrient composition of other foods becomes the key problem. This introduces many factors that affect infant nutrition, such as the amount of supplementary feed and its composition, consistency and bioavailability of nutrients.

In general, up to 6 months of age babies fed only on breast milk are supplied with the necessary micronutrients, including Fe and Zn, which are present in low concentration but with high bioavailability and absorption. After 6 months, the child can eat solid or semi-solid food

Handbook of Mineral Elements in Food, First Edition. Edited by Miguel de la Guardia and Salvador Garrigues.

in the form of porridge or mashed foods. These foods are usually milk mixed with cereals or baby rice or mixed vegetables with meat or fruit porridge. After 9 months of age, baby food includes many types of meat and the child is introduced to fish and eggs, which are not included in the diet until 1 year of age. Additionally, the consistency of food must be such that babies can pick up the food. After 12 months, the child can start eating family foods, but chopped or mashed [2, 3].

Various national and international health agencies recommend the levels of mineral elements needed for appropriate baby nutrition. Table 32.1 compares the levels recommended by different agencies for each essential element. Recommended values for 12 essential elements (Ca, Cr, Cu, Fe, Mg, Mn, Mo, P, Se, Na and Zn), together with the daily intake recommendations according to infant nutrition at three different ages (0–5, 6–11 and 12–23 months), were taken from different organizations in the USA and Canada [6], Australia and New Zealand [7], Europe [8] and the WHO [9]. From the data summarized in Table 32.1 it can be concluded that the recommended values are very similar among the different organizations. Additionally, it can be seen that essential elements can be classified according to their required amounts: K and Na at levels of around 1 g and 0.4 g/day, respectively; Ca and P at levels in the order of hundreds of milligrams per day; Mg, Fe, Zn and Mn also required at milligram per day levels; Cu at the hundreds of micrograms per day level; and Se, Mo and Cr in the low micrograms per day level. Because of this, all these elements must be carefully controlled in baby foods.

The main parameters are reported as follows.

- Estimated average requirement (EAR): the average daily nutrient intake that meets the needs of 50% of healthy individuals in a particular age and gender group.
- Recommended daily allowance (RDA), equivalent to recommended nutrient intake (RNI): the average daily nutrient intake sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a particular life stage and gender group.
- Adequate intake (AI): the recommended average daily intake based on observed or experimentally determined approximations or estimations of nutrient intake by a group of apparently healthy people that are assumed to be adequate.
- When an RDA cannot be determined, tolerable upper intake level (UL): the highest average daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population [9, 10].

32.2 Methods employed for determination of the mineral composition of baby foods

The main analytes to be determined in baby foods are those that can cause problems for the child's health, either by excessive or insufficient intake. For example, iron and zinc are the most problematic elements for baby's health, because of their relatively low bioavailability in infant food. Sodium content can also be a problem for infants, because despite its essential character an excess of this element may cause hypertension. The most common baby food samples are purées of different foods, as mixtures of meat or fish with vegetables or mixtures of fruits. The nutritional content of these purées is usually about 80–85% water, 10% carbohydrate, 3.2% protein, 2% fat, 1% fibre and 0.1% sodium.

Baby food samples have high water content, and samples can be analysed either wet or after being dried. The drying process allows pre-concentration of the elements to be determined and ensures better preservation of samples than that achieved for wet foods. The drying process can be accomplished by application of temperature in an oven (normally about 100°C), by applied vacuum or by lyophilization. For oven drying, Carbonell-Barrachina *et al.* [12] suggest placing the samples in an oven for 78 hours at 80°C. Lyophilization involves very low temperatures and pressures of around –50°C and 0.05 mbar, respectively [13], and these conditions are highly recommended in order to avoid the loss of highly volatile elements and chemical forms of minerals like Hg and chloride compounds.

The most common measurement techniques for the analysis of mineral composition in baby foods are atomic absorption and emission spectrometry. However, to carry out the mineral analysis using these methodologies requires liquid samples, so that baby food samples need previous dissolution or digestion. Figure 32.1 shows the methodology used to determine mineral elements in infant food, and also shows how mineral speciation could be accomplished using prior separation of the different mineral compounds of the same element by chromatographic or non-chromatographic techniques.

Table 32.1 Recommended values for essential element intake of infants obtained from different national and international agencies.

Element	Age (months)	Food and Nutrition Board (USA and Canada) [6]				Australia and New Zealand [7]				WHO [9]		Europe [8]
		EAR	RDA	AI	UL	EAR	RDI	AI	UL	RDI	UL	RDA
Ca (mg/day)	0–5			200	1000			210		300		
	6–11			260	1500			270		400		450
	12–23	500	700		2500	360	500		2500	500	3000	550
Cr (µg/day)	0–5			0.2				0.2				
	6–11			5.5				5.5				10
	12–23			11				11				20
Cu (µg/day)	0–5			200				200				
	6–11			220				220				400
	12–23	260	340		1000			700	1000	500*	1500*	500
Fe (mg/day)	0–5			0.27	40			0.2	20			
	6–11	6.9	11		40	7	11		20	7.7†		8
	12–23	3.0	7		40	4	9		20	4.8†		8
K (g/day)	0–5			0.4				0.4				
	6–11			0.7				0.7				0.7
	12–23			3.0				2				1.0
Mg (mg/day)	0–5			30				30		26		
	6–11			75				75		54		75
	12–23	65	80			65	80			60	65	80
Mn (mg/day)	0–5			0.003				0.003				
	6–11			0.6				0.600				0.7
	12–23			1.2	2			2.0				1.2
Mo (µg/day)	0–5			2				2				
	6–11			3				3				15
	12–23	13	17		300	13	17		300			25
P (mg/day)	0–5			100				100				
	6–11			275				275				350
	12–23	380	460		3000	380	460		3000			550

(Continued)

Table 32.1 (Continued)

Element	Age (months)	Food and Nutrition Board (USA and Canada) [6]				Australia and New Zealand [7]				WHO [9]		Europe [8]
		EAR	**RDA**	**AI**	**UL**	**EAR**	**RDI**	**AI**	**UL**	**RDI**	**UL**	**RDA**
Se (µg/day)	0–5			15	45			12	45	6		
	6–11			20	60			15	60	10		15
	12–23	17	20		90	20	25		90	17		20
Na (g/day)	0–5			0.12				0.12				
	6–11			0.37				0.17				0.3
	12–23			1.0	1.5			0.2–0.4	1			0.4
Zn (mg/day)	0–5			2	4			2.0	4	2.8‡		
	6–11	2.5	3		5	2.5	3.0		5	4‡		4
	12–23	2.5	3		7	2.5	3.0		7	4‡		5

EAR, estimated average requirement; RDA, recommended daily allowance; RDI, recommended daily intake; AI, adequate intake; UL, tolerable upper intake level.

*World Health Organization [11].

†Assuming 12% bioavailability.

‡Assuming moderate bioavailability.

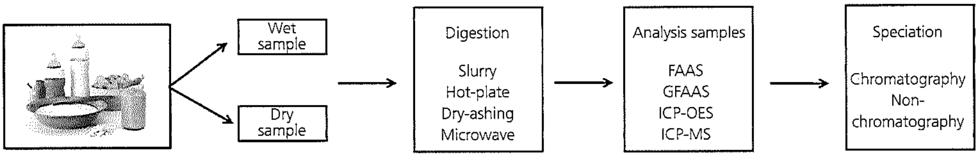

Figure 32.1 Scheme of general methodology used for mineral element analysis in baby food.

32.2.1 Mineralization and dissolution of samples

The organic nature of baby foods makes samples insoluble in water and therefore digestion of baby food samples with acid is mandatory in order to obtain a solution of the mineral compounds. Figure 32.2 shows the parameters (temperature, pressure, reagents) required for complete digestion of baby foods in order to destroy the matrix without loss of minerals.

Mineral elements can be dissolved by simply using reagents at room temperature, thus providing solutions or slurries of samples. It is true that many of the elements to be analysed are easily soluble in water or dilute acids, but such dilution of baby food samples with aqueous extraction solutions causes problems related to matrix effects during measurement and compels the use of highly sensitive techniques for determination of trace and ultratrace elements.

Dry ashing or wet ashing are the main strategies for digestion of baby food samples. Dry ashing uses basically temperature and total decomposition of the matrix, thus providing a high pre-concentration of samples to be analysed. However, it is clear that the simple use of temperature and time causes the loss of volatile elements and compounds and so ashing aids have been proposed to reduce these problems. Indeed, the combined use of temperature and acids is the most useful technique for complete dissolution of the food ash. The use of pressure is probably the best way to accelerate food sample digestion. Nowadays, pressurized microwave-assisted digestion is the most commonly used digestion process.

In some cases, direct analysis of solid samples can be performed by using simple laser ablation of samples or by direct atomization of sample slurry in a graphite furnace. Methods employed for mineralization of baby food samples are summarized in Table 32.2 and it can be seen that sample slurries, digestion with HNO_3 and HCl on a hot plate, dry ashing and microwave-assisted digestion are the most commonly used procedures.

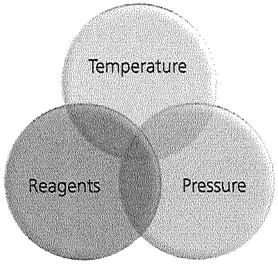

Figure 32.2 Key parameters used for the digestion of baby food samples.

Slurry sampling

The simplest sample preparation is direct analysis of samples without applying temperature or pressure or the addition of any reagent, although no studies of this kind where infant food was measured directly have been found in the literature in recent years. However, it is quite common to prepare sample slurries in water when analysing infant food, in which case it is only necessary to add aqueous solutions of acids or surfactants and then homogenize the sample. Slurry sampling has many advantages, such as reduced sample preparation time, decreased analyte losses, reduced risk of contamination, and elimination of hazards associated with the use of acids at high temperatures. Slurries can then be nebulized into a flame (FAAS) or plasma (ICP-OES/MS) or deposited in a graphite tube for GFAAS.

Baby food slurry is usually composed of a small amount of sample plus Triton X-100, nitric acid and an antifoam agent. The addition of nitric acid facilitates extraction of the analyte or analytes into the liquid phase of the slurry, while Triton X-100 serves as a dispersing agent of solid particles. Ozbek and Akman [14] reported a method for slurry analysis of baby food for Fe and Zn determination using FAAS. To prepare the slurry, 0.5 g of sample were weighed and suspended in 20 mL of a diluent containing 0.05% Triton X-114 and 0.1 M nitric acid. The slurries were homogenized using an Ultra-Turrax at 15,000 rpm for 5 min. This method

Table 32.2 Methods employed in the analytical literature for the mineralization of baby food samples.

Sample	Digestion process	Reference
Baby food reference (TUGAP 1201 R)	Slurry (Triton X-114/HNO_3) MW (HNO_3/H_2O_2)	[14]
Commercial baby food based on chicken, hake, vegetables and fruit	Slurry (Triton X-100/ HNO_3/H_2O_2) MW (HNO_3/H_2O_2)	[15]
Commercial baby food, fish based	Slurry (Triton X-100/ HNO_3/H_2O_2) MW (HNO_3/H_2O_2)	[16]
Commercial infant rice, puréed infant foods (hake and chicken based)	Dry ashing	[19]
Commercial and home-made baby food of lamb, beef and chicken	Dry ashing	[21]
Home-made baby food, meat based (chicken and veal)	Dry ashing	[23]
Commercial baby food, vegetable based	Hot-plate (HNO_3/HCl)	[17]
Total diet survey	HNO_3/HCl	[18]
Commercial infant cereals, baby food based on chicken, hake and infant rice	MW(HNO_3/H_2O_2)	[12]
Foods consumed by children	MW (HNO_3/H_2O_2)	[25]
Commercial baby food of vegetables and fruits	MW (HNO_3/H_2O_2)	[26]
Commercial baby food of meat and vegetables	MW (HNO_3/H_2O_2)	[27]
Commercial baby food of meat, fish, vegetables and fruit	MW (HNO_3)	[28]
Commercial baby food	MW (HNO_3)	[29]
Infant food	MW (HNO_3)	[30]
Commercial baby food of chicken and fish	MW (HNO_3/HCl/H_2O_2)	[31]

MW, microwave assisted.

was compared with microwave-assisted digestion, using GBW-08503 Wheat flour and IRMM-804 Rice flour, obtaining recovery percentages of 95% and 98% for Fe and 103–104% and 101–102% for Zn, respectively for slurry and microwave digestion. The total time for slurry preparation was 20 min, while the time required for microwave digestion was 135 min. For the determination of Cu, Co, Ni, Mn, Se, Pb and Cd in samples of meat, fish, vegetable and fruit baby foods and fish baby foods, Viñas *et al.* [15, 16] weighed the samples and prepared suspensions by diluting with 5 mL of a solution containing 0.1% w/v Triton X-100, 1% v/v nitric acid and 30% w/v hydrogen peroxide (1% w/v ammonium dihydrogenphosphate only for Cu), with measurement by GFAAS. Slurries were homogenized by movements of the plunger, which took about 5 min. The suspensions were sonicated for a few minutes and stirred with a magnetic stirrer before measurement by GFAAS. The accuracy of the method was verified by using the reference materials HDP Total diet, DORM-2 Dogfish muscle, SRM 1566a Oyster tissue, SRM 1577b Bovine liver, SRM 1568a Rice flour and SRM 1572 Citrus leaves. This sample preparation method was compared with microwave-assisted digestion; Student's *t*-test revealed no difference between the results obtained using either of the assayed procedures at a level of significance of 0.05.

Wet digestion

Wet digestion of baby foods uses mixtures of acids, generally nitric acid mixed with other acids or with hydrogen peroxide, which are heated on a hot plate. In a study involving the determination of eight essential elements (Ca, Cu, Fe, K, Mg, Mn, P and Zn) by ICP-OES [17], the decomposition of different weaning foods was made on a hot plate, with 0.1 g of sample being digested in 125-mL beakers with 20 mL concentrated nitric acid and 1 mL concentrated perchloric acid. Samples were covered with glass and set on a hot-plate at 120°C for 1 hour. After that, the temperature was increased to 150°C and samples refluxed overnight. Then, samples were taken to almost dryness in order to reduce the volume to approximately 1 mL, at the same temperature. Dried samples were treated with 2.5 mL of nitric/perchloric acid (4 : 1). After cooling, solutions were transferred to tubes and diluted to a 50-mL final volume with deionized water.

Thomson *et al.* [18] determined Na and Fe in the New Zealand Total Diet Survey by ICP-OES by also using wet digestion. In this study, 0.5–2.5 g of the homogenized food were digested with nitric and hydrochloric acids. The accuracy of the method was verified by measurement of NIST 1549 Non-fat milk powder, SRM 1577b

Bovine liver, RM 8414 Bovine muscle, SRM 1568a Rice flour, SRM 1515 Apple leaves and SRM 1566b Oyster tissue. The accuracy of the determinations, based on recovery from certified reference materials, was better than 85%. These results indicate the suitability of wet digestion at atmospheric pressure for the determination of trace elements in baby foods. However, the atmospheric pressure wet digestion is time-consuming, analyte contamination and losses occur, and often this kind of digestion does not allow complete decomposition of the sample matrix.

Dry ashing

Dry ashing has been commonly used in baby food analysis. This method allows ash dissolution in small amounts of reagents, whereas wet digestion uses large amounts of reagents. On the other hand, dry digestion has the drawback of time (1 day or more) and requires attention from the analyst.

Burló *et al.* [19] determined As in baby food using dry ashing at 450°C for 12 hours and HGAAS. In this method, 1 g of dry sample was weighed, adding 1 mL ashing aid and 5 mL nitric acid 50% v/v, then the mixture was evaporated on a sand bath to total dryness. Samples were mineralized as described [20]. Ashes from the digested samples were dissolved in 5 mL HCl 50% (v/v) and 5 mL KI-ascorbic acid. After 30 min, solutions were diluted to the volume with HCl 50% (v/v) and filtered. Okesli *et al.* [21] used an official method [22] for the determination of sodium by flame atomic emission spectrometry and previous incineration of the samples in a muffle furnace at 525°C, while Van den Boom *et al.* [23] used the dry digestion official method [24] for the determination of Na, K, Ca, Mg, Fe and Zn by atomic spectrometry.

Microwave-assisted digestion

Wet sample digestion can be performed on a heating plate at atmospheric pressure or inside closed vessels by microwave heating at high pressure. The use of microwave-assisted sample digestion has increased considerably in the last few decades. This technique is rapid and allows total decomposition of sample with minor risks of contamination and minor analyte losses. In the majority of published papers nitric acid and hydrogen peroxide were used for the digestion of baby food samples. The volume of nitric acid varies from 2 to 8 mL and hydrogen peroxide from 0.5 to 2 mL, depending on the study. For example, Bosscher *et al.* [25, 26] used 2 mL of nitric acid and 0.5 mL of H_2O_2 for the digestion of 0.4 g of baby food to be analysed by FAAS for determination of Ca, Fe and Zn. The accuracy of the method was verified by measurement of NIST 1573a Tomato leaves, NBS 1549 Non-fat milk powder and NBS 1571 Orchard leaves, obtaining recovery of 94, 114 and 107% for Ca, Fe and Zn, respectively. Carbonell-Barrachina *et al.* [12] used 2 mL of HNO_3 and 2 mL of H_2O_2 to digest meat and fish baby food samples, with digestion of 30 min at 95°C. The method was validated using NIST SRM 1568a Rice Flour, obtaining recoveries of 64.2–99.5% for 13 analytes studied by ICP-MS. Ozbek and Akman [14] proposed the use of 7 mL of HNO_3 and 1 mL of H_2O_2, with a digestion step at 190°C for 15 min. IRMM-804 Rice Flour and GBW-08503 Wheat flour were used as certified reference materials for evaluation of the effectiveness of the digestion. The recovery percentages obtained were 98% for Fe and 101–102% for Zn. Zand *et al.* [27] used 5 mL of HNO_3 and 0.5 mL of H_2O_2 to digest 0.5 g of wet baby food sample. The recoveries obtained for Ca, Cu, Mg, Fe, Zn, K, Na and Se were 90–112%. Other authors [28–30] used only HNO_3 for sample digestion, with acid volumes of 3–8 mL.

As can be seen, microwave digestion is very versatile, provides a digestion time lower than that required for digestion with acids at atmospheric pressure or for dry ashing, and reagent consumption is not very high, especially when working with sealed reactors. It allows fast and complete dissolution of both dry and wet baby food samples.

32.2.2 Analytical methods

Various factors should be considered when choosing an appropriate measurement technique for determination of the mineral profile in baby foods. Such factors include detection capability and sensitivity, availability of instrumentation, speed of analysis, cost and application, as well as classical features like accuracy, precision and selectivity. Table 32.3 shows a comparison of the characteristics of the main measurement techniques used to determine mineral elements in baby food. The analytical methods employed in the literature to determine minerals in baby food are indicated in Table 32.4, from which it can be seen that minor elements like Fe, Zn and Ca have been frequently determined by FAAS. Trace elements like Cu, Co, Ni, Mn, Se, Cd and Pb were analysed using GFAAS and a more or less complete mineral profile of baby foods was established using ICP-OES or ICP-MS.

Table 32.3 Comparison of the main characteristics of measurement techniques commonly employed for the determination of the mineral profile of baby foods.

Technique	Multi-element	LOD (μg/kg)	Precision (%)	Cost
FAAS	No	1–100	0.1–1	Low
GFAAS	No	0.01–1	1–5	Moderate
ICP-OES	Yes	1–100	0.1–1	High
ICP-MS	Yes	0.001–0.1	0.2–2	High

Table 32.4 Analytical methods employed in the recent literature to determine mineral elements in baby food.

Sample	Elements	Analytical technique	Reference
Baby food reference (TUGAP 1201 R)	Fe, Zn	FAAS	[14]
Foods consumed by children	Fe, Zn	FAAS	[25]
Commercial baby food of vegetables and fruits	Ca, Fe, Zn	FAAS	[26]
Commercial baby food based on chicken, hake, vegetables and fruit	Co, Cu, Ni, Mn	GFAAS	[15]
Commercial baby food, fish based	Cd, Se, Pb	GFAAS	[16]
Foods consumed by children	Cu	GFAAS	[25]
Commercial infant cereals, baby food based on chicken, hake and infant rice	Ca, Co, Cr, Cu, Fe, Mn, Na, Ni, Se, Zn	ICP-MS	[12]
Commercial baby food of meat and vegetables	Ca, Cu, Fe, K, Mg, Na, Se, Zn	ICP-OES/ICP-MS	[27]
Commercial baby food of meat, fish, vegetables and fruit	Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mo, Na, Ni, Zn	ICP-MS	[28]
Commercial baby food	Ca, Cu, Fe, Mn, Ni, Se, Zn	ICP-OES	[29]
Commercial baby food of chicken and fish	Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Se, Zn	ICP-OES/ICP-MS	[31]

Flame atomic absorption spectrometry

FAAS is a well-known technique for the analysis of trace elements in baby food samples. However, despite being a simple and robust tool for the analysis of a wide range of elements, it is a single-elemental technique.

Fe and Zn have been determined by FAAS in vegetable and fruit baby foods [25, 26]. Samples were digested with HNO_3 and H_2O_2 in a microwave oven and analysed using the method of standard addition from 0 to 1 mg/L for Fe, and from 0 to 0.4 mg/L for Zn, with a dilution factor of 4 for Fe and 5 for Zn. This method shows linearity between 0 and 10 mg/L and between 0 and 0.5 mg/L for Fe and Zn, respectively, with good detection limits of 0.06 mg/L and 0015 mg/L (Table 32.5). The reproducibility of the method for 10 measurements of vegetable jars was 2.5–15% for Fe and 8–9.4% for Zn, and for fruit jars was 4.7–14.4% and 8.9–15.8% for Fe and Zn, respectively.

Ozbek and Akman [14] used FAAS for Fe and Zn determination in baby food samples. In this case, slurry sampling with Triton X-114 and nitric acid was used for the direct analysis of these elements. For the determination of Fe a wavelength of 256.8 nm was used, with a limit of detection (LOD) of 5.46 mg/kg. In the case of Zn, a wavelength of 213.9 nm was used, with an LOD of 3.37 mg/kg (Table 32.5). These LODs are quite high, but bearing in mind that samples were not digested and were directly determined in a sample slurry, it must be accepted that the sensitivity is sufficient for screening purposes.

In infant food analysis, Fe and Zn are the two most important elements, and their lack in infant nutrition often creates greater deficiency problems in childhood. Thus it can be concluded that FAAS is a good technique if only Fe and Zn are to be measured, as it has good sensitivity and accuracy and low cost, which makes this an adequate methodology for small laboratories.

Table 32.5 Limits of detection reported in the recent literature for the determination of mineral elements in baby foods.

Element	[12] LOD (mg/kg)	[13] LOD (mg/kg)	[14] LOD (mg/kg)	[25] LOD (mg/L)	[29] LOD (mg/kg)	[31] LOD (mg/kg)
Al		0.15 (ICP-OES)				0.1 (ICP-MS)
As	0.006 (ICP-MS)	0.3 (ICP-OES)				0.003 (ICP-MS)
Ba		0.009 (ICP-OES)				0.02 (ICP-MS)
Ca	10 (AAS)	0.10 (ICP-OES)			2 (ICP-OES)	1.56 (ICP-OES)
Cd	0.007 (ICP-MS)	0.02 (ICP-OES)				0.006 (ICP-MS)
Co	0.037 (ICP-MS)	0.05 (ICP-OES)				0.0015 (ICP-MS)
Cr	0.005 (ICP-MS)	0.04 (ICP-OES)				0.0078 (ICP-MS)
Cu	0.11 (ICP-MS)	0.03 (ICP-OES)		0.0005 (GFAAS)	0.2 (ICP- OES)	0.036 (ICP-MS)
Fe	7 (AAS)	0.05 (ICP-OES)	5.46 (FAAS)	0.06 (FAAS)	0.2 (ICP- OES)	0.15 (ICP-OES)
K		3.0 (ICP-OES)				0.03 (ICP-OES)
Mg		0.03 (ICP-OES)				0.36 (ICP-OES)
Mn	0.02 (ICP-MS)	0.003 (ICP-OES)			0.04 (ICP- OES)	0.014 (ICP-MS)
Mo		0.03 (ICP-OES)				0.0069 (ICP-MS)
Na	10 (AAS)	0.5 (ICP-OES)				0.33 (ICP-OES)
Ni	0.038 (ICP-MS)	0.07 (ICP-OES)			0.2 (ICP- OES)	0.024 (ICP-MS)
Pb	0.039 (ICP-MS)	0.3 (ICP-OES)				0.0042 (ICP-MS)
Se	0.013 (ICP-MS)	1.3 (ICP-OES)			0.2 (ICP- OES)	0.0066 (ICP-MS)
Zn	0.16 (ICP-MS)	0.2 (ICP-OES)	3.37 (FAAS)	0.015 (FAAS)	0.2 (ICP- OES)	0.15 (ICP-OES)

Graphite furnace atomic absorption spectrometry

GFAAS provides a parts per billion detection capability using only microlitre samples deposited inside a graphite tube. This technique is a versatile and highly sensitive tool for food analysis. Viñas *et al.* [15] reported a method for the analysis of meat, fish, vegetable and fruit baby food slurries with regard to the determination of Cu, Co, Ni and Mn. Optimum atomization temperatures for the analytes were 1800°C for Cu, 2600°C for Co, 2600°C for Ni and 2000°C for Mn. The wavelength used to determine Cu, Co, Ni and Mn in baby food were 324.8, 240.7, 232.0 and 279.5 nm, respectively. To evaluate the precision of the method, the relative standard deviation (RSD) values were 3.7–11.2% for Cu, 0.7–1.7% for Mn, 2.4–9.9% for Ni and 6.2–9.6% for Co. Lower RSD values were found for jars of vegetables and fruits, and the highest RSD values were obtained for meat and fish jars.

Viñas *et al.* [16] also employed baby food slurries to determine toxic elements (Cd and Pb) by GFAAS. They used wavelengths of 283.3 and 228.8 nm for Pb and Cd analysis, respectively. LODs obtained by this method were 0.4 μg/kg for Cd and 3.4 μg/kg for Pb, and the corresponding RSD values were 2.5 and 2.7%.

Determination of Cu in samples of food for children aged 2–3 years was made by GFAAS, after a previous microwave-assisted digestion of samples with HNO_3 and H_2O_2 [25]. The applied dilution factor was 10, with LOD of 0.5 μg/L and RSD of 2% for 10 independent determinations in the same sample.

Inductively coupled plasma optical emission spectrometry

ICP-OES is a powerful multi-element technique for the determination of trace elements in foods and offers a very wide linear dynamic range of about five orders of magnitude. Usually, the LODs obtained by ICP-OES are comparable with those obtained by FAAS and higher than those found by GFAAS by about one or two orders of magnitude.

In a study of the mineral profile of infant food, Ca, Cu, Fe, Mn, Ni, Se and Zn were determined by ICP-OES [29]. The wavelengths selected were 393.366, 324.752, 259.939, 257.61, 231.604, 196.026 and 213.857 nm for Ca, Cu, Fe, Mn, Ni, Se and Zn, respectively. LODs obtained are shown in Table 32.5. The accuracy of the method was calculated by RSD, and the values obtained were less than 2% for Mn and Zn, less than 3% for Ca and Fe, less than 5% for Cu, less than 7% for Ni and less than 12% for Se.

In other multi-element studies of baby food samples, ICP-OES was used to determine Ca, Cu, Mg, Fe, Zn, K and Na in meat and vegetable jars [27] or to determine Ca, Fe, Mg, Mn, Na, K and Zn in meat and fish jars [31]. In both cases, microwave digestion with nitric acid and hydrogen peroxide for sample preparation were used. The limit of quantification (LOQ) data were 5.2, 0.5, 0.1, 0.048, 1.2, 1.1 and 0.5 mg/kg for Ca, Fe, Mg, Mn, K, Na and Zn, respectively.

In short it can be concluded that ICP-OES is a very good technique for evaluating the mineral profile of baby foods, because it allows a large number of elements to be determined simultaneously in a short period of time. It is a robust and sensitive technique with good LODs and the cost of analysis is not excessive despite the high consumption of gas required for plasma stabilization and analyte measurement. ICP-OES is a good choice when the aim is to determine elements at ppm levels in baby foods. Conversely, if the objective is to measure elements at ultratrace levels, the sensitivity of this technique is not sufficiently high and a pre-concentration step will be mandatory.

Inductively coupled plasma mass spectrometry

ICP-MS involves the detection of ions extracted from the sample nebulized inside a high-temperature argon plasma. ICP-MS provides multi-element data with LODs lower than those of FAAS and ICP-OES. Some of the specific advantages of ICP-MS include extremely good sensitivity and selectivity, wide linear dynamic range and very fast acquisition of quantitative information about the elements present in the dissolved samples.

In a multi-element study of infant cereal and puréed infant food, Cu, Mn, Zn, Se, Cr, Ni and Co were determined by ICP-MS [12]. LODs obtained with this method can be seen in Table 32.5. The recovery percentages obtained varied were between 80 and 120%. In this study samples from different countries (Spain, UK, China and USA) were compared.

ICP-MS was also used to determine Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mn, Mo, Na, Ni, Pb and Zn in 74 infant food products for babies aged 4–18 months [28]. The accuracy of the method was verified by analysis of a certified material NIST 1577b Bovine Liver. The concentrations found were within 95–105% of the certified values. To assess possible contamination during sample preparation, blanks were prepared using the same procedure employed for samples.

32.3 Mineral composition of baby foods

The daily mineral intake of children can be evaluated by direct determination of the mineral profile of baby foods (Figure 32.3) or by indirect strategies based on both the estimation of food consumed by babies and the mineral composition of basic foods consumed in a country. One of the most common strategies employed to evaluate the mineral intake of baby foods is based on the Feeding Infants and Toddlers Study (FITS). This provides an estimate of the elemental composition of baby foods by surveying parents about the food intake of their children and by accessing data collected in different food databases. Devaney *et al.* [32] and Heird *et al.* [33] reported data for Ca, P, Mg, Fe, Zn, Na and K for infants and toddlers aged 4–24 months. These studies consisted of three separate telephone interviews: a household interview, a 24-hour dietary recall and a second-day 24-hour dietary recall for a random subsample of respondents with supplementary questions on growth, development and feeding patterns and a second dietary recall for a random subset of samples. Data obtained with the survey were entered in the Software of Intake Distribution Estimation (Iowa State University). This program provides estimates of the percentiles of usual nutrient intake distributions, as well as estimates of the proportion above or below defined cut-off values. Six years later, Butte *et al.* [34] followed the same procedure to estimate the intake of Ca, P, Mg, Fe, Zn, Na and K in children aged 0–4 years.

32.3.1 Essential element content in baby foods

As indicated in the introduction, mineral elements play an important role in the normal development of the child. Essential elements are involved in numerous biochemical reactions in the body, affecting physiological, neuromuscular and cardiac functions. They also participate in the development and maintenance of bones and muscles. In this section we will comment in detail on the influence of each of the commonly analysed elements in baby foods.

Potassium

Potassium is the major cation of intracellular fluid and an almost constant component of lean body tissues, and maintains body water and acid balance. It plays a role in

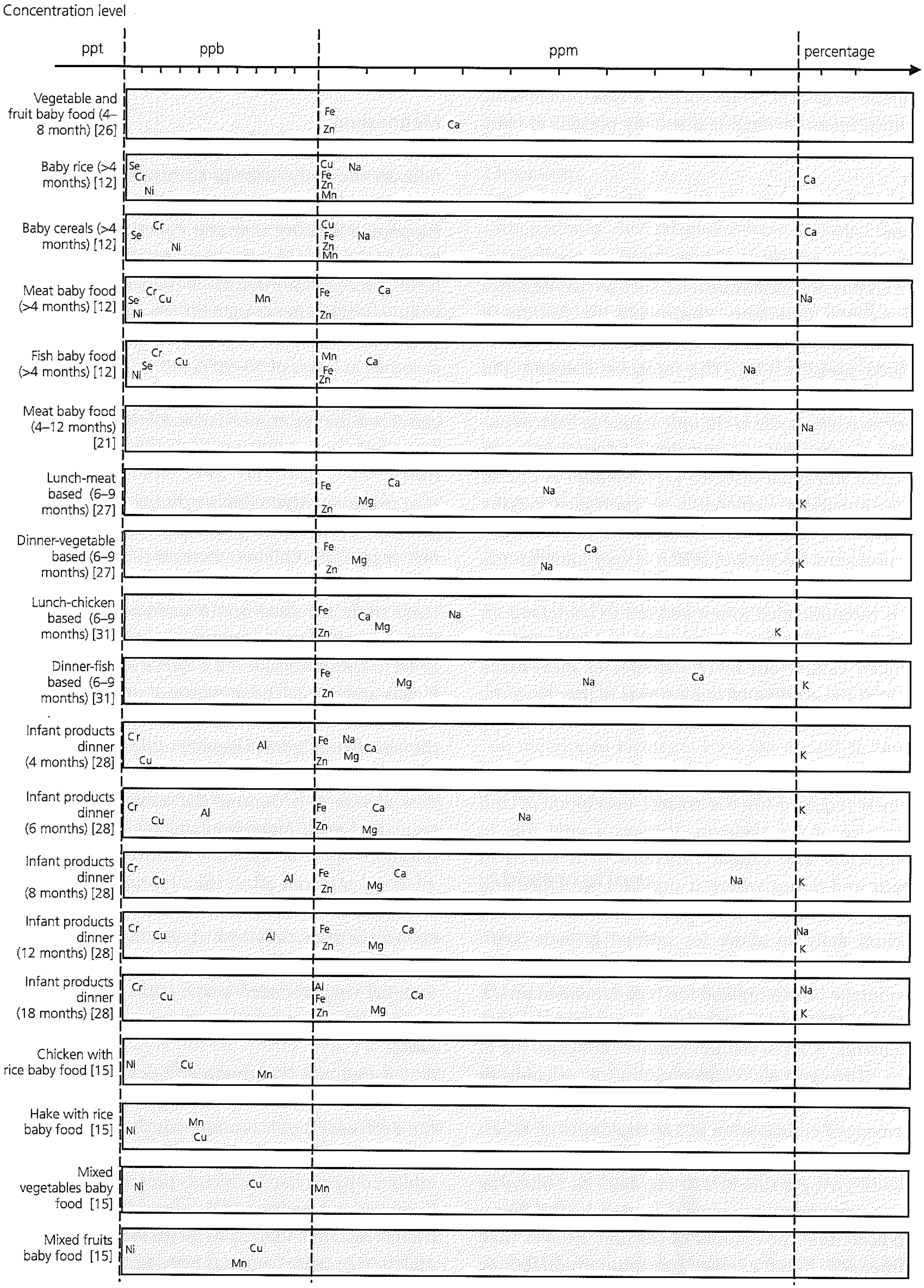

Figure 32.3 Concentrations of mineral elements in baby food samples.

transmission of nerve impulses to muscles, in muscle contraction and in the maintenance of normal blood pressure [35]. About 85% of potassium is absorbed by the human organism. Potassium is a non-problematic element in infant feeding. It is usually present in baby food in adequate quantities at recommended intake values.

Sodium

Sodium is an essential nutrient that is involved in numerous physiological functions, such as maintenance of extracellular osmolarity, controlling the volume of body water compartments and the maintenance of normal blood pressure [36]. The intake of calcium and potassium can affect sodium needs. The major adverse effect of increased sodium chloride intake in babies concerns the increase in blood pressure, a risk factor for cardiovascular and renal diseases [37]. Sodium is one of the most important elements to be controlled in baby diets, because abuse of this element can cause various health problems. Because of this, it is very important to control sodium in commercially available baby foods, and it is recommended to not add salt to baby food. In the literature, values of sodium agree well with the recommended values, but in two studies of meat-based infant food the content of sodium was higher than the recommended values [21, 23].

Calcium

Calcium is required for the correct development and maintenance of the skeleton. It plays a vital role in neuromuscular and cardiac functions. It is stored in the teeth and bones where it provides structure and strength. In the first 2 years of life, infants need 120 mg of calcium daily to allow for normal growth. With human milk, absorption of 120 mg of calcium requires a mean intake of 240 mg and the recommended intake is 300 mg [38]. Sodium intake can affect calcium requirements; 2.3 g of sodium removes about 40 mg of calcium. The average concentration of calcium in breast milk for infants aged 7–12 months is 210 mg/L. This provides a contribution of 126 mg/day from breast milk that is added to the 140 mg/day from complementary foods, giving an AI of 270 mg/day [9]. Following these recommendations, several studies in the literature indicate that the amount of calcium is lower than recommended, for an average consumption of 400 mg of baby food [12, 28]. On the other hand, Zand *et al.* [27, 31] found levels of calcium in vegetable- and fish-based infant food to be higher than those in foods based on meat.

Magnesium

Magnesium is involved in over 300 biochemical reactions in the body. It helps to maintain normal muscle and nerve function, maintains a regular heart rhythm, supports a healthy immune system, and keeps bones strong. Magnesium also helps to regulate blood sugar levels, promotes normal blood pressure, and is known to be involved in energy metabolism and protein synthesis [39]. Magnesium in breast milk or infant formula is absorbed at levels of 50–90% [40, 41]. As for potassium, there is no problem with magnesium because infants can access the recommended amounts in available foods.

Iron

Iron requirements are higher during the periods of rapid growth in early childhood and adolescence. During the first year of life, children increase their weight threefold and should bend body iron content. At 4 months of life there is an increased iron requirement of 0.78 mg/day due to rapid growth and the iron reserves could diminished to pathological levels if needs are not covered with dietary iron [36]. The presence of other nutrients, such as vitamin C and citric, lactic or malic acids, can increase the absorption from vegetables consumed at the same time. In contrast, calcium and zinc can inhibit the absorption of iron [9]. Commercial baby food products are regularly fortified with iron and ascorbic acid. These fortifications must be carefully controlled because a high intake of iron can affect the absorption of other nutrients, such as calcium or zinc. Iron is the most problematic element in terms of bioavailability. In all cases reported in the literature, the amount of Fe in baby food is lower than the recommended intake [12, 23, 25–28, 31].

Zinc

Zinc is essential for growth and development of young children. Dietary intake of iron in some supplements can decrease zinc absorption, which is of particular concern during lactation. The bioavailability of zinc is also inhibited by phytate, which is present in large amounts in cereals and legumes [42]. Absorption of zinc is higher from breast milk than from infant formula based on cow milk or soy; these formulas have a much higher content of zinc that breast milk, which compensates for the low absorption efficiency. Zinc is also problematic in terms of bioavailability, so it is necessary to control the amount of zinc in infant foods.

Manganese

Manganese is an essential element involved in the formation of bone and the metabolism of carbohydrates, cholesterol and amino acids. Manganese is present in many common foods. Therefore it is extremely rare to have a deficiency of this element in humans [43]. In infant foods based on meat, fish or fruit, manganese levels are lower than the AI or RDA, but in vegetable-based infant food Mn values agree with the recommended values [12, 15].

Copper

Copper is essential for life. It is required for infant growth, bone strength, iron transport, and cholesterol and glucose metabolism [44]. Very high levels of zinc and iron, generally taken as supplements, can affect the absorption of Cu in infants. Copper deficiency can affect the central nervous system and the immune and cardiovascular systems, notably in infants [45]. In general, the values of Cu in complementary foods are adequate to meet the recommended intake, although in some cases values of Cu are lower than the recommended values, for example in dinner products for babies aged 12–18 months analysed by Melø *et al.* [28], meat- and fish-based baby foods from Spain [12] or commercial baby foods containing chicken with rice and hake with rice from Spain [15]. However, it must be taken into account that milk also provides small amounts of Cu.

Selenium

Selenium has been implicated in the protection of body tissues against oxidative stress, the maintenance of defences against infection, and the modulation of growth and development. Cow milk-based formula provides less than one-third of the selenium content of human milk. Estimates of selenium intake by 2-month-old infants were 7.8 μg/day from cow-milk compared with 22.4 μg/day from human milk [7]. Normal content of selenium in baby foods is 50–120 μg/kg depending on type of baby food. High levels of Se have been reposted in fish-based foods [12].

Molybdenum

Molybdenum is absorbed very efficiently by passive transport. Legumes are major contributors of molybdenum in the diet, as are grain products and nuts. Animal foods, fruits and vegetables are low in molybdenum [7]. Molybdenum intakes in Asian countries are higher due to the predominantly vegetarian diet in these countries. Between weaning and 3 years of age, molybdenum intakes appear to be higher but decline thereafter [11].

Chromium

Chromium is important in the metabolism of fats and carbohydrates. Several studies have indicated the beneficial effects of chromium on circulating glucose, insulin and lipids in humans. Chromium deficiency may be seen as impaired glucose tolerance and can be found in infants with protein-calorie malnutrition. Supplementation of chromium could be recommended but Cr absorption is low and its excretion rate is high. Additionally, toxicity of this element is not common [46]. A study on the mineral and trace elements in commercial infant food [28] for infants aged 4–18 months found high values of Cr compared with the AI and RDA. The same results were found in a study of essential elements in infant foods from Spain, the UK, China and the USA [12].

From literature data summarized in Figure 32.3 it can be seen that K followed by Ca and Na are present at percentage levels in baby foods analysed worldwide, with Mg, Fe, Zn, Cu, Mn and Al at ppm levels and Ni, Cr and Se at ppb levels. However, depending on the type of food analysed and the country of origin, the concentrations of the mentioned elements vary. On the other hand, it is important to note that highly toxic elements like As, Pb, Cd or Hg have not been reported in baby foods.

Abbreviations

AI: Adequate intake
AOAC: Association of Official Analytical Chemists
EAR: Estimated average requirement
ESPGHAN: European Society of Paediatric Gastroenterology, Hepatology and Nutrition
FAAS: Flame atomic absorption spectrometry
FAO: Food and Agriculture Organization
FITS: Feeding Infants and Toddlers Study
GFAAS: Graphite furnace atomic absorption spectrometry
HGAAS: Hydride generation atomic absorption spectrometry
ICP-MS: Inductively coupled plasma mass spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry

IRMM: Institute for Reference Materials and Measurements
LOD: Limit of detection
LOQ: Limit of quantification
MW: Microwave
NBS: National Bureau of Standards
NIST: National Institute of Standards and Technology
RDA: Recommended daily allowance
RDI: Recommended daily intake
RM: Reference materials
RNI: Recommended nutrient intake
RSD: Relative standard deviation
SRM: Standard reference materials
UL: Tolerable upper intake level
UNICEF: United Nations Children's Fund
WHO: World Health Organization

References

1 ESPGHAN Committee on Nutrition. Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2008;**46**:99–110.

2 Foote KD, Marriott LD. Weaning of infants. *Arch Dis Child* 2003;**88**:488–492.

3 World Health Organization. *Infant and Young Child Feeding. Model Chapter for Textbooks for Medical Students and Allied Health Professionals.* WHO, Geneva, 2009.

4 World Health Organization. *Guiding Principles for Complementary Feeding of the Breastfed Child.* WHO, Geneva, 2003.

5 World Health Organization. *Strengthening Action to Improve Feeding of Infants and Young Children 6–23 Months of Age in Nutrition and Child Health Programmes.* WHO, Geneva, 2008.

6 Food and Nutrition Board, Institute of Medicine. *Dietary Reference Intake (DRIs): Recommended Intakes for Individuals Elements.* National Academies Press, Washington, DC, 2004.

7 National Health and Medical Research Council. Nutrient reference values for Australia and New Zealand. National Health and Medical Reserch Council, Canberra, Australia, 2005.

8 Scientific Committe on Food. Opinion of the Scientific Committee on Food on the revisión of reference values for nutrition labelling. SCF/CS/NUT/GEN/18 Final, European Commission, 2003.

9 FAO/WHO. *Vitamin and Mineral Requirements in Human Nutrition: Report of a Joint FAO/WHO Expert Consultation,* 2nd edn. Bangkok, Thailand, 2004.

10 Food and Nutrition Board. *Dietary Reference Intake: The Essential Guide to Nutrient Requirements.* National Academies Press, Washington, DC, 2006.

11 World Health Organization. *Trace-Elements in Human Nutrition and Health.* WHO, Geneva, 1996.

12 Carbonell-Barrachina AA, Ramirez-Gandolfo A, Wu X *et al.* Essential and toxic elements in infant foods from Spain, UK, China and USA. *J Environ Monit* 2012;**14**:2447–2455.

13 Mir-Marqués A, González-Masó A, Cervera ML, de la Guardia M. Mineral profile of Spanish commercial baby food. Unpublished report.

14 Ozbek N, Akman S. A slurry sampling method for the determination of iron and zinc in baby food by flame atomic absorption spectrometry. *Food Addit Contam* 2012;**29**:208–216.

15 Viñas P, Pardo-Martínez M, Hernández-Córdoba M. Determination of copper, cobalt, nickel, and manganese in baby food slurries using electrothermal atomic absorption spectrometry. *J Agric Food Chem* 2000;**48**:5789–5794.

16 Viñas P, Pardo-Martínez M, Hernández-Córdoba M. Rapid determination of selenium, lead and cadmium in baby food samples using electrothermal atomic absorption spectrometry and slurry atomization. *Anal Chim Acta* 2000;**412**:121–130.

17 Fernandez DR, Vanderjagt DJ, Williams M *et al.* Fatty acid, amino acid, and trace mineral analyses of five weaning foods from Jos, Nigeria. *Plant Food Hum Nutr* 2002;**57**:257–274.

18 Thomson BM, Vannoort RW, Haslemore RM. Dietary exposure and trends of exposure to nutrient elements iodine, iron, selenium and sodium from the 2003–4 New Zealand Total Diet Survey. *Br J Nutr* 2008;**99**:614–625.

19 Burló F, Ramírez-Gandolfo A, Signes-Pastor A, Haris P, Carbonell-Barrachina A. Arsenic contents in Spanish infant rice, pureed, infant foods and rice. *J Food Sci* 2012;**71**:T15–T19.

20 Ybañez N, Cervera ML, Montoro R, de la Guardia M. Comparison of dry mineralization and microwave-oven digestión for the determination of arsenic in mussel products by platform in furnace Zeeman-effect atomic-absorption spectrometry. *J Anal Atom Spectrom* 1991;**6**:379–384.

21 Okesli V, González-Bermúdez CA, Vidal-Guevara ML, Dalmau J, Ros G. Alimentación complementaria: ¿es igual el perfil nutricional de un pure casero que el de un tarrito commercial? *Acta Pediátr España* 2011;**69**:235–240.

22 Association of Official Analytical Chemists. *Official Methods of Analysis,* 15th edn. AOAC, Rockville, MD, 1990, pp. 931–945.

23 Van den Boom S, Kimber AC, Morgan JB. Nutritional composition of home-prepared baby meals in Madrid. Comparison with comercial products in Spain and home-made meals in England. *Acta Paediatr* 1997;**86**:57–62.

24 Association of Official Analytical Chemists. *Official Methods of Analysis,* 15th edn. AOAC, Rockville, MD, 1990, pp. 806–807.

25 Bosscher D, Van Cauwenbergh R, Robberecht H, Van Caillie-Bertrand M, Deelstra H. Daily dietary iron, zinc and copper intake of infants in Belgium. *Eur Food Res Technol* 2002;**215**:275–278.

26 Bosscher D, Van Cauwenbergh R, Van der Auwera J, Robberecht H, Deelstra H. Calcium, iron and zinc availability from weaning meals. *Acta Paediatr* 2002;**91**:761–768.

27 Zand N, Chowdhry BZ, Zotor FB, Wray DS, Amuna P, Pullen FS. Essential and trace elements content of commercial infant foods in the UK. *Food Chem* 2011;**128**:123–128.

28 Melø R, Gellein K, Evje L, Syversen T. Minerals and trace elements in commercial infant food. *Food Chem Toxicol* 2008;**46**:3339–3342.

29 Pandelova M, Lopez WL, Michalke B, Schramm K. Ca, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn contents in baby foods from the EU market: comparison of assessed infant intakes with the present safety limits for minerals and trace elements. *J Food Compos Anal* 2012;**27**:120–127.

30 Plessi M, Bertelli D, Monzani A. Determination of aluminum and zinc in infants formulas and infant foods. *J Food Compos Anal* 1997;**10**:36–42.

31 Zand N, Chowdhry BZ, Wray DS, Pullen FS, Snowden MJ. Elemental content of commercial 'ready to-feed' poultry and fish based infant foods in the UK. *Food Chem* 2012;**135**: 2796–2801.

32 Devaney B, Ziegler P, Psc S, Karwe V, Barr S. Nutrient intake of infants and toddlers. *J Am Diet Assoc* 2004;**104**: S14–S21.

33 Heird W, Ziegler P, Reidy K, Briefel R. Current electrolyte intakes of infants and toddlers. *J Am Diet Assoc* 2006;**106**: S43–S51.

34 Butte NF, Fox MK, Briefel RR *et al.* Nutrient intakes of US infants, toddlers, and preschoolers meet or exceed dietary reference intakes. *J Am Diet Assoc* 2010;**110**:S27–S37.

35 Food and Agriculture Organization. Alimentation, nutrition et agricultura, Alimentación, nutrición y agricultura. Available at www.fao.org/docrep/x2650T/x2650T00.htm.

36 Gil Hernández A, Uauy Dagach R, Dalmau Serra J. Bases for adequate complementary feeding in infants and young children [in Spanish]. *An Pediatr (Barc)* 2006;**65**:481–495.

37 Graudal NA, Galloe AM, Garred P. Effects of sodium restriction on blood pressure, renin, aldosterone, catecholamines, cholesterols and triglyceride: a meta-analysis. *JAMA* 1998;**279**:1383–1391.

38 FAO/WHO. *Human Vitamin and Mineral Requirements. Report of a Joint FAO/WHO Expert Consultation*. Bangkok, Thailand, 2002.

39 Office of Dietary Supplements. Strengthening knowledge and understanding of dietary supplements: Magnesium. Available at http://ods.od.nih.gov/factsheets/Magnesium-Consumer/.

40 Lonnerdal B. Magnesium nutrition of infants. *Magnesium* 1995;**8**:99–105.

41 Lonnerdal B. Effects of milk and milk components on calcium, magnesium and trace element absorption during infancy. *Physiol Rev* 1997;**77**:643–669.

42 Brown KH, Wuehler SE, Peerson JM. The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. *Food Nutr Bull* 2001;**22**:113–125.

43 World Health Organization. *Guidelines for Drinking-water Quality: Manganese in Drinking-water*. Available at www.who.int/water_sanitation_health/dwq/chemicals/manganese.pdf.

44 Scientific Committe on Food. *Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Copper*. SCF/CS/NUT/UPPLEV/57 Final, European Commission, 2003.

45 Turnlund JR. Copper. In: Shils ME, Olson JA, Shike M, Ross AC (eds) *Modern Nutrition in Health and Disease*, 9th edn. Williams & Williams, Baltimore, 1999, pp. 241–252.

46 Food and Nutrition Board, Institute of Medicine. Chromium in diet: MedlinePlus Medical Encyclopedia, US National Library of Medicine, 2011.

Journal of Food Composition and Analysis 27 (2012) 160–168

Contents lists available at SciVerse ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus

Alba Mir-Marqués, M. Luisa Cervera *, Miguel de la Guardia

Department of Analytical Chemistry, University of Valencia, Jeronimo Muñoz Building, 50th Dr. Moliner St., 46100 Burjassot (Valencia), Spain

ARTICLE INFO

Article history:
Received 20 December 2011
Received in revised form 16 May 2012
Accepted 17 June 2012

Keywords:
Food analysis
Food composition
Mineral composition
Inductively coupled plasma optical emission spectroscopy (ICP-OES)
Essential elements
Toxic elements
Menu samples
Multi-elemental analysis
Recommended daily intake (RDI)
Food safety

ABSTRACT

The concentration of mineral elements in complete menus has in Spain been determined in order to establish the levels of essential and toxic elements and their contribution to the maximum recommended intake. Thirteen university canteen menus obtained from the Burjassot campus, in Valencia, were analysed to establish a first approach on mineral composition in the Spanish diet. The menus were composed of a mixture of cereals, meat, fish, vegetables, fruits and dairy products. Samples were crushed, homogenized and lyophilized, and 25 elements were determinated by inductively coupled plasma optical emission spectroscopy (ICP-OES) after digestion of samples in a microwave oven. Menu samples analyzed are in general adequate for an appropriate mineral intake, providing from 33–66% of the recommended daily intake (RDI) for Ca, 34–152% for Cu, 24–133% for Fe, 20–81% for K, 28–109% for Mg, 41–93% for Mn, and 33–72% for Zn, with however excessive use of sodium chloride, 61–202% of the RDI. On the other hand, the absolute amount of non-essential elements provided by the menus studied varied from non-detectable amounts to 3 mg Al, 0.8 mg As, 0.5 mg Ba, 0.05 mg Li, 2 mg Sr or 1 mg Ti.

