Compuestos bioactivos y producción de grelos y nabizas: variación fenotípica y ambiental

Marta Francisco Candeira
Universidad de Vigo
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COMPUESTOS BIOACTIVOS Y PRODUCCIÓN DE GRELOS Y NABIZAS: VARIACIÓN FENOTÍPICA Y AMBIENTAL

Memoria presentada por **Marta Francisco Candeira** para la obtención del grado de Doctor Europeo por la Universidad de Vigo

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Fdo.: Marta Francisco Candeira

Pontevedra, Septiembre de 2010

Directores:

Elena Cartea González

Investigadora Científica

Misión Biológica de Galicia (CSIC)

Pontevedra

Pablo Velasco Pazos

Científico Titular

Misión Biológica de Galicia (CSIC)

Pontevedra

Fdo.: Elena Cartea González

Fdo.: Pablo Velasco Pazos

Pontevedra, Septiembre de 2010

Dña. Mª Elena Cartea González, Investigadora Científica del Consejo Superior de Investigaciones Científicas y D. Pablo Velasco Pazos, Científico titular del Consejo Superior de Investigaciones Científicas, ambos investigadores de la Misión Biológica de Galicia, en Pontevedra

INFORMAN:

Que el trabajo titulado "Compuestos bioactivos y producción de grelos y nabizas: Variación fenotípica y ambiental" realizado por la licenciada en Biología Dña. Marta Francisco Candeira, bajo la dirección de los Dres. Elena Cartea González y Pablo Velasco Pazos, puede ser presentado para su exposición y defensa como Tesis Doctoral en el Departamento de Ingeniería de Recursos Naturales y Medio ambiente de la Universidad de Vigo.

Considerando que se encuentra concluida damos el Vº Bº para su presentación y lectura.

Fdo.: Elena Cartea González Fdo.: Pablo Velasco Pazos

Pontevedra, Septiembre de 2010

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RESUMEN

En Galicia, los cultivos de la especie *Brassica rapa* subs. *rapa* L conocidos como nabizas y grelos tienen una larga tradición y amplia distribución. Las nabizas son las hojas vegetativas y los grelos son los brotes florales junto con las hojas que los rodean. El cultivo de estas hortalizas se realiza en pequeños minifundios y los agricultores cosechan su propia semilla año tras año. Estos hechos favorecieron la generación de numerosas variedades adaptadas a las condiciones ecológicas de cada zona así como a las preferencias de los consumidores locales lo que implica una amplia diversidad de los cultivos en toda la geografía gallega. Esta diversidad hace que sean una fuente prometedora para buscar características deseables, ya sea desde el punto de vista de la calidad, producción o resistencia a estreses.

En la presente tesis se ha llevado a cabo el estudio de diferentes caracteres agronómicos, nutritivos y sensoriales en 12 variedades locales de *B. rapa* cultivadas en diferentes ambientes. En conjunto, los resultados obtenidos demuestran que existe una gran variabilidad entre variedades locales de *B. rapa*, por lo que fue posible seleccionar aquellas más productivas en función de su uso (nabiza o grelo), o de sus cualidades nutritivas. Gracias al estudio de la interacción de los distintos genotipos con el ambiente fue posible determinar las variedades más estables así como los ambientes más idóneos para su producción. Además, se completó el estudio nutricional de nabizas y grelos con la identificación y cuantificación de compuestos beneficiosos para la salud humana. Sobre algunos de estos compuestos se realizó un estudio más detallado para determinar el efecto del genotipo, el ambiente y el procesado en su contenido final. Así mismo se definieron las cualidades organolépticas de nabizas y grelos y su relación con la presencia de determinados metabolitos secundarios. Como conclusión, los datos aportados en este trabajo pueden ser de utilidad, por un lado para profundizar en el conocimiento acerca de estos cultivos, ya sea de su calidad nutritiva u organoléptica como de su relación con el medio ambiente de cultivo, como a la hora de seleccionar variedades para futuros programas de mejora en base a preferencias tanto del agricultor como del consumidor.

ABSTRACT

In Galicia (northwestern Spain), *Brassica rapa* subs. *rapa* L. includes turnip greens and turnip tops as main crops and are widely distributed and consumed in this region. Turnip greens are the leaves harvested in the vegetative period, while turnip tops are the fructiferous stems with the flower buds and the surrounding leaves. According to the particularities of Galician agriculture (small familiar farms and tradicional cultural practices), farmers obtain their own seeds for sowing. This process has led to a great number of landraces adapted to different conditions and uses all along Galician geography. This diversity makes them a promising source to find desirable properties, either from the standpoint of quality, production or resistance to stresses.

In the present thesis the study of several agronomic, nutritional and sensory traits in 12 local varieties of *B. rapa* grown in different environments has been carried out. Overall, results showed that there is a great variability in the local *B. rapa* varieties, therefore, it was possible to select the most suitable varieties for turnip greens or turnip tops production and the varieties with enhanced and stable levels of health-beneficial compounds. The study of genotype × environment interaction allowed to determine the most stable varieties and also the suitable environments for fresh crop production. Besides, this work assessed the nutritional quality of turnip greens and turnip tops with the identification and quantification of health-beneficial compounds. On some of these compounds a detailed study to determine the effect of genotype, environment and procesing methods on the final concentrations of these metabolites was carried out. Furthermore, were defined the sensory attributes of turnip greens and turnip tops as well as their relationship with secondary metabolites. In conclusion, the data reported in this work may be usefull to deeper understanding about these crops, either for nutritional or sensory quality and its relationship with the culture environment or for selecting varieties for future breeding programs based on producers and consumers preferences.

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CAPÍTULO I

Introducción

1. INTRODUCCIÓN

Los cultivos de nabos, nabizas y grelos han sido durante muchas generaciones un pilar básico de la agricultura gallega, utilizados tanto para la alimentación animal como para el consumo humano. El nabo es la raíz o hipocotilo engrosado de la planta, las nabizas son las hojas vegetativas tiernas y los grelos son los brotes o tallos florales recolectados antes de que la planta entre en floración. Los tres cultivos pertenecen a la especie *Brassica rapa* grupo *rapa*, del género *Brassica*, el cual incluye otras plantas hortícolas de gran importancia económica como el brécol, las coles de Bruselas, la coliflor o el repollo.

Los agricultores gallegos han cultivado durante siglos diferentes variedades de esta especie y, en consecuencia, han ido seleccionando indirectamente aquellas plantas que acumulaban más sustancias de reserva en el hipocotilo para la producción de nabos, aquellas con un rápido crecimiento aéreo vegetativo para la producción de nabizas o bien aquellas con numerosas ramificaciones del tallo principal para la producción de grelos. De este modo, las variedades locales de esta especie que se conservan hoy en día en Galicia presentan una alta diversidad genética en sus formas y usos y están adaptadas a las diferentes condiciones locales de cultivo.

Las nabizas y los grelos representan uno de los productos más típicos de la gastronomía gallega, donde forman parte de la cocina tradicional. Al igual que otras hortalizas del género *Brassica*, estos cultivos aportan a la dieta cantidades importantes de vitaminas, minerales y fitoquímicos relacionados con efectos beneficiosos en la salud humana, por lo que diversos estudios realzan sus cualidades nutricionales y recomiendan su incorporación en la dieta. La recuperación de la cocina tradicional, así como una mayor diversificación en la presentación y comercialización de estos típicos productos han supuesto un apoyo adicional a su demanda. Debido a la creciente importancia de las nabizas y grelos, recientemente estos cultivos se han incluido bajo la denominación "Grelos de Galicia" en el registro de productos con indicativo de calidad (Indicación Xeográfica Protexida, IXP) en todo el territorio comunitario.

1.1. Descripción del género *Brassica*

El género *Brassica* pertenece a la familia *Brassicaceae* (también conocida como *Cruciferae*) y abarca un gran número de especies distribuidas por todo el mundo debido a su capacidad de adaptación a un amplio rango de condiciones climáticas (Rosa, 1999). La familia está compuesta por 350 géneros y en torno a 3.500 especies. Los cultivos del género se desarrollan mejor en las regiones templadas y subtropicales del hemisferio norte (Nuez y otros, 1999) aunque, en general, se consideran plantas moderadamente resistentes a las heladas (Gill y otros, 1965) y altamente resistentes a la sequía (Guerrero, 1992).

Taxonómicamente el género *Brassica* comprende un número considerable de cultivos con una gran diversidad de características biológicas, debido a sus diferentes usos así como a su domesticación (Prakash y Hinata, 1980). Durante años se llevaron a cabo estudios moleculares que trataron de esclarecer las relaciones entre las diferentes especies de este género y con géneros afines (Harberd, 1972; 1976; Gómez-Campo, 1980; Harberd y McArthur, 1980; Takahata y Hinata, 1980; 1986). De estos trabajos surgieron agrupaciones y filogenias muy distintas, reflejo de la dificultad clasificatoria del grupo como consecuencia de la fertilidad entre especies y la variación del número cromosómico entre especies que mantienen similitudes morfológicas.

Gómez-Campo (1999) presentó una clasificación completa del género *Brassica* y sus géneros afines, indicando subgéneros, secciones, especies y subespecies. En dicha clasificación, Gómez-Campo subdivide el género *Brassica* en dos subgéneros: *Brassica* y *Brassicaria*. Posteriormente, el mismo autor sugirió incluir las especies pertenecientes al subgénero *Brassicaria* bajo la denominación genérica *Guenthera* Andr. In Bess, en base varias características morfológicas. En la Tabla 1.1 se puede ver una clasificación del género *Brassica,* el cual incluye especies hortícolas de importancia económica.

Tabla 1.1. Clasificación taxonómica del género *Brassica* (Gómez-Campo, 1999; 2003).

Sección *Brassica*
B. oleracea L.
B. montana Pourret
B. incana Ten. subsp. *incana*
B. villosa Biv. subsp. *villosa*
B. rupestris Rafin subsp. *rupestris*
B. macrocarpa Guss.
B. insularis Moris
B. cretica Lam. subsp. *cretica*
B. botteri Vis. subsp. *botteri*
B. hilarionis Post.
B. carinata Braun
B. balearica Pers.
Sección *Rapa* (Miller) Salmeen
B. rapa L. subsp. *rapa*
B. napus L.
B. juncea (L.) Czern.
Sección *Micropodium* DC.
B. fruticulosa Cyr. subsp. *fruticulosa*
B. nigra (L.) Koch
B. cossoniana Boiss. and Reuter
B. spinescens Pomel
B. maurorum Durieu
B. procumbens (Poiret) O.E.Schulz
B. cadmea O.E.Schulz
B. desertii Danin and Hedge
Sección *Brassicoides* Boiss.
B. deflexa Boiss.
Sección *Sinapistrum* Willkomm
B. barrelieri (L.) Janka
B. oxyrrhina Coss.
B. tournefortii Gouan

Entre todas las especies que integran este género la importancia económica se centra de manera casi exclusiva en seis: tres diploides y tres anfidiploides. Las tres especies diploides, *Brassica nigra* (L.) Koch (2n = 16), *Brassica oleracea* L. (2n = 18) y *Brassica rapa* L. (2n = 20), forman el triángulo propuesto por U (1935) basándose en la citología del género y las relaciones entre los genomas de las especies (Fig. 1.1). *Brassica rapa* y *B. oleracea* se habrían originado a partir de un ancestro común de 6 cromosomas, mientras que *B. nigra* habría evolucionado a partir de un ancestro diferente, estando más próximo a *Sinapis alba*, un tipo de mostaza. En la naturaleza, estas especies han hibridado en diferentes combinaciones para dar lugar a las tres especies anfidiploides: *Brassica carinata* A. Braun (2n = 4x = 34), *Brassica juncea* (L.) Czern. (2n = 4x = 36) y *Brassica napus* L. (2n = 4x = 38). Los genomas de *B. rapa*, *B. nigra* y *B. oleracea* han sido denominados A, B y C, respectivamente. Los anfidiploides *B. juncea*, *B. napus* y *B. carinata* se denominan AB, AC y BC, respectivamente. La mayoría de las especies de brásicas diploides son autoincompatibles y se consideran predominantemente alógamas. Los anfidiploides o poliploides son fundamentalmente autógamas, aunque con una tasa variable de alogamia parcial (Becker y otros, 1999; Soengas y otros, 2010).

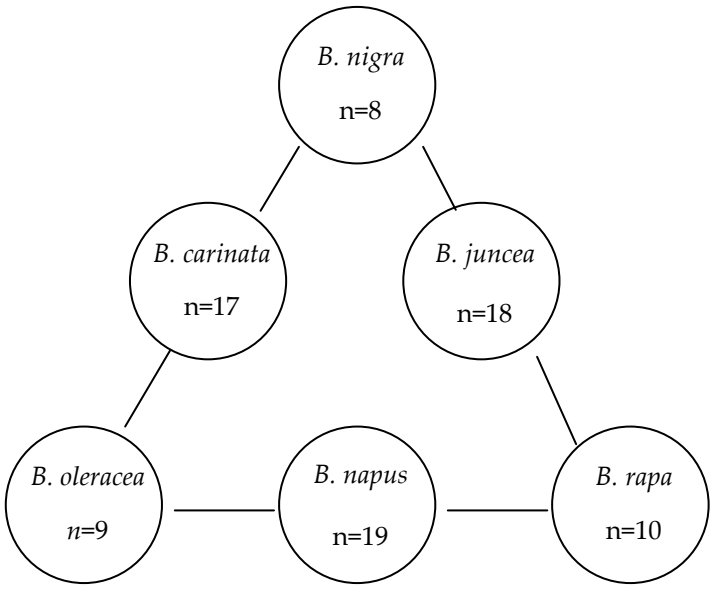


Figura 1.1. Triángulo de U (1935).

Las partes aprovechables para el consumo de las especies de este género son muy variadas y pueden ser yemas, inflorescencias, hojas, raíces, semillas y tallos.

Dependiendo de la parte de la planta utilizada sus cultivos se clasifican en oleaginosos, forrajeros, hortícolas y condimentos. La misma especie puede ser utilizada para varios usos de acuerdo con las distintas formas o tipos. Cuatro especies, *B. oleracea*, *B. rapa*, *B. napus* y *B. juncea*, contienen los cultivos con uso hortícola (Tabla 1.2).

Tabla 1.2. Especies y variedades del género *Brassica* de importancia económica.

Especie	Grupo	Nombre común
Brassica oleracea	*acephala*	Berza
	capitata capitata	Repollo
	capitata sabauda	Repollo de hojas rizadas, col de Milán
	costata	Asa de cántaro
	gemmifera	Coles de Bruselas
	botrytis botrytis	Coliflor
	botrytis italica	Brécol
	gongylodes	Colirrábano
Brassica rapa	*rapa*	Nabo, nabiza, grelo
	chinensis	'Pak-choi', 'bok-choy'
	pekinensis	Repollo chino
Brassica napus	*pabularia*	Nabicol
	napobrassica	Colinabo o rutabaga
Brassica juncea	*rugosa*	Hojas de mostaza
	capitata	Repollo de mostaza

Las especies *B. oleracea* y *B. rapa* contienen la mayoría de los cultivos hortícolas del género *Brassica* y ofrecen muchas opciones de formas comestibles (Fig. 1.2). La mayor variabilidad genética y fenotípica de *B. oleracea* se encuentra en Europa, mientras que Asia representa el principal ámbito de la diversificación de los cultivos vegetales de *B. rapa*. Los cultivos de mayor distribución mundial pertenecen a la especie *B. oleracea* e incluyen formas hortícolas y forrajeras, como la col rizada, repollo, brécol, coles de Bruselas, coliflor y otros; *B. rapa* incluye formas vegetales como el nabo, col china y 'pak-choi' junto con cultivos forrajeros y oleaginosos; los cultivos de *B. napus* se utilizan principalmente por sus semillas oleaginosas para la producción de aceites, como la colza, aunque la especie también incluye tipos forrajeros como la colza

forrajera, hortícolas de raíz como la rutabaga y hortícolas de hoja como el nabicol. Por último, *B. juncea*, se usa principalmente como condimento a partir de sus semillas, aunque también se utilizan sus hojas y las pellas o cabezas para el consumo hortícola, principalmente en los países asiáticos.

Figura 1.2. Diversidad morfológica de las brásicas hortícolas.

Los inicios del cultivo de brásicas datan de finales del neolítico y se centraron en dos regiones fundamentalmente: Asia Menor y el Mediterráneo oriental. Las primeras formas cultivadas, pertenecientes probablemente a la especie *B. rapa* (Gómez-Campo y Prakash, 1999), tuvieron un aprovechamiento hortícola, aunque también cabe destacar su utilización en procesos medicinales (Kimber y McGregor, 1995). Si bien estos cultivos fueron importantes en los imperios griego y romano, donde eran muy valorados por sus características nutritivas, la gran expansión de las brásicas a través de Europa tuvo lugar durante los primeros años de la Edad Media, llegando a constituir la principal fuente de hortalizas de la población campesina hasta la introducción de la patata en el siglo XVIII (Kimber y McGregor, 1995).

1.2. Especie *Brassica rapa*

1.2.1. Origen y evolución

Brassica rapa incluye cultivos hortícolas importantes y, en menor medida, cultivos oleaginosos y forrajeros. Esta especie se expandió de forma natural desde el Mediterráneo occidental hasta Asia Central. Una variedad de esta especie (Yellow sarson) aparece mencionada en la literatura sánscrita (Prakash, 1961), lo que prueba la antigüedad del cultivo. La amplia distribución y el consiguiente cultivo de *B. rapa* a lo largo de miles de años en diversas partes del mundo ha dado lugar a una gran variación morfológica y genética. De esta especie se aprovechan sus semillas, hojas, inflorescencias y raíces o hipocotilos (Fig. 1.3).

Figura 1.3. Diferentes aprovechamientos de la especie *Brassica rapa* según la parte de la planta utilizada: (A) semilla, (B) hoja, (C) inflorescencia, (D) hipocotilo.

Se han propuesto varios nombres y esquemas para la clasificación de los diferentes morfotipos (Gómez Campo, 1999; Diederichsen, 2001). Sin embargo, muchos aspectos de la filogenia dentro de la especie no se entienden completamente, hecho que hace necesario la creación de una nomenclatura aceptada internacionalmente de la especie y de sus grupos de cultivos. En la Tabla 1.3 se puede encontrar una descripción de los

grupos taxonómicos de *B. rapa*, el nombre y la zona principal del cultivo así como la parte de la planta utilizada.

Tabla 1.3. Descripción de los grupos taxonómicos dentro de *Brassica rapa*.

Grupo	Cultivos	Principal zona de cultivo	Parte utilizada
chinensis	col china, 'pak-choi' o 'bok-choy'	China	Hojas
dichotoma	'brown sarson', toria	India	Semilla
narinosa	'wutacai' o 'heibaicai'	China	Hojas
nipposinica	'mibuna', 'mizuna'	Japón	Hojas
oleifera	nabina o colza	China	Semilla
pekinensis	repollo chino, 'pe-Tsai'	China	Hojas
perviridis	'komatsuna' o mostaza espinaca	Japón	Hojas
rapa o *rapifera*	nabo, nabiza, grelo	Europa	Raíz, hoja e inflorescencia
parachinensis	'caixin' o 'caitai'	China	Inflorescencia
trilochularis	'yellow sarson'	India	Semilla

El grupo *oleifera* comprende cultivos oleaginosos utilizados por su semilla, conocidos en Europa como nabina o colza. En India los cultivos utilizados para la producción de aceite son los grupos *trilocularis* y *dichotoma* al que pertenecen los tipos sarson y toria. El grupo *rapa* o *rapifera*, distribuido por toda Europa, se caracteriza por el engrosamiento del hipocotilo, que puede presentar diversos colores y formas, dando lugar a la formación de un nabo. Dentro de este mismo grupo se encuentran además de los nabos, las nabizas y los grelos, ampliamente utilizados en la cocina tradicional en Galicia, Portugal (bajo el nombre de nabiça) e Italia (bajo el nombre de broccoletto cima di rapa). Las nabizas son las hojas de la planta, mientras que los grelos son los brotes florales. En China se consumen también las flores del cultivo llamado 'choy-sum' (grupo *parachinensis*), estas inflorescencias se conocen como 'caixin' o 'caitai'. Aunque estos últimos tienen un hábito de crecimiento similar a los grelos tienen un sabor muy diferente, por lo que, probablemente, evolucionaron de forma independiente.

El grupo más numeroso y diverso de cultivos de *B. rapa* está formado por las hortalizas de hoja diferenciado a su vez en varias subespecies o grupos de cultivos (Fig. 1.4). Los cultivos del grupo *pekinensis* se caracterizan por tener hojas grandes de superficie rugosa y formar cabezas de diferentes formas. Por otro lado, las hojas de 'pak-choi' o 'bok-choy', también llamados col china (grupo *chinensis*) no forman cabezas y son lisas. El 'wutacai' (grupo *narinosa*) constituye uno de los subtipos de cultivos de 'pak-choi' por su aspecto plano y abundantes hojas oscuras. En Japón los cultivos de *B. rapa* consumidos por sus hojas son muy variados, pueden tener hojas anchas y de margen entero como la 'komatsuna' (grupo *perviridis*), estrechas e incisivas como la 'mizuna' o enteras coma la 'mibuna' (ambas pertenecientes al grupo *nipposinica*) y son generalmente utilizadas crudas en ensaladas.

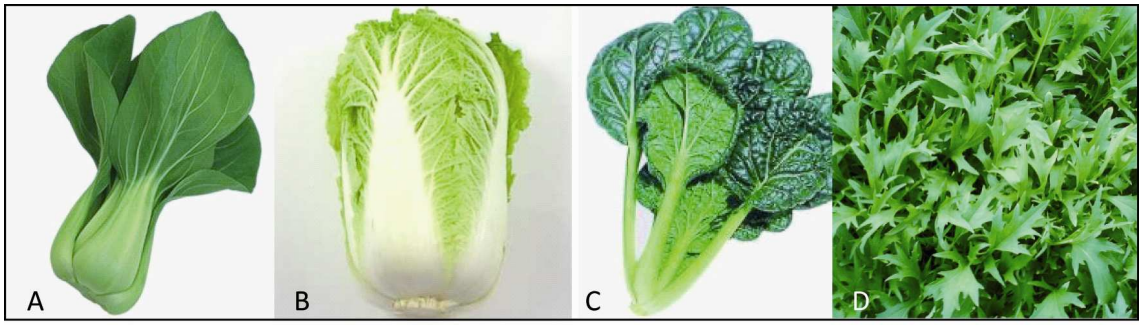


Figura 1.4. Algunos de los grupos de cultivos de hoja de la especie *Brassica rapa*: (A) grupo *chinensis*, (B) grupo *pekinensis*, (C) grupo *narinosa*, (D) grupo *nipposinica*.

Hoy en día esta especie está representada por un gran número de subespecies de importancia reconocida. Estudios basados en la morfología, distribución geográfica, isoenzimas y moleculares indican que *B. rapa* se originó a partir de dos centros de origen independientes (Gómez Campo, 1999). Europa constituiría el centro primario de origen para formas oleíferas y nabos (grupos *oleifera* y *rapa*, respectivamente) mientras que las formas orientales se diferenciarían como hortalizas de hoja en el sur de China. Por último, las formas oleaginosas de India pudieron originarse a partir de formas oleíferas europeas.

1.2.2. Importancia económica

Los cultivos de la especie *B. rapa* desempeñan un papel importante en la agricultura y horticultura mundial. Poseen una larga tradición de cultivo y son ampliamente conocidos, ya que forman parte de numerosos platos típicos en todo el mundo. La mayoría de ellos tiene un aprovechamiento hortícola a partir de sus partes verdes que son consumidas tanto en crudo (en ensaladas) como cocinadas. El nabo hortícola tiene un uso similar a la patata en múltiples recetas, principalmente en caldos y purés. En la alimentación animal, los cultivos de esta especie pueden ser, además, utilizados como suplemento en forma de forraje en verde o como concentrado proteico resultante de la extracción del aceite de la semilla. Sin embargo, a nivel mundial no se dispone de datos oficiales sobre la producción y el consumo de los diversos cultivos de *B. rapa* ya que para la gran mayoría de ellos su comercio se realiza en pequeños mercados locales.

Las estadísdicas mundiales de la FAO (FAOSTAT, 2010) sobre las brásicas hortícolas diferencian dos apartados: 'coles y otras crucíferas', por un lado y 'coliflor y brécol', por otro. Dentro del primer apartado están incluidos cultivos como el repollo, Col de Milán, col china, col de Bruselas y la col rizada, entre otros. Todos estos cultivos pertenecen a la especie *B. oleracea* con la excepción de la col china, que, como se mencionó anteriormente, pertenece a *B. rapa* por lo que probablemente este informe no incluye otros cultivos de esta misma especie como el repollo chino, los grelos o las nabizas.

Como ya se ha comentado, las partes verdes de *B. rapa* se utilizan como hortalizas de una forma importante en el este de Asia. La col china y el repollo chino son líderes en el ranking anual de la producción de hortalizas en China, especialmente en el norte. En China, el área de cultivo anual de coles abarca unos 1,8 millones de hectáreas, aproximadamente el 30% del total de las hortalizas a nivel nacional (FAOSTAT, 2010). En Corea, la col china es muy apreciada ya que se utiliza como componente principal del 'kimchi', una receta tradicional coreana. En los últimos años el consumo y producción de estas hortalizas ha crecido paulatinamente en diferentes países de Europa y América, donde cada vez es más común encontrarlos a disposición del consumidor. Sin embargo, no se dispone de datos oficiales respecto al área cultivada y producción.

Las semillas de las variedades oleaginosas de *B. rapa* son muy apreciadas por su contenido en aceite y proteínas. El aceite resultante es la base de aplicaciones industriales para margarinas y otros productos comestibles, aunque hoy en día también se utiliza como combustible para la obtención de biodiesel. Las variedades productoras de aceite poseen una larga tradición en Estados Unidos, Canadá, China e India (Zhang y otros, 2004). Durante la década de los años 70 el 75% de la superficie de canola cultivada pertenecía a la especie *B. rapa* hasta que a partir de 1990 fue desplazada por variedades de canola o colza de la especie *B. napus*, debido principalmente a que esta última especie posee un mayor rendimiento. Sin embargo, el corto período de crecimiento de *B. rapa* supone para esta especie una elección óptima en algunas zonas del centro y norte de Europa (Ofori y Becker, 2008).

Respecto a la especie *B. rapa* grupo *rapa*, los nabos (hortícolas y forrajeros) se encuentran ampliamente distribuídos en Estados Unidos, Europa y Canadá mientras que las nabizas y grelos aparecen como formas hortícolas exclusivamente en Europa. En Portugal, los grelos ocupan el sexto puesto dentro de las brásicas hortícolas con cerca de 1.000 ha cultivadas (Aires, 2009) mientras que en el sur de Italia ocupan más de 10.000 ha (de Pascale y otros, 2007). En España, las nabizas y grelos son conocidos de modo casi exclusivo en Galicia y su modo de venta y consumo es preferentemente en mercados locales; por ello, una vez más es díficil obtener cifras reales en cuanto a producción y rendimiento de estos cultivos.

1.2.3. Cultivos de *Brassica rapa* en Galicia

Los cultivos de la especie *Brassica rap*a que están presentes en Galicia pertenecen al grupo *rapa* y se conocen como nabos, nabizas y grelos (Fig. 1.5). Estas verduras han sido durante muchas generaciones un pilar básico de la agricultura gallega, tanto como actividad destinada a la obtención de productos para consumo humano como en la alimentación animal.

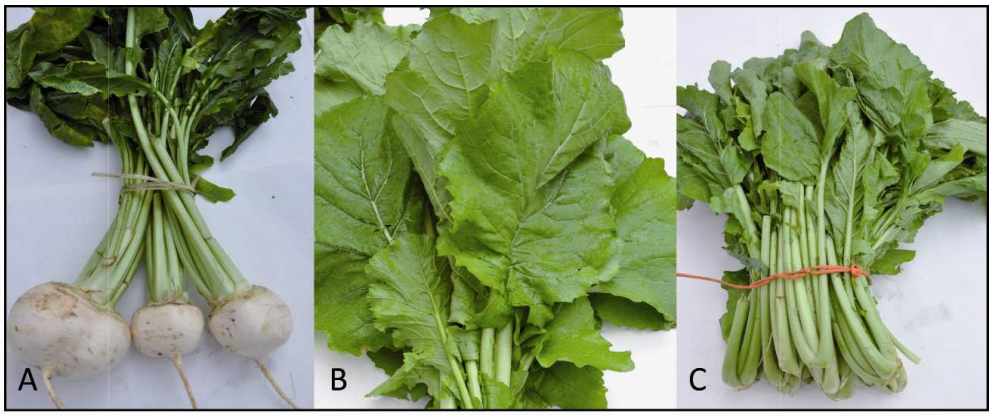


Figura 1.5. Cultivos de *Brassica rapa* grupo *rapa*: (A) nabos, (B) nabizas y (C) grelos.

1.2.3.1. Historia y situación actual

Según la Consellería de Cultura e Turismo, en los estudios arqueológicos llevados a cabo en Prado do Inferno, perteneciente a la parroquia y ayuntamiento de Muras, en el norte de la provincia de Lugo, aparecieron restos del primer cultivo de brásicas en Galicia, los cuales datan aproximadamente del 3.000 a.C. Aunque la incorporación de los cultivos de *B. rapa* a la rotación de cultivo en Galicia no aparece por primera vez documentada hasta el siglo XIII a través de diversos documentos forales y otros de compra-venta de fincas denominadas nabales. Más tarde, durante el siglo XVI, descripciones de la época hacen referencia al cultivo y en los expedientes de Hacienda del Archivo General de Simancas se ofrecen datos de producciones. '*En la tierra del Deza se cultivaron entre 1.580-1.585 un promedio de 4.745 fanegas de trigo, 14.000 de centeno y 6.100 de mijo, amén de 260 carros de nabos y unos haces de lino*' (Consellería de Cultura e Turismo, Xunta de Galicia).

Hoy en día estos cultivos siguen siendo un producto típico y forman parte de la cultura popular gallega. Son muchas las cantigas, adivinanzas, refranes y frases hechas que, en referencia a la planta o a los platos que se elaboran con ella, expresan creencias e ideas recibidas de la comunidad. Un dicho popular dice '*Do nabo sae a nabiza. Da nabiza sae o grelo. Nabo, nabiza e máis grelo, trinidade do galego. Son tres persoas distintas, un só Deus verdadeiro*' puesto que los tres productos proceden de la misma planta. De la planta se cosecha su hipocotilo o nabo, con aprovechamiento hortícola o forrajero, teniendo las hojas un aprovechamiento secundario. El nabo, cuando es tierno, se utiliza

como ingrediente en caldos, pero el destino más común es la alimentación de ganado. Las nabizas y grelos resultan ser hortalizas imprescindibles en alguno de los platos más representativos de la cocina gallega como el caldo o pote gallego, el cocido y el lacón con grelos constituyendo asimismo un elemento diferenciador con respecto a la cocina tradicional de otras regiones.

Durante la época de carnaval los grelos son el producto estrella en los mercados pero también de las tradicionales ferias y fiestas gastronómicas así como de los platos que con ellos se elaboran. Hay que destacar la 'Feira do Grelo de As Pontes' (A Coruña) que este año alcanzó su XXX edición, aunque también son muy populares las ferias de Abadín (Lugo) y Val de Xestoso (A Coruña) (Fig. 1.6).

Figura 1.6. (A) Cartel de la 'Feira do Grelo de As Pontes', y fotografías de (B) la 'Feira do Grelo de Abadín' y (C) la 'Feira do Grelo de Val de Xestoso'.

Las ferias de grelos comenzaron como encuentros sencillos que tenían como eje principal un concurso de manojos de grelos en el que participaban los agricultores de la zona. A la vista del éxito alcanzado se hacen coincidir con el carnaval, época que se considera más relacionada con el consumo de este vegetal, ya que es cuando se prepara el lacón con grelos.

1.2.3.2. Importancia económica de nabizas y grelos

En España, Galicia es el principal productor y consumidor de nabizas y grelos. Aunque en los anuarios no existen datos oficiales, se calcula que en la comunidad gallega existen unas 6.500 ha dedicadas al cultivo de nabizas y grelos. Según datos de la Consellería do Medio Rural, cada año se cosechan en Galicia cerca de 100.000 toneladas

de grelos. Lo más probable, sin embargo, es que las cifras reales sean mucho mayores ya que para estos cultivos no existe una estructura comercial establecida, se venden en fresco en los mercados locales y básicamente se cultivan en pequeñas superficies destinadas al autoconsumo. Por ello, es posible que la superficie real dedicada a cultivos de huerta en la comunidad gallega sea mayor ya que las parcelas para autoconsumo no están contabilizadas en los anuarios.

La zona de producción se extiende por todos los municipios de la Comunidad Autónoma de Galicia, aunque la provincia de Lugo es la gran productora de nabizas y grelos de Galicia. Este cultivo invernal está presente en todas las zonas de la misma. La segunda zona de producción de nabizas y grelos es el entorno de Santiago de Compostela, especialmente la zona norte de la comarca, donde gozan de gran fama los grelos de la localidad de Ordes, en la que es práctica común la venta de esta verdura a pie de carretera por los 'greleiros', término aceptado en 2008 por la Real Academia Gallega y definido como la 'persona o entidad que se dedica al cultivo de los grelos o que negocia con ellos'. Otra zona importante es el norte de la provincia de A Coruña, gozando de especial renombre los grelos de Monfero.

En resumen, las dos principales zonas de producción de Grelo en Galicia son Santiago y Lugo, por lo que se habla de dos grandes ecotipos a partir de los cuales se han obtenido las variedades comerciales registradas como 'Grelos de Santiago' y 'Globo blanco de Lugo'.

La producción de nabizas y grelos ha sido desde siempre muy artesanal y su principal forma de presentación es en fresco en mercados locales, aunque cada vez es más frecuente su comercialización en pequeñas y grandes superficies. La revalorización del producto junto con su carácter perecedero y el corto período de cosecha ha hecho que en la actualidad más de una docena de empresas gallegas y nacionales transformen grelos y nabizas, se pueden encontrar comercializados en lata, desecados y próximamente como producto de cuarta gama, así como formando parte de preparaciones culinarias tales como el caldo gallego, revueltos, etc. (Fig. 1.7).

Figura 1.7. Diferentes formas de grelo envasado disponibles en el mercado.

Una de las empresas más antiguas dedicada al envasado de grelos en Galicia es Conservas A Rosaleira, en O Rosal (Pontevedra). En el año 1967 inició la producción de grelos en conserva y fue durante muchos años la única fábrica de conservas vegetales de Galicia. Más tarde le siguieron otras empresas centenarias dedicadas a la conserva de una amplia gama de verduras y hortalizas que en los últimos años también apostaron por los grelos. Entre ellas cabe citar Marrón Glacé en Ourense y Gutarra en Villafranca (Navarra).

Cooperativas gallegas como Champivil, situada en Vilalba (Lugo), y Val Xestoso, en Monfero (A Coruña) también ampliaron mercado con la incorporación de grelos procesados en su oferta. Desde el año 2000 Champivil comercializa 70.000 kilos anuales de grelos congelados, y unos 200.000 kilos anuales enlatados. La Cooperativa Val Xestoso, por su parte, comercializa al año más de 35.000 kilos de grelos, la mayor parte de ellos a través de una cadena gallega de supermercados y el resto en restaurantes de su zona de influencia. Otra cooperativa destacada es Milhulloa, de Palas de Rei (Lugo), distinguida con varios premios en los últimos años por su innovación en el entorno rural. Esta es la única empresa que comercializa grelos y nabizas deshidratados y procedentes de la agricultura ecológica.

Ante la importancia que el cultivo de grelos tiene para Galicia, la Consellería do Medio Rural y los productores de grelos impulsaron una marca de calidad que fue reconocida por la Unión Europea a través de la correspondiente publicación de su registro en el Diario Oficial de la Unión Europea (DOUE) el 30 de Octubre de 2009. Posteriormente, tras el nombramiento del Consejo Regulador y de su publicación en el

Diario Oficial de Galicia (DOG), con fecha del 8 de enero de 2010, los grelos de Galicia obtuvieron el registro definitivo como "Indicación Xeográfica Protexida Grelos de Galicia" (IXP) (Fig. 1.8). El producto amparado por la IXP se define como "*la parte vegetativa de la especie Brassica rapa L. var. rapa de las variedades correspondientes a los ecotipos de Santiago y Lugo, así como de las variedades comerciales registradas "Grelos de Santiago" y "Globo blanco de Lugo" destinadas al consumo humano*". Estas variedades se definen por tener un color verde intenso y un sabor ligeramente ácido combinado con cierto amargor. El Instituto Galego de Calidade Alimentaria (INGACAL) será el órgano encargado del control y certificación para los productos de la indicación, mientras que la tutela administrativa le corresponderá a la Consellería de Medio Rural.

Figura 1.8. Símbolo del indicativo de calidad 'Indicación Xeográfica Protexida Grelos de Galicia'.

1.2.3.3. Descripción y condiciones del cultivo de *Brassica rapa* grupo *rapa*

Los cultivos incluidos en *B. rapa* grupo *rapa* L son anuales. Poseen una raíz pivotante, bulbosa (aplanada, alargada, cilíndrica o redondeada dependiendo de la variedad), carnosa, que se hincha hasta alcanzar tamaños que pueden llegar a los 5-15 cm de diámetro y pesar de media 150 g, aunque pueden llegar a pesar hasta 500 g (Padilla y otros, 2005). La carne de esta raíz es blanca o amarillenta, de sabor endulzado o picante, también dependiendo de la variedad. Al exterior, presenta tonalidades blanquecinas, aunque puede mostrar colores rojizos o púrpuras en el extremo superior.

La planta mide entre 30 y 120 cm de alto y el tallo es liso y ramificado, aunque el grado de ramificación depende de la variedad y de las condiciones ambientales (Padilla y otros, 2005). Las hojas vegetativas o típicas de nabizas son pilosas, pecioladas, cerosas y con lóbulos laterales anchos y pequeños, aunque el terminal es

mayor. A partir de las hojas más bajas se formarán los tallos florares y cada uno terminará en una inflorescencia. Las hojas del tallo floral o grelos son oblongo-lanceoladas con dos orejuelas grandes, redondeadas, abrazadoras y glabras (MARM, 2010) (Fig. 1.9).

Las flores aparecen agrupadas en racimos terminales, tienen cuatro pétalos de color amarillo vivo y un pedúnculo de una longitud similar a la del resto de la flor. Los frutos son silicuas de 3,5 a 8 cm de largo, con una sola hilera de semillas (Downey y otros, 1980).

Figura 1.9. Morfología de las hojas de *Brassica rapa* grupo *rapa* desde el periodo vegetativo (derecha) hasta el periodo reproductivo (izquierda).

Los cultivos de *B. rapa* son hortalizas de climas frescos, pero no soportan heladas repetidas (Rosa, 1999). Las temperaturas elevadas provocan una floración precoz a costa del desarrollo (Mainardi, 1995). El mismo efecto puede producirlo las bajas temperaturas por lo que se necesita un emplazamiento abrigado durante el invierno en zonas muy frías (Maroto, 1995). Los mejores resultados se obtienen en las regiones templado-frías y en lugares no demasiado soleados.

El suelo requiere una textura media y buen drenaje (Yuste, 1997). Deben ser suelos ricos en materia orgánica, frescos, no encharcados, sueltos y fértiles (Cordeiro y otros, 1998) siendo la llanura la situación más conveniente (Tamaro, 1968). El pH aconsejado para el suelo oscila entre 6,0 y 6,9 (Yuste, 1997).

La siembra se realiza a partir de mediados de agosto, en tiempo fresco, variando la fecha en función de la zona de producción y la precocidad de la variedad. La siembra tradicional se realiza manualmente a voleo procurando un reparto

uniforme y con una profundidad de 2-3 cm (Fig. 1.10 A). La dosis de siembra varía entre 1 y 10 kg/ha en función de la zona de producción y del manejo que se haga del cultivo (DOG, 2010). Otro modo de cultivo es mediante trasplante a partir de plántulas previamente germinadas en semilleros o macetas, al igual que se hace con otros cultivos del género como las berzas o los repollos. En este último caso, la disposición del cultivo en la parcela se realiza en surcos a una distancia entre sí de 35-50 cm y la densidad final obtenida es, por supuesto, menor (Fig. 1.10 B).

Figura 1.10. (A) Cultivo de nabizas y grelos a voleo y (B) en surcos.

El cultivo no precisa un cuidado excesivo, tan sólo escardadas eventuales para mantener el suelo limpio de malas hierbas (Mainardi, 1995). El riego se lleva a cabo en la época crítica de falta de agua que se produce en las primeras fases de cultivo, a los 4-5 días de la siembra o trasplante, durante la germinación y anclaje de las plantas en campo, respectivamente. El riego es conveniente en épocas de sequía estival que se produce de forma más acusada en zonas del interior de la comunidad.

