



Measuring trace element fingerprinting for cereal bar authentication based on type and principal ingredient

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ABSTRACT

This paper introduces a method for determining the authenticity of commercial cereal bars based on trace element fingerprints. In this regard, 120 cereal bars were prepared using microwave-assisted acid digestion and the concentrations of Al, Ba, Bi, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Se, Sn, Sr, V, and Zn were later measured by ICP-MS. Results confirmed the suitability of the analyzed samples for human consumption. Multi-elemental data underwent autoscaling preprocessing for then applying PCA, CART, and LDA to input data set. LDA model accomplished the highest classification modeling performance with a success rate of 92%, making it the suitable model for reliable cereal bar prediction. The proposed method demonstrates the potential of trace element fingerprints in distinguishing cereal bar samples according to their type (conventional and gluten-free) and principal ingredient (fruit, yogurt, chocolate), thereby contributing to global efforts for food authentication.

1. Introduction

Cereal bar is defined as a mass composed of ready-to-eat cereal that has been compressed into a bar shape with a binder system consisting of a sweetened substance and other ingredients (Sciammaro et al., 2018). Currently, the cereal bars have gained acceptance among consumers because they are considered healthy sources of energy production and constitute a balanced nutritional profile (Henchion et al., 2017). Thus, these foods are used as appropriate platform to incorporate essential nutrients such as dietary fiber, protein, vitamins, minerals, or ingredients with functional properties for persons with specific health concerns (Marques et al., 2015).

The increasing demand for cereal bars and other cereal-based products over the last years can be attributed to the growing need for healthier and more nutritious food options (Boukid et al., 2022). Consumers are becoming more conscious of their dietary choices and are actively seeking products that offer a balanced nutritional profile. Consumers, manufacturers, and health professionals alike are interested in obtaining detailed information about the food's nutritional

composition, including both essential and non-essential trace elements present in them. Cereal-based foods, especially those made from whole grains, are considered a rich source of minerals (Pinto et al., 2017). These minerals, also known as trace elements, play vital roles in various metabolic functions since they are required for maintaining proper physiological processes and overall health. Nevertheless, it is crucial to consider the possible occurrence of toxic or objectionable elements in such foods, given the potential contamination of their raw materials (principal ingredients, sweeteners, binders, flavors, and spices, among others) either during cultivation or in the preparation stages (FAO/WHO, 2016).

The quality control of cereal bars is usually measured by using chemical and physical assays (Klerks et al., 2022). Considering that essential and non-essential elements are present at trace and ultra-trace levels in foods, the determination of such elements require of analytical techniques capable of achieving extremely low detection limits and highly selective aiming to remove matrix effects. In this regard, several methods based on flame atomic absorption spectrometry (FAAS), inductively coupled plasma atomic emission spectrometry (ICP OES),

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and ICP-mass spectrometry (ICP-MS) have been developed to determine the multielemental composition of different foodstuffs focused on nutritional and toxicological assessments, as well as authentication approaches (Drivelos & Georgiou, 2012; Laursen et al., 2014; Mazarakioti et al., 2022).

Of the aforementioned techniques, ICP-MS stands out as it is a trusted analytical tool that has been extensively used for quantification of several trace elements due to its high sensitivity, wide dynamic range, low detection limit and simultaneous determination. This technique combines the advantages of ICP for element ionization with the mass spectrometry sensitivity, allowing it to obtain an element fingerprinting describing the information on the sample isotopic composition and the concentration of each analyzed element (Clases & Gonzalez de Vega, 2022). Its applications in foodstuffs encompass the analysis of honey (Gohar & Shakeel, 2021), extra-virgin olive oil (Astolfi et al., 2021), eggs (Esposito et al., 2016), almonds (von Wuthenau et al., 2022), commercial avocado fruit (Munoz-Redondo et al., 2022), wines (Pérez-Álvarez et al., 2019), pepper (Zhang et al., 2019), soybean (Nguyen-Quang et al., 2021), whisky (Gajek et al., 2022), pork (Kim et al., 2017), cowpea beans (Pérez-Rodríguez et al., 2019), truffles (Segelke et al., 2020), and gluten-free snacks (Yalçın, 2017), among others.

Chemometrics plays a key role in resolving some issues related to food authentication or traceability. Within this framework, the utilization of pattern recognition algorithms has gained significant traction for the evaluation of multielemental data, with the purpose of tackling the emerging challenges pertaining to food authenticity and identity certification. These tasks have become increasingly prevalent, driven to ensure the quality, safety, and transparency of foodstuffs. By effectively extracting concealed information from complex datasets, these methods enable enhanced interpretation of results, thereby greatly simplifying the evaluation of new sample characteristics through predictive modeling (Sarker, 2021).

Trace element fingerprinting in cereal bar authentication provides a significant advantage by establishing a unique and characteristic elemental profile for each sample, thereby offering valuable insights into the nutritional information of these products. It is interesting to note that the elemental profile of any food serves as a natural and stable biomarker, allowing to distinguish between genuine and counterfeit or adulterated products. Besides, this analytical tool is capable of detecting and identifying any variations or discrepancies in the sample elemental composition that resulting from different production systems, process operations, geographical origins of raw materials, preparation methods, or potential adulteration practices, making it a reliable indicator for authentication purposes. Likewise, the elemental profiles of cereal bars depend mainly on the ingredients used (cereals, fruits, yogurt, chocolate, among others), and the quality and safety of these foods is therefore subject to the multi-elemental composition of their ingredients.