1. Introduction

Human exposure to mineral elements is an important problem the point of view of both nutritional and environmental toxicology, because some mineral elements are essentials for life but many heavy metals are regarded as toxic to living organisms. Therefore efforts should be focused on the estimation of daily intakes of mineral elements by consumers. To accomplish this, the most informative and sensitive analytical methods are required, in order to monitor human ingestion and evaluate the risks associated with the use of foods produced in polluted areas or subjected to bad processing practices.

There are two main alternative ways to estimate the daily mineral intake in a country: one corresponds to Total Diet Study (TDS) market basket studies, and consists of representative key foods (raw, and processed using commonly used methods) (EFSA, 2011; Food Standards Australia New Zealand, 2008; French Total Diet Study 2, 2011; Millour et al., 2010, 2011a,b; U.S. Food Drug Administration, 2010; Tanase et al., 2011). The other way is based on the duplicate portion studies analysis of duplicate portions of foods in representative meals and analysis of individual food items (Bastías et al., 2010; Lee et al., 2006; Marzec and Schlegel-Zawadzka, 2004; Nöel et al., 2003). With this in mind, in the present study we determined the mineral composition of university canteen menu samples because many people eat in these places every day, and thus the data obtained could be of great value for an estimation of the total diet in a country to be compared with data from other sources.

To date, many total diet studies have been carried out to determine the quantity of mineral elements ingested with foods (Food Standards Australia New Zealand, 2008; Muñoz et al., 2005; Pennington and Schoen, 1995; Urieta et al., 1996; U.S. Food Drug Administration, 2010; Ysart et al., 1999, 2000), along with studies of specific meals to determine the amount of essential elements ingested with the foods (Cheung et al., 2008; Llobet et al., 2003; Rubio et al., 2005, 2006; Sola-Larrañaga and Navarro-Blasco, 2009) and duplicated portion studies (Mainhara et al., 1998, 2004; Nöel et al., 2003; Robberecht et al., 2002; Van Cauwenbergh et al., 1999, 2000). There are also studies in the literature on exposure to toxic elements such as As, Cd, Pb or Hg in diets (Cheung et al., 2008; Lee et al., 2006; Llobet et al., 2003; MacIntosh et al., 1996; Mainhara et al., 1998; Muñoz et al., 2005; Rubio et al., 2006). Other studies have focused on a specific essential element, for example Se (Mainhara et al., 2004; Pedrero and Madrid, 2009).

The majority of mineral components to be determined in diet samples are present at trace and ultra-trace levels; hence

---

* Corresponding author. Tel.: +34 96 354 3486; fax: +34 96 354 4845.
E-mail address: m.luisa.cervera@uv.es (M.L. Cervera).

0889-1575/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jfca.2012.06.006

analytical techniques with adequate sensitivity are required for their accurate determination. Additionally, it is important to remember that the accuracy of the quantitative analysis procedures is strongly dependent on the sampling and sample preparation steps. The techniques most employed for trace element analysis in foods are those based on atomic spectrometry and inorganic mass spectrometry. To analyze a few elements it is very common to use electrothermal atomic absorption or hydride generation atomic absorption or fluorescence spectrometry. However, when there is a general interested on the mineral composition of foods and diets, multi-elemental techniques based on inductively coupled plasma optical emission (ICP-OES) (Boeting et al., 2010; González et al., 2009, 2011; Lee et al., 2006; Sola-Larrañaga and Navarro-Blasco, 2009) or inductively coupled plasma mass spectrometry (ICP-MS) (Llobet et al., 2003; Millour et al., 2011a; Nöel et al., 2003) are the best options.

The main purpose of this study was to evaluate the nutrient profile and exposure to chemical contaminants of students at the University of Valencia. Data obtained have been used for establishing an approximation of the daily intake of essential and toxic elements by people living in the same area.

2. Materials and methods

2.1. Instruments and reagents

ICP-OES measurements were carried out using a Perkin Elmer Model Optima 5300 DV spectrometer (Norwalk, CT, USA) equipped with an auto sampler AS 93-plus, and a cross flow nebulizer. Argon C-45 (purity higher than 99.995%), supplied by Carburos Metálicos (Barcelona, Spain), was employed as plasmogen and carrier gas. The operating conditions of the ICP-OES equipment are summarized in Table 1.

A microwave laboratory system, Ethos SEL from Millestone (Sorisole, Italy), equipped with a thermocouple, for automatic temperature control, and an automatic gas leaks detector has been employed for samples digestion. The system operated at a maximum exit power of 1000 W, was employed for microwave-assisted digestion of menu samples using high pressure Teflon vessels of 100 mL inner volume. For cleaning the vessels an automatic cleaning device traceCLEAN from Millestone was used.

Other equipment included a Cryodos 50 lyophilizer Telstar (Barcelona, Spain) employed for sample preservation, and an ultrasound water bath from Selecta (Barcelona, Spain), of 9 L volume with 50 W power and 50 Hz frequency, which was used for sample sonication.

All reagents used were of analytical grade and all solutions were prepared in ultrapure water with a minimum resistivity of 18.0 MΩ cm obtained from a Milli-Q Millipore system (Bedford, MA, USA).

Stock solutions of a multi-element standard solution (100 mg L^{-1}) containing 26 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Se, Na, Sr, Tl, Ti, V and Zn) dissolved in 5% HNO_3 from Scharlau (Barcelona, Spain), and a multi-element solution containing 20 mg L^{-1} Dy, Er, Eu, Gd, Ho, Lu, Sm, Sc, Tb, Tm, Yb and Y and 100 mg L^{-1} Ce, La, Nd and Pr dissolved in 5% HNO_3, also from Scharlau, were employed to prepare the calibration standards.

Table 1
Instrumental parameters employed for ICP-OES determination of the mineral composition of menu samples.

Parameter	Value
RF power	1200 W
Gas	Argon
Plasma gas	15 L min^{-1}
Auxiliary gas	0.5 L min^{-1}
Nebuliser gas	0.7 L min^{-1}
Sample aspiration rate	1.10 mL min^{-1}
View	Axial and radial for Ca, K, Mg and Na
Background correction	2 points
Number of replicates	3
Nebuliser	Cross-flow

HNO_3 69% for trace analysis and H_2O_2 35% reagent grade, both from Scharlau, were used for sample treatment.

A 1000 mg L^{-1} Ruthenium Atomic Spectroscopy Standard Solution prepared from $RuCl_3$ in 1 M HCl Fluka (Buchs, Switzerland) and 1000 mg L^{-1} Scandium Atomic Spectroscopy Standard Solution prepared with Sc_2O_3 in 0.5 M HNO_3 Fluka were used as internal standards. A 1000 mg L^{-1} Sodium Standard Solution for AA prepared with $NaNO_3$ in 0.5 M HNO_3 Scharlau, a 1000 mg L^{-1} Potassium Standard Solution for Atomic Absorption prepared with KNO_3 in 0.5 M HNO_3 Scharlau, were also employed.

2.2. Samples and sampling plan

Samples of different menus were purchased in 2010 at two canteens of the campus of Burjassot of the University of Valencia. Menus were composed of a starter, a main dish and a dessert and also included a 50–70 g piece of bread. The foods corresponding to the starter, main course, bread and dessert were separated into different plastic containers and transported to the laboratory.

The composition of each sample menu analyzed is described in Table 2. The samples were chosen to include all the foods offered to the students in the canteen menus. Different starters and main courses were selected randomly between those offered each day in order to have a representative description of the diet followed in the area of study.

2.3. Sample storage and handling

Samples were handled immediately if possible or stored in the refrigerator until analysis for a period of less than 24 h. The edible part of the foods was weighed and mixed, and immediately after the samples were crushed and homogenized with a domestic Braun mixer (Kronberg, Germany), and finally frozen at −20 °C in a freezer. Afterwards, they were lyophilized for a minimum of 48 h at a chamber pressure of 0.05 mbar. The dried complete menu samples were pulverized with a domestic mixer, and the powdered samples were stored in polyethylene bottles until analysis.

2.4. Sample digestion

A 1 g freeze-dried menu sample was accurately weighed inside the Teflon digestion vessel and 8 mL of concentrate nitric acid were added, allowing the sample to be predigested by standing open in an ultrasound water bath for a minimum of 15 min. Then 2 mL of H_2O_2 were added and the mixture was sonicated for a further 45 min. After that, the Teflon reactors were closed and placed inside the microwave oven. The following program was run: step 1, 3 min to reach 85 °C; step 2, 12 min to reach 145 °C; step 3, 10 min to reach 180 °C; step 4, 15 min at 180 °C; and step 5, cooling down. The reactors were opened and sonicated. The resulting solution was quantitatively transferred to a plastic flask and made up to 20 mL with nanopure water.

Samples were predigested inside the reactors vessels with HNO_3 and after that with H_2O_2, by sonication in an ultrasound water bath for 1 h. This step (60 min) is time-consuming because the amount of sample employed (1 g) is higher compared with the conventional procedures for microwave-assisted digestion, but it is necessary in order to avoid foam formation and excess of gasses inside the reactors during the digestion step which could produce too much pressure. This process removes the main part of the gases

Table 2
Food composition of the menu samples analyzed through this study.

Menu	Starters	Main courses	Dessert
1	Seafood paella (rice, mussels, grouper, squid rings)	Salmon with chips	Pear
2	Vegetable pie with tomato sauce (carrots, tomatoes, peppers, beets, zucchini,. . .)	Tuna omelette with smashed potatoes	Home-made crème caramel
3	Lentil (350 g)	Ham croquettes (170 g) with chips (100 g)	Skimmed yogurt (120 g)
4	Spaghetti with carbonara sauce (380 g)	Sausages (140 g) with chips (80 g) and vegetables (peppers, cabbage and garlic) (25 g)	Fried milk with sugar a nd cinnamon (90 g)
5	Salad (lettuce, tomato, corn, carrot, eggs, cucumber, pepper, soy and olives) (300 g)	Mixed spinach and mushrooms (190 g) with steamed potatoes (90 g)	Orange (180 g)
6	Soup with bread, garlic, egg, onion (330 g)	Grilled tuna (120 g) with steamed potatoes (150 g)	Lemon yogurt (125 g)
7	Macaroon with tomato (390 g)	Ham (30 g), bacon (20 g) and sausage (50 g) grilled with potatoes (85 g), cabbage and steamed carrot (120 g)	Apple (120 g)
8	Beans (360 g)	Meatballs with sauce (peas, carrots and potatoes) (200 g) with chips (80 g)	Orange gelatine (75 g)
9	Vegetable cream (260 g)	Roast chicken (110 g) with green pepper (55 g) and chips (130 g)	Orange (80 g)
10	Spaghetti with carbonara sauce (310 g)	Ham schnitzel (85 g) with chips (70 g)	Vanilla custard (120 g)
11	Salad (lettuce, carrots, ham, soy, cheese and mayonnaise) (225 g)	Cod (130 g) with carrots, parsley and other vegetables (60 g)	Orange (200 g)
12	Beans with ham (250 g)	Zucchini gratin (180 g) with béchamel sauce, cheese and bacon (80 g)	Strawberry and orange juice (110 g)
13	Rice with squid, cuttlefish and prawns (260 g)	Pork loin (80 g) with carrots (15 g), peas (18 g) and chips (85 g)	Lemon yogurt (125 g)

Note: All menus include approximately 70–75 g (M1–M9) and 50 g (M10–M13) of bread.

formed during the decomposition of samples, and prevents leaks. Finally, reactors were introduced in the microwave oven. After digestion, the solutions were sonicated to eliminate nitrous vapours. A complete digestion for all the samples assayed was carried out, obtaining a clear and precipitate free solutions for all the samples assayed.

2.5. *ICP-OES determination*

The calibration range for the 24 trace elements studied was established from 0.05 to 5 mg L^{-1}, and for K and Na it was established from 2 to 10 mg L^{-1}. Scandium (0.5 mg L^{-1}) was used as internal standard and added to all samples and standards. Digested samples were measured with a final dilution 1:2 and making 1:100 dilution to determine the concentration of Ca, K, Mg and Na. Determinations were made using the instrument parameters indicated in Table 1.

2.6. *Quality control*

Samples were prepared and measured in triplicate. Reagent blanks were made regularly together with each batch of sample digestion, and certified reference materials were digested and measured together with the different sample batches. Additionally, control standards were measured for every series of 10 independent sample measurements.

Three different certified reference materials were analysed: NIST 1568a (Rice Flour) obtained from the NIST (National Institute of Standards and Technology, Gaithensburg, MD, USA); TORT-2 (Lobster Hepatopancreas), purchased from the NRC (National Research Council, Ottawa, Canada) and BCR-185R (Bovine liver) obtained from the IRMM (Institute for Reference Materials and Measurements, Geel, Belgium).

The limit of detection of instrument (LODi) values were calculated as the concentrations corresponding to signals equal to 3 times the standard deviation of 10 reagents blank solutions. Additionally, limits of detection of the method values (LODm) were referred to the original samples (mg kg^{-1}), taking into consideration the amount of sample digested and the final dilution employed in the recommended procedure. The limits of quantification of instrument (LOQi) were determined in the same way for a factor of 10 times the deviation of blank measurements, and the limits of quantification of the method (LOQm) were calculated in terms of concentration in the original sample.

The repeatability of the instrumental measurements was calculated as the relative standard deviation (RSD%) of 10 independent measurements of a 100 μg L^{-1} multi-elemental standard solution, except for Ca, K, Mg and Na for which a standard solution of 5 mg L^{-1} was used. The repeatability of the whole method was calculated as the relative standard deviation (RSD%), for 3 independent analysis of 3 independent portions of the same sample.

3. Results and discussion

3.1. *Sample measurements*

Wavelengths recommended by the manufacturer and additional wavelengths were employed for the determination of each element in order to verify possible interferences and to establish appropriate baseline correction at different spectral regions. In general, those lines recommended by the instrument manufacturer were chosen, as they are the most sensitive ones. Table 3 shows the selected wavelengths employed to analyze the menu samples.

In ICP-OES measurements one of the main problems is due to the high emission intensity of alkaline and alkaline-earth elements. These elements can modify excitation and ionization plasma conditions. Additionally the presence of major elements can affect the introduction rate of the aforementioned samples, through changes in the sample viscosity and surface tension, thus creating physical and matrix interferences in the trace element determinations. One way to solve problems produced by the matrix consists of diluting the samples. A study of different dilution factors: 1:10^4, 1:10^3, 1:10^2, 1:10 and 1:2 was carried out for all considered elements and data found were compared with the direct measurement of undiluted digested samples. The dilution 1:100 was chosen to measure major elements (Ca, Na, K, Mg). For the rest of the elements, studies of standard addition on the sample digested undiluted and with dilution 1:2 and 1:10 were carried out. Table 4 shows the comparison of the slopes for the

Table 3
Analytical figures of merit of ICP-OES determination of the mineral composition of menu samples.

	Spectral line (nm)	LODi (mg L^{-1})	LODm (mg kg^{-1})	LOQi (mg L^{-1})	LOQm (mg kg^{-1})	RSDi (%)
Al	396.153	0.06	2.4	0.20	8	2.1
As	188.979	0.014	0.54	0.047	1.8	8.7
Ba	233.527	0.0025	0.10	0.0083	0.33	1.6
Be	313.042	0.002	0.08	0.0067	0.27	0.2
Bi	223.061	0.01	0.4	0.033	1.3	2.2
Ca	317.933	0.25	10	0.83	33	0.8
Cd	228.802	0.0007	0.027	0.0023	0.09	1.7
Co	228.616	0.0013	0.05	0.0043	0.17	1.5
Cr	267.716	0.002	0.08	0.0067	0.27	1.7
Cu	327.393	0.001	0.04	0.0033	0.13	0.5
Fe	238.204	0.006	0.23	0.020	0.77	1.5
K	766.490	0.2	7.5	0.67	25	1.5
Li	670.784	0.00006	0.0025	0.0002	0.008	0.9
Mg	285.213	0.01	0.4	0.033	1.3	0.7
Mn	257.610	0.0012	0.05	0.004	0.17	0.4
Mo	202.031	0.0024	0.1	0.008	0.33	1.6
Na	589.592	0.26	10.6	0.87	35	0.7
Ni	231.604	0.0025	0.1	0.008	0.33	1.3
Pb	220.353	0.0032	0.13	0.011	0.43	3.5
Se	196.026	0.027	1.1	0.090	3.7	7.5
Sr	407.771	0.0006	0.026	0.002	0.09	2.2
Ti	334.940	0.0004	0.016	0.001	0.05	0.3
Tl	190.801	0.007	0.3	0.023	1.0	17
V	290.880	0.005	0.21	0.017	0.7	1.1
Zn	206.200	0.005	0.21	0.017	0.7	1.5

LODi, limit of detection of instrument; LODm, limit of detection of the method; LOQi, limit of quantification of instrument; LOQm, limit of quantification of the method; RDSi, relative standard deviation of instrumental measures.

standard addition and multi-element external calibration obtained, in order to evaluate the matrix effect and any transports and nebulization interferences.

For undiluted digested samples, relative errors higher than 10% were found for half of the elements; however, for the dilution 1:2, relative errors were lower than 10% for all elements except Al, thus this dilution (1:2) was chosen for sample measurements.

3.2. The use of internal standards

The choice of a suitable internal standard and the use of relative measurements of the emission intensities between the analyte and the internal standard can compensate for some matrix induced signal variations as well as the background drift, thus improving accuracy and repeatability. Ru and Sc were tested as internal standards for the determination of mineral elements in the menu samples. Results obtained from the analysis of samples showed higher sensitivity for Sc as compared with Ru (Sc provides 490,000–550,000 emission units while Ru provides 7500–10,000 emission units). So, the use of Sc is recommended because of the greater number of emission units obtained, resulting in the most effective the internal standard correction. This internal standard was added to all samples, calibration standards and blanks at a fixed concentration of 0.5 mg L^{-1}.

3.3. Analytical characteristics of the method

The mineral contents provided in the present study were submitted to a quality control of the measurements through certified referential materials, reagent blanks and standards

Table 4
Study of standard addition: comparison of the slope for standard addition in front of external multi-element calibration for the undiluted digested sample and for the 1:2 dilution factors.

Analyte	Slope multielemental calibration	Dilution 1:2		Undiluted	
		Slope standard addition	Relative error	Slope standard addition	Relative error
Al	47,232	57,440	22	58,741	24
As	846	829	−2	757	−10
Ba	39,620	38,372	−3	35,738	−10
Be	586,373	556,369	−5	496,472	−15
Bi	1200	1219	2	1156	−4
Cd	18,031	17,972	−0.3	16,538	−8
Co	10,122	9776	−3	8914	−12
Cr	26,613	26,411	−1	24,823	−7
Cu	92,702	101,363	9	100,994	9
Fe	21,441	20,643	−4	18,915	−12
Mn	151,774	145,985	−4	136,142	−10
Mo	6340	6291	−1	5898	−7
Ni	8593	8553	−0.5	7895	−8
Pb	1844	1820	−1	1665	−10
Se	447	449	0.5	417	−7
Sr	1,445,955	1,453,792	1	1,375,736	−5
Ti	130,737	130,239	−0.4	123,854	−5
Tl	887	870	−2	780	−12
V	29,640	29,557	−0.3	27,767	−6
Zn	7850	7853	0.04	6868	−13

Note: Relative error was calculated (((slope standard addition − slope multi-elemental calibration)/slope multi-elemental calibration) × 100).

Table 5
Evaluation of the accuracy of the method employed through the comparison between values found and those certified in a series of food reference materials.

Element	TORT-2		NIST 1568a		BCR-185R	
	Obtained ($\mathrm{mg\,kg^{-1}}$)	Certified ($\mathrm{mg\,kg^{-1}}$)	Obtained ($\mathrm{mg\,kg^{-1}}$)	Certified ($\mathrm{mg\,kg^{-1}}$)	Obtained ($\mathrm{mg\,kg^{-1}}$)	Certified ($\mathrm{mg\,kg^{-1}}$)
Al	–	–	3.5 ± 0.5	4.4 ± 1.0	–	–
As	24 ± 1	21.6 ± 1.8	<LOD (0.54)	0.29 ± 0.03	<LOD (0.54)	0.033 ± 0.002
Cd	25.9 ± 0.4	26.7 ± 0.6	<LOD (0.027)	0.022 ± 0.002	<LOD (0.027)	0.544 ± 0.017
Cr	0.64 ± 0.03	0.77 ± 0.15	–	–	–	–
Cu	109.0 ± 0.5	106 ± 10	2.55 ± 0.08	2.4 ± 0.3	284 ± 3	277 ± 5
Fe	98 ± 3	105 ± 13	7.3 ± 0.2	7.4 ± 0.9	–	–
Mn	12.7 ± 0.2	13.6 ± 1.2	18.5 ± 0.3	20.0 ± 1.6	10.7 ± 0.2	11.07 ± 0.29
Mo	0.79 ± 0.04	0.95 ± 0.1	1.33 ± 0.07	1.46 ± 0.08	–	–
Na	–	–	<LOD (10.6)	6.6 ± 0.8	–	–
Se	6.8 ± 0.6	5.63 ± 0.67	<LOD (1.1)	0.38 ± 0.04	2.1 ± 0.3	1.68 ± 0.14
Sr	47 ± 3	45.2 ± 1.9	–	–	–	–
Zn	182 ± 1	180 ± 6	18.9 ± 0.2	19.4 ± 0.5	134 ± 2	138.6 ± 2.1

Note: Samples were analyzed in triplicate.

controls in every batch sample. Table 3 shows the main analytical figures of merit; the procedures carried out were defined in Section 2.6 concerning quality control.

All RSD instrumental values were equal to or lower than 2.5%, except for the As, Pb, Se and Tl which provided a poorer repeatability, due to the low sensitivity for these elements (see slope of calibration in Table 4). The RSD of the method (for triplicate analysis of samples) was lower than 10% for all elements in all samples except for Al, As, Cr, Mo and Ti, for which it was around 20% in some cases. This may be due to the low content of the aforementioned elements in samples analyzed, which were close the limit of detection of the method.

The accuracy of the procedure was tested based on the analysis of 3 reference materials, and Table 5 shows the results obtained; it can be seen that they are in good agreement with the reference data. A statistical evaluation of the comparison between the two data populations was made on the regression between average obtained values and certified values provided a regression line $y = (0.997 \pm 0.017)x + (0.17 \pm 1.28)$, $y = (0.952 \pm 0.027)x - (0.058 \pm 0.329)$, $y = (1.02 \pm 0.02)x - (1.59 \pm 3.37)$ respectively for TORT-2, NIST 1568a and BCR-185R, which demonstrates that the intercept and the slope correspond statistically to 0 and 1, respectively, with a regression coefficient (r^2) of 0.998, 0.997 and 0.9991, respectively.

3.4. Mineral content of menu samples

Table 6 shows the concentration values of Al, As, Ba, Ca, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Sr, Ti and Zn in the different menu samples analyzed, expressed in $\mathrm{mg\,kg^{-1}}$ of dry sample. As it can be seen from the initial 25 elements investigated, only 16 are present in detectable concentrations in the edible part of the samples under consideration.

Aluminium (Al). Aluminium is a well-known neurotoxicant, but its possible connection with Alzheimer's disease has been subjected to considerable controversy in recent years. Although aluminium is ubiquitous in the environment, as well as present in all diets, little is normally absorbed from foods or beverages (Reilly, 2002). Fresh foods like fruits, vegetables, and meat contain very little aluminium. The major source of aluminium in the diet in many countries is processed food. Aluminium compounds (permitted additives) may be added during processing of foods, such as flour, coloring agents and anti-caking agents (ATSDR, 2008). The content of aluminium in the studied samples is high in those menus which include pre-processed foods (M2, M6, M7 and M8). The values of the mineral element concentrations found in this study were compared with values found in the literature and it can be concluded that Al concentrations are similar to those found in studies made in France (Nöel et al., 2003) and lower than those obtained in Italy (Gramiccioni et al., 1996) or US (Pennington and Schoen, 1995).

Arsenic (As). This element occurs in the environment in both organic and inorganic compounds in its trivalent or pentavalent state. The toxicity of arsenic is highly dependent of its chemical form; in contrast to inorganic compounds, organic arsenicals are less or non-toxic. Inorganic arsenic induces skin lesions and disturbances in the cardiac, circulatory and nervous systems (Nordberg et al., 2007). Certain fish and crustaceans contain very high levels of organic arsenic, often as arsenobetaine. In most other foodstuffs, levels of arsenic are low, but the form is not known, since their toxicological risk is still unknown. The predominant dietary source of arsenic is seafood, followed by rice, mushrooms, and poultry. The total daily intake depends on the seafood consumed. The highest levels of As correspond to samples with fish (M6 and M11). The values obtained for As are similar to those obtained in studies related to Spanish foods (Llobet et al., 2003; Martorell et al., 2011; Urieta et al., 1996), but higher than values found in other countries (MacIntosh et al., 1996; Mainhara et al., 1998; Muñoz et al., 2005; Nöel et al., 2003).

Barium (Ba). Barium is found in all foods, usually at levels of about 0.05–1.0 $\mathrm{mg\,kg^{-1}}$. There is no particularly good food source of barium with the exception of Brazil nuts; high levels are also found in pecan nuts and dry cocoa (Reilly, 2002). Some foods, such as seaweed, fish and certain plants, may also contain high amounts of barium. The amount of barium found in food and water usually is not high enough to be a health concern (ATSDR, 2007). When the menu contains many vegetables, Ba content is high, as for example in M3, M5, M8 and M11. The values obtained for Ba are similar to those obtained in studies related to the UK (Ysart et al., 1999).

Calcium (Ca). Calcium is an essential nutrient that plays a vital role in neuromuscular function, many enzyme-mediated processes, blood clotting, and providing rigidity to the skeleton via phosphate salts. Its non-structural roles require the strict maintenance of ionised calcium concentration in tissue fluids at the expense of the skeleton if necessary, and it is therefore the skeleton which is at risk if the supply of calcium falls short of requirement (FAO/WHO, 2002). The richest sources of calcium are dairy products. In countries where milk is scarce, calcium may be obtained from certain cereals (notably millets). Small saltwater and freshwater fish such as sardines and sprats supply good quantities of calcium since they are usually eaten whole, bones and all. Vegetables and pulses provide some calcium (FAO, 1997). The values of Ca are similar to data reported in the US (Egan et al., 2002), higher than in France (Nöel et al., 2003), Greenland (Deutch et al., 2007), Brazil (Avegliano et al., 2011) or the UK (Ysart et al., 1999) and less than in Sweden (Becker et al., 2011).

Chromium (Cr). Trivalent chromium is an essential nutrient for humans, which is involve in the glucose tolerance. Adequate

Table 6
Mineral composition of menu samples.

Analyte	Sample concentration (mg kg^{-1} dry weight)												
	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13
Al	7.5 ± 0.3	11.4 ± 0.2	7.9 ± 0.2	5.2 ± 0.2	5.3 ± 0.4	9.1 ± 0.2	12 ± 1	11 ± 1	4.4 ± 0.8	3.2 ± 0.3	3.4 ± 0.4	4.0 ± 0.1	3.5 ± 0.5
As	1.0 ± 0.3	0.70 ± 0.08	<0.54	<0.54	<0.54	2.72 ± 0.07	<0.54	<0.54	<0.54	<0.54	5.0 ± 0.1	1.157 ± 0.003	1.6 ± 0.1
Ba	0.25 ± 0.01	1.25 ± 0.04	1.778 ± 0.006	0.63 ± 0.02	1.46 ± 0.03	0.53 ± 0.01	0.69 ± 0.02	1.28 ± 0.05	0.91 ± 0.03	0.56 ± 0.02	1.62 ± 0.03	0.76 ± 0.01	0.26 ± 0.03
Ca	850 ± 50	2370 ± 30	1460 ± 30	1460 ± 50	2090 ± 70	1550 ± 10	970 ± 40	885 ± 8	970 ± 30	1450 ± 20	3130 ± 50	2620 ± 50	1300 ± 100
Cr	<0.08	0.23 ± 0.06	<0.08	<0.08	<0.08	0.164 ± 0.007	0.19 ± 0.03	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
Cu	1.94 ± 0.08	1.41 ± 0.02	3.55 ± 0.01	2.12 ± 0.09	2.55 ± 0.08	1.129 ± 0.007	2.19 ± 0.01	3.69 ± 0.02	2.92 ± 0.02	2.00 ± 0.04	1.74 ± 0.06	2.65 ± 0.06	2.01 ± 0.03
Fe	33 ± 1	23.2 ± 0.4	24.3 ± 0.5	15.6 ± 0.3	31.7 ± 0.5	22 ± 2	18.1 ± 0.6	29.4 ± 0.2	22.9 ± 0.2	12.8 ± 0.2	10.7 ± 0.3	24.1 ± 0.9	17.4 ± 0.6
K	5600 ± 300	6360 ± 70	8500 ± 100	5400 ± 200	10,400 ± 400	7690 ± 10	5290 ± 20	10,700 ± 100	10,800 ± 300	5640 ± 40	8800 ± 200	14,100 ± 200	6100 ± 200
Li	0.092 ± 0.004	0.120 ± 0.003	0.111 ± 0.001	0.0691 ± 0.0001	0.302 ± 0.005	0.130 ± 0.001	0.120 ± 0.002	0.1370 ± 0.0004	0.105 ± 0.001	0.048 ± 0.001	0.143 ± 0.002	0.052 ± 0.003	0.078 ± 0.002
Mg	560 ± 20	539 ± 2	710 ± 20	500 ± 10	780 ± 30	600 ± 2	477 ± 4	870 ± 7	750 ± 20	497 ± 2	720 ± 10	960 ± 10	520 ± 20
Mn	4.9 ± 0.2	4.8 ± 0.1	6.83 ± 0.03	5.06 ± 0.08	6.71 ± 0.05	4.388 ± 0.002	5.19 ± 0.09	6.44 ± 0.03	5.3 ± 0.2	4.46 ± 0.06	3.96 ± 0.06	9.5 ± 0.2	3.79 ± 0.08
Mo	<0.1	0.11 ± 0.01	0.58 ± 0.05	<0.1	0.12 ± 0.02	<0.1	<0.1	1.28 ± 0.04	0.481 ± 0.005	<0.1	<0.1	0.27 ± 0.03	<0.1
Na	9500 ± 400	11,700 ± 200	11,000 ± 100	10,300 ± 400	7900 ± 300	11,410 ± 30	13,200 ± 100	11,500 ± 100	12,200 ± 300	6560 ± 40	12,800 ± 300	9500 ± 100	9200 ± 300
Sr	5.8 ± 0.4	4.15 ± 0.02	3.67 ± 0.04	4.2 ± 0.4	10.3 ± 0.4	2.54 ± 0.04	4.13 ± 0.07	4.08 ± 0.07	4.39 ± 0.08	3.35 ± 0.03	14.1 ± 0.2	8.9 ± 0.6	3.7 ± 0.3
Ti	0.28 ± 0.03	0.120 ± 0.008	0.11 ± 0.03	0.51 ± 0.06	0.10 ± 0.03	0.237 ± 0.008	0.35 ± 0.08	0.19 ± 0.02	0.10 ± 0.03	<0.016	<0.016	<0.016	0.07 ± 0.02
Zn	13.7 ± 0.4	16.5 ± 0.3	16.5 ± 0.2	15.8 ± 0.2	19.6 ± 0.2	16.83 ± 0.07	12.3 ± 0.3	16.8 ± 0.3	21.6 ± 0.3	19.83 ± 0.08	18.0 ± 0.2	20.1 ± 0.3	21.5 ± 0.8

Note: Three independent samples of each item were analyzed. For details about each menu sample composition see Table 2.

chromium nutrition may reduce risk factors associated with cardiovascular disease as well as diabetes mellitus. Hexavalent chromium is, in contrast, extremely toxic (Reilly, 2002). The major source of trivalent chromium is meat and vegetables. But this level could be increased by migration into the food from stainless steel cooking equipment and cans. The amount of Cr is higher in menus that include canned tuna or other canned food (M2, M6 and M7). The values of Cr average values obtained through this study are similar to those found in the literature (Nöel et al., 2003; Ysart et al., 2000) but higher than Sweden (Becker et al., 2011) or Brazil (Avegliano et al., 2011).

Copper (Cu). An essential trace element for humans, copper is a vital component of several enzymes. Its absorption depends on the amount ingested, its chemical form, and the composition of other dietary components such as zinc. Drinking water has a significant contribution to dairy intake due to the use of domestic copper pipes. Offal, such as liver and kidney, contain high copper levels. Fish, fruits, cereals, nuts, and green vegetables are good sources of copper, whereas meat and dairy products contain lower levels (Nordberg et al., 2007). The amount of Cu is higher in menus that include a lot of vegetables (for example M3, M8, M9, M12). The values of Cu average values obtained through this study are similar to those found in the literature (Becker et al., 2011; Nöel et al., 2003; Ysart et al., 1999, 2000).

Iron (Fe). Iron is needed for the synthesis of blood pigments, as well as for many other essential activities of cells. The best food source of iron is animal offal, especially liver. Other animal products like red meat are also rich in iron. The levels in cereals and vegetables are generally low, with the exception of dark green vegetables, such as spinach, which are rich in iron (Reilly, 2002). The amount of Fe in each menu depends on the type of foods, and in some cases is quite low and in other cases very high. So, as much as possible variability of the foods in the diet must be recommended for an equilibrated daily mineral intake. Values obtained through this study are similar to those found in the literature (Becker et al., 2011; Egan et al., 2002; Nöel et al., 2003; Ysart et al., 1999), but in Greenland (Deutch et al., 2007) iron values are higher than in this study.

Potassium (K). Potassium is an essential mineral that works to maintain the body's water and acid balance. It plays a role in transmitting nerve impulses to muscles, in muscle contractions and in the maintenance of normal blood pressure (FAO, 1999). Potassium can be found in vegetables, fruits, potatoes, meat, bread, milk and nuts. The highest amounts have been found in menus that include several vegetables and orange fruit (M5, M8, M9, M11 and M12). The potassium average values obtained through this study are similar to those found in France (Nöel et al., 2003), Belgium (Van Cauwenbergh et al., 1999) and Sweden (Becker et al., 2011) but less than in the US (Egan et al., 2002) and higher than Brazil (Avegliano et al., 2011).

Lithium (Li). The essential role of lithium has not been established, but its use in psychiatric medicine to treat manic-depressive psychosis is well known. Lithium is present in all food, though at low levels (Reilly, 2002). The concentrations obtained in this study are much higher than those found in France (Nöel et al., 2003) or the UK (Ysart et al., 1999).

Magnesium (Mg). In spite of the structural function of magnesium in the skeleton, it has many functions in muscles and soft tissues, such as a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nerve tissues and cell membranes. Dietary deficiency of magnesium of a severity sufficient to provoke pathologic changes is rare (FAO/WHO, 2002). Magnesium is widely distributed in plant and animal foods. Most green vegetables, legume seeds, peas, beans and nuts are rich in magnesium, as are also some shellfish, spices and soya

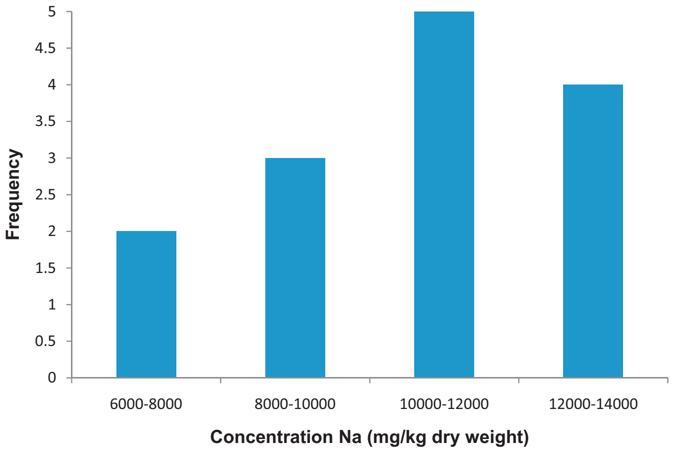

Fig. 1. Sodium concentration in menu samples in descending order of magnitude given as dry weight (mg kg^{-1}).

flour. The values of Mg were similar in all the menus, between 91 and 225 mg day^{-1}. The Mg concentrations are comparable to those found in Spain (Llobet et al., 2003) and Sweden (Becker et al., 2011) but higher than values reported in France (Nöel et al., 2003).

Manganese (Mn). This is an essential element for humans and animals. Manganese is present in all plant and animal tissues, with food the major source in human intake. The highest manganese concentrations are found in some foods of plant origin, especially wheat and rice. High concentrations are also found in nuts, tea, legumes, pineapples, and lower levels in milk products, meats, fish and eggs (Nordberg et al., 2007). The Mn concentrations are similar to those found in France (Nöel et al., 2003), the UK (Ysart et al., 1999) and Sweden (Becker et al., 2011).

Molybdenum (Mo). This is an essential constituent of several enzymes, and dietary recommendations have been established. The foods contributing the most molybdenum to the diet are grains, milk, dairy products, and legumes. In contrast, animal products generally have low levels. The Mo data are of the same order as those reported in other countries (Nöel et al., 2003; Ysart et al., 1999).

Sodium (Na). This element is necessary for humans in order to maintain balance in physical fluid systems. Sodium is also required for the operation of nerves and muscles, but an excess of sodium can damage the kidneys and increase the possibilities of hypertension. In general, the sodium content was very high in all samples and must be attributed to the addition of sodium chloride while cooking the foods. Fig. 1 shows the frequency of the values of sodium concentration in the samples in descending order of magnitude. The values found are also higher than the values reported in the literature of France (Nöel et al., 2003), Belgium (Van Cauwenbergh et al., 1999), US (Egan et al., 2002), Brazil (Avegliano et al., 2011) and Sweden (Becker et al., 2011).

Strontium (Sr). This is a non-essential trace element, which can substitute for calcium in bone. Food and drinking water are the highest sources of exposure to strontium. Grains, leafy vegetables and dairy products contribute the greatest percentage of dietary strontium to humans. Problems with bone growth may occur in children eating or drinking unusually high levels of strontium, especially if the diet is low in calcium and protein (ATSDR, 2004). The amount of Sr is higher in menus that include several vegetables (M5, M11 and M12). No data were found in the literature.

Titanium (Ti). There is no evidence that titanium is an essential element for humans. It is poorly absorbed by plants and animals. High levels of titanium could be found for vegetables from contaminated soils and in certain types of cheese from the addition of titanium dioxide as whitener and an aging accelerator (Nordberg et al., 2007). The values of Ti found are between 0.02 and 1.66 mg day^{-1}. These values are agree with data reported (about 2 mg kg^{-1}) in cereals, vegetables, dairy products and some others foods (Reilly, 2002).

Zinc (Zn). Zinc is an essential element in human nutrition; it is present in many important enzymes essential for metabolism. Zinc is present in most foods both of vegetable and of animal origin, but the richest sources tend to be protein-rich foods such as meat, seafoods and eggs (FAO, 1997). The amount of Zn is higher in menus that include vegetables and meat than in those based on pasta and fish. The values of Zn average values obtained through this study are similar to those found in the literature (Becker et al., 2011; Urieta et al., 1996; Ysart et al., 1999, 2000) and higher than Brazil (Avegliano et al., 2011).

Table 7 shows the percentage of the recommended daily intake (RDI) provided by each menu analyzed for Ca, Cu, Fe, K, Mg, Mn, Na and Zn. The percentage of RDI was calculated as milligrams of every element that gives each menu divided by the values of RDI reported in the literature and multiplied by 100. The RDI values were 800, 0.7, 6, 4700, 350, 2, 2400 and 9.4 mg day^{-1} for Ca, Cu, Fe, K, Mg, Mn, Na and Zn, respectively (FAO/WHO, 2002; Food and Nutrition Board, 2004). From comparing data found with the recommended RDI values it can be seen that the contribution of Ca, K, Mg, Mn and Zn to the daily intake is between 30 and 70% of the RDI, thus indicating that their concentration level in analyzed menus is appropriate to meet the daily needs of the aforementioned minerals. On the other hand, the contribution of sodium is very high in many cases, reaching 200% of the RDI in some samples. This level of Na can create problems for people with hypertension. The amount of Cu and Fe found in each menu depends on the type

Table 7
Contribution of the studied samples to the daily intake of essential elements.

	Percentage that provides each menu to the RDI (%)							
Sample	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
M1	41	78	133	38	63	69	137	48
M2	41	34	57	20	28	41	79	35
M3	62	143	98	60	84	96	162	58
M4	66	97	71	47	70	81	180	63
M5	43	64	80	44	54	59	68	43
M6	45	34	65	43	54	46	128	44
M7	43	91	75	41	59	75	202	44
M8	41	152	121	81	109	93	177	61
M9	33	97	76	62	70	62	140	63
M10	59	74	48	41	56	58	91	65
M11	65	40	24	36	47	32	103	36
M12	47	50	46	46	52	63	61	33
M13	54	76	67	43	57	51	125	72

Note: RDI (Recommended Daily Intake) as indicated in (FAO/WHO, 2002; Food and Nutrition Board, 2004).

of foods and in some cases is quite low and in other cases very high. Therefore it is recommended to vary as much as possible the foods in the diet to arrive at a balanced daily mineral intake.

4. Conclusions

The data results of this study demonstrate the general good quality of university canteen menus with respect to daily intake of mineral elements, as well as low levels of potentially toxic elements. On considering the average concentration of trace essential elements found in present study, it can be concluded that they are adequate as compared with the recommended daily intake by the international organisms, providing from 46 to 79% of the RDI for the essential elements, except for Na (127%). Only in the case of Na, action must be taken to reduce the excessively high levels, namely using less sodium chloride during cooking. In general, the contents of Cu and Fe are relatively high, but it should be noted that Spanish menus provide high concentrations for Ca, K and Mg as well.

The mineral contents provided in the present study have been submitted to a quality control of the measurements being employed certified referential materials, reagent blanks and standards controls in each batch samples.

Finally, in order to collect data on mineral intake from the diet, we consider that determining the mineral composition of typical menu samples in a given country can provide an alternative to studies on diet composition based on questionnaires and reference data.

Acknowledgments

Authors acknowledge the financial support of the Generalitat Valenciana Project PROMETEO 2010-055 and University of Valencia Project UVINV-AE11-42834.

References

Agency of Toxic Substances & Disease Registry (ATSDR), Public Health Statement for Al (2008), As (2007), Ba (2007), Mn (2008), Sr (2004), from: www.atsdr.cdc.gov.

Avegliano, R.P., Maihara, V.A., da Silva, F.F., 2011. A Brazilian Total Diet Study: evaluation of essential elements. Journal of Food Composition and Analysis 24, 1009–1016.

Bastías, J.M., Bermúdez, M., Carrasco, J., Espinoza, O., Muñoz, M., Galotto, M.J., Muñoz, O., 2010. Determination of dietary intake of total arsenic, inorganic arsenic and total mercury in the Chilean School Meal Program. Food Science and Technology International 0001–0008.

Becker, W., Jorhem, L., Sundström, B., Petersson Grawé, K., 2011. Contents of mineral elements in Swedish market basket diets. Journal of Food Composition and Analysis 24, 279–287.

Boeting, K., Aguilera de Benzo, Z., Cervera, M.L., de la Guardia, M., 2010. Authentication of the protected designation of origin horchata de Valencia through the chemometric treatment of mineral content. Analytical Methods 2, 1723–1728.

Cheung, S.W., Ping, K., Yau, J., Wong, W., 2008. Dietary exposure to antimony, lead and mercury of secondary school students in Hong Kong. Food Additives and Contaminants 25, 831–840.

Deutch, B., Byerberg, J., Pedersen, H.S., Aschlund, E., Hansen, J.C., 2007. Traditional and modern Greenlandic food–Dietary composition, nutrients and contaminants. Science of the Total Environment 384, 106–119.

Egan, S.K., Tao, S.S-H., Pennington, J.A.T., Bolger, P.M., 2002. US Food and Drug Administration's Total Diet Study: intake of nutrional and toxic elements 1991–96. Food Additives and Contaminants 19, 103–125.

European Food Safety Authority (EFSA), 2011. Towards a Harmonised Total Diet Study Approach: A Guidance Document, from: www.efsa.europa.eu/en/efsa-journal/doc/2450.pdf.

FAO Human nutrition in the developing world, 1997. Food and Nutrition Series No. 29 by Michael C. Latham, from www.feo.org/docrep/W0073E/w0007300.htm.

FAO Food, Nutrition and Agriculture, 1999. Alimentation, nutrition et agriculture. Alimentación, nutrición y agricultura, from www.fao.org/docrep/x2650T/x2650T00.htm.

FAO/WHO Human Vitamin and Mineral Requirements, 2002. Report of a Joint FAO/WHO Expert Consultation Bangkok, Thailand.

Food and Nutrition Board, Institute of Medicine, 2004. Dietary Reference Intake (DRIs): Recommended Intakes for Individuals Elements. National Academies Press.

Food Standards Australia New Zealand, 2008. The 22th Australian Total Diet Survey, A Total Diet Study of Five Trace Elements: I, Se, Cr, Mo, Ni, from: http://www.foodstandards.gov.au/newsroom/publications/22tdaustraliantotaldiet-study/index.cfm.

French Total Diet Study 2, 2011. Tome 1: Contaminants inorganiques, minéraux, pollutants organiques persistants, mycotoxines, phyto-estrogènes. Agence nationale de sécurité sanitaire alimentation, environnement, travall from: http://www.anses.fr/Documents/PASER2006sa0361Ra1.pdf.

Gonzálvez, A., Llorens, A., Cervera, M.L., Armenta, S., de la Guardia, M., 2009. Elemental fingerprints of wines from the protected designation of origin Valencia. Food Chemistry 112, 26–34.

Gonzálvez, A., Armenta, S., de la Guardia, M., 2011. Geographical traceability of Arròs de Valencia rice grain based on mineral element composition. Food Chemistry 126, 1254–1260.

Gramiccioni, L., Ingrao, G., Milarna, M.R., Santaroni, P., Tomassi, G., 1996. Aluminium levels in Italian diets and in selected foods from aluminium utensils. Food Additives and Contaminants 13, 767–774.

Lee, H.S., Cho, Y.H., Park, S.O., Kye, S.H., Kim, B.H., Hahm, T.S., Kim, M., Lee, J.O., Kim, C., 2006. Dietary exposure of the Korean population to arsenic, cadmium, lead and mercury. Journal of Food Composition and Analysis 19, S31–S37.

Llobet, J.M., Falcó, G., Casas, C., Teixidó, A., Domingo, J.L., 2003. Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. Journal of Agricultural and Food Chemistry 51, 838–842.

Maclntosh, D.L., Spengler, J.D., Özkaynak, H., Tsai, L., Ryan, P.B., 1996. Dietary exposures to selected metals and pesticides. Environmental Health Perspectives 104, 202–209.

Maihara, V.A., Vasconcellos, M.B.A., Cordeiro, M.B., Cozzolino, S.M.F., 1998. Estimates of toxic element intake in diets of pre-school children and elderly collected by duplicate portion sampling. Food Additives and Contaminants 15, 782–788.

Mainhara, V.A., Gonzaga, I.B., Silva, V.L., Fávaro, D.I.T., Vasconcellos, M.B.A., Cozzolino, S.M.F., 2004. Daily dietary selenium intake of selected Brazilian population groups. Journal of Radioanalytical and Nuclear Chemistry 259, 465–468.

Martorell, I., Perelló, G., Martí-Cid, R., Llobet, J.M., Castell, V., Domingo, J.L., 2011. Human exposure to arsenic, cadmium, mercury, and lead from foods in Catalonia, Spain: temporal trend. Biological Trace Element Research 142, 309–322.

Marzec, Z., Schlegel-Zawadzka, M., 2004. Exposure to cadmium, lead and mercury in the adult population from Eastern Poland, 1990–2002. Food Additives and Contaminants 21, 963–970.

Millour, S., Nöel, L., Chekri, R., Vastel, C., Kadar, A., Guérin, T., 2010. Internal quality controls applied in inductively coupled plasma mass spectrometry multi-elemental analysis in the second French Total Diet Study. Accreditation and Quality Assurance 15, 503–513.

Millour, S., Nöel, L., Kadar, A., Chekri, R., Vastel, C., Guérin, T., 2011a. Simultaneous analysis of 21 elements in foodstuffs by ICP-MS after closed-vessel microwave digestion: method validation. Journal of Food Composition and Analysis 24, 111–120.

Millour, S., Nöel, L., Kadar, A., Chekri, R., Vastel, C., Sirot, V., Leblanc, J.C., Guérin, T., 2011b. Pb, Hg, Cd, As, Sb and Al levels in foodstuffs from the 2nd French total diet study. Food Chemistry 126, 1787–1799.

Muñoz, O., Bastias, J.M., Araya, M., Morales, A., Orellana, C., Rebolledo, R., Velez, D., 2005. Estimation of the dietary intake of cadmium, lead, mercury, and arsenic by the population of Santiago (Chile) using a Total Diet Study. Food and Chemical Toxicology 43, 1647–1655.

Nöel, L., Leblanc, J.C., Guérin, T., 2003. Determination of several elements in duplicate meals from catering establishments using closed vessels microwave digestion with inductively coupled plasma mass spectrometry detection: estimation of daily dietary intake. Food Additives and Contaminants 20, 44–56.

Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T., 2007. Handbook on Toxicology of Metals, 3rd edn. Elsevier.

Pedrero, Z., Madrid, Y., 2009. Novel approaches for selenium speciation in foodstuffs and biological specimens: a review. Analytical Chimica Acta 634, 135–152.

Pennington, J., Schoen, S.A., 1995. Estimates of dietary exposure to aluminium. Food Additives and Contaminants 12, 119–128.

Reilly, C., 2002. Metal Contamination of Food, 3rd edn. Oxford Brookes University, Oxford, UK.

Robberecht, H., Van Cauwenbergh, R., Bosscher, D., Cornelis, R., Deelstra, H., 2002. Daily dietary total arsenic intake in Belgium using duplicate portion sampling and elemental content of various foodstuffs. European Food and Research Technology 214, 27–32.

Rubio, C., González-Iglesias, T., Revert, C., Reguera, J.I., Gutiérrez, A., Hardisson, A., 2005. Lead dietary intake in the Spanish Population (Canary Islands). Food Additives and Contaminants 53, 6543–6549.

Rubio, C., Hardisson, A., Reguera, J.I., Revert, C., Lafuente, M.A., González-Iglesias, T., 2006. Cadmium dietary intake in the Canary Islands, Spain. Environmental Research 100, 123–129.

Sola-Larrañaga, C., Navarro-Blasco, I., 2009. Chemometric analysis of mineral and trace elements in raw cow milk from the community of Navarra, Spain. Food Chemistry 112, 189–196.

Tanase, C.M., Griffin, P., Koski, K.G., Cooper, M.J., Cockell, K.A., 2011. Sodium and potassium in composite food samples from Canadian Total Diet Study. Journal of Food Composition and Analysis 24, 237–243.

Urieta, I., Jalon, M., Eguileor, I., 1996. Food surveillance in the Basque Country (Spain). 2. Estimation of the dietary intake of organochlorine pesticides, heavy metals, arsenic, aflatoxin M(1), iron and zinc through the total diet study, 1990/91. Food Additives and Contaminants 13, 29–56.

U.S. Food and Drug Administration Centre for Food Safety and Applied Nutrition, College Park, MD, 2010. Total Diet Study Statistics on Element Results. Market Baskets 2006-1 through 2008-4, from: http://www.fda.gov/downloads/food.-totaldietstudy/ucm184301.pdf.

Van Cauwenbergh, R., Hendrix, P., Robberecht, H.J., Deelstra, H.A., 1999. Daily dietary sodium and potassium intake in Belgium, using duplicate portion sampling. European Food and Research Technology 209, 63–67.

Van Cauwenbergh, R., Bosscher, D., Robberecht, H., Deelstra, H., 2000. Daily dietary cadmium intake in Belgium using duplicate portion sampling. European Food and Research Technology 212, 13–16.

Ysart, G., Miller, P., Crews, H., Robb, P., Baxter, M., de L'argy, C., Lofthouse, S., Sargent, C., Harrison, N., 1999. Dietary exposure estimates of 30 elements from the UK Total Diet Study. Food Additives and Contaminants 16, 391–403.

Ysart, G., Miller, P., Croasdale, M., Crews, H., Robb, P., Baxter, M., de L'Argy, C., Harrison, N., 2000. 1997 UK Total Diet Study—dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. Food and Additives and Contaminants 17, 775–786.