El momento óptimo de recolección dependerá del producto que se quiera obtener, de la precocidad de la variedad, de la época de siembra, de las condiciones edafo-climáticas y de las prácticas de cultivo. A las seis u ocho semanas se puede realizar un primer aprovechamiento de las hojas más tiernas o nabizas. Si la densidad de siembra es muy elevada se realiza un aclareo, si no simplemente se cortan las hojas y tallos. A partir de los tres meses se puede empezar a cosechar los tallos florales o grelos, justo antes de que los brotes se abran y se produzca la floración. Una vez recogidos puede haber un rebrote que se puede alargar hasta bien entrada la primavera. Son por tanto, hortalizas de alta estacionalidad con épocas de recolección específicas.

La cosecha se realiza manualmente (Fig. 1.11 A) y requiere un manejo muy cuidadoso para prevenir daños en las hojas, lo cual afecta a la apariencia y constituye una posible fuente de entrada de microorganismos causantes de enfermedades. Cada tallo secundario conforma un grelo (Fig. 1.11 B) y la forma típica de comercialización es en fresco, en tradicionales manojos de 0,5 a 1,0 kg (Fig. 1.11 C) (DOG, 2010).

Figura 1.11. (A) Cosecha manual de nabizas, (B) tallos secundarios que forman cada uno de los grelos y (C) prsentación de manojos de grelos para su venta.

1.3. Calidad de nabizas y grelos

La calidad de las nabizas y los grelos, así como de las hortalizas en general, se establece en función de criterios de apreciación visual como el tamaño, color o carencia de defectos y enfermedades, además de otros atributos sensoriales como el sabor, el aroma y la textura. Sería conveniente conjugar estos factores con otros criterios de calidad como son los parámetros referentes a su contenido en nutrientes o compuestos bioactivos, ya que al igual que otros cultivos hortícolas del género *Brassica*, la nabiza y el grelo, son una buena fuente de compuestos beneficiosos para la salud humana como minerales, fibra, glucosinolatos y antioxidantes naturales, entre los que se incluyen las vitaminas y los compuestos fenólicos (Singh y otros, 2007).

1.3.1. Cualidades nutritivas de nabizas y grelos

Desde siempre, la sabiduría popular ha atribuido a las nabizas y grelos efectos beneficiosos para la salud. Hoy en día sabemos que estos cultivos, al igual que el resto de las brásicas hortícolas, son productos con pocas calorías por su bajo contenido en grasa y proteína y son ricos en fibras, minerales y vitaminas. Si se comparan con otros cultivos de hortalizas (alcachofa, zanahoria, maíz dulce, cebolla, lechuga o espinaca),

las brásicas tienen el mayor nivel de vitamina C (USDA, 2008) causante de que estos vegetales hayan sido utilizados históricamente en medicina para combatir el escorbuto (Rosa, 1999). También son altos en vitamina E, vitamina B-6, vitamina A, β-caroteno, luteína, y vitamina K. La vitamina C, E y carotenoides tienen el potencial de prevenir y tratar enfermedades (Jahangir y otros, 2009). Además, los cultivos de brásicas muestran altos niveles de folato, que es una vitamina escasa e importante en relación con la reducción del riesgo de padecer enfermedades vasculares, cáncer y defectos del tubo neural (Jahangir y otros, 2009).

Se caracterizan además por ser ricos en minerales, particularmente en potasio, cobre, magnesio, manganeso, hierro, zinc y calcio (Kopsell y otros, 2004; Ayaz y otros, 2006). Entre éstos, lo más destacado es su alto nivel de calcio con respecto a otras hortalizas (Bicudo y otros, 1990; Farnham y otros, 2000; Kopsell y otros, 2004). Según Lucarini y otros (1999), el calcio presente en las brásicas muestra una excelente biodisponibilidad, debido a los bajos niveles de los ácidos oxálico y fítico. También muestran altos niveles de potasio, mineral esencial que participa en diferentes procesos metabólicos (síntesis de proteínas y metabolismo de los carbohidratos, entre otros), así como en el mantenimiento de la salud humana (presión arterial, enfermedades cardiacas y osteoporosis). El resto de minerales aparecen en niveles adecuados, incluyendo el selenio, que es un elemento importante desde el punto de vista biológico y sanitario (Pyrzynska, 2009).

Un factor a tener en cuenta en estos cultivos, al igual que en todas las crucíferas, es la presencia de un tipo de fitoquímicos presentes únicamente en la familia *Brassicaceae* y denominados glucosinolatos ya que éstos son los principales responsables de las cualidades organolépticas, nutritivas y medicinales atribuidas a estas hortalizas. Por otro lado, presentan una cantidad importante de compuestos fenólicos tales como los flavonoides kaempferol y quercetina con un importante papel antioxidante y derivados de los ácidos clorogénico y sinápico. En conjunto, estos compuestos presentes en nabizas y grelos grelos tienen un efecto positivo sobre la salud, ya que se ha relacionado el consumo de brásicas hortícolas con la disminución de la incidencia de ciertos tipos de cáncer y de enfermedades cardiovasculares (Rosa

1999, Mithen y otros, 2000, Fahey y otros, 2001, Smith y otros, 2005, Traka y Mithen, 2009).

1.3.1.1. Glucosinolatos y sus productos de degradación

Los glucosinolatos son una clase de glucósidos azufrados químicamente estables, localizados en el líquido intersticial celular de las semillas, raíces y partes verdes de la planta. Estos compuestos provienen del metabolismo secundario de las plantas y se sintetizan a partir de aminoácidos, directamente o previamente modificados (Rosa y otros, 1997).

La molécula de los glucosinolatos está constituída por un grupo β-D-tioglucósido unido a una oxima sulfonada y a una cadena lateral (R) derivada de un aminoácido (Fig. 1.12). Según el aminoácido del que deriven se clasifican en alifáticos (metionina), indólicos (triptófano) y aromáticos (fenilalanina y tirosina). Se han descrito más de 100 glucosinolatos distintos, que difieren en el terminal R, aunque únicamente unos 15 alcanzan niveles significativos en las brásicas (Rosa, 1999). Los glucosinolatos mayoritarios en *B. rapa* son los alifáticos y en menor medida los indólicos (Tabla 1.4).

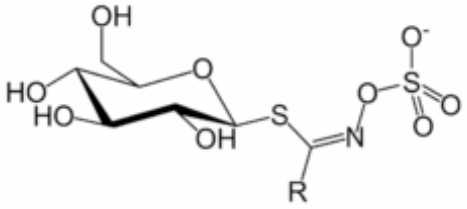


Figura 1.12. Estructura general de los glucosinolatos.

Los glucosinolatos por sí mismos no presentan actividad, pero cuando la planta sufre una agresión o mediante la flora del colon durante la ingesta, se desencadena un proceso de hidrólisis por acción de la enzima mirosinasa (tioglucósido glucohidrolasa, enzima hidrolítica endógena que se localiza en el interior de los idioblastos) y se transforman dando lugar a una serie de compuestos entre los cuales destacan los isotiocianatos, indoles, nitrilos, epi-nitrilos, oxazolidinas y tiocianatos (Verkerk y otros, 1997) que desempeñan una función de defensa en la planta, impidiendo el desarrollo de nematodos, hongos y otros microorganismos, o bien atraen o repelen a

determinadas especies de insectos (Vig y otros, 2009). Además, estos compuestos son los responsables del efecto positivo que ejercen las brásicas sobre la salud humana (Traka y Mithen, 2008).

Tabla 1.4. Glucosinolatos mayoritarios presentes en *Brassica rapa* y en otras brásicas hortícolas.

Nombre químico	Nombre común	Especie en la que predominan
Alifáticos		
2-Propenil	Sinigrina	*B. oleracea*
3-Butenil	Gluconapina	*B. rapa*, *B. napus*
4-Fentenil	Glucobrasicanapina	*B. rapa*, *B. napus*
2-Hidroxi-3-butenil	Progoitrina	*B. rapa*, *B. napus*
2-Hidroxi-4-fentenil	Gluconapoleiferina	*B. oleracea*, *B. napus*
3-Metilsulfinilpropil	Glucoiberina	*B. oleracea*, *B. rapa*
4-Metilsulfinilbutil	Glucorafanina	*B. oleracea*
5-Metilsulfinilfentil	Glucoalisina	*B. oleracea*
Indólicos		
3-Indolilmetil	Glucobrasicina	*B. oleracea*, *B. rapa*, *B. napus*
1-Metoxi-3-indolilmetil	Neoglucobrasicina	*B. oleracea*, *B. rapa*, *B. napus*
4-Metoxi-3-indolilmetil	4-Metoxiglucobrasicina	*B.oleracea*
4-Hidroxi-3-indolilmetil	4-Hidroxiglucobrasicina	*B. oleracea*, *B. rapa*
Aromáticos		
2-Feniletil	Gluconasturtina	*B. oleracea*, *B. rapa*, *B. napus*

El tipo de producto de degradación que se origine a partir de un glucosinolato concreto va a depender (entre otras casusas) de la estructura del terminal R, del pH al que tiene lugar la hidrólisis o de los iones metálicos existentes. Así, por ejemplo, los glucosinolatos alifáticos a pH 5-7 producen isotiocianatos y oxazolidina-2-tiona y los glucosinolatos indólicos se hidrolizan a isotiocianatos inestables que dan lugar a indol-3-carbinol (Fig. 1.13) (Rosa, 1999). El factor clave a la hora de definir los efectos beneficiosos de los productos de la hidrólisis de los glucosinolatos es su biodisponibilidad, que va a depender de la liberación por parte de la planta, de la

absorción en el aparato digestivo, de su distribución a través del organismo y de su metabolismo y excreción (Holst y Williamson, 2004).

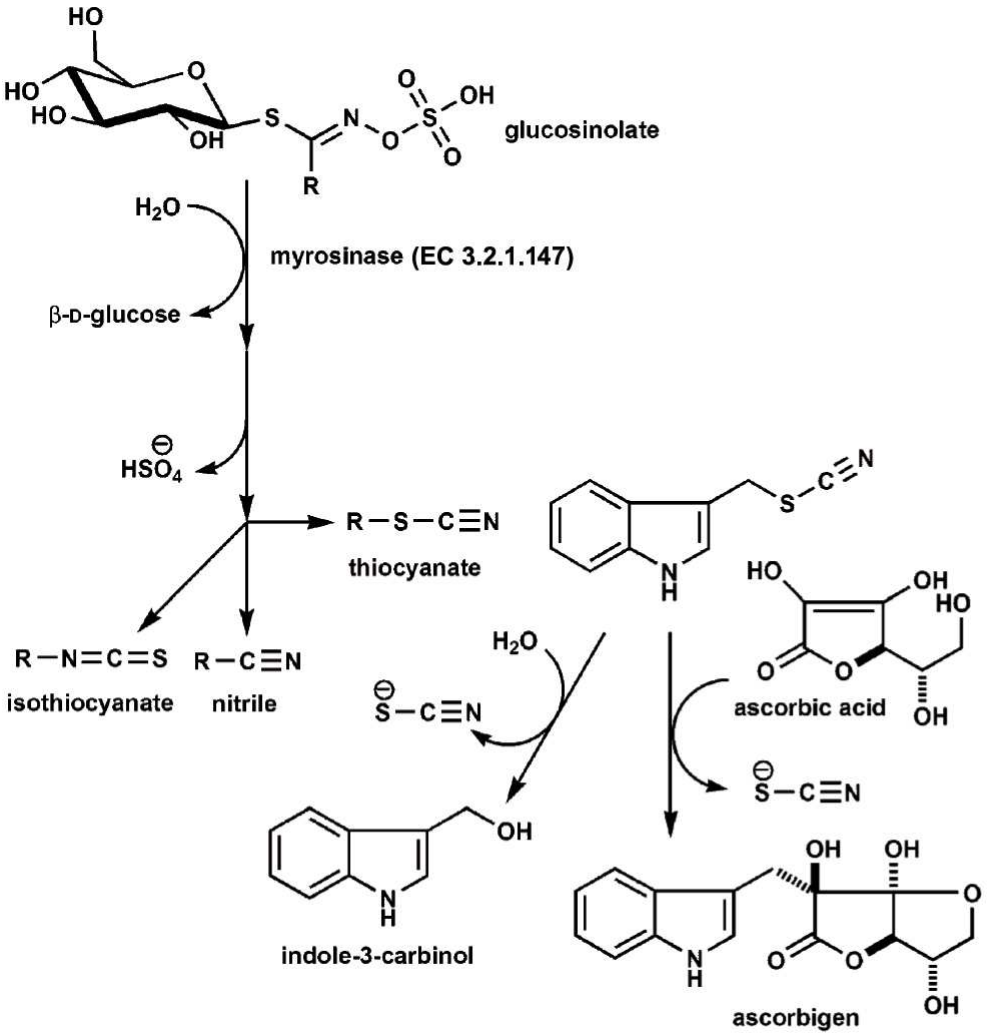


Figura. 1.13. Conversión de glucosinolatos en sus productos de degradación mediante la acción de la enzima mirosinasa (Suzuki y otros, 2006).

Los isotiocianatos e indoles son los dos grandes grupos de productos de descomposición autolítica de los glucosinolatos. Ambos presentan actividades de protección contra muchos tipos de cáncer. Estudios *in vitro* e *in vivo* han encontrado que estos compuestos afectan a muchas etapas del desarrollo del cáncer, incluyendo la inducción de las enzimas de detoxificación (enzimas de la fase II) y la inhibición de las enzimas de activación (enzimas de la fase I) (Anilakumar y otros, 2006; Jeffery y Araya, 2009; Verkerk et al., 2009). Son, además, inductores de la enzima tioredoxina-reductasa (uno de los antioxidantes más potentes presentes en los mamíferos) y poderosos

agentes reguladores de reacciones redox celulares (Bao, 2005). Los isotiocianatos que presentan un mayor efecto inductor de las enzimas de la fase II son la sulforafanina, iberina y erucina, que son productos de degradación de la glucorafanina, glucoiberina y glucoerucina, respectivamente (Fahey y Talalay, 1999; Liang y otros, 2005). De un modo especial se ha estudiado el potente efecto protector contra sustancias carcinogénicas que posee la sulforafanina, uno de los glucosinolatos mayoritarios presentes en el brécol (Cartea y Velasco, 2008; Traka y Mithen, 2008).

1.3.1.2. Compuestos fenólicos

Los compuestos fenólicos constituyen una de las principales clases de metabolitos secundarios de las brásicas, donde desempeñan diversas funciones fisiológicas. Entre otras, intervienen en el crecimiento y reproducción de las plantas y en procesos defensivos frente a patógenos, predadores o radiación ultravioleta (Duthie y Crozier, 2000). Los compuestos fenólicos presentan un anillo benceno hidroxilado como elemento común en sus estructuras moleculares, las cuales pueden incluir grupos funcionales como ésteres, metil ésteres o glicósidos (Duthie y Crozier, 2000). Aunque existe una gran variedad de compuestos fenólicos en las plantas (se conocen más de 8.000), la mayor parte de ellos tiene como origen metabólico común la ruta del ácido siquímico y el metabolismo de los fenilpropanoides (Robards y otros, 1999). En los alimentos, los compuestos fenólicos habitualmente se presentan conjugados con azúcares, ácidos, aminas o lípidos (Duthie y otros, 2003).

Según su estructura, los compuestos fenólicos se pueden dividir en dos grupos: flavonoides y no flavonoides. Los flavonoides incluyen diferentes tipos de compuestos como flavonoles, antocianos, flavan-3-oles, proantocianidinas, flavanonas, flavonas, isoflavonas y charconas. Dentro de los compuestos no flavonoides se incluyen los ácidos hidroxicinámicos, ácidos hidroxibenzoicos, taninos hidrolizables y estilbenos (Robards y otros, 1999).

Los flavonoides son compuestos de bajo peso molecular que comparten un esqueleto común de difenilpiranos (C6-C3-C6), compuesto por dos anillos de fenilos (A y B) ligados a través de un anillo C de pirano (heterocíclico). Los átomos de carbono en los anillos C y A se numeran del 2 al 8, y los del anillo B desde el 2' al 6' (Fig. 1.14).

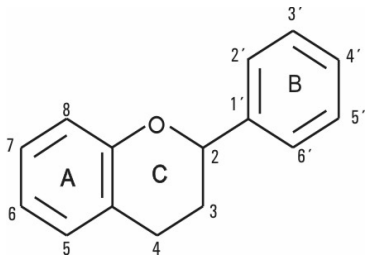


Figura 1.14. Estructura básica de los flavonoides y sistema de numeración.

A los flavonoides se unen azúcares, de forma que estos compuestos se encuentran comúnmente como *O*-glicósidos, siendo la glucosa el residuo de azúcar más frecuente. Otros residuos de azúcares son la galactosa, la ramnosa, la arabinosa, la xilosa, así como el ácido glucurónico. La parte sin azúcares de la molécula flavonoide se llama aglicona.

Los flavonoides se ubican principalmente en las hojas y en zonas aéreas. Una excepción son los tubérculos de cebolla, que contienen una gran cantidad de quercitina 4'-D-glucósidos. Los flavonoides se encuentran en frutas, verduras, semillas y flores, así como en cerveza, vino, té verde, té negro y soja, los cuales se consumen en la dieta humana de forma habitual y también pueden utilizarse en forma de suplementos nutricionales, junto con ciertas vitaminas y minerales (Scalbert y Williamson, 2000). Desempeñan un papel importante en la biología vegetal; así, responden a la luz y controlan los niveles de las auxinas reguladoras del crecimiento y diferenciación de las plantas. Otras funciones incluyen un papel antifúngico y bactericida, confieren coloración, lo que puede contribuir a los fenómenos de polinización y tienen una importante capacidad para fijar metales como el hierro y el cobre (Formica y Regelson, 1995).

En las brásicas se encuentran varios compuestos de naturaleza flavonoide, siendo los flavonoles como la quercetina, kaempferol e isorhamnetina los más abundantes (Fig. 1.15) (Podsedek, 2007). Los flavonoles se caracterizan por poseer un doble enlace entre el C2 y el C3 y por la presencia de un grupo hidroxilo en posición 3 (Price y otros, 1998).

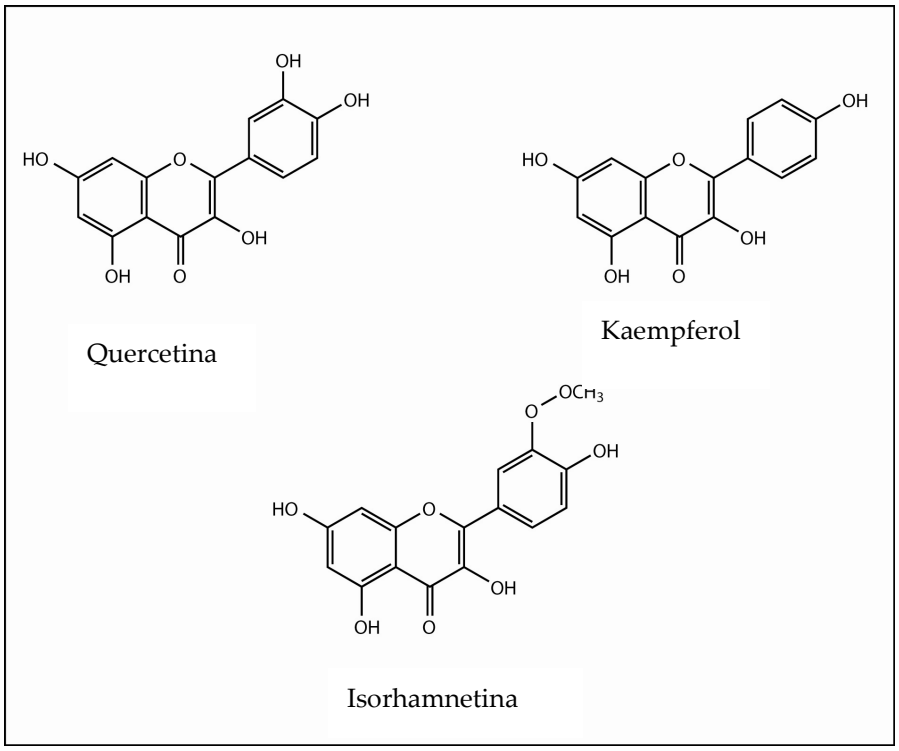


Figura.1.15. Estructura de los flavonoles más comunes en las brásicas hortícolas.

Como el resto de flavonoides suelen aparecer en forma glicosilada, unidos al menos a un azúcar que suele ser una glucosa o una ramnosa, preferentemente en la posición C3 y con menor frecuencia en la C7 del anillo A (Manach y otros, 2004). En general, en casi todos los cultivos de brásicas, los flavonoles glicosilados más abundantes son la quercetina 3-*O*-soforósido y el kaempferol 3-*O*-soforósido aunque también se han encontrado otros como la quercetina 3-*O*-glucósido y kaempferol 3-*O*-glucósido (Podsedek, 2007). En la especie *B. rapa*, son además frecuentes los flavonoles glicosilados derivados de la isorhamnetina, aglicona poco común en los cultivos de *B. oleracea* (Ferreres y otros, 2008).

El otro gran grupo de compuestos fenólicos son los de naturaleza no flavonoide. Estos compuestos se encuentran ampliamente distribuidos como conjugados en materias vegetales, incluyendo muchos alimentos y bebidas (Clifford, 2000). Se caracterizan por poseer en su estructura química el anillo aromático y el grupo hidroxílico comunes a los compuestos fenólicos, además de una función carboxílica. Los más abundantes son los ácidos fenólicos, que se diferencian a su vez en

dos grupos principales, los ácidos hidroxibenzoicos y los ácidos hidroxicinámicos, siendo estos últimos los más abundantes en los cultivos de brásicas (Podsedek, 2007).

Los ácidos hidroxicinámicos más frecuentes son el ácido *p*-coumárico (ácido 4-hidroxicinámico), el ácido cafeico (ácido 3, 4-dihidroxicinámico), el ácido sinápico (ácido 3,5-dimetoxi-4-hidroxicinámico) y en menor medida, el ácido ferúlico (ácido 3-metoxi-4-hidroxicinámico) (Fig. 1.16) (Clifford, 2000).

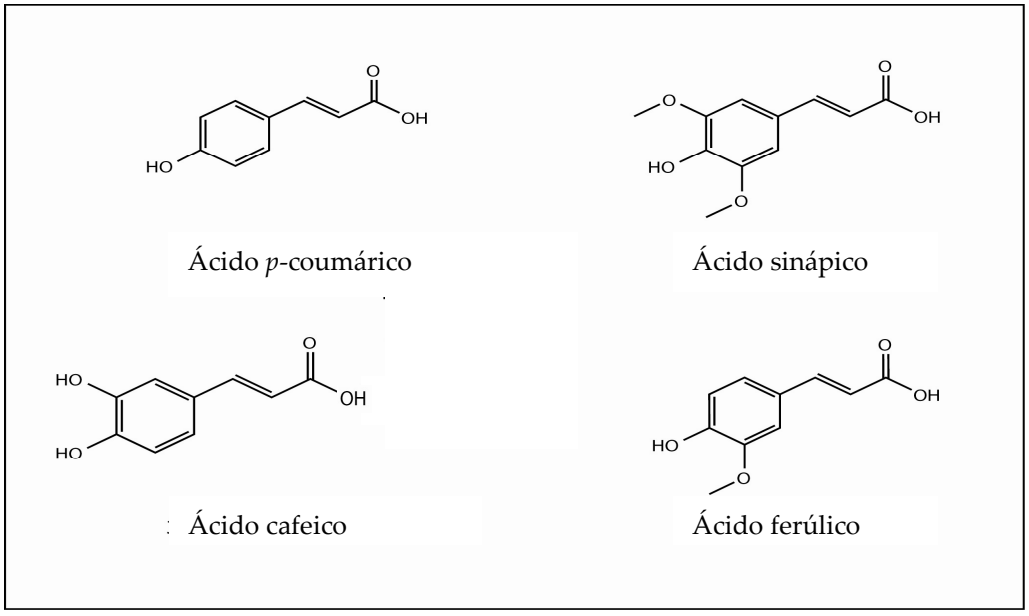


Figura 1.16. Estructura de los ácidos hidroxicinámicos más comunes en brásicas.

Salvo en el caso de alimentos procesados, raramente se encuentran como ácidos libres y de forma predominante aparecen esterificados con ácido quínico, tartárico o glucosa o bien unidos a flavonoides (Faulds y Williamson, 1999). El ácido cafeico suele aparecer esterificado, principalmente con el ácido quínico, dando lugar al ácido clorogénico (ácido 5-cafeoilquínico), compuesto presente también en las brásicas y en otras verduras (Scalbert y Williamson, 2000). En comparación con otras especies de brásicas, los cultivos de *B. rapa* contienen grandes concentraciones de ácidos hidroxicinámicos tales como el ácido clorogénico y el ácido sinápico, aunque también es muy común que estos ácidos aparezcan unidos a glucosa como el 1-sinapoil-2-feruloilgentiobiosa, el 1,2'diferuloilgentiobiosa o el 1,2,2' trisinapoilgentiobiosa (Fernandes y otros, 2007; Sousa y otros, 2008).

Durante los últimos años, los compuestos fenólicos han sido objeto de numerosos estudios debido principalmente a los efectos beneficiosos que ejercen sobre la salud humana (Manach y otros, 2004; Aron y Kennedy, 2008). Su papel más importante es el de actuar como antioxidantes naturales y esto se debe a su facilidad para ceder átomos de hidrógeno de un grupo hidroxilo aromático a un radical libre y a la posibilidad de deslocalización de cargas en el sistema de dobles enlaces del anillo aromático (Duthie y otros, 2003). Los compuestos fenólicos poseen además una estructura química ideal para captar iones metálicos (principalmente hierro y cobre) e inhibir la formación de radicales libres (Pereira y otros, 2009). Además de las propiedades antioxidantes, a estos compuestos se les atribuyen actividades biológicas beneficiosas para salud. Entre éstas destacan sus efectos vasodilatadores, anticarcinogénicos, antiinflamatorios, bactericidas, estimuladores de la respuesta inmune, antialérgicos, antivirales, efectos estrogénicos, o inhibidores de enzimas prooxidantes, como la cicloxigenasa, lipooxigenasa y xantina oxidasa (Pereira y otros, 2009).

1.3.1.3. Identificación y cuantificación de glucosinolatos y fenoles

La identificación y cuantificación de estos dos grandes grupos de fitoquímicos, glucosinolatos y fenoles, presentes en los diferentes productos de origen vegetal ha despertado un gran interés debido a su importancia nutricional en la dieta, lo que ha hecho que cada día sea más la información que se puede encontrar en la bibliografía científica sobre el perfil y contenido de estos metabolitos en los distintos alimentos.

La gran abundancia y variedad estructural de estos compuestos junto con el hecho de que cada uno produce diferentes productos de degradación hace muy complicado su análisis (Mithen y otros, 2000). En el caso de los glucosinolatos y, debido a que se encuentran coexistiendo en la planta junto con la enzima mirosinasa, el procesamiento del material vegetal fresco en presencia de agua inicia una hidrólisis rápida de los productos originales, lo que añade una complejidad al problema. Por tanto, las muestras a procesar deben estar completamente secas, lo cual se consigue mediante un proceso de liofilización del material vegetal tras una congelación previa del mismo en nitrógeno líquido y conservación a -80 ºC. Por otro lado, para realizar

una correcta identificación de los compuestos fenólicos es necesario realizar una hidrólisis alcalina del tejido vegetal (fresco o seco) extraído con agua y metanol.

Existe un gran número de técnicas analíticas para la identificación y cuantificación de glucosinolatos y fenoles. Las primeras técnicas desarrolladas fueron las espectrofotométricas. Entre las más comunes se encuentra el ensayo de Folin-Ciocalteu, muy utilizado para la cuantificación de compuestos fenólicos totales en vegetales del género *Brassica* (Sikora y otros, 2008). Estos métodos tienen interés ya que son rápidos y sencillos aunque no aportan la suficiente información para establecer un estudio más detallado. Para llevar a cabo la identificación individualizada de cada uno de los metabolitos de interés se han desarrollado técnicas más precisas, como las cromatográficas. Las primeras identificaciones se basaron en la cromatografía en papel de capa fina (Fahey y otros, 2001). Hoy en día, las técnicas de cromatografía líquida de alta resolución (HPLC) y cromatografía de gases (GC) son las más empleadas. Existen distintos soportes y fases móviles que permiten el análisis tanto de glucosinolatos intactos como desulfatados, flavonoides y ácidos hidroxicinámicos. La utilización del detector de diodos PDA (photo-diodo array) facilita la detección de estos compuestos por HPLC, al utilizar de forma conjunta el tiempo de retención y el espectro ultravioleta para la identificación de los picos.

Actualmente, el uso de HPLC con detección por PDA acoplado a un detector de masas está ampliamente utilizado para la identificación de glucosinolatos y fenoles individuales (LLorach y otros, 2003; Vallejo y otros, 2004; Ferreres y otros, 2005; Fabre y otros, 2007; Rochfort y otros, 2008; Lin y Harnly, 2010). En esta técnica, las áreas del pico de cada uno de los compuestos que se desea conocer pueden emplearse para la cuantificación, mientras que el detector de masas se utiliza para incrementar la especificidad del método y mejorar la identificación del compuesto.

Mediante el empleo de la cromatografía se puede, por tanto, determinar un gran número de metabolitos presentes en muestras vegetales. En general, esta técnica requiere la utilización de métodos de extracción y análisis optimizados para cada uno de los compuestos que se vayan a analizar. Recientemente, se ha puesto a punto un método de extracción, identificación y cuantificación para analizar de forma simultánea glucosinolatos y compuesto fenólicos intactos (Bennet y otros, 2003; 2006).

Las muestras se analizan por cromatografía líquida y espectrometría de masas (LC-MS) y cada tipo de compuesto se detecta a diferentes longitudes de onda. Por tanto, esta técnica permite una completa determinación de la composición química de la planta estudiada.

1.3.1.4. Factores que afectan al contenido de glucosinolatos y fenoles

Dada la importancia de los glucosinolatos y fenoles en la prevención de enfermedades resulta esencial definir los factores que influyen sobre su distribución y contenido en los productos vegetales. Por un lado, existen factores intrínsecos al propio vegetal (de origen genético), que llevan a que la composición en estas sustancias sea diferente no sólo entre distintos géneros o especies, sino incluso también entre variedades de la misma especie (Fenwick y otros, 1983b; Kushad y otros, 1999; Podsedek, 2007; Cartea y Velasco, 2008). Además, hay una gran variación dentro de la planta dependiendo del estado de desarrollo, ya que pueden variar en los tejidos vegetativos y florales (Rosa y otros, 1997; Vallejo y otros, 2003a; Velasco y otros, 2007). En general, se ha descrito que las semillas poseen mayor concentración de glucosinolatos, seguido por los brotes, hojas, raíces y tallos (Brown y otros, 2003).

Por otra parte, la composición en sustancias fitoquímicas va a estar influida por factores extrínsecos al vegetal, ligados a sus circunstancias de cultivo (factores agroambientales) y a las condiciones de conservación y procesamiento tras la recolección. Así, se ha demostrado que la presencia o ausencia de determinados nutrientes en el suelo y el exceso o déficit de riego pueden afectar a la composición fotoquímica de las hortalizas, tanto cualitativa como cuantitativamente. Se sabe que el calcio, el boro y el contenido en sustancias nitrogenadas del suelo tienen un efecto decisivo sobre el contenido en sustancias fenólicas antioxidantes, mientras que el contenido en compuestos azufrados influye considerablemente sobre el contenido en glucosinolatos (Stewart y otros, 2001; Kim y otros, 2002).

Las condiciones climáticas también se han descrito como factores importantes; el grado de iluminación e irradiación de las plantas y la temperatura de cultivo ejercen una influencia importante sobre el contenido en sustancias fitoquímicas. En general, las estaciones frías parecen conducir a una reducción tanto en glucosinolatos como en

flavonoides, debido a los días cortos, las condiciones más húmedas, las temperaturas frías y menos radiación (Hertog y otros, 1992; Rosa y otros, 1997; Vallejo y otros, 2003b) aunque no se ha encontrado un patrón claro. Se ha descrito también que las enfermedades y plagas influyen en la concentración de estos compuestos (Giamoustaris y Mithen, 1995; Daniel y otros, 1999; Velasco y otros, 2007).

Los procesos de manufacturación y preparación culinaria son también importantes, ya que pueden influir sobre la estabilidad y la disponibilidad de estas sustancias. Sin embargo, y a pesar de la importancia de los glucosinolatos y fenoles en la salud humana, existen pocos estudios para conocer la degradación térmica de los mismos y la mayoría de la literatura se centra en las cualidades nutritivas del producto en fresco. Aunque algunos cultivos como el brécol, la coliflor o el repollo pueden consumirse crudos en ensaladas, las nabizas y grelos se someten a un proceso de cocción antes de ser consumidos. Por tanto, se hace necesario el estudio de distintas técnicas de procesado y preparación del alimento para determinar cómo influyen en la estabilidad y disponibilidad de estos compuestos beneficiosos. En general, se ha demostrado que los métodos convencionales de cocción, tales como el hervido, cocido al vapor, a presión y en microondas reducen la concentración de glucosinolatos en aproximadamente 30 a 60%, dependiendo del método, la intensidad y tipo de compuesto (Rodrigues y Rosa, 1999; Rangkadilok y otros, 2002; Verkerk y otros, 2001; Verkerk y Dekker, 2004). En lo referente a los fenoles, se ha encontrado que el cocinado al vapor es el método que retiene mayor concentración de flavonoides y ácidos hidroxicinámicos en el brécol. Por el contrario, una cocción entre 3 y 15 minutos en microondas o hervido tradicional produce pérdidas en el contenido fenólico del 30 al 90% (Czarniecka-Skubina, 2002; Vallejo y otros, 2003c; Zhang y Hamauzu, 2004).

1.3.2. Cualidades organolépticas de nabizas y grelos

En general, la preferencia del consumidor por un tipo de hortaliza u otro depende de su sabor, aroma y olor. Las nabizas y los grelos bajo el amparo de la denominación 'IXP grelos de Galicia' se caracterizan por poseer '*un sabor ligeramente ácido combinado con cierto amargor. Son además verduras con textura ligeramente fibrosa más acusada si la variedad*

es de hoja estrecha con elevado porcentaje de peciolos y su dureza es muy baja debido al bajo contenido en fibra alimentaria'.

La textura es una cualidad sensorial muy importante en las hortalizas, hasta el punto de que una textura firme se considera índice de frescura y un factor determinante de su aceptabilidad (García-Fernández y otros, 2000). Esto resulta especialmente difícil en nabizas y grelos ya que tienen un elevado contenido en agua y requieren, por lo tanto, un extremo cuidado después de la recolección para mantener la textura original del producto a través de los canales de distribución y de venta.

Cuando un alimento se introduce en la boca y se disuelve con la saliva se genera un impulso nervioso que se transmite al cerebro y éste lo transforma mediante complejos sistemas de reconocimiento en un sabor concreto. La frecuencia con la que se repiten los impulsos indica la intensidad de sabor. Paralelamente se produce un incremento en la emisión de olores que por vía retronasal estimulan los receptores olfativos desarrollándose así la percepción del aroma que, junto con el sabor constituyen lo que se conoce como 'flavor' (Arias, 2009). El 'flavor' (o conjunto de propiedades olfativas y gustativas) de los vegetales está influenciado por factores genéticos y por la manipulación anterior, durante y posterior a la cosecha. Cuanto mayor sea el tiempo transcurrido entre la cosecha y el consumo de los vegetales mayor será la pérdida de estos atributos o 'flavor' (Kader, 2008).

El aroma característico de frutas y verduras está determinado por un complejo espectro de compuestos orgánicos. En algunos tejidos, los precursores del aroma se convierten enzimáticamente en los compuestos responsables de éste cuando se produce la rotura celular durante la masticación o por otros agentes mecánicos (Fennema, 2000). La mayoría de los aromas típicos de alimentos son una mezcla de aromas simples producidos por moléculas individuales (Belitz y otros, 2004). Cuando los tejidos de las brásicas son dañados por un corte (en el procesado) o la masticación, la enzima catalítica mirosinasa, degrada los glucosinolatos que contienen azufre, dando lugar a la liberación de los isotiocianatos, compuestos responsables en parte del sabor amargo y 'amostazado' y del olor característicos de los cultivos del género *Brassica* (Fenwick y otros, 1983a; Rosa y otros, 1997). Sin embargo, la relación entre el contenido en glucosinolatos y el amargor de las brásicas no está muy estudiada; lo más

probable es que las cualidades organolépticas de estas hortalizas se deban a una función sinérgica compleja de los glucosinolatos junto con otros metabolitos (Padilla y otros, 2007).

El metabolismo de los ácidos orgánicos a partir del acetil-Co-A en el ciclo de Krebs también se relaciona con la síntesis de compuestos fenólicos, lípidos y de las sustancias volátiles responsables del aroma y del sabor más o menos ácido de las hortalizas (Fennema, 2000). Algunos flavonoides son los compuestos responsables del sabor amargo en los cítricos, como la naringina, que es el componente amargo mayoritario del pomelo. Además, ciertos flavonoides glicosilados amargos o insípidos pueden transformarse por apertura del anillo en calconas (con sabor dulce), las cuales por hidrogenación posterior se transforman en compuestos con poder edulcorante igual o superior al de la sacarina (Belitz y otros, 2004). Otros compuestos fenólicos como los ácidos hidroxicinámicos y sus derivados entre los que destaca el ácido clorogénico, han sido asociados al sabor amargo de la cerveza, el vino y la sidra (Shahidi y Naczk, 1995).

1.4. Antecedentes y justificación del trabajo

En Galicia, el cultivo de nabizas y grelos se realiza en pequeños minifundios siendo productos generalmente de autoconsumo producidos en huertas familiares donde los agricultores cosechan su propia semilla año tras año. De este modo, se ha favorecido la generación de numerosas variedades adaptadas a las condiciones ecológicas de cada zona así como a las preferencias de los consumidores locales, lo que implica una diversidad del cultivo en toda la geografía gallega. Por otra parte, este hecho dificulta el conocimiento del número de variedades locales, sus características cualitativas y potencial agronómico de cada una de ellas. Por esta razón, el grupo de Mejora y Genética de Brásicas de la Misión Biológica de Galicia (MBG), comenzó en los años 80 un programa para recolectar y estudiar las variedades locales de grelos y de otros cultivos de brásicas en Galicia que permitiera conservar la variabilidad de las variedades locales y utilizarlas como reservorios de genes interesantes para la mejora genética. Esta prospección de material originó la creación de un banco de germoplasma

de brásicas. En la actualidad se mantienen 212 entradas o variedades locales de *B. rapa*, además de 259 entradas de *B. oleracea* y 49 entradas de *B. napus*.

En los últimos años, el grupo de Brásicas de la MBG ha conseguido avanzar de manera notoria en el estudio de los cultivos de *B. rapa*. Inicialmente, los trabajos en esta especie se basaron en la caracterización morfo-agronómica de la variabilidad de 30 variedades locales llevada a cabo por Baladrón y Ordás (1989). Posteriormente, estos estudios se ampliaron a la caracterización agronómica y nutricional de 120 variedades de *B. rapa*, trabajo realizado por Padilla y otros (2005), quienes evaluaron de modo exhaustivo este material en dos ambientes utilizando un diseño aumentado de Petersen (Petersen, 1985). Este trabajo determinó una gran variabilidad intravarietal para todos los caracteres morfo-agronómicos estudiados, hecho que permitió efectuar un primer cribado, seleccionando un conjunto de variedades que destacaron por su buen comportamiento agronómico para cada uno de los tres tipos de cultivo: nabo, nabiza y grelo. Sin embargo, hasta la fecha, existe un escaso conocimiento de la aptitud de una determinada variedad para la producción en fresco de nabizas y/o grelos y de la influencia de factores ambientales, del genotipo y su interacción en el rendimiento.

En esta especie también se llevó a cabo la evaluación de parámetros de calidad como el análisis de desulfoglucosinolatos en hojas de 113 variedades de *B. rapa* (Padilla y otros, 2007). En este estudio, se identificaron los glucosinolatos presentes en las hojas y se encontró una gran variabilidad entre variedades para el contenido en glucosinolatos totales que osciló entre 12 y 74 μmol g^{-1} ps. No obstante, hasta el momento no se ha estudiado el perfil y el contenido de estos metabolitos en los brotes o grelos así como la influencia ambiental en el contenido en glucosinolatos en las hojas y en los brotes. Respecto a los compuestos fenólicos, no se ha llevado a cabo ningún estudio para conocer cuál es la composición de los mismos en estos cultivos, siendo bastante escasa la información disponible en la bibliografía sobre el contenido fenólico en los cultivos de *B. rapa*.