Considering the foregoing, this paper focuses on utilizing trace element fingerprinting data modeling to ascertain the authenticity of commercial cereal bars based on their type (conventional and gluten-free) and principal ingredient (fruit, yogurt, and chocolate). For this aim, 120 cereal bar samples were prepared following a microwave-assisted acid digestion procedure and the concentrations of elements Al, Ba, Bi, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Se, Sn, Sr, V and Zn were quantified using ICP-MS, thus obtaining a trace element fingerprinting of each sample analyzed. Afterwards, PCA, CART and LDA were applied to the input data towards verifying the genuineness of commercial cereal bars, which contributed for sample authentication and therefore to protect consumers.

2. Materials and methods

2.1. Reagents and analytical standards

Nitric acid 65% (w/v) and hydrogen peroxide 30% (w/v) were purchased from Sigma (St. Louis, MO, USA). Nitric acid was additionally

purified by sub-boiling distillation. A Milli-Q Plus Water purification system (Millipore Corp., Molsheim, France) was used to obtain the ultrapure water (18.2 MΩ cm at 25 °C) with which all the standard and working solutions were prepared. Calibration solutions were prepared by properly dissolving a TraceCERT® CRM multi-element standard solution which was acquired from Merck (Darmstadt, Germany). Standard solutions of ¹¹⁵In and ⁸⁰Y were obtained from Agilent (Santa Clara, CA). Argon (99.998% purity) was supplied by Praxair (Córdoba, Argentina).

2.2. Cereal bar samples

A total of 120 cereal bars were purchased from the Argentinean markets between July and May 2021–2022. The cereal bars under study were categorized based on their type (conventional and gluten-free) and main ingredient. The classification for cereal formulation was divided into six categories: conventional cereal bars with fruit (Fruit), conventional cereal bars with chocolate (Chc), conventional cereal bars with yogurt (Yog), gluten-free cereal bars with fruit (Fruit-GF), gluten-free cereal bars with chocolate (Chc-GF) and gluten-free cereal bars with yogurt (Yog-GF). Additional ingredients such as wheat, oat, rice, corn, soybean, sunflower seeds, amaranth seeds, quinoa seeds, flax seeds, preservatives, conditioning agents, emulsifiers, stabilizers, and coloring or flavoring agents were also included in the formulations but were not considered in this work.

2.3. Sample preparation

Cereal bar samples were grinded in a cryogenic mill to reduce particle sizes. The set grinding program included two steps: freezing (2 min each) and grinding (3 min) and by using two cycles it was possible to complete the sample homogenization process. Later, about 500 mg of each ground sample was placed directly into a microwave-closed vessel, and 2 mL of 30% (w/v) H₂O₂ and 4 mL of 65% (w/v) HNO₃ were added it. The digestion procedure was programmed as follows: Stage 1, 25–200 °C for 15 min; Stage 2, 200 °C for 15 min; and Stage 3, 200–110 °C for 15 min. Finally, the digested samples were diluted to a final volume of 25 mL with ultrapure water and stored until analysis by ICP-MS.

2.4. Apparatus

A cryogenic mill from Spex 6750 (Metuchen, NJ, USA) was used for grinding the cereal bar samples and their acid digestion was performed using an Ethos One (Milestone, Chicago, USA) microwave oven.

The determination of 22 trace elements has been carried out by an Agilent 7700 Series (Agilent Technologies, Japan) ICP-MS spectrometer powered by a 27.12 MHz radiofrequency solid-state generator at 1500 W. The instrument was comprised of a MicroMist glass concentric nebulizer combined with a cooled double-pass spray chamber made of quartz. ICP torch was a Fassel-type torch which consists of a three-cylinder assembly, with injector diameter 2.5 mm. Ni sampler and skimmer cones of 1.0 mm and 0.4 mm were used. To suppress polyatomic interferences originating from sample matrix, octopole reaction system with 5 mL min⁻¹ He as collision gas and kinetic energy discrimination mode was used (collision mode). It was equipped with off-axis ion lens, a quadrupole mass analyzer, and an electron multiplier detector. All instrument parameters were optimized daily while aspirating the tuning solution. The selected isotopes for measurement were: ²⁷Al, ⁷⁵As, ¹³⁷Ba, ²⁰⁹Bi, ^{111,114}Cd, ⁵⁹Co, ⁵³Cr, ⁶³Cu, ⁵⁷Fe, ⁷¹Ga, ⁷Li, ⁵⁵Mn, ⁹⁵Mo, ⁶²Ni, ^{206,208}Pb, ⁸⁵Rb, ⁸⁰Se, ¹¹⁸Sn, ⁸⁸Sr, ^{203,205}Tl, ⁵¹V, and ⁶⁶Zn.

2.5. Analytical performance and quality control measurements

All digested samples and blank solutions were measured by direct nebulization. The monitored isotopes for the ICP-MS analysis were

chosen according to previous studies. Two internal standards (^{115}In and ^{80}Y) were added to all digested solutions aiming at accurately quantifying the elemental composition of samples and correcting any instrument drift during analysis.