Trends in Analytical Chemistry 82 (2016) 457–467

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Mineral analysis of human diets by spectrometry methods

Alba Mir-Marqués, M. Luisa Cervera, Miguel de la Guardia *

Department of Analytical Chemistry, University of Valencia, Jeronimo Muñoz Research Building, 50th Dr. Moliner str. Burjassot, 46100 Valencia, Spain

ARTICLE INFO

Keywords:
Human diets
Mineral element determination
Sampling diet
Sample treatment of foods
Atomic spectroscopy
Ion spectroscopy

ABSTRACT

Mineral element determination in human diets is very important for human health, due to the presence in foods of essential and toxic elements or their incorporation in the manipulation and cooking food process. Different instrumental techniques have been used to determine mineral elements in human diets, but atomic spectroscopy and mass spectrometry based ones are the most commonly employed. Sampling procedures for diet analysis are the main critically step for mineral element determination, being employed different standardised protocols. This review summarised critically the state-of-the-art of mineral analysis in human diets, considering sampling, sample preparation and determination procedures. Results obtained from different countries are compared between them and with recommended daily intake values established by international agencies for the preservation of human health.

Contents

1. Introduction

The general population is exposed to a wide range of mineral elements through food, water, air and other environmental sources. The diet together with tap water is the main route of exposure to toxic elements, but also the way to obtain essential and non-essential elements [1,2]. Food, as consumed, contains a lot of different compounds which some are necessary and other unnecessary for a correct human health. Nutrients are a natural part of food and are the basis for human nutrition. In some cases, especially in processed foods, they may also be added to ensure the optimal functionality of the final product and to provide specific qualities [3]. The amounts that can be added to processed foods are regulated by maximum permitted levels defined in food legislation for each food category and each country to ensure the consumer safety [4]. Essential elements are very important for the correct functioning of human body, because of their interactions in genetic and physiological processes. A deficiency of essential elements could bring on structural and physiological abnormalities, while a higher concentration than recommended ones may be harmful.

Potentially toxic elements may be also found in foods but, fortunately, in lower concentrations than essential ones. The presence of toxic and non-essential elements in foods is attributed to natural sources; such as raw materials or contamination during their production and processing. Arsenic, cadmium, mercury, lead, aluminium, barium, antimony and tin can accumulate in foods creating

---

* Corresponding author. Tel.: +34 963544838; fax: +34 963544845.
E-mail address: miguel.delaguardia@uv.es (M. de la Guardia).

http://dx.doi.org/10.1016/j.trac.2016.07.007

potentially dangerous situation for the consumers. Based on the aforementioned considerations, it is very important to control the concentration of mineral elements in foods.

Daily intake for essential elements is recommended by international organisms and the maximum permitted levels of toxic ones are regulated by the legislation concerning each food category to avoid hazards for human health [5]. To accomplish a complete characterisation of the mineral profile of foods and human diets, highly informative and sensitive analytical methods are required. To monitor human ingestion and to evaluate the risks associated with the use of foods produced or subjected to bad processing practices. An evaluation element by element and food by food of the human mineral intake is a very complex task and cannot provide a realistic picture about the lack of essential elements and the risks associated to the toxic ones in the different communities all around the world. Because of that, the control of diets seems the most appropriate approach for screening the human consumption of mineral elements.

The majority of mineral components to be determined in diet samples are present at trace levels; hence analytical techniques with adequate sensitivity are required for their accurate determination. Additionally, it is important to remember that the accuracy of the quantitative analysis procedures is strongly dependent on the sampling and sample preparation steps, and thus, these two aspects must be controlled in food analysis together with the most employed techniques for trace element analysis in foods.

Another point to consider is the bioavailability of minerals in food, as not all the minerals that human ingest are available for the human organism, only a fraction of them present in the food matrix is bioavailable and can be absorbed and utilised by the human body. The fraction which can be bioaccessible to the human body depends on the speciation of the elements, on the behaviour of organometallic species and complexes in the gastrointestinal tract, and on interactions with the food matrix [6]. Because of that, in recent years some diet studies are focusing on study the minerals bioavailability in the human diet, not only in total mineral content of food.

On the other hand, the new approaches in analytical chemistry are focused on finding more environmental friendly techniques including in food chemistry. In the literature, it can find enough scientific articles regarding green techniques for analysis of minerals in foods, while they are not often applied to the analysis of complete diets.

The main objective of this review has been to provide an update of methods proposed in the literature to determine essential, non-essential and toxic elements in human diets, being that the most direct way to evaluate mineral element intake of humans.

2. Mineral composition of diet samples and their contribution to the daily intake

The control of elements ingested through the diet is very important to avoid levels of toxic elements that can cause health damages and moreover to ensure the daily recommended intake of essential elements, since their deficiency might also cause health troubles.

2.1. Toxic and non-essential elements

Toxic elements are those can cause hazardous effects in human health. It is not always possible to clearly differentiate between toxic elements, elements that play essential roles in human body functions and ones with unknown effects. However, it must be taken into account that at high concentration levels practically all the mineral element present deleterious effects on the health. The most relevant toxic elements in the diet are As, Cd, Pb and Hg, and similarly exposure to minor toxic metals as Al, Ba, Be, Cr (VI), Sb, Sn, Tl and U must be taken into consideration and, in some cases, are considered in the legislation about maximum tolerable levels in food. Fig. 1 summarised the concentration levels found for toxic and non-essential elements in studies concerning human diet analysis all around the world.

Some international agencies as the World Health Organization (WHO), the European Food Safety Authority (EFSA) and the Agency for Toxic Substances and Disease Registry (ATSDR) are evaluating the adverse health effects of heavy metals and other elements. The most frequently parameters used are the acceptable daily intake (ADI), tolerable daily intake (TDI), provisional tolerable daily intake (PTDI) or provisional tolerable weekly intake (PTWI) and the tolerable upper level intake (UL). PTWI for As, Cd, Hg and Pb are 15, 7, 5 and 25 μg per kg body weight per week, respectively [7,8]. PTWI values represent permissible human weekly exposure to contaminants unavoidably associated with the consumption of foods. The term ‘provisional’ is related to the lack of a deep knowledge on the consequences of human exposure at levels approaching those established by the Joint FAO/WHO Expert Committee of Food Additives (JECFA) [9].

The contribution of arsenic, cadmium, mercury and lead to the PTWI found in the literature are lower than 100% in all cases. The highest values of PTWI (>80%), found in the literature, for Cd and Hg were to a duplicate diet study in a restaurant from Tarragona (Spain) [10]; and for Pb were to a total diet study from Chilean population [11].

From the data reported for non-essential elements in diets, it can be say that the content of elements like aluminium, barium, antinomy and tin, in different kind of menus and total diet studies, is very low and therefore, being not a problem for the population. The weekly intake values of Ba, Sn and Sb found in the literature are lower than 20% of the established PTWI. In the case of Al, the weekly intake are lower than 40% of the PTWI in all cases, except to diet analysis made in Tokio (Japan) [12] and Canary Island (Spain) [13], but no way are these values dangerous from human health.

2.2. Essential elements

Calcium, iron, potassium, magnesium, sodium and zinc are considered essential elements and their intake are required at milligrams per kilogram level to maintain the human organisms free from deficiency effects. Additionally, copper, cobalt, chromium, manganese, molybdenum, nickel and selenium are needed at trace levels in the human diets. Fig. 2 shows the range of concentration of essential elements found in the recent literature regarding diet analysis.

Unfortunately, sodium is an essential element present in diets at g day^{-1} level followed by potassium, calcium and magnesium. High sodium intake is associated with high risk of incident stroke, fatal stroke and fatal coronary heart disease. WHO recommends a reduction to less than 2 g day^{-1} of sodium (5 g day^{-1} salt) in adults [14]. In the 40% of the data found in the literature, sodium content exceeds this value, especially in human diets obtained from restaurants. On the other hand, WHO recommends an increase in potassium intake from food in order to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart disease in adults, mitigating the negative effects of elevated sodium consumption [15,16]. WHO suggests a potassium intake of at least 3510 mg day^{-1} for adults. Calcium is essential for the correct development and maintenance of the skeleton, muscular function, nerve conduction, and it is involved in multiple intracellular pathways and enzymatic reactions. The recommended daily intake for calcium or recommended daily allowance is 1000 mg day^{-1} for adults older than 19 years [17]. Fortunately, levels of calcium reported in diet studies are appropriated, because the most of the diets provide between 75 to 110% of the recommended daily intake. The main role of magnesium is the potassium fluxes regulation and its involvement in the metabolism of calcium. Magnesium content is abundant in most

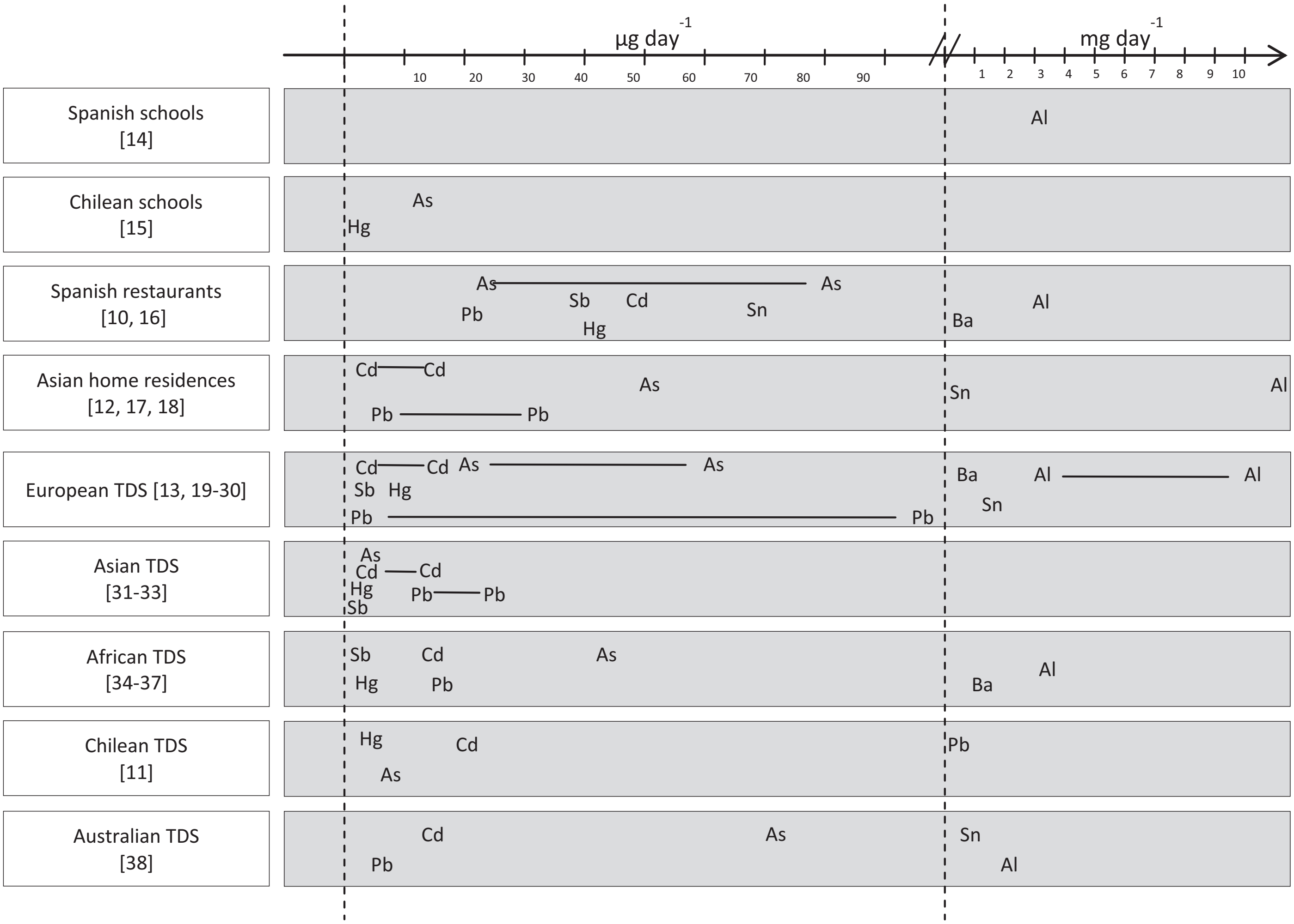

Fig. 1. Daily intake of toxic and non-essential elements in human diets obtained from published data during the last ten years.

green vegetables, legume seeds, beans, and nuts, some shellfish, spices, and soya flour, which usually contain more than 500 mg kg^{-1} in fresh weight. The recommended daily intake of magnesium is between 220 and 260 mg day^{-1} for adult females and males, respectively [17].

The concentration found in the literature for Fe, Zn, Mn and Cu are lower than 100 mg day^{-1}. Iron has several vital functions in the body, being calcium the only dietary factor that negatively influences in their mechanism of absorption. The recommended daily intake of iron depends on its bioavailability and varies between 19.6 to 58.5 mg day^{-1} for females and 9.1 until 27.4 mg day^{-1} for males [17]. Taking into account the bioavailability of iron from ingested food, the iron content in diets is very low for women and can cause anaemia, WHO reported that 30% of non-pregnant women suffer from anaemia [18]. Zinc is an essential component of a more than 300 enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. The recommended daily intake of zinc depends on its bioavailability, for a low bioavailability (15%) the RDI value is 9.8 and 14 mg day^{-1}, for a medium bioavailability (30%) is 4.9 and 7 mg day^{-1} and for high bioavailability (50%) is 3 and 4.2 mg day^{-1} for females and males, respectively [17]. In the case of zinc, the intake in reported studies is generally higher than the recommended values, especially when the bioavailability of zinc in foods is medium or high. Manganese is present in all plant and animal tissues, being food the major source of manganese for humans [19]. The RDI value is 1.8 and 2.3 mg day^{-1} for females and males, respectively. These values are lower than most data found in the literature for diet analysis. Copper is an essential trace element as a vital part of several enzymes. Fish, fruits, cereals, nuts, and green vegetables are good sources of copper [19]. WHO suggested a daily intake of copper of 1.2 and 1.3 mg day^{-1} for females and males, respectively [20]. Most data found in the literature about copper intake in diets reach the recommended values by the WHO.

In almost studies found in the literature Cr, Se, Mo, Co and Ni are present at levels below 400 µg day^{-1}. For Cr, the 50% of the data reported in the recent literature concerning the contribution to the recommended daily intake values exceeded this limit [21–24]. In most cases, levels of molybdenum and selenium reported in diet studies are higher than recommended values, and for Co and Ni values found in the literature agree with the values of recommended daily intake.

2.3. *Mineral bioavailability in diets*

To evaluate the real intake of essential micronutrients from the diet, the determination of the potentially bioaccessible fraction from food must be taken into account. There are factors which enhancing or inhibiting the bioaccessibility of minerals, like interactions between different foods and physiological ones (sex, age, and nutritional status). Scarce studies of bioavailability of minerals in complete human diets are found in the literature. Lazarte et al. [25]

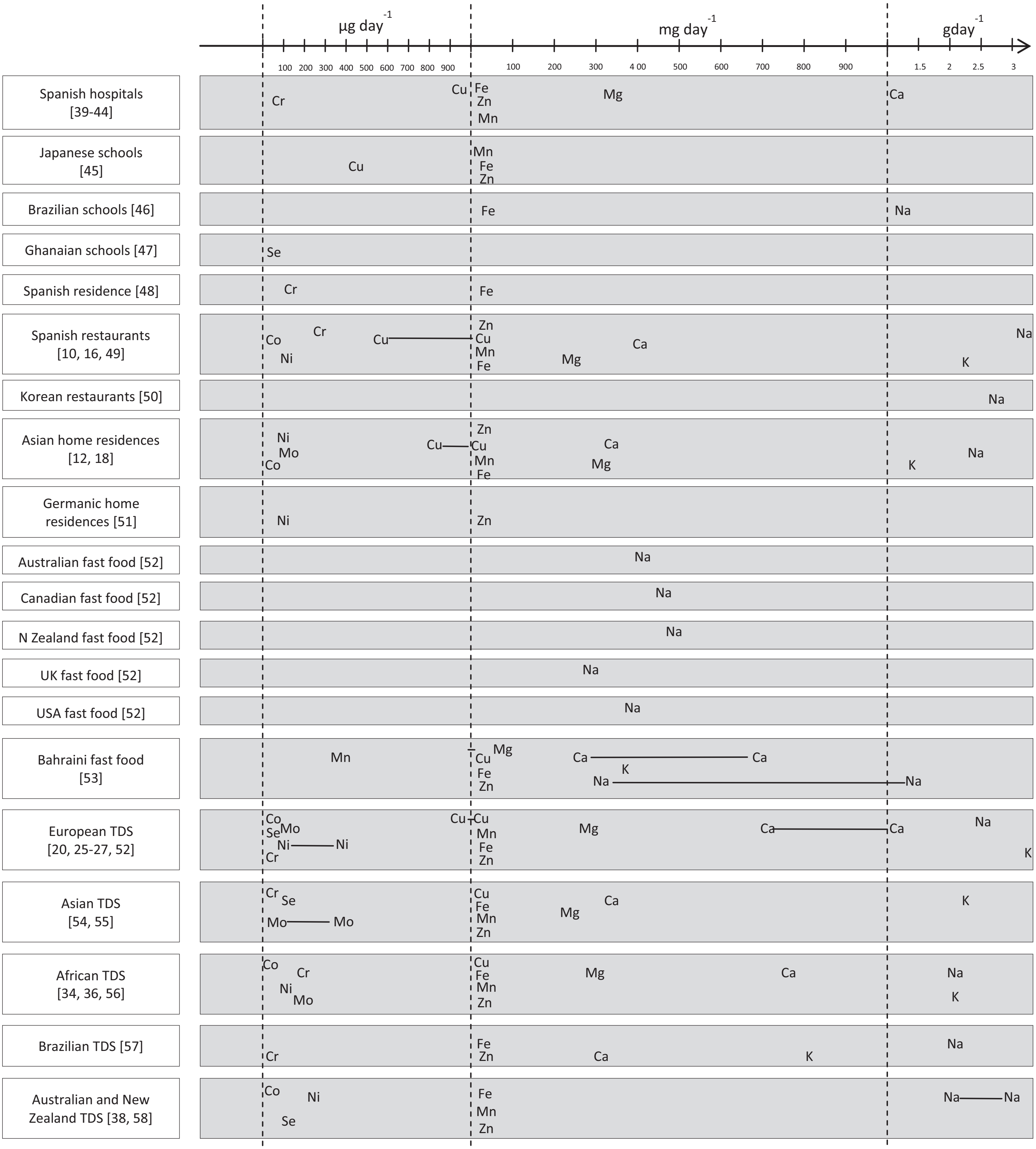

Fig. 2. Daily intake of essential elements inhuman diets obtained from published data during the last ten years.

analysed the content of zinc, iron, calcium and phytate in the 16 most consumed foods from five villages in a tropical rural area of Bolivia, and they evaluated the mineral bioavailability by calculation the molar ratio of phytate and each mineral. They concluded that phytate may be one of the main factors cause zinc and iron deficiencies.

Singh et al. [26] reported the total content of Fe and Zn and their bioavailability in the food samples which are commonly consumed by Fiji population. This study showed that there are many factors which affect minerals bioavailability like mineral content of soil, climatic conditions, storage, ripping system and cooking methods of foods. The absorption of Fe and Zn mineral from foods depends on their contents and other factors (phytate, tannin, etc.) available in the food samples.

Moreover, it can be found several references in the literature on mineral bioavailability, generally in vegetables, which have a high content of certain minerals but low bioavailability [27,28].

3. Sample treatment for determination of the mineral composition of diets

The most common methods employed for the analysis of mineral composition of human diets are those based on atomic and mass spectrometry. However, to carry out the mineral analysis using these methodologies samples must be previously dissolved or digested in order to obtain appropriate solutions, and to do it, sampling and sample preparation procedures must be critically discussed.

3.1. Diet sampling methods

Sampling is the most important step in the analytical process in order to obtain reliable analytical results. It is essential that samples are taken from the lot in a representative way [29]. Sampling methods for diet analyses are very complex, because diet samples include many ingredients from liquids to solid with different textures. Because of that, there are detailed sampling protocols for diets in the literature as it can be seen in Fig. 3.

Sampling methods employed in diet analysis can be separated in two groups, direct and indirect approaches. Direct sampling allows the incorporation of a large number of personal exposure monitoring and biological markers of exposure. These methods are simple and accurate. **Duplicate portion** method provides an useful methodology that supplies the most accurate information on the ingested dose of mineral elements in a diet [30]. This approach is based on the preparation of twice the usual portions of food that would be consumed by an individual within the home or institution. Residential homes [12,31–35], schools [23,36–41], hospitals [22,42–46], cafeterias [10,32,47–49] and fast food restaurants [50–53] have been reported as the basis through with duplicate portion studies were organised. Aung et al. [12] did their study during seven consecutive days with 25 pairs of Japanese children and their parents in the city centre and in the suburb regions of Tokyo; Wittsiepe et al. [33] also carried out their study during seven consecutive days in young children living in urban and rural areas of Germany, both in residential homes. Cabrera-Vique et al. [36,52] and Adotey et al. [40] also did their sampling during seven consecutive days in female university residences from Spain and adolescent orphanages from Ghana, respectively. Liu et al. [31,34] and Sun et al. [35] did their study with 30 children and 30 adults from south-east of China during three consecutive days. Raghunat et al. [32] did the sampling only during one day but extended to 250 volunteers belonged to the middle class from Mumbai (India). Although some authors prefer to do the sampling during many days, especially when they have few places for do it. Weber & Morais [39] performed their sampling during four weeks in 15 canteens from public and private primary schools in Brazil. In a hospital from Motril (Spain) it was made a sampling during 36 consecutive days by Velasco-Reynold et al. [22,42–46]. Duplicate portion is a suitable method for institutional groups and for small surveys but, because of the cost and time involved, it is not appropriate for large-scale food consumption studies [54].

Total diet study, food supply data, household data, and individual dietary surveys are included within indirect methods for assessment of dietary intake. Indirect methods make predictions of personal exposure to mineral elements using exposure factor information, modelling and questionnaires combined with the analysis of selected foods or database mineral content of foods. **Total diet study** (TDS) also known as a market basket survey (MBS) is the most common approach to evaluate daily intake of mineral elements from the analysis of individual food items. A list which includes foods that constitutes the largest part of the total diet of the studied population is compiled, and then they are purchased and prepared as usually consumed by the population using the most common local practices and combining them into composite samples before

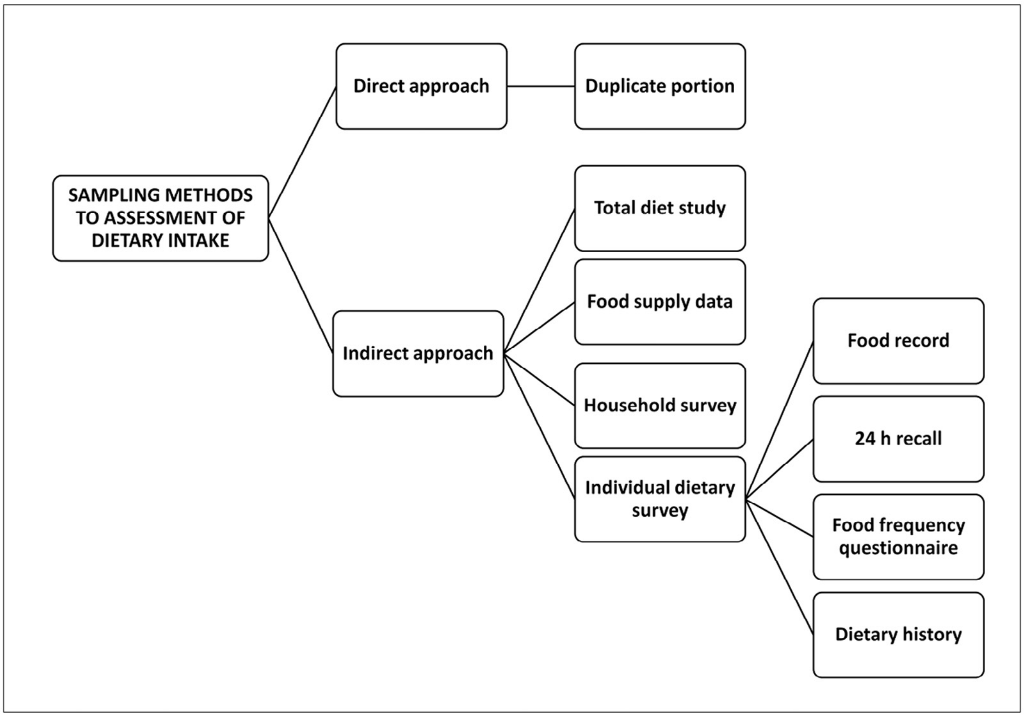

Fig. 3. Scheme about different kind of sampling methods in human diet analysis.

analysis for contaminants. Food consumption and content data must be combined to evaluate the intakes within the population. This distribution is compared with international reference recommendation for essential elements and toxicological values in order to assess the safety of food consumed by the population [1]. It is very common that health agencies from different countries are responsible to carry out or supervise the total diet studies like Australia, US, Canada, New Zealand and China [55–59].

Food supply data approach, also known as food balance sheets (FBS), has been developed because of the need for assessing the amount of food and nutrients available for human consumption. Results are an estimation of the average value per head of the population classified for age or gender. However, food supply data do not indicate any difference in food consumption levels between different regions, occupations or income levels. Becker et al. [60] based their study on food supply data of 1998 from Sweden. They did a shopping list where separated categories into food items using data on their market shares. Food items consumed on average less than 0.5 kg per person in one year were excluded from the list. The shopping list finally consisted of 116 foods and beverages and covered approximately 90% of the total annual consumption expressed in kg per person.

In contrast to food supply data, **household surveys** provide information about food consumption levels in different regions, occupations or income levels, although not provide information on how food is handled within the household, or members' consumption. Data on the quantity of consumed food may be collected by record keeping, by interviews or by both methods. Gimou et al. [24,61,62] carried out their diet sampling based on the 2nd Cameroonian Household Budget Survey from 2001. They purchased a total of 1773 foods representing 203 food items that were prepared as consumed in Cameroon. Avegliano et al. [63] did the sampling survey of 2017 household, based on the food consumption data base of the National Household Food Budget Survey from 2003. They purchased 5440 food items and prepared them at the university restaurant of University of Sao Paulo (Brazil).

To obtain information on average food and nutrition intake and their distribution over groups of individuals it has been used **individual dietary surveys**. Methods to collect dietary intake data at an individual level can be divided into two categories, record and recall methods. Record methods collect information on intake over one or more days. Recall methods reflect the past consumption, varying from intake of previous days (24 hour recall) to usual food intake (food frequency or dietary history). So, the following methodologies have been applied in the literature: i) *Food record*, which is kept for a specific time period, usually 1–7 days. The food records include all foods and beverages consumed at meals and in quantified amounts, as well as the inedible waste, the total cooked weight of meals, the cooked weight of the individual portion and dish waste, ii) In *recall method* for which the subject does a list of foods and beverages consumed the previous day or the 24 hours prior to the recall interview, and describes the kind and amounts of all foods and beverages ingested during this period of time. Food quantities are usually assessed by using household measures, food models or photographs, iii) *Food frequency questionnaire*, a method used as a tool in assessing the frequency of individual foods or food group's intake over extended periods of time (weeks, months, or years). The questionnaire, in this case, may include questions regarding the amount of food consumed, usual food preparation methods, including the use of dietary supplements and iv) in *dietary history method*, the main purpose is obtaining a picture of dietary consumption habits of the studied population. A trained interviewer assesses an individual's total usual food intake and meal pattern.

The main part of studies use 24 h recall to obtain daily intake information, because this method provides detailed quantitative assessment of intake and thus, these data can be used to estimate acute or chronic exposure, offer a possibility of calculating average and distribution of exposure and estimates that can be produced for specific population groups [11,30,64–69].

In order to collect data on mineral intake from the diet in small scale we consider that the best solution is the duplicate portion approach which is faster and easier than total diet studies. The determination of mineral composition of typical menu samples in a given country can provide an alternative to the studies on diet composition based on questionnaires and reference data.

3.2. Diet sample pre-treatment

Sample pre-treatment should involve as few steps as possible, reducing the probability of sample and analyte losses, or alteration of species. Sample pre-treatment procedures used in diet analysis by atomic spectrometry and mass spectrometry methods include digestion of target samples by dry-ashing and wet-digestion procedures. Other available techniques; such as infrared or X-ray fluorescence, only require drying of samples for mineral determination [48].

Diet samples required preservation, avoiding microbiological degradation by drying or freezing. Due to high degree of heterogeneity in diet samples is recommended pulverise and homogenise the sample previously.

In general, results of mineral analysis in diet are expressed on a dry-weight basis, because drying is the way to preserve samples during extended period of time and allows pre-concentration of the elements to be determined. Samples usually were dried inside the oven until constant weight at different temperatures, according to the literature, as 40°C [61,62,66,70–73], 50°C [11], 65°C [37], 60–80°C [13,22,44,45], 100°C [65], 102°C [39] or 105°C [74,75].

On the other hand, freeze-drying is also commonly used to dry diet samples before their analysis because it permits that the original structure of the products remain unaltered and only water is removed during this process [12,33,40,47,63,69,76,77]. To start the freeze-drying process, it is necessary to freeze the raw material at −18°C approximately. The second step of the process is the extraction of the water from the raw material, for that, the food is placed in a vacuum chamber. Under very low pressure, the frozen water contained in its structure is removed in the form of vapour. Some authors use different methods like microwave [23,36] or infrared lamp [64] to dry the samples. It has been reported that during food drying steps some compounds could be lost and, because of that, other authors prefer to treat directly wet samples for their mineral analysis in diet. In these cases, samples are just frozen to improve its preservation until their analysis [10,21,35,38,41,60,68,78–82].

Sample matrix treatment can be carried out by dry-ashing or wet digestion procedures that permit the decomposition of organic compounds and produce a residue of inorganic compounds followed for the elemental analysis by atomic spectroscopy methods.

3.2.1. Dry-ashing of diets

Ashing methods ensure the complete decomposition of organic matter by heating the sample at high temperatures inside a muffle furnace. Samples are heated between 400°C [37] to 550°C [68], being the most common temperature used 450°C [11,13,41,65,67]. Samples heating at high temperature can involve losses of some elements by volatilisation, like Hg, As, Se, Te, Sb or halogen compound. So, this problem may be avoided by addition of ashing aids, as magnesium nitrate or magnesium oxide [11,37].

Ashes are dissolved with nitric acid or hydrochloric acid, in variable concentrations depending of authors (see Table 1) and, in some cases, solutions are filtered before their analysis [11].

Dry-ashing methodology uses few millilitres of reagents for completely dissolve the ashes, but requires long mineralisation time.

Table 1
Methods reported in the literature for the mineralisation of human diet samples

Digestion process	References
Dry-ashing followed by dissolution of ashes with HNO_3	[13,32,60,64,65]
Dry-ashing followed by dissolution of ashes with HNO_3-HCl	[30,37,67,83]
Dry-ashing followed by dissolution of ashes with HNO_3-H_2O_2	[11,38,84]
Dry-ashing followed by dissolution of ashes with HCl	[68]
Wet digestion in hot-plate with HNO_3	[10,12,81,85,86]
Wet digestion in hot-plate with HNO_3-HCl	[87]
Wet digestion in hot-plate with HNO_3-H_2O_2-HCl	[76]
Wet digestion in MW with HNO_3-H_2O_2	[31,33,35,47,69,70, 73–75,88]
Wet digestion in MW with HNO_3-H_2O	[24,61,62,71,72,79]
Wet digestion in MW with HNO_3-$HClO_4$	[36,53,85,89]
Wet digestion in MW with HNO_3-H_2SO_4	[66]
Wet digestion in MW with HNO_3-HCl	[80]
Wet digestion in MW with HNO_3-HCl-HF	[78]

MW microwave-assisted digestion.

3.2.2. Wet digestion of diets

Wet digestion requires lower temperatures than those applied for dry-ashing methods, being carried out wet digestion until clear solutions, at temperatures in the range 80–120°C in hot plate [10,76,85,86] or between 180 and 210°C in microwave oven [31,35,47,88], until to clear solutions.

A pre-digestion step at the beginning, can be employed for accelerate sample dissolution during a time of 15 min to 8 h. Ultrasonication can be also applied directly to the samples or indirectly through the walls of the sample container using a water bath for pre-digestion [90].

Sample amounts and reagents used in microwave digestion methodologies are similar to those employed in hotplate digestion. In the case of wet sample treatment, the amount of sample varies between 0.2 to 3 g and, in the case of dry samples 0.1 to 1 g of sample diet were treated. Generally, the most widely used combination reagents in microwave-assisted digestion treatment is nitric acid with hydrogen peroxide in varying proportions (see Table 1).

The aforementioned wet digestion methodologies at atmospheric pressure are time-consuming, and analyte contamination or losses can occur. So, the use of microwave-assisted digestion (MW) could be a solution to provide a rapid total decomposition of diet sample with reduced risks of contamination and analyte losses [91].

The most employed methods reported in the literature for the mineralisation of human diets are summarised in Table 1.

4. Analytical methods for mineral analysis of diets

Total concentration of elements in complete diet samples are mainly determined by atomic spectrometry and mass methods, including inductively coupled plasma mass spectrometry (ICPMS), inductively coupled plasma optical emission spectroscopy (ICPOES), graphite furnace atomic absorption spectroscopy (GFAAS) and flame atomic absorption spectroscopy (FAAS). One of the complexities in food analysis is that the essential elements like K, Mg, Ca, Na, Fe and Zn are present at relatively high concentration levels, while toxic elements as Pb, Cd, Hg and As should be present at trace or ultra-trace levels. So, for toxic elements and trace minerals determination in diets ICPMS and GFAAS are often used due the capability to work at ppb level with limit of detection (LOD) values very low, being the working range for ICPOES and FAAS at ppm level (see Fig. 4).

Looking at the recently published works (see Table 2) it can be appreciated that in 36% of cases ICPMS was employed as analysis technique, 24% used GFAAS, 24% used FAAS and 16% used ICPOES. To choose an appropriate measurement technique for determination of the mineral profile of diet samples various factors, such as detection capability, sensitivity, speed of analysis, cost and applicability, must be taken into account. ICP-based techniques are particularly effective in the multi-elemental analysis of diet samples within wide concentration ranges. ICPMS is considered the most suitable technique for elemental food analysis, because it allows quantify a big amount of contaminants, minerals and nutrients whose concentration ranges varied widely. GFAAS and FAAS are more time-consuming than ICP-based techniques due to their mono-elemental detection, but still quite often applied when studies are focused on a few number of elements, because of their relatively low cost. Table 3 shows LODs found in the literature to determine elements in human diets.

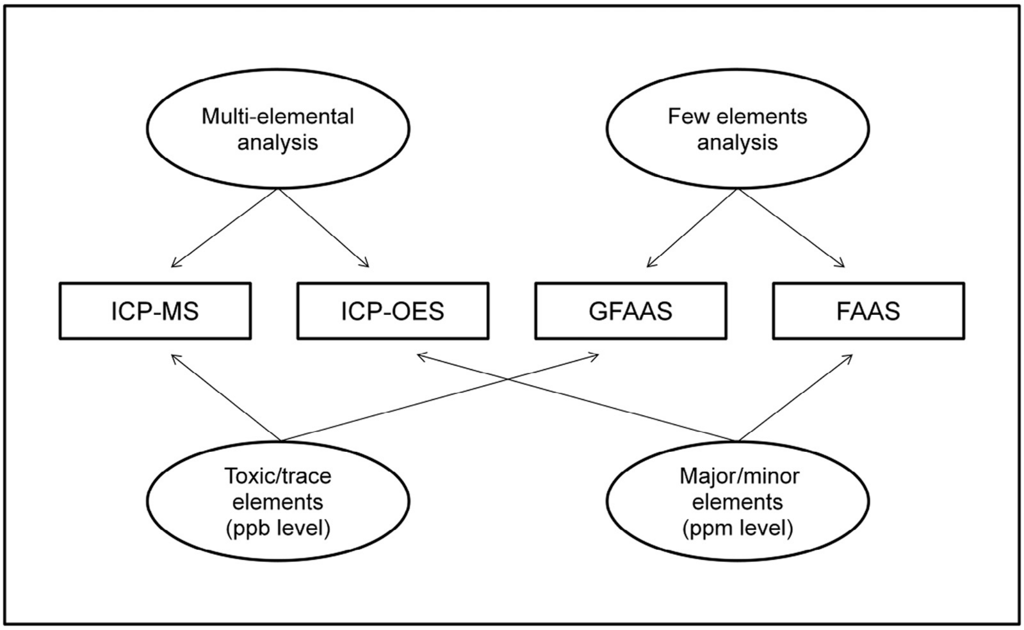

Fig. 4. Principal uses of atomic and mass spectrometry techniques.

Table 2
Common analytical techniques reported in the literature for the elemental analysis of human diets

Element	Analytical Techniques				
	ICPMS	ICPOES	GFAAS	FAAS	Other technique
Al	[12,61,62,71,72,79–81]	[13,47,78]	[36]		
As	[10,24,62,71,72,79–81,85,86,88]	[68]			HGAFS [11,31,37,70]
Ba	[61,69,72,78,80]	[21,47]			
Ca	[69,79]	[47,60,77]		[24,32,41,43,62,83]	INAA [63], MIR, NIR and XRF [48]
Cd	[10,12,24,62,71,72,74,75,78–80,85,86,88]	[68]	[11,34,60,65,70]	[32,67]	
Co	[10,24,62,69,71,73,74,78,79,81]		[60]	[32]	
Cr	[10,12,24,62,69,73,79,80,86]	[44]	[21,60]	[22,23]	INAA [63]
Cu	[10,12,61,62,69,73,74,78–81]	[47,89]	[45,60]	[32,38,41]	
Fe	[24,61,62,74]	[47,78,87]	[23,60]	[32,38,39,41,42,83,84]	INAA [63], MIR, NIR and XRF [48]
Hg	[10,24,62,71,72,75,76,79–81,85,88]				CVAFS [11,66], HGAFS [35,37,70]
K	[69,79]	[47,60,77]		[24,32,62,82]	INAA [63], MIR, NIR and XRF [48]
Li	[61,62,73,78,79]	[21,47]			
Mg	[60,69,79]	[47,77]		[24,32,43,62]	MIR, NIR and XRF [48]
Mn	[10,12,61,62,69,73,74,79–81,86]	[47,78]		[32,38,44,60]	
Mo	[12,61,62,69,73,78–80,89]	[47]			
Na	[49,79]	[47,60,87]		[24,32,39,62,82]	INAA [63], MIR and NIR [48]
Ni	[10,12,61,62,67,69,71,72,74,78,79,81,86]		[33,60]	[32,67]	
Pb	[10,12,41,61,62,69,71,72,74,75,78–81,85,86,88]	[68]	[11,34,60,64,70]	[32,67]	
Sb	[10,41,61,62,69,71,73,76,79,80]				
Se	[12,24,62,73,79–81,89]	[60,87]			INAA [40]
Sn	[10,12,78,80,86]				
Sr	[61,62,69,77,80]	[21,47]			
Zn	[10,12,24,62,69,73,74,78–81]	[47]	[33,46]	[32,38,41,60,83,84]	INAA [63], MIR,NIR and XRF [48]

Inductively coupled plasma mass spectrometry ICPMS; inductively coupled plasma optical emission spectroscopy ICPOES; graphite furnace atomic absorption spectroscopy GFAAS; flame atomic absorption spectroscopy FAAS; hydrogen generation atomic fluorescence spectroscopy HGAFS; cold vapour atomic fluorescence spectroscopy CVAFS; middle infrared spectroscopy MIR; near infrared spectroscopy NIR; x-ray fluorescence XRF; instrumental neutron activation analysis INAA.

In order to reduce time and cost of analysis, spectrometric methods that combine fast analysis together with a simple sample preparation have a great interest for the analysis of food samples. Energy dispersive X-ray fluorescence (EDXRF) and infrared spectroscopy (IR) eliminate the need of sample pre-treatment because they can be applied to solid samples and providing rapid measurements. The combination of infrared spectroscopy or XRF measurements with chemometric data treatment could offer increasing possibilities for the direct determination of mineral components in food samples. In the literature, partial least squares (PLS) or modified-partial lest squares (MPLS) have been employed for developing models for quantitative determination of mineral elements in foods [92–96] and diet analysis [48]. Unfortunately, XRF and IR suffer from relatively low sensitivity and strong inter-element and matrix effects.

Essential and toxic elements in diet samples can be also determined using instrumental neutron activation analysis (INAA) which is a really effective technique to measure major, minor and trace elements in foods [40,63]. However, this technique has been rarely used, probably due to its expensive costs, the use of big facilities, additionally than the long time required. Generally, the potential toxic elements as cadmium and lead are analysed by GFAAS [11,34,60,64,65,70], while arsenic and mercury are analysed by HGAFS [11,31,35,37,66,70]. However, some authors prefer to determine the last four elements by ICPMS [10,62,73,79–81,85,86,88].

Table 3
Detection limits reported in the literature for the elemental analysis of human diets

Element	LOD in μg kg^{-1} except for elements Al, Ca, Fe, K, Mg and Na in mg kg^{-1}				
	ICPMS	ICPOES	GFAAS	FAAS	HGAFS
Al	0.01–0.29 [8,24–26,38,46,89]	2.4 [47]			
As	1–200 [24,25,30,34,35,45,46,50,80,89]	10–540 [47,68]			2.2–10 [31,37,70]
Ba	7–45 [26,38,46,50]	100 [47]			
Ca	8–160 [25,50]	10 [47]		12.5 [24,62]	
Cd	0.13–25 [8,15,16,24–26,30,34,35,38,46,50,89]	5–27 [47,68]	1–22 [60,70]	2 [67]	
Co	1.5–16 [15,25,45,46,50,63,89]	50 [47]	3.5 [60]		
Cr	3–140 [25,45,46,50,63,89]	80 [47]	5 [60]		
Cu	0.5–40 [15,25,26,46,50,63]	40 [47]	84 [60]		
Fe	0.043^{a}–1.1 [15,45,46,50]	0.23 [47]	0.14 [60]		
Hg	0.5–100 [16,24–26,30,35,45,46,89]				0.02–0.12 [35,37,70]
K	83 [25]	7.5 [47]		1.5–4.5 [24,62,72,82]	
Mg	0.246–1.6 [24,25,45,46,50]	0.4 [47]		2.5 [24,62,72]	
Mn	0.5–28 [8,15,26,34,38,46,50,63]	50 [47]		20 [60]	
Mo	0.02–61 [8,26,38,46,50,54,63]	100 [47]			
Na	2.9 [24,25,45,46]	10.6 [47]		2.4–4.15 [62,72,77]	
Ni	0.5–110 [8,24,26,34,38,46,50,51,89]	100 [47]	18 [60]	0.8 [67]	
Pb	0.5–25 [8,15,16,25,26,30,34,35,38,46,50,89]	10–130 [47,68]	3.5–5 [60,70]	0.8 [67]	
Sb	0.1–9.9 [24–26,38,46,50,89]				
Se	0.6–50 [8,26,45,46,54,63]	110 [47]			
Sn	0.19–50 [8,26,34]				
Sr	2.9–20 [26,38,46,50]	26 [47]			
Zn	0.5–110 [8,15,25,26,45,46,50,63]	210 [47]			

[a] With cell collision.

5. Conclusions

The analysis of the mineral composition of complete diets offers the best way to obtain true values on the daily intake of minerals by a target population. It must be preferred in front of those approaches based on food surveys and average data on the concentration of mineral elements in each kind of foods.

Regarding the sample treatment microwave assisted digestion employing nitric acid hydrogen peroxide is the most used procedure, being also employed dry-ashing and hot-plate digestion.

In spite of the new developments in analytical chemistry, we have confirmed that for mineral analysis of diet samples atomic spectroscopy and mass spectrometry based techniques are the most employed to obtain the available data for human diets (36% for ICPMS, 24% for GFAAS, 24% for FAAS and 16% for ICPOES).

Future directions in mineral analysis of diets are likely to focus on the development of improved sample preparation, mineral bioavailability, direct analytical techniques and the implementation of green direct analytical tools that could provide screening information about the presence of essential, non-essential and toxic elements in both, the main foods which compose the human diets and the complete diets.

Acknowledgement

Authors acknowledge the financial support the Ministerio de Economía y Competitividad (project CTQ2012-38635 and project CTQ2014-52841) and Generalitat Valenciana (project PROMETEO-II 2014-077). Alba Mir-Marqués acknowledges the grant ‘Atracció de Talent’ (PREDOC13-110514) provided by the University of Valencia.

References

[1] Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO), Principles and Methods for the Risk Assessment of Chemicals in Food, Geneva, 2009. <http://www.who.int/foodsafety/publications/chemical-food/en/>.

[2] World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), Food Safety Risk Analysis: A Guide for National Food Safety Authorities, Rome, 2007. <http://www.fao.org/docrep/012/a0822e/a0822e00.htm>.

[3] R. Kroes, D. Müller, J. Lambe, M.R. Löwik, J. van Klaveren, J. Kleiner, et al., Assessment of intake from the diet, Food Chem. Toxicol. 40 (2002) 327–385.

[4] World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), Codex Alimentarius, 2015. <http://www.codexalimentarius.org/>.

[5] Joint FAO/WHO Expert Committee on Food Additives, World Health Organization (WHO), Evaluation of Certain Food Additives and Contaminants: Seventy-Fourth Report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, 2011. <http://apps.who.int/iris/bitstream/10665/44788/1/WHO_TRS_966_eng.pdf>.

[6] R.B. Khouzam, P. Pohl, R. Lobinski, Bioaccessibility of essential elements from white cheese, bread, fruit and vegetables, Talanta 86 (2011) 425–428.

[7] Joint FAO/WHO Expert Committee on Food Additives, Food and Agriculture Organization of the United Nations, World Health Organization, Evaluation of Certain Contaminants in Food: Seventy-Second Report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, Switzerland, 2011. <http://apps.who.int/iris/bitstream/10665/44514/1/WHO_TRS_959_eng.pdf>.

[8] Joint FAO/WHO Expert Committee on Food Additives and World Health Organization, Evaluation of Certain Food Additives and Contaminants: Seventy-Third Report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, Switzerland, 2011. <http://apps.who.int/iris/bitstream/10665/44515/1/WHO_TRS_960_eng.pdf>.

[9] Food and Agriculture Organization of the United Nations, World Health Organization, Working Document for Information and Use in Discussions Related to Contaminants and Toxins in the GSCTFF, Geneva, 2011. <ftp://ftp.fao.org/codex/meetings/CCCF/cccf5/cf05_INF.pdf>.

[10] J.L. Domingo, G. Perelló, J. Giné Bordonaba, Dietary intake of metals by the population of Tarragona County (Catalonia, Spain): results from a duplicate diet study, Biol. Trace Elem. Res. 146 (2012) 420–425.

[11] O. Muñoz, J.M. Bastias, M. Araya, A. Morales, C. Orellana, R. Rebolledo, et al., Estimation of the dietary intake of cadmium, lead, mercury, and arsenic by the population of Santiago (Chile) using a Total Diet Study, Food Chem. Toxicol. 43 (2005) 1647–1655.

[12] N.N. Aung, J. Yoshinaga, J.I. Takahashi, Dietary intake of toxic and essential trace elements by the children and parents living in Tokyo Metropolitan Area, Japan, Food Addit. Contam. 23 (2006) 883–894.

[13] D. González-Weller, A.J. Gutiérrez, C. Rubio, C. Revert, A. Hardisson, Dietary intake of aluminum in a Spanish population (Canary Islands), J. Agric. Food Chem. 58 (2010) 10452–10457.

[14] World Health Organization, Guideline: Sodium Intake for Adults and Children, Geneva, 2012. <http://www.who.int/nutrition/publications/guidelines/sodium_intake_printversion.pdf>.

[15] D.B. Young, Role of Potassium in Preventive Cardiovascular Medicine, sixth ed., Kluwer Academic Inc., Boston, 2001.

[16] World Health Organization, Guideline: Potassium Intake for Adults and Children, Geneva, 2012. <http://www.who.int/nutrition/publications/guidelines/potassium_intake_printversion.pdf>.

[17] World Health Organization, Food and Agriculture Organization of the United Nations, Vitamin and Mineral Requirements in Human Nutrition, second ed., Rome, 2004. <http://apps.who.int/iris/bitstream/10665/42716/1/9241546123.pdf>.

[18] B. De Benoist, World Health Organization, Centers for Disease Control and Prevention (U.S.), Worldwide Prevalence of Anaemia 1993–2005 of WHO Global Database of Anaemia, Geneva, 2008. <http://apps.who.int/iris/bitstream/10665/43894/1/9789241596657_eng.pdf>.

[19] G.F. Nordberg, Handbook on the Toxicology of Metals, third ed., Elsevier, Amsterdam, 2007.

[20] Scientific Committee on Food European Commission, Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Copper, Brussels, 2003. <http://ec.europa.eu/food/fs/sc/scf/out176_en.pdf>.

[21] D. González-Weller, C. Rubio, A.J. Gutiérrez, G.L. González, J.M.C. Mesa, C.R. Gironés, et al., Dietary intake of barium, bismuth, chromium, lithium, and strontium in a Spanish population (Canary Islands, Spain), Food Chem. Toxicol. 62 (2013) 856–868.

[22] C. Velasco-Reynold, M. Navarro-Alarcón, H. Lopez-Ga de la Serrana, V. Perez-Valero, M.C. Lopez-Martinez, Determination of daily dietary intake of chromium by duplicate diet sampling: *in vitro* availability study, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 25 (2008) 604–610.

[23] C. Cabrera-Vique, M. Mesías, Chromium and iron content in duplicate meals at a university residence: daily intake and dialysability, Br. J. Nutr. 105 (2011) 1546–1552.

[24] M.M. Gimou, U.R. Charrondière, J.C. Leblanc, L. Noël, T. Guérin, R. Pouillot, Dietary exposure and health risk assessment for 11 minerals and trace elements in Yaoundé: the Cameroonian Total Diet Study, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 30 (2013) 1556–1572.

[25] C.E. Lazarte, N.G. Carlsson, A. Almgren, A.S. Sandberg, Y. Granfeldt, Phytate, zinc, iron and calcium content of common Bolivian food, and implications for mineral bioavailability, J. Food Compost. Anal. 39 (2015) 111–119.

[26] P. Singh, S. Prasad, W. Aalbersbe, Bioavailability of Fe and Zn in selected legumes, cereals, meat and milk products consumed in Fiji, Food Chem. 207 (2016) 125–131.

[27] R.L. Bertin, H.F. Maltez, J.S. Gois, D.L.G. Borges, G. Borges, L. Gonzaga, et al., Mineral composition and bioacessibility in Sacocornia ambigua using ICP-OES, J. Food Compost. Anal. 47 (2016) 45–51.

[28] M. Vaz-Tostes, T. Verediano, E.G. de Mejia, N. Brunoro, Evaluation of iron and zinc bioavailability of beans targeted for biofortification using in-vitro and in-vivo models and their effect on the nutrition status of preschool children, J. Sci. Food Agric. 96 (2016) 1326–1332.

[29] L.D. Martínez, R.A. Gil, P.H. Pacheco, S. Cerutti, Elemental composition analysis of food by FAES and ICP-OES, in: M. de la Guardia, S. Garrigues (Editors), Handbook of Mineral Elements in Food, John Wiley & Sons Inc., Chichester, UK, 2015, pp. 219–238.

[30] J. Zukowska, M. Bizuk, Methodological evaluation of method for dietary heavy metal intake, J. Food Sci. 73 (2008) R21–R29.

[31] P. Liu, C.N. Wang, X.Y. Song, Y.F. Yu, Y.N. Wu, Dietary intake of arsenic by children and adults from Jinhu area of China, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 27 (2010) 1128–1135.

[32] R. Raghunath, R. Tripathi, B. Suseela, S. Bhalke, V. Shukla, V. Puranik, Dietary intake of metals by Mumbai adult population, Sci. Total Environ. 356 (2006) 62–68.

[33] J. Wittsiepe, K. Schnell, A. Hilbig, P. Schrey, M. Kersting, M. Wilhelm, Dietary intake of nickel and zinc by young children – results from food duplicate portion measurements in comparison to data calculated from dietary records and available data on levels in food groups, J. Trace Elem. Med. Biol. 23 (2009) 183–194.

[34] P. Liu, C.N. Wang, X.Y. Song, Y.N. Wu, Dietary intake of lead and cadmium by children and adults – result calculated from dietary recall and available lead/cadmium level in food in comparison to result from food duplicate diet method, Int. J. Hyg. Environ. Health 213 (2010) 450–457.

[35] J. Sun, C. Wang, X. Song, Y. Wu, B. Yuan, P. Liu, Dietary intake of mercury by children and adults in Jinhu area of China, Int. J. Hyg. Environ. Health 214 (2011) 246–250.