Como ya se ha expuesto anteriormente, en el contenido final de estos metabolitos influyen, además del genotipo, diferentes factores como las condiciones edafo-climáticas, las prácticas culturales o la parte de la planta evaluada. Los únicos estudios que han contrastado el efecto del genotipo respecto al efecto del ambiente

sobre la concentración final de los glucosinolatos y fenoles presentes en las brásicas se han centrado en la glucorafanina, compuesto mayoritario en el brécol (Rosa y Rodrigues, 2001; Farnham y otros, 2004). Por tanto, no hay datos sobre el efecto ambiental en otros compuestos mayoritarios de otros cultivos de brásicas, como es el caso de *B. rapa*. Por otra parte, los tratamientos efectuados durante la conservación tras la recolección así como las distintas técnicas de procesado y preparación del alimento van a influir en la estabilidad y disponibilidad de estos compuestos beneficiosos. Por tanto, es importante conocer cómo varían estos compuestos para así mantener los niveles de glucosinolatos beneficiosos después de la cosecha y proporcionar el método de cocción más apropiado que asegure beneficios óptimos en la salud. Diferentes estudios han demostrado las ventajas e inconvenientes de diferentes técnicas de cocción si bien la mayoría de estos trabajos se han centrado una vez más en el brécol y en el contenido en glucorafanina (Rodrigues y Rosa, 1999; Verkerk y otros, 2001; Vallejo y otros, 2003c). Por ello, se hace necesario conocer el efecto de los tratamientos térmicos en las sustancias bioactivas presentes en nabizas y grelos.

El análisis sensorial es una técnica que aporta una valiosa información, ya que permite un conocimiento más completo de las características de los alimentos y posibilita su adecuada elaboración con objeto de satisfacer el gusto de los consumidores a los que van destinados. El Departamento de Química Analítica, Nutrición y Bromatología de la Universidad de Santiago ha desarrollado una ficha de cata, compuesta por 17 descriptores, para realizar el análisis sensorial de nabiza y grelo, aunque no se ha probado con diferentes variedades procedentes de varias zonas de producción. Tampoco se ha estudiado si existe una relación directa entre los caracteres sensoriales y el contenido en metabolitos secundarios.

1.5. Objetivos

El principal objetivo de la presente Tesis Doctoral es ampliar el conocimiento agronómico, nutritivo y organoléptico de los cultivos de nabizas y grelos, a través del estudio de caracteres enfocados a la producción de cada cultivo, la identificación y cuantificación de los compuestos bioactivos presentes en estas hortalizas y el estudio de los efectos fenotípicos y ambientales sobre ellos.

Este objetivo general consta de los siguientes objetivos parciales:

1. Determinar las variedades locales de *B. rapa* más idóneas para la producción en fresco de nabiza o grelo y aquellas variedades que puedan tener un doble aprovechamiento. Estudiar el efecto del ambiente sobre el rendimiento y la estabilidad de los genotipos.

2. Establecer el perfil nutricional de *B. rapa* mediante la identificación y cuantificación de compuestos bioactivos presentes en nabizas y grelos.

3. Estudiar el efecto del genotipo, el ambiente y el procesado en el contenido final de compuestos bioactivos.

4. Definir los atributos sensoriales que mejor describen las cualidades organolépticas de *B. rapa* y relacionarlos con el contenido en metabolitos secundarios.

CAPÍTULO II

Material y métodos

2. MATERIAL Y MÉTODOS

2.1. Material vegetal

Para la realización del presente estudio se han utilizado 12 variedades locales de *B. rapa* conservadas en el banco de germoplasma de la MBG. Cada variedad local se identifica con las siglas MBG-BRS y el número que le corresponde por orden de entrada en el banco. De todas las variedades estudiadas, diez de ellas se eligieron a partir de evaluaciones previas (Padilla y otros, 2005) y en base a su comportamiento agronómico para la producción de nabizas y/o grelos y dos de ellas proceden de tres ciclos de selección masal por rendimiento en fresco de nabizas. A continuación se hace una relación del origen de todas ellas (Tabla 2.1; Fig. 2.1).

Tabla 2.1. Procedencia de las variedades locales gallegas de *Brassica rapa* utilizadas en este estudio.

Identificación	Provincia	Localidad	Proc*	Nombre local
MBG-BRS0082	Pontevedra	Vilar, Forcarei	L	Nabizas tempranas
MBG-BRS0143	A Coruña	Lama, Boqueixón	L	Nabizas tempranas
MBG-BRS0163	Ourense	Barcia, Melón	S	Nabizas
MBG-BRS0173	Ourense	Valongo, Cortegada	L	Nabizas
MBG-BRS0184	A Coruña	Carballo	L	Nabos tardíos
MBG-BRS0197	Ourense	Arnoia	S	Nabizas
MBG-BRS0401	A Coruña	San Xiao, Coirós	L	Nabos
MBG-BRS0433	A Coruña	Oroso	L	Nabizas de Santiago
MBG-BRS0451	A Coruña	O Val, Narón	L	Nabo, nabiza o grelo
MBG-BRS0461	Lugo	Castro de Rei, Guitiriz	L	Nabos tardíos
MBG-BRS0472	A Coruña	Porta, Sobrado	L	Nabos
MBG-BRS0550	A Coruña	Trazo	L	Nabizas tardías

*Proc= Procedencia, L= procedencia local; S= proceden de tres ciclos de selección masal por rendimiento en fresco de nabiza.

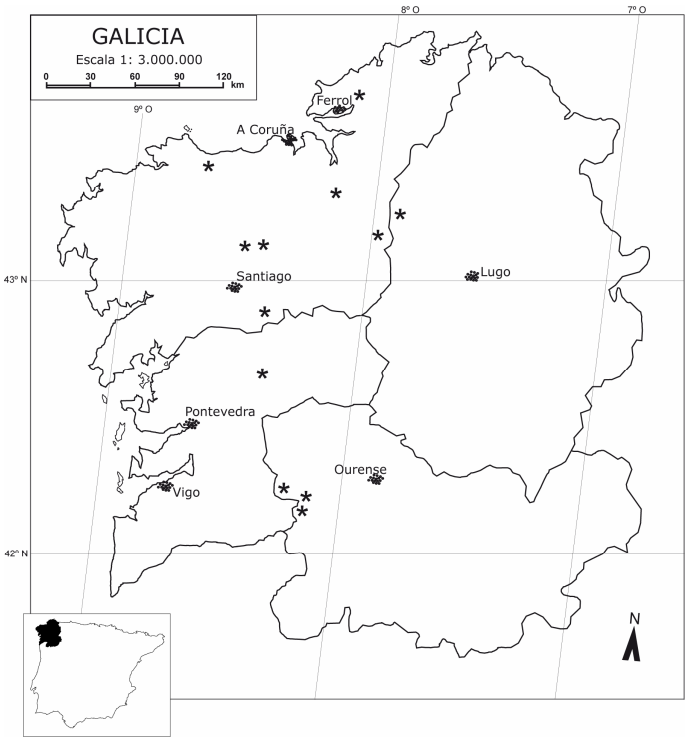


Figura 2.1. Origen geográfico de las variedades de *Brassica rapa* utilizadas en este estudio.

Las variedades MBG-BRS0163 y MBG-BRS0197 procedentes de sendos programas de selección masal fueron incluidas a partir del segundo año de estudio, momento en el que se completaron tres ciclos de selección. Por otro lado, la variedad MBG-BRS0433 fue eliminada del estudio a partir del segundo año por presentar una alta heterogeneidad intravarietal marcada principalmente por una baja sincronía de formación de brotes y floración y, por tanto, dificultar la toma de datos de grelo.

2.2. Fincas empleadas y labores culturales

Las siembras se realizaron en invernadero bajo condiciones de temperatura y humedad controladas. Como recipientes de siembra se utilizaron bandejas de plástico de 40 alvéolos acanalados de 100cc con turba mixta rubia y negra, a razón de 3-4 semillas por alvéolo (Fig. 2.2).

Figura 2.2. Plántulas de *Brassica rapa* creciendo en el semillero.

Las variedades fueron evaluadas durante tres ciclos de cultivos (2006/07, 2007/08 y 2008/09) en tres localidades representativas de las zonas de producción de *B. rapa* en el noroeste de España: Oroso (A Coruña) (43° 1'N, 8° 26'W, 280 msnm) , Guitiriz (Lugo) (43° 12 'N, 7° 53'W, 516 msnm) y Salcedo (Pontevedra) (42° 24'N, 8° 38'W, 20 msnm). En cada localidad cada ensayo abarcó una superficie aproximada de 1000 m^2. La semana anterior al trasplante se realizaron los siguientes trabajos:

-Pase de grada de discos para picar las hierbas y facilitar el posterior pase de vertedera.

-Pase de arado de vertedera a 30 cm para eliminar restos de un cultivo anterior.

-Abonado con abono mineral complejo granulado 8-15-15 a razón de 412 kg/ha, lo que supone un aporte de 33Kg/ha de N y 62 Kg/ha de P_2O_5 y de K_2O.

-Enterrado con grada de discos.

-Preparación del lecho de plantación con fresadora a una profundidad de 15 cm.

Para el control de plagas se utilizaron Aphox® contra áfidos y Laidan® contra la mosca de la col (*Delia radicum* L). El insecticida Force® fue añadido en el momento del trasplante para prevenir el posible ataque de los insectos del suelo.

Las siembras en invernadero se realizaron a finales del mes de julio en 2007 y durante el mes de agosto en 2006 y 2008. El trasplante se realizó manualmente en campo en los meses de septiembre y octubre, cuando las plantas alcanzaron un desarrollo de 5-6 hojas verdaderas. Las fechas de siembra para cada localidad se encuentran en las tablas 2.2, 2.3 y 2.4. En la localidad de Pontevedra, en Salcedo, los ensayos de dos años, 2006/07 y 2007/08, se perdieron. En 2006 el ensayo se perdió debido a las desfavorables condiciones climáticas a causa de las elevadas precipitaciones provocando el encharcamiento y anegación de la parcela y, por

consiguiente, el marchitamiento radicular y la muerte de la planta. En 2007 la pérdida del cultivo se debió al intenso ataque ocasionado por la plaga de la mosca de la col, aproximadamente un mes después del trasplante. Las condiciones climáticas (precipitaciones intensas seguidas de altas temperaturas) ocurridas durante la época de siembra-trasplante favoreció el desarrollo de esta plaga en campo. Las larvas de este insecto atacan y se alimentan de las raíces, destruyendo en muy poco tiempo la parcela de ensayo (Fig. 2.3).

Figura 2.3. Aspecto que presentaban las plantas tras el ataque sufrido por *Delia radicum* L. en la parcela de Salcedo durante el cultivo de 2007/08.

2.3. Diseño experimental

Cada parcela elemental constó de tres surcos con 15 plantas por surco. La distancia entre los surcos fue de 0,8 m y entre plantas de cada surco de 0,5 m (Fig. 2.4). En todos los ambientes las parcelas se dispusieron según un diseño en bloques al azar, con tres repeticiones cada uno. Para cada uno de los ensayos se realizó un sorteo distinto. El objetivo de este agrupamiento es lograr que las unidades en un bloque sean lo más uniformes posibles para minimizar la variabilidad dentro de cada bloque y maximizar la diferencia entre bloques. De este modo se reduce el error experimental y se mejora la precisión del experimento para detectar diferencias significativas.

Figura 2.4. Parcelas experimentales entre tres y cuatro semanas después del trasplante.

En el momento óptimo de consumo de cada uno de los cultivos (nabiza y grelo) se llevó a cabo la recogida de material para los análisis nutricional y sensorial. Las fechas de recogida de material están detalladas en las tablas 2.2, 2.3 y 2.4.

2.4. Caracteres estudiados

La presente tesis será presentada por compendio de artículos, de modo que las metodologías utilizadas para cada uno de los análisis serán convenientemente explicadas en cada artículo.

2.4.1. Caracteres morfológicos y agronómicos

A lo largo del ciclo de cultivo se tomaron diversos caracteres agronómicos y morfológicos que se encuentran detallados en el capítulo III de esta tesis. Estos caracteres se adaptaron a partir de los descriptores para *Brassica* y *Raphanus* del Instituto Internacional de Recursos Fitogenéticos (IBPGR, 1990) y de la Unión Internacional para la Protección de las Obtenciones Vegetales (UPOV, 2001).

2.4.2. Caracteres nutricionales y sensoriales

La metodología sobre la determinación de proteína, fibra, minerales y aniones presentes en las partes comestibles de *B. rapa* se encuentra en el capítulo III de la tesis. La metodología sobre identificación de glucosinolatos y fenoles está explicada en el capítulo IV y la cuantificación de estos compuestos se encuentra en los capítulos IV, V y VI. La cuantificación de Vitamina C se detalla en el segundo apartado del capítulo VI.

Los diferentes descriptores organolépticos utilizados en la caracterización sensorial se detallan en el capítulo VII.

Tabla 2.2. Fechas de traplante y recogida de material para el análisis nutricional y sensorial, en cada variedad y localidad durante el ciclo de cultivo 2006/07.

[1]Localidad

Variedad	Loc[1]	Trasplante	Análisis nutricional de nabiza	Análisis sensorial de nabiza	Análisis nutricional de grelo	Análisis sensorial de grelo
MBG-BRS0082	Guitiriz	19/10/2006	17/01/2007	17/01/2007	25/04/2007	25/04/2007
MBG-BRS0143	Guitiriz			09/01/2007	01/02/2007	01/02/2007
MBG-BRS0173	Guitiriz			25/01/2007	28/02/2007	28/02/2007
MBG-BRS0184	Guitiriz			17/01/2007	25/04/2007	25/04/2007
MBG-BRS0401	Guitiriz			09/01/2007	28/02/2007	28/02/2007
MBG-BRS0433	Guitiriz			25/01/2007	03/05/2007	03/05/2007
MBG-BRS0451	Guitiriz			25/01/2007	29/03/2007	29/03/2007
MBG-BRS0461	Guitiriz			17/01/2007	25/04/2007	25/04/2007
MBG-BRS0472	Guitiriz			09/01/2007	07/03/2007	07/03/2007
MBG-BRS0550	Guitiriz			09/01/2007	03/05/2007	03/05/2007
MBG-BRS0082	Oroso	10/10/2006	14/12/2006	13/12/2006	18/05/2007	18/05/2007
MBG-BRS0143	Oroso			14/12/2006	01/02/2007	01/02/2007
MBG-BRS0173	Oroso			20/12/2006	28/02/2007	28/02/2007
MBG-BRS0184	Oroso			13/12/2006	25/04/2007	25/04/2007
MBG-BRS0401	Oroso			20/12/2006	28/02/2007	28/02/2007
MBG-BRS0433	Oroso			20/12/2006	18/05/2007	18/05/2007
MBG-BRS0451	Oroso			14/12/2006	29/03/2007	29/03/2007
MBG-BRS0461	Oroso			14/12/2006	25/04/2007	25/04/2007
MBG-BRS0472	Oroso			20/12/2006	07/03/2007	07/03/2007
MBG-BRS0550	Oroso			13/12/2006	18/05/2007	18/05/2007

Tabla 2.3. Fechas de trasplante y recogida de material para el análisis nutricional y sensorial, en cada variedad y localidad durante el ciclo de cultivo 2007/08.

Variedad	Loc[1]	Trasplante	Análisis nutricional de nabiza	Análisis sensorial de nabiza	Análisis nutricional de grelo	Análisis sensorial de grelo
MBG-BRS0082	Guitiriz	11/09/2007	05/11/2007	05/11/2007	28/03/2008	08/04/2008
MBG-BRS0143	Guitiriz			25/10/2007	18/12/2007	16/01/2008
MBG-BRS0163	Guitiriz			25/10/2007	23/01/2008	04/02/2008
MBG-BRS0173	Guitiriz			05/11/2007	29/01/2008	30/01/2008
MBG-BRS0184	Guitiriz			05/11/2007	18/03/2008	25/03/2008
MBG-BRS0197	Guitiriz			05/11/2007	19/01/2008	23/01/2008
MBG-BRS0401	Guitiriz			25/10/2007	23/01/2008	23/01/2008
MBG-BRS0433	Guitiriz			25/10/2007	16/01/2008	16/01/2008
MBG-BRS0451	Guitiriz			25/10/2007	03/03/2008	11/03/2008
MBG-BRS0461	Guitiriz			05/11/2007	25/03/2008	02/04/2008
MBG-BRS0472	Guitiriz			05/11/2007	18/02/2008	27/02/2008
MBG-BRS0550	Guitiriz			25/10/2007	18/04/2008	22/03/2008
MBG-BRS0082	Oroso	04/09/2007	07/11/2007	13/11/2007	02/04/2008	08/04/2008
MBG-BRS0143	Oroso			07/11/2007	18/12/2007	16/01/2008
MBG-BRS0163	Oroso			07/11/2007	30/01/2008	04/02/2008
MBG-BRS0173	Oroso			13/11/2007	20/01/2008	30/01/2008
MBG-BRS0184	Oroso			13/11/2007	16/03/2008	25/03/2008
MBG-BRS0197	Oroso			13/11/2007	20/01/2008	22/01/2008
MBG-BRS0401	Oroso			07/11/2007	20/01/2008	30/01/2008
MBG-BRS0433	Oroso			07/11/2007	.[2]	.[2]
MBG-BRS0451	Oroso			07/11/2007	20/02/2008	11/03/2008
MBG-BRS0461	Oroso			13/11/2007	28/03/2008	02/04/2008
MBG-BRS0472	Oroso			13/11/2007	18/02/2008	27/02/2008
MBG-BRS0550	Oroso			07/11/2007	16/04/2008	22/04/2008

[1]Localidad; [2]Datos perdidos para la variedad MBG-BRS0433 debido a que presentó una alta heterogeneidad intravarietal, hecho que dificultó la toma de datos de grelos.

Tabla 2.4. Fechas de trasplante y recogida de material para el análisis nutricional en cada variedad y localidad durante el ciclo de cultivo 2008/09.

Variedad	Localidad	Trasplante	Análisis nutricional de nabiza	Análisis nutricional de grelo
MBG-BRS0082	Guitiriz	24/09/2008	25/11/2008	10/04/2009
MBG-BRS0143	Guitiriz			22/01/2009
MBG-BRS0163	Guitiriz			11/02/2009
MBG-BRS0173	Guitiriz			11/02/2009
MBG-BRS0184	Guitiriz			24/03/2009
MBG-BRS0197	Guitiriz			11/02/2009
MBG-BRS0401	Guitiriz			11/02/2009
MBG-BRS0451	Guitiriz			09/03/2009
MBG-BRS0461	Guitiriz			24/03/2009
MBG-BRS0472	Guitiriz			09/03/2009
MBG-BRS0550	Guitiriz			20/04/2009
MBG-BRS0082	Oroso	18/09/2008	11/11/2008	30/03/2009
MBG-BRS0143	Oroso			22/01/2009
MBG-BRS0163	Oroso			11/02/2009
MBG-BRS0173	Oroso			11/02/2009
MBG-BRS0184	Oroso			24/03/2009
MBG-BRS0197	Oroso			11/02/2009
MBG-BRS0401	Oroso			11/02/2009
MBG-BRS0451	Oroso			09/03/2009
MBG-BRS0461	Oroso			24/03/2009
MBG-BRS0472	Oroso			09/03/2009
MBG-BRS0550	Oroso			15/04/2009

MBG-BRS0082	Salcedo	16/09/2008	18/11/2008	17/04/2009
MBG-BRS0143	Salcedo			22/12/2008
MBG-BRS0163	Salcedo			11/02/2009
MBG-BRS0173	Salcedo			11/02/2009
MBG-BRS0184	Salcedo			25/03/2009
MBG-BRS0197	Salcedo			11/02/2009
MBG-BRS0401	Salcedo			11/02/2009
MBG-BRS0451	Salcedo			13/03/2009
MBG-BRS0461	Salcedo			25/03/2009
MBG-BRS0472	Salcedo			17/02/2009
MBG-BRS0550	Salcedo			30/04/2009

2.5. Análisis estadísticos

Con los datos agronómicos, sensoriales y de la cuantificación de cada compuesto analizado se realizaron análisis de varianza individuales y combinados. La comparación entre variedades, estados de la planta, localidades y años se hizo mediante la comparación de medias con la mínima diferencia significativa protegida de Fisher a $p = 0.05$ (Steel y otros, 1997). Estos análisis se realizaron en base al diseño experimental en bloques al azar.

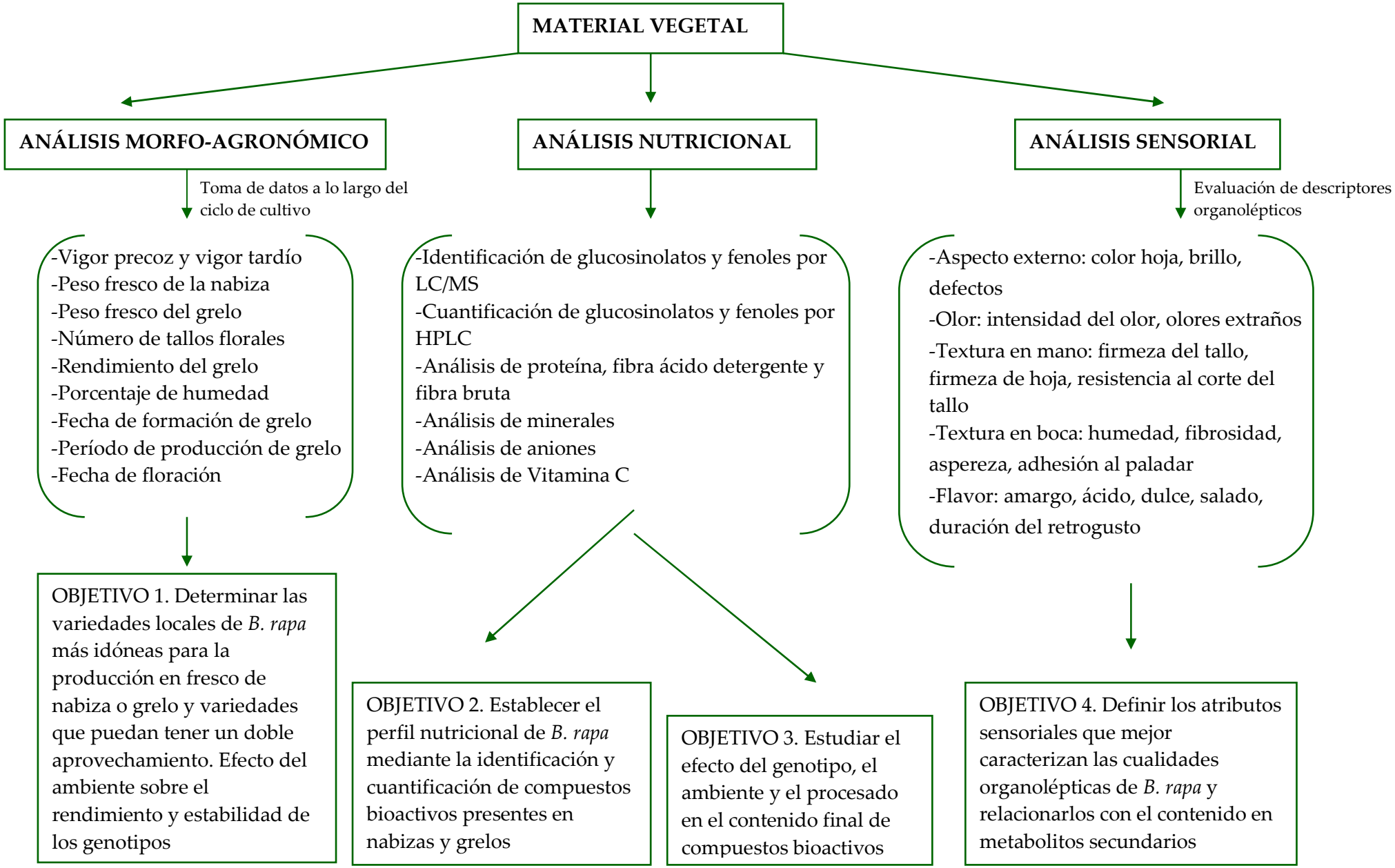
Para determinar el efecto del genotipo (G), del ambiente (E) y de su interacción (GE) sobre la producción en fresco y sobre el contenido en metabolitos secundarios de nabiza y grelo se realizó un análisis de regresión SREG (Sites Regresion) (Crossa y Cornelius, 1997). En el modelo SREG los términos lineales de genotipos no se consideran individualmente y se adicionan al término multiplicativo de la interacción GE. En este método se realiza una estandarización usando el error estándar de la media de cada genotipo dentro de ambientes (Cornelius y Crossa, 1997); además, permite la representación simultánea de la variabilidad de genotipos y ambientes, basada en el análisis de componentes principales (Yan y otros, 2000). Cada ambiente fue definido como la combinación de un año por localidad. El biplot GGE (G+GE) se construye a

partir de las primeras dos componentes principales (CP) del modelo SREG. La primera componente, cuando se encuentra altamente correlacionada con el efecto principal de G, representa la proporción del carácter medido que se debe sólo a las características del G. La segunda componente representa la parte del carácter debida a la interacción GE. Los genotipos cercanos entre sí en el biplot GGE presentan patrones similares de respuesta a través de los ambientes. Los ambientes cercanos entre sí, dado por el ángulo agudo entre sus vectores, indican asociación ambiental positiva, es decir, patrones similares de respuesta en el comportamiento relativo de un conjunto de genotipos (Yan y otros, 2000). Las variedades y los ambientes fueron representados en el mismo gráfico.

En cada localidad se tomaron datos de temperatura y precipitación de estaciones meteorológicas situadas en cada una de las parcelas de experimentación. Con estos datos climáticos, se realizó un análisis de correlaciones simples para determinar su relación con los caracteres agronómicos por un lado y con el contenido en metabolitos secundarios por otro. El análisis de correlaciones también fue empleado para estudiar la relación existente entre los caracteres sensoriales y el contenido en metabolitos secundarios presentes en nabizas y grelos.

Todos los análisis estadísticos llevados a cabo en este trabajo se realizaron con el programa estadístico SAS (2007).

A continuación se muestra un esquema que resume todos los análisis llevados a cabo en base a cada uno de los objetivos planteados.


MATERIAL VEGETAL
ANÁLISIS MORFO-AGRONÓMICO
ANÁLISIS NUTRICIONAL
ANÁLISIS SENSORIAL
Toma de datos a lo largo del ciclo de cultivo
Evaluación de descriptores organolépticos
-Vigor precoz y vigor tardío
-Peso fresco de la nabiza
-Peso fresco del grelo
-Número de tallos florales
-Rendimiento del grelo
-Porcentaje de humedad
-Fecha de formación de grelo
-Período de producción de grelo
-Fecha de floración
-Identificación de glucosinolatos y fenoles por LC/MS
-Cuantificación de glucosinolatos y fenoles por HPLC
-Análisis de proteína, fibra ácido detergente y fibra bruta
-Análisis de minerales
-Análisis de aniones
-Análisis de Vitamina C
-Aspecto externo: color hoja, brillo, defectos
-Olor: intensidad del olor, olores extraños
-Textura en mano: firmeza del tallo, firmeza de hoja, resistencia al corte del tallo
-Textura en boca: humedad, fibrosidad, aspereza, adhesión al paladar
-Flavor: amargo, ácido, dulce, salado, duración del retrogusto
OBJETIVO 1. Determinar las variedades locales de B. rapa más idóneas para la producción en fresco de nabiza o grelo y variedades que puedan tener un doble aprovechamiento. Efecto del ambiente sobre el rendimiento y estabilidad de los genotipos
OBJETIVO 2. Establecer el perfil nutricional de B. rapa mediante la identificación y cuantificación de compuestos bioactivos presentes en nabizas y grelos
OBJETIVO 3. Estudiar el efecto del genotipo, el ambiente y el procesado en el contenido final de compuestos bioactivos
OBJETIVO 4. Definir los atributos sensoriales que mejor caracterizan las cualidades organolépticas de B. rapa y relacionarlos con el contenido en metabolitos secundarios

CAPÍTULO III

Producción en fresco y valor nutritivo de nabizas y grelos

Environmental influence on agronomic and nutritional value of *Brassica rapa*

Marta Francisco, Pablo Velasco, Margarita Lema, María Elena Cartea

Misión Biológica de Galicia (CSIC), PO Box 28, E-36080· Pontevedra, Spain

ABSTRACT

In Galicia (northwestern Spain), *Brassica rapa* subsp. *rapa* L. includes turnip greens and turnip tops as main crops. Twelve varieties of this species grown were agronomical and nutritionally evaluated in seven environments to determine the best and stable genotypes for future breeding programs focused on turnip greens and/or turnip tops fresh production as well as to assess their nutritional characteristics. Significant differences were found among varieties, years and locations for most agronomical traits. Interactions between the genotype main effect (G) plus the genotype by environment interaction (GE) and fresh production were established using a SREG analysis model. The GGE interaction explained 46% and 58% of fresh production for turnip greens and tops, respectively. The production and stability of genotypes and environments were studied by GGE biplot. Varieties evaluated in this work displayed enough variability to determine the appropriate varieties for each one of the distinct crops. The varieties MBG-BRS0550, MBG-BRS0082 and MBG-BRS0184 had good agronomical performance as turnip greens whereas the most suitable varieties for turnip tops production were MBG-BRS0472 and MBG-BRS0143. Salcedo 2008 was the most productive and stable environment for both crops. Varieties differed significantly for crude fibre and ADF content and they showed higher concentration of the anions Cl^-, NO^{3-} and $SO4^{2-}$ in turnip greens than in turnip tops. Both plant organs showed high contents of Ca, K, Mg and P. Therefore, *B. rapa* varieties evaluated proved to be a valuable source of minerals, protein and fibre.

INTRODUCTION

Brassica rapa L. is an important species of the genus *Brassica* widely cultivated in the world, which includes a variety of vegetables and oilseed crops. A number of studies based on morphology, geographic distribution, isozymes and molecular data indicate that *B. rapa* originates from two independent centers (Gómez-Campo, 1999). Europe is proposed as one primary centre of origin for oil and turnip types, which were further developed in Russia, Central Asia and the Near East. East Asia is proposed as another primary centre of origin for Indian oil types and Chinese leafy vegetables. The cultivation of this species for many centuries in different parts of the world caused a large variation in the plant organs that are consumed, which has resulted in the human selection of different morphotypes depending on local preferences (Zhao et al., 2005).

In Galicia (Northwestern Spain) and in the coldest regions of Portugal, *B. rapa* subsp. *rapa* includes three different crops: turnips, turnip greens and turnip tops. Turnips are the thickened roots, turnip greens are the young leaves harvested in the vegetative period and turnip tops are the floral shoots and surrounding leaves. They have been under cultivation for a very long time since they were among the first vegetables to be introduced into the Western Iberian Peninsula (Gómez-Campo, 1999). According to the particularities of Galician agriculture (small familiar farms and traditional cultural practices), farmers obtain their own seeds for sowing. This process has led to a great number of landraces adapted to different conditions and uses all along Galician geography.

A collection of *B. rapa* subsp. *rapa* from Northwestern Spain is currently kept at 'Misión Biológica de Galicia' (CSIC, Spain). These landraces are a valuable resource since they are adapted to the climatic conditions of the area. Nutritional evaluations of this collection were carried out by Padilla et al. (2007) and Francisco et al. (2009) finding that these varieties are a valuable source of bioactive compounds such as glucosinolates and phenolics, related to human health and the reduction of the risk of certain cancers and cardiovascular diseases (Rice-Evans et al., 1996; Cartea and Velasco, 2008). Nevertheless, it would be necessary to evaluate other nutritional parameters in order to complete the nutritional characterization of turnip greens and tops.

In a preliminary work, 120 *B. rapa* varieties of this collection were evaluated based on agronomical and morphological traits (Padilla et al., 2005). These authors found that the same landrace can be sown for more than one purpose as turnips, turnip greens, and/or turnip tops. This fact prevents the fixation of the three uses mentioned above but also allows the existence of local populations with high levels of variability. However, the potential yield of these varieties and the stability of performance have yet not been explored. Due to the health beneficial compounds found in brassicas, turnip greens and turnip tops have good commercial perspectives. The high genetic diversity described within *B. rapa* species (Hirai and Matsumoto, 2007) would be useful to select varieties with high production and improved nutritional value.

The growth and development of plants is dependent on the genotype and also on abiotic and biotic factors (Boyer, 1982). Abiotic factors include the environmental conditions and biotic factors include animals, insects, and diseases. Each plant has certain environmental requirements. To attain the highest potential yields, a crop must be grown in an environment that meets these requirements. Unfavorable environmental conditions can produce a stress on plants resulting in lower yields (Diepenbrock, 2000). Therefore, cultivars with higher production and a wide range of adaptability to edaphic and climatic conditions are essential for breeding programs. Evaluation of different genotypes at different locations across years will provide us valuable information about the performance of these genotypes and allow identify superior cultivars on several environments.

Different methods have been used by several authors to study yield stability, including AMMI analysis (Gauch, 1992). Among them, the Sites Regression (SREG) method has been suggested as the appropriate model when large variation is due to environments (Crossa and Cornelius, 1997). The multiplicative method considers genotype (G) and genotype × environment interaction (GE) effects simultaneously (Yan et al., 2000). The SREG method supplies a graphical display called GGE (G plus GE interaction) biplot that facilitates visual cultivar evaluation.

Thus, the objectives of this study were: i) to determine the best and stable varieties for future breeding programs focused on turnip greens and/or turnip tops fresh production and ii) to assess the nutritional characteristics of these varieties.

MATERIAL AND METHODS

Plant material. Twelve local varieties of *B. rapa* were evaluated in this study (Table 1). From these, ten varieties were chosen based on their agronomic performance for turnip tops and/or turnip greens production and two varieties derived from three cycles of masal selection by fresh yield.

Table 1. Local varieties of *Brassica rapa* evaluated in this study.

Identification	Origin	Source [1]	Type [2]
MBG-BRS0082	Forcarei, Salcedo, Pontevedra	MBG	L
MBG-BRS0143	Lama, Boqueixón, A Coruña	MBG	L
MBG-BRS0163	Barcia, Melón, Ourense	MBG	S
MBG-BRS0173	Valongo, Cortegada, Ourense	MBG	L
MBG-BRS0184	Carballo, A Coruña	MBG	L
MBG-BRS0197	Arnoia, Ourense	MBG	S
MBG-BRS0401	San Xiao, Coirós, A Coruña	MBG	L
MBG-BRS0433	Oroso, A Coruña	MBG	L
MBG-BRS0451	O Val, Narón, A Coruña	MBG	L
MBG-BRS0461	Castro de Rei, Guitiriz, Lugo	MBG	L
MBG-BRS0472	Porta, Sobrado, A Coruña	MBG	L
MBG-BRS0550	Trazo, A Coruña	MBG	L

[1]Germplasm Bank at the Misión Biológica of Galicia (MBG-CSIC).
[2] L= Local variety (without selection), S= Variety derived from three cycles of masal selection by fresh yield.

The varieties were evaluated in three years (2006, 2007 and 2008) at three locations representative of the *B. rapa* production areas in northwestern Spain: Oroso (A Coruña) (43°1'N, 8°26'W, 280 m.a.s.l.), Guitiriz (Lugo) (43°12'N, 7°53'W, 516 m.a.s.l) and Salcedo (Pontevedra) (42°24'N, 8° 38'W, 20 m.a. s.l). At Salcedo, two trials were lost due to unfavorable climatic conditions in 2006 and plant damages caused by *Delia radicum* L. immediately after transplanting in 2007. The varieties were planted in multipot-trays and seedlings were transplanted into the field at the five or six leaves stage. Transplanting dates were from September 1st to October 19th. Varieties were transplanted in a randomized complete block design with three replications. The

experimental plots consisted of three rows with ten plants per row. Rows were spaced 0.8 m apart and plants within rows 0.5 m apart. Cultural operations, fertilization, and weed control were made according to local practices. For pest control were used Aphox against aphids and Laidan against root or turnip fly (*Delia radicum*) L. Force® was added at the time of transplantation to combat soil insects.

Morphological and agronomical traits were recorded along maturity cycle of varieties. Traits are described in Table 2 and were adapted from the International Board for Plant Genetic Resources *Brassica* L. and *Raphanus* L. descriptors list (IBPGR, 1990). Early vigor was taken one month after transplanting as a visual subjective scale from 1 (very poor) to 5 (excellent). Rapid early development of leaf area and above ground biomass was denoted as early vigor. Late vigor was taken three months after transplanting when plants reach their optimal vegetative growth and before they comes into turnip tops production. Late vigor helps to increment plant production since increases the available water by shading soil surface and reducing evaporation, and trend to make plants more competitive again weeds, resulting in higher biomass production. The turnip top production period is the difference between time to flowering and time to turnip top production. This is an important trait because it provides the production time to obtain edible turnip tops.

Soil analyses and climate data. Soil samples were collected at the three locations (Guitiriz, Oroso and Salcedo). Samplings were carried out using a hollow cylindrical corer with an internal diameter of 7 cm. Six subsamples, each 25 cm deep, were taken following a zigzag path across the center of each plot. Subsamples were mixed to obtain homogeneous samples, about 500-1000 g, to be analyzed. The soil properties examined were pH, percentage of organic matter, available phosphorus, available potassium, exchangeable magnesium, exchangeable cations (Ca, Mg, Na, K, and Al), and cation exchange capacity. Soil analyses were performed at "Estación Fitopatológica do Areeiro" (Pontevedra, Spain). Climatic data (temperature, degree days and precipitation) were obtained from meteorological stations located close from trials.

Table 2. Agronomic traits used in the evaluation of local varieties of *Brassica rapa* from northwestern Spain.

Agronomic traits	Description
Early vigor	Subjective scale from 1 (very poor) to 5 (excellent)
Late vigor	Subjective scale from 1 (very poor) to 5 (excellent)
Turnip green fresh matter	Average fresh weight of a leaf (g) (mean of 25 leaves per plot)
Turnip top fresh matter	Average fresh weight of a turnip top (g) (mean of all turnip tops taken from 5 plants per plot)
Turnip green moisture	Percentage of fresh weight of a fresh leaf (%)
Turnip top moisture	Percentage of fresh weight of a fresh turnip top (%)
Secondary stems	Average number of secondary stems per plant (mean of 5 plants per plot)
Turnip top fresh production	Turnip top fresh matter × number of secondary stems (kg)
Time to turnip top production	Days from transplanting until 50% of plants have the first turnip top
Turnip top production period	Difference between the time to flowering and the time to turnip top production
Time to flowering	Days from transplanting until 50% of plants have the first flower

Nutritional traits. The recorded nutritional quality data were crude protein, acid detergent fibre (ADF), crude fibre, the following minerals: Boron (B), Calcium (Ca), Zinc (Zn), Copper (Cu), Phosphorus (P), Iron (Fe), Magnesium (Mg), Manganese (Mn) and Potassium (K) and the following anions: chloride (Cl^-), nitrate (NO^{3-}), sulphate (SO_4^{2-}), and phosphate (PO_4^{3-}). For the protein, ADF, crude fibre and mineral determination, a sample of about 0.5 kg of turnip greens and turnip tops from each block were randomly harvested during 2007/08 at two locations and during 2008/09 at three locations. Samples were placed in paper-bags and dried in a heater at 80 ºC for at least 48 h. Determinations of anions were made on two samples of turnip greens and turnip tops from five plants per plot at each location in 2006/07. After harvesting on dry ice, the material was immediately transferred to the laboratory and frozen at -80 °C, prior to their lyophilisation. The dried material was ground for subsequent laboratory analysis. The ADF content was analysed by Van-Soest method and leaf protein concentration by Kjedhal method following the protocols of AOAC (2000). Mineral analyses were made by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). The anion analyses were carried out by Ion Chromatography and concentrations were determined by comparing peak areas with those of known standards. Nutritional analyses were performed at "Centro de Investigaciones Agrarias de Mabegondo (CIAM)" (A Coruña, Spain) and "Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC)" (Murcia, Spain).

Statistical analysis. Individual and combined analyses of variance over locations, years and plant organs were made for each trait according to a randomized complete block design. Varieties, years and locations were considered as fixed effects. Comparisons of means among plant organs were performed for each trait using the Fisher's protected least significant difference (LSD) at $P \leq 0.05$ (Steel et al., 1997). Simple correlations ($P < 0.05$) between agronomical traits and climatic factors were made in order to establish the relationships between them.

To study the genotype × location (GE) interaction, the Sites Regression method (SREG) was used (Crossa and Cornelius, 1997). Each environment was defined as the combination of a year and a location, resulting in seven different environments under study. Since this method does not allow missing data and since some varieties or plant

organs were not evaluated in all years and/or all locations, 11 varieties were evaluated for turnip greens production and seven varieties for turnip tops at five environments. For this method, principal components (PC) analysis is made on residuals of an additive model with environments as the only main effects. A two-dimensional biplot (Gabriel, 1971) called GGE biplot (G plus GE interaction) of the two first PCs was used to display the performance of each genotype on each environment (Yan et al., 2000). Each genotype and environment was defined by the scores of genotypes and locations on the two PCs, respectively. All statistical analyses were made by a SAS (SAS Institute, 2007) program.