Calibration curves were obtained at five different concentration levels in triplicate. Least squares linear regression analysis was used to obtain the coefficients of determination (R^2). The limits of detection (LOD) and quantification (LOQ) were calculated according to the AOAC guidelines (Latimer Jr, 2016), i.e., as 3 and 10 times, respectively, the standard deviation of measurements of 10 blank solutions, divided by the slope of the calibration curve. Analytical quality control measurements were verified using recovery and precision assays. Samples were fortified with studied elements at $0.01 \mu\text{g g}^{-1}$ and $0.1 \mu\text{g g}^{-1}$ and the percentages (%) recovery were determined using matrix-matched standard calibration curves. The variability of the determinations was expressed as the relative standard deviation (% RSD).

2.6. Multivariate data analysis

The original data based on trace element fingerprints were transformed into a matrix format $X (120 \times 19)$, in which the rows corresponded to the 120 cereal bar samples analyzed by ICP-MS, and the columns, to the 19 elemental predictors. Data matrix was first auto-scaled and mean centered to suppress any variability in the different concentration scales registered. Next, principal component analysis (PCA), classification and regression trees (CART) and linear discriminant analysis (LDA) were applied to the preprocessed data. PCA was used to visualize the data natural distribution looking for clustering trends among the sample classes. Instead, CART and LDA were used to evaluate the discrimination of the cereal bar samples according to their type (conventional and gluten-free) and principal ingredient (fruit, yogurt, chocolate). To build the classifiers, the sample set was randomly split into two subsets (training and test). The training subset was made up of 70% of the total samples and served to create classifiers, while test subset comprised the remaining 30%, which was used to validate the built models. The splitting up into subsets was carried out in a stratified manner to maintain the distributions of subsets matching to the original set. Modeling performance was evaluated by measuring the correct classification rate, which was calculated as the ratio between all correct predictions and the total number of examined cases and was expressed as a percentage (%). All calculations were carried out using R-project software version 3.6.3 (R Core Team, 2020) with partykit package (Team, 2020).

3. Results and discussion

3.1. Analytical performance parameters of the ICP-MS method

The multi-element calibration curves were obtained at five different concentration levels. The coefficients of determination (R^2) fitted were greater than or equal to 0.9983, displaying good linearity of ICP-MS method in the selected concentration range for each element. Similar results were obtained for the calibration curves constructed using standard solutions of each element and by spiking the commercial cereal bars matrix with the standard solutions (described earlier). Digested samples that were beyond the linear range were diluted and reanalyzed. The main figures of merit for the method proposed are summarized in Table 1. As can be seen, the LOD values ranged from 0.001 to $0.09 \mu\text{g g}^{-1}$ and those of LOQ were within the range of $0.004\text{--}0.31 \mu\text{g g}^{-1}$. Recovery assays were performed with known samples spiked aiming to assure analytical data reliability. The average recoveries obtained ranged from 92% to 106%, from which it can be inferred that the no significant loss of elements occurs during the microwave digestion step. Regarding the method precision, the RSD (%) values corresponding to the intra-day and inter-day determinations were in the ranges 2.3–6.1% and 4.0–9.8%, respectively. These results indicated that the measurements

Table 1

Figures of merit, precisions, and recoveries obtained for ICP-MS method.

Element	R^2	LOD ($\mu\text{g g}^{-1}$)	LOQ ($\mu\text{g g}^{-1}$)	Precision (RSD %)		Recovery (%)
				Intra-day ^a	Inter-day ^b	
Al	0.9991	0.080	0.270	2.3	4.0	92
As	0.9987	0.005	0.018	5.2	7.1	101
Ba	0.9995	0.040	0.140	5.0	6.8	104
Bi	0.9989	0.001	0.004	5.9	9.7	100
Cd	0.0094	0.002	0.007	3.4	5.2	98
Co	0.9993	0.004	0.014	5.8	9.5	101
Cr	0.9985	0.015	0.052	4.2	8.1	95
Cu	0.9998	0.020	0.060	5.8	7.4	97
Fe	0.9993	0.070	0.240	6.1	9.2	102
Ga	0.9985	0.004	0.012	5.6	9.1	99
Li	0.9991	0.003	0.010	4.8	8.5	104
Mn	0.9992	0.060	0.200	2.9	7.1	106
Mo	0.9983	0.020	0.060	3.8	7.5	99
Ni	0.9985	0.018	0.054	5.4	9.8	100
Pb	0.9994	0.004	0.013	5.3	9.5	98
Rb	0.9987	0.020	0.067	3.8	6.9	96
Se	0.9996	0.003	0.010	4.5	7.8	95
Sn	0.9989	0.001	0.004	5.2	9.0	103
Sr	0.0097	0.010	0.033	6.0	9.3	104
Tl	0.9995	0.003	0.009	5.1	7.9	102
V	0.9988	0.008	0.027	4.6	8.2	100
Zn	0.9983	0.090	0.301	5.7	9.5	98

^a Average of three determinations ($n = 3$).