[36] C. Cabrera-Vique, M. Mesías, Content and bioaccessibility of aluminium in duplicate diets from Southern Spain: aluminium in duplicate diets from Spain, J. Food Sci. 78 (2013) T1307–T1312.

[37] J.M. Bastias, M. Bermudez, J. Carrasco, O. Espinoza, M. Munoz, M.J. Galotto, et al., Determination of dietary intake of total arsenic, inorganic arsenic and total mercury in the Chilean School Meal Program, Food Sci. Technol. Int. 16 (2010) 443–450.

[38] M. Goshima, T. Murakami, H. Nakagaki, T. Shibata, T. Sugiyama, K. Kato, et al., Iron, zinc, manganese and copper intakes in Japanese children aged 3 to 5 years, J. Nutr. Sci. Vitaminol. 54 (2008) 475–482.
[39] M.L. Weber, T.B. Morais, Nutritional composition, assessed by chemical analyses, of prepared foods available for primary-school children: a comparison of public and private schools, Public Health Nutr. 13 (2010) 1855–1862.
[40] D.K. Adotey, V. Stibilj, Y. Serfor-Armah, B.J.B. Nyarko, R. Jaćimović, Dietary supply of selenium for adolescents in three residential care orphanages in Southern Ghana, Sci. Total Environ. 410 (2011) 72–79.
[41] F. Cámara, M.A. Amaro, R. Barberá, G. Clemente, Bioaccessibility of minerals in school meals: comparison between dialysis and solubility methods, Food Chem. 92 (2005) 481–489.
[42] C. Velasco-Reynold, M. Navarro-Alarcon, H. Lopez-Ga de la Serrana, V. Perez-Valero, M.C. Lopez-Martinez, Iron dialyzability from hospital duplicate meals: daily intake, Biol. Trace Elem. Res. 130 (2009) 241–248.
[43] C. Velasco-Reynold, M. Navarro-Alarcon, H. Lopez-Ga de la Serrana, V. Perez-Valero, A. Agil, M.C. Lopez-Martinez, Dialysability of magnesium and calcium from hospital duplicate meals: influence exerted by other elements, Biol. Trace Elem. Res. 133 (2010) 313–324.
[44] C. Velasco-Ryenold, M. Navarro-Alarcón, H. Lopez-Ga de la Serrana, V. Perez-Valero, M.C. Lopez-Martinez, Total and dialyzable levels of manganese from duplicate meals and influence of other nutrients: estimation of daily dietary intake, Food Chem. 109 (2008) 113–121.
[45] C. Velasco-Reynold, M. Navarro-Alarcon, H. López-Ga de la Serrana, V. Perez-Valero, M.C. Lopez-Martinez, Analysis of total and dialyzable copper levels in duplicate meals by ETAAS: daily intake, Eur. Food Res. Technol. 227 (2008) 367–373.
[46] C. Velasco-Reynold, M. Navarro-Alarcon, H. López-Ga de la Serrana, V. Perez-Valero, M.C. Lopez-Martinez, In vitro determination of zinc dialyzability from duplicate hospital meals: influence of other nutrients, Nutrition 24 (2008) 84–93.
[47] A. Mir-Marqués, M.L. Cervera, M. de la Guardia, A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus, J. Food Compost. Anal. 27 (2012) 160–168.
[48] A. Mir-Marqués, S. Garrigues, M.L. Cervera, M. de la Guardia, Direct determination of minerals in human diets by infrared spectroscopy and X-ray fluorescence, Microchem. J. 117 (2014) 156–163.
[49] H.R. Park, G.O. Jeong, S.L. Lee, J.Y. Kim, S.A. Kang, K.Y. Park, et al., Workers intake too much salt from dishes of eating out and food service cafeterias; direct chemical analysis of sodium content, Nutr. Res. Pract. 3 (2009) 328.
[50] E. Hobin, C. White, Y. Li, M. Chiu, M.F. O'Brien, D. Hammond, Nutritional quality of food items on fast-food "kids" menus': comparisons across countries and companies, Public Health Nutr. 17 (2014) 2263–2269.
[51] A.O. Musaiger, J.H. Al-Jedah, R. D'souza, Proximate, mineral and fatty acid composition of fast foods consumed in Bahrain, Br. Food J. 110 (2008) 1006–1018.
[52] C. Cabrera-Vique, M. Mesías, P.R. Bouzas, Nickel levels in convenience and fast foods: in vitro study of the dialyzable fraction, Sci. Total Environ. 409 (2011) 1584–1588.
[53] C. Cabrera-Vique, P.R. Bouzas, Chromium and manganese levels in convenience and fast foods: in vitro study of the dialyzable fraction, Food Chem. 117 (2009) 757–763.
[54] World Health Organization, Guidelines for the Study of Dietary Intakes of Chemical Contaminants, Geneva, 1985. <http://apps.who.int/iris/handle/10665/39255>.
[55] Food Standards Australia New Zealand, 24th Australian Total Diet Study, Kingston, 2014. <http://www.foodstandards.gov.au/publications/Pages/24th-Australian-Total-Diet-Study.aspx>.
[56] US Food and Drug Administration, Total Diet Study Statistics on Element Results – 2006–2011, College Park, Maryland, 2014. <http://www.fda.gov/downloads/Food/FoodScienceResearch/TotalDietStudy/UCM184301.pdf>.
[57] Bureau of Chemical Safety and Health Products and Food Branch, Canadian Total Diet Study, Ottawa, Ontario, 2007. <http://www.hc-sc.gc.ca/fn-an/surveill/total-diet/index-eng.php>.
[58] Ministry of Agriculture and Forestry, 2009 New Zealand Total Diet Study. Agricultural Compound Residues, Selected Contaminant and Nutrient Elements, Crown, 2011. <http://www.foodsafety.govt.nz/science-risk/programmes/total-diet-survey.htm>.
[59] China National Center for Food Safety Risk Assessment, China Total Diet Study, Beijing, 2013. <http://www.chinafoodsafety.net/detail.aspx?id=2F8F16B8AB6EB2D2D514FFBE794B3FB98C5610E779E432B5>.
[60] W. Becker, L. Jorhem, B. Sundström, K.P. Grawé, Contents of mineral elements in Swedish market basket diets, J. Food Compost. Anal. 24 (2011) 279–287.
[61] M.M. Gimou, R. Pouillot, U.R. Charrondiere, L. Noël, T. Guérin, J.C. Leblanc, Dietary exposure and health risk assessment for 14 toxic and essential trace elements in Yaoundé: the Cameroonian total diet study, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 31 (2014) 1064–1080.
[62] M.M. Gimou, U.R. Charrondière, J.C. Leblanc, R. Pouillot, L. Noël, T. Guérin, Concentration data for 25 elements in foodstuffs in Yaoundé: the Cameroonian Total Diet Study, J. Food Compost. Anal. 34 (2014) 39–55.
[63] R.P. Avegliano, V.A. Maihara, F.F. da Silva, A Brazilian Total Diet Study: evaluation of essential elements, J. Food Compost. Anal. 24 (2011) 1009–1016.
[64] C. Rubio, T. González-Iglesias, C. Revert, J.I. Reguera, A.J. Gutiérrez, A. Hardisson, Lead dietary intake in a Spanish population (Canary Islands), J. Agric. Food Chem. 53 (2005) 6543–6549.
[65] C. Rubio, A. Hardisson, J. Reguera, C. Revert, M. Lafuente, T. Gonzaleziglesias, Cadmium dietary intake in the Canary Islands, Spain, Environ. Res. 100 (2006) 123–129.
[66] C. Rubio, A.J. Gutiérrez, A. Burgos, A. Hardisson, Total dietary intake of mercury in the Canary Islands, Spain, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 25 (2008) 946–952.
[67] Z. Marzec, W. Koch, A. Marzec, W. Żukiewicz-Sobczak, Dietary exposure to cadmium, lead and nickel among students from the south-east region of Poland, Ann. Agric. Environ. Med. 21 (2014) 825–828.
[68] H.S. Lee, Y.H. Cho, S.O. Park, S.H. Kye, B.H. Kim, T.S. Hahm, et al., Dietary exposure of the Korean population to arsenic, cadmium, lead and mercury, J. Food Compost. Anal. 19 (2006) S31–S37.
[69] H. Marcussen, B.H. Jensen, A. Petersen, P.E. Holm, Dietary exposure to essential and potentially toxic elements for the population of Hanoi, Vietnam, Asia Pac. J. Clin. Nutr. 22 (2013) 300–311.
[70] C. Chen, Y. Qian, Q. Chen, C. Li, Assessment of daily intake of toxic elements due to consumption of vegetables, fruits, meat, and seafood by inhabitants of Xiamen, China, J. Food Sci. 76 (2011) T181–T188.
[71] N. Arnich, V. Sirot, G. Rivière, J. Jean, L. Noël, T. Guérin, et al., Dietary exposure to trace elements and health risk assessment in the 2nd French Total Diet Study, Food Chem. Toxicol. 50 (2012) 2432–2449.
[72] S. Millour, L. Noël, A. Kadar, R. Chekri, C. Vastel, V. Sirot, et al., Pb, Hg, Cd, As, Sb and Al levels in foodstuffs from the 2nd French total diet study, Food Chem. 126 (2011) 1787–1799.
[73] L. Noël, R. Chekri, S. Millour, C. Vastel, A. Kadar, V. Sirot, et al., Li, Cr, Mn, Co, Ni, Cu, Zn, Se and Mo levels in foodstuffs from the Second French TDS, Food Chem. 132 (2012) 1502–1513.
[74] L. Nasreddine, O. Nashalian, F. Naja, L. Itani, D. Parent-Massin, M. Nabhani-Zeidan, et al., Dietary exposure to essential and toxic trace elements from a Total diet study in an adult Lebanese urban population, Food Chem. Toxicol. 48 (2010) 1262–1269.
[75] L. Nasreddine, N. Hwalla, O. El Samad, J.C. Leblanc, M. Hamzé, Y. Sibiril, et al., Dietary exposure to lead, cadmium, mercury and radionuclides of an adult urban population in Lebanon: a total diet study approach, Food Addit. Contam. 23 (2006) 579–590.
[76] S.W. Cheung Chung, K.P. Kwong, J. Yau, W. Wong, Dietary exposure to antimony, lead and mercury of secondary school students in Hong Kong, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 25 (2008) 831–840.
[77] A.G. Gharib, M.G. Gharib, Intakes of radiologically important trace and minor elements from Iranian daily diets, Iran J. Sci. Technol. Trans. A Sci. 34 (2010) 227–236.
[78] G. Turconi, C. Minoia, A. Ronchi, C. Roggi, Dietary exposure estimates of twenty-one trace elements from a Total Diet Study carried out in Pavia, Northern Italy, Br. J. Nutr. 101 (2009) 1200.
[79] J.C. Leblanc, T. Guérin, L. Noël, G. Calamassi-Tran, J.L. Volatier, P. Verger, Dietary exposure estimates of 18 elements from the 1st French Total Diet Study, Food Addit. Contam. 22 (2005) 624–641.
[80] M. Rose, M. Baxter, N. Brereton, C. Baskaran, Dietary exposure to metals and other elements in the 2006 UK Total Diet Study and some trends over the last 30 years, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 27 (2010) 1380–1404.
[81] A. Callan, A. Hinwood, A. Devine, Metals in commonly eaten groceries in Western Australia: a market basket survey and dietary assessment, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 31 (2014) 1968–1981.
[82] C.M. Tanase, P. Griffin, K.G. Koski, M.J. Cooper, K.A. Cockell, Sodium and potassium in composite food samples from the Canadian Total Diet Study, J. Food Compost. Anal. 24 (2011) 237–243.
[83] M. Umeta, C.E. West, H. Fufa, Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia, J. Food Compost. Anal. 18 (2005) 803–817.
[84] A.M. Karunaratne, P.H. Amerasinghe, V.M. Sadagopa Ramanujam, H.H. Sandstead, P.A.J. Perera, Zinc, iron and phytic acid levels of some popular foods consumed by rural children in Sri Lanka, J. Food Compost. Anal. 21 (2008) 481–488.
[85] R. Martí-Cid, J.M. Llobet, V. Castell, J.L. Domingo, Dietary intake of arsenic, cadmium, mercury, and lead by the population of Catalonia, Spain, Biol. Trace Elem. Res. 125 (2008) 120–132.
[86] A. Bocio, M. Nadal, J.L. Domingo, Human exposure to metals through the diet in Tarragona, Spain: temporal trend, Biol. Trace Elem. Res. 104 (2005) 193–202.
[87] B.M. Thomson, R.W. Vannoort, R.M. Haslemore, Dietary exposure and trends of exposure to nutrient elements iodine, iron, selenium and sodium from the 2003–4 New Zealand Total Diet Survey, Br. J. Nutr. 99 (2008) 614–625.
[88] I. Martorell, G. Perelló, R. Martí-Cid, J.M. Llobet, V. Castell, J.L. Domingo, Human exposure to arsenic, cadmium, mercury, and lead from foods in Catalonia, Spain: temporal trend, Biol. Trace Elem. Res. 142 (2011) 309–322.
[89] M.K. Choi, M.H. Kang, M.H. Kim, The Analysis of copper, selenium, and molybdenum contents in frequently consumed foods and an estimation of their daily intake in Korean adults, Biol. Trace Elem. Res. 128 (2009) 104–117.
[90] M.D.G. Andrade Korn, E.S. da Boa Morte, D.C.M. Batista dos Santos, J.T. Castro, J.T.P. Barbosa, A.P. Teixeira, et al., Sample preparation for the determination of metals in food samples using spectroanalytical methods – a review, Appl. Spectrosc. Rev. 43 (2008) 67–92.
[91] A. Mir-Marques, M.L. Cervera, M. de la Guardia, Baby foods, in: M. de la Guardia, S. Garrigues (Editors), Handbook of Mineral Elements in Food, John Wiley & Sons Inc., Chichester, UK, 2015, pp. 709–723.

[92] C. Collell, P. Gou, P. Picouet, J. Arnau, J. Comaposada, Feasibility of near-infrared spectroscopy to predict aw and moisture and NaCl contents of fermented pork sausages, Meat Sci. 85 (2010) 325–330.
[93] D. Cozzolino, W. Cynkar, N. Shah, P. Smith, Quantitative analysis of minerals and electric conductivity of red grape homogenates by near infrared reflectance spectroscopy, Comput. Electron. Agric. 77 (2011) 81–85.
[94] A. Lucas, D. Andueza, E. Rock, B. Martin, Prediction of dry matter, fat, pH, vitamins, minerals, carotenoids, total antioxidant capacity, and color in fresh and freeze-dried cheeses by visible-near-infrared reflectance spectroscopy, J. Agric. Food Chem. 56 (2008) 6801–6808.
[95] J. Moros, I. Llorca, M.L. Cervera, A. Pastor, S. Garrigues, M. de la Guardia, Chemometric determination of arsenic and lead in untreated powdered red paprika by diffuse reflectance near-infrared spectroscopy, Anal. Chim. Acta 613 (2008) 196–206.
[96] A. Mir-Marqués, M. Martínez-García, S. Garrigues, M.L. Cervera, M. de la Guardia, Green direct determination of mineral elements in artichokes by infrared spectroscopy and X-ray fluorescence, Food Chem. 196 (2016) 1023–1030.

Microchemical Journal 117 (2014) 156–163

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Direct determination of minerals in human diets by infrared spectroscopy and X-ray fluorescence

Alba Mir-Marqués, Salvador Garrigues, M. Luisa Cervera, Miguel de la Guardia *

Department of Analytical Chemistry, University of Valencia, Jeronimo Muñoz Research Building, 50th Dr. Moliner str. 46100 Burjassot, Valencia, Spain

ARTICLE INFO

Article history:
Received 19 June 2014
Accepted 27 June 2014
Available online 4 July 2014

Keywords:
Mineral elements
Human diets
Infrared spectroscopy
X-ray fluorescence
Partial least squares

ABSTRACT

The use of near and mid infrared spectroscopy and X-ray fluorescence (XRF) to determine the concentration of mineral elements in Spanish human diets was investigated. Thirty-five commercial baby foods, 6 children fast food menus and 13 university canteen menu samples were analysed by infrared and XRF spectroscopy and spectra evaluated by using reference data obtained by inductively coupled plasma optical emission spectroscopy (ICP-OES). Models for calcium, potassium, iron, magnesium, sodium and zinc determination were built and validated. Spectra were pre-treated by using different pre-processing algorithms (multiplicative scatter correction, standard normal variation, first derivate, orthogonal signal correction, smoothing and mean centre) prior to developing calibration models using partial least squares and were evaluated by cross-validation and external validation. The highest coefficients of determination in validation (R^2_{val}) and the lowest relative root-mean-square error of prediction were obtained for potassium determination by MIR (0.86 and 11%), NIR (0.9 and 11%) and XRF (0.9 and 10%).

1. Introduction

In the last years, significant changes in the lifestyle have improved the consume of baby jars in the period from 6 till 12 months, and modified the nutrition habits, moving from home meals to the frequent use of fast food and canteen menus. Because of that, analytical control of this kind of diets has an increased importance. Additionally, the mineral profile of foods is very important in order to assure the recommended daily intake of essential elements and to control excessive consume of salt, frequently added to cooked foods [1–3].

The determination of the mineral profile of foods and diets is commonly made by using atomic spectroscopy and mass spectrometry methods, being the most common used techniques for this purpose inductively coupled plasma optical emission (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) [4–8]. These techniques are very useful for the multi-elemental analysis of samples in a few minutes. However, to do it, additionally than the use of high cost and non-portable instrumentation it is required a previous sample digestion and dissolution, thus involving the use of long and tedious sample pretreatment with the use of strong acids and oxidants and a lot of energy, also providing non-degradable wastes.

Green alternatives to the aforementioned techniques could be based on direct measurements by infrared spectroscopy [9,10] and X-ray fluorescence (XRF) [11]. These techniques permit a direct and fast analysis of solid samples without any chemical sample pre-processing, thus avoiding reagent consumption and toxic waste generation, making these analyses very easy and cheap. Another advantage is the reduced cost of the new IR and XRF portable instrumentation as compared with the atomic and mass spectrometry ones. However, the main disadvantage of both infrared and XRF is their low sensitivity and high matrix effects. Therefore, it is essential to use chemometric tools to built appropriate calibration models from the spectra of well characterised samples in order to increase the prediction capability of measurements made [12]. Being those reasons for which IR and XRF methods have been mainly considered as semi-quantitative tools.

The combination of middle infrared (MIR), near infrared (NIR) and XRF measurements with chemometric data treatment could offer increasing possibilities for the direct determination of mineral components in food samples [13] and to do it, partial least squares (PLS) or modified-partial lest squares (MPLS) have been employed for building models proposed in the literature to quantitative determine mineral elements in foods [14–19]. So, the main purpose of this study was to develop chemometric based methods to extract information about as much as possible mineral elements in different human diets from the MIR, NIR and XRF spectra of solid freeze dried samples.

2. Materials and methods

2.1. Apparatus and instruments

Reference values on the mineral composition of samples were determined by ICP-OES after microwave-assisted digestion of samples. It was used an optima 5300 DV ICP-OES Perkin Elmer (Norwalk, CT, USA) equipped with an auto sampler AS 93-plus and a cross flow nebuliser. Samples were previously digested using a Milestone Ethos

---

* Corresponding author.
E-mail address: miguel.delaguardia@uv.es (M. de la Guardia).

http://dx.doi.org/10.1016/j.microc.2014.06.028
0026-265X/© 2014 Elsevier B.V. All rights reserved.

SEL microwave laboratory system (Sorisole, Italy), equipped with a thermocouple probe for automatic temperature control, and an automatic gas leak detector using high pressure Teflon vessels of 100 mL inner volume. Teflon vessels were cleaned with vapours of nitric acid (69%), using the Trace CLEAN from Milestone (Sorisole, Italy) to avoid cross-contamination.

To obtain the MIR spectra it was employed a model Tensor 27 Fourier transform mid-infrared (FT-MIR) spectrometer from Bruker (Bremen, Germany) equipped with a DLaTGS detector and a dry-air purge in-compartment DuraSampleIR accessory for attenuated total reflectance measurements (ATR) from Smiths Detection Inc. (Warrington, UK) with a three reflection diamond/ZnSe DuraDisk plate. For instrument control and data acquisition, OPUS software 6.5 from Bruker was used.

For NIR measurements it was employed a MPA model Multipurpose Analyser Fourier transform near-infrared (FT-NIR) spectrometer from Bruker equipped with an integrating sphere, used to diffuse reflectance spectra acquisition. For instrument control and data acquisition, the OPUS software 6.5 from Bruker was used.

X-ray fluorescence spectra were obtained using a portable model S1 Titan energy dispersive X-ray fluorescence (ED-XRF) spectrometer from Bruker equipped with a Rhodium X-ray tube and X-Flash® SDD detector. For instrument control S1RemoteCtrl and S1Sync software from Bruker were employed, and for spectra treatment, the ARTAX software from Bruker was used.

2.2. Reagents

For the reference procedure argon C-45 (purity higher than 99.995%) supplied by Carburos Metálicos (Barcelona, Spain) was used as plasmogen, and stock solution of a multi-element standard solution (100 mg L^{-1}) containing 26 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Se, Na, Sr, Tl, Ti, V and Zn) dissolved in 5% HNO_3 obtained from Scharlau (Barcelona, Spain), a 1000 mg L^{-1} Scandium Standard Solution for ICP, HNO_3 69% for trace analysis and H_2O_2 35% reagent grade, all from Scharlau were used. Ultra-pure water with a minimum resistivity of 18.2 MΩ cm, obtained using a Milli-Q plus Millipore system (Molsheim, France) was employed to prepare the standard and sample solution.

2.3. Sample collection

A total of 54 samples of different types were purchased in Spain from different markets, restaurants and university canteens, including 35 samples of commercial baby food jars, 6 samples of fast food for children and 13 samples of university canteen menus.

Jar samples were composed by meat with vegetables, fish with vegetables, only vegetables or only fruit. Baby foods were recommended for children aged of 6 and 12 months. Fast foods for children were composed by meat, cheese, chips, bread, ketchup and mustard, dairy products and beverage. University canteen menus were composed by entries, a main dish and dessert and also included a piece of 50–70 g of bread. Detailed composition of all food samples used in this study is indicated in Supplementary material.

In the case of menus, the edible part of the foods was weight and mixed. After that, samples were crushed and homogenised with a domestic Braun mixer (Kronberg, Germany), and finally frozen at −20 °C in a freezer. Afterwards, they were lyophilised in a Cryodos 50 lyophiliser Telstar (Barcelona, Spain) for a minimum of 48 h at a chamber pressure of 50 Pa. The dried complete samples were pulverised and homogenised with a domestic mixer, and the powdered samples were stored in polyethylene bottles until their analysis.

2.4. Reference procedure

Reference data were obtained by ICP-OES after microwave-assisted digestion. For samples digestion, 1 g portions of freeze-dried sample were accurately weight inside Teflon reactors and 8 mL of concentrated nitric acid and 2 mL of H_2O_2 were added. Reactors were closed and placed inside the microwave oven with a digestion step of 15 min at 180 °C (700 W). The resulting solution was quantitatively transferred to a plastic flask and made up to 20 mL with nanopure water and preserved at 4 °C until to be analysed.

Digested samples were measured with a final dilution to 40 mL. Additional, control standards were measured for every series of 10 independent sample measurements. Scandium (0.5 mg L^{-1}) was used as internal standard and added to all samples, reagent blanks and standards. The average concentration of blanks was subtracted from the analytical signals of digested samples after interpolation on the calibration graphs. Results were expressed as a basis of dry sample weight.

To establish precision and accuracy of the reference method, CRMs were analysed under the same conditions than samples: NIST 1568a Rice Flour (National Institute of Standards and Technology, Gaithensburg, MD, USA); TORT-2 Lobster Hepatopancreas (National Research Council, Ottawa, Canada) and IAEA-359 Cabbage (International Atomic Energy Agency, Vienna, Austria). A statistical evaluation based on the regression between average obtained values and certified values provided a regression lines of $y = (0.996 \pm 0.005)x - (7 \pm 20)$ for IAEA-359, $y = (0.998 \pm 0.012)x + (0.12 \pm 0.14)$ for NIST 1568a and $y = (1.007 \pm 0.017)x + (0.096 \pm 1.433)$ for TORT-2, evidencing that the intercept and the slope values correspond, in all cases, statistically to 0 and 1, with regression coefficients (r^2) of 0.9999, 0.998 and 0.998, respectively. Detection limits (LOD) were calculated as three times the standard deviation of 10 reagent blanks, taking into account the analytical sensitivity and sample mass. LODs for calcium, potassium, iron, magnesium, sodium and zinc were 0.25, 0.2, 0.06, 0.01, 0.26 and 0.005 mg kg^{-1} respectively. Reproducibility was calculated as the relative standard deviation of method (RSD) for three independent analyses of three independent portions of the same sample. RSD for all elements was lower than 4%.

2.5. General procedures

2.5.1. MIR measurements

Direct ATR-FTIR measurements were performed by placing approximately 100 mg of dry sample directly onto the diamond ATR crystal and pressed with the corresponding accessory. Spectra were recorded in the range between 4000 and 600 cm^{-1} by accumulating 50 scans per spectrum, at a resolution of 4 cm^{-1}. The background spectrum was acquired from a spectrum of the clean ATR crystal, obtained immediately before acquiring the spectrum of each sample. After measurement, samples were aspirated and the ATR crystal cleaned with cellulose. ATR correction was applied to all spectra.

2.5.2. NIR measurements

Approximately 0.5–1 g of dry samples were placed inside glass vials of 20 mL (22 mm of internal diameter and 44 mm high) to obtain their NIR spectra by diffuse reflectance. The vials were rotated to obtain triplicate measurements for each sample. Diffuse reflectance near spectra (DRIFT) were recorded in Kubelka–Munk units, in the 14,000–4000 cm^{-1} spectral region, using a resolution of 4 cm^{-1} and cumulating 50 scans per spectrum. The background spectrum was acquired from the closed integrating sphere using the same instrumental conditions than those employed for samples.

2.5.3. XRF measurements

Approximately 3 g of dry samples were compacted in a special holder of stainless steel and were pressed to obtain pellets of a diameter of 40 mm using a hydraulic press for 1 min at a pressure of 120 psi. Pellets were covered with a thin window of Mylar® to protect and to avoid hydration from air humidity during manipulation, and spectra were obtained by triplicate using 50 kV voltage, 11 μA current and 60 s as acquisition time (30 s for high atomic number elements, and 30 s for low atomic number elements).

2.6. Chemometric data treatment

Models for the prediction of the mineral elements content from spectral measurements of samples were performed using partial least squares regression (PLS) using Matlab 2013a software from Mathworks (Natick, MA, USA) and the PLS Toolbox 6.2 from Eigenvector Research Inc. (Wenatchee, WA, USA). Kennard–Stone (KS) algorithm was assayed to select the most appropriate sample calibration set. The number of samples in the calibration set was fixed as 40 and the validation set was composed by 14 additional samples, being used the same number of samples in sets for each one of the three considered methodologies (MIR, NIR and XRF). KS is a well-known method for the selection of a representative subset of samples based only on the instrumental signals. The selected subset should cover the experimental region homogeneously by choosing objects which are uniformly distributed in the experimental space.

For building the best PLS models, different spectral regions were tested. Interval PLS (iPLS) was used to develop local PLS models on equidistant subintervals of the whole spectrum in order to provide an overall picture of the relevant information in different spectral subdivisions and thereby narrowing the important spectral variables [20]. Local PLS models were developed on spectral subintervals of equal width, and the prediction performance of these local models and that based on the whole spectrum were compared, based on the use of root mean square error of cross-validation (RMSECV). Other parameters such as squared correlation coefficient (R^2), slope and offset were also consulted to ensure a comprehensive model overview.

Prior to PLS model calculation, several pre-treatments of spectra were tested for normalisation, filtering, scaling and centring. Pretreatment models assayed were multiplicative scatter correction (MSC), standard normal variation (SNV), first derivate (FD), orthogonal signal correction (OSC), smoothing (SMO) and mean centre (MC) also combination of them. The best one was selected for each element on the basis of the lowest root mean square error of calibration (RMSEC) and cross-validation (RMSECV). The optimum number of PLS factors was determined by cross-validation using venetian blinds with (m/5) splits in order to obtain the minimum value of RMSECV [21]. Samples from the validation set were then analysed in the selected conditions. Predictive power of assayed models was evaluated through the use of the root mean square of prediction (RMSEP). PLS models were evaluated for all elements with available reference data but only the most significant information about mineral content of diet samples is presented through this paper.

3. Results and discussion

3.1. MIR spectra of diet samples

Fig. 1 shows the typical ATR-FTIR spectra between 4000 and 600 cm^{-1} for baby foods (B), children fast food (F) and adult menu samples (M). The broad and strong peak from 3600 to 3200 cm^{-1} was due to O–H and N–H stretching vibrations. Peaks at 2925 and 2855 cm^{-1} correspond to CH_2 asymmetric and symmetric stretching vibration of the fatty acids, respectively. The band at 1745 cm^{-1} can be associated with the C = O stretching, in this region dominate protein and peptide bands. The C = C stretching vibration provided showed a peak at 1650 cm^{-1}. The region between 1500 and 1200 cm^{-1} is a mixed region containing vibrational bands of fatty acids, proteins and polysaccharides. The region at 1200–900 cm^{-1} is related to polysaccharides and sucrose. Finally, the absorbance bands obtained under 900 cm^{-1} correspond to the fingerprint [22,23].

3.2. PLS models for determination of minerals by ATR-FTIR

For the selection of the two sets of samples to be employed for ATR-FTIR determination of minerals as calibration and validation ones, Kennard–Stone algorithm was used; remaining samples were considered for validation purpose. For calibration set, 25 baby foods, 5 infant fast food and 10 menus were selected. The mean, concentration range and standard deviation for the concentration of assayed elements in diet samples for both, the calibration and validation, sets employed for ATR-FTIR analysis are shown in Table 1.

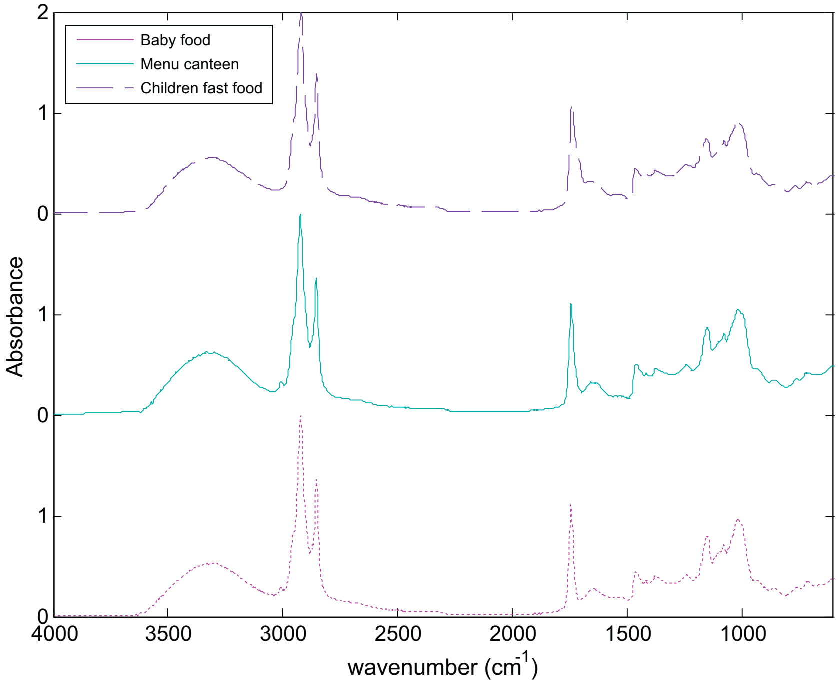

Fig. 1. Mean ATR-FTIR spectra of baby foods, children fast food and adult menus. NOTE: Spectra were shift in the Y axes to improve their presentation.

Table 1
Statistics of PLS- ATR-FTIR models employed for the determination of mineral elements in human diets, also including the reference data of sample sets used for calibration and validation.

Element	Set	Min	Max	Mean	SD	Pre-process	LV	R^2_{cal}	R^2_{CV}	R^2_{val}	RMSEC	RMSECV	RMSEP	RRMSEP
Ca	CAL	288	4000	1426	872	OSC + MC	4	0.97	0.22	0.63	146	1147	263	29
	VAL	365	1635	891	426									
K	CAL	3860	15190	9329	3204	MSC + FD	8	0.86	0.52	0.86	1189	2219	1010	11
	VAL	5290	12000	9069	2489									
Fe	CAL	2.1	33	17	8	SNV + FD	8	0.87	0.46	0.76	2.98	6.48	3.4	19
	VAL	6.9	30	19	7									
Mg	CAL	402	1120	692	192	OSC	6	0.92	0.4	0.57	52	155	102	16
	VAL	461	920	637	134									
Na	CAL	45	14400	7111	3558	OSC + MC	2	0.994	0.73	0.95	266	1887	1606	30
	VAL	179	13200	5293	4115									
Zn	CAL	3.99	47	18	9	SNV	6	0.72	0.41	0.82	4.68	7.22	3.63	19
	VAL	3.73	46	21	11									

NOTE: Minimum (min), maximum (max), mean and standard deviation (SD) in mg kg^{-1} dry weight for calibration (CAL) and validation (VAL) sets. RMSEC, root-mean-square error of calibration in mg kg^{-1}; RMSECV, root-mean-square error of cross-validation in mg kg^{-1}; RMSEP, root-mean-square error of prediction in mg kg^{-1}; RRMSEP, relative root-mean-square error of prediction in percentage (%).

Statistics of the PLS calibration models developed for calcium, potassium, iron, magnesium, sodium and zinc in diet samples using ATR-FTIR and pre-processing models were built and compared in order to evaluate their prediction capability. The parameters chosen for the best model for each mineral element are also reported in Table 1. For every element, all MIR spectra (4000–600 cm^{-1}) were used to build PLS models. Coefficients of determination in calibration (R^2_{cal}) and cross-validation (R^2_{cv}) were fairly good; for calibration R^2 varied between 0.72 and 0.994, and for cross-validation were lower than previous ones (0.22–0.73). Regarding the number of required latent variables (LV) only two were employed for sodium PLS model and the cumulative variance percentage explained was 99.4% in contrast to potassium and iron PLS models which needed eight LV to explain 86 and 87% of cumulative variance, respectively (see Table 1). The highest coefficients of determination in cross-validation (R^2_{cv}) and the lowest errors of cross-validation (RSECV) were obtained for potassium (0.52 and 2219 mg k^{-1}), magnesium (0.4 and 155 mg k^{-1}) and sodium (0.73 and 1887 mg k^{-1}).

3.3. Prediction capability of PLS-ATR-FTIR for mineral elements determination in diets

Cross-validation does not inform about the capability of the developed models to be used for the prediction of unknown samples, different than those included in the calibration. Because of that, the prediction capability of PLS-ATR-FTIR methods has been evaluated by using an independent external validation set of diet samples, not employed during the calibration stage. Coefficients of determination in validation (R^2_{val}) were quite good; being between 0.57 for Mg and 0.95 for Na (see Table 1). The highest coefficients of determination in validation (R^2_{val}) and the lowest relative root-mean-square error of prediction (RRMSEP) were obtained for potassium (0.86 and 11%), iron (0.76 and 19%) and zinc (0.82 and 19%). The minimum RRMSEP was obtained for potassium.

The prediction capability of the PLS-ATR-FTIR method was good for almost all considered elements, RRMSEP being lower than 20% for potassium, magnesium, iron and zinc. However, for calcium and sodium this error was higher than 25%, and Fig. 2 shows the regression between predicted values for potassium concentration and those obtained by reference method.

3.4. NIR spectra of diet samples

Fig. 3 shows the typical near DRIFT spectra obtained between 14000 and 4000 cm^{-1} for baby foods (B), children fast food (F) and adult menu samples (M).

The broad and strong peak from 8300 cm^{-1} was due to second overtone C–H stretching vibrations. At 7000–6400 cm^{-1} it can be seen a broad peak which corresponds to the first overtone of N–H and

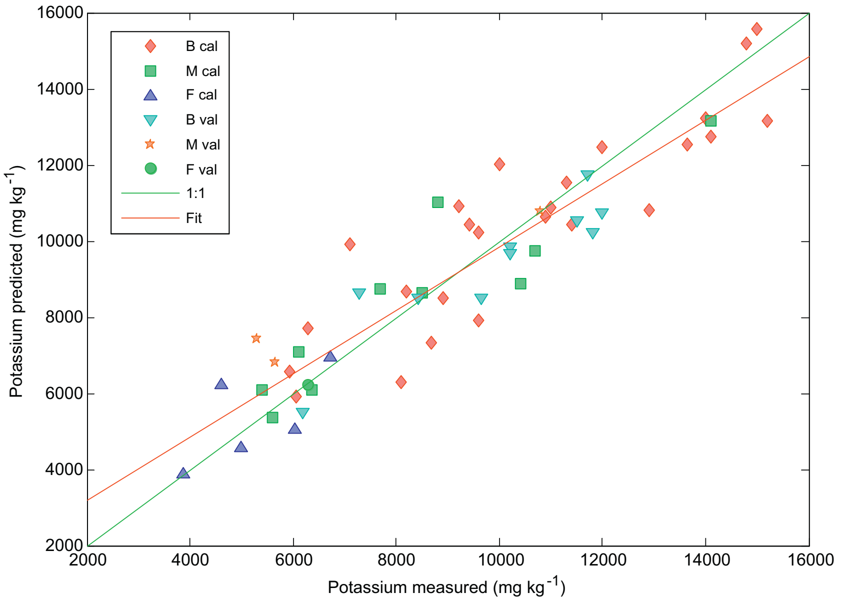

Fig. 2. Predicted vs. measured potassium values for the PLS-ATR-FTIR model employed for the analysis of baby foods and diet samples.

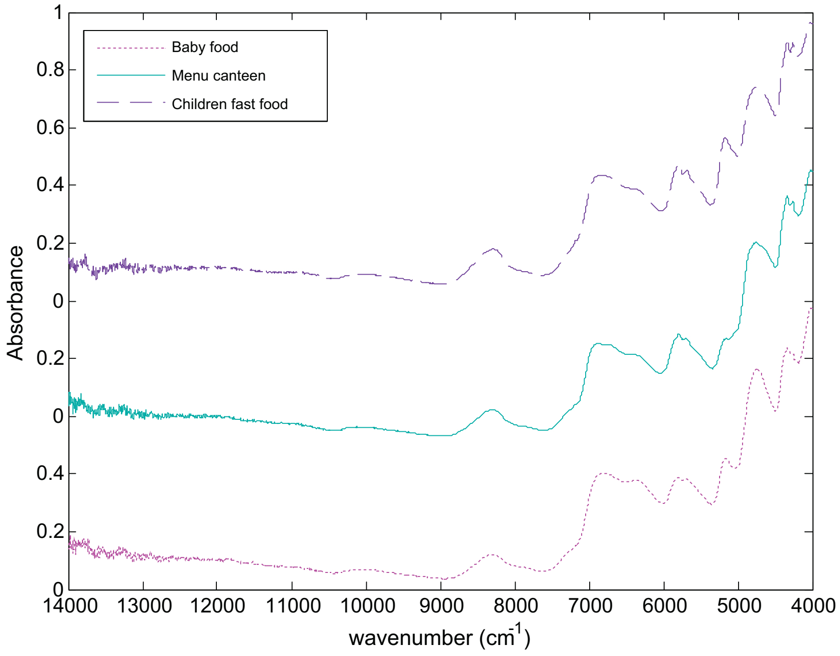

Fig. 3. Mean DRIFT spectra of baby foods, children fast food and adult menus. NOTE: Spectra were shift in the Y axes for clarity.

the O–H stretching of water. Peaks at 5700 and 5800 cm^{-1} correspond to first overtone C–H stretching. O–H first and second overtone can be seen at 5155 cm^{-1}. A strong peak at 4732 cm^{-1} was due to N–H and O–H stretching. Double peak at 4330 and 4260 cm^{-1} was due to combination of C–H stretching [14,23].

3.5. PLS models for the determination of minerals by NIR

For the selection of the two sets of samples, to be used for calibration and validation, the same methodology than that employed for MIR was used. For calibration, 24 baby foods, 5 infant fast foods and 11 adult menus were selected; remaining samples constitute the validation set. The mean, concentration range and standard deviation for the elements measured in diet samples in both sets employed for NIR analysis are shown in Table 2.

Several spectral regions and different pre-processing strategies were assayed to build the best models and those compared in order to evaluate their prediction capability. Statistics for the PLS-NIR determination of calcium, iron, magnesium and zinc, using the NIR wavenumber region (14000–4000 cm^{-1}) are shown in Table 2. For potassium and sodium the best spectral range used was 9000–4000 cm^{-1}. The most common pre-treatment used was OSC with mean centring.

The highest coefficients of determination in cross-validation (R^2_{cv}) and the lowest RMSECV were obtained for potassium (0.7 and 18%) and magnesium (0.71 and 15%). Coefficients of determination in calibration varied between 0.9 and 0.997, and coefficients of cross-validation were lower than previous ones. Regarding the number of LV selected, with only three LVs for calcium PLS model the cumulative variance percentage explained was 99.7% but RMSECV was high, being the same for iron. For zinc and sodium PLS models needed eight or nine LV to explain 99 and 90% of cumulative variance, respectively (see Table 2).

3.6. Prediction capability of PLS-NIR for mineral elements determination in diets

External validation was used to evaluate the PLS-NIR methodology, using an independent set of diet samples, not employed during the calibration step. Coefficients of determination in validation were good and varied between 0.41 for Mg and 0.9 for K (see Table 2). The minimum RRMSEP obtained was for potassium, only 11%, the same as that obtained by MIR procedure (see Fig. 4). However, the prediction capability of PLS-NIR was lower than that of PLS-ATR-FTIR and only for potassium and magnesium RRMSEP was lower than 20%; for calcium, iron, sodium

Table 2
Statistics of PLS-NIR models employed for the determination of mineral elements in human diets, also including the reference data of sample sets used for calibration and validation.

Element	Set	Min	Max	Mean	SD	Pre-process	LV	R^2_{cal}	R^2_{CV}	R^2_{val}	RMSEC	RMSECV	RMSEP	RRMSEP
Ca	CAL	288	4000	1275	790	OSC + MC	3	0.997	0.22	0.42	44	797	668	51
	VAL	351	3400	1324	905									
K	CAL	4600	15190	9293	3085	OSC + MC	8	0.9	0.7	0.9	961	1711	997	11
	VAL	3860	14000	9172	2910									
Fe	CAL	2.1	33	18	8	OSC	7	0.991	0.355	0.41	0.76	6.75	6.39	37
	VAL	6.9	29.4	16	8									
Mg	CAL	402	1120	675	190	MSC + MC	9	0.988	0.689	0.405	19.8	104	119	19
	VAL	477	920	685	150									
Na	CAL	45	14400	6874	3774	OSC + MC	9	0.9	0.64	0.8	1173	2413	1711	30
	VAL	485	13200	5967	3762									
Zn	CAL	3.7	47	20	10	OSC + MC	8	0.985	0.62	0.69	1.22	6.29	4.41	25
	VAL	4.23	28.69	17	7									

NOTE: Minimum (min), maximum (max), mean and standard deviation (SD) in mg kg^{-1} dry weight for calibration (CAL) and validation (VAL) sets. RMSEC, root-mean-square error of calibration in mg kg^{-1}; RMSECV, root-mean-square error of cross-validation in mg kg^{-1}; RMSEP, root-mean-square error of prediction in mg kg^{-1}; RRMSEP, relative root-mean-square error of prediction in percentage (%).

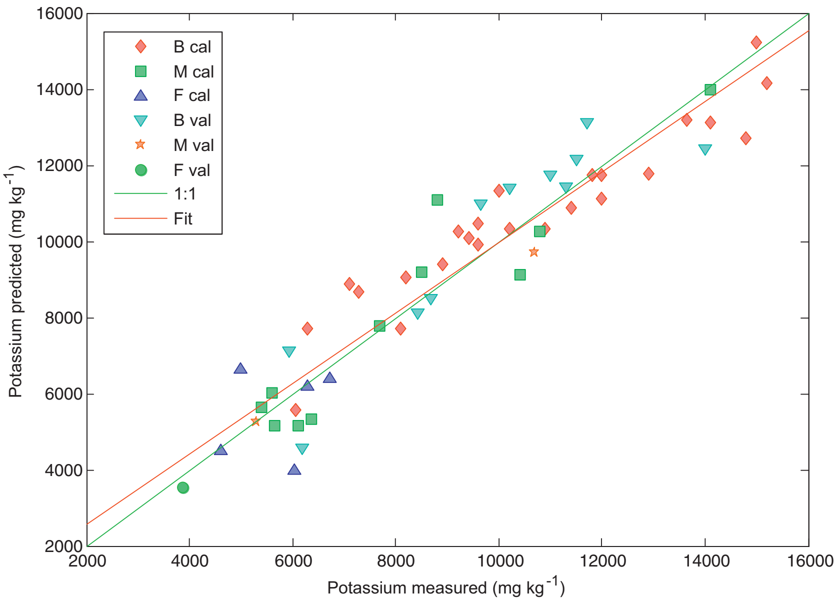

Fig. 4. Predicted vs. measured potassium values for PLS-NIR model employed for the analysis of baby foods and diet samples.

and zinc this value was higher than 20%. These results demonstrate the good predictive capability of the PLS-NIR models developed for potassium and magnesium determination, while a poor prediction was found for the other considered elements in diet samples.

3.7. XRF spectra of diet samples

Fig. 5 shows the typical EDXRF spectra between 0 and 30 keV for baby foods (B), children fast food (F) and adult menu (M) samples. All elements studied in this paper provided bands at energies lower than 10 keV, except sodium, which cannot be determined using this portable instrument. The Kα lines of magnesium, potassium, calcium and zinc are at 1.25, 3.31, 3.69 and 8.64 keV, respectively. Peaks higher than 15 keV correspond to the radiation due to the XR tube.

3.8. PLS models for the determination of mineral elements in diets by XRF

For the selection of the two sample sets used for calibration and validation, the Kennard–Stone method was used. For calibration set, 22 baby foods, 5 infant fast food and 13 adult menus were selected, and remaining 14 samples were used for validation. The mean, concentration range and standard deviation for the elements determined in diet samples in both, calibration and validation, sets employed for XRF are shown in Table 3.

Several spectral regions and different pre-processing strategies were assayed to build the models and compared in order to evaluate their prediction capability. iPLS was used to select the spectral range for each considered element. Spectral regions selected included in all cases the Kα energy corresponding to each element. In general XRF

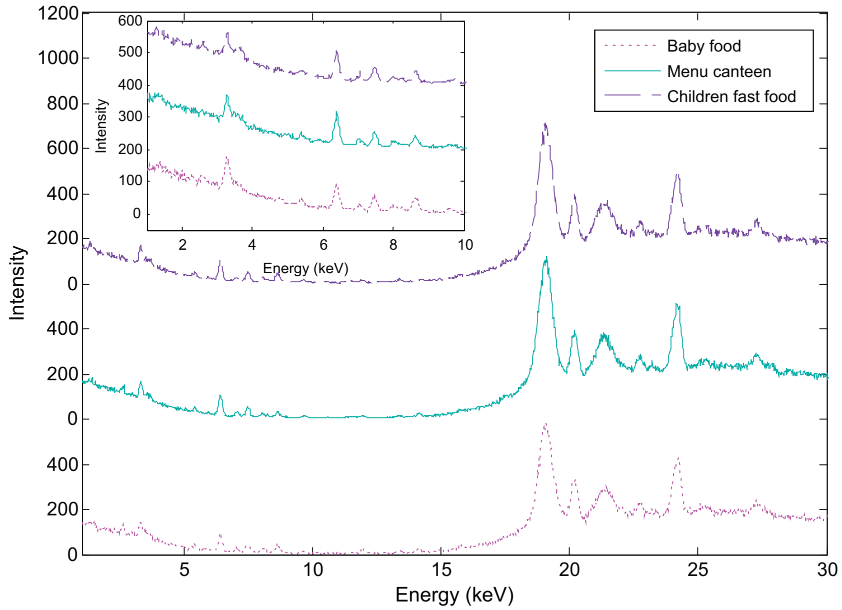

Fig. 5. XRF spectra of baby foods, children fast food and adult menus. Inset of the region between 1 and 10 keV. NOTE: Spectra shift in the Y axes for clarity.

Table 3
Statistics of PLS-XRF models employed for the determination of mineral elements in human diets, also including the reference data of sample sets used for calibration and validation.

Element	Set	Min	Max	Mean	SD	Pre-process	LV	R^2_{cal}	R^2_{CV}	R^2_{val}	RMSEC	RMSECV	RMSEP	RRMSEP
Ca	CAL	288	4000	1438	887	SMO + MSC	4	0.77	0.62	0.86	417	535	226	19
	VAL	351	2600	1268	561									
K	CAL	3860	15190	8866	3225	OSC + FD	3	0.94	0.77	0.92	795	1530	1037	10
	VAL	4600	15000	9911	2983									
Fe	CAL	2.1	33	20	7	SMO + OSC	6	0.94	0.22	0.6	1.5	7.3	6.1	34
	VAL	4.5	31.4	18	9									
Mg	CAL	402	1120	680	180	SMO + OSC	2	0.999	0.76	0.63	2	89	130	18
	VAL	460	1110	709	208									
Zn	CAL	8.8	46	20	8	SMO + MSC	5	0.97	0.9	0.84	1.3	2.4	4.2	3
	VAL	10.53	47	22	10									

NOTE: Minimum (min), maximum (max), mean and standard deviation (SD) in mg kg^{-1} dry weight for calibration (CAL) and validation (VAL) sets. RMSEC, root-mean-square error of calibration in mg kg^{-1}; RMSECV, root-mean-square error of cross-validation in mg kg^{-1}; RMSEP, root-mean-square error of prediction in mg kg^{-1}; RRMSEP, relative root-mean-square error of prediction in percentage (%).

spectra were smoothed, being also employed OSC or MSC as pre-processing of signals (see Table 3).

Coefficients of determination in calibration varied between 0.7 and 0.999, and coefficients of cross-validation between 0.22 and 0.9. Regarding the number of LV, few factors, two for magnesium, three for potassium, four for calcium, five for zinc and six for iron, were necessary to obtain good models, with cumulative variance percentage explained around 77–99%. EDXRF provided RMSECV values lower than those found by IR spectroscopy, except for iron (see Tables 1–3). The highest R^2_{CV} and the lowest RSECV were obtained for zinc being 0.9 and 2.4 mg k^{-1}, which evidenced an excellent coherence of the developed model.

3.9. Prediction capability of PLS-XRF for the determination of mineral elements in diets

An external sample set was used for the validation of the PLS-XRF developed methodology, using an independent set of samples, not employed during the calibration step. Coefficients of determination in validation were quite good, between 0.6 for Fe and 0.92 for K (see Table 3). The minimum RRMSEP obtained was 3% for zinc, better than that found for this element by MIR and NIR (see Fig. 6). The prediction capability of PLS-XRF was higher than PLS-ATR-FTIR and PLS-NIR; for every element considered RRMSEP was lower than 20% in all cases except for iron. These results demonstrate a good predictive capability of the PLS-XRF models developed to predict the concentration of calcium, potassium, magnesium and zinc in different diets. PLS-XRF models for sodium were not created because the EDXRF portable instrument only measured elements with atomic number higher than 12.

3.10. Comparison of MIR, NIR and XRF for mineral analysis of diet samples

PLS models were established to determine mineral elements in human diets using MIR, NIR and XRF spectra. Tables 1–3 show prediction results for calcium, potassium, iron, magnesium, sodium and zinc in human diets based on MIR, NIR and XRF spectra. In general, XRF provided much better results than MIR and NIR spectra with lower LVs, and potassium was the best element prediction except in the case of sodium determination for which it must be used IR spectroscopy.