RESULTS AND DISCUSSION

Agronomical traits

The combined analysis of variance showed significant differences among years, locations and varieties for most traits (Table 3). The variety × location, variety × year and year × location interactions were also significant for some of them. Therefore, individual analyses of variance for each year and location were made (data not shown). Comparing the results from the individual analyses, varieties showed a similar performance across years and locations, i.e., varieties with the extreme values were the same at different locations and years for most traits. Locations were also similar in each year. Then, magnitude changes rather than rank changes contributed to these interactions.

Varieties showed significant differences for all agronomical traits (Table 3). The means of combined data across environments are showed in Table 4. Agronomical traits will allow us to define the most appropriate varieties for turnip greens and turnip tops fresh production. Varieties suitable for early turnip greens production should have a fast and vigorous growth of the leaves. Numerous secondary stems and early flowering are desirable characteristics for turnip tops production (Monteiro and Dias, 1996; Padilla et al., 2005). Early vigor represents the ability of a variety to compete with weeds, which are abundant in Galician agriculture, as they are favored by the warm temperature and humidity that normally occur during the first stages of vegetative development. The most vigorous varieties were MBG-BRS0550 and MBG-BRS0472

(Table 4). Turnip greens fresh matter ranged from 14.84 g to 28.49 g, being the best varieties MBG-BRS0163, MBG-BRS0550, MBG-BRS0082, MBG-BRS0451 and MBG-BRS0184 (Table 4). In a previous evaluation carried out by Padilla et al (2005) authors also found that MBG-BRS0082 and MBG-BRS0184 were appropriate varieties for turnip greens production. The variety MBG-BRS0163 is derived from three cycles of masal selection by fresh yield, threrefore it is expected good agronomical performance.

The turnip tops fresh production depends on the number of secondary stems and on the turnip tops fresh matter. Local varieties MBGBRS0550 and MBGBRS0082 showed the highest turnip tops fresh production (more than 1.5 kg) differing significantly from all other varieties, although MBGBRS0472 and MBGBRS0184 presented also high values (Table 4). Moreover, MBGBRS0550 and MBGBRS0472 reached the best values for late vigor. From the point of view of the farmer-producer, the flowering periods are important traits. The latest varieties delay the turnip greens production while the earliest varieties allow offering turnip tops on the market before their usual dates. Besides, earliest varieties have greater flexibility to grow in different growing seasons due to lower cold and photoperiod requirements. MBG-BRS0550 showed the longest time to flowering (220 days) and it was the latest variety for turnip tops production (204.5 days), although it not differed significantly from MBG-BRS0082 and MBG-BRS0433. Contrarily, MBG-BRS0143 was the earliest variety (106 days to turnip tops production and 136 days to flowering), differing significantly from all other varieties. Another important trait to consider for selecting a variety is the turnip top production period, i.e., when the plant starts producing edible turnip tops. The variety MBG-BRS0143 had the longest period (29.6 days) opposite to MBG-BRS0082 (14.6 days). Short periods are normally associated to synchrony for production among plants of the same variety, which allows a uniform or a single harvest while long periods are normally associated to low synchrony among plants, that can delay the turnip tops harvesting time.

As a conclusion, varieties MBG-BRS0550 and MBG-BRS0082 had good agronomical performance for turnip greens and turnip tops production because of their high values of fresh matter, while the earlier variety MBG-BRS0143 and the

vigorous and productive variety MBG-BRS0472 were the most suitable for turnip top production.

There were significant differences between locations and years for most agronomical traits (Table 3). Varieties flowered earlier in Oroso than in the other two locations while turnip top production period was longer in Salcedo. The average turnip green fresh matter was 37.6g, 22.05g, and 17.03g for Salcedo, Oroso and Guitiriz, respectively. The average turnip top fresh production was 1.44g, 0.95g and 0.69 kg for Salcedo, Guitiriz and Oroso, respectively. Plants at Salcedo had better agronomic performance than those in Guitiriz or Oroso. Temperature, solar radiation, air humidity and soil conditions are the main determinants for crop yield (Rötter and van de Geijn, 1999). The pH soil analysis were from pH=5.3 at Guitiriz to pH=5.6 at Salcedo. Low pH restricts nitrification rates and increases the content of certain elements known to be toxic to many plants (e.g. aluminum). Available phosphorus was high in Salcedo and Guitiriz and medium in Oroso. The available potassium was high in Oroso and Salcedo and medium in Guitiriz. The increasing availability of nutrients (N, P, K) can bring an increase in biomass production (Vermeer and Berendse, 1983).

Table 3. Mean squares of the combined analysis of variance of the agronomic traits taken in the *Brassica rapa* varieties from northwestern Spain evaluated at three locations and three years.

	Location (L)	Year (Y)	Variety (V)	L×Y	L×V	Y×V	L×Y×V	Error
Early vigor	0.21	0.09	4.14**	0.06	0.67**	0.51	0.64*	0.33
Turnip green fresh matter	2022.57**	4629.43**	447.61**	415.08	56.04**	54.51**	23.08	26.08
Turnip green moisture	96.13**	9.93	10.83**	76.18**	3.77	10.915**	4.03	2.98
Late vigor	0.08	0.13	5.89**	0.08	0.53*	0.81*	0.56	0.41
Turnip top fresh matter	2253.90*	94727**	8569.25**	2821.48**	1113.55**	3213.29**	581.55	400.64
Turnip top moisture	13.33	0.88	7.61**	10.16	4.52*	3.85	6.31**	2.58
Secondary stems	277.12**	7.74	45.04**	82.89*	19.39**	34.19**	18.57*	7.45
Turnip top fresh production	4.47**	1.62**	1.43**	0.67*	0.43**	0.47**	0.22	0.10
Time to turnip top production	1404.43**	1843.37**	16662**	2496.44**	193.71**	155.41	60.40	95.97
Time to flowering	1299.02**	8688.62**	13694**	2598.17**	109.51	184.59**	120.30	76.83
Turnip top production period	458.83**	2747.07**	367.80**	10.36	102.82**	100.35**	68.87*	34.07

*, ** Significant at $P \leq 0.05$ and 0.01, respectively

Table 4. Means of several agronomical traits of 12 local varieties of *B. rapa* evaluated in three locations and three years in northwestern Spain.

Variety	Early vigor	Turnip green fresh matter	Turnip green moisture	Late vigor	Turnip top fresh matter	Turnip top moisture	Secondary stems	Turnip top fresh production	Time to turnip top production	Time to flowering	Turnip top producion period
MBG-	(1-5)	(g)	(%)	(1-5)	(g)	(%)	(Nº)	(kg/plant)	(days)	(days)	(days)
BRS0082	3.1	26.64	89	3.1	63.17	91	15.72	1.50	198.8	213.4	14.6
BRS0143	2.8	16.69	89	3.0	49.08	91	14.60	0.93	106.3	136.0	29.6
BRS0163	2.9	28.49	90	3.1	58.69	91	13.14	0.77	143.7	167.3	23.7
BRS0173	2.4	15.61	90	2.3	40.42	90	13.22	0.72	141.0	158.1	17.2
BRS0184	3.2	25.20	89	3.0	75.61	91	10.83	1.20	188.6	205.1	16.4
BRS0197	2.7	19.28	90	3.1	52.70	90	13.44	0.68	138.9	160.1	21.2
BRS0401	2.2	16.42	91	2.2	33.19	90	11.26	0.48	137.3	155.5	18.2
BRS0433	3.3	14.84	90	3.1	24.21	92	-(1)	-(1)	194.8	212.0	17.2
BRS0451	3.4	25.79	89	3.1	63.92	91	10.79	0.94	171.7	192.4	20.7
BRS0461	3.2	23.17	89	3.2	75.81	91	9.17	0.92	181.5	203.7	22.1
BRS0472	3.5	22.95	89	3.9	92.06	92	10.96	1.30	158.5	179.0	20.5
BRS0550	3.6	28.29	90	4.1	110.32	91	9.57	1.57	204.5	220.0	16.0
Mean	3.0	22.12	90	3.1	63.02	91	12.13	0.97	162.3	181.9	19.7
LSD (0.05)	0.3	3.35	1	0.4	14.32	1	2.14	0.24	6.8	6.1	4.1

LSD: Least Significant Difference, (1) These traits were not taken in 2006/2007 when the variety MBG-BRS0433 was evaluated.

Since climatic conditions can be the cause of the differences among locations and years reported here, simple correlations between agronomical traits and climatic factors were made. Results showed that some traits evaluated seem to have important relationships with some climatic parameters (Table 5).

For instance, early vigor had significant and positive correlations with mean of the maximum temperatures, mean of the minimum temperatures and mean of the mean temperatures (ranging from r = 0.76 to r = 0.90).Therefore, turnip greens were more vigorous at higher temperatures. Olesen and Grevsen (1997) have reported linear responses of temperature and leaf expansion rate in broccoli and cauliflower, with an optimum temperature of 21 ºC. On the other hand, turnip top fresh matter showed a significant negative correlation with mean of the minimum temperature (r = -0.90) while the turnip top production period had positive and high correlations with the mean of maximum temperatures (r = 0.87) and with degree days of maximum temperatures (r=0.89). *Brassica rapa* is a winter crop; therefore, low temperatures favor turnip tops fresh matter while high temperatures enlarge the turnip tops production period.

The site regression (SREG) model was used to select varieties that perform stably across environments with different climates and to select the best environments for turnip greens and/or turnip tops fresh production. The GGE refers to the genotype main effect (G) plus the genotype by environment interaction (GE), which are the two sources of variation of the SREG model (Yan et al., 2000). For selecting a suitable variety for turnip tops production, it should be taken into account that this crop was not obtained in some varieties in some years and locations. For this reason, varieties MBG-BRS0082, MBG-BRS0184, MBG-BRS0433, MBG-BRS0451 and MBG-BRS0550 were eliminated in the SREG analysis. Most of them were late varieties meaning that a variety growing out of the usual dates are not capable of producing edible turnip tops. Results of the analysis of variance for SREG are presented in Table 6, which shows the relative magnitudes of E and GGE variance terms.

Table 5. Simple correlation coefficients among eight agronomical traits taken on 12 varieties of *Brassica rapa* and 7 climatic factors taken at three locations and three years in northwestern Spain.

Climatic factors	Early vigor	Turnip green fresh matter	Late vigor	Turnip top fresh matter	Time to turnip top production	Time to flowering	Turnip top production period	Secondary stems
Mean of the mean temperatures	0.90**	0.58	0.05	-0.66	-0.49	-0.42	-0.25	-0.05
Mean of the maximum temperatures	0.76*	0.69	-0.20	0.77	0.43	0.75	0.87*	0.60
Mean of the minimum temperatures	0.78*	-0.04	0.09	-0.90*	-0.53	-0.74	-0.76	-0.53
Degree days of mean temperatures	0.55	0.17	-0.11	-0.53	-0.40	-0.23	-0.01	-0.02
Degree days of maximum temperatures	0.62	0.47	-0.27	0.70	0.39	0.73	0.89*	0.53
Degree days of minimum temperatures	0.30	-0.34	0.13	-0.47	-0.44	-0.28	-0.06	0.19
Precipitation	-0.21	-0.64	0.35	0.65	0.10	0.32	0.44	0.70

*, ** Significant at $P \leq 0.05$ and 0.01, respectively

Table 6. Analysis of variance of the sites regression (SREG) multiplicative model for 11 *Brassica rapa* varieties evaluated for turnip green production and for 7 *Brassica rapa* varieties evaluated for turnip top production at five environments.

Source of variation	Degrees of freedom	Sum of squares	Mean Squares	Cumulated % variability
Turnip greens				
E	4	6963.85	1740.96**	
GGE	50	7258.88	161.31**	
PC1	13	5547.84	426.76**	76.43
PC2	11	877.97	79.81**	88.52
PC3	9	668.60	74.28**	97.73
PC4	7	155.13	22.16	99.87
PC5	5	9.34	1.68	100.00
Error	105	3383.66	32.22	
Turnip tops				
E	4	6447361.98	1611840.50**	
GGE	30	9255523.02	1542587.17**	
PC1	9	6702270.55	744696.73**	72.01
PC2	7	1673354.97	239050.71**	90.00
PC3	5	891242.89	178248.58**	99.85
PC4	3	31386.27	10462.09	99.10
PC5	1	8443.41	8443.41	100.00
Error	62	4559204.72	73535.55	

*, ** Significant at $P \leq 0.05$ or 0.01, respectively.
E = Environmental main effects, where one E is the combination of a location and year.
GGE = Genotype plus genotype × environment interaction effects.

Leaf fresh matter was significantly affected by E, which explained 44% of the total variation, while GGE accounted for 46% of total sum of squares. Genotype main effects (G) accounted for the 69% of the GGE variation. Percentage of variation due to G was larger than that due to GE interaction, but GE interaction was significant, meaning that differences among genotypes vary across environments. The first two PCs of the SREG which make up a GGE biplot, explained 88.52% of GGE variation.

Yan et al. (2000) and Crossa et al. (2002) stated that if the primary effects of sites from the SREG model are all of the same sign in the two-dimensional biplot, as it was found in the present study for leaf fresh matter (Fig. 1a), the PC1 reflects the mean

performance plus non-crossover GE interaction. Therefore, a genotype with a larger PC1 score has a greater average production in direct proportion to the environment PC1 scores. The two dimensional biplot for turnip greens production (Fig 1a) showed that MBG-BRS0550, MBG-BRS0082 and MBG-BRS0184 were the best genotypes in most environments. Therefore, these varieties could be recommended for future breeding programs. On the other hand, the varieties MBG-BRS0451 and MBG-BRS0163 had the highest production only at Oroso 2008 (E3). Varieties MBG-BRS0143, MBG-BRS0173, MBG-BRS0197 and MBG-BRS0401 had negative PC1 scores, suggesting poor average performance at all environments. The low genotypic PC2 score found for MBG-BRS0472 represents a proportionate response of this variety across environments, which means a stable genotype. The ideal test environments, should have small (absolute) PC2 scores (more representative of the overall environments) and large PC1 scores (more power to discriminate genotypes in terms of the genotypic main effect) (Yan et al., 2000; Yan and Rajcan, 2002). Therefore, for turnip greens production, Guitiriz 2008 (E4) and Salcedo 2008 (E5) were the most stable and productive locations, respectively.

For turnip tops fresh production, E explained 40% of the total variation and GGE explained 58%. About 64% of the proportion explained by GGE accounted for G while the remaining 36% was due to GE effects. Likewise it happened for turnip greens production, the variation due to G was significant and larger than due to the GE interaction. The first two PCs of the SREG model explained 90% of GGE variation. The primary effects of sites from the SREG model are all of the same sign which means a non-crossover GE interaction. The two dimensional biplot (Fig. 1b) showed that MBG-BRS0472 and MBG-BRS0143 had the highest performance for turnip tops yield at all locations over years. Besides MBG-BRS0472 was a stable variety, suitable characteristic for future crop breeding programs. The variety MBG-BRS0163 was the most stable genotype, but presented low yield in all environments. Likewise it happened in the SREG analysis for turnip greens production, the varieties MBG-BRS0173, MBG-BRS0197 and MBG-BRS0401 had a poor performance. Salcedo 2008 (E5) and Guitiriz 2008 (E4) were the most stable environments and Oroso 2008 (E3) appeared to be the best location for turnip tops production.

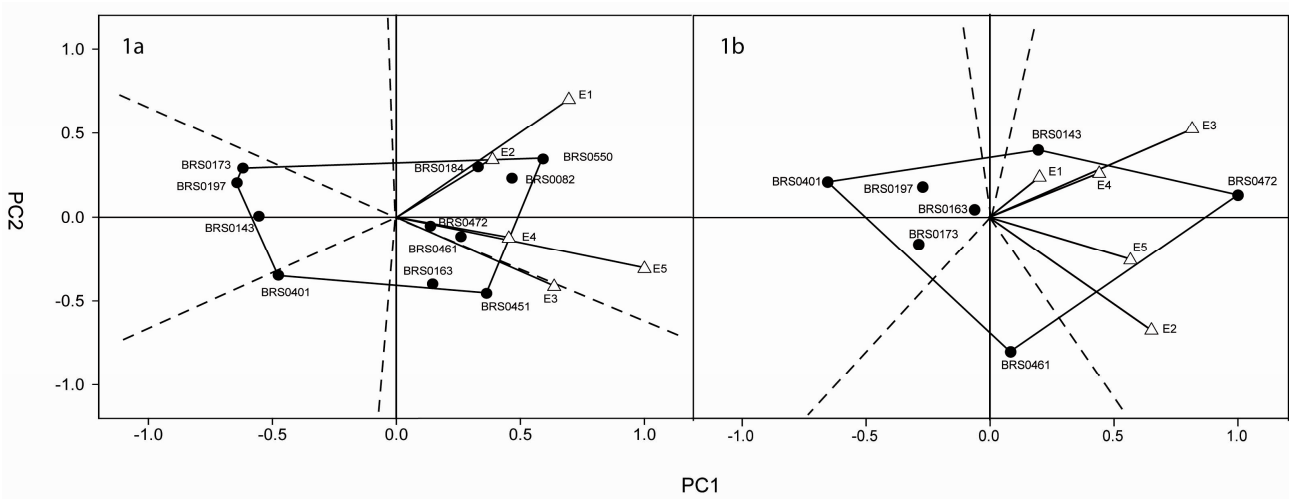


Figure 1. The G + GE interaction (GGE) biplot based on the turnip greens yield performance of 11 *Brassica rapa* varieties at five environments (Fig. 1a) and on the turnip tops yield performance of five varieties at five environments (Fig. 1b). Environments are E1 (Oroso 2007), E2 (Guitiriz 2007), E3 (Oroso 2008), E4 (Guitiriz 2008), E5 (Salcedo 2008). The polygon shown with tiny dots was made joining the genotypes which are on vertex. The intermediate sized dotted lines are the perpendicular lines to each side of the polygon; it shows which genotype(s) were grouped together as the most promising in a specific environment(s).

Nutritional quality of turnip greens and turnip tops

In order to assess the nutritional value of *B. rapa,* the concentration of different minerals (B, Ca, Zn, Cu, P, Fe, Mg, Mn, K), anions (Cl^-, NO^{3-}, PO_4^{3-} , SO_4^{2-}) as well as crude fibre, FAD and crude protein were studied.

It is well known that the nutritional content of brassica crops is variable and depends on environmental but also of the degree of maturity of the plant at harvest time (Grattan and Grieve, 1999). Since plant organ differed significantly for all traits, means for each mineral and anion are separately shown for each plant organ (Table 7). The Ca, K, Mn, Fe, and B concentrations were found in higher levels in turnip greens whereas P and Zn contents were higher in turnip tops (Table 7). The same trend was found by Rosa and Almeida (1996) in different tissues of several brassica crops.

Table 7. Mineral and anion content (mean, minimum and maximum) of turnip greens and turnip tops from 12 Galician *Brassica rapa* varieties evaluated at three locations (Salcedo, Oroso and Guitiriz) during 2007/2008 and 2008/2009.

	Turnip greens (100 g of fresh weigth)			Turnip tops (100 g of fresh weigth)		
Minerals (mg)	**Mean**	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**
K	351	325	392	317	248	282
Ca	133	121	151	101	69	116
P	53	45	56	62	59	71
Mg	22	19	37	23	21	26
Fe	2.70	2.03	3.36	1.13	0.75	2.69
B	0.97	0.87	1.12	0.21	0.17	0.26
Mn	1.10	0.97	1.29	0.66	0.55	0.80
Zn	0.47	0.40	0.52	0.53	0.45	0.67
Cu	0.04	0.03	0.05	0.05	0.04	0.06
Anions (mmol)						
Cl^-	0.115	0.080	0.147	0.042	0.025	0.061
NO^{3-}	0.010	0.004	0.021	0.004	0.000	0.013
SO_4^{2-}	0.052	0.044	0.063	0.047	0.039	0.052
PO_4^{3-}	0.036	0.029	0.041	0.050	0.040	0.061

Both plant organs (turnip greens and turnip tops) showed high contents of Ca, K, Mg and P (Table 7) with regard to those obtained in other brassica crops such as broccoli, Brussels sprouts, kale or Chinese cabbage reviewed by Fahey (2003). The Ca is an essential mineral for human health, participating in the biological functions of several tissues (musculoskeletal, nervous and cardiac system, bones and teeth, and parathyroid gland) and it is involved in the maintenance of the mineral homeostasis and physiological performance in general (Martínez-Ballesta et al., 2010). According to Lucarini et al. (1999) Ca present in brassicas shows an excellent bioavailability, because of the low levels of oxalic and phytic acids, which make brassicas a good source of this mineral. Varieties also show high levels of K, P and Mg, which are involved in different metabolic processes as well as in the maintenance of human health (Martínez-Ballesta et al., 2010).

Other elements such as Fe, Cu, Mn, Zn and B were found in trace quantities. These minerals have been considered as essential micronutrients. Iron plays an essential role in the formation of hemoglobin, which is regarded as a major oxygen

transporter (Huskisson et al., 2007). The Fe contents in vegetables and fruits are low, varying from 0.13 to 3.01 mg/100 g. Varieties evaluated in this study had high levels of Fe in turnip greens (2.70 mg 100 g^{-1} fw) and turnip tops (1.13 mg 100 g^{-1} fw). The iron found in vegetable sources, known as non-heme iron, is less available to the body. Although the Vitamin C present in brassica vegetables help to increase its absorption.

Varieties showed higher concentration of the anions Cl^-, NO^{3-} and SO_4^{2-}, in turnip greens than in turnip tops (Table 7), which are in agreement with the fact that plants minimized the concentration of toxic ions in their reproductive organs (Hachicha et al., 2000). On the contrary, the PO_4^{3-} content was higher in turnip tops than in turnip greens. The sulphate content of *Brassica* species is related with their high content of secondary sulphur compounds called glucosinolates. The Cl^- and NO^{3-} ions play an interchangeable role in osmoregulation. The NO^{3-} levels found in our *B. rapa* varieties were lower than those found in other brassica crops (De Pascale et al., 2007; López-Berenguer et al., 2009) and also they are below the limits imposed by EU regulations for other leafy vegetables (Santamaria, 2006). The review made by Anjana et al. (2007) pointed out that high concentrations of NO^{3-} anion are the cause of a large number of harmful effects on human health; therefore it seems reasonable to take preventive measures to decrease accumulation of nitrate in plants and its subsequent consumption by humans.

Brassica crops are used as feed and fodder; therefore, crude protein and fibre content are also important parameters in traditional farming systems, where residual postharvest leaves are usually used as fodder. Brassica crops are relatively low in fibre and are readily digested, providing good concentrations of energy for ruminants. Fibre and protein analyses were carried out in turnip greens because they are used for animal consumption. Varieties differed significantly ($P \leq 0.001$) for crude fibre and ADF concentration. MBG-BRS0082 and MBG-BRS0451 had the highest crude fibre content (about 12% dw) and along with MBG-BRS0184 also had the highest percentage of ADF. The values of crude protein and ADF found in turnip greens were higher than those found in leaf rape (*B. napus*) and kales (*B. oleracea*) crops grown in Galicia (Cartea et al., 2008; Vilar et al., 2008). In contrast, crude fibre content was lower than those found in kales. The analysis of variance for crude fibre, ADF and protein showed

significant differences ($P \leq 0.001$) among locations (data not showed). In Salcedo were found the highest levels of crude fibre (12.9% dw) and ADF (23.5% dw); protein was found in higher content in Oroso (29.3% dw) than in the other two locations. The good values of dietary fibre and protein quality found in brassica crops are some of the arguments used to increase their consumption as part of the daily diet or as feed.

CONCLUSIONS

The *B. rapa* varieties evaluated showed a wide range of intervarietal diversity to determine the most appropriate for either turnip greens or turnip tops fresh production. The SREG model analysis allowed evaluating the significance and magnitude of the E, G and GE interaction effects on turnip greens and turnip tops production. Environmental factors, as soil parameters and climatic factors appear to have influence in some traits related to production, earliness and adaptation; plants were more vigorous and productive at higher temperatures. The GGE biplots showed a graphical representation of the best performing variety and environment for each crop. The varieties MBG-BRS0550, MBG-BRS0082 and MBG-BRS0184 had good agronomical performance as turnip greens. The most suitable varieties for turnip tops production were MBG-BRS0472 and MBG-BRS0143. The most stable genotypes across locations and years were MBG-BRS0472 and MBG-BRS0163 for turnip greens and turnip tops production, respectively. For turnip greens and turnip tops production, Salcedo 2008 and Oroso 2008 were the most productive while Salcedo 2008 and Guitiriz 2008 were the most stable locations being Salcedo 2008 the best location for stability and production. Furthermore, the identification of the cultivar that behaves best at a specific growing environment would be useful to breeders and producers.

Since the importance of the quality and nutritional properties of vegetables for consumers has continuously increased, the present study assessed that *B. rapa* local varieties are a good source of minerals, fibre and protein and thus, they could be used as feed and fodder crops. Therefore, like other brassica vegetables, they remain among the best sources of the dietary components and should be consumed regularly as part of a diet rich in fruit and vegetables.

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CAPÍTULO IV

Identificación de metabolitos secundarios en nabizas y grelos

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Simultaneous identification of glucosinolates and phenolic compounds in a representative collection of vegetable *Brassica rapa*

Marta Francisco[a], Diego A. Moreno[b], María Elena Cartea[a,*], Federico Ferreres[b], Cristina García-Viguera[b], Pablo Velasco[a]

[a] *Misión Biológica de Galicia (CSIC), PO Box 28, E-36080 Pontevedra, Spain*
[b] *Department of Food Science and Technology, CEBAS-CSIC, Campus Universitario de Espinardo, PO Box 164, Espinardo, E-30100 Murcia, Spain*

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ABSTRACT

Brassica rapa rapa group is widely distributed and consumed in northwestern Spain. The consumption of Brassica vegetables has been related to human health due to their phytochemicals, such as glucosinolates and phenolic compounds that induce a variety of physiological functions including antioxidant activity, enzymes regulation and apoptosis control and the cell cycle. For first time in *Brassica* crops, intact glucosinolates and phenolic compounds were simultaneously identified and characterized. Twelve intact glucosinolates, belonging to the three chemical classes, and more than 30 phenolic compounds were found in *B. rapa* leaves and young shoots (turnip greens and turnip tops) by LC–UV photodiode array detection (PAD)–electrospray ionization (ESI). The main naturally occurring phenolic compounds identified were flavonoids and derivatives of hydroxycinnamic acids. The majority of the flavonoids were kaempferol, quercetin and isorhamnetin glycosylated and acylated with different hydroxycinnamic acids. Quantification of the main compounds by HPLC-PAD showed significant differences for most of compounds between plant organs. Total glucosinolate content value was 26.84 μmol g^{-1} dw for turnip greens and 29.11 μmol g^{-1} dw for turnip tops; gluconapin being the predominant glucosinolate (23.2 μmol g^{-1} dw). Phenolic compounds were higher in turnip greens 51.71 μmol g^{-1} dw than in turnip tops 38.99 μmol g^{-1} dw, in which flavonols were always the major compounds.

1. Introduction

In Galicia (northwestern Spain), *Brassica rapa rapa* group is widely distributed and the edible parts are represented by the leaves and the young shoots. Turnip greens are the leaves harvested in the vegetative period, while turnip tops are the fructiferous stems with the flower buds and the surrounding leaves which are consumed before opening and while still green. Both edible parts are consumed boiled, generally as meat companions. These two products are part of traditional recipes and represent two important commodities. They are characterized by a particular bitter and pungent taste, which differentiate them from other *Brassica* vegetables, such as cabbage, broccoli, and cauliflower.

The consumption of Brassica vegetables has been related to human health and to reduction of the risk of suffering certain type of chronic diseases including cardiovascular problems and different types of cancers [1,2]. This association is often attributed to their phytochemicals, such as glucosinolates [3,4] and phenolic compounds [5,6] that induce a variety of physiological functions including antioxidant activity, regulating enzymes and controlling apoptosis and the cell cycle.

Glucosinolates are nitrogen and sulphur-containing plant secondary metabolites that occur mainly in the *Brassicaceae* family. Glucosinolates are β-thioglucoside N-hydroxysulphates containing a side chain and a β-D glucopyranosyl moiety. Upon cellular disruption, glucosinolates are hydrolyzed to various bioactive breakdown products by the endogenous enzyme myrosinase (thioglucohydrolase; E.C. 3.2.1.147). Isothiocyanates and indole glucosinolate metabolites (in particular indol-3-carbinol) are two major groups of autolytic breakdown products of glucosinolates. Both of them exhibit protective activities against many types of cancer. *In vitro* and *in vivo* studies have reported that these compounds may affect many stages of cancer development, including the induction of detoxification enzymes (Phase II enzymes) and the inhibition of activation enzymes (Phase I enzymes) [4,7].

Phenolic compounds are a large group of secondary metabolites widespread in plant kingdom. They are categorized into classes depending on their structure and subcategorized within each class according to the number and position of hydroxyl group and the presence of other substituents. The most widespread and diverse group of the polyphenols are the flavonoids which are built

* Corresponding author. Tel.: +34 986854800; fax: +34 986841362.
E-mail address: ecartea@mbg.cesga.es (M.E. Cartea).

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upon C_6–C_3–C_6 flavone skeleton. Flavonoids and hydroxycinnamic acid derivatives are widely distributed in plants and are important biologically active constituents of the human diet. In *Brassica* foods the flavonoids are complex, with up to five sugar residues present, and these may be further substituted with hydroxycinnamic residues [8,9]. The bioavailability and activity of different glycosides depends on their substituents [10]. For this reason, it is important to characterize and quantify the different derivatives of phenolic compounds. These compounds have direct antioxidant and free radical-scavenging activities but can also induce expression of various genes encoding metabolic enzymes thought to decrease the risk of various diseases and disorders [11]. Besides, phenolic compounds, glucosinolates have beneficial properties on human health and synergic effects could appear between both classes of metabolites.

The Brassicaceae family has been widely investigated for glucosinolates [12–15] and for phenolic composition [8,9,16–19]. Nowadays, the profile of different brassica species is well established. The analysis of these compounds by different methods is laborious and time consuming. For this reason, a method to extract and analyze these compounds at the same time would be very useful. Bennet et al. [11,20] used a method for analyzing both kinds of compounds on different species. As yet, the method has not been used for *Brassica* crops, and particularly in *B. rapa* species.

At the Misión Biológica de Galicia (Spanish Council for Scientific Research), a collection of local varieties of *B. rapa* [*rapa* group] is kept as part of the *Brassica* genus germplasm bank. In previous reports, this collection was evaluated and classified based on morphological and agronomical traits [21] and the profile of desulphoglucosinolates in leaves was studied [15] but to date, no information is available on the content of intact glucosinolates and phenolic compounds in these varieties. Therefore for a more comprehensive assessment, that allows the nutritional study, the objectives of this work were identification and quantification of glucosinolates, flavonoids and hydroxycinnamic acids in turnip greens and turnip tops in a set of *B. rapa* varieties. Identification was carried out by LC–UV photodiode array detection (PAD)-electrospray ionization (ESI) MSn and quantification by HPLC-PAD.

2. Experimental

2.1. Plant material

Ten local varieties of *B. rapa* were evaluated in this study. These varieties represent the variability of the *B. rapa* germplasm collection of northwestern Spain and were selected based on previous agronomic and nutritional evaluations [15,21]. The populations were evaluated at two locations in: Oroso (A Coruña) (43°1′N, 8°26′W, 280 m a.s.l.) and Guitiriz (Lugo) (43°12′N, 7°53′W, 516 m a.s.l.). Both locations represent standard *B. rapa* production areas in northwestern Spain. The populations were planted in multipot-trays and seedlings were transplanted into the field at the five or six leaves stage. Transplanting dates were on the 10th and 19th October 2006, in Oroso and Guitiriz, respectively. Populations were evaluated during the crop cycle, which was from autumn to spring, in a randomized complete block design with three replications. The experimental plot consisted of three rows with 10 plants per row. Rows were spaced 0.8 m apart and plants within rows 0.5 m apart. Cultural operations, fertilization, and weed control were made according to local practices. For the study of glucosinolates and phenolic compounds, turnip greens were harvested three months after transplanting and turnip tops samples were harvested just after flower buds formation, before opening and while still green, in this case from February to May 2007 according to the earliness of each variety. Three samples of healthy leaves and young shoots were used from five plants per plot. After harvesting on dry ice, the material was immediately transferred to the laboratory and frozen at −80 °C, prior to their lyophilization. The dried material was powdered using a IKA-A10 (IKA-Werke GmbH & Co.KG) mill and the powder was used for analysis.

2.2. Sample preparation

Extraction and the LC gradient for glucosinolate and phenolic analyses is a multi-purpose chromatographic method that simultaneously separates glucosinolates and phenolics [20]. Fifty milligrams of each sample were extracted in 1.5 mL 70% MeOH at 70 °C for 30 min with vortex mixing every 5 min to facilitate the extraction. The samples were centrifuged (13,000 × *g*, 15 min, 4 °C). The supernatants were collected and methanol was completely removed using a rotary evaporator under vacuum at 37 °C. The dry material obtained was redissolved in 1 mL of ultrapure water and filtered through a 0.20 μm syringe PTFE filters (Anotop™, Whatman International Ltd., UK).

2.3. Alkaline hydrolysis

For the study of acyl flavonoid derivatives, an alkaline hydrolysis was carried out to eliminate acid moieties like p-coumaroyl (*m*/*z* 146) and caffeoyl (*m*/*z* 162), which coincide with those of rhamnosyl and hexosyl residues respectively and, therefore, a miss-assignation can occur in MS analysis. Saponification was performed as follows: 1 mL of the extract with 2 M NaOH (up to pH 9–10) for 12 h at room temperature in a stoppered test tube under N_2 atmosphere. The alkaline hydrolysis products were acidified with concentrated HCl (up to pH 1–2) and directly analyzed by LC/UV-PAD/ESI-MSn.

2.4. LC/UV-PAD/ESI-MSn analyses

Chromatographic analyses were carried out on a Luna C18 column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex, Macclesfield, UK). The mobile phase was a mixture of (A) Trifluoro acetic acid (TFA) 0.1% and (B) acetonitrile/TFA (99.9:0.1). The flow rate was 1 mL min^{-1} in a linear gradient starting with 0% B at 0–5 min, reaching 17% B at 15–17 min, 25% B at 22 min, 35% B at 30 min, 50% B at 35 min, 99% B at 50 min and at 55–65 min 0% B. The flow rate was 1 mL min^{-1}, and the injection volume 20 μL. Chromatograms were recorded at 330 nm for flavonoid glycosides and acylated derivatives and 227 nm for glucosinolates. The LC/UV-PAD/ESI-MSn analyses were carried out in an Agilent HPLC 1100 series equipped with a photodiode array detector and mass detector in series (Agilent Technologies, Waldbronn, Germany). The HPLC consisted of a binary pump (model G1312A), an autosampler (model G1313A), a degasser (model G1322A), and a photodiode array detector (model G1315B). The HPLC system was controlled by a ChemStation software (Agilent, v. 08.03). The mass detector was an ion trap spectrometer (model G2445A) equipped with an electrospray ionisation interface and was controlled by LCMSD software (Agilent, v. 4.1). The ionisation conditions were adjusted at 350 °C and 4 kV for capillary temperature and voltage, respectively. The nebulizer pressure and flow rate of nitrogen were 65.0 psi and 11 L min^{-1}, respectively. The full scan mass covered the range from *m*/*z* 100 up to *m*/*z* 1500. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 up to 2 V. Mass spectrometry data were acquired in the negative ionisation mode. MSn is carried out in the automatic mode on the more abundant fragment ion in MS(n^{-1}).

2.5. HPLC-PAD analyses

For quantitative analysis of glucosinolates and phenolic compounds, 20 μL of the sample was analyzed using the same column and conditions mentioned in the previous paragraph, in an HPLC system (Waters Cromatografía SA, Barcelona, Spain) consisting of a W600E multisolvent delivery system, an in-line degasser, a W717Plus autosampler and a W2996 photodiode array detector. Cromatograms were recorded at 227 nm for glucosinolates and 330 nm for phenolic compounds. Glucosinolates were quantified using sinigrin (sinigrin monohydrate from Phytoplan, Diehm & Neuberger GmbH, Heidelberg, Germany) as standard. Caffeoyl-quinic and *p*-coumaroyl-quinic acids derivatives were quantified as chlorogenic acid (5-caffeoyl-quinic acid, Sigma–Aldrich Chemie GmbH, Steinheim, Germany), flavonoids as quercetin 3-rutinoside (Merck, Darmstadt, Germany) and sinapic acid and derivatives as sinapic acid (Sigma). The calibration curves were made, at least with six data points, from 0.06 to 1 mM for sinigrin, from 0.01 to 2 mM for quercetin 3-rutinoside and 0.02 to 1 mM for chlorogenic acid and sinapic acid. The average regression equation for sinigrin, chlorogenic acid, quercetin 3-rutinoside and sinapic acid were $y = 1.07 \times 10^6\ x$ ($R^2 = 0.99$), $y = 3.74 \times 10^6\ x$ ($R^2 = 0.99$), $y = 1.44 \times 10^6\ x$ ($R^2 = 0.99$) and $y = 5.20 \times 10^6\ x$ ($R^2 = 0.99$) respectively.

2.6. Statistical analyses

Analyses of variance were made according to a randomized complete block design. Varieties were considered as random effect while plant organ was considered as fixed effect. Comparison of means among plant organ was performed for each trait using the Fisher's protected least significant difference (LSD) at $p = 0.05$ [22]. These analyses were made using the GLM procedure from SAS [23].

3. Results and discussion

The study of aqueous lyophilized extract of turnip greens and turnip tops allows the detection of intact glucosinolates and phenolic compounds by LC/UV-PAD/ESI-MSn and the quantification of the main compounds by HPLC-PAD.

3.1. Glucosinolates LC/UV-PAD/ESI-MSn analyses

The study of MS2[M−H]$^-$ fragmentation of *B. rapa* glucosinolates showed specific product ions at *m*/*z* 195, 241, 259 and 275 which correspond to the fragment ions from the aglycone side chain (Table 1), that have been found by other authors [24,25]. The fragmentation of the ion *m*/*z* 259 (MS3[(M−H)→259]$^-$) gave rise to the fragments at *m*/*z* 139, 97 (corresponding to the sulphate group [SO_3H]$^-$) and *m*/*z* 81. Identification of the intact glucosinolates was based on the detection of compounds with a constant neutral loss of 97 amu which with fragment ion *m*/*z* 259 are the most consistently ions produced by the disassociation of glucosinolates in the ion trap mass spectrometer. The structures of identified glucosinolates were confirmed according their retention times and molecular masses, MS/MS fragmentation as well as from the characteristic product ions. The mass spectral information of the intact glucosinolates identified is summarized in Table 1. Glucosinolates **A** and **B** had the same deprotonated molecular ion at *m*/*z* 388 and their MS2[M−H]$^-$ fragmentation produced a ion at *m*/*z* 332 (29%). These two glucosinolates could not be differentiated from each other by MS/MS data. Instead, this pair could be differentiated by their different retention times as progoitrin (**A**) and epiprogoitrin (**B**). Glucosinolate **C** with [M−H]$^-$ at *m*/*z* 436, fragmented mainly to *m*/*z* 372 (100%) due to the loss of the methyl sulphoxide moiety, was identified as glucoraphanin. The MS2[M−H]$^-$ fragmentation of compound **E** (*m*/*z* 372) showed the loss of *m*/*z* 196 that can be attributed to the elimination of a neutral thioglucose moiety give rise to an ion at *m*/*z* 176 described by [24] as characteristic ion of gluconapin. The compound **G** was found at 15 min, with a deprotonated molecular ion at *m*/*z* 374 which had a mass not known for any glucosinolate described, however the MS2[M−H]$^-$ presented typical product ions of the common moiety of glucosinolates, *m*/*z* 275, 259, 241 and 195 and the product ions of the variable side chain gave rise to an ion at *m*/*z* 294 due to the loss of sulphate group, whereas the *m*/*z* 132 fragment is a consequence of the combined loss of sulphur trioxide and anhydroglucose ([(M−H)−242]$^-$), the ion at *m*/*z* 178 attributed to the elimination of a neutral thioglucose moiety was different by two mass units from the *m*/*z* 176 diagnostic ion of gluconapin. This glucosinolate, described for first time in this work, was tentatively identified as a dihydrogluconapin. Compounds **F**, **J** and **L** were characterized by odd masses for the aglycone fragment, and they were thus indicative of a structure with nitrogen atoms of indolic glucosinolates [24]. The fragments at *m*/*z* 267 and 251 for compounds **F** and **J** respectively due to losses of thioglucose (196 amu) and the product ions of these fragments that implying the loss of nitrogen fragments allowed the identification as 4-hydroxyglucobrassicin (**F**) and glucobrassicin (**J**). Glucosinolate **L** gives rise to a *m*/*z* 446 as base peak due to the loss of methoxy group implying a radical anion corresponding to the glucobrassicin radical anion, this compound was identified as neoglucobrassicin. With respect to **I**, with [M−H]$^-$ at *m*/*z* 408 was identified as glucotropaeolin and the MS2[M−H]$^-$ show a ion at *m*/*z* 212 due to the elimination of thioglucose and the other at *m*/*z* 166 resulting from the combined loss of sulphur trioxide and anhydroglucose ([(M−H)−242]$^-$). Peaks **D** with [M−H]$^-$ at *m*/*z* 402, **H** at *m*/*z* 386 and **K** at *m*/*z* 422 were identified by comparison of retention time and [M−H]$^-$ as gluconapoleiferin (**D**), glucobrassicanapin (**H**) and gluconasturtiin (**K**).