^b Average of nine determinations ($n = 9$).

by ICP-MS were sufficiently sensitive, precise, and accurate for making the determination of trace elements in commercial cereal bar samples.

Instrumental drifts resulting from possible matrix interferences were also redressed by fortifying matrix with internal standards like ^{115}In and ^{80}Y because they are not detected in cereal bar samples. These assays also followed the digestion procedure used for the real samples and the average recovery of the added elements (97% for ^{115}In and 95% for ^{80}Y) was determined by using matrix-matched standard calibration curves. All results agree with the acceptance criteria for the studied parameters according to the AOAC regulations (Latimer Jr, 2016), i.e., the RSD (%) values were $\leq 15\%$ and the recovery percentages (%) were within range 80–110%. The obtained data indicated that the performance of the ICP-MS method proposed was satisfactory for its purposes. Furthermore, it is proved here that the major components of the commercial cereal bar samples did not significantly interfere in determining of trace elements after performing an appropriate sample digestion, evidencing thus the absence of matrix effects.

3.2. Trace element concentrations

The concentrations of 22 elements (Al, As, Ba, Bi, Cd, Co, Cr, Cu, Fe, Ga, Li, Mn, Mo, Ni, Pb, Rb, Se, Sn, Sr, Tl, V and Zn) were determined by ICP-MS. Table 2 shows the trace element contents found in cereal bar samples. The obtained data are expressed as average values of three measurements along with the corresponding standard deviation (SD).

As seen in Table 2, the trace element composition obtained is quite variable among different sample types, which can be attributed to the nature of the samples analyzed. According to the data obtained, the trace element content showed the following order of abundance: Rb > Mn > Fe > Li > Zn > Al > u > Sr > Ba > Mo > Ni > Bi > Ob > Cr > Se > Co > Cd > Sn for Chc samples, Rb > Mn > Fe > Cu > Li > Al > Zn > Sr > Ba > Ni > Mo > Bi > Pb > Se > Co > Cd > Cr > V > Sn for Chc-GF samples, Fe > Rb > Al > Mn Cu > Zn > Li > Sr > Cr > Ba > Ni > Mo > Bi > Se > Co > Pb > Cd > V > Sn for Fruit samples, Rb > Fe > Mn > Li > Al = Zn > Cu > Sr > Ba > Ni > Mo > Bi > Pb > Cr > Cd > Co > Se > V > Sn for Fruit-GF samples, Fe > Rb > Mn > Al > Cu > Zn > Li > Sr > Mo > Ba > Ni > Cr > Bi > Co > Pb > Se > V > Cd > Sn for Yog samples, and Fe > Rb > Li > Zn > Mn > Cu > Sr > Al > Mo > Ni > Ba > Bi > Cr > Co > Se > Pb > V > Cd > Sn for Yog-GF samples.

Table 2
Trace element concentration profiles found in commercial cereal bar samples.

Element $\mu\text{g g}^{-1}$	Sampling cereal bars (average \pm SD)					
	Chc ^a (n = 18)	Chc-GF ^b (n = 21)	Fruit ^c (n = 20)	Fruit-GF ^d (n = 20)	Yog ^e (n = 20)	Yog-GF ^f (n = 21)
Al	3.31 \pm 0.98	4.20 \pm 1.51	8.52 \pm 3.64	7.21 \pm 3.76	5.40 \pm 2.83	2.23 \pm 0.52
Ba	1.20 \pm 0.25	0.91 \pm 0.42	0.53 \pm 0.42	0.60 \pm 0.35	0.51 \pm 0.007	0.30 \pm 0.008
Bi	0.17 \pm 0.01	0.12 \pm 0.05	0.10 \pm 0.01	0.15 \pm 0.01	0.12 \pm 0.04	0.12 \pm 0.04
Cd	0.017 \pm 0.011	0.051 \pm 0.013	0.031 \pm 0.019	0.055 \pm 0.015	0.019 \pm 0.013	0.016 \pm 0.012
Co	0.041 \pm 0.007	0.053 \pm 0.019	0.062 \pm 0.007	0.040 \pm 0.006	0.067 \pm 0.011	0.039 \pm 0.011
Cr	0.064 \pm 0.008	0.037 \pm 0.009	0.537 \pm 0.130	0.063 \pm 0.025	0.282 \pm 0.119	0.044 \pm 0.010
Cu	3.12 \pm 1.48	4.50 \pm 1.57	7.81 \pm 1.47	2.63 \pm 1.05	4.80 \pm 1.75	2.79 \pm 1.63
Fe	9.6 \pm 4.6	6.3 \pm 3.7	21.4 \pm 3.1	10.6 \pm 1.9	15.6 \pm 4.0	18.8 \pm 4.4
Li	4.71 \pm 1.82	4.40 \pm 1.86	4.33 \pm 1.28	8.70 \pm 2.06	3.81 \pm 1.83	8.72 \pm 0.81
Mn	12.1 \pm 6.0	7.9 \pm 5.1	8.1 \pm 4.3	9.8 \pm 4.2	12.2 \pm 5.1	5.5 \pm 2.5
Mo	0.40 \pm 0.09	0.19 \pm 0.02	0.203 \pm 0.002	0.43 \pm 0.28	0.520 \pm 0.005	0.44 \pm 0.11
Ni	0.38 \pm 0.07	0.38 \pm 0.04	0.42 \pm 0.03	0.44 \pm 0.07	0.40 \pm 0.08	0.34 \pm 0.06
Pb	0.077 \pm 0.044	0.086 \pm 0.064	0.058 \pm 0.041	0.072 \pm 0.033	0.066 \pm 0.037	0.035 \pm 0.026
Rb	13.3 \pm 3.3	15.6 \pm 3.8	13.4 \pm 3.5	11.6 \pm 3.1	13.2 \pm 3.4	13.6 \pm 2.6
Se	0.049 \pm 0.010	0.062 \pm 0.009	0.068 \pm 0.013	0.029 \pm 0.008	0.056 \pm 0.012	0.038 \pm 0.013
Sn	0.014 \pm 0.001	0.010 \pm 0.005	0.008 \pm 0.002	0.011 \pm 0.007	0.009 \pm 0.005	0.012 \pm 0.005
Sr	2.10 \pm 0.75	2.61 \pm 0.41	2.82 \pm 0.48	1.50 \pm 0.34	1.82 \pm 0.40	2.41 \pm 0.86
V	0.020 \pm 0.004	0.031 \pm 0.006	0.030 \pm 0.003	0.022 \pm 0.003	0.021 \pm 0.005	0.033 \pm 0.003
Zn	3.60 \pm 1.19	2.91 \pm 1.36	5.70 \pm 2.31	7.21 \pm 0.70	4.50 \pm 0.93	8.62 \pm 0.81