For calcium determination PLS-XRF ($R^2_{\mathrm{val}} = 0.86$, RMSEP = 226 mg kg^{-1}) model provided better results than PLS-ATR-MIR ($R^2_{\mathrm{val}} = 0.63$, RMSEP = 263 mg kg^{-1}) and PLS-NIR ($R^2_{\mathrm{val}} = 0.42$, RMSEP = 668 mg kg^{-1}). For potassium results found by the three techniques were similar ($R^2_{\mathrm{val}} = 0.92$, RMSEP = 1037 mg kg^{-1} for PLS-XRF, $R^2_{\mathrm{val}} = 0.86$, RMSEP = 1010 mg kg^{-1} for ATR-MIR-PLS and $R^2_{\mathrm{val}} = 0.9$, RMSEP = 997 mg kg^{-1} for NIR-PLS). However, PLS-XRF model was built with three LVs, while MIR and NIR models needed eight LVs. For prediction of iron content PLS-ATR-MIR was the best

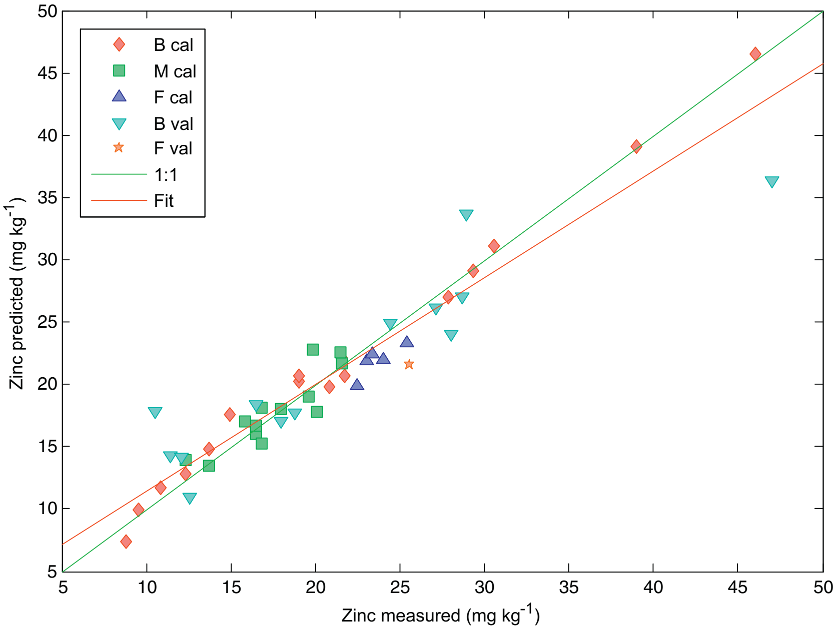

Fig. 6. Predicted vs. measured zinc values for PLS-XRF model employed in baby foods and diet sample analysis.

alternative ($R^2_{\mathrm{val}} = 0.76$, RMSEP = 3.4 mg kg^{-1}) with eight LVs. This element is very important for infants, because after 4 months of life the iron requirements increase due to the rapid growth of baby and iron reserves diminished till pathological levels if the needs are not covered with dietary iron [24]. For determination of magnesium, PLS-XRF only with two LVs provided $R^2_{\mathrm{val}} = 0.63$ and RMSEP = 130 mg kg^{-1}, thus, better results than MIR or NIR models. For sodium prediction, PLS-XRF model was not able but PLS-ATR-MIR and PLS-NIR models were very similar. However, for MIR two LVs were used, while in NIR nine LVs were used. For zinc determination, the best method was PLS-XRF which provided very good values ($R^2_{\mathrm{val}} = 0.84$ and RMSEP = 4.2 mg kg^{-1}).

4. Conclusions

MIR, NIR spectroscopy and X-ray fluorescence techniques provided fast and green alternatives to determine calcium, potassium, iron, magnesium and zinc in human diets, being XRF the best choice in the main part of cases. Potassium and zinc content were easily predicted with all the techniques, obtaining good results. For iron, MIR was the best technique to do it and for calcium XRF was the best. Results found through this study indicate that XRF, MIR and NIR spectroscopy, combined with chemometrics, could be applied as a rapid and green method for the determination of the main minerals in human diets. For sodium, XRF cannot be used and for magnesium content prediction using XRF, NIR and MIR must be further investigated. In short, these studies evidenced the strengths and weaknesses of direct spectroscopy methods for the determination of the major minerals presents in diets and offer fast procedures for the valuation of these samples.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.microc.2014.06.028.

Acknowledgements

The authors acknowledge the financial support of the Ministerio de Economia y Competitividad-Feder (projects CTQ2011-25743 and CTQ2012-38635). Alba Mir-Marqués acknowledges a grant ‘Atracció de Talent’ provided by the University of Valencia.

References

[1] FAO/WHO, Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation, 2nd edition, 2004.
[2] Food and Nutrition Board, Dietary Reference Intake: the essential guide to nutrient requirements, National Academies Press, 2006.
[3] WHO, Trace-elements in human nutrition and health, 1996.
[4] A.A. Carbonell-Barrachina, A. Ramirez-Gandolfo, X. Wu, G.J. Norton, F. Burlo, C. Deacon, A.A. Meharg, Essential and toxic elements in infant foods from Spain, UK, China and USA, J. Environ. Monit. 14 (2012) 2447–2455.
[5] R. Melo, K. Gellein, L. Evje, T. Syversen, Minerals and trace elements in commercial infant food, Food Chem. Toxicol. 46 (2008) 3339–3342.
[6] A. Mir-Marques, M.L. Cervera, M. de la Guardia, A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus, J. Food Compos. Anal. 27 (2012) 160–168.
[7] L. Noël, J.-C. Leblanc, T. Guérin, Determination of several elements in duplicate meals from catering establishments using closed vessel microwave digestion with inductively coupled plasma mass spectrometry detection: estimation of daily dietary intake, Food Addit. Contam. 20 (2003) 44–56.
[8] N. Zand, B.Z. Chowdhry, F.B. Zotor, D.S. Wray, P. Amuna, F.S. Pullen, Essential and trace elements content of commercial infant foods in the UK, Food Chem. 128 (2011) 123–128.
[9] M. de la Guardia, S. Garrigues (Eds.), Handbook of green analytical chemistry, John Wiley & Sons, Chichester, 2012.
[10] M. de la Guardia, S. Garrigues (Eds.), Challenges in green analytical chemistry, RSC Publishing, Cambridge, 2011.
[11] P.T. Palmer, R. Jacobs, P.E. Baker, K. Ferguson, S. Webber, Use of fiel-portable XRF analyzers for rapid screening of toxic elements in FDA-regulated products, J. Agric. Food Chem. 57 (2009) 2605–2613.
[12] M. de la Guardia, S. Garrigues (Eds.), Handbook of mineral elements in food, John Wiley & Sons, Chichester, 2014, (in press).
[13] S. Schmitt, S. Garrigues, M. de la Guardia, Determination of the mineral composition of foods by infrared spectroscopy: a review of a green alternative, Crit. Rev. Anal. Chem. 44 (2014) 186–197.
[14] C. Collell, P. Gou, P. Picouet, J. Arnau, J. Comaposada, Feasibility of near-infrared spectroscopy to predict aw and moisture and NaCl contents of fermented pork sausages, Meat Sci. 85 (2010) 325–330.
[15] D. Cozzolino, A. Moron, Exploring the use of near infrared reflectance spectroscopy (NIRS) to predict trace minerals in legumes, Anim. Feed Sci. Technol. 111 (2004) 161–173.
[16] D. Cozzolino, W. Cynkar, N. Shah, P. Smith, Quantitative analysis of minerals and electric conductivity of red grape homogenates by near infrared reflectance spectroscopy, Comput. Electron. Agric. 77 (2011) 81–85.
[17] I. Gonzalez-Martin, C. Gonzalez-Perez, J. Hernandez-Mendez, N. Alvarez-Garcia, Mineral analysis (Fe, Zn, Ca, Na, K) of fresh Iberian pork loin by near infrared reflectance spectrometry - Determination of Fe, Na and K with a remote fibre-optic reflectance probe, Anal. Chim. Acta. 468 (2002) 293–301.
[18] A. Lucas, D. Andueza, E. Rock, B. Martin, Prediction of dry matter, fat, pH, vitamins, minerals, carotenoids, total antioxidant capacity, and color in fresh and freeze-dried cheeses by visible-near-infrared reflectance spectroscopy, J. Agric. Food Chem. 56 (2008) 6801–6808.
[19] J. Moros, I. Llorca, M.L. Cervera, A. Pastor, S. Garrigues, M. de la Guardia, Chemometric determination of arsenic and lead in untreated powdered red paprika by diffuse reflectance near-infrared spectroscopy, Anal. Chim. Acta. 613 (2008) 196–206.
[20] L. Nørgaard, A. Saudland, J. Wagner, J.P. Nielsen, L. Munck, S.B. Engelsen, Interval partial least squares regression (iPLS): a comparative chemometric study with an example from near-infrared spectroscopy, Appl. Spectrosc. 54 (2000) 413–419.
[21] D.A. Burns, E.W. Ciurczak, Handbook of Near-Infrared Analysis, Marcel Dekker, New York, 1992.
[22] J. Moros, F.A. Iñón, M. Khanmohammadi, S. Garrigues, M. de la Guardia, Evaluation of the application of attenuated total reflectance–Fourier transform infrared spectrometry (ATR–FTIR) and chemometrics to the determination of nutritional parameters of yogurt samples, Anal. Bioanal. Chem. 385 (2006) 708–715.
[23] D. Wu, Y. He, J. Shi, S. Feng, Exploring near and midinfrared spectroscopy to predict trace iron and zinc contents in powdered milk, J. Agric. Food Chem. 57 (2009) 1697–1704.
[24] A. Gil Hernández, R. Uauy Dagach, J. Dalmau Serra, Bases para una alimentación complementaria adecuada de los lactantes y los niños de corta edad, An. Pediatr. 65 (2006) 481–495.

Food Chemistry 172 (2015) 291–297

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Mineral profile of kaki fruits (*Diospyros kaki* L.)

Alba Mir-Marqués, Ana Domingo, M. Luisa Cervera *, Miguel de la Guardia

Department of Analytical Chemistry, University of Valencia, Jerónimo Muñoz Building, 50th Dr. Moliner St., 46100 Burjassot (Valencia), Spain

ARTICLE INFO

Article history:
Received 19 April 2013
Received in revised form 8 September 2014
Accepted 13 September 2014
Available online 20 September 2014

Keywords:
Kaki fruit
Diospyros kaki L.
Mineral profile
Inductively coupled plasma optical emission spectroscopy (ICP-OES)
Inductively coupled plasma mass spectroscopy (ICP-MS)
PDO fruit

ABSTRACT

The main objective of this study was the determination of the mineral profile of 167 kaki fruit (*Diospyros kaki* L.) samples produced from different regions of Spain, including samples with the protected designation of origin (PDO) 'Kaki Ribera del Xúquer' Valencia (Spain). Samples were analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Consumption of one piece of kaki fruit (200–400 g) would give a mineral intake providing 1–10% of the recommended daily allowance (RDA) for calcium, 1–30% for copper and potassium, 1–15% from iron and magnesium, up to 1% of sodium, and up to 4% of zinc. ANOVA analysis indicates differences between samples from different Spanish region, thus offering a way for authentication of PDO sample origin.

1. Introduction

Persimmon (*Diospyros kaki* L.) is a fruit native to China, but cultivated in warm regions all around the world. Rojo Brillante is an astringent persimmon and this cultivar originates in la Ribera del Xúquer (Valencia) region of Spain. It has the protected designation of origin (PDO) 'Kaki Ribera del Xúquer', which includes more than 95% of persimmon grown in the region, production being around 25 million kg/year (CRDO, 2011; Orihuel-Iranzo, Miranda, Zacarías, & Lafuente, 2010; Plaza, Colina, de Ancos, Sanche-Moreno, & Cano, 2012).

Much of the kaki farmland is located in the low plain of the rivers Xúquer and Magro, and has compact and rich soil. Moreover, in the lowlands of the valley, there are soils with the capacity to sustain intensive exploitation. The region enjoys a mild climate favourable for the cultivation of persimmon with an average temperature of 17 °C, low cloud cover, and average annual rainfall of 400–500 mm. Furthermore, the surrounding mountains protect crops against frost especially in the valleys. These characteristics determine the quality and character of the 'Kaki Ribera del Xúquer' fruits. The shape of the kaki fruit depends not only the variety, but also the climate. The ratio height to diameter is larger and more pointed in hot–dry climates, which provides a point of differentiation from fruits not protected by PDO status (see Fig. 1) (CRDO, 2011).

The cultivation of persimmon is very important in the 'Ribera del Xúquer' for economic and traditional reasons. Rojo Brillante is highly sought after because of its excellent nutritional and sensorial qualities. It is important to know the chemical composition of this fruit, particularly the content of minerals and trace elements, since this reflects the soil type and the growing environment, and may offer a route for authenticating fruit produced inside the PDO. In addition, the mineral composition of foods has a vital role in human health and adequate intakes of many elements are key to a healthy diet.

Essential elements are those needed in the human diet to maintain normal physiological functions. Thus, risk assessment in terms of dietary intake concerns toxic substances, which must be absent, and essential elements, high intakes of which could result in toxic effects and low intakes in nutritional inadequacy (Goldhaber, 2003). It is important a thorough analysis of mineral profiles in foods, including essential and toxic elements, is carried out in order to know the contribution of foods to the recommended daily amount (RDA). The RDA is the 'amount of a nutrient a healthy person should eat each day on average through diet to maintain a good health' (Food & Nutrition Board, 2004). To achieve this, analytical techniques with sufficient sensitivity are required.

Different techniques, such as flame atomic absorption spectroscopy (FAAS) or graphite furnace atomic absorption spectroscopy (GFAAS) (Gorinstein et al., 2001; Radwan & Salama, 2006), have been employed for the determination of mineral elements in fruits. However, inductively coupled plasma optical emission

---

* Corresponding author. Tel.: +34 96 354 3486; fax: +34 96 354 4845.
E-mail address: m.luisa.cervera@uv.es (M.L. Cervera).

http://dx.doi.org/10.1016/j.foodchem.2014.09.076
0308-8146/

spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are useful techniques for complete mineral profile determination of foods (Moreno-Rojas, Sanchez-Segarra, Camara-Martos, & Amaro-Lopez, 2010; Terrab, Hernanz, & Heredia, 2004; Wall, 2006; Zeiner, Steffan, & Cindric, 2005) and have been used as a basis for authentication of PDO products (Ariyama et al., 2007; Boeting, Aguilera de Benzo, Luisa Cervera, & de la Guardia, 2010; Brescia, Monfreda, Buccolieri, & Carrino, 2005; Gonzalvez, Llorens, Cervera, Armenta, & de la Guardia, 2009; Matos-Reyes, Simonot, Lopez-Salazar, Cervera, & de la Guardia, 2013). These techniques provide a wide linear dynamic range and permit the determination of several elements in a same sample.

The main purpose of this study was to determine the mineral profile of persimmon from different areas in Spain. Data were used to categorise (adequate, insufficient or toxic) daily intake of minerals following regular consumption of kaki fruit, and characterisation of samples produced under de PDO 'Kaki Ribera del Xúquer'.

2. Materials and methods

2.1. Apparatus

An ICP-OES Optima 5300 DV inductively coupled plasma optical emission spectrometer Perkin Elmer (Norwalk, CT, USA) equipped with an auto sampler AS 93-plus, and a ultrasonic nebuliser U6000AT$^+$ Cetac (Nebraska, EEUU) and ICP-MS Perkin-Elmer SCIEX Elan 6100 DRCII apparatus, equipped with a Meinhard nebuliser and an autosampler Perkin-Elmer AS-91, were used for mineral determination.

The apparatus employed for sample pre-treatment was a microwave laboratory system, Ethos SEL from Milestone (Sorisole, Italy), equipped with a thermocouple for automatic temperature control, an automatic gas leaks detector and high pressure Teflon vessels of 100 mL inner volume. For cleaning the vessels, an automatic cleaning device, traceCLEAN from Milestone (Sorisole, Italy), was used.

A LyoAlfa Plus 10–55 lyophiliser Telstar (Barcelona, Spain) employed for sample drying and preservation, and an ultrasound water bath from Selecta (Barcelona, Spain, 9 L, 50 W, 50 Hz) was used for sample sonication.

2.2. Reagents

Stock solutions of a multi-element standard solution (100 mg L^{-1}) containing 26 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Se, Na, Sr, Tl, Ti, V and Zn) dissolved in 5% nitric acid, a multi-element solution containing 20 mg L^{-1} Dy, Er, Eu, Gd, Ho, Lu, Sm, Sc, Tb, Tm, Yb and Y and 100 mg L^{-1} Ce, La, Nd and Pr dissolved in 5% nitric acid, a 1 g L^{-1} potassium standard solution for atomic absorption in 0.5 mol L^{-1} nitric acid, a 1 g L^{-1} calcium standard solution for atomic absorption in 0.5 mol L^{-1} nitric acid, a 1 g L^{-1} magnesium standard solution for atomic absorption in 0.5 mol L^{-1} nitric acid all from Scharlau (Barcelona, Spain), were employed to prepare the calibration standards.

Nitric acid (69% for trace analysis) and hydrogen peroxide (35% reagent grade), both from Scharlau, and ultra-pure water with a minimum resistivity of 18.2 MΩ cm, obtained using a Milli-Q plus Millipore system (Molsheim, France), were used for sample digestion and dilution.

A 1 g L^{-1} ruthenium standard solution for ICP in hydrochloric acid 5%, and a 1 g L^{-1} rhodium standard solution for ICP in hydrochloric acid 5%, both from Scharlau (Barcelona, Spain) were used as internal standards. Argon C-45 (purity higher than 99.995%), supplied by Carburos Metálicos (Barcelona, Spain), was employed as plasmogen and carrier gas.

2.3. Sampling, sample storage and pre-treatment

167 kaki fruit samples were collected from different agricultural cooperatives and producers in Spain during 2010 and 2011. Samples (113) were produced under the PDO 'Kaki Ribera del Xúquer', four samples were from Alicante, 11 from Andalucía, 10 from Castellón, three from Extremadura, 13 from the Ribera area (outside the PDO), and 13 from Valencia. Regions where kaki fruit samples were obtained are shown in Fig. 1. The edible parts of the fruit were cut into pieces and frozen at −20 °C for a minimum of 24 h. Subsequently, they were lyophilised for a minimum of 48 h under 50 Pa. Dried samples were pulverised with a domestic Braun mixer (Kronberg, Germany), and the powdered samples stored in polyethylene bottles until analysis. In order to have a fixed reference for mineral elements concentration, the water content of samples was determined, and found to be 81 ± 2% for all the samples analysed.

2.4. Sample digestion

The microwave-assisted treatment was adapted from that employed by us for the determination of the mineral profile of diets (Mir-Marqués, Cervera, & de la Guardia, 2012) according to the microwave laboratory system manufacturer recommendations and our own experience.

A mass of 0.5 g freeze-dried sample was weighed accurately in the Teflon digestion vessel and 8 mL of nitric acid (69%) added. Samples were allowed to pre-digest in an ultrasound water bath for 30 min at room temperature. Then, 2 mL of hydrogen peroxide (35%) were added and the mixture was sonicated for further 45 min. After that, the Teflon reactors were closed and placed inside the microwave oven. The following program was run: step (1) 3 min to reach 85 °C (500 W), step (2) 12 min to reach 145 °C (500 W), step (3) 10 min to reach 180 °C (700 W), step (4) 15 min at 180 °C (700 W), and step (5) cooling down. After cooling to ambient temperature, the reactors were opened and sonicated to eliminate the nitrous vapours. The resulting solution was quantitatively transferred to a plastic flask and made up to 20 mL with ultra-pure water. Teflon digestion vessels were previously cleaned in nitric acid solution to avoid cross-contamination. All samples were analysed in triplicate to eliminate any specific error, and to verify the homogeneity of samples and to evaluate the repeatability of the procedure. Blanks were prepared in the same way, but the sample omitted, for each batch of samples, and the certified reference material was also digested using the same method.

2.5. ICP-OES determination

The operating conditions of the ICP-OES equipment were as follow 15 L min^{-1} of argon plasma gas flow rate, 0.2 L min^{-1} of auxiliary gas flow rate, 0.8 L min^{-1} nebuliser gas flow rate, 1300 W of radio frequency (RF) power, 1.10 mL min^{-1} of sample flow rate. All elements were detected in axial mode except calcium, magnesium, lithium, potassium, sodium and strontium, which were detected in radial mode. For the signal integration four points per peak were used while for the background correction two points were employed. All analytes were measured in two different emission lines. In order to check for matrix effects on the sensitivity and selectivity, a scanning of the emission lines was obtained for a standard, a digested sample and a digested spiked sample. The emission line was selected, taking into account non-spectral interferences and the best signal to background ratio for all elements, and background corrected manually for all emission lines selected (see Table 1).

The calibration standards were prepared in 0.5% nitric acid. The calibration range for all elements was evaluated from 0.05 to

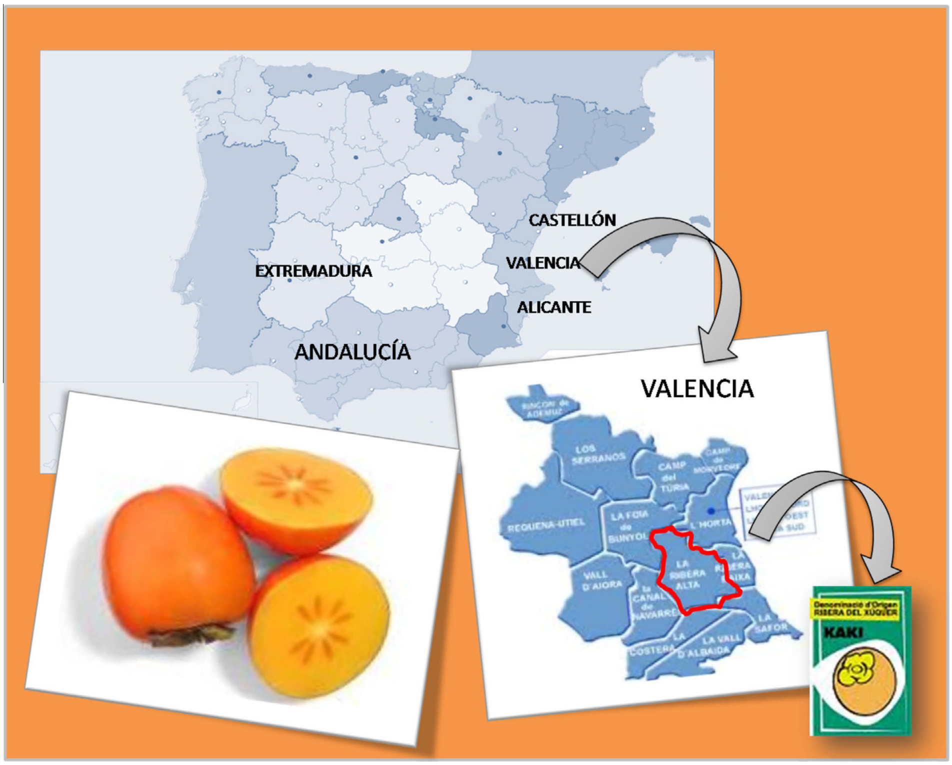

Fig. 1. Map of Spain indicating the different regions where the kaki fruit samples employed through this study were produced, the area covered by PDO 'Kaki Ribera del Xúquer' from Valencia and the PDO label.

Table 1
Analytical figures of merit of ICP-OES and ICP-MS determination of the mineral composition of kaki fruit samples.

Elements	ICP-OES			ICP-MS		
	Wavelength (nm)	LODm (mg kg^{-1})	LOQm (mg kg^{-1})	m/z	LODm (μg kg^{-1})	LOQm (μg kg^{-1})
Al	396.153	0.12	0.4	–	–	–
As	188.979	0.3	0.8	–	–	–
Ba	233.527	0.07	0.2	138	3	9
Be	313.042	0.05	0.16	–	–	–
Bi	223.061	0.3	0.9	209	0.5	2
Ca	317.933	3	9	–	–	–
Cd	228.802	0.011	0.04	114	2	7
Co	228.616	0.016	0.05	59	1.5	5
Cr	267.716	0.07	0.2	52	71	237
Cu	327.393	0.05	0.17	65	25	82
Fe	238.204	0.3	0.9	–	–	–
K	766.490	2	7	–	–	–
Li	670.784	0.005	0.017	7	8	26
Mg	285.213	0.09	0.3	–	–	–
Mn	257.610	0.011	0.04	55	34	113
Mo	202.031	0.04	0.13	95	10	34
Na	589.592	1.2	4	–	–	–
Ni	231.604	0.10	0.3	60	58	194
Pb	220.353	0.05	0.15	206	7	22
Se	196.026	0.4	0.3	–	–	–
Sr	407.771	0.02	0.07	88	5	16
Ti	334.940	0.04	0.13	47	66	219
Tl	190.801	0.09	0.3	205	0.2	0.8
V	290.880	0.05	0.16	51	3	9
Zn	206.200	0.14	0.5	66	93	310

Note: LOD and LOQ data are referred to dry sample taking into account the sample mass 0.5 g and dilution carried out for the measurement.

2 mg L^{-1} except calcium and magnesium for which calibration curves were prepared from 2 to 10 mg L^{-1} and potassium for which the calibration was from 20 to 100 mg L^{-1}. Ruthenium (1 mg L^{-1}) was used as internal standard and added to all samples, reagent blanks and standards. Digested samples were diluted 1:2, 1:5, 1:10 and 1:100, 1:10 and, additionally, undiluted samples were analysed by using the standard addition method in order to evaluate the presence of matrix effect and its correction. From the results, a final dilution of 1:2 was selected. The average value for blank samples was subtracted from the analytical signals of

digested samples after interpolation on the corresponding calibration graphs.

2.6. ICP-MS determination

For the analysis by ICP-MS, a solution (prepared daily) containing 10 μg L^{-1} of barium and 1 μg L^{-1} of magnesium, aluminium, chromium, manganese, copper, rhodium, cadmium, indium, cerium, lead, and thorium was used to optimise the operating conditions. A radio frequency (RF) power of 1125 W, argon plasma gas of 15 L min^{-1}, auxiliary gas of 1.2 L min^{-1}, nebuliser gas flow rate of 0.95 L min^{-1}, and lens voltage of 7 V were automatically selected by the instrument during the optimisation step. The dwell time was 50 ms. The selected mass numbers employed to analyse the kaki fruit samples are shown in Table 1.

The calibration standards were prepared in 0.5% nitric acid. The calibration range for all studied elements was from 2 to 400 μg L^{-1}. Rhodium (1 μg L^{-1}) was used as internal standard and added to all samples and standards. Digested samples were measured with a final dilution 1:5. The average of blank ICP-MS signals was subtracted from analytical signals of digested samples after interpolation on the corresponding calibration graphs.

2.7. Quality control

Kaki fruit samples were prepared and measured in triplicate. Reagent blanks were made regularly together with each batch of sample digestion, and certified reference materials were digested and measured together with the different sample batches in order to control the trueness of data found. Additionally, control standards were measured for every series of ten independent sample measurements.

For the precision and trueness control of the method, a kaki fruit sample was spiked with two concentration levels (0.1 and 0.5 mg L^{-1}), and a certified reference material of NIST 1573a (Tomato leaves), obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA), was analysed under the same conditions as the samples.

Detection limits of instrument (LODi) were calculated as the concentrations corresponding to signals equal to three times the standard deviation of ten reagents blank measurements. Additionally, detection limits of the method (LODm) were calculated based on the original samples (mg kg^{-1} in dry weight), taking into consideration the amount of sample digested and the final dilution employed in the recommended procedure. Quantification limits of instrument (LOQi) were determined in the same way for a factor of ten times the deviation of blank measurements, and the quantification limits of the method (LOQm) were calculated in terms of concentration in the original sample in dry weight.

3. Results and discussion

3.1. Method quality assurance

Table 1 shows the main analytical figures of merit, LODm and LOQm for ICP-OES and ICP-MS measurements. In general, ICP-OES LODm and LOQm were higher than those obtained for ICP-MS. Thus, we used ICP-MS for lithium, nickel, titanium and zinc because it is not easy to determined these by ICP-OES.

The results found for certified reference material (NIST 1573a) were statistically compared with certified values. The regression line of average content versus certified values for each element was $y = (1.013 \pm 0.016)x - (2.917 \pm 3.25)$, which indicates the intercept and slope correspond to 0 and 1, and the regression coefficient was 0.9997. The percentage of recoveries evaluated by comparison between concentration data found and the certified values of the reference material provided recovery values from 97% for Na to 114% for Mg. It can be seen in Table 2 that the recovery percentages for samples spiked at two concentrations (0.1 mg L^{-1} and 0.5 mg L^{-1}) were 91–111%.

3.2. Mineral content of samples

Minimum, maximum, mean and median concentration of elements Al, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Sr, Ti and Zn found in kaki fruit samples are shown in Table 3. Data were classified as a function of the production regions (PDO Ribera del Xúquer, Alicante, Andalucía, Castellón, Extremadura, Ribera outside the PDO and Valencia) and expressed in mg kg^{-1} of dry weight. Data found for each assayed sample are included in the supplementary material. As indicated by the initial investigation of 42 elements, only 14 were present at detectable concentrations in the edible portion of the samples analysed.

The concentration of potassium in kaki samples (between 6500 and 15,500 mg kg^{-1} dry weight) provided values comparable with those obtained in bananas (Hardisson et al., 2001; Paul, Rodríguez, Darias, & Díaz, 2002). Thus, kaki fruits may be a good source of potassium, which is essential to maintain body water content and acid balance. Levels of calcium and magnesium were 400 mg kg^{-1}. Calcium allows the correct formation of the skeleton during childhood and has an essential role in preventing osteoporosis in later life. The values of calcium found through this study were lower than data reported for kakis by Farran, Zamora, and

Table 2
Evaluation of the trueness of the method employed for ICP-OES and ICP-MS determination of the mineral profile of kaki fruit.

Element	Certified (mg kg^{-1})	Obtained (mg kg^{-1})	Instrument	Recovery[b](%)	Recovery[c] (%)
Al	598 ± 12	587 ± 6	ICP-OES	91	103
Ba	63	60 ± 3	ICP-MS	107	107
Ca	5.05 ± 0.09[a]	5 ± 0.5[a]	ICP-OES	–	–
Cu	4.70 ± 0.14	4.7 ± 0.4	ICP-MS	–	–
Fe	368 ± 7	363 ± 2	ICP-OES	103	104
K	2.70 ± 0.05[a]	3.02 ± 0.08[a]	ICP-OES	–	–
Mg	1.2[a]	1.23 ± 0.04[a]	ICP-OES	–	–
Mn	246 ± 8	248 ± 10	ICP-MS	106	111
Na	136 ± 4	155 ± 5	ICP-OES	–	–
Ni	1.59 ± 0.07	1.66 ± 0.12	ICP-MS	105	105
Sr	85	87 ± 3	ICP-MS	96	101
Zn	30.9 ± 0.7	30 ± 3	ICP-MS	107	111

Note: Data obtained were found as the average of 15 independent analysis made in different sessions by the proposed method.
[a] Data reported in percentage (%).
[b] Spiked level (0.1 mg/L).
[c] Spiked level (0.5 mg/L).

Table 3
Mineral composition of kaki fruit samples from different Spain production areas.

		Al	Ba	Ca	Cu	Fe	K	Li	Mg	Mn	Na	Ni	Sr	Ti	Zn
PDO "Kaki Ribera del Xúquer"	Min	<0.12	0.4	210	0.1	<0.3	6800	<0.005	240	0.4	<1.2	<0.10	0.3	<0.04	0.4
	Max	8.6	4.8	540	2.2	11.0	12,600	1.0	500	14.2	200	2.3	9.8	3.6	4.5
	Mean	2.6 ± 1.9	1.7 ± 0.8^{b}	$380 \pm 70^{b,c}$	$1.1 \pm 0.5^{a,b}$	2.6 ± 2.3	9600 ± 1400^{b}	0.16 ± 0.22^{b}	$400 \pm 50^{a,b}$	5.4 ± 3.3^{a}	50 ± 30^{a}	0.7 ± 0.4^{b}	4.6 ± 1.7^{a}	2.3 ± 0.7	$1.7 \pm 0.6^{a,b}$
	Median	2.4	1.6	380	1.0	2.4	9700	0.09	400	4.4	40	0.7	4.5	2.5	1.6
Alicante	Min	1.2	0.14	500	1.32	0.57	9400	<0.005	420	4.3	40	0.85	1.8	2.6	1.70
	Max	2.7	7.8	1000	2.10	3.68	11,600	0.06036	540	11.1	110	2.55	3.0	3.3	2.77
	Mean	1.9 ± 0.8	$3.2 \pm 3.4^{a,b}$	800 ± 200^{a}	1.60 ± 0.34^{a}	2.00 ± 1.60	$10,300 \pm 900^{a,b}$	$0.015 \pm 0.030^{a,b}$	480 ± 50^{a}	$7.6 \pm 2.8^{a,b}$	$70 \pm 30^{a,b}$	1.66 ± 0.69^{a}	$2.3 \pm 0.5^{a,b}$	3.0 ± 0.3	2.37 ± 0.48^{a}
	Median	1.9	2.4	800	1.49	1.87	10,100	0	480	7.5	70	1.63	2.3	3.0	2.50
Andalucía	Min	0.5	<0.07	230	0.3	0.6	9200	<0.005	380	2	30	0.3	0.2	1.4	1.1
	Max	9.3	4.0	550	2.0	8.7	15,517	0.56	610	35	90	2.5	5.1	3.7	3.0
	Mean	3.4 ± 3.1	$2.2 \pm 1.2^{a,b}$	$400 \pm 80^{b,c}$	$1.1 \pm 0.5^{a,b}$	4.1 ± 2.2	$11,200 \pm 1800^{a}$	$0.16 \pm 0.20^{a,b}$	440 ± 70^{a}	$9 \pm 10^{a,b}$	$60 \pm 20^{a,b}$	$0.9 \pm 0.6^{a,b}$	2.6 ± 1.6^{b}	2.6 ± 0.6	$1.8 \pm 0.5^{a,b}$
	Median	2.6	2.3	400	1.3	4.2	10,600	0.09	410	6	50	0.8	3.0	2.6	1.7
Castellón	Min	<0.12	0.46	240	0.19	0.33	6500	<0.005	330	3.1	10	<0.10	2.0	1.0	<0.14
	Max	2.60	2.51	430	1.04	4.05	11,200	0.194	420	11.6	40	0.75	4.1	3.2	1.44
	Mean	1.08 ± 1.02	1.45 ± 0.68^{b}	$350 \pm 60^{b,c}$	0.70 ± 0.26^{b}	2.17 ± 1.38	9000 ± 1300^{b}	0.070 ± 0.081^{b}	360 ± 30^{b}	5.6 ± 2.4^{b}	$20 \pm 10^{a,b}$	0.35 ± 0.27^{b}	$3.1 \pm 0.7^{a,b}$	2.3 ± 0.6	0.76 ± 0.54^{c}
	Median	0.69	1.51	340	0.72	2.10	8900	0.041	360	5.0	20	0.39	3.2	2.3	0.95
Extremadura	Min	0.3	2.59	230	0.25	4.0	9300	<0.005	290	7.0	4.7	<0.10	1.07	1.28	0.83
	Max	0.7	4.08	270	1.45	8.2	9900	0.08	350	19.7	12.4	0.44	1.22	1.58	1.12
	Mean	0.5 ± 0.2	3.53 ± 0.82^{a}	250 ± 20^{c}	$0.72 \pm 0.64^{a,b}$	6.0 ± 2.1	$9600 \pm 300^{a,b}$	$0.03 \pm 0.05^{a,b}$	320 ± 30^{b}	15.4 ± 7.2^{a}	$9.7 \pm 4.4^{a,b}$	0.20 ± 0.22^{b}	1.16 ± 0.08^{b}	1.40 ± 0.16	$0.97 \pm 0.15^{b,c}$
	Median	0.7	3.91	250	0.45	5.6	9700	0	300	19.4	12.1	0.17	1.19	1.33	0.97
Ribera ouside PDO "Kaki Ribera del Xúquer"	Min	<0.12	<0.07	210	0.7	<0.3	7600	<0.005	330	3	20	<0.10	1.2	1.5	1.0
	Max	10	3	520	2.1	13	11,100	1.0	500	10	130	1.2	7	3	3
	Mean	3 ± 4	$2 \pm 0.8^{a,b}$	$410 \pm 90^{b,c}$	$1.2 \pm 0.4^{a,b}$	4 ± 3.5	9000 ± 1100^{b}	0.38 ± 0.26^{a}	$410 \pm 60^{a,b}$	7 ± 2^{b}	$50 \pm 30^{a,b}$	$0.8 \pm 0.3^{a,b}$	$4 \pm 1.6^{a,b}$	3 ± 0.7	$2 \pm 0.5^{a,b,c}$
	Median	2	2	400	1.2	3	9100	0.43	400	7	50	0.8	3	3	1.4
Valencia	Min	<0.12	0.5	270	0.6	<0.3	8000	<0.005	340	0.7	3	0.4	1.0	1.7	0.7
	Max	14.3	5.1	660	3.3	6.3	12,300	0.77	630	22	60	5.1	9.3	4.2	2.9
	Mean	3.2 ± 4.1	$2.0 \pm 1.2^{a,b}$	450 ± 130^{b}	1.4 ± 0.7^{a}	2.0 ± 1.8	$10,600 \pm 1400^{a,b}$	$0.18 \pm 0.24^{a,b}$	440 ± 90^{a}	$7.7 \pm 6.5^{a,b}$	22 ± 20^{b}	1.3 ± 1.2^{a}	$3.2 \pm 2.4^{a,b}$	2.5 ± 0.7	$1.9 \pm 0.6^{a,b}$
	Median	2.1	1.7	400	1.3	1.7	11,100	0.03	400	5.1	20	1.0	2.4	2.4	1.8

Note: concentration in mg/kg dry weight. Mean values in the same column with different superscripts (a–c) are significantly different ($p < 0.05$) with respect of the origin of kaki fruits by Tukey's test.

Table 4
Contribution of one kaki fruit per day to the recommended daily allowance of essential elements.

Element	RDA	RDA percentage provided by one piece of kaki fruit						
		PDO "Ribera del Xúquer"	Alicante	Andalucía	Castellón	Ribera outside PDO	Extremadura	Valencia
Ca	800	1–6	3–10	1–6	1–5	1–6	1–4	1–7
Cu	0.7	1–29	7–21	2–22	1–13	5–20	1–20	3–35
Fe	6	0–14	0–4	0–13	0–7	0–15	3–10	0–8
K	4700	5–23	6–22	7–26	7–20	6–20	9–20	6–25
Mg	350	3–12	4–11	4–14	4–10	4–13	4–10	4–15
Na	2400	0–1	0–0.3	0–0.3	0–0.2	0–0.4	0–0.05	0–0.2
Zn	9.4	0–4	1–2	0–3	0–1	0–3	0–1	0–3

Note: RDA values in mg, obtained from FAO/WHO (2002) and Food and Nutrition Board (2004).

Cervera (2004) and Souza, Argenta, Rombaldi, Souza, and Almarante (2012), and very similar to values reported by Gorinstein et al. (2001) with exception of samples from Alicante. Magnesium is also essential for humans; it is needed for more than 300 biochemical reactions in the body, helps to regulate blood sugar levels and is involved in energy metabolism and protein synthesis. The values of magnesium found through this study are higher than ones reported from Brazil (Souza et al., 2012). Sodium concentrations found in this study were lower than those reported by Gorinstein et al. (2001). Sodium is also an essential element, but is only necessary in small amounts. High intakes of this element are associated with increased blood pressure and risk of cardiovascular disease (WHO Guideline: Sodium intake for adults, 2012). Therefore, the kaki fruits could be beneficial because its consumption is not associated with hypertension.

Manganese, strontium, iron, aluminium and titanium concentration levels in kaki samples were less than 5 mg kg^{-1} but, in general, greater than 2 mg kg^{-1}. Manganese content was very similar to that reported by Gorinstein et al. (2001), except in the case of kaki fruit from Extremadura. The values for iron found in this study were the same order as those reported by Gorinstein et al. (2001), but lower than those reported by Farran et al. (2004). Manganese and iron are essential nutrients for humans. An adequate supply of iron is very important for females between 14 and 50 years old and for babies in the first months of life. Strontium, aluminium and titanium are not essential for humans, but it is important to monitor their levels in foods, since excess could cause health problems. Barium, zinc, copper and nickel concentrations were around 1 mg kg^{-1} in the kaki fruits analysed. Lithium was determined between 0.005 and 1 mg kg^{-1}. Copper and zinc content were, in general, higher than levels found by Gorinstein et al. (2001). In all cases, the content of lead and cadmium were lower than the LOD values obtained by ICP-MS, using the proposed method, at 2 and 7 μg kg^{-1}, respectively. Therefore, lead and cadmium content do not exceed the maximum levels permitted in fruits (0.10 and 0.05 mg kg^{-1} respectively) according to the European Commission (EC, 2006).

The contribution of calcium, copper, iron, potassium, magnesium, sodium and zinc to the recommended daily intake provided by one piece of kaki fruit (200–400 g fresh weight) are shown in Table 4. The percentage of RDA was calculated as milligrams of essential elements provided by each sample divided by the recommended values of RDA (FAO/WHO, 2002; Food & Nutrition Board, 2004) and multiplied by 100. The contribution of calcium, iron and magnesium is between 1% and 15% of the RDA. The percentage of copper and potassium is 5–30% whereas the contribution of sodium and zinc is negligible (1% and 4%, respectively).

We performed a one-way ANOVA for each analyte to see whether there were significant differences between mineral contents from different regions. This analysis indicates the differences were statistically significant at a probability level of 95% for all the elements determined, except aluminium. Additionally, Tukey's test was applied with $p < 0.05$ significance level and samples classified in groups with respect to their mean values (see Table 3). This information could for the basis of kaki fruit PDO authentication.

4. Conclusions

The mineral contents indicated in the present study were submitted to quality control using certified referential materials, reagent blanks and standards controls in each batch samples. Data obtained in this study, demonstrate the nutritional quality of kaki fruits produced in Spain, and their contribution to daily intake of essential mineral elements as well as the potential intake of low levels of toxic elements. It can be concluded that the intake of calcium, iron and magnesium are adequate compared with the RDA. Kaki fruits provide high levels of potassium and copper, and very low levels of zinc and sodium. Kaki fruits could be a healthy option for people with heart diseases and hypertension as well as the winder population. Also, our data suggests the mineral profile of kaki fruits could be used for the authentication of fruit covered by PDO.

Acknowledgements

Authors acknowledge the financial support of the Generalitat Valenciana (Spain) (project PROMETEO 2010-055) and the Ministerio de Economia y Competitividad (Spain)-Feder (project CTQ2011-25743 and project CTQ2012-38635). Also acknowledge for "Coagri (Alginet)", "Agrícola de Catadau", "Coop. Agrícola del Marquesat (Llombai)", "Canso (L'Alcúdia)", "Coop. Agrícola San Felipe Benicio (Benimodo)", "Copal (Algemesí)", "Coop. Carlet", "Coalfa (Alfarp)", "Coagal (Almussafes)", "Alzicoop (Alzira)", "Cohoca (Benifaió)", "Frutas Teresí Alegre (Alzira)", "Hnos Gimenez (Alzira)", "Cofrudeca (Belgida)", "Coop. Agrícola Cristo de la Salud (Navarrés)", "Coop. San Isidro Labrador (Segorb)", "Vale Marc (Pego)", "Naturcrex (Cáceres)", "Ecotiendadelavera (Cáceres)", "Grupo Medina (Huelva)", "Agrícola Antoluz (Huelva)" and "Supra Cañamas (Sevilla)" to provide kaki fruit samples. A. Mir-Marqués acknowledge the 'Atracció de Talent' grant provided by the University of Valencia (Spain).

References

Ariyama, K., Aoyama, Y., Mochizuki, A., Homura, Y., Kadokura, M., & Yasui, A. (2007). Determination of the geographic origin of onions between three main production areas in japan and other countries by mineral composition. *Journal of Agricultural and Food Chemistry, 55*, 347–354.

Boeting, K., Aguilera de Benzo, Z., Luisa Cervera, M., & de la Guardia, M. (2010). Authentication of the protected designation of origin horchata de valencia through the chemometric treatment of mineral content. *Analytical Methods, 2*, 1723–1728.

Brescia, M., Monfreda, M., Buccolieri, A., & Carrino, C. (2005). Characterisation of the geographical origin of buffalo milk and mozzarella cheese by means of analytical and spectroscopic determinations. *Food Chemistry, 89*, 139–147.

CRDO (2011). Consejo Regulador de la Denominación de Origen ‘‘Kaki Ribera del Xúquer’’. Available from: http://www.kakifruit.com.

EC (2006). Commission of the European Communities. Commission Regulation (EC) No. 1881/2006 Regulation of setting maximum levels for certain contaminants in foodstuffs. Official Journal European Union L364–5/L364-24.

FAO/WHO Human Vitamin and Mineral Requirements (2002). Report of a joint FAO/WHO expert consultation Bangkok, Thailand.

Farran, A., Zamora, R., Cervera, P. (2004). Tabla de composición de alimentos del CESNID, from: Base de Datos Española de Composición de Alimentos www.bedca.net.

Food and Nutrition Board. (2004). *Dietary Reference Intake (DRIs): Recommended intakes for individuals elements*. Institute of Medicine, National Academies Press.

Goldhaber, S. (2003). Trace element risk assessment: Essentiality vs. toxicity. *Regulatory Toxicology and Pharmacology, 38*, 232–242.

Gonzalvez, A., Llorens, A., Cervera, M. L., Armenta, S., & de la Guardia, M. (2009). Elemental fingerprint of wines from the protected designation of origin Valencia. *Food Chemistry, 112*, 26–34.

Gorinstein, S., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., Zemser, M., et al. (2001). Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples. *Journal of Agricultural and Food Chemistry, 49*, 952–957.

Hardisson, A., Rubio, C., Baez, A., Martin, M., Alvarez, R., & Diaz, E. (2001). Mineral composition of the banana (*Musa acuminata*) from the island of Tenerife. *Food Chemistry, 73*, 153–161.

Matos-Reyes, M. N., Simonot, J., Lopez-Salazar, O., Cervera, M. L., & de la Guardia, M. (2013). Authentication of Alicante’s Mountain cherries protected designation of origin by their mineral profile. *Food Chemistry, 141*, 2191–2197.

Mir-Marqués, A., Cervera, M. L., & de la Guardia, M. (2012). A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus. *Journal of Food Composition and Analysis, 27*, 160–168.

Moreno-Rojas, R., Sanchez-Segarra, P. J., Camara-Martos, F., & Amaro-Lopez, M. A. (2010). Multivariate analysis techniques as tools for categorization of southern spanish cheeses: Nutritional composition and mineral content. *European Food Research and Technology, 231*, 841–851.

Plaza, L., Colina, C., de Ancos, B., Sanche-Moreno, C., & Cano, M. P. (2012). Influence of ripening and astringency on carotenoid content of high-pressure treated persimmon fruit (*Diospyros kaki* L.). *Food Chemistry, 130*, 591–597.

Orihuel-Iranzo, B., Miranda, M., Zacarías, L., & Lafuente, M. T. (2010). Temperature and ultra low oxygen effects and involvement of ethylene in chilling injury of ‘‘Rojo Brillante’’ Persimmon fruit. *Food Science and Technology International, 16*, 159–167.

Paul, M., Rodríguez, E., Darias, J., & Díaz, C. (2002). Statistical differentiation of bananas according to their mineral composition. *Journal of Agricultural and Food Chemistry, 50*, 6130–6135.

Radwan, M. A., & Salama, A. K. (2006). Market basket survey for some heavy metals in Egyptian fruits and vegetables. *Food and Chemical Toxicology, 44*, 1273–1278.

Souza, E. L., Argenta, L. C., Rombaldi, C. V., Souza, A. L. K., & Almarante, C. V. T. (2012). Diagnosis of fruit quality and mineral contents of ‘Fuyu’ persimmon produced in southern Brazil. *Acta Horticulturae, 934*, 775–781.

Terrab, A., Hernanz, D., & Heredia, F. (2004). Inductively coupled plasma optical emission spectrometric determination of minerals in thyme honeys and their contribution to geographical discrimination. *Journal of Agricultural and Food Chemistry, 52*, 3441–3445.

Wall, M. (2006). Ascorbic acid, vitamin A, and mineral composition of banana (musa sp.) and papaya (carica papaya) cultivars grown in hawaii. *Journal of Food Composition and Analysis, 19*, 434–445.

WHO Guideline: Sodium intake for adults and children (2012). World Health Organization (WHO), Geneva.

Zeiner, M., Steffan, I., & Cindric, I. (2005). Determination of trace elements in olive oil by ICP-AES and ETA-AAS: A pilot study on the geographical characterization. *Microchemical Journal, 81*, 171–176.

CIENCIA 24(3), 153-164, 2016
Maracaibo, Venezuela

Authentication of the protected designation of origin “Kaki Ribera del Xúquer” from its mineral profile

Alba Mir-Marqués, María Luisa Cervera* y Miguel de la Guardia

Department of Analytical Chemistry, University of Valencia, Jerónimo Muñoz Building, 50th Dr. Moliner St., 46100 Burjassot (Valencia), Spain

Recibido: 24-11-15 Aceptado: 29-07-16

Abstract

Protected designation of origin (PDO) “Kaki Ribera del Xúquer” samples were discriminated from those produced in other five origins concerning: towns surrounding Valencia, Alicante, Andalucía, Castellón and also Ribera, produced outside the PDO frame. Multivariate analysis tools; such as principal components analysis (PCA), hierarchical cluster analysis (HCA), linear discriminate analysis (LDA) and classification and regression trees (CARTs) were evaluated in order to achieve a correct sample classification of the six sample origin groups considered. Chemometric analysis was conducted with 14 variables (content of different elements in mg kg-1, determined by ICP-OES and ICP-MS). HCA and CART cannot authenticate the origin of kaki fruits at 100 %; CART provided a separation of 79 % for the PDO samples. PCA only discriminated well the samples as a function of their different harvest but LDA provided quite satisfactory results obtaining a classification rate of 100 % for samples of the calibration set and 67 % for those included in the validation set.

Keywords: Protected designation of origin (PDO), mineral profile, inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), kaki fruit.

Autenticación de la denominación de origen protegida “Kaki Ribera del Xúquer” a partir de su perfil mineral

Resumen

Muestras de caqui con denominación de origen protegida (DOP) “Kaki Ribera del Xúquer” fueron discriminadas de otras con distinta procedencia española: de ciudades cerca de Valencia, Alicante, Andalucía, Castellón y la Ribera producidas fuera de la DOP. Se usaron herramientas de análisis multivariante como el análisis de componentes principales (PCA), análisis jerárquico de clústers (HCA), análisis discriminante lineal (LDA) y árboles de clasificación y regresión (CARTs) para evaluar la correcta clasificación de los seis grupos de origen. Para el análisis quimiométrico se utilizaron 14 variables (contenido mineral en mg kg-1, determinado por ICP-OES e ICP-MS). HCA y CARTS no permiten la total autentificación de caquis; CART proporciona una separación del 79 % para las muestras de DOP, mientras que PCA sólo permite discriminar bien las muestras a partir de su año de cosecha. LDA proporciona unos resultados bastante satisfactorios ya que se obtiene una clasificación correcta del 100 % para el set de calibración y de 67 % para el set de validación.

---

*Corresponding author: m.luisa.cervera@uv.es

1. Introduction

Kaki is a seasonal fruit whose origin is China and Japan, and it was brought to Spain during the 19th century. One of the main geographical locations of kaki fruit production is the river Xúquer region (Valencia, Spain). The local variety “Rojo Brillante” has the protected designation of origin (PDO) “Kaki Ribera del Xúquer” from the European Union.

Authenticity is a quality criterion for food which importance increases within Europe as a result of legislative protection of regional foods, based on the approval of different labels (1,2). The PDO label is used to describe foodstuffs, with regional identity, that are produced, processed and prepared in a specific geographical area (3,4). From the economic point of view, food authenticity has great importance, for the sectors involved in food production and also for the consumer. This is a very important factor because the authenticity helps to guarantee the characteristics and quality of food products and to prevent overpayment (5,6).

The use of analytical techniques is the best way to authenticate foods without any doubt in order to determine their geographical origin. There are many papers that try to discriminate foods between PDO areas to those produced outside the PDO, using different analytical methods. The most commonly analytical techniques used are GC-MS (7-11) and HPLC (12-14) for the determination of organic compounds, but organic compounds vary easily due to a lot of factors: fertilization, climatic conditions... So, it is sometimes very difficult to have definitive authentication. The mineral profile of foods is characteristic due to the soil type and the climatic conditions. Therefore, the determination of the mineral profile is a good technique to assure the geographical origin of food samples (5). The most commonly techniques employed to determine mineral elements in food in order to do their authentication are flame atomic absorption spectrometry FAAS (15,16), inductively coupled plasma optical emission spectrometry ICP-OES (17-20) and inductively coupled plasma mass spectrometry ICP-MS (21,22).