Twelve glucosinolates, belonging to the three chemical classes were detected (Fig. 1). The GS profile was similar in both organs (turnip greens and turnip tops) and it was similar to those reported by other authors in *B. rapa* leaves [15,26], except for the presence of

Table 1
List of identified glucosinolates with the corresponding retention times and MS data.

Compounds	Rt (min)	[M−H]$^-$	MS2[M−H]$^-$, *m*/*z* (%)
(**A**) Progoitrin/(**B**) Epiprogoitrin	5.3/6.3	388	332(29), 308(12), 301(17), 275(40), 259(100), 240(18) 210(78), 195(14), 192(24), 154(16),136(42), 130(30)
(**C**) Glucoraphanin	6.9	436	420(6), 372(100), 356(1), 291(2), 275(1), 259(3), 194(2), 162(4)
(**D**) Gluconapoleiferin	11.6	402	306(53), 275(20), 259(100), 240(7), 225(10), 215(24), 195(10), 163(13), 160(9), 145(18), 140(23), 120(9)
(**E**) Gluconapin	12.0	372	292(4), 275(30), 259(100), 241(37), 227 (9), 195(25), 176(8), 139 (11),130(10)
(**F**) 4-Hydroxyglucobrassicin	14	463	403(2), 383(10), 365(6), 300(6), 285(73), 267(100), 259(17), 240(25), 220(17), 169(30), 160(23), 132(5)
(**G**) Dihydrogluconapin	15	374	294(3), 275(19), 259(100), 241(37), 227(1), 212(4), 195(3), 178(2), 163(1), 145(3), 139(3), 132(6)
(**H**) Glucobrassicanapin	15.1	386	306(4), 275(21), 259(100), 241(33), 208(12), 195(6), 163(4), 144(18) 139(8)
(**I**) Glucotropaeolin	15.5	408	348(9), 312(16), 286(12) 275(10), 259(100), 212(18), 166(8)
(**J**) Glucobrassicin	17.1	447	367(22), 291(6), 275(34), 259(100), 251(17), 241(11), 224(3), 205(28), 195(11),144(9)
(**K**) Gluconasturtiin	18.7	422	342(9), 275(24), 259(100), 244(7), 229(8), 195(13), 180(24), 169(1), 163(5), 145(4), 140(6), 119(4)
(**L**) Neoglucobrassicin	22.7	477	447(68), 446(100), 429(16), 385(5), 273(12), 259(16), 241(26), 224(4), 205(5)

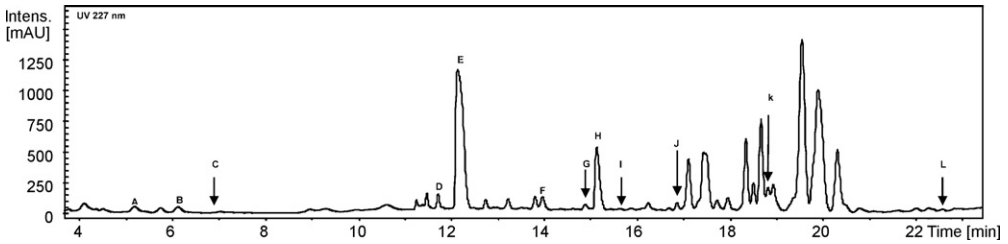

Fig. 1. HPLC-PAD chromatogram of glucosinolate profile of turnip tops. Variety: MBG-BRS0163. Location: Guitiriz (Lugo). Detection at 227 nm. Peaks: (A) progoitrin, (B) epiprogoitrin, (C) glucoraphanin, (D) gluconapoleiferin, (E) gluconapin, (F) 4-hydroxyglucobrassicin, (G) dihydrogluconapin, (H) glucobrassicanapin, (I) glucotropaeolin, (J) glucobrassicin, (K) gluconasturtiin and (L) neoglucobrassicin.

glucotropaeolin, glucosinolate not identified before in *B. rapa* and generally used as internal standard.

3.2. Phenolics LC/UV-PAD/ESI-MSn analyses

The study of *B. rapa* phenolics showed glycosylated flavonoids and most of them acylated with hydroxycinnamic acids. The most frequent ions which characterise the fragmentation of these flavonoid *O*-glycosides are shown in Tables 2 and 3. Other ions were found but they have not been included due to their low significance on the MS analysis. Fig. 2 shows a characteristic phenolic compound chromatogram from *B. rapa*.

3.2.1. Deacylated flavonoids

The study of saponificated extracts from turnip greens and turnip tops showed a characteristic UV spectra of flavonols with a sugar substitutions (*O*-glycoside) at the 3 position (Band I, ≈350 nm; See Table 2; compound **1**–**9**) and flavonols with a free hydroxyl in the 3 position (**10**–**12**) (Band I, ≈365–370 nm; see Table 2), with monohydroxy substitution in the B-ring (**2**, **3**, **5**, **8**, **9**, and **11**) (Band II, ≈266 nm) or dihydroxy substituent in the B-ring (**1**, **4**, **6**, **7**, **10** and **12**) (Band II, ≈255, 267 sh nm; see Table 2) [27]. The presence of the ion at *m*/*z* 285/284 $[Agl-H/2H]^-$ as base peak for compounds **2**, **3**, **5**, **8**, **9** and **11** show them as kaempherol derivatives (3,5,7,4′-tetrahydroxyflavone), while on compounds **1**, **4**, **7**, and **10** this ion was *m*/*z* 301/300 indicative of quercetin derivatives (3,3′, 4′, 5,7-pentahydroxyflavone) and *m*/*z* 315 for **6** and **12** isorhamnetin derivatives (3,4′,5,7-tetrahydroxy-3′-methoxyflavone) (Table 2). The MS2$[M-H]^-$ fragmentation of compounds **1**–**6** showed ions $[M-H-162]^-$ as base peak, indicated a loss of glycosidic residue at 7 position [28]. The fragmentation MS3$[(M-H)\rightarrow(M-H-162)]^-$ of **1**–**3** showed losses to come from interglycosidic fragmentations at position 3 of the ring which agree with [28], indicate $(1\rightarrow 2)$ interglycosidic linkage. Compounds **4**–**6** are flavonoid with two sugar moieties linked to different phenolic hydroxyl (di-*O*-glycosidics). The appearance of $[Agl-H]^-$ as base peak in the MS2$[M-H]^-$ from the compounds **7**–**9**, together with the observed fragmentations, indicating them as flavonol-*O*-diglycosidics. The UV spectra and MS fragmentation for compounds **7** and **8** show that they are Kaempferol-3-*O*-dihexosides isomers. The $[M-H-180]^-$ ion was not observed in the fragmentation of **8**; while this ion is very important for the compound **9**, indicating a interglycosidic linkage $(1\rightarrow 2)$ for this compound.

Using as reference previous works on *Brassica* spp. [8,9,16,17,29] these compounds have been tentatively characterized as: (**1**) quercetin-3-*O*-sophoroside-7-*O*-glucoside; (**2**) kaempferol-3-*O*-triglucoside-7-*O*-glucoside; (**3**) kaempferol-3-*O*-sophoroside-7-*O*-glucoside; (**4**) quercetin-3,7-di-*O*-glucoside; (**5**) kaempferol-3,7-di-*O*-glucoside; (**6**) isorhamnetin-3,7-di-*O*-glucoside; (**7**) quercetin-3-*O*-sophoroside; (**8**) kaempferol-3-*O*-diglucoside; (**9**) kaempferol-3-*O*-sophoroside; (**10**) quercetin-7-*O*-glucoside; (**11**) kaempferol-7-*O*-glucoside; (**12**) isorhamnetin-7-*O*-glucoside.

In native extracts we observed acyl compounds previously described and deacylated compounds **1** and **3**–**9**. The compound **2** is found in trace quantities. Compounds **10**–**12** (flavonol-7-*O*-hexosides) are handling artifacts produced during the process of saponification.

3.2.2. Acylated flavonols

Compounds **13**–**26** showed UV spectra that resemble the superposition of UV flavonoid with the hydroxycinnamic acid predominating the last one with a maximum absorbance about 330 nm and a small inflexion peak at 269 nm, and other signal around 250 nm could be observed in the derivatives with two substituents in the B-ring (Table 3). The MS2$[M-H]^-$ fragmentation of compounds **13**–**23** showed a base peak resulting from the *m*/*z* 162 loss $[M-H-162]^-$ and other ions resulting from the loss of the corresponding acyl radical. This fragmentation is typical from flavonid-3-*O*-(acyl)glycoside-7-*O*-hexoside and has been widely described in different *Brassicas* [8,9,16,17,29]. The event MS3$[(M-H)\rightarrow(M-H-162)]^-$ showed as base peak the resultant ion from the radical acyl loss to give the aglycone attached to the glycosidic fraction in position 3, the ion for the deprotonated aglycone could also be observed (Table 3). These compounds had been characterized as acylated derivatives of quercetin-3-*O*-sophoroside-7-*O*-glucoside with methoxycaffeoyl (**13**), caffeoyl (**14**), sinapoyl (**17**), feruloyl (**18**), *p*-coumaroyl (**19**), and kaempferol-3-*O*-sophoroside-7-*O*-glucoside with methoxycaffeoyl (**15**), caffeoyl (**16** and **23**), sinapoyl (**20**), feruloyl (**21**) and *p*-coumaroyl (**22**).

Compounds **24**–**27** presented a fragmentation MS2$[M-H]^-$ similar to the MS3 of previous compounds (Table 3) that is expected of flavonoids with glycosilation on a single phenolic hydroxyl. In addition, an ion resulting from loss of the acyl radical and fragment *m*/*z* 180 (162 + 18) $[(M-H)\text{-acyl}-180]^-$ that comes from the interglycosidic breakdown is observed in their fragmentations, confirming the structure of flavonoid-*O*-diglycosides and non flavonoid-di-*O*-diglycosides. These compounds have been characterized as acyl derivatives of kaempferol-3-*O*-sophoroside with methoxycaffeoyl (**24**), caffeoyl (**25**), sinapoyl (**27**) and (**26**) quercetin-3-*O*-(feruloyl) sophoroside.

The flavonoid composition of the turnip greens and turnip tops samples studied is qualitatively similar to that observed in *B. rapa rapa* group leaves [29] and it differs only by the presence or absence of a flavonoid in trace amounts. Most of the acyled flavonols were quercetin and kaempferol derivatives but in contrast to *Brassica oleracea* vegetables one of the main aglycone flavonol found in the saponificated extract was isorhamnetin. Isorhamnetin-3,7-di-*O*-glucoside and their derivatives were also found in others works in *B. rapa* [18,29].

3.2.3. Hydroxycinnamic acids

The following derivatives of hydroxycinnamic acids were detected, 3-caffeoyl quinic acid (**3CQA**) (Rt 17.3 min; UV 295sh, 325 nm; MS: 353, MS2(353): 191 (100%), 179 (62%), 3-*p*-coumaroyl

Table 2
Rt, UV and -MS: $[M-H]^-$, -MS2$[M-H]^-$ and -MS3$[(M-H)\rightarrow (M-H-162)]^-$ data of glycosilated flavonoid not acylates from turnip greens and turnip tops Flavonoid-3-*O*-diglucoside/triglucoside-7-*O*-glucoside, -3,7-di-*O*-glucoside, -3-*O*-diglucoside and -7-*O*-glucoside.

Compounds[a]	Rt (min)	UV (nm)	$[M-H]^-$ (*m*/*z*)	-MS2$[M-H]^-$ (*m*/*z*) (%)	-MS3$[(M-H)\rightarrow (M-H-162)]^-$ (*m*/*z*) (%)		
		Flavonoid-3-*O*-trihexoside-7-*O*-hexoside					
				(−162)	(−162)	(−342)	$[Agl-H/2H]^-$ (−486)
2 Kaempf-3-*O*-triglc-7-*O*-glc	17.0	266, 316sh, 348	933	771(100)	609(45)	429(26)	285(100)
		Flavonoid-3-*O*-dihexoside-7-*O*-hexoside					
				(−162)	(−162)	(−180)	(−324)
1 Querc-3-*O*-soph-7-*O*-glc	16.9	255, 267sh, 351	787	625(100)	463(23)		301(100)
3 Kaempf-3-*O*-soph-7-*O*-glc	17.4	266, 317sh, 347	771	609(100)	447(44)	429(29)	284(50)[b]
		Flavonoid-3,7-di-*O*-hexoside					
				-MS2$[M-H]^-$ (*m*/*z*) (%)			
					$[Agl-H]^-$		
				(−162)	(−324)		
4 Querc-3,7-di-*O*-glc	18.6	255, 267sh, 353	625	463(100)	301(28)		
5 Kaempf-3,7-di-*O*-glc	19.6	265, 319sh, 347	609	447(100)	285(40)		
6 Isorhmnt-3,7-di-*O*-glc	19.9	255, 267sh, 352	639	477(100)	315(28)		
		Flavonoid-3-*O*-dihexoside					
				(−162) (−180)	(−324)		
7 Querc-3-*O*-soph	22.8	–	625	463(15) 445(8)	300(100)[b]		
8 Kaempf-3-*O*-diglc	23.0	266, 297sh, 347	609	447(47)	285(100)		
9 Kaempf-3-*O*-soph	24.5	266, 295sh, 347	609	447(8) 429(65)	285(100)		
		Flavonoid-7-*O*-hexoside					
					(−162)		
10 Querc-7-*O*-Glc	26.0	255, 267sh, 370	463		301(100)		
11 Kaempf-7-*O*-Glc	28.1	266, 320sh, 365	447		285(100)		
12 Isorhmnt-7-*O*-Glc	28.4	255, 265sh, 369	477		315(100)		

Main observed fragments. Other ions were found but they have not been included.
[a] Kaempf: Kaempferol; Querc: Quercetin; Isorhmnt: Isorhamnetin; Soph: Sophoroside; Sophtr: Sophorotrioside; Glc: Glucoside.
[b] Fragments from homolytic cleavage of the glycosidic bond ($[Aglycon-2H]^{\bullet -}$) [40].

quinic acid (**3*p*CoQA**) (Rt 19.1 min; UV 311 nm; MS: 337, MS2(337): 191 (7%), 179 (100%)) [30], sinapoylglucoside (**SG**) (Rt 20.5 min; UV 329 nm; MS: 285, MS2(285): 291 (100%), 223 (85%)), and sinapic acid (**SA**) (Rt 27.3 min; UV 329 nm; MS: 223, MS2(223): 208 (35%), 179(30%), 164(100%)). We also found other identified hydroxycinnamic acid derivatives corresponding to sinapic and ferulic acids esterified carrying more than one hexose moiety (compounds **A1**–**A4**). The loss of 224 amu from the deprotonated molecular ion, corresponding to sinapic acid could be observed in all cases. Compound **A2** and **A4** also presented ferulic acid and displayed the loss of this acid (194 amu). By comparison with data reported earlier in other *Brassicas* [8,17] these compounds were tentatively identified as: 1,2-disinapoylgentiobioside (**A1**) (Rt 28.1 min; UV 330 nm; $[M-H]^-$: 753, MS2(753): 529 (100%), 223 (1%), MS3 (529): 223(100%), 205(59%)); 1-sinapoyl-2-feruloylgentiobioside (**A2**) (Rt 28.7 min; UV 330 nm; $[M-H]^-$: 723, MS2(723): 529 (11%), 499 (100%), 259(4%), MS3(499): 259(28%), 246(20%), 217(53%), 193(100%), 175(58%)); 1, 2, 2′-trisinapoylgentiobioside (**A3**) (Rt 30.5 min; UV 330 nm; $[M-H]^-$: 959, MS2(959): 735 (100%), MS3(735): 717(15%), 529(100%)); 1,2′-disinapoyl-2-feruloylgentiobioside (**A4**) (Rt 31.2 min; UV 330 nm; $[M-H]^-$: 929, MS^2 (929): 735 (4%), 705 (100%), MS^3(735): 499

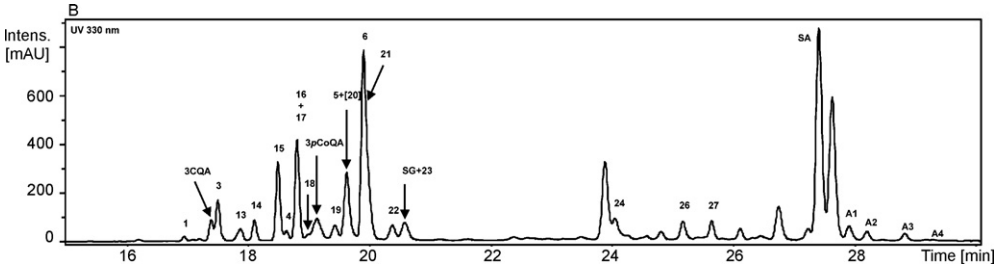

Fig. 2. HPLC-PAD chromatogram of phenolic profile of turnip greens. Variety: MBG-BRS0401. Location: Oroso (A Coruña). Detection at 330 nm. Peaks: (1) quercetin-3-*O*-sophoroside-7-*O*-glucoside; (2) kaempferol-3-*O*-triglucoside-7-*O*-glucoside; (3) kaempferol-3-*O*-sophoroside-7-*O*-glucoside; (4) quercetin-3,7-di-*O*-glucoside; (5) kaempferol-3,7-di-*O*-glucoside; (6) isorhamnetin-3,7-di-*O*-glucoside; (7) quercetin-3-*O*-sophoroside; (8) kaempferol-3-*O*-diglucoside; (9) kaempferol-3-*O*-sophoroside; (10) quercetin-7-*O*-glucoside; (11) kaempferol-7-*O*-glucoside; (12) isorhamnetin-7-*O*-glucoside; (13) quercetin-3-*O*-(methoxycaffeoyl)sophoroside-7-*O*-glucoside; (14) quercetin-3-*O*-(caffeoyl)sophoroside-7-*O*-glucoside; (15) kaempferol-3-*O*-(methoxycaffeoyl) sophoroside-7-*O*-glucoside; (16) kaempferol-3-*O*-(caffeoyl)sophoroside-7-*O*-glucoside; (17) quercetin-3-*O*-(sinapoyl)sophoroside-7-*O*-glucoside; (18) quercetin-3-*O*-(feruloyl)sophoroside-7-*O*-glucoside; (19) quercetin-3-*O*-(*p*-coumaroyl)sophoroside-7-*O*-glucoside; (20) kaempferol-3-*O*-(sinapoyl)sophoroside-7-*O*-glucoside; (21) kaempferol-3-*O*-(feruloyl)sophoroside-7-*O*-glucoside; (22) kaempferol-3-*O*-(*p*-coumaroyl)sophoroside-7-*O*-glucoside; (23) kaempferol-3-*O*-(caffeoyl)sophoroside-7-*O*-glucoside (isomer); (24) kaempferol-3-*O*-(methoxycaffeoyl)sophoroside; (25) kaempferol-3-*O*-(caffeoyl)sophoroside; (26) quercetin-3-*O*-(feruloyl)sophoroside; (27) kaempferol-3-*O*-(sinapoyl)sophoroside. (3CQA) 3-caffeoyl quinic acid; (3*p*CoQA) 3-*p*-coumaroylquinin acid; (SG) sinapoylglucoside; (CA) caffeic acid; (SA) sinapic acid; (**A1**) 1,2-disinapoylgentiobioside; (**A2**) 1-sinapoyl-2-feruloylgentiobioside; (**A3**) 1, 2, 2′-trisinapoylgentiobioside; (**A4**) 1,2′-disinapoyl-2-feruloylgentiobioside.

Table 3
Rt, UV and -MS: $[M-H]^-$, -MS2$[M-H]^-$ and -MS3$[(M-H)\rightarrow(M-H-162)]^-$ data of acylated derivatives of glycosilated flavonoid from native hidroalcoholic extract of turnip greens and turnip tops.

Compounds[a]	Rt (min)	UV (nm)	$[M-H]^-$ (m/z)	-MS2$[M-H]^-$ (m/z) (%)										-MS3$[(M-H)\rightarrow(M-H-162)]^-$ (m/z) (%)					
				−146 -*p*.Coum	−162 -Glc	−176 -Fer	−192 -MeOCaf	−206 -Sinp	−308 -G-*p*C	−324 -G-C	−338 -G-F	−354 -G-MC	−368 -G-S	−146 -*p*.Coum	−162 -Caf	−176 -Fer	−192 -MeOCaf	−206 -Sinp	[Agl−H/2H]$^-$
Acylated derivatives from 1: Quercetin-3-*O*-sophoroside-7-*O*-glucoside																			
13 1-MeOCaf	17.9	251, 269sh, 337	979		817(100)		787(70)					625(70)					625(100)		300(10)[b]
14 1-Caf	18.1	253, 269sh, 299sh, 337	949		787(100)					625(50)					625(100)				
17 1-Sinp	18.8	Coeluting with **16**	993		831(100)			787(60)					625(66)					625(100)	
18 1-Fer	19.0	251, 269sh, 337	963		801(100)	787(27)					625(30)					625(100)			300(15)[b]
19 1-*p*.Coum	19.4	250, 269, 297sh, 326	933	787(7)	771(100)				625(46)					625(100)					
Acylated derivatives from 3: Kaempferol-3-*O*-sophoroside-7-*O*-glucoside																			
15 3-MeOCaf	18.4	269, 331	963		801(100)							609(7)					609(100)		285(12)
16 3-Caf	18.8	Coeluting with **17**	933		771(100)					609(7)					609(100)				285(9)
20 3-Sinp	19.6	Coeluting with **5**	977		815(100)								609(2)					609(100)	284(11)[b]
21 3-Fer	20.0	Coeluting with **6**	947		785(100)						609(6)					609(65)[c]			
22 3-*p*.Coum	20.3	269, 317	917		755(100)				609(5)					609(100)					284(8)[b]
23 3-Caf (isomer16)	20.6	Coeluting with Sinp-Glc	933		771(100)						609(9)				609(100)				

				Acylated derivatives from: Flavonol-3-*O*-Sophorosides -MS2$[M-H]^-$ (m/z) (%)						
				−162 -Caf	−176 -Fer	−192 -MeOCaf	−206 -Sinp	-Acyl -180	[Agl−H]$^-$	
24 9-MeOCaf	24.0	Coeluting with other Acs	801			609(100)		429(5)	285(10)	
25 9-Caf	24.7	269, 330	771	609(100)				429(3)	285(8)	
27 9-Sinp	25.6	269, 330	815				609(100)	429(3)	285(7)	
26 7-Fer	25.1	269, 330	801		625(100)			445(2)	301(4)	

Main observed fragments. Other ions were found but they have not been included.

[a] G (Glc): Glucosyl. *p*C (*p*.Coum): *p*-Coumaroyl. F (Fer): Feruloyl. S (Sinp): Sinapoyl. MC (Me Caf): MethoxyCaffeoyl. **1**: Quercetin-3-*O*-Sophoroside-7-*O*-Glucoside. **3**: Kaempferol-3-*O*-Sophoroside-7-*O*-Glucoside. **11**: Kaempferol-3-*O*-Sophoroside.

[b] Fragments from homolytic cleavage of the glycosidic bond ($[Aglycon-2H]^{\bullet-}$) [40].

[c] Compound **21** (feruloyl derivative) showed an abundant ion at m/z 623 (base peak) resultant from the loss of 162u (176-14).

Table 4
Mean squares of the combined analysis of variance for the glucosinolates, flavonoids and hydroxycinnamic acids content in the *Brassica rapa* varieties from northwestern Spain evaluated.

Traits	Location (L)	Variety (V)	Plant organ (P)	L × V	L × P	V × P	L × V × P	Error
Glucosinolates								
PRO	3.05	2.69	6.56*	2.00	0.05	1.28	1.05	4.64
GNA	35.83	322.02*	114.15	50.53	5.70	41.79	15.91	62.37
4-OHGBS	0.20	0.13	3.92**	0.02	0.09	0.11*	0.03	0.09
GBN	1.25	5.37	37.89*	1.44	1.43	4.63	2.16	4.54
GBS	18.38	6.50	5.98	5.16	0.33	4.62	5.69	8.59
NGBS	3.56	1.87	2.61	0.57	0.22	0.68	0.23	5.41
Total glucosinolates	212.73	312.69	299.93	69.41	0.00	69.14	29.93	259.95
Flavonoids								
1	0.02	0.03	0.40**	0.03*	0.00	0.02	0.01	0.13
3	0.53	1.44	8.84*	0.60	4.95*	1.24	0.50	0.62
15	1.87	1.22	37.34**	0.99	0.40	0.95	0.67	1.04
16	0.42	2.09	79.98**	0.63	1.22	0.78	0.40	3.01
17	0.11	2.20	2.77	1.04	3.06	0.60	0.86	33.24
5 + 20	17.99	11.23	137.16**	0.99	27.66*	9.37	3.33	4.10
6 + 21	49.40	18.65	22.19	4.00	0.20	24.40	16.32	8.98
26	0.09	0.05	0.18	0.07	0.01	0.06	0.05	0.06
Total flavonoids	131.68	109.27	39.70	7.22	61.74	78.92	31.01	−26.71
Hydroxycinnamics								
3-CQA	2.41*	0.54*	0.00	0.15*	0.00	0.05	0.04	0.20
3*p*-CoQA	0.45	5.30	17.56*	0.99	1.58	2.39	1.63	2.18
SA	0.03	7.50	2735.09**	1.51	28.99**	10.81	2.61	16.79
A1	0.01	0.48	0.69*	0.18*	0.39*	0.09	0.04	0.56
A2	0.32	2.75	11.00**	0.66	2.82**	0.69	0.25	2.04
A3	0.00	0.04	0.09	0.02	0.22*	0.06	0.02	0.06
Total hydroxycinnamics	0.22	27.42	3524.02**	2.99	59.11**	9.15	4.41	4.04
Total phenolics	142.46	170.03	4311.75**	13.27	0.03	109.74	40.32	−27.71

PRO: progoitrin; GNA: gluconapin; 4-OHGBS: 4-hydroxyglucobrassicin; GBN: glucobrassicanapin; GBS: glucobrassicin; NGBS: neoglucobrassicin; 1: quercetin-3-*O*-sophoroside-7-*O*-glucoside; 3: kaempferol-3-*O*-sophoroside-7-*O*-glucoside; 5: kaempferol-3,7-di-*O*-glucoside; 6: isorhamnetin-3,7-di-*O*-glucoside;15: kaempferol-3-*O*-(methoxycaffeoyl)sophoroside-7-*O*-glucoside; 16: kaempferol-3-*O*-(caffeoyl)sophoroside-7-*O*-glucoside; 17: quercetin-3-*O*-(sinapoyl)sophoroside-7-*O*-glucoside; 20: kaempferol-3-*O*-(sinapoyl)sophoroside-7-*O*-glucoside; 21: kaempferol-3-*O*-(feruloyl)sophoroside-7-*O*-glucoside; 26: quercetin-3-*O*-(feruloyl)sophoroside; 3CQA: 3-caffeoyl quinic acid; 3*p*CoQA: 3-*p*-coumaroylquinin acid; SA: sinapic acid; **A1**: 1,2-disinapoylgentiobioside; **A2**: 1-sinapoyl-2-feruloylgentiobioside; **A3**: 1, 2, 2′-trisinapoylgentiobioside.
* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.

(100%)). These results are in accordance with compounds detected in other *Brassica* species, like turnip tops [18], tronchuda cabbage [17], and broccoli [9].

3.3. HPLC-DAD quantitative analyses of glucosinolates and phenolic compounds

3.3.1. Glucosinolates quantification

Six major glucosinolates (progoitrin, gluconapin, 4-hydroxyglucobrassicin, glucobrassicanapin, glucobrassicin and neoglucobrassicin) were quantitatively determined in *B. rapa*. A combined analysis of variance was made to evaluate the interactions between locations, varieties, and plant organs (Table 4). No glucosinolate showed significant interactions among location, variety, and plant organ, except for 4-hydroxyglucobrassicin, a glucosinolate present in small quantities in *B. rapa* varieties. There were significant differences among plant organs for progoitrin, 4-hydroxyglucobrassicin and glucobrassicanapin, but not for gluconapin, the main glucosinolate in these crops. With regard to glucosinolates, there were no differences for total glucosinolate content nor for gluconapin, which means that in this experiment the nutritional value of both products is similar. Turnip tops are the flower buds with the surrounding leaves which can explain the similarity with the vegetative leaves. For gluconapin, there were significant differences among varieties (Table 4). This means that there is enough genetic variability in the local varieties representing the germplasm collection of NW Spain to select for high or low gluconapin content varieties.

Aliphatic glucosinolates were predominant, representing almost 93% of the total analyte of glucosinolates, as found in previous studies with a similar glucosinolate pattern in leaves [15,26,31] and in flower buds [31,32].

Total glucosinolate content ranged from 19.40 to 36.79 μmol g^{-1} dw for turnip greens and from 18.43 to 41.67 μmol g^{-1} dw for turnip tops, with an average value of 26.84 μmol g^{-1} dw for turnip greens and 29.11 μmol g^{-1} dw for turnip tops. These contents are consistent with those previously found by Padilla et al. [15], but lower than the data reported in Kim et al. [31].

The predominant glucosinolate, gluconapin, represented 84% of the total glucosinolate content. The mean value of this glucosinolate was 23.31 μmol g^{-1} dw with a minimum of 17.78 μmol g^{-1} dw and a maximum of 36.12 μmol g^{-1} dw (Table 5). For this glucosinolate there were significant differences among varieties. Therefore, this would be useful for future breeding programs focused on varieties of high or low glucosinolate contents.

With respect to glucobrassicin and glucobrassicanapin, each one represents 5% of the total glucosinolate content. Glucobrassicanapin was significatively higher in turnip tops (2.05 μmol g^{-1} dw) than in turnip greens (0.87 μmol g^{-1} dw). The mean value of glucobrassicin was 1.46 μmol g^{-1} dw (Table 5). This glucosinolate is the parent compound of indole-3-carbinol, which along with the sulphoraphane has been proved as the most potent anticancer compounds found in cruciferous vegetables (Zhang and Talalay [33]).

The rest of the glucosinolates were found in lower amounts. Progoitrin described as a potentially goitrogenic glucosinolate, was

Table 5
Mean (μmol g^{-1} dw) for the glucosinolates, flavonoids and hydroxycinnamic acids content in the *Brassica rapa* varieties from northwestern Spain evaluated.

Traits	Turnip greens	Turnip tops	LSD (5%)
Glucosinolates			
PRO	0.35	0.91	0.30
GNA	22.16	24.33	1.93
4-OHGBS	0.00	0.40	0.11
GBN	0.87	2.05	0.51
GBS	1.71	1.17	0.81
NGBS	0.52	0.22	0.29
Total glucosinolates	26.84	29.11	2.58
Flavonoids			
1	0.40	0.25	0.06
3	2.05	1.47	0.26
15	2.60	1.47	0.28
16	3.99	2.24	0.38
17	2.63	2.95	0.59
5 + 20	5.01	7.36	0.87
6 + 21	11.09	10.15	1.67
26	0.30	0.22	0.10
Total flavonoids	29.70	28.44	2.54
Hydroxycinnamics			
3-CQA	0.75	0.75	0.1
3*p*-CoQA	3.41	2.55	0.44
SA	12.46	2.14	0.73
A1	1.43	1.57	0.16
A2	3.19	2.47	0.49
A3	0.39	0.44	0.1
Total hydroxycinnamics	22.08	10.20	0.89
Total phenolics	51.71	38.99	3.05

PRO: progoitrin; GNA: gluconapin; 4-OHGBS: 4-hydroxyglucobrassicin; GBN: glucobrassicanapin; GBS: glucobrassicin; NGBS: neoglucobrassicin; 1: quercetin-3-*O*-sophoroside-7-*O*-glucoside; 3: kaempferol-3-*O*-sophoroside-7-*O*-glucoside; 5: kaempferol-3,7-di-*O*-glucoside; 6: isorhamnetin-3,7-di-*O*-glucoside;15: kaempferol-3-*O*-(methoxycaffeoyl)sophoroside-7-*O*-glucoside; 16: kaempferol-3-*O*-(caffeoyl)sophoroside-7-*O*-glucoside; 17: quercetin-3-*O*-(sinapoyl)-sophoroside-7-*O*-glucoside; 20: kaempferol-3-*O*-(sinapoyl)sophoroside-7-*O*-glucoside; 21: kaempferol-3-*O*-(feruloyl)sophoroside-7-*O*-glucoside; 26: quercetin-3-*O*-(feruloyl)sophoroside; 3CQA: 3-caffeoyl quinic acid; 3*p*CoQA: 3-*p*-coumaroylquinin acid; SA: sinapic acid; **A1**: 1,2-disinapoylgentiobioside; **A2**: 1-sinapoyl-2-feruloylgentiobioside; **A3**: 1, 2, 2′-trisinapoylgentiobioside.

significantly lower in turnip greens (0.35 μmol g^{-1} dw) than in turnip tops (0.91 μmol g^{-1} dw) and 4-hydroxyglucobrassicin was only detected in turnip tops (0.40 μmol g^{-1} dw) (Table 5).

3.3.2. Phenolic compounds quantification

The HPLC-PAD analysis allowed the quantification of 16 phenolic compounds indentified in turnip greens and turnip tops of *B. rapa*. They are 10 flavonols: compounds **1**, **3**, **5** coeluting with **20**, **6** coeluting with **21**, **15**, **16**, **17**, **26**, and six hydroxycinnamic acids and derivatives: **3CQA**, **3*p*CoQA**, **SA**, **A1**, **A2**, **A3**.

For flavonoids, the combined analysis of variance did not show significant interactions—plant organ × location, and plant organ × variety, except for compounds kaempferol-3,7-di-*O*-glucoside coeluting with kaempferol-3-*O*-(sinapoyl)sophoroside-7-*O*-glucoside (Table 4). For this reason, this compound was analyzed separately and in that analysis the effect of location was not significant. The analysis of the rest of flavonoids was made combining locations and plant organs. Significant differences were detected between plant organs for most of them, but not among varieties or locations. This means that flavonoids are more stable among environments and there is not enough variability among varieties to select in our germplasm collection for a particular compound.

Most of the sinapic acid derivatives found in turnip greens and tops had a significant location × plant organ or variety × plant organ interaction. Then, these traits for each plant organ, were analyzed separately. For turnip greens, there were significant differences among varieties for sinapic acid, but not for the rest of sinapic acid derivatives. The effect of location was not significant for any trait. For turnip tops, there were significant differences between locations for sinapic acid, while two of four of the sinapic acid derivatives (1,2-disinapoylgentiobioside and 1-sinapoyl-2-feruloylgentiobioside) showed significant differences among varieties.

Quantification of phenolic compounds in the *B. rapa* samples analyzed revealed higher amount of these compounds in turnip greens 51.71 μmol g^{-1} dw (ranging from 52.12 to 49.92 μmol g^{-1} dw) than in turnip tops 38.99 μmol g^{-1} dw (ranging from 33.25 to 49.33 μmol g^{-1} dw), in which flavonols were always the major compounds (Table 5). The values of total phenolic content of turnip greens and turnip tops are quite high, even higher than those for all other *Brassicaceae* [34,35] and similar to those found in turnip tops by Fernandes et al. [36] and Sousa et al. [19].

Turnip greens and turnip tops exhibited a similar phenolic profile, in which the peaks containing kaempferol-3,7-di-*O*-glucoside coeluting with kaempferol-3-*O*-(sinapoyl)sophoroside-7-*O*-glucoside and isorhamnetin-3,7-di-*O*-glucoside coeluting with kaempferol-3-*O*-(feruloyl)sophoroside-7-*O*-glucoside were the flavonoid compounds present in higher contents, each peak corresponding to 9–26% of total phenolics. The pair isorhamnetin-3,7-di-*O*-glucoside with kaempferol-3-*O*-(feruloyl)sophoroside-7-*O*-glucoside had an average concentration of 10.63 μmol g^{-1} dw (Table 5). Isorhamnetin diglucoside, isolated from mustard leaf (*B. juncea*), showed a strong activity in reducing serum levels of glucose in Diabetes Mellitus through an antioxidant activity test [37].

In this work, the acyl flavonoids are derived mainly from quercetin-3-*O*-sophoroside-7-*O*-glucoside and kaempferol-3-*O*-sophoroside-7-*O*-glucoside. With respect to two of the major acylated flavonoids derived from kaempferol, turnip tops showed significantly lower amounts of the pair kaempferol-3-*O*-(metoxycaffeoyl)sophoroside-7-*O*-glucoside (1.47 μmol g^{-1} dw) and kaempferol-3-*O*-(caffeoyl)sophoroside-7-*O*-glucoside (2.24 μmol g^{-1} dw) than turnip greens with 2.60 μmol g^{-1} dw and 3.99 μmol g^{-1} dw (Table 5), respectively. These compounds are reported to have high radical scavenging capacity due to the presence of an *O*-dihydroxy structure in the caffeoyl moiety, which confers great stability to the radical form and participates in the electron delocalisation [38].

In these *B. rapa* varieties the contribution of phenolic acids is very high, representing 36% of the total polyphenols, being higher than 26% found by Sousa et al. [19] in *B. rapa* var. *rapa* and clearly distinct from the 2% from *B. oleraceae var. costata* and *B. oleraceae var. acephala* described by the authors mentioned above. The concentration of hydroxycinnamics acids in *B. rapa* showed significant differences among plant stage, in this case in turnip greens the amount of total hydroxycinnamics (22.08 μmol g^{-1} dw) corresponding to twice the amount exhibited by turnip tops (10.2 μmol g^{-1} dw). These differences are mainly due to the high amount of sinapic acid, whose concentration in turnip greens was very much higher than in turnip tops, 12.46 μmol g^{-1} dw and 2.14 μmol g^{-1} dw respectively. In addition, the content of sinapic acid was the major hydroxycinnamic acid and the main phenolic compound in turnip greens. Differences were apparent among environments, the samples from the Guitiriz location (1.78 μmol g^{-1} dw) had lower concentration of sinapic acid than at Oroso (2.60 μmol g^{-1} dw). Other hydroxycinnamic acid, 3-caffeoyl quinic acid was significatively higher at Oroso (0.92 μmol g^{-1} dw) than at Guitiriz (0.59 μmol g^{-1} dw). This variation can result from variations in environmental and agronomic factors such as water availability, soil composition as well as fertilization [39].

4. Conclusions

A method to simultaneously extract and identify glucosinolates and phenolic compounds [20] is presented for first time in *Brassica* crops. Twelve intact glucosinolates and more than 30 phenolic compounds in *B. rapa* turnip greens and turnip tops were characterized simultaneously by LC/UV-PAD/ESI-MSn.

Identification by mass spectrometry is more sensitive and gives greater resolution than UV–LC. The practical importance to quantify these compounds using HPLC-DAD lies in the utility of this method to characterize high volume of samples of different plant species and varieties with commercial importance. For agricultural experiments and agro-industrial purposes, it is necessary to analyze a high number of samples normally from different locations and over more than one season. Therefore, the use of HPLC-DAD can be less precise data than the mass spectrometry, but this is robust enough to identify the main metabolites present in the samples and give us the opportunity to run and evaluate a high number of samples in a system available in more laboratories than a mass spectrometer.

Almost identical profiles of glucosinolates and phenolic compounds were found in the two plant organs, but the quantification of the main compounds in 10 Galician varieties showed significant differences for most of the compounds between turnip greens and turnip tops. Total glucosinolate contents were similar in the two plant organs and gluconapin was the predominant glucosinolate. A significant content of glucobrassicin was also found in the two plant organs. Based on the results obtained, this method is useful for identifying the main secondary metabolites in *B. rapa.* The same metabolites were identified when compared with separate methods for glucosinolates and for phenolic compounds, and in similar quantities. For this reason, this could become a reference method to work in the nutritional quality of vegetable brassicas, because the information obtained is comparable to that obtained by using the standard methods and, additionally it is a less laborious and time consuming method.

This study shows that *B. rapa* is a good source of phenolic antioxidants. The main naturally occurring phenolic compounds identified were flavonols and hydroxycinnamic acids. The majority of the flavonoids found in Galician varieties are kaempferol, quercetin and isorhamnetin glycosylated and acylated with different hydroxycinnamic acids. Quercetin and kaempherol are the most prevalent flavonoids in the *Brassicaceae* family. Kaempherol is known to be a strong antioxidant and quercetin also a potent free radical scavenger and is considered to be a protective against cardiovascular disease. It is remarkable that the presence of isorhamnetin as one of the major flavonoids which are not present in the *B. oleracea* family, but described in several *B. rapa* characterizations, may serve as a biochemical marker of these varieties.