^a Conventional cereal bars with chocolate.

^b Gluten-free cereal bars with chocolate.

^c Conventional cereal bars with fruit.

^d Gluten-free cereal bars with fruit.

^e Conventional cereal bars with yogurt.

^f Gluten-free cereal bars with yogurt.

As expected, the Fruit samples showed the highest concentrations for most elements regardless of whether the sample contained gluten or not. This suggests that the incorporation of dried fruits to the formulation of commercial cereal bars can serve as an important mineral source. Essential elements Cr and Cu presented higher concentrations in most samples with gluten. The levels of Al, Cr, Cu, Fe, and Sr found agreed with those reported for commercial gluten-free amaranth bars produced in Argentina (Hidalgo et al., 2015). Most of the gluten-free samples showed the highest concentrations of Li. The values obtained for Ni were higher than those found in infant foods from France (Chekri et al., 2019) and cereal bars and cakes from Spain (Zurita-Ortega et al., 2020).

Yog samples present highest concentrations of Co, Mn, and Mo. Co and Mn levels were higher than those reported for pasta bread, porridge, and pasta samples from Brazil (Pedron et al., 2016), while Mo content was similar to those determined in meal replacement shakes (Zurita-Ortega et al., 2020). Instead, Yog-GF samples showed the highest Zn values, which were lower than those found in rice and corn flour samples (de Souza et al., 2019). This element is frequently monitored in food samples since it is necessary for the functioning of over 300 different enzymes in the human body (Sangeetha et al., 2022).

In Chc samples, Ba, Bi, and Sn concentrations were significantly

higher than in the other sample types analyzed. Chekri et al. (Chekri et al., 2019) reported infant foods with lower Ba contents and higher Sr contents than those found in this work. In turn, the Bi concentrations were lower than the amount reported for cereal products from Spain (González-Weller et al., 2013).

Regarding toxic trace elements, As, Cd, Pb, and Tl are cause for great concern due to the harmful effects they cause to human health when ingested or inhaled, so their presence in food must be strictly monitored. In this work, As and Tl contents were below the LOD in all the analyzed samples, whereas Cd and Pb concentrations ranged from 0.01 to 0.05 $\mu\text{g g}^{-1}$ and from 0.03 to 0.08 $\mu\text{g g}^{-1}$, respectively. The highest average Cd concentration was found in Fruit-GF samples (0.055 $\mu\text{g g}^{-1}$) followed by Chc-GF samples (0.051 $\mu\text{g g}^{-1}$). The levels of Pb were the highest in the Chc-GF samples followed by Chc samples. Some authors suggest that these heavy elements can be incorporated into the cereal through the presence of the element in the raw cereals, addition of ingredients like honey, cacao, and dehydrated fruits, or contamination during processing from friction with equipment or metal utensils (Hernández-Martínez & Navarro-Blasco, 2012). The concentrations of Cd and Pb found were higher than those reported for common cereal bars (Zurita-Ortega et al., 2020) and lower than those determined in gluten-free cereal bars (Hidalgo et al., 2015). According to European Food Safety Authority (EFSA) regulations, the tolerable upper intake for Cd is of 2.5 $\mu\text{g kg}^{-1}$ b.w. per week (EFSA, 2012a) and for Pb is of 0.63 $\mu\text{g kg}^{-1}$ b.w. per day for adults (EFSA, 2012b). The levels of Cd and Pb found in all cereal bar samples were below the maximum tolerance limits specified by EFSA, indicating that the samples analyzed were suitable for human consumption.