The main purpose of this study is the authentication of kaki fruit samples produced from PDO “Kaki Ribera del Xúquer” using their mineral profile information together with multivariate analysis.

2. Material and methods

2.1. Apparatus

An ICP-OES Optima 5300 DV inductively coupled plasma optical emission spectrometer Perkin Elmer (Norwalk, CT, USA) equipped with an auto sampler AS 93-plus, and an ultrasonic nebuliser U6000AT$^+$ Cetac (Nebraska, EEUU) was used for mineral determinations. ICP-MS measurements were carried out with a Perkin-Elmer SCIEX Elan 6100 DRCII apparatus, equipped with a Meinhard nebuliser and an autosampler Perkin-Elmer AS-91 was used for sample introduction.

The apparatus employed for sample pre-treatment was a microwave laboratory system, Ethos SEL from Milestone (Sorisole, Italy), equipped with a thermocouple for automatic temperature control, and an automatic gas leaks detector.

2.2. Reagents

Argon C-45 (purity higher than 99.995%) supplied by Carburos Metálicos (Barcelona, Spain), was employed as plasmogen and carrier gas for ICP-OES and ICP-MS.

Stock solutions of a multi-element standard solution (100 mg L^{-1}) containing 26 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Se, Na, Sr, Tl, Ti, V and Zn) dissolved in 5% HNO_3 obtained from Scharlau (Barcelona, Spain), and a multi-element solution containing 20 mg L^{-1} Dy, Er, Eu, Gd, Ho, Lu, Sm, Sc, Tb, Tm, Yb and Y and 100 mg L^{-1} Ce, La, Nd and Pr dissolved in 5% HNO_3, also from Scharlau, were employed to prepare the calibration standards.

For sample digestion it was employed HNO_3 69 % for trace analysis and H_2O_2 35 % reagent grade, both from Scharlau, and nanopure water with a minimum resistivity of 18.0 MΩ cm, obtained from a Milli-Q Millipore system (Bedford, MA, USA).

A 1000 mg L^{-1} ruthenium standard solution for ICP and a 1000 mg L^{-1} rhodium standard solution for ICP both from Scharlau, were used as internal standards.

2.3. Samples

162 kaki fruit samples from different Spanish agricultural cooperatives were collected during 2010 and 2011 harvests. Each sample corresponds to a mixture of 2-4 pieces of kaki fruit obtained from the same producer but from different trees. 114 samples were produced under the PDO "Kaki Ribera del Xúquer", four samples from Alicante, 12 from Andalucía, 10 from Castellón, nine from Ribera (outside the PDO) and 13 from Valencia (see Figure 1). The samples were taken from October to December, for each harvest. The edible parts were lyophilised, pulverised and stored in polyethylene bottles until analysis.

Figure 1. Map of Spain indicating the different regions where the kaki fruit samples employed through this study were produced

The microwave-assisted treatment was adapted from that provided by Mir-Marqués et al., 2012 (23) according to the microwave laboratory system manufacturer recommendations and our experience.

Freeze-dried samples (0.5 g) were digested with 8 mL of concentrated nitric acid and 2 mL of H_2O_2 into the Teflon vessel inside the microwave oven. The digestion step of 15 min at 180 °C (700 W) was employed. The resulting solution was quantitatively transferred to a plastic flask and made up to 20 mL with ultrapure water. All samples were analysed in triplicate to eliminate any specific error, to verify the homogeneity of samples and to evaluate the repeatability of the procedure. Blanks and certified material (NIST 1573a Tomato Leaves from the National Institute of Standards and Technology (Gaithensburg, MD, USA)) were prepared in the same way than the samples.

2.4. Analytical procedures

The calibration range for all the 42 elements evaluated was established from 0.05 to 2 mg L^{-1} and ruthenium (1 mg L^{-1}) was used as internal standard and added to all samples, blanks and standards for ICP-OES analysis. Digested samples were measured with a final dilution of 40 mL. For ICP-MS analysis, the calibration range for all the 42 elements evaluated was from 2 to 400 µg L^{-1} and rhodium (10 µg L^{-1}) was used as internal standard and added to samples, blanks and standards. In this case, digested samples were measured with a final dilution of 100 mL. Additional, control standards were measured for every series of 10 independent sample measurements and a reference material every series.

The average measurement of blanks was subtracted from the analytical signals of digested samples after interpolation on the calibration graphs. Results were expressed as a basis of dry weight.

2.5. Statistical analysis

Both, univariate and simple multivariate methodologies were employed through this study for the authentication of the origin of samples from their mineral profile.

Univariate analysis

Analysis of variance was applied to all variables studied considering only those elements present in the samples over the detection limit of the method employed. In cases where the concentration was below the detection limit, this value was taken as data for the chemometric studies. The mean values obtained for samples with different origin were compared by using one-way ANOVA and two-way ANOVA including the production year.

Multivariate analysis

Statistical multivariate analysis was performed by using the software XLSTAT2008 from Addinsoft (Barcelona, Spain). Principal component analysis (PCA), hierarchical cluster analysis (HCA), linear discriminate analysis (LDA) and classification and regression trees (CART) were performed on the mineral elements concentration of the studied samples.

Although 42 elements were evaluated in the 162 kaki fruit samples analysed, calculations were performed using only 14 variables considering the content of Al, Ba, Ca, Cu, Fe, Li, K, Mg, Mn, Na, Ni, Sr, Ti and Zn, in mg kg^{-1}, determined by ICP-OES and ICP-MS. The concentrations of other elements were lower than the detection limit (LOD) in almost samples analysed in this study and thus they were not used in data treatment.

3. Results and discussion

3.1. Mineral content of kaki fruit

Table 1 shows the maximum, minimum, mean and median concentration of elements (Al, Ba, Ca, Cu, Fe, Li, K, Mg, Mn, Na, Ni, Sr, Ti and

Zn) present in all samples at concentration levels higher than the LOD as a function of their production region (Alicante, Andalucía, Castellón, PDO Ribera del Xúquer, Ribera outside PDO, Valencia) expressed in mg kg^{-1} of dry weight.

3.2. Statistical analysis

3.2.1. Univariate analysis

ANOVA was used to compare elemental profiles of food in different origin (6). We performed a one-way ANOVA for each one of the 14 analytes present in all samples over the LOD of the employed method: Al, Ba, Ca, Cu, Fe, Li, K, Mg, Mn, Na, Ni, Sr, Ti and Zn, to find whether there were significant differences between samples produced in different regions. ANOVA analysis shows that the differences were statistically significant at a probability level of 95 % ($p<0.05$) since F calculated is greater than F critic (F critic 2.16) for all elements except for

Table 1. Mineral composition of kaki fruit samples from different Spain production

	Al	Ba	Ca	Cu	Fe	K	Li	Mg	Mn	Na	Ni	Sr	Ti	Zn
min	< 0.12	0.4	210	0.1	< 0.3	6800	< 0.005	240	0.4	< 1.2	< 0.10	0.3	< 0.04	0.4
max	8.6	4.8	540	2.2	11.0	12600	1.0	500	14.2	200	2.3	9.8	3.6	4.5
mean	3 ± 2	1.7 ± 0.8	380 ± 70	1.1 ± 0.5	3 ± 2	9600 ± 1400	0.2 ± 0.2	400 ± 50	5 ± 3	50 ± 30	0.7 ± 0.4	4.6 ± 1.7	2.3 ± 0.7	1.7 ± 0.6
median	2.4	1.6	380	1.0	2.4	9700	0.09	400	4.4	40	0.7	4.5	2.5	1.6
min	1.2	0.14	500	1.32	0.57	9400	< 0.005	420	4.3	40	0.85	1.8	2.6	1.70
max	2.7	7.8	1000	2.10	3.68	11600	0.06036	540	11.1	110	2.55	3.0	3.3	2.77
mean	1.9 ± 0.8	3 ± 3	800 ± 200	1.6 ± 0.3	2.0 ± 1.6	10300 ± 900	0.02 ± 0.03	480 ± 50	8 ± 3	70 ± 30	1.7 ± 0.7	2.3 ± 0.5	3.0 ± 0.3	2.4 ± 0.5
median	1.9	2.4	800	1.49	1.87	10100	0	480	7.5	70	1.63	2.3	3.0	2.50
min	0.5	< 0.07	230	0.3	0.6	9200	< 0.005	380	2	30	0.3	0.2	1.4	1.1
max	9.3	4.0	550	2.0	8.7	15517	0.56	610	35	90	2.5	5.1	3.7	3.0
mean	3 ± 3	2.2 ± 1.2	400 ± 80	1.1 ± 0.5	4 ± 2	11200 ± 1800	0.2 ± 0.2	440 ± 70	9 ± 10	60 ± 20	0.9 ± 0.6	2.6 ± 1.6	2.6 ± 0.6	1.8 ± 0.5
median	2.6	2.3	400	1.3	4.2	10600	0.09	410	6	50	0.8	3.0	2.6	1.7
min	< 0.12	0.46	240	0.19	0.33	6500	< 0.005	330	3.1	10	< 0.10	2.0	1.0	< 0.14
max	2.60	2.51	430	1.04	4.05	11200	0.194	420	11.6	40	0.75	4.1	3.2	1.44
mean	1.1 ± 1.0	1.5 ± 0.7	350 ± 60	0.7 ± 0.3	2.2 ± 1.4	9000 ± 1300	0.07 ± 0.08	360 ± 30	6 ± 3	20 ± 10	0.4 ± 0.3	3.1 ± 0.7	2.3 ± 0.6	0.8 ± 0.5
median	0.69	1.51	340	0.72	2.10	8900	0.041	360	5.0	20	0.39	3.2	2.3	0.95
min	< 0.12	< 0.07	210	0.7	< 0.3	7600	< 0.005	330	3	20	< 0.10	1.2	1.5	1.0
max	10	3	520	2.1	13	11100	1.0	500	10	130	1.2	7	3	3
mean	3 ± 4	2.0 ± 0.8	410 ± 90	1.2 ± 0.4	4 ± 4	9000 ± 1100	0.4 ± 0.3	410 ± 60	7 ± 2	50 ± 30	0.8 ± 0.3	4.0 ± 1.6	3.0 ± 0.7	2.0 ± 0.5
median	2	2	400	1.2	3	9100	0.43	400	7	50	0.8	3	3	1.4
min	< 0.12	0.5	270	0.6	< 0.3	8000	< 0.005	340	0.7	3	0.4	1.0	1.7	0.7
max	14.3	5.1	660	3.3	6.3	12300	0.77	630	22	60	5.1	9.3	4.2	2.9
mean	3 ± 4	2.0 ± 1.2	450 ± 130	1.4 ± 0.7	2.0 ± 1.8	10600 ± 1400	0.2 ± 0.2	440 ± 90	8 ± 7	22 ± 20	1.3 ± 1.2	3 ± 2	2.5 ± 0.7	1.9 ± 0.6
median	2.1	1.7	400	1.3	1.7	11100	0.03	400	5.1	20	1.0	2.4	2.4	1.8

NOTE: concentration in mg kg^{-1} dry weight. n= number of samples aluminium. So, this information evidences that the mineral profile could be important as a basis of the kaki fruit PDO authentication. We also performed a two-way ANOVA including the production year, in this case samples from Alicante and Castellon had not considered because we did not harvested samples in 2010 (see Table 2). As can be seen in Table 2 from the two-way ANOVA there were differences between harvest areas for all the hand Cu, Li, K, Mg, Mn, Na, Ni, Sr, Ti and Zn did not present differences among production year.

Table 2. Two way ANOVA evaluation of the comparability of the mineral profile of kaki samples produced inside and outside the PDO and the production year

Element	F_A	F_B	Element	F_A	F_B
Al	47.16	9.78	Mg	14.47	0.86
Ba	55.2	11.4	Mn	19.02	2.74
Ca	28.01	4.98	Na	15.26	1.75
Cu	11.94	0.86	Ni	5.35	0.67
Fe	26.38	4.25	Sr	16.86	2.21
Li	19.04	2.47	Ti	10.81	0.53
K	10.60	0.42	Zn	47.16	9.78

NOTE: F_A is calculated Fisher’s F to different origin, F value critic is 2.14. F_B is calculated Fisher’s F to different production year, F value critic is 2.75.

3.2.2. Multivariate analysis

In this study, the concentration of 14 elements was used to evaluate the fruits coming from PDO “Kaki Ribera del Xúquer” produced in a restricted area near Valencia and to discriminate between different regions of kaki fruit production close to the zone covered by the PDO label. In this sense, mineral content of the edible part of the samples was used as chemical descriptor for building the statistical methods, in order to establish differences between PDO kaki fruit and the other ones. As it has been indicated, PCA, HCA, LDA and CART models were employed for chemometric treatment. Results obtained from this chemometric models were compared between them, in order to look for the best discrimination processing tool.

Principal component analysis

Principal component analysis is an unsupervised technique that allows visualising the information of the data set in a few principal components retaining the maximum possible variability within that set.

From the loadings of original variables in the two first considered principal components (see Table 3).

Table 3. Loadings of the variables for the two first Principal Components established from the mineral profile of kaki samples produced in different parts of Spain during the period 2010-2011

Variable	PC1	PC2	Variable	PC1	PC2
Al	-0.131	-0.312	Mg	0.620	0.579
Ba	0.543	-0.531	Mn	0.277	-0.011
Ca	0.590	-0.128	Na	0.095	0.444
Cu	0.731	-0.288	Ni	0.692	-0.104
Fe	-0.020	-0.284	Sr	0.609	-0.342
K	0.284	0.856	Ti	0.480	0.455
Li	0.390	0.061	Zn	0.703	-0.108

Principal component 1 (PC1) represents 24.80 % of the total variance. Cu, Ni, Mg and Sr were identified as the dominant variables in the PC1 while Fe and Na show the lowest values. PC2 explains 15.54 % of the total variance. Revising loadings, K, Mg and Ba result as the most dominant variables in PC2, while Li and Mn show the lowest dominant values. PC1 and PC2 account for 40.34 % of the total variance, and it allows differentiating two groups by harvest years, 2010 and 2011 (see Figure 2). However, it is really difficult to discriminate between samples produced in different areas during the two evaluated years, neither within each of the considered harvest years. The values of total variance explained are very similar than to values reported in bananas from Tenerife and Ecuador (24) and different banana flours (25).

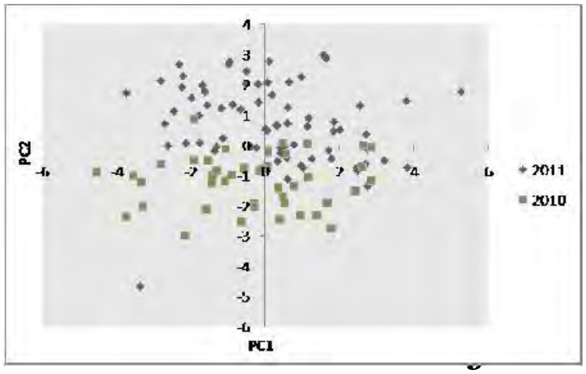

Figure 2. Principal components analysis (PC1 vs. PC2) of the mineral profile of kaki samples produced in different parts of Spain.

Hierarchical cluster analysis is an unsupervised technique that uses the information obtained from the measuredmineral elements, to determine if there are cluster association between investigated samples with the same origin. Based on the previous results obtained by PCA analysis, HCA was applied to the whole sample population and was separately used in order to evaluate samples produced in 2010 and 2011. As it can be seen in Figure 3, seven clusters were obtained from the 162 samples considered. However, clusters A1 and A2 concerns both the Alicante samples and because of their vicinity they were cumulated in a single cluster A. Cluster B contains two samples from PDO, one sample from Ribera and another sample from Valencia. Cluster C includes samples of four different origins (PDO (8), Castellón (1), Ribera (4) and Valencia (2)). Cluster D contains a single sample from PDO. Cluster E contains the main part of samples produced from the PDO (65 samples) which are accompanied by 25 samples from different origins (Alicante (1), Andalucía (7), Castellón (7), Ribera (2) and Valencia (8), thus providing also a bad classification of kaki fruit from their origin. Cluster F contains 49 samples (PDO (36), Andalucía (4), Castellón (2), Ribera (2) and Valencia (5)). So it can be concluded that the mineral composition of kaki fruits produced in different parts of Spain during the period 2010-2011 cannot be described correctly by HCA in order to authenticate their origin.

The HCA of the mineral profile of samples corresponding to 2010 and 2011 harvest provided five and seven clusters respectively. However, the distribution of the 56 samples collected in 2010 between the five clusters was arbitrary, and as an example 38 samples of the PDO were classified in all clusters. Similar results were found applying HCA to samples collected in 2011. Only one of the clusters correctly classified Alicante samples. The remaining clusters contained samples of the other origins. Therefore, it can be concluded that HCA is an unsuitable tool to do the authentication of kaki fruits from their mineral profile. Compared with other authors, Foster et al., 2002 (24) differentiated between Tenerife and Ecuador bananas by HCA, this may be due to the large distance between the two origins, because they belong to different continents with very different characteristics. According to the investigated kaki fruits, all samples belong to the same country and all regions analyzed show very similar characteristics in both climatic and soil conditions.

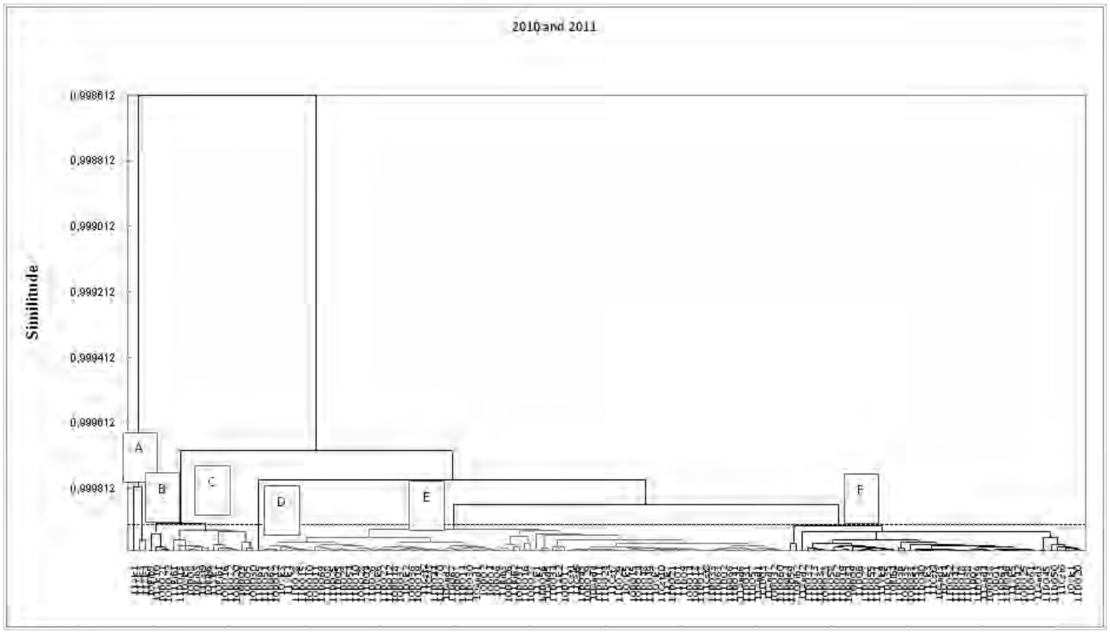

Figure 3. Cluster classification of kaki fruit samples obtained from their mineral profile

Linear discriminate analysis

Linear discriminate analysis is a supervised technique for classifying a set of observations into predefined classes. LDA maximises the variance between categories, minimises the variance within categories, and suppresses the correlation among variables.

LDA was performed by separating the samples in 6 classes: Alicante, Andalucía, Castellón, PDO Kaki Ribera del Xúquer, Ribera (outside the PDO) and Valencia. The kaki samples were divided in two groups: a training set consisting in 108 samples (2 from Alicante, 8 from Andalucía, 6 from Castellón, 78 from PDO, 6 from Ribera outside PDO and 8 from Valencia) and another set for validation containing 54 samples (2 from Alicante, 4 from Andalucía, 4 from Castellón, 36 from PDO, 3 from Ribera outside the PDO and 5 from Valencia). The division into two groups was made randomly. Results were satisfactory, obtaining a classification rate for calibration of 100 % and 67 % for validation (see Table 4) for the validation set.

In Table 4 it is also included the confusion matrix for the validation samples. For the 2010 harvest, a total of 56 samples were divided into 2 sets; one for calibration, containing 36 samples (3 from Andalucía, 23 from PDO, 7 from Ribera and 3 from Valencia), and another set for validation, containing 20 samples (1 from Andalucía, 16 from PDO, 3 from Ribera and 0 from Valencia). In this case a correct classification rate of 85 % was obtained for validation.

Table 4. LDA confusion matrix of validation set for the classification of kaki fruit samples from their mineral profile

	Assigned group **Original group**	**Alic**	**And**	**Cast**	**PDO**	**Rib**	**Val**	**Total**	**% Correct**
All samples (harvest 2010 and 2011)	Alic	2	0	0	0	0	0	2	100%
	And	0	3	0	1	0	0	4	75%
	Cast	0	0	2	1	0	1	4	50%
	PDO	0	1	2	25	5	3	36	**69%**
	Rib	0	0	0	2	1	0	3	33%
	Val	0	0	1	1	0	3	5	60%
	Total	2	4	5	30	6	7	54	67%

	Assigned group **Original group**	**And**	**PDO**	**Rib**	**Val**	**Total**	**% Correct**
Samples of the 2010 harvest	And	1	0	0	0	1	100%
	PDO	1	14	1	0	16	**87%**
	Rib	0	1	2	0	3	67%
	Val	0	0	0	0	0	0%
	Total	2	15	3	0	20	85%

	Assigned group **Original group**	**Alic**	**And**	**Cast**	**PDO**	**Rib**	**Val**	**Total**	**% Correct**
Samples of the 2011 harvest	Alic	2	0	0	0	0	0	2	100%
	And	0	2	0	1	0	0	3	67%
	Cast	0	0	3	1	0	1	5	60%
	PDO	0	2	5	17	1	0	25	**65%**
	Rib	0	0	0	0	1	0	1	100%
	Val	0	0	0	1	0	3	4	75%
	Total	2	4	8	20	2	4	40	68%

In the case of the 2011 harvest, a total of 106 samples were divided into a set of calibration, containing 66 samples (2 from Alicante, 4 from Andalucía, 5 from Castellón, 47 from PDO, 2 from Ribera and 6 from Valencia), and a set of validation with 40 samples (2 from Alicante, 3 from Andalucía, 5 from Castellón, 25 from PDO, one from Ribera and 4 from Valencia). As it can be seen in Table 3 a correct classification rate of 68 % was obtained.

So, as it can be seen, the capability of LDA for a correct classification of kaki fruit samples from their mineral profile is quite good and does not depends dramatically on the harvest. So, it is possible authentication of Kaki Ribera del Xúquer at a 69 % correct level; being those samples produced in the same area, but outside the protected label, the most critical group to obtain a correct differentiation. Other authors obtained a correct classification of 100 % in bananas and cherries (20,25) but they do not explained if it corresponds to classification set or validation set, in this case we could think that they have not performed external validation sets, so their data are comparable with ours.

Classification analysis regression trees

CART technique was applied to the samples collected during the two harvests (2010 and 2011) in order to check the capability of classification of determined concentration of mineral elements in kaki fruits.

This technique divides the data into sub-groups (nodes) that are more homogeneous with respect to the response of the initial data set. When performing the CART data with both harvests, we obtain a separation of 79 % for the PDO, with 4 nodes (see Figure 4), been characterised these samples by low concentrations for calcium and nickel and high concentrations for sodium and strontium. This separation is not pure, and does not let us to separate completely the PDO samples from other ones produced in the Ribera (7), Valencia (2), Andalucía (5) and Castellón (2).

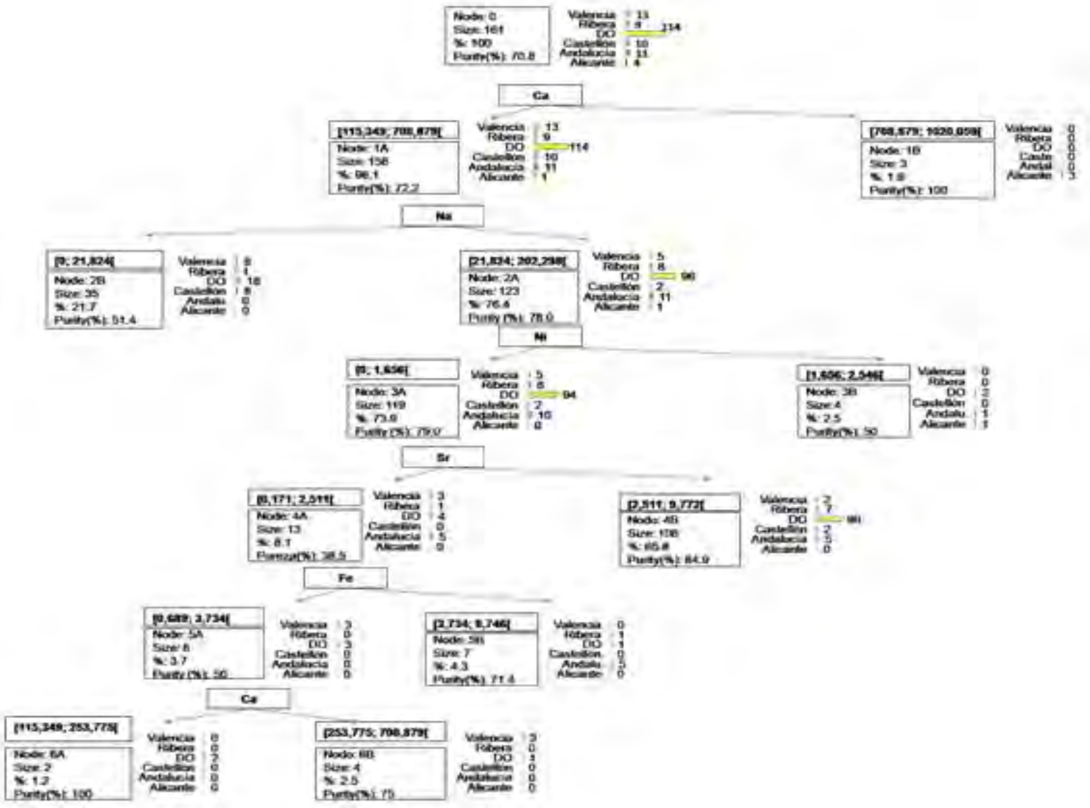

Figure 4. Classification analysis regression trees for kaki fruit samples collected in 2010 and 2011 harvests

On the other hand, CART analysis of samples collected in the 2010 harvest provided a correct classification of 84 % of samples produced inside the PDO with two nodes corresponding to low concentrations of Ca and Li. However once again, the separation was not complete. It was the same for samples produced in 2011, for which the separation was 85 % with 5 nodes corresponding to low concentrations of Ca and Ni, and high concentrations of Zn and Sr.

So, it can be concluded that CART provided coherent results with those suggested by LDA.

Conclusions

Mineral profile of kaki fruit samples from different origin produced in Spain cannot be clearly discriminate by their production area. Because of that, kaki fruits produced inside the PDO "Kaki Ribera del Xúquer" cannot be totally authenticate, being obtained uncorrected classification of other samples produced in Alicante, Andalucía, Castellón, Ribera outside PDO and Valencia by using HCA, PCA and CART chemometric treatment. LDA provided satisfactory results to the authentication of PDO "Kaki Ribera del Xúquer", obtaining a classification rate of 100 % for samples of the calibration set and 67 % for validation set. Although, additional data must be required for a complete description of the fruit PDO and new efforts are in due course to use improved chemometric tools for data treatment.

Acknowledgements

Authors acknowledge the financial support the Ministerio de Economia y Competitividad-Feder (project **CTQ2012-38635**) and Generalitat Valenciana (project **PROMETEO II 2014-077**). "Coagri (Alginet)", "Agrícola de Catadau", "Coop. Agrícola del Marquesat (Llombai)", "Canso (L'Alcúdia)", "Coop. Agrícola San Felipe Benicio (Benimodo)", "Copal (Algemesí)", "Coop. Carlet", "Coalfa (Alfarp)", "Coagal (Almussafes)", "Alzicoop (Alzira)", "Cohoca (Benifaió)", "Frutas Teresí Alegre (Alzira)", "Hnos Gimenez (Alzira)", "Cofrudeca (Belgida)", "Coop. Agrícola Cristo de la Salud (Navarrés)", "Coop. San Isidro Labrador (Segorb)", "Vale Marc (Pego)", "Naturcrex (Cáceres)", "Ecotiendadelavera (Cáceres)", "Grupo Medina (Huelva)", "Agrícola Antoluz (Huelva)" and "Supra Cañamas (Sevilla)" to provide kaki fruit samples. Alba Mir-Marqués acknowledges a grant "**Atracció de Talent**" provided by the University of Valencia.

References

1. EU Regulation. European Union Regulation (EEU) 2081/1992
2. EU Regulation. European Union Regulation (EEU) 1151/2012
3. KELLY S., HEATON K., HOOGEWERFF J. ***Trends Food Sci Tech*** 16: 555-567. 2005.
4. DE LA GUARDIA M., GONZÁLVEZ A. (Eds). ***Food Protected Designation of Origin: methodologies and applications***. Elsevier. Amsterdam (Netherland). 2013.
5. GONZÁLVEZ A., ARMENTA S., DE LA GUARDIA M. ***Trac-trend Anal Chem*** 28: 1295-1311. 2009.
6. DRIVELOS S.A., GEORGIOU C.A. ***Trac-trend Anal Chem*** 40: 38-51. 2012.

7. BIANCHI F., CARERI M., CHIAVARO E., MUSCI M., VITTADINI E. ***Food Chem*** 110: 787-793. 2008.
8. AQUILANTI L., SANTARELLI S., BABINI V., OSIMANI A., CLEMENTI F. ***Int Dairy J*** 29: 42-52. 2013.
9. INNOCENTE N., MUNARI M., BIASUTTI M. ***J Dairy Sci*** 96: 26-32. 2013.
10. KARALI F., GEORGALA A., MASSOURAS T., KAMINARIDES S. (2013). ***J Sci Food Agr*** 93: 1845-1851. 2013.
11. MONTERO-PRADO P., BENTAYEB K., NERIN C. ***Food Chem*** 138: 724-731. 2013.
12. GALAUP P., FLAMIN C., CARLET E., DUFOSSÉ L. ***Food Res Int*** 28: 855-860. 2005.
13. BARREIRA J.C.M., CASAL S., FERREIRA I.C.F.R., PERES A.M., PEREIRA J.A., OLIVEIRA M.B.P.P. ***J Agr Food Chem*** 60: 9697-9704. (2012).
14. RUSSO R., SEVERINO V., MENDEZ A., LLIBERIA J., PARENTE A., CHAMBERY A. ***J Mass Spectrom*** 47: 1407-1414. 2012.
15. ARIYAMA K., HORITA H., YASUI A. ***Anal Sci*** 20: 871-877. 2004.
16. MORENO-ROJAS R., SANCHEZ-SEGARRA P.J., CAMARA-MARTOS F., AMARO-LOPEZ M.A. ***Eur Food Res Tech*** 231: 841-851. 2010.
17. ANDERSON K., SMITH B. ***J Agr Food Chem*** 53: 410-418. 2005.
18. ARIYAMA K., AOYAMA Y., MOCHIZUKI A., HOMURA Y., KADOKURA M., YASUI A. ***J Agr Food Chem*** 55: 347-354. 2007.
19. GONZÁLVEZ A., ARMENTA S., DE LA GUARDIA M. ***Food Chem*** 126: 1254-1260. 2011.
20. MATOS-REYES M.N., SIMONOT J., LÓPEZ-SALAZAR O., CERVERA M.L., DE LA GUARDIA M. ***Food Chem*** 141: 2191-2197. 2013.
21. DI GIACOMO F., DEL SIGNORE A., GIACCIO M. ***J Agr Food Chem*** 55: 860-866. 2007.
22. COSTAS-RODRIGUEZ M., LAVILLA I., BENDICHO C. ***Anal Chim Acta*** 664: 121-128. 2010.
23. MIR-MARQUÉS A., CERVERA M.L., DE LA GUARDIA M. ***J Food Compos Anal*** 27: 160-168. 2012.
24. FOSTER M., RODRIGUEZ E., MARTIN J., ROMERO C. ***J Agr Food Chem*** 50: 6130-6135. 2002.
25. ALKARKHI A.F.M., BIN RAMLI S., EASA A.M. ***Int J Food Sci Nutr*** 60: 116-125. 2009.

Journal of Food Engineering 142 (2014) 17–22

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Classification of persimmon fruit origin by near infrared spectrometry and least squares-support vector machines

M. Khanmohammadi [a], F. Karami [a], A. Mir-Marqués [b], A. Bagheri Garmarudi [a], S. Garrigues [b], M. de la Guardia [b,*]

[a] *Chemistry Department, Faculty of Science, IKIU, Qazvin, Iran*
[b] *Department of Analytical Chemistry, University of Valencia, Jerónimo Muñoz Building, 50th Dr. Moliner St., 46100 Burjassot, Valencia, Spain*

A R T I C L E I N F O

Article history:
Received 1 February 2014
Received in revised form 30 May 2014
Accepted 2 June 2014
Available online 11 June 2014

Keywords:
Persimmon
Fourier transform near infrared spectrometry
Least squares-support vector machines
Chemometrics
Geographical origin
Protected designation of origin

A B S T R A C T

The main objective of this work has been the authentication by Fourier transform near infrared (FT-NIR) spectrometry of the origin of persimmon fruits cultivated in different regions of Spain. In order to achieve this goal, 166 persimmon samples from 7 different regions of Spain were analyzed by FT-NIR spectrometry. By splitting the spectral data in training and independent test sets, a classification model was built using least squares support vector machines chemometric technique. Orthogonal signal correction and principal component analysis were performed prior to conduct the classification strategy. The verified model was applied for the prediction of the origin of 50 samples from the independent test set being the prediction error 2%.

1. Introduction

Quality control in horticultural products is a serious concern for food industry. It is an important issue for every producer and buyer, whether dealing with manufactured commodities to farmed fruits and products. Even local farmers must organize their activities on high quality reputation to sustain development, fruitfulness, profitability and competitiveness.

Experimental inspection regulations lay down stringent requirements which would guarantee the quality of products based on declared standards. In addition, there are some quality schemes which help in identification of specifications of products and foodstuffs farmed and produced. Quality control in this field consists of different subtitles. Considering the probable contamination of fruit products with chemical pollutants e.g. pesticides, there are several analytical approaches for evaluation of the quality of fruits prior to be consumed in the market. On the other hand there are several important nutritional parameters, affecting the quality of fruits. Mineral profile of the fruits together with other food components e.g. vitamins are the main quality parameters influencing the product.

The characteristics of foods are highly depending on the geographical region and farming practices in which the fruit garden has been located. Thus the origin of a fruit product is an important factor, affecting its quality. The protected designation of origin (PDO) is a status given by the name of a specific geographical area, used as a designation for an agricultural product or a foodstuff which has been produced, prepared or processed there. Thus the final product acquires unique properties and sometimes its quality is exclusively determined via its origin geographical environment i.e. natural and human factors (De la Guardia and Gonzálvez, 2013).

Persimmon is a native Chinese fruit, cultivated in warm regions worldwide. There are several different persimmon species farmed in Spain; such as Rojo Brillante astringent type. Mild climate is a favorable requirement for cultivation of persimmon. The main land areas in which this type of fruit is grown in Spain are La Ribera del Xúquer with European PDO register label, Alicante, Andalucía, Castellón, Extremadura, Ribera without PDO and Valencia.

Climate characteristics of the production area are important factors influencing the quality and features of the fruits. The variety of persimmon fruit and climate conditions also determine the shape of it (Kaki Ribera del Xúquer, 2011). In addition, the soil chemical composition is an important parameter in the nutritional profile of persimmon. All of these effective parameters are mainly

---

* Corresponding author. Tel./fax: +34 96 3544838.
E-mail address: miguel.delaguardia@uv.es (M. de la Guardia).

http://dx.doi.org/10.1016/j.jfoodeng.2014.06.003

depending on the geographical location and for this reason it is favorable to determine the origin of the fruit and also to discriminate the fruit samples from different origins while some may be PDO stated.

Generally, there are few reliable methods available for origin recognition of fruits. The main technique for this aim is to use 26s rDNA and 28s rDNA analysis (El Sheikha et al., 2009) and free amino acid distribution (Licciardello et al., 2011) as the features to do the differentiation of samples. However, there are numerous analytical approaches for quality control of fruits, introduced to enable the authorities in reliable evaluation of unknown origin samples. Quantitative determination of phenolic compounds in fruit is a common tool for discrimination of fruit species (Silva et al., 2000). Isorhamnetin glycosides and phloridzin are typical contents of pear and apple have been also employed for their authentication (Hvattum, 2002), food authenticity control has been also performed by NMR spectroscopy (Colquhoun, 1998) together with fractionation and characterization of cell wall polysaccharides (Feugel et al., 2004).

Spectroscopy techniques have been widely used for qualitative and quantitative determination of different components in fruit samples. They have been utilized for the quantitative determination of several compounds. Also, the output data from these techniques are useful tools to access reliable conclusions on their origin. One of the spectroscopy techniques used in food chemistry is Fourier transform infrared (FTIR) spectroscopy especially in the near infrared (NIR) spectral region (800–2500 nm) (Norris, 1964; Nicolaï et al., 2007; Davies and Grant, 1987; Birth et al., 1985; Roy et al., 1993; Terdwongworakul et al., 2012; Fan et al., 2009).

Obtained data from NIR spectroscopy measurements are very advantageous when well-defined data processing is performed. Nowadays, chemometrics data processing methods are important tools to obtain information and data from NIR spectra. NIR spectroscopy has been a beneficial technique in determination of the quality of persimmon fruit (Mowat and Poole, 1997).

Main chemometrics methods used in analytical chemistry concern calibration, both supervised and unsupervised, classification and experimental design. Versatile combination of chemometrics and IR spectroscopy has been applied in food industry, agriculture and horticulture; such as partial least squares discrimination analysis (PLS-DA) which is a reliable classification technique in chemometrics and mid infrared (mid-IR) diffuse reflectance spectroscopy (3–5 μm) (Wang and Mizaikoff, 2008; Ribeiro et al., 2010; Sârbu et al., 2012; Gómez-Caravaca et al., 2013; Ni et al., 2011). Among several chemometric techniques, support vector machine (SVM) is a classification approach, found to be very useful for different aims (Vapnik, 2000; Xu et al., 2006). Considering the current interest in development of SVM based classification models in chemometrics and also the verified capabilities of NIR spectroscopy, in this work we have used multi class least squares SVM for treatment of NIR spectral data of persimmon samples as a tool for discriminating the origin of fruits cultivated in different regions of Spain.

2. Materials and methods

2.1. Sampling and analysis

166 Samples produced in Spain were collected from different agricultural cooperatives and producers during the harvests of 2010 and 2011. Among these samples 122 were produced under the PDO status as "Ribera del Xúquer", 4 samples from Alicante, 11 samples from Andalucía, 10 samples from Castellón, 3 samples from Extremadura, 13 samples from the Ribera area but produced without PDO status, and 13 samples from Valencia. Geographical distribution of the regions from which persimmon samples used in this study were provided is shown in Fig. 1. The edible parts of fruits were frozen and lyophilized during 72 h and after that samples were pulverized and stored until their analysis. The NIR spectra were acquired by using a Bruker® MPA (Bremen, Germany) FT-NIR analyzer equipped with an air cooled NIR source, TE-InGaAs detector and a quartz beam splitter. The scanner of the interferometer was operated at a He–Ne laser modulation frequency of 10 kHz and total reflectance spectra of samples were obtained in glass vials of 20 mL with an internal diameter of 23 mm. The spectral range considered covered between 740 and 2700 nm spectral region and spectra were obtained with a resolution of 4 cm^{-1} and cumulating 50 scans.

2.2. Data set and treatment

NIR spectra of 166 persimmon samples from 7 regions of Spain (see Fig. 2) were splitted in 2 subsets. In this regards, 116 samples were randomly selected from the data set to form the training set and the remaining 50 samples were used as independent test set for the external evaluation of the different models built. Chemometric data processing was performed by using MATLAB Ver. 8.0 and appropriate toolboxes. The first step in chemometric data treatment was to evaluate the data set by principal component analysis (PCA). Then the capabilities of cluster analysis (CA) in data classification were evaluated. Next step was to conduct the Orthogonal Signal Correction (OSC) pre-treatment and to track the origin pattern recognition of persimmon samples by least squares-support vector machine (LS-SVM). In order to classify the NIR spectra, samples from Kaki Ribera del Xúquer were labeled as class 1, the Ribera area samples produced outside the PDO were class 2, Andalucía samples were class 3, while Valencia, Alicante, Castellón and Extremadura samples were class 4, 5, 6 and 7 respectively.

3. Results and discussion

3.1. Initial NIR spectra evaluation

As observed in Fig. 2 absorbance bands obtained from total reflectance of lyophilized kaki samples measured inside glass vials are due to overtones and combination vibrations. There is a weak signal around 1400 nm due to first stretching vibration overtone

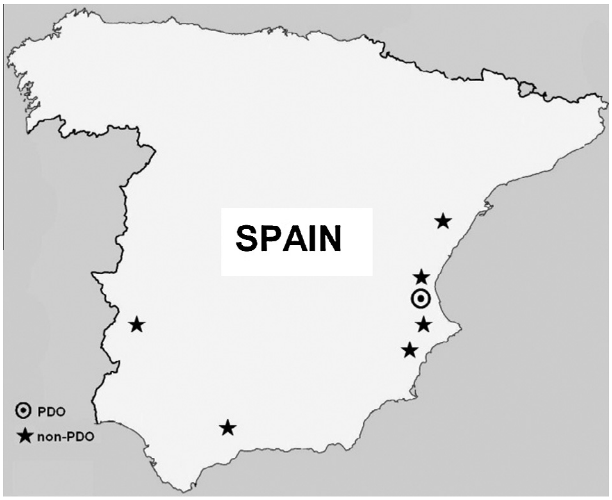

Fig. 1. Map of Spain indicating the different regions where the persimmon fruit samples employed through this study were collected.

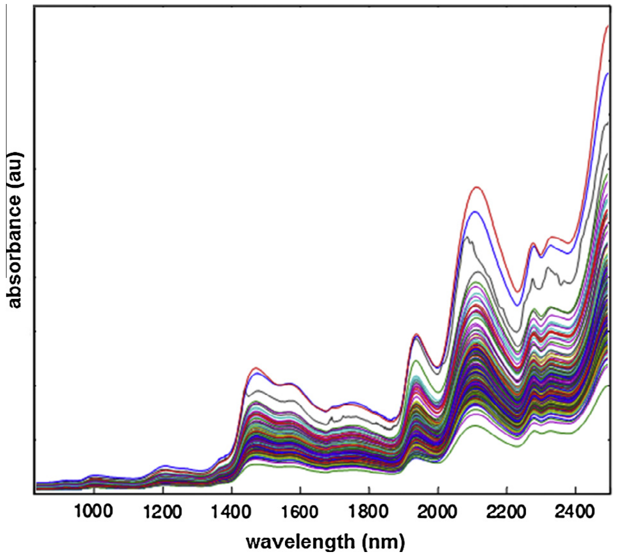

Fig. 2. Raw FT-NIR spectra of persimmon samples employed through this study.

of O–H. This peak is related to phenolic compounds and organic acids that they are main ingredients in persimmon (Park et al., 2006). Also the amine based structure provided signals around 2100 nm. In data processing all of the wavelengths were used because the results showed that application of the domain completely, decreases the error of prediction. In order to study the general possibility for locating the persimmon samples in different sub-spaces, all the spectra were projected in a 2 dimension space based on the use of 2 different wavelengths randomly selected (see Fig. 3). The allocating plot of data by using the aforementioned two variables, 2010.6 and 2497.7 nm, confirmed that there is no adequate separation between classes based only on the crude spectra.

3.2. Orthogonal Signal Correction (OSC)

Multivariate analysis based on NIR spectra often needs pre-processing as an essential step. Systematic variation with NIR spectra unrelated to responses may disturb the multivariate modeling and cause prediction errors. Systematic variation in the data can be removed by differentiation and signal correction methods. The only concern dealing with these methods may be the probable removal of some useful information from original data set. Orthogonal Signal Correction (OSC) (Wold et al., 1998) is a method that performs uncorrelated signal removing by considering the response. It also removes information which is uncorrelated to the response. The spectra after OSC are shown in Fig. 4 in which it can be also seen the presence of one outlier.

3.3. Cluster analysis (CA) and principal component analysis (PCA)

The CA discrimination is an unsupervised classification technique (Almeida et al., 2007; Sharma, 1996) in chemometrics and it is successful after OSC pre-processing in discrimination among classes. The CA discrimination was performed utilizing Mahalanobis distance, the parameter which determines the similarity between samples (see Fig. 5). Regions B and D are those parts of the data set in which the CA classification had been successful while in A and C regions, there are several misclassified samples. In the A region, samples from classes 2 and 3 are complicatedly present while the B region consists of only class 1 samples. Samples from class 4 are in the C region while classed 5, 6 and 7 are disordered in the D region.

As it can be observed in Fig. 5 the overall scheme of classification after OSC treatment of persimmon NIR spectra is a binary system for distances higher than 0.6 and the absolute distance in which the performance of discrimination is visually realized would be seriously doubtful. It is obvious from the dendrogram that the quality of classification is not reliable enough to be evaluated as a practical way for origin based authentication of persimmon samples.

PCA is a well-known chemometric technique (Flury and Riedwyl, 1988) for size reduction in multivariate data analysis. It is commonly used for constructing new dimensions of data set by reducing the total number of variables into a small one displaying most of the original variability of data in a reduced space. The elements of the new space are linear combinations of the original variables, orthogonal and contain the maximum variance within them. The PCA output consists of a spectrum like patterns, called loading matrix, representing the principal components (PC's) and

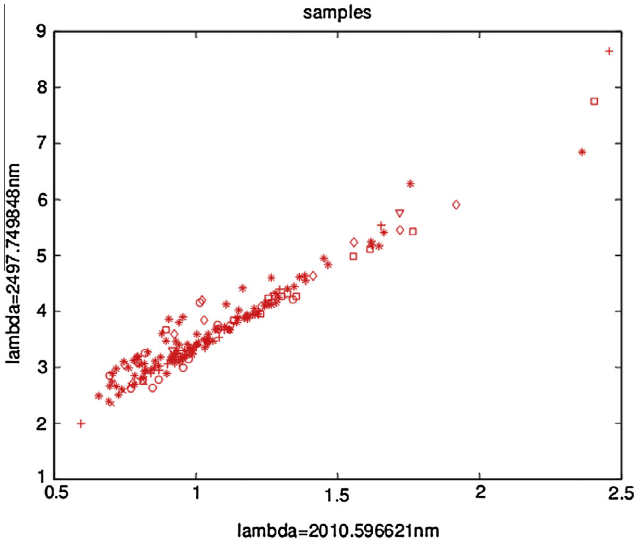

Fig. 3. NIR spectra data distribution plot obtained by using the two wavelengths 2010.6 and 2497.7 nm. Note: identification of sample classes: class 1 (✱), class 2 (□), class 3 (+), class 4 (○), class 5 (×), class 6 (◇) and class 7 (▽).

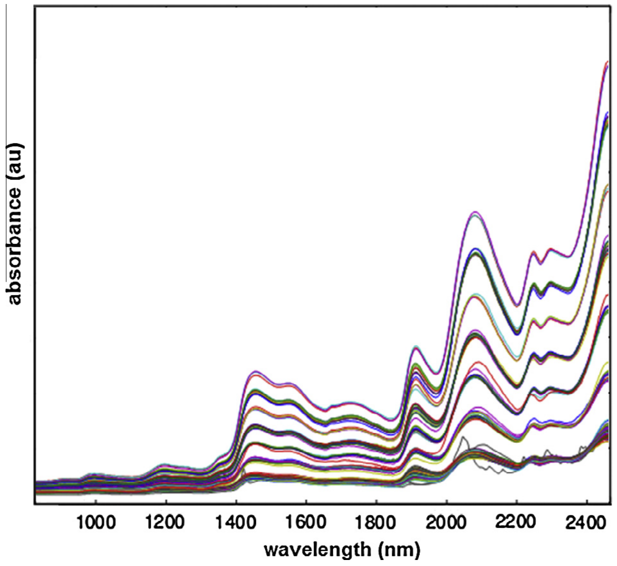

Fig. 4. FT-NIR spectra of persimmon samples obtained after OSC.

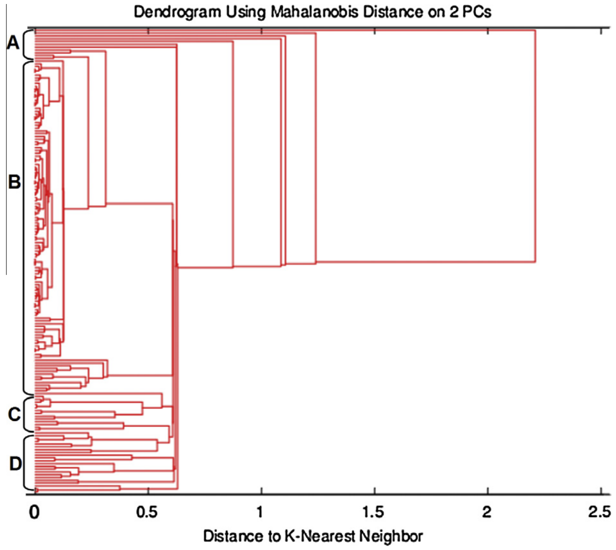

Fig. 5. Dendrogram of CA on persimmon NIR spectra after OSC.

a score matrix, which provides the coordinates of the original spectra on the new axes determined by the corresponding PC's.

However, constructing the PCA model based on the OSC treated data; an initial separation between the 7 classes would be clearly obtained (see Fig. 6). Score plot for OSC treated data shows a justifiable separation within classes in a well-defined scheme that correlates well with geographical distances between them. As an example, samples from Alicante, Castellón and Valencia, which are close regions are also close in the PCA graph. Scores of samples in class 1, class 2 and class 3 are less distributed across the 2nd PC. On the other hand, both PDO and non-PDO samples from Ribera region are also close located. The only deviation is related to Extremadura and Andalucía samples that in spite of the proximity of their geographical origin would far PC location.

3.4. Least squares-SVM (LS-SVM)

Support vector machines (SVM) is a powerful method for classification and regression aims (Vapnik, 1995). Training process in SVM is a quadratic programming problem (Tao et al., 2009) and the technique is originally developed for binary classification. It maps the original data points from original data space to high or infinite dimensional feature space. Then, a hyper plane is constructed to discriminate the classes (Lutsa et al., 2010; Suykens and Vandewalle, 1999). Data set is $\{x_i, y_i\}_{i=1}^{N}$ with $x_i \in R^d$ that they are input vectors and $y_i \in \{-1, +1\}$ that they are the class labels. Mapping of data from real space to feature space is performed by using kernel function which could be a linear or non-linear function or radial basis function (RBF). The most popular kernel function used in SVM is RBF. Each kernel function consists of a set of tunable parameters. In the case of RBF there is only one parameter (σ) which is radial width of kernel function. In the SVM classification approaches C and σ are two mathematical parameters that they should be optimized. Where C is positive regularization constant and σ radial width of kernel function.

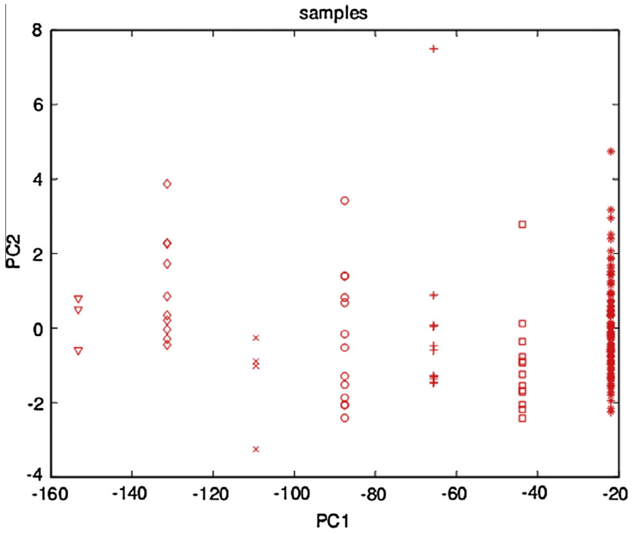

Fig. 6. PCA score pot obtained for persimmon sample NIR spectra after performing OSC. Note: identification of sample classes: class 1 (❋), class 2 (□), class 3 (+), class 4 (○), class 5 (×), class 6 (◇) and class 7 (▽).