Varieties of turnip greens and turnip tops from NW Spain are an appreciable source of health-protective compounds. Our results showed the diversity in the content of certain phytochemicals found among different geographical locations that could be useful for studying the relationship between the content of these compounds and environmental and agronomic factors, and many of the compounds showed great differences among varieties that can be used for future breeding programs.

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CAPÍTULO V

Variación fenotípica y ambiental de glucosinolatos y fenoles

Effect of genotype and environmental conditions on health-promoting compounds in *Brassica rapa*

Marta Francisco, María Elena Cartea, Pilar Soengas, Pablo Velasco

Misión Biológica de Galicia (CSIC), PO Box 28, E-36080· Pontevedra, Spain

ABSTRACT

Brassica crops are rich in glucosinolates and phenolic compounds, metabolites related with human health. It is well know that a variety of factors (genetic and environmental) affects the ultimate metabolite levels in vegetables, although there is still little information about the role that genetics and environment play on these levels. The objetives were i) to evaluate the content and distribution of glucosinolates and phenolic compounds in two distinct plant organs, turnip greens (leaves) and turnip tops (shoots), from *Brassica rapa* varieties grown in different production areas and ii) to study the environmental influence on those health-promoting bioactive compounds. Total glucosinolates were more abundant in turnip tops (26.02 μmol g^{-1} dw) than in turnip greens (17.78 μmol g^{-1} dw). On the other hand, total phenolics content was found in higher quantities in turnip greens (43.81 μmol g^{-1} dw) than in turnip tops (37.53 μmol g^{-1} dw). The SREG analysis model allowed to study the effects of genotype (G), environment (E) and genotype × environment on metabolite content. Aliphatic glucosinolates were clearly regulated by G, in contrast, the effects of E and GE on the content of indolic glucosinolates and phenolics compounds appeared as the main effects of variation. Climate factors and soil parameters were related with metabolite levels. Varieties MBG-BRS0163, MBG-BRS0197, MBG-BRS0173 and MBG-BRS0143 were the most promising varieties for future breeding programs focussed on varieties with high glucosinolate contents. Besides MBG-BRS0143 and MBG-BRS0401 could be also good candidates for their high content of flavonoids. The identification of genotypes with enhanced and stable levels of these compounds would provide a valued-added opportunity for marketing this crop with superior health promotion to consumers.

INTRODUCTION

Brassica vegetables commonly known as crucifers, include a variety of horticultural crops (e.g., broccoli, Brussels sprouts, cabbage, turnip), which play a significant role in worldwide vegetable production and consumption. Brassica vegetables are low fat and protein content and have a high content in vitamins, fibre and minerals (Jahangir et al., 2009). Besides, they show high quantities of phytochemicals such as glucosinolates and phenolic compounds widely studied for its beneficial properties (Cartea and Velasco, 2008; Crozier et al., 2009; Traka and Mithen, 2009).

Glucosinolate diversity varies widely in families and species, suggesting that diversification has accompanied speciation (Rosa et al., 1997). Distribution of glucosinolates and phenolic compounds have been the target of several comprehensive reviews (Cartea and Velasco, 2008; Fenwick et al., 1983; Kushad et al., 1999; Podsedek, 2007) showing that the profile and amount of these phytochemicals varies widely in families, species and cultivars. Besides, the type and content of glucosinolate and phenolics levels depends on the plant part and can vary in vegetative and floral tissues during ontogeny (Rosa et al., 1997; Vallejo et al., 2003b; Velasco et al., 2007). In addition, there are many environment factors that play a role in regulating expression of these metabolites. Nitrogen and sulfur applications to the soil have a different effect on glucosinolate and phenolic content in brassica edible parts (Kim et al., 2002; Stewart et al., 2001). Related to climatic conditons, winter or autumn seasons seem to lead to lower both glucosinolate and flavonoid levels, due to short days, wetter conditions, cool temperatures and less radiation (Hertog et al., 1992; Rosa et al., 1997; Vallejo et al., 2003a). Moreover, it has been reported that higher disease and pest pressure influenced the concentration of these compounds (Daniel et al., 1999; Giamoustaris and Mithen, 1995; Velasco et al., 2007).

The Sites Regression (SREG) (Crossa and Cornelius, 1997) has been suggested as an appropriate model to study the influence of genotype (G), environment (E) and genotype × environment interaction (GE) when large variation is due to E (Yan et al., 2000). Although this method was mainly used for yield studies, nowadays it has been extended to other types of studies conducted in breeding programs to study host-pathogen relationship (Yan and Falk, 2002) or QTL environment interactions (Yan and

Tinker, 2005), as well as data from diallel crosses (Yan and Hunt, 2002) or gene × environment correlations (Lee et al., 2003; Yan and Rajcan, 2002). The SREG method supplies a graphical display called GGE (G plus GE interaction) biplot that facilitates visual cultivar evaluation.

In northwestern Spain and Portugal, *Brassica rapa* subsp. *rapa* L. includes turnip greens and turnip tops for culinary profit as well as turnips for fodder (Padilla et al., 2005). Turnip greens are the leaves harvested in the vegetative period while turnip tops are the fructiferous stems with the flower buds and the surrounding leaves which are consumed before opening and while still green (Rosa, 1997). In those countries, agriculture is still very traditional and even today farmers continue to grow landraces in vegetable gardens for their own consumption.

A collection of *B. rapa* subsp. *rapa* from northwestern Spain is currently kept at 'Misión Biológica de Galicia' (CSIC, Spain). In a preliminar work, part of this collection was evaluated based on agronomical and morfological traits (Padilla et al., 2005), finding that in many cases, the same landrace is sown for more than one purpose. This fact allows the existence of local populations with high levels of variability. Further studies determined the variation of desulphoglucosinolates among varieties (Padilla et al., 2007) and recently, Francisco et al.(2009b) determined the profile of intact glucosinolates and phenolic compounds in two different organs, leaves and shoots, on representative varieties of this collection. However, little information is available about the stability of glucosinolate and phenolic compounds profiles between varieties across environments and developmental stages.

Since a variety of factors affects the ultimate bioactive compounds levels in vegetables it is necessary to study the role that genetics and environment play on these levels for improving the health benefit of functional foods. This study will determine which plant part contains the highest concentration of these beneficial compounds for human health and their environmental influence. The objectives of this study were i) to evaluate the content and distribution of glucosinolates and phenolic compounds in two distinct turnip edible parts (turnip greens and turnip tops) from *B. rapa* varieties grown in different production areas and ii) to study the environmental influence on those health-promoting bioactive compounds.

MATERAL AND METHODS

Plant material. Twelve local varieties of *B. rapa* were evaluated in this study. From these, ten varieties were chosen based on the study carried out by Padilla et al., (2005) for their agronomic performance to produce turnip tops and/or turnip greens: MBG-BRS0082, MBG-BRS0143, MBG-BRS0173, MBG-BRS0184, MBG-BRS0401, MBG-BRS0433, MBG-BRS0451, MBG-BRS0461, MBG-BRS0472, MBG-BRS0550 and two varieties derived from three cycles of masal selection for fresh yield: MBG-BRS0163(S)C3 and MBG-BRS0197(S)C3. Varieties were transplanted in three years (2006, 2007 and 2008) at three locations that represent standard *B. rapa* production areas in Northwestern Spain: Oroso (A Coruña) (43°1'N, 8°26'W, 280 m.a.s.l.), Guitiriz (Lugo) (43°12'N, 7°53'W, 516 m.a.s.l) and Salcedo (Pontevedra) (42°24'N, 8° 38'W, 20 m. a s.l). At Salcedo, trials were lost due to unfavourable climatic conditions in 2006 and plant damages caused by *Delia radicum* L. immediately after transplanting in 2007. The varieties were planted in multipot-trays and seedlings were transplanted into the field at the five or six leaves stage. Transplanting dates were from September to October. Varieties were transplanted in a randomized complete block design with three replications. The experimental plots consisted of three rows with 10 plants per row. Rows were spaced 0.8 m apart and plants within rows 0.5 m apart. Cultural operations, fertilization, and weed control were made according to local practices. Three samples of healthy leaves (turnip greens) and young shoots (turnip tops) were used from five plants per plot. Turnip greens harvest ranged from 44 to 64 days after planting while turnip tops harvest ranged from 98 to 229 days after planting according to the maturity cycle of each variety at the optimum time for consumption, just after flower buds formation, before flower opening. After harvesting on dry ice, the material was immediately transferred to the laboratory and frozen at -80 °C, prior to their lyophilisation. The dried material was powdered using an IKA-A10 (IKA-Werke GmbH & Co.KG) mill and the powder was used for analysis.

Extraction and determination of glucosinolates and phenolic compounds. The LC gradient for glucosinolate and phenolic analyses is a multipurpose chromatographic method that simultaneously separates glucosinolates and phenolics and it was recently applied to Galician brassicas (Francisco et al., 2009b; Velasco et al., 2010). A portion of

150 mg of each sample was extracted in 4 mL 70% MeOH at 70 °C for 30 min with vortex mixing every 5 min to facilitate the extraction. The samples were centrifuged (13,000g, 15 min), 1 mL of supernatants was collected and methanol was completely removed using a sample concentrator (DB-3D, Techne, UK) at 70 ºC. The dry material obtained was redissolved in 1 mL of ultrapure water and filtered through a 0.20 μm syringe filters (Acrodisc® Syringe Filters, Pall Life Sciences). Chromatographic analyses were carried out on a Luna C18 column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex, Macclesfield, UK). The mobile phase was a mixture of: (A) ultrapure water/trifluoro acetic acid (TFA) (99.9:0.1) and (B) methanol/TFA (99.9:0.1). The flow rate was 1 mL min^{-1} in a linear gradient starting with 0% B at 0–5 min, reaching 17% B at 15–17 min, 25% B at 22 min, 35% B at 30 min, 50% B at 35 min, 99% B at 50 min and at 55–65 min 0% B. The injection volume was 20 μL and chromatograms were recorded at 330 nm for phenolics derivatives and 227 nm for glucosinolates in a Model 600 HPLC instrument (Waters) equipped with a Model 486 UV tunable absorbance detector (Waters). Glucosinolates were quantified using sinigrin (sinigrin monohydrate from Phytoplan, Diehm and Neuberger GmbH, Heidelberg, Germany) as standard. Caffeoyl-quinic and *p*-coumaroyl-quinic acids derivatives were quantified as chlorogenic acid (5-caffeoyl-quinic acid, Sigma–Aldrich Chemie GmbH, Steinheim, Germany), flavonoids as kaempferol 3-rutinoside (Extrasynthese, Genay, France) and sinapic acid and derivatives as sinapic acid (Sigma).

Soil Analyses and Climate Data. Soil samples were collected at the three above mentioned environments. Samplings were carried out using a hollow cylindrical corer with an internal diameter of 7 cm. Six subsamples, each 25 cm deep, were taken following a zigzag path across the center of each plot. Subsamples were mixed to obtain a homogeneous sample, about 500-1000 g, to be analyzed. The soil properties examined were pH, percentage of organic matter, available phosphorus, available potassium, exchangeable magnesium, exchangeable cations (Ca, Mg, Na, K, and Al), and cation exchange capacity. Soil analyses were performed at 'Estación Fitopatológica do Areeiro"' (Salcedo, Spain). Glucosinolates and phenolics content were related to several environmental covariables: precipitation, degree days, mean of the maximum temperatures, mean of the minimum temperatures, mean of the mean temperatures,

number of days with max temperature over 30 and 20ºC, number of days with mean temperature over 20 and below 10ºC, and number of days with minimum temperature below 10 and 0ºC. Climatic data were obtained from meteorological stations located close to the experimental fields.

Statistical analyses. Analyses of variance were performed for each trait according to a randomized complete block design. Years, locations and varieties were considered as fixed effect. Comparisons of means among varieties in each plant organ were performed using the Fisher's protected least significant difference (LSD) at P=0.05 (Steel et al., 1997). Simple correlation coefficients ($P < 0.05$) between secondarry metabolites and climatic data were made in order to establish the relationsips between them. To study the genotype × location (GE) interaction the Sites Regression method (SREG) was used (Crossa and Cornelius, 1997). Each environment was defined as the combination of a year and a location resulting in seven different environments under study. Since this method does not allow missing data, 11 varieties were evaluated for turnip greens assessment and nine varieties for turnip tops at five locations. For this method, principal components (PC) analysis was made on residuals of an additive model with locations as the only main effects. A two-dimensional biplot (Gabriel, 1971) called GGE biplot (G plus GE interaction) of the two first PCs was used to display the genotypes and the environments simultaneously (Yan et al., 2000). Genotypes and locations were displayed in the same plot. Each genotype and location was defined by the scores of genotypes and locations on the two PCs, respectively. All statistical analyses were made by a SAS (SAS Institute, 2007).

RESULTS AND DISCUSSION

The chemical profile of *B. rapa* varieties studied in this work was composed by eight glucosinolates belonging to the three chemical classes (progoitrin, glucoraphanin, gluconapin, 4-hydroxyglucobrassicin, glucobrassicanapin, glucobrassicin, neoglucobrassicin and gluconasturtiin) and 17 phenolic compounds, from which nine were flavonoids and eight were hydroxycinnamic acids. Flavonoids were glycosylated in the 3-position with sophoroside, with some compounds simultaneously acylated with cinnamic acids and in the 7-position with glucose: (**1**) quercetin-3-*O*-(methoxycaffeoyl) sophoroside-7-*O*-glucoside; (**2**) quercetin-3-*O*-(caffeoyl) sophoroside-7-*O*-glucoside; (**3**) kaempferol-3-*O* (methoxycaffeoyl) sophoroside-7-*O*-glucoside; (**4**) kaempferol-3-*O* (caffeoyl) sophoroside-7-*O*-glucoside; (**5**) quercetin-3,7-di-*O*-glucoside; (**6**) kaempferol-3-*O*-(sinapoyl) sophoroside-7-*O*-glucoside; (**7**) kaempferol-3-*O*-(feruloyl) sophoroside-7-*O*-glucoside; (**8**) kaempferol-3,7-di-*O*-glucoside; (**9**) isorhamnetin-3,7-di-*O*-glucoside. Hydroxycinnamic acids were quinic acids and sinapic acids derivatives: (**3CQA**) 3-caffeoyl quinic acid; (**3*p*CoQA**) 3-*p*-coumaroylquinic acid; (**SA**) sinapic acid; (**SG**) sinapoylglucoside; (**A1**) 1, 2-disinapoylgentiobioside; (**A2**) 1-sinapoyl-2-feruloylgentiobioside; (**A3**) 1, 2, 2′-trisinapoylgentiobioside; (**A4**) 1, 2′-disinapoyl-2-feruloylgentiobioside.

The combined analysis of variance showed significant differences among varieties for seven glucosinolates (Table 1) and for most of phenolic compounds mentioned above (Table 2). The year × variety, locality × variety, and year × locality × variety interactions were highly significant for most of these compounds (Tables 1 and 2), showing the great environmental influence on these compounds. Bible and Chong (1975) showed that climate can influence amounts of glucosinolates in root radish. Velasco et al. (2007) in kale and Ciska et al. (2000) in different cruciferous vegetables found that low temperatures caused a reduction on glucosinolate content while high average temperature increased in a significant way the glucosinolate concentration. Moreover, lower average rainfall increased glucosinolate content (Rosa and Heaney 1996; Rosa et al. 1997). Regarding to phenolic compounds, there are lack of information about the influence of climatic effects of these compounds in Brassica crops, although Vallejo et al (2003) found that flavonoid content in broccoli was influenced by season.

Table 1. Mean squares of the combined analysis of variance for the individual and total glucosinolates content in the *B. rapa* varieties evaluated in northwestern Spain.

	Glucosinolates										
Traits	**PRO**	**GRA**	**GNA**	**OHGBS**	**GBN**	**GBS**	**GST**	**NGBS**	**ALIPH**	**INDOL**	**GLUCT**
Location (L)	1.14	0.29	3740**	0.05	3.03	0.89**	0.32*	0.15	482.33**	0.93**	513.68**
Year (Y)	9.89**	6.99**	102.71*	9.38**	2.41	10.68**	4.37**	6.69**	168.85**	65.24**	322.36**
Variety (V)	6.48**	0.34**	513.20**	0.30**	8.12**	0.20**	0.11**	0.05	440.81**	0.36*	443.99**
Plant organ (P)	23.81**	0.60*	5000**	0.17*	21.29**	0.33*	0.82**	0.89**	6551**	0.01	6408**
L×Y	0.02	0.41	150.84**	0.24*	7.64**	0.12	0.56**	0.05	217.12**	0.04	224.40**
V×L	0.44	0.18**	6.43	0.039	0.44	0.04	0.05	0.10*	7.62	0.29*	6.8
V×Y	1.27**	0.21**	24.82**	0.07*	1.78**	0.11	0.07	0.06	40.35**	0.29*	43.09**
P×L	1.00**	1.20**	25.16	0.05	0.35	0.19	0.31**	0.47**	16.03	1.49**	25.13
P×Y	3.49**	0.06	802.67**	0.88**	18.15**	3.06**	0.13	3.16**	46.44**	14.93**	1422**
P×V	2.84**	0.14	61.37**	0.09**	1.48**	0.17**	0.15**	0.04	104.75**	0.32*	48.58**
V×L×Y	0.51*	0.08	44.59**	0.033	1.20**	0.05	0.09**	0.03	44.49**	0.14	47.95**
P×L×Y	1.18*	0.04	75.90**	0.17**	4.63**	0.09	0.24**	0.01	20.72	0.58*	110.46**
P×V×L	0.72**	0.12	17.15	0.03	0.71	0.05	0.03	0.13**	23.51	0.32**	22.65
P×V×Y	1.05**	0.18*	20.36*	0.07**	1.05**	0.07	0.16**	0.05	17.32	0.23	27.32*
P×V×L×Y	0.5	0.15	14.57	0.02	0.91*	0.07	0.08*	0.04	32.90**	0.16	19.52
Error	0.43	0.15	23.05	0.05	0.85	0.10	0.07	0.05	32.53	0.12	35.21

PRO: progoitrin; GRA: glucoraphanin; GNA: gluconapin; 4-OHGBS: 4-hydroxyglucobrassicin; GBN: glucobrassicanapin; GBS: glucobrassicin; GNT: gluconasturtiin; NGBS: neoglucobrassicin; ALIPH: total aliphatics; INDOL: total indolics; GLUCT: total glucosinolates.

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$

Table 2. Mean squares of combined analysis of variance for the major individual and total flavonoids and hydroxycinnamic acids content in the *B. rapa* varieties evaluated in northwestern Spain.

	Phenolic compounds												
Traits	**3**	**4**	**6**	**7**	**8**	**9**	**FLAVt**	**SG**	**SA**	**A1**	**A2**	**HIDRt**	**PHENt**
Location (L)	6.25*	5.42*	5.58**	2.48*	6.73**	22.69**	255.24**	38.05**	192.03*	4.60	5.52	440.24**	1291**
Year (Y)	24.87**	69.84**	3.26**	1.35	38.30**	61.79**	866.30**	380.06	233.28*	19.54**	55.06**	477.02**	2739**
Variety (V)	1.99**	2.16**	3.24**	1.18	3.01**	1.79	53.83**	2.45**	40.68*	3.89**	14.51**	23.78	106.80
Plant organ (P)	2.17	39.54**	1.29*	0.14	201.73**	0.28	40.69	157.62**	15170**	244.72**	441.42**	4412**	3432**
L×Y	1.25	1.34	0.25	0.09	1.10	2.58	16.49	3.29	174.17*	3.03	0.26	135.79	281.13
V×L	0.67	0.53	0.15	0.30	0.94	0.95	9.03	2.27**	18.47	0.89	1.67	24.25	44.96
V×Y	1.21*	0.80	0.33	0.50	1.21	1.69	17.86	0.55	23.11	1.28	4.31**	51.92	110.53
P×L	2.03	1.39	1.43**	0.90	7.26**	6.02**	89.83**	4.72**	232.30**	2.86	2.16	131.19*	151.57
P×Y	6.93**	33.53**	7.21**	10.42**	22.23**	0.03	464.39**	86.84**	704.31**	51.71**	121.00**	2159**	4464**
P×V	1.14	0.29	0.49**	0.6*	0.92	1.77	12.32	2.37**	23.25	1.25	1.92	34.31	68.42
V×L×Y	1.27*	1.12*	0.60**	0.45	1.14*	1.63	22.31*	0.36	29.40	1.26	1.45	56.99*	126.47
P×L×Y	2.35*	1.93	1.09**	0.02	7.64**	3.51	55.70*	1.00	102.88**	4.79	8.84**	179.26**	354.62**
P×V×L	0.49	0.53	0.22	0.30	1.02	0.88	7.83	2.49**	10.32	1.31	1.98	15.68	36.02
P×V×Y	1.51	0.79	0.47*	0.27	1.43**	1.33	23.14*	0.77	14.67	0.93	2.18	26.76	87.05
P×V×L×Y	0.40	0.31	0.20	0.20	0.88	1.59	3.81	0.57	11.84	0.59	1.89	23.56	35.55
Error	1.06	1.23	0.35	0.40	0.81	1.19	19.18	0.97	48.72	2.85	3.35	61.86	138.71

3: kaempferol-3-*O* (methoxycaffeoyl) sophoroside-7-*O*-glucoside; 4: kaempferol-3-*O* (caffeoyl) sophoroside-7-*O*-glucoside; 6: kaempferol-3-*O*-(sinapoyl) sophoroside-7-*O*-glucoside; 7: kaempferol-3-*O*-(feruloyl) sophoroside-7-*O*-glucoside; 8: kaempferol-3,7-di-*O*-glucoside; 9: isorhamnetin-3,7-di-*O*-glucoside; FLAVt: Total flavonoids; SA: sinapic acid; SG: sinapoylglucoside; A1: 1,2-disinapoylgentiobioside; A2: 1-sinapoyl-2-feruloylgentiobioside; HIDRt: total hydroxycinnamic acids; PHENt: total phenolics.

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$

Furthermore, significant differences were found between plant organs (turnip greens and turnip tops) for most compounds (glucosinolates and phenolic compounds) analyzed. As it has been recently reported, the concentration of glucosinolate and phenolics in *B. rapa* vary across stage development (Bellostas et al., 2007; Fernandes et al., 2007). Similarly to what happened with varieties, the year × plant organ, locality × plant organ, and year × locality × plant organ interactions were significant for most compounds (Tables 1 and 2), showing once again the great environmental influence on these compounds. In spite of these interactions, varieties and plant organs showed similar behavior across locations and years for most compounds, mainly for total glucosinolate and gluconapin content as well as for total phenolics and SA content which are the major glucosinolate and phenolic found in both organs, respectively. Therefore, a combined analysis of variance, for each plant organ, was made, focusing on the main effects which are location, year and variety (data not showed).

Variation of glucosinolates between plant organs

In the individual analysis of variance by each plant organ significant differences ($P \leq 0.01$) were found among varieties, locations and years for most individual and total glucosinolate content in both plant organs. The year × plant organ interactions were also significant, these interactions were mainly due to the different climatic conditions in each location and year, all along the crop cycle (see figure 1). Total glucosinolate content was higher in turnip tops than in turnip greens. For turnip greens, total glucosinolate content ranged from 14.35 to 23.60 μmol g^{-1} dw with a mean value of 17.78 μmol g^{-1} dw. For turnip tops, total glucosinolate content ranged from 20.18 to 36.36 μmol g^{-1} dw with a mean value of 26.02 μmol g^{-1} dw (Table 3). Similar values were found by Francisco et al. (2009b) and Padilla et al. (2007) in *B. rapa* varieties from northwestern Spain. These differences on glucosinolate concentrations among different plant organs have been also reported by other authors those showed that total glucosinolate content increased from vegetative to reproductive stages and maturity (Brown et al., 2003; Bellostas et al., 2007; Velasco et al., 2007).

Glucosinolate quantification showed that aliphatic glucosinolates were predominant, representing 72 and 82% of the total glucosinolates content in turnip greens and turnip tops, respectively. Gluconapin was by far the most abundant

glucosinolate in these cultivars followed by glucobrassicanapin. Yang and Quiros (2010) studied the glucosinolate variation in more than 80 crops of *B. rapa* and they found that the major glucosinolate was gluconapin. In our varieties, gluconapin levels represented between 49-68% and 56-78% of the total glucosinolate content in turnip greens and turnip tops, respectively. The mean value of gluconapin was 10.21 μmol g^{-1} dw in trunip greens and 17.39 μmol g−1 dw in turnip tops. These contents are consistent with those previously found by Francisco et al. (2009b) and Padilla et al. (2007) but lower than those reported by Kim et al. (2003). Some reports found that the pungent and bitter flavour of some Brassica crops are related with gluconapin content (Fenwick et al., 1983; Francisco et al., 2009a; Padilla et al., 2007; Rosa et al., 1997). The second glucosinolate in abundance, glucobrassicanapin, represented between 5-15% of total glucosinolate content in both plant stages. The mean value of this glucosinolate was 1.90 μmol g^{-1} dw in turnip greens and 2.38 μmol g^{-1} dw in turnip tops.

Other aliphatic glucosinolates such as glucoraphanin and progoitrin were found in minor quantities (Table 3). Among the glucosinolates presents in *Brassica* crops, the most studied is glucoraphanin, the main glucosinolate in broccoli, which is related as a good source of cancer protective compound (Mithen et al., 2003). It is well know that glucoraphanin, progoitrin and gluconapin are in the same pathway of the biosynthesis of the aliphatic glucosinolates (Giamoustaris and Mithen, 1995). The fact that our varieties are very rich in gluconapin could offer future perspectives to further modify the glucosinolate composition and get glucoraphanin accumulating plants as a source of anticarcinogens. Biosynthesis of gluconapin requires a functional allele *Brgsl-Alk* that converts glucoraphanin to its alkenyl homolog, gluconapin. Li and Quiros (2003) obtained *Arabidopsis* plants with reduced concentration of glucoraphanin, which was converted into gluconapin. Some approaches to develop a variety of *B. rapa* containing glucoraphanin is to produce *Brgsl-Alk* knockout lines to efficiently accumulate glucoraphanin in the side-chain modification pathway, or use gene silencing methods such as RNAi to accomplish the same objective (Yang and Quiros, 2010).

The indol group of glucosinolates represented between 19% and 13% of the total glucosinolate content in turnip greens and turnip tops, respectively.

Glucosinolates belonging to this class, found in our samples were 4-hydroxyglucobrassicin, glucobrassicicin and neoglucobrassicin. For this group of compounds differences between plant organs were not found, with means of 3.30 μmol g^{-1} dw in turnip greens and 3.21 μmol g^{-1} dw in turnip tops. It is interesting to note that, when these classes of compounds are hydrolysed give rise to a range of involatile indole compounds which have been implicated in the anti-carcinogenic activities of brassica vegetables (Aggarwal and Ichikawa, 2005).

Gluconasturtiin was the unique aromatic glucosinolate found in a concentration of 1.52 μmol g^{-1} dw and 1.43 μmol g^{-1} dw in turnip greens and turnip tops, respectively. Phenethyl isothiocyanate (PEITC) is the degradation product of the gluconasturtiin wich occurs in large quantities in watercress. A protective effect of PEITC has been reported as a inhibitor of tumour growth inducing apoptosis human prostate cancer cells (Dong et al., 2006).

The cancer chemopreventive effect of cruciferous vegetables is mainly attributed to the degradation products of glucosinolates (Traka and Mithen, 2009). The most promising varieties for future breeding purposes would be those with the highest total glucosinolate content and, particularly, glucosinolates with beneficial effects related to human health. In the present study we found a high variability on glucosinolate content among varieties in both plant organs, being MBG-BRS0197, MBG-BRS0163 and MBG-BRS0173 the varieties that showed the highest values on total glucosinolate, total aliphatic and gluconapin contents for both plant organs (Table 3). The variety MBG-BRS0163 had aslo the highest content of indolic glucosinolates (Table 3). In turnip greens, the varieties with the highest gluconasturtiin content were MBG-BRS0184 and MBG-BRS0550 while in turnip tops were the varieties MBG-BRS0197 and MBG-BRS0163. Besides, variety MBG-BRS0143 had high levels of this glucosinolate at the two plant organs (Table 3). Apart from the medicinal value of isothiocyanates, these compounds also play a significant organoleptic role in brassica products. Some reports found that the pungent and bitter flavour of some Brassica crops are related with gluconapin content (Fenwick et al., 1983; Francisco et al., 2009a; Padilla et al., 2007; Rosa et al., 1997) .

Table 3. Mean (μmol g^{-1} dw) for the glucosinolates content in turnip greens and turnip tops from the *B. rapa* varieties evaluated in northwestern Spain.

Variety	Glucosninolates										
MBG-	PRO	GRA	GNA	OHGBS	GBN	GBS	GST	NGBS	ALIPH	INDOL	GLUCT
Turnip greens											
BRS0082	0.88	0.36	8.47	0.86	2.09	1.31	1.37	1.04	11.81	3.21	16.38
BRS0143	0.73	0.25	8.27	0.96	1.53	1.18	1.61	1.06	10.78	3.21	15.60
BRS0163	0.63	0.24	13.52	1.22	2.26	1.53	1.49	1.09	16.65	3.83	21.98
BRS0173	0.62	0.29	15.35	0.99	1.69	1.19	1.43	1.05	17.94	3.23	22.60
BRS0184	1.06	0.30	9.15	0.98	2.78	1.41	1.63	1.05	13.31	3.43	18.37
BRS0197	0.86	0.40	14.99	1.20	2.27	1.27	1.51	1.09	18.53	3.55	23.60
BRS0401	0.75	0.14	10.93	0.71	1.70	1.20	1.50	1.06	13.52	2.97	17.99
BRS0433	0.78	0.00	7.07	1.01	1.80	1.22	1.42	1.08	9.64	3.29	14.35
BRS0451	0.59	0.09	9.55	1.03	1.94	1.22	1.59	1.07	12.18	3.32	17.09
BRS0461	0.99	0.24	8.16	1.08	1.57	1.23	1.49	1.07	10.96	3.38	15.84
BRS0472	0.67	0.09	8.52	0.91	1.58	1.22	1.55	1.09	10.86	3.21	15.61
BRS0550	1.16	0.24	8.57	1.05	1.62	1.22	1.61	1.05	11.59	3.31	16.52
Mean	0.80	0.22	10.21	0.98	1.90	1.26	1.52	1.06	12.96	3.30	17.78
LSD (5%)	0.19	0.09	1.19	0.07	0.19	0.05	0.08	0.05	1.29	0.11	1.39

Table 3 (Continued)

Turnip tops											
BRS0082	2.02	0.48	12.29	0.95	2.64	1.25	1.37	1.03	17.43	3.22	22.02
BRS0143	1.38	0.46	12.42	0.94	1.78	1.52	1.58	0.96	16.02	3.40	21.01
BRS0163	0.75	0.28	24.58	1.26	3.16	1.64	1.58	0.90	28.78	3.79	34.15
BRS0173	0.60	0.23	24.87	1.04	1.70	1.23	1.48	0.85	27.39	3.11	31.98
BRS0184	1.42	0.44	15.85	1.06	3.73	1.31	1.42	0.70	21.44	3.05	25.91
BRS0197	0.72	0.35	28.21	1.20	2.09	1.38	1.62	0.80	31.36	3.38	36.36
BRS0401	0.41	0.08	20.21	0.84	1.85	1.37	1.38	0.83	22.55	3.04	26.97
BRS0433	1.63	0.06	13.18	0.80	2.53	0.94	1.15	0.98	17.40	2.71	21.26
BRS0451	1.03	0.16	14.25	1.02	2.17	1.13	1.45	0.89	17.60	3.02	22.08
BRS0461	1.28	0.40	14.87	1.02	2.76	1.32	1.37	0.86	19.31	3.20	23.88
BRS0472	0.99	0.57	12.70	0.91	1.70	1.12	1.41	0.79	15.95	2.82	20.18
BRS0550	2.49	0.57	15.25	1.14	2.46	1.18	1.29	0.91	20.77	3.23	25.30
Mean	1.28	0.35	17.39	1.02	2.38	1.30	1.43	0.89	21.38	3.21	26.02
LSD (5%)	0.20	0.13	1.31	0.06	0.33	0.13	0.07	0.11	1.38	0.18	1.42

PRO: progoitrin; GRA: glucoraphanin; GNA: gluconapin; 4-OHGBS: 4-hydroxyglucobrassicin; GBN: glucobrassicanapin; GBS: glucobrassicin; GNT: gluconasturtiin; NGBS: neoglucobrassicin; ALIPH: total aliphatics; INDOL:total indolics; total GS: total glucosinolates.

LSD: Least Significant Difference

Variation of phenolic compounds between plant organs

For turnip tops, the variance analysis showed significant differences ($P \leq 0.01$) among varieties, locations and years for most of individual and total phenolics. For turnip greens, significant differences ($P \leq 0.01$) were found among the main effects for most of individual phenolic compounds. Nevertheless, at this plant organ, no differences were found between varieties for total flavonoids, total hydroxycinnamic acids and total phenolics. Likewise it happened on glucosinolate analysis, variety × year interaction was also significant at both plant organs. Total phenolic content was found in higher quantities in turnip greens than in turnip tops and values ranged from 41.16 to 47.58 μmol g^{-1} dw with a mean value of 43.81 μmol g^{-1} for turnip greens and from 29.50 to 41.72 μmol g^{-1} dw with a mean value of 37.53 μmol g^{-1} for turnip tops (Table 4). These values were higher than those reported by other authors in different *B. oleracea* crops (Price et al., 1998; Zhang et al., 2003) and similar to those found in turnip tops by other authors (Fernandes et al., 2007; Francisco et al., 2009b; Sousa et al., 2008). According with our results, Fernandes et al.(2007) found different concentrations of individual flavonoids and hydroxycinnamic acids between leaves and stems of *B. rapa*.

The hydroxycinnamic acids were the major phenolic compounds in *B. rapa* varieties evaluated in this work, representing 62% and 54% of the total phenolics in turnip greens and turnip tops, respectively. These percentages were higher than those reported by Sousa et al. (2008) in the inflorescences of *B. rapa*. Hydroxycinnamic acids are found in higher quantities in turnip greens than in turnip tops, determined by the content of SA, which reaches 74% and 33% of total hydroxycinnamic acids in turnip greens and turnip tops, respectively. In turnip greens, SA content was twice to five times the amount exhibited by turnip tops, whith a mean value of 20.25 μmol g^{-1} dw and 6.63 μmol g^{-1} dw, respectively (Table 4). This variation can be ascribed to the fact that the production of phenolics is usually lower during rapid growth of the younger leaves, increasing significantly thereafter, when the photosynthetic capacity of the newly matured leaves is highest (Riipi et al., 2002). On the contrary, sinapoyl derivatives (A1, A2, A3 and A4) were higher in turnip tops than in turnip greens, providing an added nutritional value to turnip tops. Plumb et al. (1997) reported that the sinapoyl derivatives are highly effective at preventing lipid damage.

Related to flavonoids, the most abundants were the kaempferol derivatives which varied between 64 and 75% of total flavonoid content in both plant organs. Flavonoids **3**, **4**, and **8** were the major kaempferol derivatives representing each one from 14 to 17% of total flavonoid content, while quercetin derivatives (**1**, **2** and **5**) were minor compounds in all varieties. In spite of not founding significant differences for total flavonoid content between plant organs, individual flavonoids were significantly different between these two plant organs. In turnip greens, flavonoids **3** and **4** reached the maximum values. The mean values of flavonoid **3** was 3.11 μmol g^{-1} dw in turnip greens and 2.98 μmol g^{-1} dw in turnip tops and the mean value for flavonoid **4** was 2.79 μmol g^{-1} dw in turnip greens and 2.04 μmol g^{-1} dw in turnip tops (Table 4). These flavonoids were acylated with caffeic acid. The presence of an O-dihydroxy structure in the caffeoylmoiety confers great stability to their radical scavenging capacity (Braca et al., 2003). On the other hand, flavonoid **8** reached the maximum values in turnip tops being also the major flavonoid at this plant organ with a mean value of 3.42 μmol g^{-1} dw in turnip tops and 1.90 μmol g^{-1} dw in turnip greens (Table 4). In contrast to other Brassica vegetables, *B. rapa* varieties showed high concentration of isorhamnetin (compound **9**) being for most varieties the second flavonoid in abundance, which represented between 15-20% of total flavonoid content. This flavonoid did not show differences between plant organs (Table 4). Isorhamnetin diglucoside, isolated from mustard leaf (*B. juncea*) showed a strong activity in reducing serum levels of glucose in Diabetes Mellitus through an antioxidant activity test (Yokozawa et al., 2002).

Because phenolic compounds are important in human nutrition as health protective agents, it would be useful the development of varieties with improved nutritional value. *B. rapa* varieties evaluated in this work showed similar contents of total phenolic, total hydroxycinnamic acids and total flavonoid in turnip greens over years. Nevertheless, turnip tops showed differences among varieties. The varieties with the highest levels of total phenolic compounds were MBG-BRS143, MBG-BRS0197 and MBG-BRS0163 (Table 4). If we focus only on each group of phenolics, MBG-BRS0143 and MBG-BRS0163 were the varieties that showed the highest flavonoids and hydroxycinnamic acids concentrations, respectively (Table 4).

Table 4. Mean (μmol g^{-1} dw) for the major flavonoids and hydroxycinnamic acids content in turnip greens and turnip tops from the *B. rapa* varieties evaluated in northwestern Spain.

Variety	Phenolic compound														
MBG-	3	4	6	7	8	9	FLAVt	3CQA	SG	SA	A1	A2	A3	HIDRt	PHENt
Turnip greens															
BRS0082	3.49	3.45	1.85	1.92	2.04	3.08	18.01	0.32	1.19	21.75	1.91	3.07	0.70	29.65	47.58
BRS0143	3.26	2.85	2.07	1.98	1.91	2.75	17.55	0.46	1.45	19.67	1.48	2.20	0.67	26.85	44.26
BRS0163	3.13	2.86	1.80	1.95	1.78	2.75	16.36	0.37	1.70	17.90	1.85	1.68	0.75	24.90	41.26
BRS0173	2.50	2.51	1.70	1.45	1.82	3.10	15.54	0.45	1.18	22.29	1.49	1.38	0.56	28.07	43.50
BRS0184	3.06	2.81	1.91	1.70	2.01	3.04	17.10	0.60	1.46	21.07	1.78	2.20	0.85	28.82	45.80
BRS0197	3.28	3.03	1.66	1.70	1.85	3.34	17.22	0.39	1.29	20.34	1.48	1.33	0.54	25.98	43.20
BRS0401	3.06	2.48	1.90	1.90	2.33	2.93	16.83	0.45	1.17	20.79	1.11	1.57	0.68	26.51	43.21
BRS0433	2.61	2.46	1.80	2.27	1.49	2.99	15.89	0.62	0.64	18.39	2.11	3.00	0.71	26.32	41.91
BRS0451	3.00	3.03	2.21	1.81	1.79	2.59	17.00	0.46	1.57	18.50	2.15	2.82	0.71	27.03	43.89
BRS0461	3.12	2.52	1.62	1.65	1.75	2.36	14.94	0.45	0.93	20.22	1.51	1.68	0.60	26.37	41.16
BRS0472	3.51	2.75	2.47	1.82	2.10	2.78	17.69	0.50	0.92	22.30	1.80	1.77	0.53	28.67	46.23
BRS0550	3.09	2.74	2.06	1.73	1.85	2.78	16.47	0.58	1.18	18.45	2.14	2.10	0.57	25.76	42.10
Mean	3.11	2.79	1.93	1.80	1.90	2.88	17.73	0.47	1.22	20.25	1.73	2.08	0.66	27.21	43.81

Table 4. (Continued)

Turnip tops															
BRS0082	2.99	2.49	2.08	1.79	3.51	2.97	17.94	0.45	2.12	6.24	4.26	5.30	1.47	20.80	38.73
BRS0143	3.23	1.95	2.60	2.23	3.57	3.39	19.78	0.50	3.26	7.54	3.43	4.82	1.38	21.95	41.72
BRS0163	2.90	2.16	2.01	2.17	3.00	2.84	17.33	0.37	3.43	7.86	3.97	4.96	1.72	23.34	40.66
BRS0173	2.83	1.70	1.70	1.59	2.87	2.52	15.46	0.37	2.25	7.48	3.63	3.93	1.17	19.68	35.14
BRS0184	2.66	2.20	1.72	1.52	3.43	3.32	16.75	0.67	2.05	5.98	3.65	4.66	1.45	19.46	36.21
BRS0197	3.14	2.00	1.86	1.91	3.46	2.82	17.76	0.38	4.53	8.85	3.49	3.84	1.32	23.27	41.03
BRS0401	2.76	1.66	1.99	1.94	3.94	3.11	17.78	0.42	3.39	7.67	2.86	3.94	1.22	20.40	38.18
BRS0433	1.99	1.53	1.69	1.30	2.45	2.61	13.42	0.59	0.60	5.93	2.99	3.93	1.15	16.07	29.50
BRS0451	3.43	2.18	2.54	2.08	3.49	3.14	19.20	0.42	3.03	4.02	3.67	5.21	1.31	18.59	37.79
BRS0461	2.81	1.86	1.49	1.39	2.93	2.54	14.95	0.47	2.15	7.41	2.60	3.14	1.13	17.89	32.84
BRS0472	3.04	1.92	2.43	1.61	3.62	2.87	17.34	0.44	3.16	5.58	2.83	3.55	0.89	17.33	34.66
BRS0550	3.28	1.86	2.54	1.97	3.95	2.70	18.20	0.55	3.58	4.94	3.56	4.78	1.38	19.71	37.91
Mean	2.98	2.04	2.10	1.82	3.42	2.96	17.49	0.47	2.83	6.63	3.47	4.40	1.30	20.04	37.53
LSD (5%)	0.28	0.27	0.18	0.15	0.29	0.33	1.16	0.05	0.30	0.98	0.45	0.54	0.17	1.40	2.20

3: kaempferol-3-*O* (methoxycaffeoyl) sophoroside-7-*O*-glucoside; 4: kaempferol-3-*O* (caffeoyl) sophoroside-7-*O*-glucoside; 6: kaempferol-3-*O*-(sinapoyl) sophoroside-7-*O*-glucoside; 7: kaempferol-3-*O*-(feruloyl) sophoroside-7-*O*-glucoside; 8: kaempferol-3,7-di-*O*-glucoside; 9: isorhamnetin-3,7-di-*O*-glucoside; FLAVt: Total flavonoids; 3CQA: 3-caffeoyl quinic acid; SA: sinapic acid; SG: sinapoylglucoside; A1: 1,2-disinapoylgentiobioside; A2: 1-sinapoyl-2-feruloylgentiobioside; A3: 1, 2, 2'-trisinapoylgentiobioside; HIDRt: total hydroxycinnamic acids; PHENt: total phenolics.