3.3. Authentication of commercial cereal bars

The authenticity of the commercial cereal bar samples was evaluated based on their type (conventional and gluten-free) and main ingredient (fruit, yogurt, chocolate) by employing principal component analysis (PCA), classification and regression trees (CART), and linear discriminant analysis (LDA) on the trace element fingerprints obtained by ICP-MS, which included the concentrations of elements Al, Ba, Bi, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Se, Sn, Sr, V and Zn. However, as the contents of As, Ga, and Tl in all cereal bar samples were found to be below the limits of detection (i.e., 0.005 $\mu\text{g g}^{-1}$ for As, 0.003 $\mu\text{g g}^{-1}$ for Ga, and 0.004 $\mu\text{g g}^{-1}$ for Tl), these elements could not be considered for sample predictive modeling.

3.3.1. Exploratory and clustering analysis

PCA is a multivariate technique widely used to detect variations in the original data through linear transformations into a small number of principal components (PCs) (Bro & Smilde, 2014). In this work, PCA models were developed considering the entire set of samples as an exploratory tool to ascertain the distribution and possible grouping of the data, as well as to find outliers and/or patterns standing out. Fig. 1 shows the loadings of the original variables for the first two PCs. PC1 explained 48.1% of the total variance. This component was positively correlated with the values of Li, Zn, Mo, and negatively with Cu, Cr, Sr, and Se. In turn, PC2 explained 18.6% of the total variance, so that the first two PCs provided the 66.7% explanation of the data variability. Ba and Fe contents showed the greatest influence in the direction of PC2. The elements Co, Ni, Rb, Cd, Bi, and Sn presented small loadings around the coordinate origin, showing little influence on data structure.

The score plot for the first two PCs is presented in Fig. 2. As can be seen, the scores corresponding to the samples were grouped according to the main ingredient of their formulations, with a large overlap between groups of Chc, Chc-GF, Yog and Yog-GF samples. The scores of Fruit samples were clearly separated from the Fruit-GF samples in PC1. Fruit-GF samples exhibited positive scores probably due to the high content of Li, Mo, and Zn, whereas the Fruit samples showed negative scores, as a consequence of the high concentration of Cr, Cu, and Se. PC1 was responsible for the separation of Fruit samples and Fruit-GF samples,

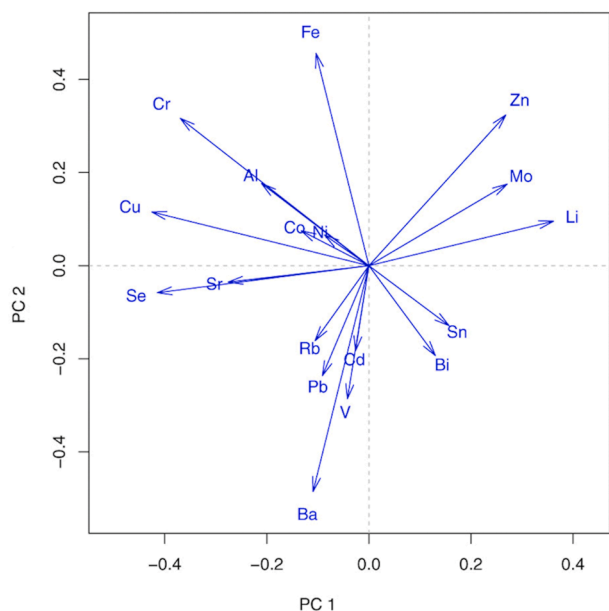


Fig. 1. Loading plot of the element predictors in the first two principal components (PCs).

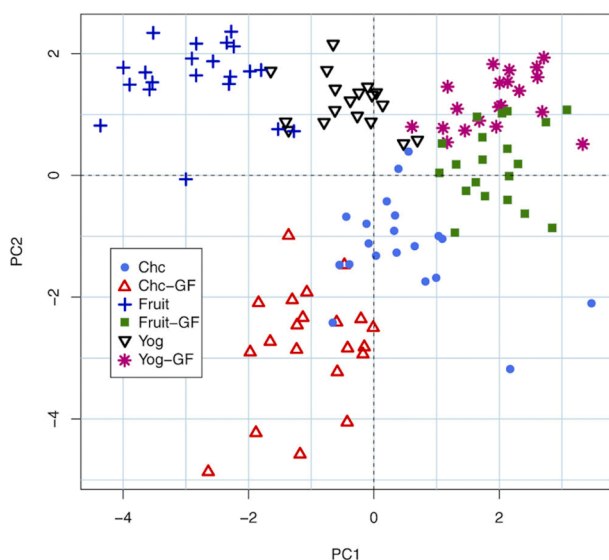


Fig. 2. Score plot of the first principal component (PC1) versus the second principal component (PC2). Chc: Conventional cereal bars with chocolate; Chc-GF: Gluten-free cereal bars with chocolate; Fruit: Conventional cereal bars with fruit; Fruit-GF: Gluten-free cereal bars with fruit; Yog: Conventional cereal bars with yogurt; Yog-GF: Gluten-free cereal bars with yogurt.

while in the PC2 the Yog and Yog-GF samples had positive scores, which were separated from the Chc samples and Chc-GF samples with negative scores.