SVM is originally a binary classification method but by utilizing some strategies it can be used for discriminating more than two classes. In one of main strategies the multiclass problem is divided into several binary class problems and the results of all binary classifications are combined. In every stage discrimination of one class is performed from other classes. This strategy is named one against all. In another strategy, k classes of multiclass problem are converted to $k(k-1)/2$ binary classifiers and each classifier separates two classes of the total k classes. This strategy is named one against one. In least-squares SVM (LS-SVM) solution of problem is obtained by determining a set of linear equations instead of quadratic programming (Vapnik, 2000). Utilizing this method, the complexity is reduced significantly. In LS-SVM positive regularization constant is shown by γ.

3.5. Recognition of the origin of persimmon samples

LS-SVM was performed for predicting the geographical origin of persimmon samples based on their NIR spectra. Training set was used for model construction with and without OSC pre-processing while PCA was previously conducted. First two PCs were used and LS-SVM parameters (γ and σ) were tuned by simplex and leave one out cross validation methods. The interval used for tuning of each of the parameters was [exp(−10) exp(10)]. Different combination or binary set of the aforementioned parameters were used to make LS-SVM model to get minimum error of cross validation. Considering these routes, the LS-SVM optimized parameters were 0.2 for γ and 2.8 for σ in raw LS-SVM while they were 2.6 for γ and 1.3 σ OSC supported LS-SVM. One against one strategy was utilized for this multiclass recognition case, trying to locate each spectrum in a unique position in the LS-SVM space. The statistical results for this classification issue are shown in Table 1, demonstrating the dramatic effect of OSC on SVM output. Comparing the schematic output of SVM (Fig. 7A) with that of OSC-SVM (Fig. 7B), it is obviously concluded that role of OSC is highly important in achieving a robust classification mode. One of the main properties of the developed method is that we can easily and exactly report the error of the work in comparison with other reported methods which determine the origin qualitatively. Also, the results of proposed method

Table 1
Statistical output of SVM different models for classification of persimmon samples.

	SVM	OSC-SVM
Training error	28%	0%
Prediction error	24%	2%
Training R^2	0.33	1.00
Prediction R^2	0.15	0.99

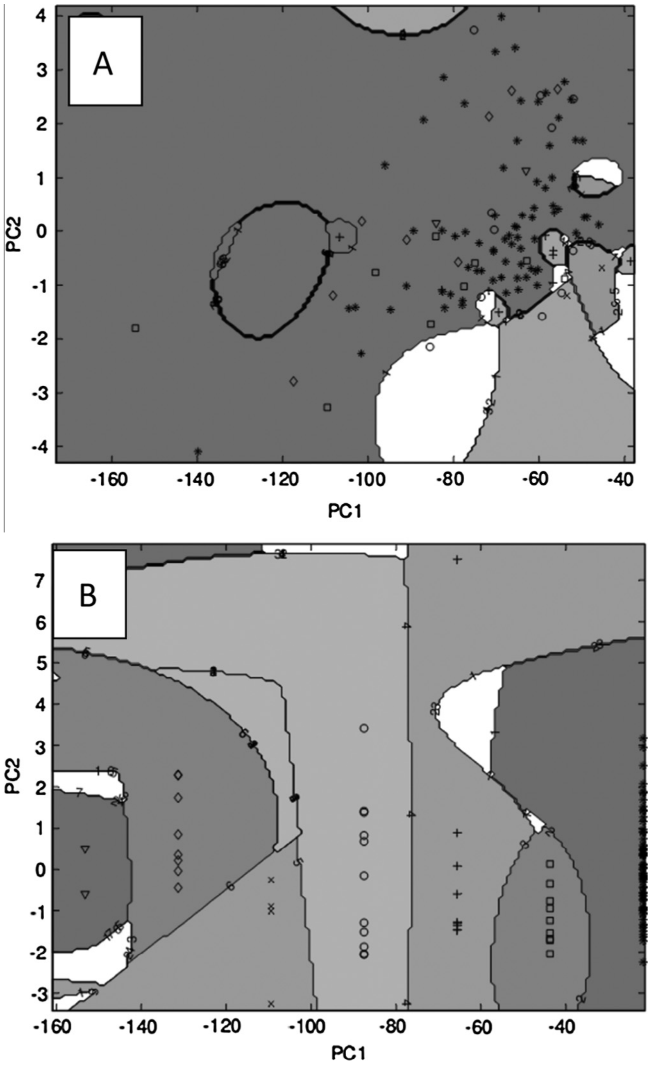

Fig. 7. Classification of NIR persimmon data by LS-SVM without performing OSC (A) and after performing OSC (B). Note: identification sample classes: class 1 (✱), class 2 (□), class 3 (+), class 4 (○), class 5 (×), class 6 (◇) and class 7 (▽).

are not influenced by some parameters such as pollution of the skin of fruits during the transportation.

4. Conclusion

Authentication of the origin, nutritional characteristics and performing quality control of fruits is one of the most important needs in food industry. In order to achieve this goal it could be utilized different methods. Most of these techniques employ complicated chemical analysis. Consequently, they are time consuming and expensive. Also, they use chemicals, such as solvents for separating ingredients, causing environmental concerns and for this reason they are not environmental green methods. Thus developing new methods for persimmon evaluating is an important task. This study evidenced that LS-SVM classification method, after OSC treatment of NIR spectra for noise removing from signals, can be used as an easy and fast approach with high reliability for the authentication of the geographical origin of persimmon fruits cultivated in various regions of Spain, thus providing a green alternative to other authentication procedures based on the determination of chemical parameters of samples after their digestion or dissolution. Additionally, it is clear that the developed methodology could be also suitable for the authentication of other foods produced in different areas and covered by protected designation of origin (PDO) or protected geographical indications (PGI).

References

Almeida, J.A.S., Barbosa, L.M.S., Pais, A.A.C.C., Formosinho, S.J., 2007. Improving hierarchical cluster analysis: a new method with outlier detection and automatic clustering. Chemom. Intelligent Lab. Syst. 87 (2), 208–217.

Birth, G.S., Dull, G.G., Renfroe, W.T., Kays, S.J., 1985. Nondestructive spectrophotometric determination of dry matter in onions. J. Am. Soc. Horticultural Sci. 110 (2), 297–303.

Colquhoun, I.J., 1998. High resolution NMR spectroscopy in food analysis and authentication. Spectrosc. Europe 10 (1), 8–18.

Davies, A.M., Grant, A., 1987. Review: near infra-red analysis of food. Int. J. Food Sci. Technol. 22 (3), 191–207.

De la Guardia, M., Gonzálvez, A. (Eds.), 2013. Food Protected Designation of Origen: Methodologies and Applications. Elsevier, Amsterdam.

El Sheikha, A.F., Condur, A., Métayer, I., Nguyen, D.D., Loiseau, G., Montet, D., 2009. Determination of fruit origin by using 26S rDNA fingerprinting of yeast communities by PCR-DGGE: preliminary application to Physalis fruits from Egypt. Yeast 26 (10), 567–573.

Fan, G., Zha, J., Du, R., Gao, L., 2009. Determination of soluble solids and firmness of apples by Vis/NIR transmittance. J. Food Eng. 93 (4), 416–420.

Feugel, R., Carle, R., Schieber, A., 2004. A novel approach to quality and authenticity control of fruit products using fractionation and characterisation of cell wall polysaccharides. Food Chem. 87 (1), 141–150.

Flury, B., Riedwyl, H., 1988. Multivariate Statistics—A Practical Approach. Chapman & Hall, London.

Gómez-Caravaca, A.M., Maggio, R.M., Verardo, V., Cichelli, A., Cerretani, L., 2013. Fourier transform infrared spectroscopy–Partial Least Squares (FTIR–PLS) coupled procedure application for the evaluation of fly attack on olive oil quality. Lebensmittel-Wissenschaft & Technologie 50 (1), 153–159.

Hvattum, E., 2002. Determination of phenolic compounds in rose hip (Rosa canina) using liquid chromatography coupled to electrospray ionisation tandem mass spectrometry and diode-array detection. Rapid Commun. Mass Spectrom. 16 (7), 655–662.

CRDO Consejo Regulador de la Denominación de Origen “Kaki Ribera del Xúquer”. Available (2011) from: <http://www.kakifruit.com>.

Licciardello, F., Muratore, G., Avola, C., Tomaselli, F., Maccarone, E., 2011. Geographical origin assessment of orange juices by comparison of free aminoacids distribution. In: ISHS Acta Horticulture 892: II International Symposium on Citrus Biotechnology <http://www.actahort.org/books/892/892_48.htm>.

Lutsa, J., Ojeda, F., Van de Plas, R., De Moor, B., Van Huffel, S., Suykens, J.A.K., 2010. A tutorial on support vector machine-based methods for classification problems in chemometrics. Anal. Chim. Acta 665 (2), 129–145.

Mowat, A.D., Poole, Philip R., 1997. Non-destructive discrimination of persimmon fruit quality using visible-near infrared reflectance spectrophotometry. Acta Hort. (ISHS) 436, 159–164, http://www.actahort.org/books/436/436_17.htm.

Ni, Y., Mei, M., Kokot, S., 2011. Analysis of complex, processed substances with the use of NIR spectroscopy and chemometrics: classification and prediction of properties – the potato crisps example. Chemom. Intelligent Lab. Syst. 105 (2), 147–156.

Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., Lammertyn, J., 2007. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: a review. Postharvest Biol. Technol. 46 (2), 99–118.

Norris, K.H., 1964. Design and development of a new moisture meter. Agric. Eng. 45 (7), 370–372.

Park, Y., Jung, S., Kang, S., Delgado-Licon, E., Ayala, A.L.M., Tapia, M.S., Martin-Belloso, O., Trakhtenberg, S., Gorinstein, S., 2006. Drying of persimmons (*Diospyros kaki* L.) and the following changes in the studied bioactive compounds and the total radical scavenging activities. LWT 39 (7), 748–755.

Ribeiro, J.S., Salva, T.J., Ferreira, M., 2010. Chemometric studies for quality control of processed Brazilian coffee using DRIFTS. J. Food Quality 33 (2), 212–227.

Roy, S., Anantheswaran, R., Shenk, J., Westerhaus, M.O., Beelman, R., 1993. Determination of moisture content of mushrooms by Vis—NIR spectroscopy. J. Sci. Food Agric. 63 (3), 355–360.

Sârbu, C., Nascu-Briciu, R.D., Kot-Wasik, A., Gorinstein, S., Wasik, A., Namiesnik, J., 2012. Classification and fingerprinting of kiwi and pomelo fruits by multivariate analysis of chromatographic and spectroscopic data. Food Chem. 130 (4), 994–1002.

Sharma, S., 1996. Applied Multivariate Techniques. Wiley, New York.

Silva, B.M., Andrade, P.B., Valentao, P., Mendes, G.C., Seabra, R.M., Ferreira, M.A., 2000. Phenolic profile in the evaluation of commercial quince jellies authenticity. Food Chem. 71 (2), 281–285.

Suykens, J.A.K., Vandewalle, J., 1999. Least squares support vector machine classifiers. Neural Process. Lett. 9 (3), 293–300.

Tao, S., Chen, D., Zhao, W., 2009. Fast pruning algorithm for multi-output LS-SVM and its application in chemical pattern classification. Chemom. Intelligent Lab. Syst. 96 (1), 63–69.

Terdwongworakul, A., Nakawajana, N., Teerachaichayut, S., Janhiran, A., 2012. Determination of translucent content in mangosteen by means of near infrared transmittance. J. Food Eng. 109 (1), 114–119.

Vapnik, V.N., 1995. The Nature of Statistical Learning Theory, second ed. Springer-Verlag, New York.

Vapnik, V.N., 2000. The Nature of Statistical Learning Theory, second ed. Springer, New York.

Wang, L., Mizaikoff, B., 2008. Application of multivariate data-analysis techniques to biomedical diagnostics based on mid-infrared spectroscopy. Anal. Bioanal. Chem. 391 (5), 1641–1654.

Wold, S., Antti, H., Lindgren, F., Ohman, J., 1998. Orthogonal signal correction of near-infrared spectra. Chemom. Intelligent Lab. Syst. 44 (1–2), 175–185.

Xu, Y., Zomer, S., Brereton, R.G., 2006. Support vector machines: a recent method for classification in chemometrics. Critical Rev. Anal. Chem. 36 (3–4), 177–188.

Food Chemistry 196 (2016) 1023–1030

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Green direct determination of mineral elements in artichokes by infrared spectroscopy and X-ray fluorescence

Alba Mir-Marqués, Maria Martínez-García, Salvador Garrigues, M. Luisa Cervera, Miguel de la Guardia *

Department of Analytical Chemistry, University of Valencia, Jeronimo Muñoz Research Building, 50th Dr. Moliner Str. 46100 Burjassot, Valencia, Spain

ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form 2 October 2015
Accepted 11 October 2015

Keywords:
Cynara Scolymus L.
Near infrared spectroscopy (NIR)
X-ray fluorescence (XRF)
Mineral elements
Partial least squares (PLS)
Green methods

ABSTRACT

Near infrared (NIR) and X-ray fluorescence (XRF) spectroscopy were investigated to predict the concentration of calcium, potassium, iron, magnesium, manganese and zinc in artichoke samples. Sixty artichokes were purchased from different Spanish areas (Benicarló, Valencia and Murcia). NIR and XRF spectra, combined with partial least squares (PLS) data treatment, were used to develop chemometric models for the prediction of mineral concentration. To obtain reference data, samples were mineralised and analysed by inductively coupled plasma optical emission spectrometry (ICP-OES). Coefficients of determination obtained for the regression between predicted values and reference ones for calcium, potassium, magnesium, iron, manganese and zinc were 0.61, 0.79, 0.53, 0.77, 0.54 and 0.60 for NIR and 0.96, 0.93, 0.80, 0.79, 0.76 and 0.90 for XRF, respectively. Both assayed methodologies, offer green alternatives to classical mineral analysis, but XRF provided the best results in order to be used as a quantitative screening method.

1. Introduction

The determination of the mineral profile in foods is commonly made by using atomic spectroscopy and mass spectrometry methods, being the most common used techniques for this purpose inductively coupled plasma optical emission (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) (dos Santos et al., 2013; Kelly & Bateman, 2010; Mahmood, Anwar, Iqbal, Bhatti, & Ashraf, 2012; Mir-Marqués, Domingo, Cervera, & de la Guardia, 2015; Mitic et al., 2012). Mineral analysis in artichokes has been done commonly by the aforementioned techniques (Harmankaya, Al Juhaimi, & Musa Özcan, 2012; Paudino, Lombardo, & Mauromicale, 2011; Terzic et al., 2012). These techniques are very useful for the multi-elemental analysis of samples in a few minutes. However, to do it, a costly instrumentation is required and liquid samples that involves the use of strong acids and other reagents, apparatus, energy and time-consuming, also generating a lot of wastes. A green alternative for the evaluation of the presence of mineral elements in solid foods could be a methodology that combined fast sample preparation, or no sample pre-treatment, easy detection of the target analysis and minimal generation of residues. Near infrared (NIR) spectroscopy has been employed as an alternative to wet chemistry-based atomic spectroscopy procedures for food analysis (Schmitt, Garrigues, & de la Guardia, 2014). Although correlations between spectra and mineral element contents seem unreliable, prediction of some major mineral by NIR may be possible through their association with the organic matrix and based on the use of chemometrics to model the NIR spectra from a series of samples well characterised in order to determine their mineral profile in a series of samples, including some foods (de la Guardia & Garrigues, 2015). Other green alternative for the analysis of minerals in foods could be based on X-ray fluorescence (XRF). This technique is very commonly employed in geological materials, archaeology objects, and environmental samples, but it is not very often used to food and beverage analysis (West et al., 2010). XRF, specially the techniques based on energy dispersion (EDXRF), bring great advantages to the analysis of mineral elements by ICP-OES or ICP-MS, as: the elimination of sample chemical pre-treatment, the lack of use of reagents and acids, the direct and rapid measurement of spectra and the fact that XRF provides a non-destructive sample technique thus deleting the waste generation and reducing drastically the environmental side effects and costs (de la Guardia & Garrigues, 2015).

However, the main disadvantage of both, NIR and XRF, is their low sensitivity and high matrix effects. Therefore, it is essential to use chemometric tools to built appropriate calibration models from the spectra of well characterised samples in order to increase the prediction capability of measurements made (de la Guardia & Garrigues, 2015; Schmitt et al., 2014).

---

* Corresponding author.
E-mail address: miguel.delaguardia@uv.es (M. de la Guardia).

http://dx.doi.org/10.1016/j.foodchem.2015.10.048
0308-8146/

The combination of NIR and XRF measurements with chemometric data treatment could offer exciting possibilities for the direct determination of mineral components in food samples. Partial least squares (PLS) or modified-partial lest squares (MPLS) have been employed for building models proposed in the literature to do the quantitative determine mineral elements in vegetables (Cozzolino, Cynkar, Shah, & Smith, 2011; Cozzolino & Moron, 2004; Manhas Verbi Pereira, Rodrigues Pereira-Filho, & Maretti Silveira Bueno, 2006; Moros et al., 2008).

Artichoke (*Cynara Scolymus* L.) is one of the oldest cultivated plants, and it is used as a food since the 15th century. There are different types of artichokes and Spain is one of the most important producers in the European Union. The 'Blanca de Tudela' variety is labelled as protected designation of origin (PDO) 'Alcachofa de Benicarló' (Castellón, Spain) and artichokes are also produced in different areas of the Basc country, Aragon, Valencia and Murcia. Artichoke is a very good food for diabetics and also favours the fat and urea metabolism, contributing to the regulation of liver and kidney functions (CRDO., 2014). Artichokes contain a lot of minerals such as calcium, potassium, magnesium, iron, manganese and zinc. Controlling minerals in artichoke is important to maintain and verify the quality control of these products before reaching the consumer and to generate a correct daily intake of essential elements.

The main purpose of this study was the development of a green analytical methodology for mineral element determinations in artichokes, based on NIR and XRF spectra with PLS treatment in order to provide a green methodology useful for quality control of essential minerals in solid foods.

2. Materials and methods

2.1. Samples

60 artichoke samples from three different Spanish origins were analysed, 38 samples were produced under the PDO 'Alcachofa de Benicarló' (Castellón, Spain), 12 samples from Valencia (Spain) and 10 from Murcia (Spain). Each sample was a combination of 2–4 artichokes of the same field. Samples were washed with ultrapure water with a resistivity of 18.2 MΩ cm, obtained using a Milli-Q plus Millipore system (Molsheim, France), and the edible parts were lyophilised in a Cryodos 50 lyophiliser Telstar (Barcelona, Spain) for a minimum of 48 h at a chamber pressure of 50 Pa. Then, samples were pulverised and homogenised with a domestic Braun mixer (Kronberg, Germany). Finally, samples were sieved through a particle size of 250 μm and stored in polyethylene bottles until their analysis.

2.2. Reference procedure

Reference data were obtained by ICP-OES using an Optima 5300 DV Perkin Elmer (Norwalk, CT, USA), equipped with an auto sampler AS 93-plus and a GemCone nebuliser, after microwave-assisted digestion using a Milestone Ethos SEL microwave laboratory system (Sorisole, Italy), equipped with a thermocouple probe for automatic temperature control, and an automatic gas leak detector, using high pressure Teflon vessels of 100 mL inner volume. For samples digestion, 0.5 g of each freeze-dried sample were accurately weight inside Teflon reactors and 10 mL of concentrated HNO_3 (69% for trace analysis, Scharlau, Barcelona, Spain) were added. Reactors were closed and placed inside the microwave oven using a digestion step of 15 min at 180 °C (700 W) (Mir-Marqués, Cervera, & de la Guardia, 2012). Digested samples were measured with a final dilution volume of 50 mL. The calibration range for iron, manganese and zinc was established from 0.01 to 2 mg L^{-1}, for calcium and magnesium it was made from 10 to 100 mg L^{-1} and for potassium was made from 20 to 900 mg L^{-1}. Rhenium (1 mg L^{-1}) was used as internal standard and added to all samples, reagent blanks and standards. The average concentration of blanks was subtracted from the analytical signals of digested samples after interpolation on the calibration graphs. Results were expressed as a basis of dry sample weight. All samples were analysed in duplicate together with blanks and certified reference materials, Tomato Leaves (NIST 1573a, National Institute of Standards and Technology, Gaithersburg, MD, USA) and Cabbage (IAEA-359, International Atomic Energy Agency, Vienna, Austria), were measured in each session in order to control possible contaminations and to verify the accuracy of the methods. Limits of detection (LOD) were calculated as three times the standard deviation of reagent blanks. LODs for calcium, potassium, magnesium, iron, manganese and zinc were 0.22, 0.24, 0.07, 0.06, 0.08 and 0.017 mg kg^{-1} respectively, taking into account the amount of samples assayed and the final dilution employed for determinations.

2.3. NIR procedure

0.5–1 g of dry samples were placed inside glass vials. The vials were rotated to obtain triplicate measurements for each sample. For acquisition data diffuse reflectance infrared Fourier transform (DRIFT) were recorded in Kubelka–Munk units, in the range of 12500–3500 cm^{-1}, with spectral resolution of 4 cm^{-1} and cumulating 50 scans per spectrum using a MPA model Multipurpose Analyser Fourier transforms near-infrared (FT-NIR) spectrometer, from Bruker (Bremen, Germany), equipped with an integrating sphere. The background spectrum was acquired from the closed integrating sphere using the same instrumental conditions than those employed for samples. For instrument control and data acquisition, the OPUS software 6.5 from Bruker was used.

2.4. XRF procedure

0.3 g of dry samples were placed inside a pellet box and were compacted using a hydraulic press for 1 min at a pressure of $5.1 \cdot 10^3$ kg. Pellets were stored inside desiccators to avoid hydration from air humidity. XRF spectra were obtained by triplicate at 50 kV, 11 μA and 60 s acquisition time (30 s for high atomic number elements, and 30 s for low atomic number elements) using a portable model S1 Titan energy dispersive X-ray fluorescence (ED-XRF) spectrometer from Bruker equipped with a Rhodium X-ray tube and X-Flash® SDD detector. For instrument control S1RemoteCtrl and S1Sync software from Bruker were employed; and for spectra treatment the ARTAX software from Bruker was used.

2.5. Chemometrics data treatment

Models for the prediction of mineral element content of artichoke samples from their spectral measurements were performed using partial least squares regression (PLS) by Matlab 2014a software from Mathworks (Natick, MA, USA) and the PLS Toolbox 6.2 from Eigenvector Research Inc. (Wenatchee, WA, USA). PLS regression is a technique used to evaluate data that contain correlated variables. This technique creates new predictor variables, known as latent variables, as linear combinations of the original variables, while considering the observed response values, leading to a model with reliable predictive power. So, PLS analysis is based on the use of combinations of the predictors that have a large covariance with the response values (Wise et al., 2006). In this study, PLS was used to build the relationship between the spectral data matrix (X) and reference concentration of elements matrix (Y) for predicting the concentration of a separate set of samples with the smallest number of latent variables (LV) (Wold, Sjöström, & Eriksson, 2001). For a reliable estimation of the prediction capability of chemometric models built, the preliminary data set, including the spectra matrix (X) and the reference concentration data (Y), must be divided into separate calibration and validation sets. Cross-validation was used to validate the quality of the models and to prevent over-fitting of the calibration. For a given set of data, cross-validation involves a series of steps called sub-validation ones in which a subset of objects is removed from the set of data, to build a model using the remaining objects as the set of data, and then applying the resulting model to the removed objects (Wise et al., 2006). In all cases, leave-one-out cross validation (CV) was employed for the selection of the optimum number of latent variables of the models according to the root mean square error of cross validation (RMSECV) values. After selection of the best parameters as variable and data pre-processing, the model must be evaluated using an independent set of measurements obtained from samples included in the validation set, which were not employed in building and optimizing the model. So, it permits to obtain a non-overoptimistic value of the prediction error.

Kennard–Stone (KS) algorithm was assayed to select the most appropriate sample calibration set. KS is a method for the selection of a representative subset of samples based only on the instrumental signals. The selected subset should cover the experimental region homogeneously by choosing objects which are uniformly distributed in the experimental space (Kennard & Stone, 1989). Different data pre-treatment, such as, mean centring (MC), standard normal variate (SNV), Savitzky–Golay first derivate (FD) and also combinations of them, were evaluated for NIR and XRF measurements. Samples from the validation set were analysed in the selected conditions. Mean-centring calculates the mean of each variable and subtract this from the obtained values. Another way of interpreting mean-centred data is that, after mean-centring, each row of the mean-centred matrix data include only how that row differs from the average sample in the original data matrix. The SNV normalization method is a weighted normalization, in order to reflect that not all points contribute to the normalization equally. SNV calculates the standard deviation of all the pooled variables for the given sample. The entire sample is then normalized by this value. First derivative is based on the use of first order derivative signals instead of zero order ones. It permits to remove the signal which is the same between the two variables and leaves only the part of the signal which is different. Derivatives de-emphasize low frequencies and emphasize high frequencies, they tend to accentuate noise and for this reason, the Savitzky–Golay algorithm is often used to simultaneously smooth the data as greatly improving the utility of derivative signals (Wise et al., 2006).

3. Results and discussion

3.1. NIR spectra of artichokes

Fig. 1 shows the typical near DRIFT spectra obtained between 9000 and 4000 cm^{-1} for artichoke samples from different Spanish origin. The first spectrum is the average of those found for Benicarló samples, the second that is corresponds to Murcia samples and the last one is that obtained for Valencia samples. The spectra regions between 12500–9000 cm^{-1} and 4000–3500 cm^{-1} were removed for data processing. The broad and strong peak around 8300 cm^{-1} was due to the second overtone C—H stretching vibrations. At 7000–6800 cm^{-1} it can be seen a broad peak, which corresponds to the combination of C—H and the N—H stretch first overtone. Peak at 5800 cm^{-1} corresponds to the C—H stretch first overtone. O—H first and second overtone can be seen at 5155 cm^{-1}. A strong peak at 4732 cm^{-1} was due to C—O stretch combination and O—H bend, characteristics of carbohydrates. Double peak at 4350 and 4250 cm^{-1} were due to C—H and CH_2 bend second overtone, corresponding to proteins (Burns & Ciurczack, 2007).

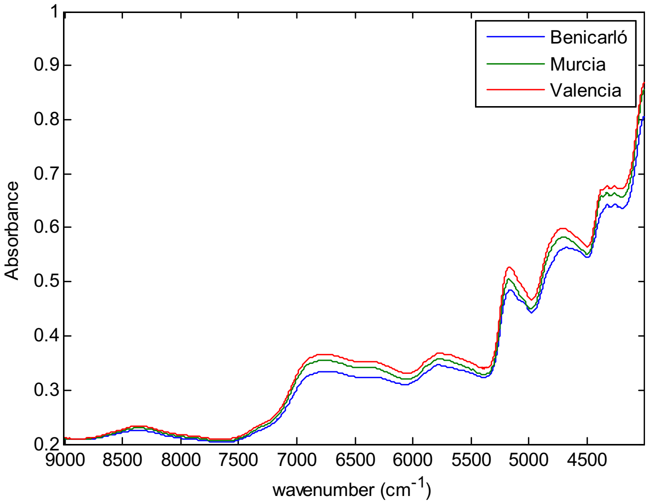

Fig. 1. Mean DRIFT spectra of artichoke samples.

3.2. PLS-NIR models for determination of mineral elements in artichokes

The complete data set of spectra was divided in two independent sets, calibration and validation, with 43 and 17 samples, respectively, using KS algorithm. In order that the calibration set could be representative of all the samples considered, it was composed by 24 Benicarló samples, 10 Valencia samples and 9 Murcia samples. The concentration range, mean and standard deviation for the elements determined in artichokes for both, calibration and validation sets, are shown in Table 1. In all PLS-NIR models, from the total spectral region (4667 variables) only 2796 variables (4000–9000 cm^{-1}) were used to build the models. These variables were removed because they contain useless or irrelevant information for the mineral element determinations, such as noise and background, which can worse the predictive ability of PLS-NIR models. For the selection of the optimum number of LV, several parameters; such as the predictive residual error sum of squares (PRESS), root mean square error for cross-validation (RMSECV) and X and Y explained variances were evaluated (see Table 1). For the micronutrients, iron, manganese and zinc, 2, 3 and 10 LV, respectively, were necessary. The RMSECV values were 7.2 (iron), 3.1 (manganese) and 8.2 mg kg^{-1} (zinc), and the explained variances for X and Y were 97.4% and 83.4% for iron, 96.3% and 79.6% for manganese and 99.9% and 95.3% for zinc, respectively. For the macronutrients, calcium, potassium and magnesium, 13, 4 and 10 LV were needed to build the corresponding PLS models. Explained variances for X and Y were 99.7% and 97.1% for calcium, 97.4% and 78.0% for potassium and 97.4% and 97.8% for magnesium.

Ratio of performance to deviation (RPD) values was calculated for all models built (see Table 1). RPD was calculated as standard deviation of the samples divided by root mean squared error for each model. According to Bellon-Maurel, Fernandez-Ahumada, Palagos, Roger, and McBratney (2010) if RDP is higher than 2 the model can be considered as excellent, if RPD values is between 1.4 and 2 the model is fair but if RPD is lower than 1.4 the model

Table 1
Reference data and statistics of mineral content of artichokes used for calibration and validation sets employed for PLS-NIR determination.

Element	Set	Samples	Range	Mean	SD	Pre-process	LV	R^2	RMSE	RPD	RRMSEP
Ca	CAL	43	900–3384	1715	594	MC + FD	13	0.97[a]	94[a]	6.3[a]	19.2
	VAL	17	970–3368	1850	623			0.61[b] (0.52)[c]	355[b] (393)[c]	1.8[b] (1.5)[c]	
K	CAL	43	25882–50541	39935	6814	MC + FD	4	0.77[a]	3116[a]	2.2[a]	4.5
	VAL	17	32923–47329	42267	4148			0.79[b] (0.61)[c]	1900[b] (4070)[c]	2.2[b] (1.7)[c]	
Mg	CAL	43	1816–3523	2834	447	MC + FD	10	0.98[a]	59[a]	7.6[a]	9.4
	VAL	17	2325–3533	2874	325			0.53[b] (0.70)[c]	271[b] (226)[c]	1.2[b] (2.0)[c]	
Fe	CAL	43	17.7–70.5	47.9	15.1	MC	2	0.83[a]	6.2[a]	2.4[a]	11.7
	VAL	17	27.0–78.7	53.9	13.3			0.77[b] (0.77)[c]	6.3[b] (7.2)[c]	3.7[b] (2.1)[c]	
Mn	CAL	43	6.7–28.9	18.1	6.3	MC	3	0.80[a]	2.6[a]	2.4[a]	14.3
	VAL	17	10.5–24.7	19.6	4.1			0.54[b] (0.72)[c]	2.8[b] (3.1)[c]	1.5[b] (2.0)[a]	
Zn	CAL	43	11.7–61.6	32.9	12.8	MC	10	0.90[a]	3.9[a]	3.3[a]	21.1
	VAL	17	19.1–60.0	38.0	12.1			0.60[b] (0.61)[c]	8.0[b] (8.2)[c]	1.5[b] (1.6)[c]	

NOTE: Range, mean and standard deviation (SD) in mg kg^{-1} dry weight for calibration (CAL) and validation (VAL) data sets. R^2, coefficient of regression for: calibration[a], external validation[b] and cross-validation[c]. RMSE, root-mean-square error for: calibration[a], external validation[b] and cross-validation[c] in mg kg^{-1}; RPD, ratio of performance to deviation for: calibration[a], external validation[b] and cross-validation[c]. RRMSEP, relative root-meansquare error of prediction in percentage (%).

must be considered as non-reliable. For calibration models built from NIR spectra RPD values were greater than 2, in all cases. On the other hand, for calcium, potassium and magnesium, a Savitzky–Golay first derivate, with a window of 11 points and a second order polynomial, and mean centring treatment were chosen as signal pre-processing. For iron, manganese and zinc mean centring was chosen. Additionally, concentration data were mean centred and auto scaled in all cases.

3.3. Prediction capability of PLS-NIR for mineral element determination in artichokes

External validation was used to evaluate the PLS-NIR methodology, using an independent set of artichoke samples, not employed during the calibration step. Predicted values of analytes versus those obtained by the reference method are shown in Fig. 2. In general, it can be seen that the best models are not good enough, since the calibration and validation samples do not fit the regression line with zero intercept and one slope. Good accuracy was obtained in the validation step for potassium and magnesium and acceptable accuracy calcium, iron, manganese and zinc. Average relative prediction errors of models were 18.6% for calcium, 3.9% for potassium, 8.0% for magnesium, 10.4% for iron, 14.0% for manganese and 18.5% for zinc, with coefficients of determination between 0.53 for magnesium and 0.79 for potassium. The precision of the models built was very high. For potassium, iron and manganese, relative standard deviation (RSD) values were lower than 2.5%, and in all cases RSD was lower than 10%. The minimum relative root mean square error of prediction (RRMSEP) obtained was 4.5% for potassium and in all the cases it was lower than 21% (see Table 1). These results, together with RPD values, demonstrate a good predictive capability of the PLS-NIR models developed to predict the concentration of potassium and iron in artichoke samples together with an acceptable prediction for calcium, manganese and zinc concentration, but a non-acceptable prediction of magnesium concentration. Thus is reduced the capability of PLS-NIR as a green alternative for the direct determination of the aforementioned elements in dried samples without a previous sample digestion.

3.4. XRF spectra of artichokes

Fig. 3 shows the typical EDXRF spectra between 0 and 14 keV of artichoke samples from different Spanish origin. The first spectrum corresponds to the average spectrum of Benicarló samples, the second is that corresponds to Valencia samples and the last one is that of Murcia samples. All elements studied provided specific Kα and Kβ bands at lower energies than 10 keV. The Kα lines of magnesium, potassium, calcium, manganese, iron and zinc are at 1.25, 3.31, 3.69, 5.90, 6.40 and 8.64 keV, respectively.

3.5. PLS-XRF models for determination of minerals in artichokes

To build PLS models, 43 samples were used for calibration set (24 Benicarló samples, 10 Valencia samples and 9 Murcia samples) and remaining 17 samples were used for validation set (14 Benicarló samples, two Valencia samples and one Murcia sample). The concentration range, mean and standard deviation for each one of elements determined in artichokes for both, calibration and validation, sets are shown in Table 2. In all PLS-XRF models, from the total spectral region (2047 variables) only 708 variables those corresponding to the energy range (0.9–14 keV) were used for built the models. For the selection of the optimum number of LV, several parameters such as PRESS, RMSECV and *X* and *Y* explained variances were evaluated, the same as those used for the NIR methodology.

For iron, manganese and zinc, six, four and five LV, respectively, were necessary to built good models. Explained variances for *X* and *Y* were 96.9% and 95.5% for iron, 96.3% and 90.8% for manganese and 90.1% and 95.9% for zinc, respectively. For calcium, potassium and magnesium, it was needed five, two and five LV, respectively, to build the best models. Explained variances for *X* and *Y* were 95.0% and 96.7% for calcium, 97.2% and 96.1% for potassium and 92.9% and 91.2% for magnesium. For calibration models built from XRF spectra, RPD values were greater than 2, in all cases. Therefore, these calibration models could be considered as excellent. For potassium, iron, manganese and zinc first derivate and mean centring were used for spectra pre-processing, and for calcium and magnesium only mean centred was chosen. Concentration data were mean centred and auto scaled in all cases. The most important calibration parameters of the PLS-XRF models are summarised in Table 2.

3.6. Prediction capability of PLS-XRF for mineral element determination in artichokes

External validation was used to evaluate the PLS-XRF methodology, using 17 artichoke samples not chose to create the corresponding models. Predicted values of analytes versus those obtained by the reference method are shown in Fig. 4. As it can be seen, calibration and validation sample points were distributed near the optimum regression line between predicted and measured values with zero intercept and one slope. Coefficients of determination in the validation set were good and varied between

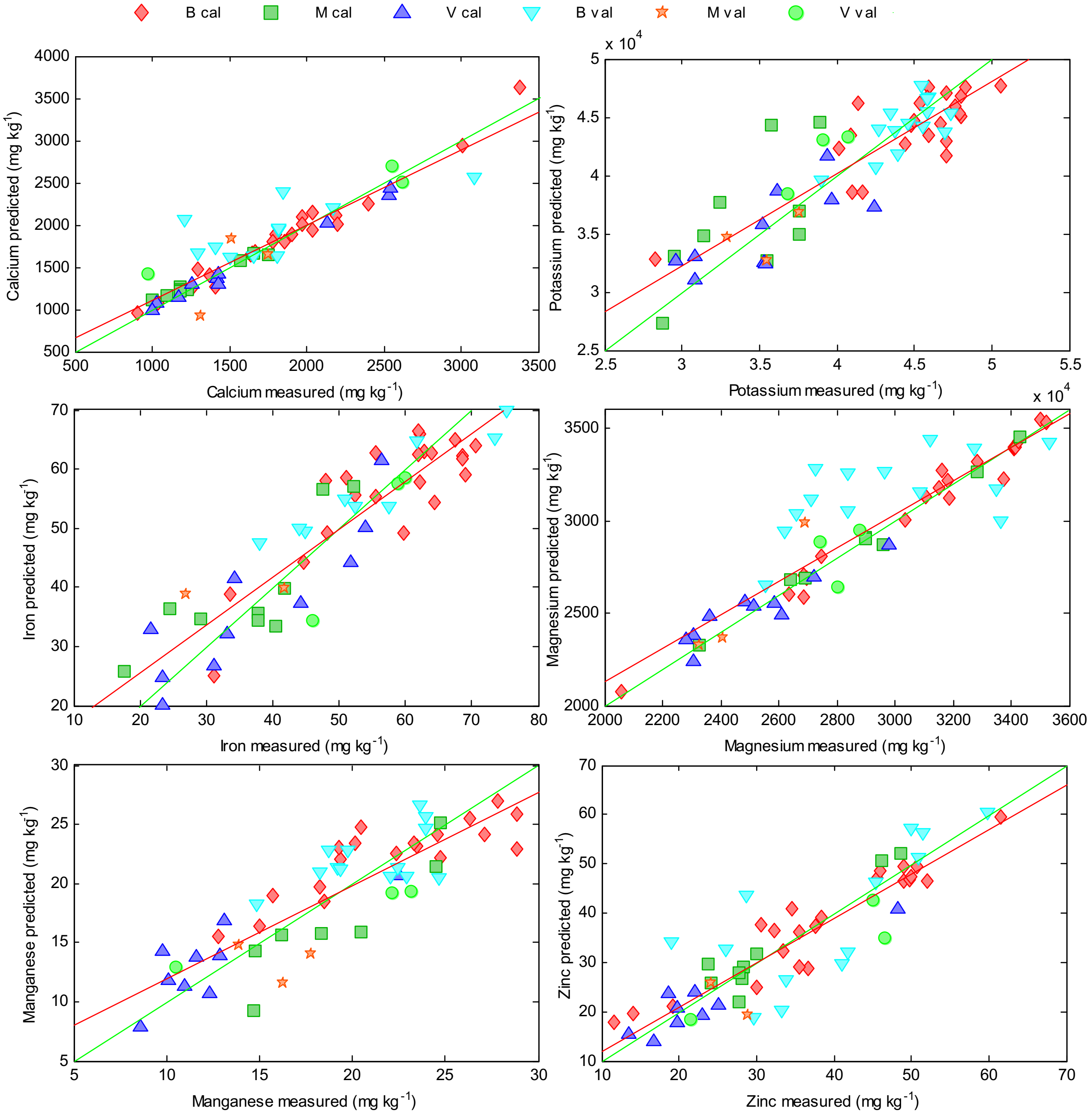

Fig. 2. Predicted values by PLS-NIR vs. reference values of mineral elements in artichoke samples. *Note*: B samples from Benicarló, M samples from Murcia, V samples from Valencia, cal samples from calibration set, val samples from validation set.

0.76 for manganese and 0.96 for calcium (see Table 2). The differences found in the external validation set between predicted concentrations and reference values vary from an average value between 8.4% for calcium, 2.5% for potassium, 4.4% for magnesium, 14.7% for iron, 14.1% for manganese and 13.2% for zinc. So, it can be concluded that the accuracy of the validation is good for calcium, potassium and magnesium, but it is only acceptable for iron, manganese and zinc, since the latter prediction errors are greater than 10%. The precision of the models built was very high, for potassium and manganese with RSD values lower than 4%, and in all cases this parameter was lower than 9%. The minimum RRMSEP obtained was 3.1% for potassium and in all cases it was lower than 18% (see Table 2). These results, together with the obtained RPD values, demonstrate the excellent predictive capability of the PLS-XRF models developed to predict the concentration of calcium, potassium, iron, magnesium and zinc in artichokes, and a good predictive capability for manganese concentration.

3.7. Comparison of NIR and XRF for mineral analysis of artichokes

PLS models were established to determine mineral elements in artichokes using NIR and XRF spectra. Prediction results for calcium, potassium, iron, magnesium, manganese and zinc in artichokes are shown in Tables 1 and 2. For calcium determination PLS-XRF ($R^2_{\text{val}} = 0.96$, RMSEP = 150 mg kg^{-1}) model provided better results than PLS-NIR ($R^2_{\text{val}} = 0.61$, RMSEP = 355 mg kg^{-1}). Additionally, PLS-XRF model was built with five LVs while NIR model needed thirteen LVs. For prediction of potassium content PLS-XRF was the best alternative ($R^2_{\text{val}} = 0.93$, RMSEP = 1280 mg kg^{-1}) with two LVs. For determination of magnesium, PLS-XRF with five LVs provided

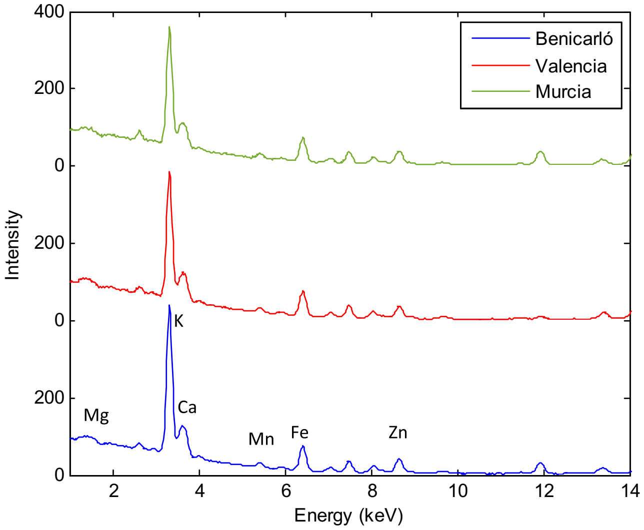

Fig. 3. Mean XRF spectra of artichoke samples produced in different areas. *Note*: spectra were shift in the Y axe for clarity.

$R^2_{\mathrm{val}} = 0.80$ and RMSEP = 149 mg kg^{-1}, thus, better results than the NIR model. For iron prediction, PLS-XRF and PLS-NIR models were very similar. However, for NIR two LVs were used while in XRF six LVs were needed. For manganese results found by XRF were $R^2_{\mathrm{val}} = 0.76$ and RMSEP = 3.25 mg kg^{-1}, while PLS-NIR prediction results were $R^2_{\mathrm{val}} = 0.54$ and RMSEP = 2.8 mg kg^{-1}. For zinc determination, the best method was PLS-XRF which provided very good values ($R^2_{\mathrm{val}} = 0.90$ and RMSEP = 5.10 mg kg^{-1}).

From the results obtained, it can be concluded that XRF presents lower calibration, cross validation, and prediction errors than NIR, with better coefficients of prediction for the determination of all assayed mineral elements which, in fact is quite correct taking into account that XRF bands are close related to the presence of mineral elements being NIR bands related to the overtones of the main molecular stretching.

3.8. Contribution of artichokes to the daily mineral intake of essential elements

The contribution of artichoke consumption to the calcium, iron, potassium, magnesium, manganese and zinc to daily intake was evaluated from the recommended daily allowance (RDA) and adequate intake (AI), considering the consume of three pieces of artichoke of approximately 43 g of fresh weight each one. The percentage of RDA or AI was calculated as milligrams of essential elements provided a sample taking into account that the RDA values for men between 19 and 50 years are 1000, 410, 8 and 11 mg day^{-1} for calcium, magnesium, iron and zinc; and for women, in the same period of age, are 1000, 320, 18 and 8 mg day^{-1}, respectively. The adequate intake values for men are 4700 and 2.3 mg day^{-1} for potassium and manganese; and for women 4700 and 1.8 mg day^{-1} (Food and Nutrition Board, 2011). So, the contribution of calcium and zinc can be between 4% and 9% of the RDA, and for iron 6% regarding women consumption, being the aforementioned values a little bit low. The percentage of iron for men, potassium, magnesium and manganese are 14, 19, 15–19% and 17–22%, respectively. These values could be considered as adequate. However, it is clear that artichoke consumption is not the main source of studied minerals for humans.

3.9. Greenness of the methods employed for mineral elements determination in artichokes

The greenness of the methodologies employed were evaluated by using the eco-scale proposed by Van Aken, Strekowski, and Patiny (2006), developed by Galuszka, Konieczka, Migaszewski, and Namiésnik (2012) and modified by Armenta, de la Guardia, and Namiesnik (in press) to establish the green certificate of analytical methods. The modified system includes a new criterion to quantify the penalty point values as a function of the volume of consumed reagents and generated wastes through the use of mathematical expressions and to associate the eco-scale value to a category class, from A to G, in the so called Green Certificate. The general form of employed equations is as following

$$(y = a \times x^b) \tag{1}$$

being $a = 0.61 \pm 0.05$ and $b = 0.31 \pm 0.02$ for reagents consumption and $a = 1.50 \pm 0.08$ and $b = 0.40 \pm 0.02$ for waste generation. So, the penalty points obtained for reagents consumption should be multiplied by coefficients of hazards: the absence of pictograms in the Globally Hazardous System Classification and Labelling of Chemicals concerning 0 penalty points, less severe hazard, described by warning pictogram, concerns 1 penalty point and more severe hazard indicated by danger pictogram involves 2 penalty points.

The energy consumes, together with the amount of reagents employed and their toxicity, were evaluated, also considering the amount of wastes generated and the occupational hazard of method application. From the initial scale of 100 points, NIR

Table 2
Reference data and statistics of mineral content of artichokes used for calibration and validation sets employed for PLS-XRF determination.

Element	Set	Range	Mean	SD	Pre-process	LV	R^2	RMSE	RPD	RRMSEP
Ca	CAL	998–3384	1805	630	MC	5	0.97^{a}	113^{a}	5.6^{a}	9.0
	VAL	900–3087	1668	556			0.96^{b} (0.91)^{c}	150^{b} (200)^{c}	3.7^{b} (3.2)^{c}	
K	CAL	28268–50541	40460	6013	MC + FD	2	0.96^{a}	1175^{a}	5.1^{a}	3.1
	VAL	25882–48021	41767	6122			0.93^{b} (0.95)^{c}	1280^{b} (1350)^{c}	4.8^{b} (4.8)^{c}	
Mg	CAL	2058–3533	2851	418	MC	5	0.91^{a}	122^{a}	3.4^{a}	5.2
	VAL	1816–3503	2855	404			0.80^{b} (0.55)^{c}	149^{b} (280)^{c}	2.7^{b} (1.5)^{c}	
Fe	CAL	17.7–78.7	50.5	14.6	MC + FD	6	0.96^{a}	2.9^{a}	5.0^{a}	14.3
	VAL	21.5–68.6	48.6	14.1			0.79^{b} (0.76)^{c}	7.0^{b} (6.7)^{c}	2.0^{b} (2.2)^{c}	
Mn	CAL	6.7–28.8	18.4	5.6	MC + FD	4	0.91^{a}	1.7^{a}	3.3^{a}	17.7
	VAL	9.8–28.9	18.4	6.0			0.76^{b} (0.71)^{c}	33^{b} (3.0)^{c}	1.9^{b} (1.9)^{c}	
Zn	CAL	16.7–60.0	34.0	12.0	MC + FD	5	0.96^{a}	2.4^{a}	5.0^{a}	14.5
	VAL	11.7–61.6	35.1	15.5			0.90^{b} (0.84)^{c}	5.1^{b} (4.8)^{c}	3.0^{b} (2.5)^{c}	

NOTE: Range, mean and standard deviation (SD) in mg kg^{-1} dry weight for calibration (CAL) and validation (VAL) data sets. R^2, coefficient of regresion for: calibration^{a}, external validation^{b} and cross-validation^{c}. RMSE, root-mean-square error for: calibration^{a}, external validation^{b} and cross-validation^{c} in mg kg^{-1}; RPD, ratio of performance to deviation for: calibration^{a}, external validation^{b} and cross-validation^{c}. RRMSEP, relative root-meansquare error of prediction in percentage (%).

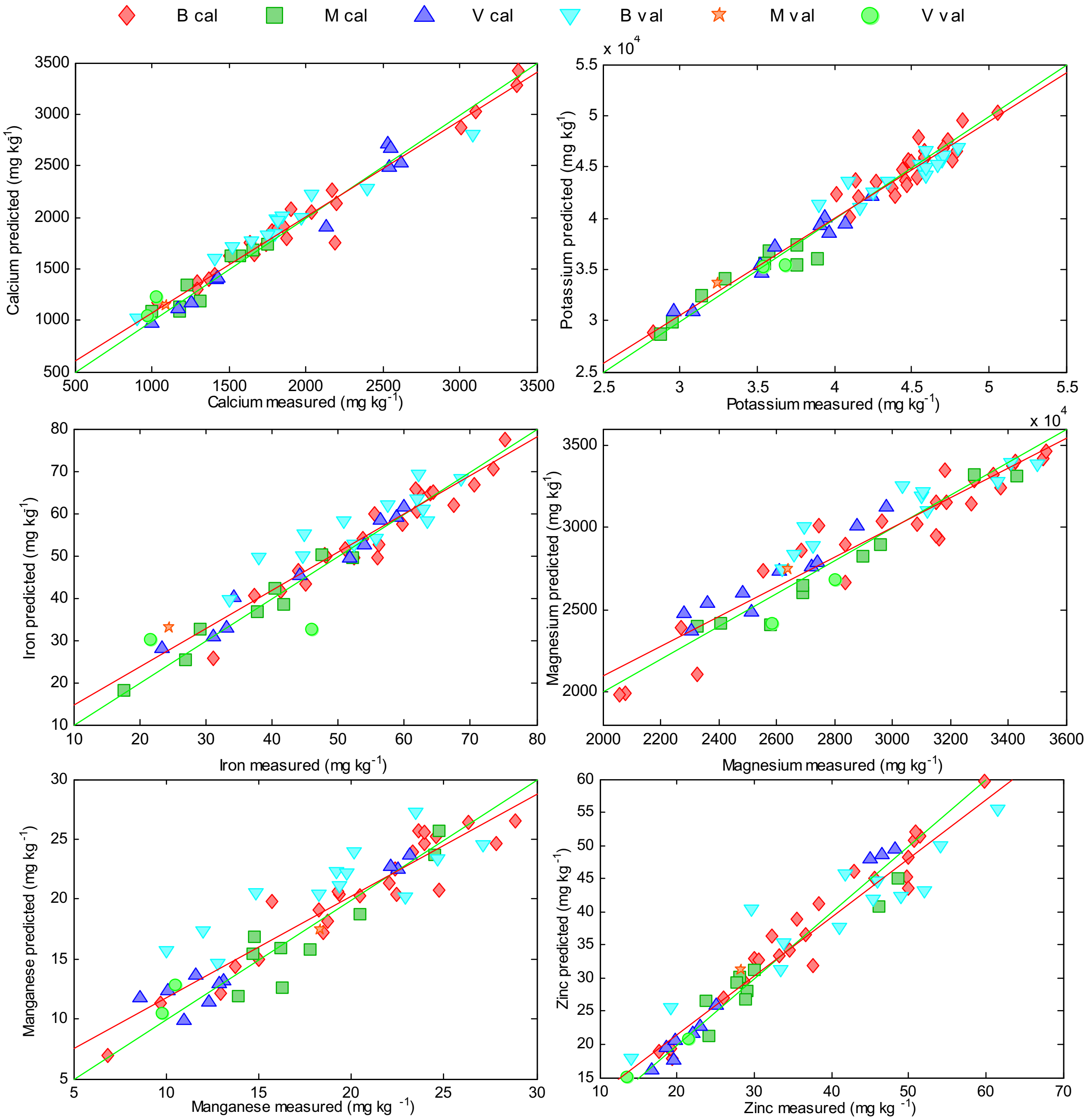

Fig. 4. Predicted values by PLS-XRF vs. reference values of mineral elements in artichoke samples. *Note*: B samples from Benicarló, M samples from Murcia, V samples from Valencia, cal samples from calibration set, val samples from validation set.

methodology and XRF methodology only involve 1 penalty point for the consume of energy, due to the use of sample lyophilisation. So, it can be concluded that these are ideal green methods, with a category class of green certificate A. ICP-OES methodology, from an initial scale of 100 points, involves 3 penalty points due to the use of HNO_3 for the treatment of samples, the use of standard solutions for calibration and argon in plasma generation; the energy consume by the lyophiliser, microwave oven and ICP-OES provided additional penalty of 3 points. Extra points from occupational hazard and wastes involve 8.5 penalty points. So, the final score of the method was 85.5, obtaining a B class in green certificate. So, it can be concluded that NIR and XRF methods evaluated through this study are clearly green alternatives to the determination of mineral elements in artichoke samples.