LSD: Least Significant Difference.

Climate and soil effects on glucosinolate and phenolic content

Soil and climate differences across environments could be the cause of significant differences between environments and plant organ × environment interaction found for some traits. Climatic conditions all along the crop cycle (between September and May) over the three years were very different at each location. The mean temperatures and precipitation between the cycle crop at 2006/07, 2007/08 and 2008/09 are shown in Fig. 1

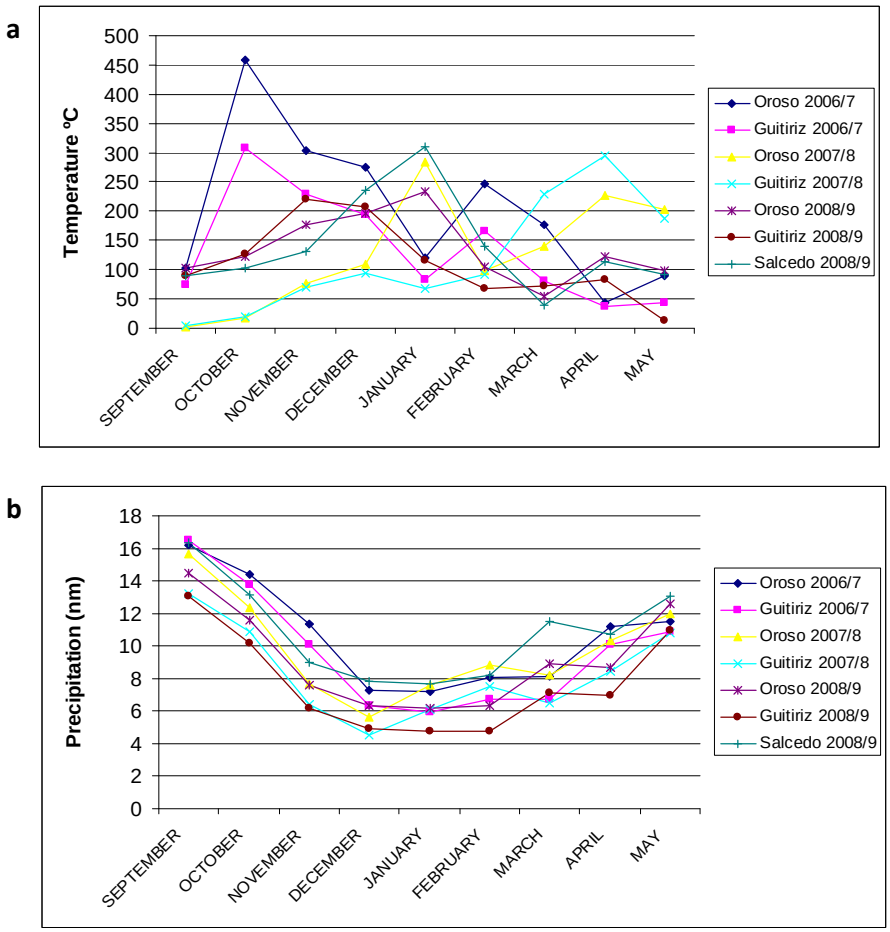

Figure 1. Mean temperatures (a) and precipitations (b) from September to May in seven environments from northwestern Spain (Oroso, Guitiriz, and Salcedo) during three years (2006 to 2009).

Simple correlations were made in order to study the relationships between climatic factors and secondary metabolite levels. Results showed that in turnip greens, the number of days with a minimum temperature under 0 ºC was negatively correlated (ranging from R = –0.67 to R= –0.76*) with total aliphatic, total glucosinolate and gluconapin contents. On the other hand, in turnip tops, these traits were positively and

highly correlated with the number of days with a maximum temperature over 20 ºC, the mean of maximum temperatures and degree days of maximum temperatures (ranging from R = 0.72 to R = 0.85*). In turnip tops, the total indolic glucosinolates were correlated with the number of days with a minimum temperature under 10 ºC (R = 0.79*). For both plant organs it was found that precipitation had negative correlations with indolic glucosinolates, being highly significant in turnip greens (R = –0.90**). These results are in agreement of those reported by other authors who found that Brassica crops grown under cool temperatures and abundant rainfall seem to have lower total glucosinolate content (Ciska et al., 2000; Rosa et al., 1997; Velasco et al., 2007).

Regarding to phenolics, no correlations between these compounds and climatic factors were found in turnip greens. On the contrary, for turnip tops there was a clear relationship between the number of days with a minimum temperature under 0 ºC and 10 ºC (ranging from R = 0.77* to R = 0.95**) with total phenolics, total hydroxycinnamic acids and total flavonoids. Being a winter crop, much of the growing cycle takes place at temperatures below 10 ºC and minimum temperatures under 0 ºC. Stefanowska et al (2002) observed large phenolic deposits in the plasma membrane and membrane-bound organelles of winter oilseed (*B. napus*) plants grown at cold and freezing temperatures. This was related with pronounced ultrastructural changes of leaf epidermal and mesophyll cells due to low temperatures.

Differences in the soil parameters were proved by edaphic analyses. The main characteristic of soils used in this study was their high acidity, with an average pH value of 5.3 in Guitiriz, 5.5 in Pontevedra and 5.6 in Oroso. Soils were rich in organic matter with an average content ranging from 6,8% in Salcedo to 13.4% in Oroso. Available phosphorus was high in Guitiriz and Salcedo and medium in Oroso. The available potassium was high in Oroso and Salcedo and medium in Guitiriz. Results showed that both aliphatic and indolic glucosinolates presented the highest levels in Salcedo along with Oroso. Phenolic compounds levels were also higher at Oroso. Therefore, for most of compounds, the highest content of glucosinolates and phenolic compounds occurred in locations with the highest soil pH and available potassium, suggesting some type of relation between glucosinolate and phenolic content and soil

effect. In addition, other soil factors can influence the content of these metabolites. Kim et al. (2002) found that in turnip, glucosinolate levels were strongly regulated by nitrogen and sulfur application. In field experiments an increase in nitrogen availability favored the hydroxylation step on aliphatic pathway (Zhao et al., 1994). In the same way, an experiment on greenhouse-grown leaf mustard has demonstrated a decrease in total phenolic levels in response to increased supply of nitrogen in the nutrient solution (Li et al., 2008). Nitrate availability was shown to directly affect the enzyme activity in the phenylpropanoid pathway (Fritz et al., 2006). On the other hand, it have been reported that flavonols of kaempferol and quercetin derivates in *Brassica rapa* L. subsp. *Sylvestris* were reduced by sulphur availability (De Pascale et al., 2007).

Genotype × environment interaction (SREG).

Results of analyses of variance for SREG are presented in Table 5. At two plant organs both aliphatic and indolic glucosinolates were significantly affected by E and GGE. For aliphatic glucosinolates, in turnip greens and turnip tops E main effect explained 53% and 17% of the total variation, while GGE accounted for 47% and 83% of total sum of squares, respectively. Genotype main effects (G) were also significant and accounted for 63% and 70% of the GGE. The variation due to G was larger than the variation due to the GE interaction, and also in turnip greens this interaction was not significant, meaning that genotypes had similar behaviour across environments. Respect to indolic glucosinolates the main effect E explained 25% and 71% of total variation in turnip greens and tops, respectively. The main effect G accounted for 45% and 21% of the GGE in turnip greens and turnip tops, respectively. In spite of the fact that GE interaction was not significant at any plant organ, the total percent of sums of squares attributable to GE was much higher than that attributable to G. Besides, for turnip tops, E had a larger influence, suggesting that these kinds of glucosinolates were highly influenced by the environmental conditions.

Table 5. Analysis of variance of he sites regression (SREG) multiplicative model for aliphatic glucosinolates, indolic glucosinolates, flavonoids and hydroxycinnamic acids on trunip greens and turnip tops of *B.rapa* varieties evaluated in five different environments.

	Turnip greens			Turnip tops		
	D. f	**S. S**	**M.S**	**D. f**	**S.S**	**M.S**
Aliphatic glucosinolates						
E	4	2463.76	615.93**	4	807.60	201.90**
GGE	50	2161.46	43.22**	40	4512.31	112.81**
Error	108	1482.87	13.73	81	1780.07	21.97
Indolic glucosinolates						
E	4	3.36	0.84**	4	49.50	12.38**
GGE	50	10.15	0.20**	40	20.17	0.50*
Error	108	11.24	0.10	81	25.76	0.31
Flavonoids						
E	4	585.12	146.28**	4	897.34	224.33**
GGE	50	669.86	13.39	40	1331.47	33.29**
Error	108	2157.50	19.97	81	990.20	12.22
Hydroxycinnamic acids						
E	4	2285.00	571.25**	4	758.87	189.71**
GGE	50	1942.53	38.85	40	1733.94	38.35**
Error	108	6282.71	58.17	81	1557.74	19.23

E= Environmental main effects, where one E is the combination of a location and year; GGE= Genotype plus Genotype × Environment interaction effects; Df= Degree of freedom; SS= Sum of Squares; MS= Mean of Squares

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$

For aliphatic and indolic gluocosinolates, the PC1 and PC2 together, which make up a GGE biplot, explained more than 90% of the total GGE variation at two plant organs (Table 5). The two-dimensional biplot showed that the varieties MBG-BRS0197 and MBG-BRS0163 for turnip greens (Fig. 2A) and MBG-BRS0173 for turnip tops (Fig. 3A) presented the highest total aliphatic content at most of environments. Besides MBG-BRS0197 for turnip greens appeared as a high and stable genotype because it showed a large PC1 score and a near-zero PC2 score (Fig. 2A). Regard to indolic glucosinolates, the varieties MBG-BRS0163 and MBG-BRS0143 had the highest mean of of these compounds at turnip greens and tops, respectively (Fig. 2B and 3B). The ideal test

environments, should have small (absolute) PC2 scores (more representative of the overall environment) and large PC1 scores (more power to discriminate genotypes in terms of the genotypic main effect (Yan and Rajcan, 2002; Yan et al., 2000). Therefore, at Oroso 2008 (E4) turnip greens showed the highest aliphatic content while Salcedo 2008 (E5) was the most discriminative. In turnip tops, Guitiriz 2008 (E3) was the highest and most stable environment. For indolic glucosinolates Guitiriz 2007 (E1) was the best environment for both crops.

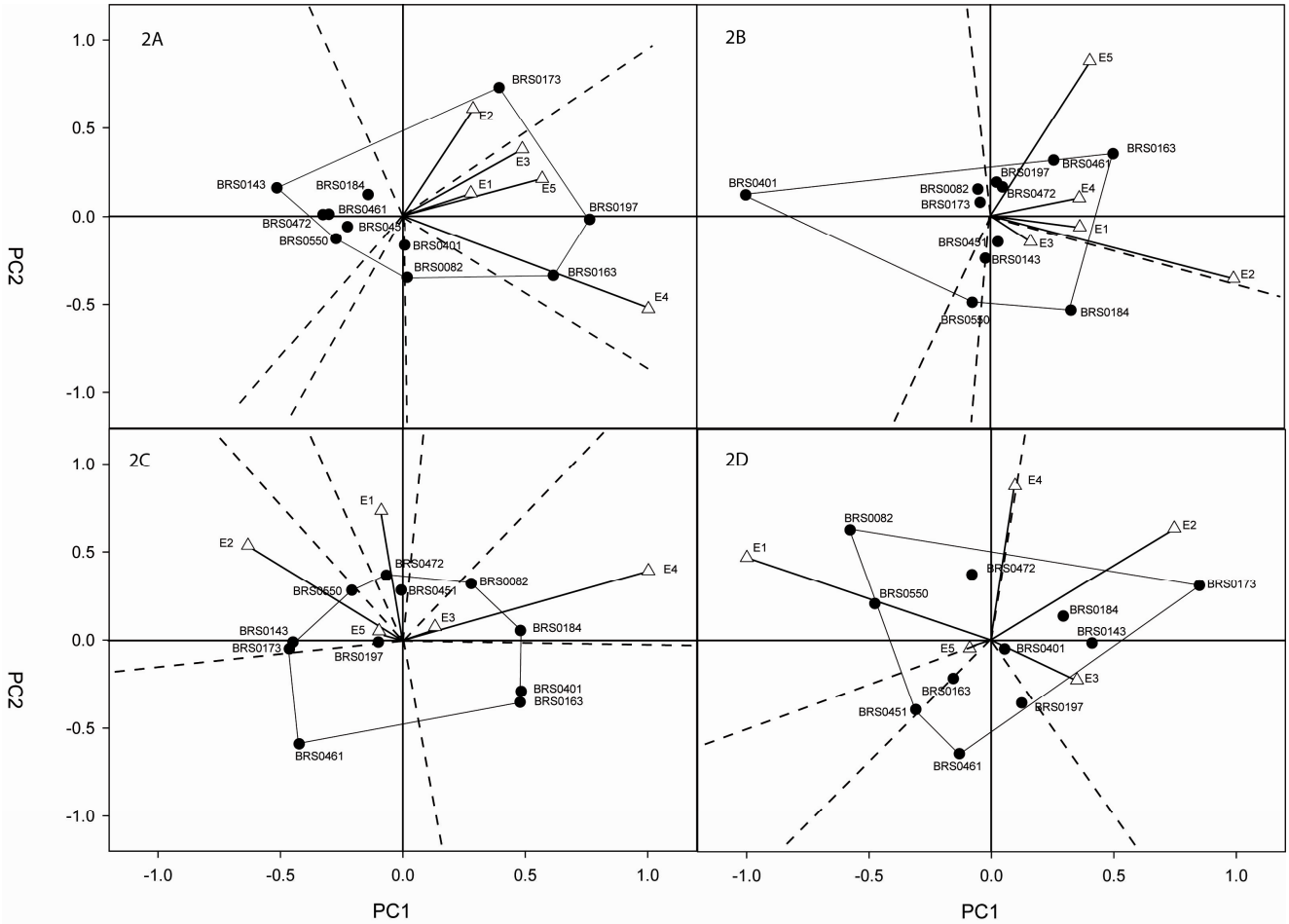

Figure 2. The G + GE interaction (GGE) biplot based on the metabolite content of 11 *B. rapa* varieties for turnip greens at five environments. Environments are E1 (Guitiriz 2007), E2 (Oroso 2007), E3 (Guitiriz 2008), E4 (Oroso 2008), E5 (Salcedo 2008). Metabolites are total alphatic glucosinolates (**2A**), total indolic glucosinolates (**2B**), total flavonoids (**2C**) and total hydroxycinnamic acids (**2D**). The polygon shown with tiny dots was made by joining with the genotypes which are on vertex.

Few studies have contrasted the genetic *vs*. the environmental contribution to glucosinolate concentration and most of them are focussed on genetic effects on glucoraphanin content (Farnham et al., 2004; Rosa and Rodrigues, 2001). Further studies carried out by Brown et al. (2002) evaluated of a subset of 10 broccoli varieties grown over 4 seasons founding that indolic glucosinolates were regulated very differently to the aliphatic ones. They reported that synthesis of aliphatic glucosinolates was clearly regulated by G (60%), with E and GE interactions exerting smaller effects (5% and 10%, respectively). In contrast, regulation of indolic glucosinolates content was primarily environmental (G 12%, E 33% and GE 21%). Results from the current study confirm the relative importance of G in expression of aliphatic glucosinolates whereas the indolic glucosinolates were mostly influenced by E and GE. These results indicate that turnip greens and tops should respond well to selection for increasing aliphatic glucosinolates concentration.

Regarding to phenolic compounds, the analyses of variance for SREG showed that, in turnip greens, total flavonoids and total hydroxycinnamic acids were significant affected only by the main effect E, which explained 46% and 53% of total variation (Table 5), respectively. The GGE interation was not significant at this plant organ. For turnip tops, E and GGE were significant for both kinds of compounds. The main effect E explained 40% and 30% of total variation of flavonoids and hydroxycinnamic acids content, respectively. The main effect G and GE interaction were significant and accounted for 38% and 62% for flavonoids and 17% and 85% for hydroxycinnamic acids, respectively.

The study of GGE biplot of phenolic compounds showed that for both plant organs, the PC1 and PC2 together explained more than 70% of the total GGE variation of total flavonoids and total hydroxycinnamics, respectively (Table 5). Except for flavonoids in turnip tops, different genotypes produced the highest metabolite content in different environments (Fig. 2C, 2D and 3D). This fact complicates the selection of varieties for future breeding programs. For turnip tops, it was possible identify the best genotypes for total flavonoids (Fig. 3C). The variety MBG-BRS0143 presented the highest flavonoid levels at most of environments and MBG-BRS0451 reached good

levels of these compounds and also was the most stable one. For these compounds, Oroso 2008 (E4) was the ideal test environment.

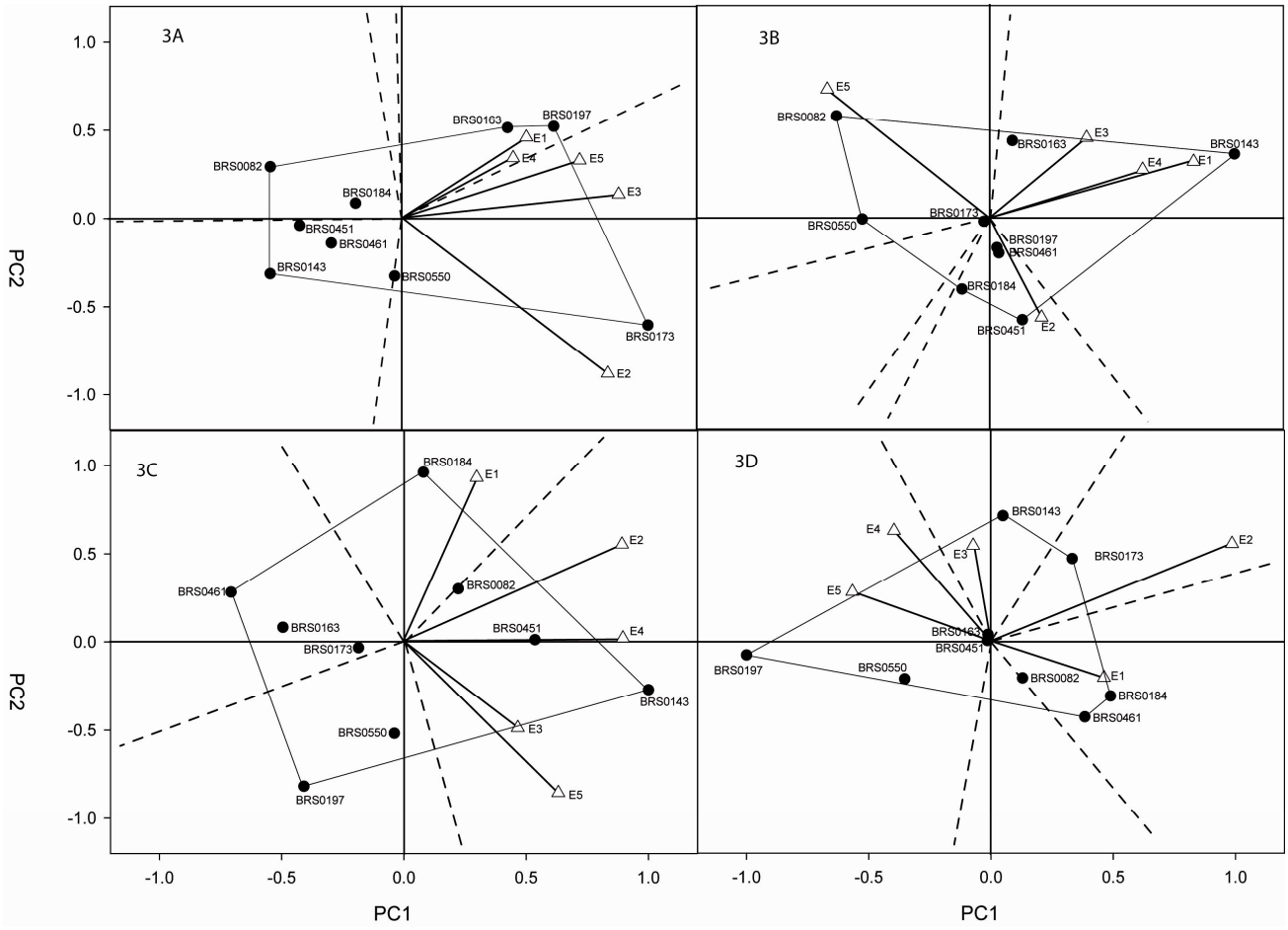

Figure 3. The G + GE interaction (GGE) biplot based on the metabolite content of nine *B. rapa* varieties for turnip tops at five environments. Environments are E1 (Guitiriz 2007), E2 (Oroso 2007), E3 (Guitiriz 2008), E4 (Oroso 2008), E5 (Salcedo 2008). Metabolites are total alphatic glucosinolates (**3A**), total indolic glucosinolates (**3B**), total flavonoids (**3C**) and total hydroxycinnamic acids (**3D**). The polygon shown with tiny dots was made by joining the genotypes which are on vertex.

Several studies, reviewed by Parr and Bolwell (2000) have demonstrated that the change in phenolic composition of plant leaves is a consequence of environmental effects (biotic and abiotic estress). Detailed examination by molecular biological approaches has indicated that the phenomenon is largely due to enhanced transcription of the phenolic biosynthetic genes following exposure to the inducing stimulus. On the other hand, genetic factors within crop populations may have important effects on the phenolic content of vegetables (Tomás-Barberán and Espín,

2001). Neverthelss there are few works which study independently the influence of genotype and environment effects or the interaction between both effects. In the present study we found that both flavonoids and hydroxycinnamic acids are highly influenced by the E effects and GE interactions, which were predominant respect to the G main effect. Therefore, if much of the variability is due to the environment, probably the heritability of these compounds is low and selection strategies must take this into account.

CONCLUSIONS

The present work is an important step forward in the knowledge of the role that G, E and their interaction GE play in the final concentration of glucosinolates and phenolics compounds. Besides, was also possible identify varieties with high and stable metabolite content. Varieties MBG-BRS0163, MBG-BRS0197, MBG-BRS0173 and MBG-BRS0143 were the most promising varieties for future breeding programs focussed on varieties with high glucosinolate contents. Moreover, due to their stability and high content of flavonoids, the varieties MBG-BRS0143 and MBG-BRS0401 could be also good candidates for breeding. Since the bioactivity of turnip greens and tops is putatively associated with concentration of glucosinolates and phenolic compounds, identification of genotypes with enhanced and stable levels of these compounds would provide a valued-added opportunity for marketing this crop with superior health promotion to consumers.

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CAPÍTULO VI

Efecto del cocinado sobre compuestos bioactivos

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Cooking methods of *Brassica rapa* affect the preservation of glucosinolates, phenolics and vitamin C

Marta Francisco [a,*], Pablo Velasco [a], Diego A. Moreno [b], Cristina García-Viguera [b], María Elena Cartea [a]

[a] Misión Biológica de Galicia (CSIC), PO Box 28, E-36080 Pontevedra, Spain
[b] Department of Food Science and Technology, CEBAS-CSIC, Campus Universitario de Espinardo, PO Box 164, Espinardo, E-30100 Murcia, Spain

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ABSTRACT

Cooking *Brassica* vegetables as a domestic processing method has a great impact on health-promoting bioactive compounds: glucosinolates (GLS), flavonoids, hydroxycinnamic acids, and vitamin C. In Galicia (NorthWestern Spain), one of the most consumed horticultural crops is *Brassica rapa*, by using the leaves (turnip greens) and the young sprouting shoots (turnip tops) in different culinary preparations. In order to determine the effect of cooking, on turnip greens and turnip tops, bioactive GLS, flavonoids, hydroxycinnamic acids and vitamin C were analysed and simultaneously determined. The level of retention of each individual compound after cooking procedures was evaluated in the edible organs, and also in the cooking water, in order to compare their composition to a fresh uncooked control. Steaming, conventional boiling, and high-pressure cooking, traditional processing methods of this kind of vegetables, were the three domestic processing methods used in this work. Results showed that total GLS and phenolics were significantly affected by the cooking procedure and the loss rate varied among individual compounds. Steaming was the method that better preserved GLS and phenolic compounds. Conventional boiling and high-pressure cooking methods presented similar rate of losses of total GLS content (64%) and total phenolic content (more than 70%). Degradation among glucosinolate classes, aliphatic or indolic, was similar. The total flavonoids lost in turnip greens were 64% and 67% for conventional boiling and high-pressure, respectively. The main losses were caused by leaching into the cooking water. The concentration of vitamin C suffered a drastic loss in the process of sample handling and after cooking. Despite the fact that any cooking procedure affected negatively the nutritional composition of the turnip greens and tops, our results showed high retentions of individual compounds in steaming, and the lowest retentions were obtained in the traditional high-pressure cooking. High retention of health-promoting compounds in the cooking water should be considered for increasing the intake of properties of *B. rapa*.

1. Introduction

The *Brassicaceae* family includes a wide range of horticultural crops, many of them with economic significance and extensively consumed as commodities and used in the industry worldwide. *Brassica rapa* is one of the oldest cultivated vegetables that has been used for human consumption since prehistoric times (Liang et al., 2006) which comprises several morphologically diverse crops, including Chinese cabbage, pak choi, turnip and broccoletto, as well as oilseeds that include yellow and brown sarsons (Gómez-Campo, 1999). In the coldest regions of Portugal and Spain the edible parts of *B. rapa* includes turnip greens and turnip tops for culinary profit as well as turnips for fodder (Padilla, Cartea, Rodríguez, & Ordás, 2005) and they constitute a unique supply of vegetables during the winter (Rosa, 1997). Turnip greens are the leaves harvested in the vegetative period while turnip tops are the fructiferous stems with the flower buds and the surrounding leaves which are consumed before opening and while still greens. Turnip edible parts are commonly consumed as a boiled vegetable generally as meat companions.

The consumption of *Brassica* vegetables has been related to human health and to reduction of the risk of certain cancers and cardiovascular diseases. This association is often attributed to the presence of glucosinolates (GLS), phenolic compounds and vitamins (Podsedek, 2007; Sies & Stahl, 1995; Traka & Mithen, 2009; Verhoeven, Verhagen, Goldbohm, vandenBrandt, & vanPoppel, 1997).

Thermal treatment causes denaturation of enzymes that can catalyse breakdown of nutrients and phytochemicals. When *Brassica* vegetables are chewed or cut, tissues will disrupt and the GLS will come into contact with myrosinase (thioglucoside glucohydrolase EC 3.2.1.147), leading the conversion to isothiocyanates, nitriles, thiocyanates, epithionitriles, oxazolidine-2-thiones, and

* Corresponding author. Tel.: +34 986854800; fax: +34 986841362.
E-mail address: mfrancisco@mbg.cesga.es (M. Francisco).

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epithioalkanes (Grubb & Abel, 2006). The number of hydrolysis products, mostly formed simultaneously during storage and processing, as well as the myrosinase activity of the intestinal microbial floral may affect to the total content and bioavailability of these compounds (Verkerk et al., 2009).

It has been generally shown that conventional cooking methods such as boiling, steaming, pressure cooking and microwaving reduce the intake of glucosinolates by approximately 30–60%, depending on the method, intensity and type of compound (Rangkadilok et al., 2002; Rodrigues & Rosa, 1999; Verkerk & Dekker, 2004; Verkerk, Dekker, & Jongen, 2001). Some reports have focused mainly on the preservation of phenolic compounds in broccoli and vitamin C in broccoli and Brussels sprouts (Czarniecka-Skubina, 2002; Howard, Wong, Perry, & Klein, 1999; Vallejo, Tomás-Barberán, & García-Viguera, 2003; Zhang & Hamauzu, 2004). These studies reported that steaming led to the retention of the highest levels of flavonoids and hydroxycinnamic acids in broccoli. On the contrary, cooking from 3 to 15 min by microwave and conventional boiling caused losses on phenolic content approximately 30–90%. Related to vitamin C were reported losses from 3% to 10% after cooking Brussels sprouts in a microwave oven and pressure cooker (Czarniecka-Skubina, 2002). Conventional cooking in broccoli florets at 0.5, 1.5 and 5 min caused loss by 19.2%, 47.5%, and 65.9% of vitamin C, respectively (Zhang & Hamauzu, 2004).

At the Misión Biológica de Galicia (CSIC), a collection of local varieties of *B. rapa* [*rapa* group] is kept as part of the *Brassica* genus germplasm bank. In previous reports, this collection was evaluated based on nutritional traits (Francisco et al., 2009; Padilla, Cartea, Velasco, de Haro, & Ordás, 2007). Since these crops are thermally processed prior to consumption, the objective of this study was to determine the changes on the content of total and individual GLS, flavonoids, hydroxycinnamic acids and vitamin C in a representative set of turnip greens and turnip tops with three different cooking methods: high-pressure cooking, steaming and conventional boiling.

2. Material and methods

2.1. Plant material

Five local varieties of *B. rapa* were evaluated in this study. From these, four varieties were chosen based on their agronomic performance for turnip tops and/or turnip greens and one variety derived from three cycles of masal selection by fresh yield. The varieties were evaluated in 2007 at two environments in NorthWestern Spain: Oroso (A Coruña) (43°1′N, 8°26′W, 280 m.a.s.l.) and Guitiriz (Lugo) (43°12′N, 7°53′W, 516 m.a.s.l.). Both environments represent standard *B. rapa* production areas in NorthWestern Spain. The varieties were planted in multipot-trays and seedlings were transplanted into the field at the five or six leaves stage. Transplanting dates were on the 01st and 04th September in Oroso and Guitiriz, respectively. Varieties were transplanted in a randomized complete block design with three replications. The experimental plots consisted of three rows with 10 plants per row. Rows were spaced 0.8 m apart and plants within rows 0.5 m apart. Cultural operations, fertilization, and weed control were made according to local practices. Leaf harvest ranged from 44 to 64 days after planting while sprouting shoot harvest ranged from 127 to 229 days after planting according to the maturity cycle of each variety at the optimum time for consumption.

2.2. Processing

Three different cooking methods were tested: conventional boiling, steaming and high-pressure cooking. A total of 1.5 kg of leaves (turnip greens) and sprouting shoots (turnip tops) of each variety and environment were randomly selected. Samples were immediately transported on ice to the laboratory, where they were vacuum packed, frozen, and stored for further cooking. For turnip greens, three cooking procedures were replicated two times in each variety sample and environment. For turnip tops, only samples from Lugo were used due to low yields from Santiago and two methods were performed (conventional boiling and steaming). Each sample was divided in several portions of 150 g for subsequent cooking and the analysis of health-promoting bioactives. For each variety, two portions of 150 g were kept as uncooked fresh control. The cooking settings (time, temperature and water) were chosen according to recipes. For conventional boiling, fresh portion was added to 1500 mL of boiling water and cooked for 15 min. For high-pressure cooking, the leaves were fully dipped in 1500 mL of cold water and cooked during 5 min under high-pressure in a pressure cooker (Fagor™ Rapid-Express, Fagor Electrodomésticos S.C., Mondragon, Guipuzkoa, Spain). For steaming, the portion of vegetable was placed on a steaming rack over boiling water in a closed water bath (1500 mL) during 15 min. Of each method, 45 mL of the cooking water was kept for further analysis. After cooking and drained, cooked portions, water samples and fresh control were flash frozen using liquid N_2 and kept at −80 °C prior to their lyophilization (Christ Alpha 1-4D, Christ, Osterode am Harz, Germany). The dried material was powdered using an IKA-A10 (IKA-Werke GmbH and Co. KG) mill and the powder was used for analysis.

2.3. Extraction and determination of GLS and phenolic compounds

The HPLC gradient for glucosinolate and phenolic analyses is a multipurpose chromatographic method that simultaneously separates glucosinolates and phenolics (Bennett et al., 2003) and it was recently applied to Galician turnip tops and greens (Francisco et al., 2009). Briefly, a portion of 150 mg of each sample were extracted in 4 mL of 70% MeOH at 70 °C for 30 min with vortex mixing every 5 min to facilitate the extraction. The samples were centrifuged (13,000*g*, 15 min), and 1 mL of supernatant was collected to completely remove methanol using a sample concentrator (DB-3D, Techne, UK) at 70 °C. The dry material obtained was redissolved in 1 mL of ultrapure water and filtered through a 0.20 μm syringe filters (Acrodisc® Syringe Filters, Pall Life Sciences). Chromatographic analyses were carried out on a Luna C18 column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex, Macclesfield, UK). The mobile phase was a mixture of: (A) ultrapure water/trifluoro acetic acid (TFA) (99.9:0.1) and (B) methanol/TFA (99.9:0.1). The flow rate was 1 mL min^{-1} in a linear gradient starting with 0% B at 0–5 min, reaching 17% B at 15–17 min, 25% B at 22 min, 35% B at 30 min, 50% B at 35 min, 99% B at 50 min and at 55–65 min 0% B. The injection volume was 20 μL and chromatograms were recorded at 330 nm for phenolics derivatives and 227 nm for GLS in a Model 600 HPLC instrument (Waters) equipped with a Model 486 UV tunable absorbance detector (Waters). Glucosinolates were quantified using sinigrin (sinigrin monohydrate from Phytoplan, Diehm and Neuberger GmbH, Heidelberg, Germany) as standard. Caffeoyl-quinic and *p*-coumaroyl-quinic acids derivatives were quantified as chlorogenic acid (5-caffeoyl-quinic acid, Sigma–Aldrich Chemie GmbH, Steinheim, Germany), flavonoids as kaempferol 3-rutinoside (Extrasynthese, Genay, France) and sinapic acid and derivatives as sinapic acid (Sigma).

2.4. Extraction and determination of vitamin C

Ascorbic (AA) and dehydroascorbic (DHAA) acid contents were determined as described by Zapata and Dufour (1992) with some modifications (Gil, Ferreres, & Tomás-Barberán, 1999; González-

Molina, Moreno, & García-Viguera, 2008). For the determination in fresh, 5 g of fresh weight sample were homogenised in a an Ultra-Turrax T25 (Janke & Kunkel, Germany) for 30 s on an ice bath with 20 mL extractant solution, consisting of MeOH and H_2O (5:95), and 2.1% (v:v) dissolved citric acid, 0.05% (v:v) EDTA, and 0.01% (v:v) NaF. For freeze-dried samples 50 mg were homogenised in a vortex stirrer for 20 s with 10 mL of extractant solution. The homogenate was filtered through a four-layer cheesecloth. The extract (1 mL) was centrifuged (3600*g* for 15 min at 4 °C), and the supernatant was recovered and filtered through a C18 Sep-Pack cartridge (Waters, Milford, MA) previously activated with 10 mL of methanol followed by 10 mL of deionized water, and then 10 mL of air. The collected extract was filtered through a 0.45 μm polyethersulfone filter (Millex-HV, Millipore, Bedford, MA). Then, 250 μL of 1,2-phenilenediamine dihydrochloride (OPDA) solution (18.8 mM) were added to 750 μL of extract for dehyhroascorbic acid derivatization into the fluorophore 3-(1,2-dihydroxietyl)furo[3,4-b]quinoxaline-1-one (DFQ). After 37 min in darkness, the samples were analysed by HPLC. Ascorbic acid and dehydroascorbic acid was evaluated using an HPLC system (Merck-Hitachi, Tokyo, Japan), equipped with a L-6000 pump, injection valve and sample loop 20 μL (Rheodyne, CA, USA) and coupled to a L-4000 UV detector. Samples were analysed on a Lichrospher 100 RP-18 reversed-phase column (250 × 4 mm, particle size 5 μm) (Teknokroma, Barcelona, España) with a C_{18} precolumn (Teknokroma, Barcelona, España). The mobile phase was MeOH/H_2O (5:95, v/v), 5 mM cetrimide, and 50 mM KH_2PO_4 (pH = 4.59). The flow rate was kept at 0.9 mL min^{-1}. The detector wavelength was initially set at 348 nm, and after DFQ eluted, it was manually shifted to 261 nm, for ascorbic acid detection. L-AA y el L-DHAA were identified and quantified by comparison with pattern areas from L-AA and L-DHAA.

2.5. Statistical analyses

All analyses were made separately for each plant organ (turnip greens and turnip tops). The content of each metabolite (individual and total GLS and phenolic compounds) was determined in two ways: (i) in the fresh (raw) and cooked vegetable tissue and (ii) in the sum in the cooked vegetable tissue plus the cooking water (CW). Individual analyses of variance were performed for each compound. Varieties were considered as random factors. Comparison of means among cooking methods was made by Fisher's protected least significant difference (LSD) at $P = 0.05$ (Steel, Torrie, & Dickey, 1997). All statistical analyses were made using SAS (SAS Institute., 2007).

3. Results and discussion

3.1. Effect of cooking on total and individual glucosinolates (GLS)

3.1.1. Effect on vegetable tissues

Total GLS content in *B. rapa* varieties was very similar in both organs (12.99 μmol/g^{-1} dw in fresh turnip greens and 12.84 μmol/g^{-1} dw in fresh turnip tops). Seven major GLS were found in both organs: progoitrin (PRO), gluconapin (GNA), glucobrassicanapin (GBN), 4-hydroxyglucobrassicin (4-OHGBS), glucobrassicin (GBS), neoglucobrassicin (NGBS) and gluconasturtiin (GNT). Aliphatic GLS were the most abundant (66% of total GLS) followed by indolic (25%) and aromatic (9%). In agreement with data published by other authors (Francisco et al., 2009; Kim, Kawaguchi, & Watanabe, 2003; Padilla et al., 2007) the predominant GLS in *B. rapa* crops was GNA, which represents 51% and 77% of total GLS and total aliphatic contents, respectively.

In turnip greens, significant differences among cooking methods were found for all GLS ($P \leqslant 0.01$). Varieties did not show any significant differences among them. The variety × cooking method interaction was not significant for any GLS, which is indicative of the stability of different genotypes. In the same way, in turnip tops, significant differences among cooking methods were found for total GLS content ($P \leqslant 0.01$) as well as for most of the individual GLS. Varieties were significantly different for GBS and total GLS content. Differences in harvest time according to the maturity state of each variety could influence the final content of GLS. No GLS showed any significant variety × cooking method interaction.

Total and individual GLS concentrations were significantly reduced by the cooking method used and these losses were similar in turnip greens and turnip tops (Table 1, Fig. 1). Conventional boiling and high-pressure methods presented similar loss rate, by about 64% of total GLS content in comparison with fresh samples. Rosa and Heaney (1993) and Pereira et al. (2002) found losses from 40% to 80% of total GLS in Portuguese cabbage after boiling. Similar degradation rates of total GLS contents (58–77%) were described by Song and Thornalley (2007) after boiling differents brassicas during 30 min. Ciska and Kozlowska (2001) also observed a time course decrease of GLS content from 35% after 5 min of cooking to 87% after 30 min in white cabbage. In coincidence with previous results in broccoli (Vallejo, Tomás-Barberán, & García-Viguera, 2002; Volden, Wicklund, Verkerk, & Dekker, 2008), in the present work the steaming method was found to be the preferred cooking method for better preservation (or higher level of retention) of individual GLS content, because the losses ranged only by 9% in turnip greens and 21% in turnip tops (Fig. 1).