Even though the results obtained by PCA showed a tendency of grouping among the cereal bars, the systematic separation of samples is not clear. Consequently, the utilization of supervised pattern recognition tools becomes essential here for developing classification models that enable reliable differentiation of cereal bar samples based on their type and main ingredient. In this regard, CART and LDA were also used to evaluate the element fingerprint data. These methods are known for their effectiveness in elucidating the role of predictors in explaining the modeling outcome, including possible interactions among them.

3.3.2. Sample classification

CART analysis is a nonparametric technique in which the results are formed into a tree structure. CART splits the information into subsets, called nodes. These nodes are more homogeneous with respect to the classes in the initial set. Node splitting continues until terminal nodes, or leaves, are obtained. This method is not subject to the assumptions of a parametric statistic, which is its biggest advantage. Therefore, CART can select among input variables the most important for predicting a given outcome without regard for the underlying relationships among variables (Loh, 2014).

Fig. 3 shows tree obtained from CART modeling. Based on the results obtained, the classification of commercial cereal bars was obtained by using only four levels of trace elements, i.e., Cd, Cr, Mo, and Zn. The content of Cr ($>0.11 \mu\text{g/g}$) allowed to discriminate Fruit and Yog samples from the others. Also, Fruit samples were characterized by the low concentrations of Mo ($<0.37 \mu\text{g/g}$) in relation to Yog samples. On the other side, the samples presenting low contents of Cr ($<0.11 \mu\text{g/g}$) were grouped according to the Zn levels ($>6.1 \mu\text{g/g}$). In fact, the latter allowed to separate the Fruit-GF and Yog-GF samples from Chc and Chc-GF samples. Cd concentration below $0.025 \mu\text{g/g}$ made possible the differentiation between Yog-GF and Fruit samples. In turn, Chc-GF samples were characterized by the low levels of Mo ($0.21 \mu\text{g/g}$) regarding Chc samples. It is interesting to highlight that the results obtained by CART are consistent with the score distribution observed previously by PCA. Lastly, the success rates accomplished for sample classification varying from 72 to 85% in test step. Although the prediction rates obtained are quite good, a more accurate model is required for cereal bar authentication.

LDA is a supervised pattern recognition method that provides discriminant functions (DFs) through linear combinations of the original variables. These DFs are used to separate the data into categories by minimizing the within-class and between-class ratio from the sum of squares (Sharma & Paliwal, 2015). Aiming to improve the classification performance and prevent data overfitting, LDA modeling was carried out considering all the variables available in the dataset (Al, Ba, Bi, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Se, Sn, Sr, V, and Zn). As an outcome, all samples of cereal bars were predicted based on their six types (Fruit, Chc, Yog, Fruit-GF, Chc-GF, Yog-GF) by using 10-fold cross-validation method with the aim of evaluating the recognition ability of this algorithm. Considering the percentage (%) of the objects belonging to the training set correctly classified, the success rate achieved was 96% because two Chc samples were incorrectly assigned to Chc-GF group and one Fruit-GF sample was identified as Yog-GF. Instead, three samples were misclassified in the testing step, which resulted in a success rate of 92%, indicating a satisfactory performance of this model for the classification of cereal bar samples. Thereby, LDA model was able to achieve correct classification rate with a proper accuracy and reproducibility as the variances between categories were maximized and the variances within categories were minimized, allowing to predict the new samples according to the studied categories using the elemental fingerprints measured.

The effectiveness of LDA modeling can also be visualized in Fig. 4, from which the projection of the samples in the plot defined by the first two discriminant functions (DFs) is illustrated. Score distribution reveals a remarkable separation of samples into well-defined groups. In particular, Chc and Chc-GF samples were associated with negative scores on LD1 and positive scores on LD2, while Fruit-GF and Yog-GF exhibited negative scores in both LD1 and LD2. Instead, Fruit and Yog samples were grouped showing positive scores in LD1. Based on the obtained results, it was confirmed that the variations detected in trace element concentrations can reliably distinguish cereal bar samples according to their type and principal ingredient. These findings reaffirm the potential of utilizing ICP-MS-based fingerprints as a trustworthy approach for authenticating the principal ingredient of commercial cereal bars and accurately identifying whether they are conventional or gluten-free products.