4. Conclusions

NIR and XRF spectroscopy techniques were evaluated to determine calcium, potassium, magnesium, iron, manganese and zinc in artichoke samples. PLS models built from NIR and XRF spectra allowed the determination of mineral concentrations with quite good results in a green way. Artichoke samples can be analysed without any chemical sample preparation except physical lyophilisation. Proposed methodologies are greener than conventional ICP-OES methodology from the reagents, time-consuming and analysis cost.

Therefore, from the obtained results, it can be seen that NIR spectroscopy and X-ray fluorescence techniques offer fast and green alternatives to determine calcium, potassium, magnesium, iron, manganese and zinc in artichokes, being XRF the best choice in the main part of cases.

Acknowledgements

Authors acknowledge the financial support the Ministerio de Economia y Competitividad-Feder (project CTQ2012-38635 and project CTQ2014-52841) and Generalitat Valenciana (project PROMETEO-II 2014-077). Alba Mir-Marqués acknowledges the grant 'Atracció de Talent' (PREDOC13-110514) provided by the University of Valencia.

References

Armenta, S., de la Guardia, M., & Namiesnik, J. (in press). Green Microextraction. In M. Valcarcel (Ed.), *Analytical Microextraction Techniques*. Bentham Science.

Bellon-Maurel, V., Fernandez-Ahumada, E., Palagos, B., Roger, J. M., & McBratney, A. (2010). Critical review of chemometric indicators commonly used for assessing the quality of the prediction of soil attributes by NIR spectroscopy. *Trac-Trend in Analytical Chemistry, 29*, 1073–1081.

Burns, D. A., & Ciurczack, E. W. (2007). *Handbook of near-infrared analysis*. New York: CRC Press.

Cozzolino, D., Cynkar, W., Shah, N., & Smith, P. (2011). Quantitative analysis of minerals and electric conductivity of red grape homogenates by near infrared reflectance spectroscopy. *Computers and Electronics in Agriculture, 77*, 81–85.

Cozzolino, D., & Moron, A. (2004). Exploring the use of near infrared reflectance spectroscopy (NIRS) to predict trace minerals in legumes. *Animal Feed Science and Technology, 111*, 161–173.

CRDO. (2014). Consejo Regulador de la Denominación de Origen "Alcachofa de Benicarló". Available from: <www.alcachofabenicarlo.com>.

de la Guardia, M., & Garrigues, S. (2015). *Handbook of mineral elements in food*. Chichester: John Wiley & Sons.

Food and Nutrition Board, Institute of Medicine. (2011). *Dietary reference intakes (DRIs): Recommended dietary allowances and adequate intakes, elements*. National Academies Press.

dos Santos, A. M. P., Lima, J. S., Anunciacao, D. S., Souza, A. S., dos Santos, D. C. M. B., & Matos, G. D. (2013). Determination and evaluation employing multivariate analysis of the mineral composition of broccoli (*Brassica oleracea* L. var. Italica). *Food Analytical Methods, 6*, 745–752.

Galuszka, A., Konieczka, P., Migaszewski, Z. M., & Namiésnik, J. (2012). Analytical eco-scale for assessing the greenness of analytical procedures. *Trac-Trend in Analytical Chemistry, 37*, 61–72.

Harmankaya, M., Al Juhaimi, F., & Musa Özcan, M. (2012). Mineral contents of Jerusalem artichoke (*Helianthus tuberosus* L.) growing wild in Turkey. *Analytical Letters, 45*, 2269–2275.

Kelly, S. D., & Bateman, A. S. (2010). Comparison of mineral concentrations in commercially grown organic and conventional crops – Tomatoes (*Lycopersicon esculentum*) and lettuces (*Lactuca sativa*). *Food Chemistry, 119*, 738–745.

Kennard, R. W., & Stone, L. A. (1989). Computer aided design of experiments. *Technometrics, 11*, 137–148.

Mahmood, T., Anwar, F., Iqbal, T., Bhatti, I. A., & Ashraf, M. (2012). Mineral composition of strawberry, mulberry and cherry fruits at different ripening stages as analyzed by inductively coupled plasma-optical emission spectroscopy. *Journal of Plant Nutrition, 35*, 111–122.

Manhas Verbi Pereira, F., Rodrigues Pereira-Filho, E., & Maretti Silveira Bueno, M. I. (2006). Development of a methodology for calcium, iron, potassium, magnesium, manganese, and zinc quantification in teas using X-ray spectroscopy and multivariate calibration. *Journal of Agricultural and Food Chemistry, 54*, 5723–5730.

Mir-Marqués, A., Cervera, M. L., & de la Guardia, M. (2012). A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus. *Journal of Food Composition and Analysis, 27*, 160–168.

Mir-Marqués, A., Domingo, A., Cervera, M. L., & de la Guardia, M. (2015). Mineral profile of kaki fruits (*Diospyros kaki* L.). *Food Chemistry, 172*, 291–297.

Mitic, S. S., Obradovic, M. V., Mitic, M. N., Kostic, D. A., Pavlovic, A. N., Tosic, S. B., & Stojkovic, M. D. (2012). Elemental composition of various sour cherry and table grape cultivars using inductively coupled plasma atomic emission spectrometry method (ICP-OES). *Food Analytical Methods, 5*, 279–286.

Moros, J., Llorca, I., Cervera, M. L., Pastor, A., Garrigues, S., & de la Guardia, M. (2008). Chemometric determination of arsenic and lead in untreated powdered red paprika by diffuse reflectance near-infrared spectroscopy. *Analytica Chimica Acta, 613*, 196–206.

Paudino, G., Lombardo, S., & Mauromicale, G. (2011). Mineral profile in globre artichoke as affected by genotype, head part and environment. *Journal of Science and Food Agricultural, 91*, 302–308.

Schmitt, S., Garrigues, S., & de la Guardia, M. (2014). Determination of the mineral composition of foods by infrared spectroscopy: A review of a green alternative. *Critical Reviews in Analytical Chemistry, 44*, 186–197.

Terzic, S., Atlagic, J., Maksimovic, I., Zeremski, T., Zoric, M., Miklic, V., & Balalic, I. (2012). Genetic variability for concentrations of essential elements in tubers and leaves of Jerusalem artichoke (*Helianthus tuberosus* L.). *Scientia Horticulturae, 136*, 135–144.

Van Aken, K., Strekowski, L., & Patiny, L. (2006). EcoScale, a semi-quantitative tool to select an organic preparation based on economical and ecological parameters. *Beilstein Journal of Organic Chemistry, 2*, 3.

West, M., Ellis, A. T., Potts, P. J., Streli, C., Vanhoof, C., Wegrzynek, D., & Wobrauschek, P. (2010). Atomic spectrometry update-X-ray fluorescence spectrometry. *Journal of Analytical Atomic Spectrometry, 25*, 1503–1545.

Wise, B. M., Shaver, J. M., Gallagher, N. B., Windig, W., Bro, R., & Koch, R. S. (2006). Chemometrics Tutorial for PLS_Toolbox and Solo Eigenvector Research Inc, Wenatchee, USA.

Wold, S., Sjöström, M., & Eriksson, L. (2001). PLS-regression: A basic tool of chemometrics. *Chemometrics and Intelligent Laboratory Systems, 58*, 109–130.

Food Control 59 (2016) 74–81

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Authentication of protected designation of origin artichokes by spectroscopy methods

Alba Mir-Marqués, Cristina Elvira-Sáez, M. Luisa Cervera, Salvador Garrigues, Miguel de la Guardia*

Department of Analytical Chemistry, University of Valencia, Jerónimo Muñoz Building, 50th Dr. Moliner St., Burjassot, Valencia 46100, Spain

A R T I C L E I N F O

Article history:
Received 4 February 2015
Received in revised form
15 April 2015
Accepted 4 May 2015
Available online 9 May 2015

Keywords:
Protected designation of origin foods (PDO)
Mineral profile
Inductively coupled plasma optical emission spectrometry (ICP-OES)
Near infrared spectroscopy (NIR)
X-ray fluorescence (XRF)
Artichoke samples

A B S T R A C T

Artichoke samples with the protected designation of origin (PDO) ‘Alcachofa de Benicarló’ were discriminated from those produced in other towns surrounding Valencia and Murcia, outside the PDO frame. Inductively coupled plasma (ICP-OES), near infrared (NIR) and X-ray fluorescence (XRF) were assayed in order to achieve a correct sample classification of the three different origin sample groups (Benicarló, Valencia and Murcia). Chemometrics was used to compare the ability of classification of artichoke from their origin by using concentration data of mineral elements, determined by ICP-OES, NIR spectra and XRF spectra. Multivariate analysis tools, as principal component analysis (PCA), hierarchical cluster analysis (HCA) and partial least square discriminant analysis (PLSDA), were used for chemometric data treatment. This study evidenced that the mineral profile of artichokes is a good way for the correct identification of sample origin and that the direct use of XRF spectra of the lyophilised edible part of artichoke can be used as a cheap and green alternative for the authentication of samples produced in the PDO ‘Alcachofa de Benicarló’ and to differentiate from those produced in near areas.

1. Introduction

Authenticity is a quality criterion for food which has an increasing interest in Europe as a result of legislative protection of regional foods, based on the approval of different labels (EU Regulation, 2012). The PDO label identifies foodstuffs, with regional identity, that are produced, processed and prepared in a specific geographical area and protects regional foods in front of the concurrency of a cheapest production of the same food from other areas or countries (de la Guardia & Gonzálvez, 2013; Kelly, Heaton, & Hoogewerff, 2005). From the economic point of view, food authenticity has a great importance for the sectors involved in food production and also for consumers. This is important because the food authentication helps to guarantee the characteristics and quality of food products and to prevent overpayment (Drivelos & Georgiou, 2012; Gonzálvez, Armenta, & de la Guardia, 2009).

Artichoke (*Cynara Scolymus* L.) is one of the oldest cultivated plants, and it is used as a food since the 15th century. There are different types of artichokes but only ‘Blanca de Tudela’ variety is labelled as PDO ‘Alcachofa de Benicarló’. The area of cultivation of the ‘Alcachofa de Benicarló’ includes the areas of Benicarló, Vinaròs, Càlig and Penyíscola (Castellón, Spain). The cultivation land is very flat with average slopes lower than 5%, and is practically at the sea level. This leads to a special micro clime that makes the production of the ‘Blanca de Tudela’ in the extra and first categories and permits the compaction of the fruit with a consequent increase of product quality, which translates into good quotes of the market. It must be also highlighted its unique dimple shape and its duration without sacrificing quality offered (CRDO, 2014).

The use of analytical techniques is the best way to authenticate foods without any doubt in order to determine their geographical origin. There are many papers that discriminate foods produced inside PDO areas to those produced outside the covered region, using different analytical methods. The most commonly analytical techniques used are gas chromatography mass spectrometry (GC-MS) (Aquilanti, Santarelli, Babini, Osimani, & Clementi, 2013; Karali, Georgala, Massouras, & Kaminarides, 2013; Montero-Prado, Bentayeb, & Nerin, 2013) and high performance liquid chromatography (HPLC) (Barreira et al., 2012; Russo et al., 2012) generally employed for the determination of organic compounds. However, the concentration of organic compounds in foods varies easily due to many

---

* Corresponding author.
E-mail address: miguel.delaguardia@uv.es (M. de la Guardia).

http://dx.doi.org/10.1016/j.foodcont.2015.05.004

factors including weather conditions additionally than land composition. So, it is sometimes very difficult to have a definitive authentication based on the organic profile of foods. Because of that, isotope relation (Kelly & Bateman, 2010; Swoboda et al., 2008) and the mineral profile of foods (Gonzálvez et al., 2009) have been used to characterise foods from the soil type and the climatic conditions. The most commonly techniques employed to determine mineral elements in foods are flame atomic absorption spectrometry (FAAS) (Ariyama, Horita, & Yasui, 2004; Moreno-Rojas, Sanchez-Segarra, Camara-Martos, & Amaro-Lopez, 2010), inductively coupled plasma optical emission spectrometry (ICP-OES) (Ariyama et al., 2007; Boeting, de Benzo, Cervera, & de la Guardia, 2010; Matos-Reyes, Simonot, López-Salazar, Cervera, & de la Guardia, 2013) and inductively coupled plasma mass spectrometry (ICP-MS) (Costas-Rodriguez, Lavilla, & Bendicho, 2010; Di Giacomo, Del Signore, & Giaccio, 2007).

On the other hand, in recent years vibrational spectroscopy has been explored as a successfully alternative to classify and authenticate foods with advantages based on the direct acquisition of signals from untreated samples, or with a minimum preparation, which provides green analytical tools. Complementary, vibrational spectroscopy offers information about major and minor components (Garrigues & de la Guardia, 2013). Additionally, direct fluorescence signals obtained by energy dispersion X-ray (ED-XRF) provides also a potential way for the characterisation of foods (Garrigues & de la Guardia, 2015).

The correct characterisation of PDO food generally involves the determination of many analytical features or the consideration of several variables included in the sample spectral fingerprinting and therefore requires the deployment of complex mathematical processes in order to obtain the relevant information. This is the main reason of the extended use of multivariate pattern recognition approaches to built successful models to verify PDO claims. Chemometric tools generally used for PDO authentication are principal component analysis (PCA), as unsupervised method (Gori, Maggio, Cerretani, Nocetti, & Caboni, 2012; Zhang et al., 2008), and linear discriminant analysis (LDA), partial least squares discriminant analysis (PLSDA) and soft independent modelling of class analogy (SIMCA) as supervised methods (Bevilacqua, Bucci, Magri, Magri, & Marini, 2012; Boeting et al., 2010; Korifi, Le Dreau, Molinet, Artaud, & Dupuy, 2011; Matos-Reyes et al., 2013).

The main purpose of this study has been the authentication of artichoke samples produced from PDO ‘Alcachofa de Benicarló’ using their mineral profile information, and also the XRF and NIR spectra to differentiate between samples produced inside the PDO and those coming from near regions.

2. Materials and methods

2.1. Apparatus

An ICP-OES Optima 5300 DV inductively coupled plasma optical emission spectrometer Perkin Elmer (Norwalk, CT, USA), equipped with an auto sampler AS 93-plus and a GemCone nebuliser, was used for mineral profile determination. Samples were digested inside Teflon vessels of 100 mL inner volume using a microwave laboratory system, Ethos SEL from Millestone (Sorisole, Italy), equipped with a thermocouple for automatic temperature control and an automatic gas leaks detector.

For NIR measurements, it was employed a Multipurpose Analyser Fourier transform near-infrared (FT-NIR) spectrometer MPA model from Bruker (Bremen, Germany) equipped with an integrating sphere, used for the acquisition of diffuse reflectance spectra. For instrument control and data acquisition, the OPUS software 6.5 from Bruker was used.

X-ray fluorescence spectra were obtained using a portable model S1 Titan energy dispersive X-ray fluorescence (ED-XRF) spectrometer from Bruker equipped with a Rhodium X-ray tube and X-Flash® SDD detector. For instrument control, the S1RemoteCtrl and S1Sync software, from Bruker, were employed, and spectra were treated by the ARTAX software also from Bruker.

2.2. Reagents

Nitric acid 69% for trace analysis from Scharlau (Barcelona, Spain) was employed for sample digestion and ultrapure water, with a minimum resistivity of 18.2 MΩ cm, obtained using a Milli-Q plus Millipore system (Molsheim, France), was used for dilution of samples and standards.

Argon C-45 (purity higher than 99.995%), supplied by Carburos Metálicos (Barcelona, Spain), was employed as plasmogen and carrier gas for ICP-OES.

Stock solutions of a multi-element standard solution (100 mg L^{-1}) containing 26 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Se, Na, Sr, Tl, Ti, V and Zn) dissolved in 5% HNO_3 and a multi-element solution containing 20 mg L^{-1} Dy, Er, Eu, Gd, Ho, Lu, Sm, Sc, Tb, Tm, Yb and Y and 100 mg L^{-1} Ce, La, Nd and Pr dissolved in 5% HNO_3, both from Scharlau, were employed to prepare the calibration standards. Additionally, a 1 g L^{-1} calcium standard solution for atomic absorption, a 1 g L^{-1} magnesium standard solution for atomic absorption, a 1 g L^{-1} sodium standard solution for atomic absorption and a 1 g L^{-1} potassium standard solution for atomic absorption, all from Scharlau, were employed for calibration. A 1 g L^{-1} rhenium standard solution for ICP, from Scharlau, was used as internal standard.

Certified references materials as NIST 1573a (Tomato Leaves) and IAEA-359 (Cabbage) obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA) and from the International Atomic Energy Agency (Vienna, Austria) respectively, were used to control the accuracy of the method employed by determinations of the artichoke mineral profile by ICP-OES.

2.3. Samples

60 artichoke samples from different Spanish agriculture cooperatives were collected during the same period of time. Each sample corresponds to a mixture of 2–4 pieces of artichoke. 38 samples were produced under the PDO ‘Alcachofa de Benicarló’ (Castellón, Spain), 10 samples from Valencia (Spain) and 12 from Murcia (Spain). Samples were washed with ultrapure water and the edible parts were lyophilised, pulverised, sieved through a particle size of 250 μm and stored in polyethylene bottles until their analysis.

2.4. Analytical procedures

2.4.1. ICP-OES measurements

0.5 g of freeze-dried sample were digested according to a previous protocol developed for food analysis by Mir-Marqués, Cervera, & de la Guardia (2012) using 10 mL of concentrate nitric acid and a digestion step of 15 min at 180 °C (700 W) inside a microwave oven. All samples were analysed in duplicate and blanks and certified reference materials were measured in each session in order to control possible contaminations and to assure the accuracy of the method. Digested samples were measured with a final dilution volume of 50 mL.

The calibration range for all the 42 elements evaluated was established from 0.01 to 2 mg L^{-1} for all elements except calcium, magnesium, sodium (10–100 mg L^{-1}) and potassium

(20–900 mg L^{-1}). Rhenium (1 mg L^{-1}) was used as internal standard and added to all samples, blanks and standards for ICP-OES analysis. Additionally, control standards were measured for every series of 10 independent sample measurements and a reference material was analysed in each series.

The average measurement of blanks was subtracted from the analytical signals of digested samples after interpolation on the calibration graphs. Results were expressed as a basis of dry weight.

2.4.2. NIR measurements

Approximately, 0.5–1 g of dry samples were placed inside glass vials of 20 mL (22 mm of internal diameter and 44 mm high) to obtain their NIR spectra by diffuse reflectance. The vials were rotated to obtain triplicate measurements for each sample. Diffuse reflectance near spectra (DRIFT) were recorded in Kubelka-Munk units, in the range of 12,500–3500 cm^{-1}, using a spectral resolution of 4 cm^{-1} and cumulating 50 scans per spectrum. The background spectrum was acquired from the closed integrating sphere using the same instrumental conditions than those employed for samples. The signals obtained were employed for artichoke authentication.

2.4.3. XRF measurements

0.12 g of dry samples were placed inside a pellet box and were compacted using a hydraulic press for 1 min at a pressure of $5.1 \cdot 10^3$ kg. Pellets were stored inside desiccators to avoid hydration from air humidity. Spectra were obtained by triplicate using 50 kV voltage, 11 μA current and 60 s acquisition time (30 s for high atomic number elements, and 30 s for low atomic number elements). Signals obtained, in the aforementioned conditions, were used for sample authentication.

2.5. Statistical analysis

Statistical multivariate analysis was performed with the Matlab software 2013a from Mathworks (Natick, MA, USA) and the PLS Toolbox 6.2 from Eigenvector Research Inc. (Wenatchee, WA, USA). Principal component analysis (PCA), hierarchical cluster analysis (HCA) and partial least squares discriminant analysis (PLSDA) were used to process the data of concentration of the mineral elements determined by ICP-OES and also to process NIR and XRF spectra of the studied samples.

Leave-one-out cross validation and external validation were assayed to validate the prediction quality of employed models and to prevent overfitting of the calibration. Additional samples, not included in the calibration set, were used for external validation. Kennard-Stone (KS) algorithm was used to select the most appropriate calibration and validation sample sets. Different data pretreatments, such as, mean centred, autoscale, standard normal variate, first derivate and also combinations of them, were evaluated for NIR, ICP-OES and XRF measurements.

3. Results and discussion

3.1. NIR analysis of artichoke samples

The diffuse reflectance spectra of artichokes were employed as descriptor of samples collected from three different production areas and evaluated in order to be used for authentication purpose.

3.1.1. NIR spectra of artichoke samples

Fig. 1 shows the typical near DRIFT spectra obtained between 9000 and 4000 cm^{-1} for Benicarló (B), Valencia (V) and Murcia (M) samples. Spectra regions between 12,500–9000 cm^{-1} and 4000–3500 cm^{-1} were removed for data processing. The broad and strong peak around 8300 cm^{-1} was due to the 2nd overtone C–H stretching vibrations. At 7000–6800 cm^{-1} it can be seen a broad peak, which corresponds to the combination of C–H and the N–H stretch 1st overtone. Peak at 5800 cm^{-1} corresponds to the C–H stretch 1st overtone. O–H 1st and 2nd overtone can be seen at 5155 cm^{-1}. A strong peak at 4732 cm^{-1} was due to C–O stretch combination and O–H bend, characteristics of carbohydrates. Double peak at 4350 and 4250 cm^{-1} were due to C–H and CH_2 bend 2nd overtone, corresponding to proteins (Burns & Ciurczack, 2007).

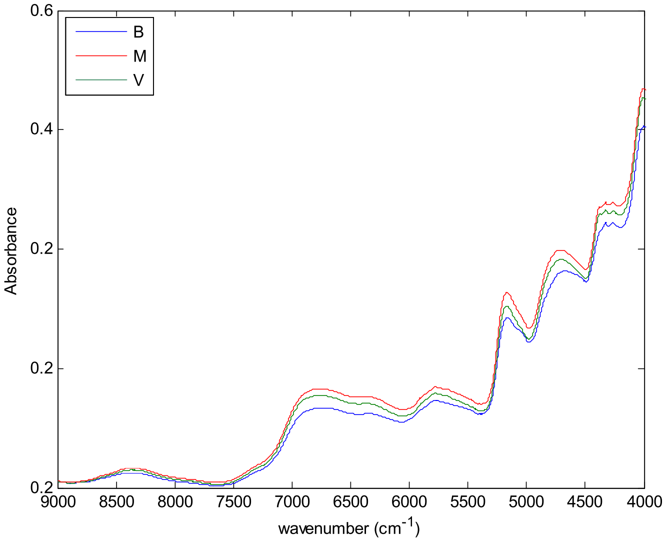

Fig. 1. Mean DRIFT spectra of artichoke samples.

3.1.2. Chemometric treatment of NIR spectra

NIR spectra of artichoke samples were pre-processed using first derivate and mean-centring before the application of modelling procedures.

An exploratory analysis was initially performed in order to observe the existence of natural groups. For this purpose, PCA and HCA were applied to the spectral data set. PCA and HCA analysis did not show clear differences between the origin groups of samples considered.

The authentication of PDO ‘Alcachofa de Benicarló’ samples was performed by PLSDA. 44 artichokes (28 samples from PDO and 16 from not PDO) and 16 artichoke samples (10 samples from PDO and 6 from not PDO) were selected for calibration and validation sets by using the Kennard-Stone algorithm. Results found provided classification rates of 100% for calibration, 93% for cross validation and 88% for external validation in order to discriminate samples produced inside and outside the PDO (see Table 1), with five latent variables (LV), 95 and 87% of explained variance of X and Y blocks, and regression coefficients of 0.87, 0.57 and 0.59 for calibration, cross validation and external validation, respectively. It must be noticed that two samples not produced inside the PDO were confused as coming from Benicarló.

Additionally, a study to identify the three origins of samples (Benicarló, Murcia and Valencia) was made. In this case, results were worse those obtained in the study of two classes. These results provided a correct classification of 98% for calibration, 82% for cross validation and 80% for external validation (see Table 1). To build this model, six LV were chosen, with 95 and 74% of explained variance of X and Y blocks, respectively. One sample from Murcia was confused as coming from Benicarló and two samples from Valencia were identified as produced in Murcia and Benicarló. So,

Table 1
PLSDA external validation confusion matrix for the classification of artichoke samples from their NIR spectra.

Assigned group Original group	PDO	Not PDO	Total	% Correct
PDO	10	0	10	100%
Not PDO	2	4	6	67%
Total	12	4	16	88%

Assigned group Original group	Benicarló	Murcia	Valencia	Total	% Correct
Benicarló	10	0	0	10	100%
Murcia	1	1	0	2	50%
Valencia	1	1	1	3	33%
Total	12	2	1	15	80%

for a complete classification of artichoke samples origin from their NIR spectra, it can be seen the restricted capability of PLSDA.

3.2. Mineral profile of artichoke samples obtained by ICP-OES

The mineral profile of artichoke samples was used as a descriptor of their relationship with the production area, based on the interaction of plant-soil-weather conditions and from these data it was evaluated the capability of trace elements for the discrimination between samples produced in Murcia, Valencia and Benicarló.

3.2.1. Mineral content of artichoke samples by ICP-OES

Table 2 shows the minimum, maximum and mean concentration of 15 elements (Al, B, Ba, Cu, Fe, Mn, Mo, Ni, Sr, Ti, V, Zn, Ca, K, Mg and Na) which were present in almost samples at concentration levels higher than the limit of detection (LOD) of the methodology classified, as a function of their production region. Although 42 elements were evaluated in the 60 artichoke samples analysed, chemometric calculations were performed using only 6 variables considering the content of Al, Fe, K, Na, Sr and V, in mg kg^{-1}, determined by ICP-OES which were identified as the most relevant variables in the PCA study.

3.2.2. Chemometric treatment of mineral composition of artichoke samples

PCA and HCA were applied to the data set of the concentration of the 6 selected elements to observe the existence of natural groups. For building the PLSDA method it was used the mineral content of the edible part of artichoke as variables. All data were auto-scaled and mean-centred before modelling procedures.

3.2.2.1. Hierarchical cluster analysis. As it can be seen in Fig. 2, three clusters were obtained from the 60 samples considered for a variance weighted distance of 10. Cluster 1 concerns only the Murcia samples. Cluster 2 includes 14 samples from Benicarló PDO, one sample from Murcia and 12 samples from Valencia thus providing a difficult differentiation between artichoke production areas. Cluster 3 includes only samples from the PDO area. So, it can be seen that HCA allows a clear discrimination of samples from Murcia to those produced in Valencia and Benicarló, but does not differentiate between Valencia samples and Benicarló ones, thus being unable for PDO artichoke authentication from their mineral profile.

3.2.2.2. Principal component analysis. The loadings of original mineral profile variables in the two first considered principal components were used to discriminate artichoke samples (see Table 3). Principal component 1 (PC1) represents 52.30% of the total variance, including strontium, vanadium, sodium and potassium, which were identified as the dominant variables in the PC1, while iron and aluminium show the lowest values. PC2 explains 20.04% of the total variance. Evaluating the loadings, potassium, iron and sodium resulted as the most dominant variables in PC2. The two first principal components take into account 72.34% of the total variance of the mineral profile of artichokes, and it allows differentiating three groups by origin almost 100%, except two samples of Benicarló and one sample of Murcia which were grouped from Valencia.

3.2.2.3. Partial least squares discriminant analysis. Authentication of artichokes with protected designation of origin from its mineral profile was carried out by building PLSDA models. Two groups of samples were selected by using the Kennard-Stone algorithm, a first group to calibrate the model and another group to validate it. The calibration set consisted of 44 samples, of which 28 belonged to the PDO and 16 samples from Valencia and Murcia. For the validation set 16 samples were selected, 10 from the PDO and 6 samples outside the PDO. Results found for this model, with five latent variables and an explained variance of 95 and 78% for X and Y blocks, respectively, were 100, 93.4 and 100% of correct classification rate for calibration, cross validation and validation external (see Table 4).

To differentiate between the three geographical areas assayed (Benicarló, Murcia and Valencia), following the above methodology,

Table 2
Minimum, maximum and mean concentration of mineral elements determined in artichoke samples from different Spain production areas (expressed in mg kg^{-1} dry weight).

	Benicarló			Valencia			Murcia		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Al	<1.1	38	7 ± 10	<1.1	4.4	2.1 ± 1.0	<1.1	3.5	1.5 ± 0.7
B	<4	25	5 ± 4	<4	6.5	4.3 ± 0.8	<4	6.6	4.2 ± 0.8
Ba	<0.1	4	1.0 ± 0.8	<0.1	2.0	0.7 ± 0.6	<0.1	2.5	0.7 ± 0.8
Cu	1	12	7 ± 3	2.7	6.9	5.0 ± 1.5	3	11	5 ± 3
Fe	31	75	55 ± 11	22	60	43 ± 14	18	79	40 ± 17
Mn	7	29	20 ± 5	9	23	14 ± 6	14	25	18 ± 4
Mo	0.14	0.64	0.36 ± 0.11	0.22	0.41	0.31 ± 0.05	0.28	0.48	0.36 ± 0.06
Ni	<0.3	1.0	0.4 ± 0.2	<0.3	0.52	0.34 ± 0.09	<0.3	<0.3	<0.3
Sr	2	13	6 ± 3	11	41	25 ± 12	19	78	42 ± 20
V	<2	4.2	2.2 ± 0.3	2.2	4.6	3.2 ± 0.9	4	12	8 ± 3
Zn	12	62	37 ± 13	13	48	27 ± 13	24	49	31 ± 9
Ca	900	3400	1900 ± 600	1000	2600	1700 ± 700	1000	1800	1400 ± 300
K	26000	51000	44000 ± 5000	30000	42000	37000 ± 4000	29000	39000	34,000 ± 4000
Mg	1800	3500	3000 ± 400	2300	3000	2600 ± 200	2300	3400	2800 ± 400
Na	400	2200	1000 ± 400	300	2000	1200 ± 600	1000	6000	4000 ± 2000

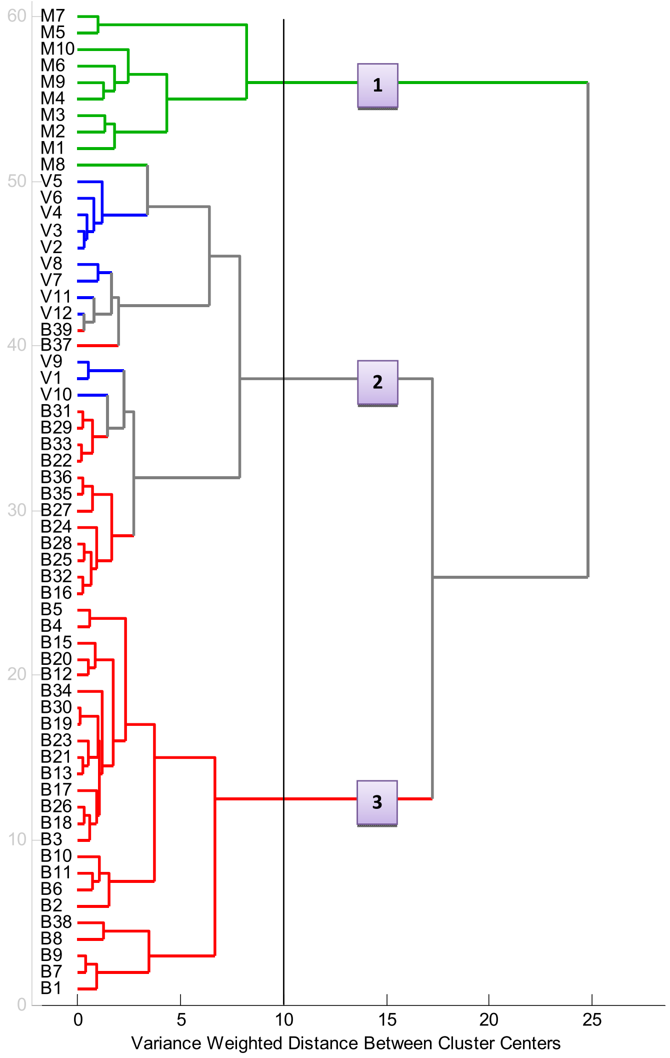

Fig. 2. Cluster classification of artichoke samples obtained from their mineral profile. NOTE: B, V and M indicate samples from Benicarló, Valencia and Murcia origin, respectively.

with 45 samples used for calibration (28 samples from Benicarló, 8 from Murcia and 9 from Valencia) and 15 samples for external validation (10 samples from Benicarló, 2 from Murcia and 3 from Valencia), results were similar to those mentioned above, getting classification coefficients of 100, 93.4 and 100% for calibration, cross-validation and external validation, respectively (see Table 4). In this case the explained variances for X and Y blocks were 97 and 71%, with five LV. So, it can be concluded that the capability of PLSDA for a correct authentication of artichoke samples from their mineral profile is very good, being thus possible to discriminate correctly the artichokes produced inside the PDO at a 100% level, in front of those produced in close areas.

Table 3
Loadings of the variables considered for the two first Principal Components established from the mineral profile of artichoke samples produced in different parts of Spain.

Variable	PC1	PC2	Variable	PC1	PC2
Al	−0.21491	0.07135	Na	0.47213	0.36608
Fe	−0.28216	0.70526	Sr	0.50000	0.28655
K	−0.41374	0.48772	V	0.48010	0.20702

Table 4
PLSDA external validation confusion matrix for the classification of artichoke samples from their mineral profile.

Assigned group Original group	PDO	Not PDO	Total	% Correct
PDO	10	0	10	100%
Not PDO	0	6	6	100%
Total	10	6	16	100%

Assigned group Original group	Benicarló	Murcia	Valencia	Total	% Correct
Benicarló	10	0	0	10	100%
Murcia	0	2	0	2	100%
Valencia	0	0	3	3	100%
Total	10	2	3	15	100%

3.3. *XRF analysis of artichoke samples*

The energy dispersive X-ray fluorescence spectra are directly related to the mineral composition of foods and were employed, in this study, as a fingerprint of artichoke samples in order to do their origin authentication.

3.3.1. *XRF spectra of artichoke samples*

The XRF spectra of artichoke samples of different origin are similar. However, as it can be seen in Fig. 3, spectra present some differences that allow their discrimination. Peak at 3.3 KeV, corresponding to Kα of potassium, in Benicarló samples is higher than in the other origin samples. In addition, Murcia and Benicarló samples have a peak at 12 KeV corresponding to Kα of bromine, while in Valencia samples this peak does not appear. So, it indicates that bromine could be a discriminating element. On the other hand, samples from Valencia and Murcia have a fairly strong peak at 14.3 KeV that belongs to Kα of strontium and another less intense at 2.7 KeV belonging to Kα of chlorine which could make differentiation from Benicarló samples. Other peaks present in the spectra of artichoke samples correspond to Kα of calcium (3.7 KeV), chromium (5.45 KeV), copper (8 KeV), nickel (7.6 KeV) and zinc (8.6 KeV) with intensities very similar between samples from different sources with the exception of calcium, which has a peak height a little bit higher in artichokes coming from Benicarló than that found in samples from Murcia and Valencia.

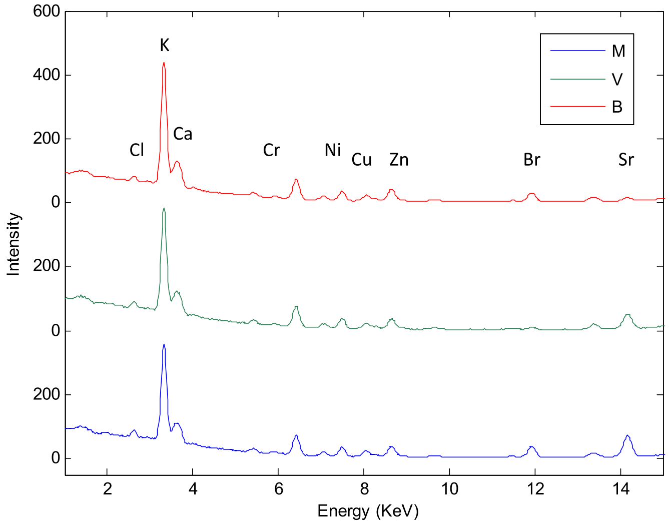

Fig. 3. Mean XRF spectra of artichoke samples produced in different areas. NOTE: spectra were shift in the Y axe for clarity.

3.3.2. *Chemometric treatment of XRF spectra of artichoke samples*

The XRF spectra were used directly to discriminate between PDO 'Alcachofa de Benicarló' and other ones. In this sense, XRF spectra of the edible part of the samples were used as chemical descriptor for building the PLSDA method. PCA and HCA were also performed, based just on the XRF spectra, in order to observe the existence of natural groups. All data were auto-scaled and mean-centred before to make the modelling procedures.

3.3.2.1. Hierarchical cluster analysis. Four clusters were obtained, for a variance distance of 100, for the 60 samples considered. Cluster 1 concerns 23 samples of Benicarló, cluster 2 contains six samples from Murcia and five samples from Valencia, cluster 3 includes 14 samples from the PDO and one from Valencia and cluster 4 contains samples from Benicarló (1), Valencia (6) and Murcia (4). So, it can be concluded that HCA of the XRF spectra of artichokes allows the discrimination of the main part of samples produced from the Benicarló PDO to those produced in Valencia and Murcia samples. However, the distribution of the Benicarló samples in cluster 2 and 4 and the difficulty for a correct differentiation between Valencia and Murcia artichoke samples, evidences that it is not a good chemometric tool for the treatment of XRF spectra and other tools must be assayed for the treatment of these signals.

3.3.2.2. Principal component analysis. From the loadings of original variables corresponding to the two first considered principal components obtained for the evaluation of the XRF spectra of artichoke samples, it was observed that PC1 represents 86.36% of the total variance, being identified signals of strontium, bromine, iron and potassium, in the XRF spectra, as the dominant variables, while chromium and nickel peaks show the lowest values. PC2 explains 2.50% of the total variance. Potassium and strontium fluorescence areas result as the most dominant variables in PC2. PC1 and PC2 take account 88.86% of the total variance, and allow differentiating between samples produced in Benicarló and those coming from Murcia and Valencia, but it does not discriminate the samples between Murcia and Valencia origin as it can be seen in Fig. 4.

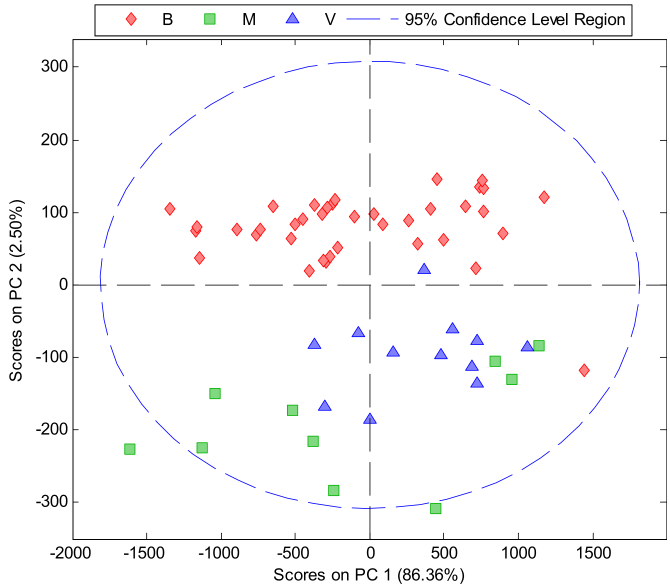

Fig. 4. Principal components analysis of the XRF spectra of artichoke samples produced in different parts of Spain.

3.3.2.3. Partial least squares discriminant analysis. To discriminate samples from PDO 'Alcachofa de Benicarló' and samples from other Spanish origin, PLSDA models were built. Two sets of samples were used, one for calibration and another for external validation of the model; for which 44 (28 samples from PDO and 16 from not PDO) and 16 artichoke samples (10 samples from PDO and 6 samples from not PDO) were chosen, respectively. This model was built with two LV and 68 and 87% explained variance in the X and Y blocks, obtaining a correct classification ratio of 100, 98 and 100% for calibration, cross-validation and external validation, and coefficients of regression between 0.73 and 0.90 (see Table 5).

Furthermore, PLSDA was performed by separating the samples in the three classes corresponding to Benicarló, Murcia, and Valencia. Results obtained after PLSDA treatment of XRF spectra were satisfactory, obtaining a classification rate of 100, 89 and 93% for calibration, cross validation and external validation, respectively (see Table 5). Three latent variables were chosen with explained variances of 79 and 88% for X and Y blocks.

As it can be seen, the capability of PLSDA for a correct classification of artichoke samples from their XRF spectra is quite good and comparable to that found based on the mineral profile obtained by ICP-OES with the advantage of the lack of sample treatment no signal calibration. This methodology allows the authentication of PDO 'Alcachofa de Benicarló' at 100% correct level in spite that one samples from Murcia was incorrectly identified as produced in Valencia.

3.4. *Evaluation of green parameters of the assayed methods*

The greenness of the methodologies employed were evaluated by using the eco-scale proposed by Van Aken, Strekowski, & Patiny (2006) and developed by Galuszka, Konieczka, Migaszewski, & Namiésnik (2012) for evaluation of analytical methods. The energy consumes, together with the amount of reagents employed and their toxicity, were evaluated, also considering the amount of wastes generated and the occupational hazard of method application. From the initial scale of 100 points, NIR methodology and XRF methodology only involve 1 penalty point for the consume of energy, due to the sample lyophilisation. So, it can be concluded that these are ideal green methods. ICP-OES methodology, from an initial scale of 100 points, involves 11 penalty points due to the use of HNO_3 for the treatment of samples, the use of standard solutions for calibration and argon in plasma generation; the energy consume by the lyophiliser, microwave oven and ICP-OES provided additional penalty of 3 points. Extra points from occupational hazard and wastes involve 8 penalty points. So, the final score of the method was 78 which

Table 5
PLSDA external validation confusion matrix for the classification of artichoke samples from their XRF spectra.

Assigned group Original group	PDO	Not PDO	Total	% Correct
PDO	10	0	10	100%
Not PDO	0	6	6	100%
Total	10	6	16	100%

Assigned group Original group	Benicarló	Murcia	Valencia	Total	% Correct
Benicarló	10	0	0	10	100%
Murcia	0	2	0	2	100%
Valencia	0	1	2	3	66.6%
Total	10	3	2	15	93.4%

Table 6
Summary of obtained results by PLSDA.

Technique	N classes	LV	% Variance X block	% Variance Y block	R^2_{cal}	R^2_{CV}	R^2_{pred}	% Correct of calibration	% Correct of cross validation	% Correct of external validation
NIR	2	5	95	87	0.87	0.57	0.59	100	93	88
	3	6	95	74	0–62–0.88	0.12–0.55	0.10–0.62	98	82	80
ICP-OES	2	5	95	78	0.78	0.67	0.79	100	96	100
	3	5	97	71	0.55–0.83	0.4–0.7	0.67–0.87	100	93	100
XRF	2	2	68	87	0.90	0.73	0.83	100	98	100
	3	3	70	88	0.84–0.91	0.3–0.75	0.44–0.82	100	89	93
NIR + XRF	2	4	85	92	0.92	0.58	0.89	100	96	100
	3	6	87	94	0.91–0.96	0.25–0.66	0.37–0.85	100	91	93

mean an excellent green analysis although clearly worse than that based on NIR and XRF spectra.

3.5. Comparison of NIR, ICP-OES and XRF for origin authentication of artichokes

In view of the obtained results in this study, which are summarised in Table 6, if it want to authenticate the samples belonging to the PDO 'Alcachofa de Benicarló', both, ICP-OES and XRF provide satisfactory results at 100% level. So, XRF technique can be used as an alternative to the ICP-OES, as it is a much greener technology, as it can be seen in the 3.4 section, much faster, as it requires little sample preparation and it is cheaper than ICP-OES.

However, if we want to discriminate perfectly the production origin of each sample, XRF provides satisfactory results to 93% of the samples included in the external validation set; which is quite good results, but if we want a perfect differentiation between the three origins studied, we would need to use ICP-OES.

Additionally, XRF and NIR spectra were used together to build PLSDA models and the results obtained are shown in Table 6. Results found were similar to those obtained only by XRF spectra. So it could conclude that the joint use of NIR and XRF is not necessary since XRF provided satisfactory enough results.

4. Conclusions

Studies carried out evidenced the possibility to authenticate artichoke samples produced from the PDO 'Alcachofa de Benicarló' based on its mineral profile, established by ICP-OES, and sample XRF spectra after the appropriate chemometric treatment. On comparing these two analytical approaches it can be seen that the direct use of XRF spectra of the lyophilised edible part of artichokes provides a cheap direct and green way for the characterisation of the sample origin and the clear discrimination of samples produced inside the PDO to other samples coming from close areas. On the other hand, the use of NIR spectra is not powerful enough to do a correct identification of artichoke sample origin.

Acknowledgements

Authors acknowledge the financial support the Ministerio de Economia y Competitividad-Feder (project CTQ2011-25743 and project CTQ2012-38635) and Generalitat Valenciana (project PROMETEO-II 2014-077). Alba Mir-Marqués acknowledges the grant '**Atracció de Talent**' (PREDOC13-110514) provided by the University of Valencia.

References

Aquilanti, L., Santarelli, S., Babini, V., Osimani, A., & Clementi, F. (2013). Quality evaluation and discrimination of semi-hard and hard cheeses from the Marche region (Central Italy) using chemometric tools. *International Dairy Journal, 29*, 42–52.

Ariyama, K., Aoyama, Y., Mochizuki, A., Homura, Y., Kadokura, M., & Yasui, A. (2007). Determination of the geographic origin of onions between three main production areas in Japan and other countries by mineral composition. *Journal of Agricultural and Food Chemistry, 55*, 347–354.

Ariyama, K., Horita, H., & Yasui, A. (2004). Chemometric techniques on inorganic elements composition for the determination of the geographic origin of welsh onions. *Analytical Sciences, 20*, 871–877.

Barreira, J. C. M., Casal, S., Ferreira, I. C. F. R., Peres, A. M., Pereira, J. A., & Oliveira, M. B. P. P. (2012). Supervised chemical pattern recognition in almond (*Prunus dulcis*) portuguese PDO cultivars: PCA- and LDA-based triennial study. *Journal of Agricultural and Food Chemistry, 60*, 9697–9704.

Bevilacqua, M., Bucci, R., Magri, A. D., Magri, A. L., & Marini, F. (2012). Tracing the origin of extra virgin olive olis by infrared spectroscopy and chemometrics: a case study. *Analytica Chimica Acta, 717*, 39–51.

Boeting, K., de Benzo, Z. A., Cervera, M. L., & de la Guardia, M. (2010). Authentication of the protected designation of origin horchata de Valencia through the chemometric treatment of mineral content. *Analytical Methods, 2*, 1723–1728.

Burns, D. A., & Ciurczack, E. W. (Eds.). (2007). *Handbook of near-infrared analysis* (3rd ed.). New York: CRC press.

Costas-Rodriguez, M., Lavilla, I., & Bendicho, C. (2010). Classification of cultivated mussels from galicia (northwest Spain) with European protected designation of origin using trace element fingerprint and chemometric analysis. *Analytica Chimica Acta, 664*, 121–128.

CRDO. (2014). *Consejo Regulador de la Denominación de Origen "Alcachofa de Benicarló"*. Available from: www.alcachofabenicarlo.com.

Di Giacomo, F., Del Signore, A., & Giaccio, M. (2007). Determining the geographic origin of Potatoes using Mineral and trace element content. *Journal of Agricultural and Food Chemistry, 55*, 860–866.

Drivelos, S. A., & Georgiou, C. A. (2012). Multi-element and multi-isotope-ratio analysis to determine the geographical origin of foods in the european union. *TrAC Trends in Analytical Chemistry, 40*, 38–51.

EU Regulation. (2012). In *European Union regulation (EEU) 1151/2012*.

Galuszka, A., Konieczka, P., Migaszewski, Z. M., & Namiésnik, J. (2012). Analytical eco-scale for assessing the greenness of analytical procedures. *TrAC Trends in Analytical Chemistry, 37*, 61–72.

Garrigues, S., & de la Guardia, M. (2013). Vibrational spectroscopy. In M. de la Guardia, & A. González (Eds.), *Food protected designation of origin: Methodologies and applications* (pp. 101–122). Amsterdam: Elsevier.

Garrigues, S., & de la Guardia, M. (Eds.). (2015). *Handbook of mineral elements in food*. Chichester: John Wiley & Sons.

Gonzálvez, A., Armenta, S., & de la Guardia, M. (2009). Trace-element composition and stable-isotope ratio for discrimination of foods with Protected Designation of Origin. *TrAC Trends in Analytical Chemistry, 28*, 1295–1311.

Gori, A., Maggio, R. M., Cerretani, L., Nocetti, M., & Caboni, M. F. (2012). Discrimination of grated cheeses by Fourier transform infrared spectroscopy coupled with chemometric techniques. *International Dairy Journal, 23*, 115–120.

de la Guardia, M., & González, A. (Eds.). (2013). *Food protected designation of origin: Methodologies and applications*. Amsterdam: Elsevier.

Karali, F., Georgala, A., Massouras, T., & Kaminarides, S. (2013). Volatile compounds and lipolysis levels of kopanisti, a traditional greek raw milk cheese. *Journal of the Science of Food and Agriculture, 93*, 1845–1851.

Kelly, S. D., & Bateman, A. S. (2010). Comparison of mineral concentrations in commercially grown organic and conventional crops – Tomatoes (*Lycopersicon esculentum*) and lettuces (*Lactuca sativa*). *Food Chemistry, 119*, 738–745.

Kelly, S., Heaton, K., & Hoogewerff, J. (2005). Tracing the geographical origin of food: the application of multi-element and multi-isotope analysis. *Trends in Food Science & Technology, 16*, 555–567.

Korifi, R., Le Dreau, Y., Molinet, J., Artaud, J., & Dupuy, N. (2011). Composition and authentication of virgin olive oil from French PDO regions by chemometric treatment of Raman spectra. *Journal of Raman Spectroscopy, 42*, 1540–1547.

Matos-Reyes, M. N., Simonot, J., López-Salazar, O., Cervera, M. L., & de la Guardia, M. (2013). Authentication of Alicante's Mountain cherries protected designation of origin by their mineral profile. *Food Chemistry, 141*, 2191–2197.

Mir-Marqués, A., Cervera, M. L., & de la Guardia, M. (2012). A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus. *Journal of Food Composition and Analysis, 27*, 160–168.

Montero-Prado, P., Bentayeb, K., & Nerin, C. (2013). Pattern recognition of peach cultivars (*Prunus persica* L.) from their volatile components. *Food Chemistry, 138*, 724–731.

Moreno-Rojas, R., Sanchez-Segarra, P. J., Camara-Martos, F., & Amaro-Lopez, M. A. (2010). Multivariate analysis techniques as tools for categorization of southern Spanish cheeses: nutritional composition and mineral content. *European Food Research and Technology, 231*, 841–851.

Russo, R., Severino, V., Mendez, A., Lliberia, J., Parente, A., & Chambery, A. (2012). Detection of buffalo mozzarella adulteration by an ultra-high performance liquid chromatography tandem mass spectrometry methodology. *Journal of Mass Spectrometry, 47*, 1407–1414.

Swoboda, S., Brunner, M., Boulyga, S. F., Galler, P., Horacek, M., & Prohaska, U. (2008). Identification of Marchfeld asparagus using Sr isotope ratio measurements by MC-ICP-MS. *Analytical and Bioanalytical Chemistry, 390*, 487–494.

Van Aken, K., Strekowski, L., & Patiny, L. (2006). EcoScale, a semi-quantitative tool to select and organic preparation based on economical and ecological parameters. *Beilstein Journal of Organic Chemistry, 2*, 3.

Zhang, Z. X., Liu, P., Kang, H. J., Liao, C. C., Chen, Z. L., & Xu, G. D. (2008). A study of the diversity of different geographical populations of *Emmenopterys henryi* using FTIR based on principal component analysis and cluster analysis. *Spectroscopy and Spectral Analysis, 28*, 2081–2086.