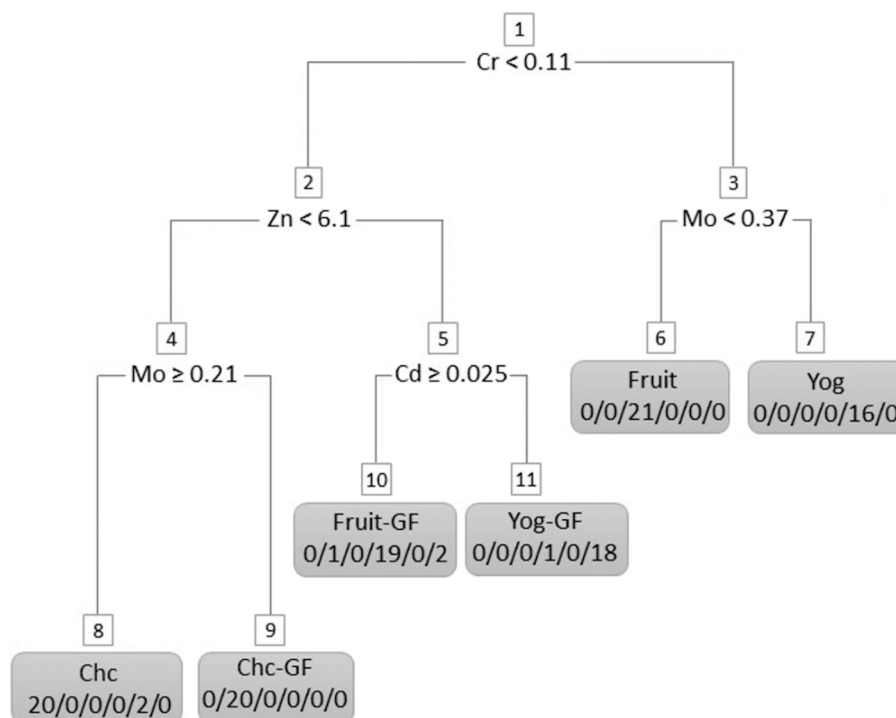


Fig. 3. Classification tree of cereal bar samples according to the element predictors using CART modeling. Chc: Conventional cereal bars with chocolate; Chc-GF: Gluten-free cereal bars with chocolate; Fruit: Conventional cereal bars with fruit; Fruit-GF: Gluten-free cereal bars with fruit; Yog: Conventional cereal bars with yogurt; Yog-GF: Gluten-free cereal bars with yogurt.

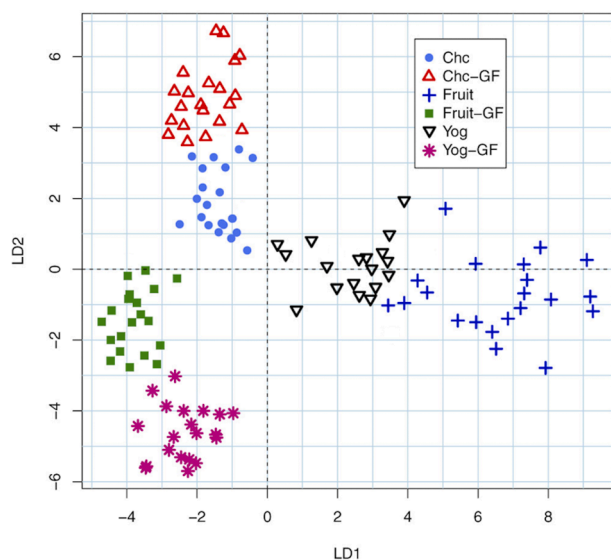


Fig. 4. Scatter plot of the cereal bar samples on the plane defined by the first two discriminant functions obtained by analyzing the trace element fingerprints. Chc: Conventional cereal bars with chocolate; Chc-GF: Gluten-free cereal bars with chocolate; Fruit: Conventional cereal bars with fruit; Fruit-GF: Gluten-free cereal bars with fruit; Yog: Conventional cereal bars with yogurt; Yog-GF: Gluten-free cereal bars with yogurt.

Considering an overall perspective, ensuring the safety and authenticity of cereal bars requires manufacturers to implement effective quality control measures throughout the production process. This involves sourcing raw materials from trusted suppliers, conducting regular testing for contaminants. It is important to emphasize that health professionals and regulatory authorities play a crucial role in monitoring and enforcing food safety regulations to safeguard consumer health. By

maintaining a balance between providing essential trace elements and minimizing potential contamination, cereal bar manufacturers can meet the growing demand for healthier and more nutritious products.

4. Conclusions

Trace element fingerprinting obtained by ICP-MS were employed for assessing the authenticity of commercial cereal bars. Essential elements like Cr, Cu, Fe, Mn, Mo, and Zn were found at concentrations providing significant nutritional contributions. Regarding toxic trace elements, Cd and Pb, were below the maximum tolerance limits set by EFSA, indicating that the analyzed samples are suitable for human consumption since they comply with food quality control guidelines related to these heavy metals. Pattern recognition tools, including PCA, CART, and LDA, was implemented for sample discrimination by analyzing the multi-elemental data. PCA modeling revealed clustering trends among the different sample classes and identified the key element predictors contributing to accurately distinguish each sample group. The CART model achieved correct classification rates ranging from 72% to 85%, while the LDA model further improved the accuracy to 92%, so the latter was chosen for the reliable prediction of cereal bars. This study demonstrated the potential of trace element fingerprints for the simultaneous classification of cereal bar samples either according to their type (conventional and gluten-free) or their main ingredient (fruit, yogurt, chocolate). The findings reported here greatly contribute to the certification process and provide valuable insights into ensuring the authenticity of food products.

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CRediT authorship contribution statement

Michael Pérez-Rodríguez: Conceptualization, Formal analysis, Data curation, Validation, Visualization, Writing – original draft, Writing – review & editing. **Melisa Jazmin Hidalgo:** Conceptualization, Investigation, Methodology, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Alberto Mendoza:** Funding acquisition, Writing – review & editing, Supervision. **Lucy T. González:** Formal analysis, Writing – review & editing. **Francisco Longoria Rodríguez:** Formal analysis, Writing – review & editing. **Héctor Casimiro Goicoechea:** Resources, Project administration, Writing – review & editing. **Roberto Gerardo Pellerano:** Resources, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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