After cooking, the relative distribution of the three classes of GLS (aliphatic, indolic, and aromatic) did not change (Table 1). In turnip greens, the total aliphatic GLS content was reduced by 14% in steamed, a 60% in conventional boiling, and by 61% in high-pressure cooking. Similarly, in turnip tops, the aliphatic GLS content reductions were 25% in steamed and 63% in conventional boiling. In turnip greens, total indole GLS content was reduced by about 60%, both after high-pressure and conventional boiling cooking while in boiled turnip tops this loss was a 52%. Aliphatic GLS are generally reported as being more thermostable than indole GLS and under different cooking treatments (Ciska & Kozlowska, 2001; Goodrich, Anderson, & Stoewsand, 1989; Vallejo et al., 2002). However, in this work we found similar degradation rates between total aliphatic and total indole GLS eventhough the loss rates varied among individual GLS. GNA, the most abundant aliphatic GLS, was reduced after steaming by 14% and 23% in turnip greens and turnip tops, respectively, while it was reduced about 60% after high-pressure and conventional boiling cookings in both turnip tissues (Fig. 1). Loss rates of PRO were notably higher in turnip greens than in turnip tops. The greatest reductions after high-pressure and conventional boiling were found for two indolic GLS (4-OHGBS and GBS) and for the aromatic GNT with losses close to 100%. Other authors found that GBS, PRO and 4-OHGBS are very susceptible to heat treatments showing a great reduction after cooking (Rosa & Heaney, 1993; Volden et al., 2008). In the edible part of steamed turnip greens, we found an increase of 85% on the initial value of the indolic 4-OHGBS. The increase of GLS levels after steaming was reported previously (Gliszczynska-Swiglo et al., 2006) and also Verkerk and Dekker (2004) found more than 70% higher levels of indolic GLS after microwave treatment who explained it by an increase in chemical extractability from the plant tissue after heating.

3.1.2. Effect on the summatory of vegetable tissues and cooking water (CW)

Glucosinolates are water-soluble compounds and are usually lost during conventional cooking because of leaching into sur-

Table 1
Mean (μmol/g^{-1} dw) for the individual and total GLS, flavonoid and hydroxycinnamic acid content in turnip greens and turnip tops before (control) and after three cooking methods.

	Turnip greens					Turnip tops			
	Control	Steaming	Conventional boiling	High-pressure	LSD (5%)	Control	Steaming	Conventional boiling	LSD (5%)
GLS									
PRO	0.83a	0.57ab	0.30bc	0.23c	0.28	0.13a	0.12a	0.05a	0.44
GNA	6.27a	5.38b	2.48c	2.49c	0.80	7.25a	5.43a	2.48b	2.47
GBN	1.44a	1.43a	0.61b	0.63b	0.28	1.31a	1.01a	0.60b	0.44
Total aliphatics	8.55a	7.38b	3.38c	3.35c	1.12	8.69a	6.56a	3.13b	2.08
4-OHGBS	0.47b	0.87a	0.09c	0.26c	0.26	0.42a	0.40a	0.00a	0.37
GBS	1.50a	1.14b	0.35ce	0.20c	0.17	1.54a	0.90b	0.52b	0.42
NGBS	1.24a	1.16a	0.83b	0.80b	0.21	1.18a	1.16a	0.97a	0.25
Total indolics	3.21a	3.17a	1.27b	1.25b	0.40	3.14a	2.46b	1.49c	0.45
GNT	1.23a	1.20a	0.00b	0.03b	0.18	1.21a	0.88a	0.27b	0.52
Total GLS	12.99a	11.80a	4.66b	4.64b	1.32	12.84a	10.02b	4.56c	2.75
Flavonoids									
F1	2.18a	2.32a	0.85b	089b	0.31	2.00a	1.48a	0.65b	0.01
F2	1.85a	1.67a	0.57b	0.57b	0.22	1.47a	1.21a	0.51b	0.28
F3	1.23a	1.41a	0.16c	0.65b	0.27	1.93a	1.44a	0.60b	0.49
F4	1.44a	1.38a	0.50b	0.64b	0.20	1.75a	1.22a	0.57b	0.56
F5	0.96a	0.86a	0.40b	0.19c	0.17	0.97a	0.37b	0.11b	0.35
F6	1.75a	1.47a	0.78b	0.78b	0.52	2.14a	1.37b	0.70c	0.62
F7	3.29a	3.55a	1.32b	1.28b	0.46	1.76a	1.15ab	0.52b	0.71
Total flavonoids	13.85a	13.10a	4.58b	5.00b	1.63	13.10a	8.40b	3.70c	3.13
Hydroxycinnamics									
3CQAc	0.41a	0.31b	0.01c	0.03c	0.06	0.19a	0.33a	0.16a	0.21
3*p*CoQAc	0.33a	0.32a	0.00b	0.01b	0.05	0.15a	0.26a	0.16a	0.19
Total quinic acids	0.75a	0.63b	0.01c	0.04c	0.09	0.40a	0.35a	0.32a	0.39
SA	12.27a	9.58b	3.04c	3.26c	1.58	0.68a	0.52a	0.25b	0.18
A1	1.48a	0.93b	0.14c	0.07c	0.30	0.21a	0.13b	0.00c	0.46
A2	1.73a	1.60a	0.12b	0.19b	0.30	0.25a	0.17a	0.00b	0.09
A3	1.68a	0.51b	0.01c	0.03c	0.11	0.17a	0.14a	0.00b	0.11
A4	0.43a	0.24b	0.03c	0.04c	0.08	0.08a	0.01b	0.00c	0.01
Total sinapics	16.59a	12.86b	3.33c	3.60c	0.62	1.78a	1.15b	0.29c	0.35
Total phenols	31.51a	26.87b	7.93c	8.67c	3.37	14.80a	9.60b	4.02c	3.23

PRO: progoitrin; GNA: gluconapin; 4-OHGBS: 4-hydroxyglucobrassicin; GBN: glucobrassicanapin; GBS: glucobrassicin; GNT: gluconasturtiin; NGBS: neoglucobrassicin; F1: kaempferol-3-O-sophoroside-7-O-glucoside; F2: kaempferol-3-O-(caffeoyl)sophoroside-7-O-glucoside; F3: kaempferol-3-O-(sinapoyl)sophoroside-7-O-glucoside; F4: kaempferol-3-O-(feruloyl)sophoroside-7-O-glucoside; F5: kaempferol-3-O-(p-coumaroyl)sophoroside-7-O-glucoside; F6: kaempferol-3,7-di-O-glucoside; F7:isorhamnetin-3,7-di-O-glucoside; 3CQAc: 3-caffeoyl quinic acid; 3*p*CoQAc: 3-*p*-coumaroylquinin acid; SA: sinapic acid; A1: 1,2-disinapoylgentiobioside; A2: 1-sinapoyl-2-feruloylgentiobioside; A3: 1,2,2′-trisinapoylgentiobioside; A4: 1,2′-disinapoyl-2-feruloylgentiobioside.
Means with the same letter in the same row are not significant different.

rounding water due to cell lysis. Analysis of the water remains after boiling indicated that all GLS were leached out into the cooking water. The analysis of GLS in CW of turnip greens and CW of turnip tops showed significant differences among cooking methods for total GLS content ($P \leqslant 0.01$) as well as for same GLS. Other GLS did not show any significant differences among cooking methods indicating low or no degradation of these compounds.

After steaming, total GLS content of CW in both plant organs was not significantly different from the total GLS content in fresh vegetables (Table 2, Fig. 2), which means that the amounts of GLS recovered were not significantly different from the initial GLS content of the fresh vegetable. On the contrary, after conventional boiling and high-pressure, there were recovered 67% and 52%, respectively of the total GLS content in fresh turnip greens (Table 2, Fig. 2). In turnip tops, this recovery was 62% after conventional boiling (Table 2, Fig. 2). The most stable GLS in both plant organs after cooking were GBN, 4-OHGBS and NGBS. In turnip greens, total recoveries of compounds with the largest reductions, i.e. PRO, GBS, and GNT were 35%, 41%, and 13%, respectively after conventional boiling, and 67%, 29%, and 4%, respectively after high-pressure cooking. Different behaviour was found for 4-OHGBS, which suffered high reductions after cooking and it was recovered completely into the cooking water. In turnip tops, the highest loss after conventional boiling was detected in GNT which was recovered only 21%. These results are not consistent with other studies in which recoveries were over 80% for all GLS (Rosa & Heaney, 1993; Vallejo et al., 2002; Volden et al., 2008). GLS losses can be explained because the breakdown of cellular membranes during cooking allows the contact between glucosinolates and myrosinase. The myrosinase mediated hydrolysis of glucosinolates generates an unstable aglycone intermediate, thiohydroxamate-O-sulfonate, which is immediately converted to a wide range of bioactive metabolites, including isothiocyanates, thiocyanates, nitriles and oxazolidines (Bones & Rossiter, 1996; Fenwick, Heaney, & Mullin, 1983). Some of them are volatile metabolites associated with the typical bitter and hot flavour of *Brassica* foods (Fenwick et al., 1983). Isothiocyanates and indoles exhibit protective activities against many types of cancer in humans (Fahey, Zalcmann, & Talalay, 2001; Mithen et al., 2003; Zhang & Talalay, 1994).

3.2. Effect of cooking on phenolic compounds

3.2.1. Effect on vegetable tissues

The HPLC-DAD analysis allowed the quantification of 14 phenolic compounds including flavonoids, quinic acid derivatives and sinapic acids derivatives: kaempferol-3-O-sophoroside-7-O-glucoside (F1); kaempferol-3-O-(caffeoyl)sophoroside-7-O-glucoside (F2); kaempferol-3-O-(sinapoyl)sophoroside-7-O-glucoside (F3); kaempferol-3-O-(feruloyl)sophoroside-7-O-glucoside (F4); kaempferol-3-O-(*p*-coumaroyl)sophoroside-7-O-glucoside (F5); kaempferol-3,7-di-O-glucoside (F6); isorhamnetin-3,7-di-O-glucoside (F7); 3-caffeoyl quinic acid (3CQAc); 3-*p*-coumaroyl quinin acid

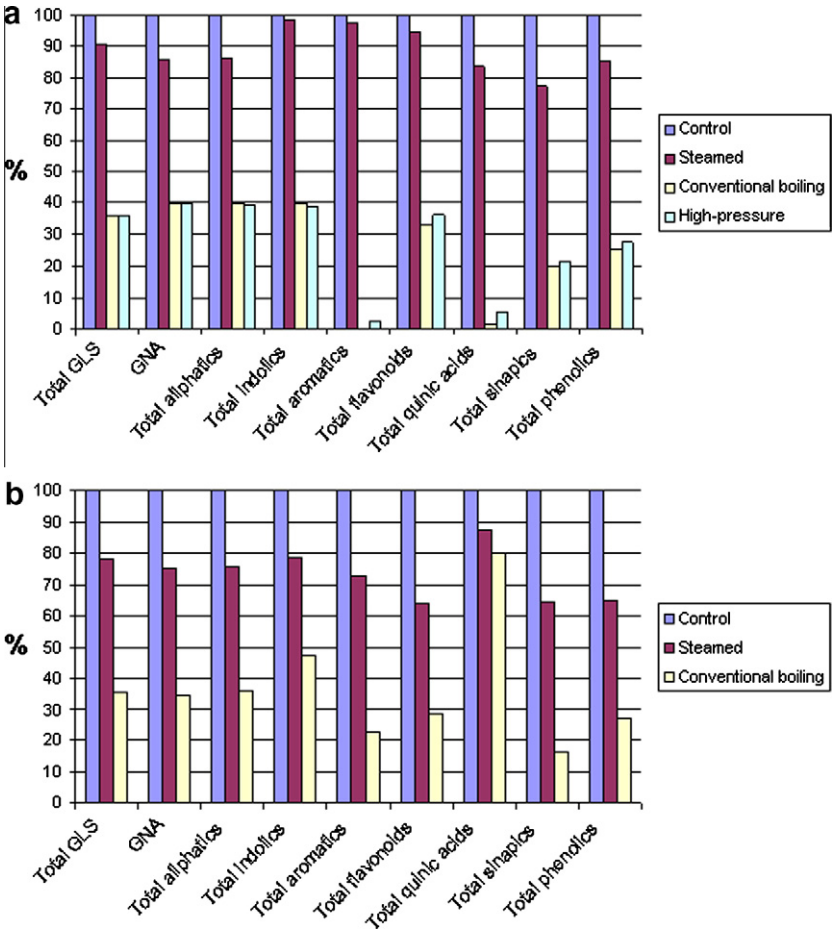

Fig. 1. Content (% of the fresh uncooked control) of the main compounds after different cooking methods in the edible parts of turnip greens (a) and turnip tops (b).

(3*p*CoQAc); sinapic acid (SA); 1,2-disinapoylgentiobioside (A1); 1-sinapoyl-2-feruloylgentiobioside (A2); 1,2,2′-trisinapoylgentiobioside (A3); 1,2′-disinapoyl-2-feruloylgentiobioside (A4). Results of total phenolic content revealed higher amount of these compounds in turnip greens (31.51 $\mu mol/g^{-1}$ dw), than in turnip tops (14.80 $\mu mol/g^{-1}$ dw). These differences are probably due to the high amount of SA in turnip greens, compound present in lower quantities in turnip tops. Total phenolic content found in our study was similar to those found in turnip tops by other authors (Fernandes et al., 2007; Francisco et al., 2009; Sousa et al., 2008).

In turnip greens, the analysis of variance showed significant differences among cooking methods ($P \leqslant 0.01$) for all of the flavonoids and hydroxycinnamic acids evaluated. No significant differences among varieties were found for any compound. Variety × cooking method interaction was significantly different ($P \leqslant 0.01$) for A1, total quinic acids derivatives, total phenolics and 3CQAc may be due to similar degradation rates found between high-pressure and conventional boiling methods. In turnip tops, the analysis of variance for phenolic compounds showed significant differences between cooking methods for total phenolic compounds and for most individual compounds ($P \leqslant 0.05$). No significant differences among varieties were found for any compound. Variety × cooking method interaction was significantly different ($P \leqslant 0.01$) for F2, F6 and A4.

After cooking, total phenolics content in turnip greens was reduced in 15%, 75% and 72% in steaming, high-pressure and conventional boiling, respectively (Fig. 1). In turnip tops, total phenolics were reduced 35% in steaming and 73% in conventional boiling (Fig. 1). During steaming, the temperature is lower than in the other two methods and the edible portions were not into contact with the cooking water. Therefore, the phenolic content was less affected. In agreement with Wachtel-Galor, Wong, and Benzie (2008), boiling and high-pressure cooking had strong effects on total phenolics content (Table 1). The depletion of total phenolics content after cooking could be due to their breakdown or by leached into the cooking water (Vallejo et al., 2003).

The amount of flavonoid glycosides lost in the cooked tissue of turnip greens were 5%, 64% and 67% for steaming, conventional boiling and high-pressure, respectively. In turnip tops, the loss of flavonoid glycosides was a 36% after steaming and a 72% after conventional boiling (Fig. 1). Our results indicate higher levels of total flavonoids in the edible part after cooking than those previously reported by Price, Casuscelli, Colquhoun, and Rhodes (1998) and Vallejo et al. (2003) which found that boiled broccoli lost a 80% of its initial flavonoid content. This better retention in turnip could be explained by the different flavonoid profile of *B. oleracea* and *B. rapa*. The studies mentioned before are focused on total phenolic content on broccoli but, as far as we are aware, there are no data available about rates of degradation on individual flavonoids presents on brassica vegetables after domestic cooking. Regarding to individual flavonoids, in the present work we focused on the study of seven major flavonoids of *B. rapa* (Table 1). Compounds F1, F2, F3, F4, F5 and F6 are flavonoids derivatives from kaempferol that have been described in other brassica vegetables such as cabbage, pak choi and broccoli (Ferreres et al., 2006; Harbaum et al., 2007; Vallejo, Tomás-Barberán, & Ferreres, 2004). Compound F7 is a flavonoid derived from isorhamnetin that was described in high quantities in *B. rapa* crops (Francisco et al., 2009).

Table 2
Mean (μmol/g^{-1} dw) for the individual and total GLS, flavonoid and hydroxycinnamic acid content in CW of turnip greens and turnip tops as compared to the uncooked tissue (control) and after three cooking methods.

	Turnip greens					Turnip tops			
	Control	Steaming (CW)	Conventional boiling (CW)	High-pressure cooking (CW)	LSD (5%)	Control	Steaming (CW)	Conventional boiling (CW)	LSD (5%)
GLS									
PRO	0.83a	0.63ab	0.56ab	0.29b	0.38	0.13a	0.22a	0.10a	0.47
GNA	6.27a	5.44a	4.05b	3.40b	0.93	7.25a	5.58b	3.68c	2.70
GBN	1.44a	1.52a	1.13a	0.90a	0.25	1.31a	1.07a	0.97a	0.49
Total aliphatics	8.55a	7.59a	5.74b	4.59b	0.95	8.69a	6.87b	4.75c	2.10
4-OHGBS	0.47a	0.93a	0.38a	0.57a	0.29	0.42a	1.15a	0.23a	0.27
GBS	1.50a	1.23b	0.62c	0.43c	0.27	1.54a	1.16a	1.01a	0.57
NGBS	1.24a	1.21a	1.32a	1.06a	0.25	1.18a	1.44a	1.20a	0.20
Total indolics	3.21a	3.37a	2.32b	2.06b	0.46	3.14a	3.75a	2.44b	0.62
GNT	1.23a	1.27a	0.16b	0.05b	0.20	1.21a	1.01a	0.26b	0.49
Total GLS	12.99a	12.26a	8.24b	6.71c	1.51	12.84a	10.42ab	7.93b	3.10
Flavonoids									
F1	2.18b	2.61b	4.20a	4.50a	0.54	2.00a	2.30a	2.45a	0.86
F2	1.85a	1.91a	1.74a	0.79b	0.64	1.47a	1.10a	1.89a	0.70
F3	1.23b	1.44ab	1.90a	1.18b	0.61	1.93a	1.44a	1.07a	0.45
F4	1.44b	1.74ab	2.16a	1.33b	0.59	1.75a	1.22a	1.13a	0.58
F5	0.96a	1.09b	1.98a	0.47c	0.49	0.97a	0.51a	0.37a	0.61
F6	1.75b	1.83b	4.13a	3.63a	0.92	2.14a	2.01a	2.96a	0.72
F7	3.29c	4.02c	6.90a	5.35b	1.11	1.76a	1.52a	1.84a	0.62
Total flavonoids	13.85b	15.20b	23.81a	17.76b	4.10	13.10a	10.75a	13.40a	3.13
Hydroxycinnamics									
3CQAc	0.41a	0.35a	0.24ab	0.10b	0.17	0.19a	0.33a	0.30a	0.22
3*p*CoQAc	0.33a	0.37a	0.44a	0.14b	0.14	0.15a	0.26a	0.32a	0.24
Total quinic acids	0.75a	0.72a	0.68a	0.24b	0.31	0.40a	0.59a	0.62a	0.46
SA	12.27a	9.74b	5.07c	4.72c	1.65	0.68a	0.75a	1.40a	0.28
A1	1.48a	0.96c	0.26c	1.11b	0.32	0.21a	0.13a	0.04a	0.09
A2	1.73a	1.64a	0.36b	0.27b	0.34	0.25a	0.16a	0.06a	0.09
A3	1.68a	0.54b	0.20c	0.12c	0.14	0.17a	0.14a	0.07a	0.10
A4	0.43a	0.24b	0.15b	0.18b	0.11	0.08a	0.07a	0.01a	0.03
Total sinapics	16.59a	13.11b	3.04c	5.40c	1.96	1.78a	1.38a	1.70a	0.50
Total phenols	31.51a	30.40a	32.40a	23.44b	5.08	14.80a	14.20a	13.00a	3.33

PRO: progoitrin; GNA: gluconapin; 4-OHGBS: 4-hydroxyglucobrassicin; GBN: glucobrassicanapin; GBS: glucobrassicin; GNT: gluconasturtiin; NGBS: neoglucobrassicin; F1: kaempferol-3-O-sophoroside-7-O-glucoside; F2: kaempferol-3-O-(caffeoyl)sophoroside-7-O-glucoside; F3: kaempferol-3-O-(sinapoyl)sophoroside-7-O-glucoside; F4: kaempferol-3-O-(feruloyl)sophoroside-7-O-glucoside; F5: kaempferol-3-O-(p-coumaroyl)sophoroside-7-O-glucoside; F6: kaempferol-3,7-di-O-glucoside; F7:isorhamnetin-3,7-di-O-glucoside; 3CQAc: 3-caffeoyl quinic acid; 3*p*CoQAc: 3-*p*-coumaroylquinin acid; SA: sinapic acid; A1: 1,2-disinapoylgentiobioside; A2: 1-sinapoyl-2-feruloylgentiobioside; A3: 1,2,2′-trisinapoylgentiobioside; A4: 1,2′-disinapoyl-2-feruloylgentiobioside.
Means with the same letter in the same row are not significant different.

Results showed that the same cooking method have different effects on different types of flavonoids, even within the same class. Besides, the loss rates of individual flavonoids varied among cooking methods and plants stages. High losses, from 80% to 90% were detected on F5 after high-pressure and conventional boiling. Compound F3 has different behaviour between cooking methods. After conventional boiling more than 86% of F3 was lost, however after high-pressure the same compound was the less reduced, only by 47%. In turnip greens F6 and F7 showed good retention levels with losses between 55% and 60% after both cooking methods, conventional boiling and high-pressure. After steaming, low hydroxycinnamic acid levels were lost in both plant organs, between 0% and 15% of total quinic acids derivatives and between 22% and 35% of total sinapic acid derivatives (Fig. 1). These minor losses could be due because during steaming inactivation of oxidative enzymes occurs (Vallejo et al., 2003). By contrast, high-pressure and conventional boiling produced losses close to 100% of total quinic acids derivatives in turnip greens (Table 1, Fig. 1). In turnip tops, 3CQAc and 3*p*CoQAc did not show significant losses after conventional boiling. Total sinapic derivatives were lost about 80% in both organs after high-pressure and conventional boiling (Table 1, Fig. 1). The loss rates of hydroxycinnamic acids found in this work were higher than those reported in boiled broccoli by other authors (Gliszczynska-Swiglo et al., 2006; Price et al., 1998; Vallejo et al., 2003). In plants, phenolic compounds occur in soluble forms as well as in combination with cell wall components. Hence, large surface area in contact with the cooking water at high temperature and the long cooking time may have been responsible of the disruption of the cell walls and the compound breakdown causing greater losses of these compounds.

3.2.2. Effect on the summatory of vegetable tissues and cooking water (CW)

The study of CW indicated that all phenolic compounds were recovered after boiling (Table 2, Fig. 2). The analysis of variance of phenolic content in CW showed that in turnip greens there were significant differences among cooking methods for total phenolics content and for most of phenolic compounds ($P \leqslant 0.01$). On the contrary, the analysis of turnip tops did not show differences among cooking methods, which means that the amounts of phenolic compounds recovered were not significantly different from the initial phenolic content of the fresh vegetable. Results showed that total flavonoid recoveries were 100% in steaming samples. After cooked at high-pressure and conventional boiling increases from 5% to 70% in CW in both plant organs were found (Table 2, Fig. 2). The deacylated compounds F1, F6 and F7 are the main contributors to the increase in the concentration of flavonoids in CW respect to the fresh portion due to a greater amount of these flavonoids into the processing water. The high retention of these compounds may be due the conversion of acylated flavonoids into their glycosylated form. Contrary to this, some hydroxycinnamic acids were lost during the cooking process (Table 2, Fig. 2). In tur-

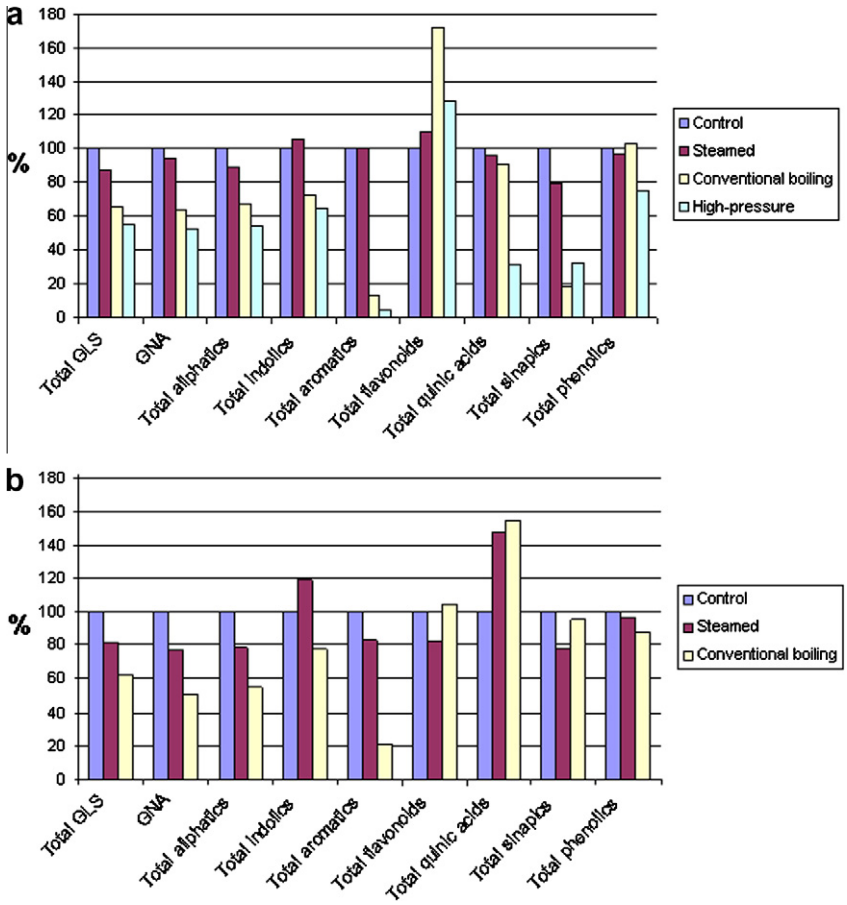

Fig. 2. Content (% of the fresh uncooked control) of the main compounds after different cooking methods in CW turnip greens (a) and CW turnip tops (b).

nip greens, after high-pressure only a 32% of total quinic acids derivatives were recovered while in turnip tops increased the amount of 3CQAc and 3*p*CoQAc specially in CW of high-pressure cooking. Total sinapics in turnip greens were recovered by 80%, 32%, and 18% after steaming, high-pressure, and conventional boiling, respectively. In turnip tops, almost all hydroxycinnamic acids were recovered. Total phenolics levels were recovered almost 100% in both plants organs except after high-pressure cooking. Traditional home cooking of turnip greens and turnip tops is carried out under long cooking times. Zhang and Hamauzu (2004) showed that a 10-fold (from 0.5 to 5 min) prolongation of the conventional cooking time caused up 2-fold total phenolic losses in broccoli and, therefore stability of phenolics strongly depended on cooking time.

3.3. Effect of cooking on vitamin C

The concentration of vitamin C (ascorbic acid, the predominant form of vitamin C) was dramatically reduced by the processing method. The content of vitamin C in fresh turnip greens and turnip tops was 62 mg/100 g fw and 46 mg/100 g fw, respectively. Similar results were described by Mondragón-Portocarrero, Pena-Martínez, Fernández-Fernández, Romero-Rodríguez, and Vázquez-Oderiz (2006) in fresh turnip greens. The fresh material suffered various manipulations before analysis (i.e. freezing, freeze-drying, and grounding) that definitively affected the content of vitamin C in the samples causing a dramatic lost respect to the fresh material (96%). With respect to cooked samples, as expected, vitamin C was decreased after all cooking methods. After steaming treatment, the loss was 64% respect to untreated fresh material and after high-pressure and conventional boiling, vitamin C was not found in the edible parts. Mondragón-Portocarrero et al. (2006) reported loss by 61% after blanching turnip greens in water for 2 min. Other authors showed that the content of ascorbic acid in broccoli declined dramatically during cooking (Vallejo et al., 2002; Zhang & Hamauzu, 2004) having the cooking time a higher influence on ascorbic acid level than any cooking method (Zhang & Hamauzu, 2004). The results obtained in the present study showed that the content of ascorbic acid not only was declined dramatically during the cooking but also in the process of sample handling.

4. Conclusions

Brassica foods include different crops such us cabbage, cauliflower, broccoli, Brussels sprouts, turnips and kale. These vegetables are consumed all year around, and represent worldwide used ingredients of different salads either as raw or frozen vegetables or after domestic processing (cooking). Conventional methods of cooking reduce the intake of potentially health-promoting compounds. Most of reports that studied the effects of cooking methods on *Brassica* vegetables are focused mainly on the preservation of total GLS and phenolic compounds. In this work we conducted a comprehensive study about more than 20 individual GLS and phenolic compounds. The quantification was carried out with a multipurpose method for the simultaneous identification of GLS and phenolics. Results have given us information on the effect of cooking on flavonoids levels, some of them have been studied for first time in this work. It can be concluded that steaming cooking resulted in high retention of the GLS and phenolic compounds. No contact of the vegetables with water during steaming prevents leaching and solubilization of these metabolites in the

cooking water. The other two methods caused similar loss rates, although in high-pressure method, plant material was less time into contact with water. Varieties were affected in the same way by the cooking methods.

In this study we found that the greatest loss of vitamin C happened throughout sample management. This indicates that not only the cooking process but also the manipulation affects the retention of ascorbic acid in the tissues, due to its high degree of water solubility and low stability.

Thus, an appropriate method might be sought for *B. rapa* domestic processing is key to better retain its nutritional value at the maximum level. Our study may help consumers to make their choice of the cooking practices to retain the nutritional quality of turnip greens and turnip tops. In this regards, it is likely that *B. rapa* vegetables cooked by steaming will be better for human consumption than other cooking methods. Although since both phenolic compounds and GLS were present in high quantities in the cooking water after boiling and high-pressure, the use of this water for either soups or gravies should also be considered for increasing the intake of these health-beneficial compounds into the diet.

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CAPÍTULO VII

Estudio sensorial de nabizas y grelos y su relación con metabolitos secundarios

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ORIGINAL PAPER

Sensory quality of turnip greens and turnip tops grown in northwestern Spain

Marta Francisco · Pablo Velasco · Ángeles Romero · Lourdes Vázquez · María Elena Cartea

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Abstract In Galicia (northwestern Spain), *Brassica rapa* var. *rapa* L. includes turnip greens and turnip tops as vegetable products that are characterized by a particular sulfurous aroma, pungent flavor, and a bitter taste. In this work, 12 local varieties grown as turnip greens and turnip tops were evaluated to define the sensory attributes, to relate them with secondary metabolites, and to select those sensorial traits that better describe these crops. Results showed differences in the sensory profiles of *B. rapa* varieties. Turnip greens were significantly different regarding aroma intensity, leaf color, and salty taste, while turnip tops were for color and firmness of leaves, moistness and fibrosity in mouth, sharpness, and bitter taste. Secondary metabolites as glucosinolates in turnip greens and phenolic compounds in turnip tops were highly correlated with texture and flavor. Glucosinolates, especially progoitrin (in turnip greens) and gluconapin (in turnip tops), showed correlation with bitter taste and aftertaste persistence. Correlation between sensory traits showed highest values between leaf firmness and stalk firmness ($R = 0.94$**), leaf firmness and fibrosity ($R = 0.92$**), aftertaste persistence and bitterness ($R = 0.91$**), and between bitterness and moistness ($R = -0.89$**).

Keywords *Brassica rapa* · Turnip greens · Turnip tops · Sensory quality · Flavor

M. Francisco · P. Velasco (✉) · M. E. Cartea
Misión Biológica de Galicia (CSIC),
PO Box 28, 36080 Pontevedra, Spain
e-mail: pvelasco@mbg.cesga.es

Á. Romero · L. Vázquez
Áreas de Nutrición y Bromatología y de Tecnología de Alimentos.
Departamento de Química Analítica, Nutrición y Bromatología,
Universidad de Santiago de Compostela, Facultad de Ciencias.
Campus de Lugo, 27002 Lugo, Spain

Introduction

Brassica rapa vegetable crops from Asian countries as Chinese cabbage, pak Choi or bock Choi have been extensively studied regarding different attributes (agronomic and nutritional) because of the importance of these crops in the Asian diet. Nevertheless, in Europe most research has been focused on different *Brassica oleracea* crops as cabbage, broccoli, or cauliflower since these have a great economic importance in this continent and consequently, studies on nutritional quality of *B. rapa* types are minor. However, leafy forms of *B. rapa* crops are very popular in farming and diet in some European countries as Portugal [1], Spain [2] or Italy [3] where they are traditionally known as 'nabiças, or grelos' and cima di rapa, or Italian turnip', respectively.

In Galicia (northwestern Spain), Brassica crops have been the main source of vegetables for human consumption and also for winter fresh fodder. According to the particularities of Galician agriculture (small familiar farms and traditional cultural practices), farmers obtain their own seeds for sowing. This process has led to a great number of Brassica landraces adapting to different conditions and to different uses all along Galician geography. In this region, *B. rapa* var. *rapa* L. includes turnip greens and turnip tops as vegetable products for culinary profit as well as turnips for fodder. Turnip greens are the young leaves, harvested in the vegetative period, which are characterized by hairy lower leaves, petiolated with broad lateral lobes, which become larger at the top. Turnip tops are the fructiferous stems with the flower buds and the surrounding leaves. Upper flower spike leaves of an oblong spear shape, with two large rounded auricles, hairless and embracing the stem. The harvest of turnip tops occurs in late winter when the flower buds are formed, which are consumed before

opening and while still green. Both are boiled and generally consumed as meat companions. They are characterized by a particular sulfurous aroma, pungent flavor, and a bitter taste, which differentiate them from other Brassica vegetables [4, 5].

Like all *Brassica* species, *B. rapa* crops contain secondary plant metabolites, mainly glucosinolates (which are found almost exclusively in Brassicaceae family) and phenolic compounds including flavonoids, and hydroxycinnamic acids. The presence of these compounds in the diet has increased during the past years because of their beneficial health properties [6]. Moreover, these compounds have been related to the sensorial and nutritional qualities of vegetables. In fact, total glucosinolate content and their breakdown products were associated with sensory attributes in Brassica crops [7–9]. Other authors [5, 10] have reported that bitterness is considerably affected by the gluconapin, an aliphatic glucosinolate.

The cultivation of *B. rapa* takes place during the winter season. In many cases, the same variety can be exploited for several uses (turnips, turnip greens, and turnip tops), preventing the fixing of standard morphological characteristics and allowing the existence of local varieties with high levels of variability. A collection of 200 varieties collected from northwestern Spain was previously evaluated for their agronomic performance [2] as well as for their nutritional value focused on glucosinolate, fiber, and protein content [5]. Besides, a first evaluation regarding sensorial attributes (bitterness and flavor) was carried out with the aim to discard those varieties that did not fit the normal parameters of this crop. As result, varieties were classified based on their morphological and agronomic attributes by using the Ward-MLM method [2]. Based on this previous classification, some varieties, suitable for turnip tops or/and for turnip greens fresh production were selected. Galician local varieties are maintained by local farmers based on their agronomic behavior, but sensory quality was not a criterion to maintain them.

In later years, the importance of the quality of vegetables for consumers has continuously increased. Main criteria are sensory characteristics and higher health benefits. Descriptive sensory analysis can be considered as the first step in the sensory characterization of a food product, providing a pre-defined terminology for describing sensory perceptions as objectively as possible [11]. Sensory profiles in Brassica crops have been determined mainly for *B. oleracea* crops such as Brussels sprouts, broccoli, and cauliflower cultivars [8, 10, 12–14]. However, little information has been reported about descriptive sensory analysis for *B. rapa* crops as turnip tops and turnip greens. Only Jones and Sanders [15] defined a panel based on flavor and aroma traits and found differences among turnip greens varieties and maturity.

The objectives of this study were (1) to define the sensory attributes of a set of *B. rapa* varieties grown as turnip tops and turnip greens in NW Spain, (2) to relate them with the content of secondary metabolites, and (3) to select those sensorial traits that better describe these crops.

Materials and methods

Plant material

Twelve local varieties of *B. rapa* were evaluated in this study (Table 1). From these, ten varieties were chosen based on their agronomic performance for turnip tops and/or turnip greens and two varieties derived from three cycles of masal selection by fresh yield. The variety designation as well as their geographical and source of origin are shown in Table 1. The varieties were evaluated in 2 years (2006 and 2007) at two locations in northwestern Spain: Oroso (A Coruña) (L1) (43°1′N, 8°26′W, 280 m.a.s.l.) and Guitiriz (Lugo) (L2) (43°12′N, 7°53′W, 516 m.a.s.l.). Both locations represent standard *B. rapa* production areas in northwestern Spain. The varieties were planted in multiplot-trays and seedlings were transplanted into the field at the five- or six-leaf stage. Transplanting dates were on the 10th and 19th October in 2006 and on the 01st and 04th September in 2007, in Oroso and Guitiriz, respectively. Varieties were transplanted in a randomized complete block design with three replications. The experimental plots consisted of three rows with ten plants per row. Rows were spaced 0.8 m apart and plants within rows 0.5 m apart. Transplanting was carried out manually according to local practice. A complex mineral fertilizer was added to the soil (8–15–15) at the rate

Table 1 Local varieties of *B. rapa* evaluated in this study

Code name	Origin	Source[1]	Type[2]
MBG-BRS0082	Vilar, Forcarei, Pontevedra	MBG	L
MBG-BRS0143	Lama, Boqueixón, A Coruña	MBG	L
MBG-BRS0163	Barcia, Melón, Ourense	MBG	S
MBG-BRS0173	Valongo, Cortejada, Ourense	MBG	L
MBG-BRS0184	Carballo, A Coruña	MBG	L
MBG-BRS0197	Arnoia, Ourense	MBG	S
MBG-BRS0401	San Xiao, Coirós, A Coruña	MBG	L
MBG-BRS0433	Santiago, A Coruña	MBG	L
MBG-BRS0451	O Val, Narón, A Coruña	MBG	L
MBG-BRS0461	Castro de Rei, Lugo	MBG	L
MBG-BRS0472	Porta, Sobrado, A Coruña	MBG	L
MBG-BRS0550	Trazo, A Coruña	MBG	L

[1] Germplasm bank of the Misión Biológica of Galicia (MBG)

[2] *L* Local variety (without selection), *S* variety derived from three cycles of masal selection by fresh yield

of 412 Kg/ha (33 K/ha N, 62 Kg/ha P_2O_5 and K_2O). For pest control were used Aphox against aphids and Laidan against *Delia radicum* L. Force® was added at the time of transplantation against soil insects. Weed control were made according to local practices. Twenty-five to 40 leaves and shoots from each variety were harvested at each environment. Since trained panel must be done on several days, plant material (leaves and shoots) was sequentially harvested on each environment according to the maturity cycle of each variety at the optimum time for consumption. Leaf harvest ranged from 44 to 98 days after planting while shoot harvest ranged from 114 to 224 days after planting.

Sample preparation

Plant material was collected and immediately carried to the laboratory for the sensory evaluation. The trained panel evaluation lasted several months as no more than three or four varieties per day could be tasted. Samples were cleaned with water, selected, and cut. After this, they were cooked in boiling water (no salt) for 45 min, with 1,000 W heat-plates—in a 1,100-g sample/2 L water proportion. Once the samples were cooked, the excess water was drained off and servings of approximately 100 g were presented to each taster in plates coded with three random digits. The samples were distributed in a complete block design. Evaluation was performed in individual sensory booths with controlled humidity and temperature.

Sensory analysis

Descriptive sensory analyses were carried out according to Alonso-Fernández et al. [16]. Thirteen trained panelists were selected for turnip green and turnip top sensory evaluation in accordance with [17]. Fifteen attributes were considered according to ISO norms [18]: aroma intensity, leaf color, leaf brightness, stalk and leaf firmness, resistance to cutting, moistness and fibrosity in mouth, sharpness, sticks to palate, bitter, acidic, sweet, and salty tastes, aftertaste persistence, and abnormal aroma. All descriptors were quantified using 10-cm no structured intensity scales [19], except abnormal aroma which were evaluated on two-point scales. Reference values for each attribute are shown in Table 2. In all cases a rating of 1 was considered 'slight' and a rating of 10 as 'high'.

Statistical analyses

A combined analysis of variance across environments was performed for each sensory trait. Analysis were made independently for each of the two plant organs evaluated (leaves and shoots). Varieties were considered as fixed effects and environments were considered as random factors. Comparison of means among varieties was made by

Table 2 Sensory traits evaluated in this study using a 10-cm non-structured intensity scales (ISO 4121:1987) according to Alonso-Fernández et al. [16]

Trait	Intensity scale	
	1	10
External aspect		
Leaf color[1]	3,975u	5,815u
Leaf brightness	Dry olive	Wet olive
Aroma		
Aroma intensity	Weak	Strong
Texture in hand		
Stalk firmness	Spaghuetti 5′	Spaghuetti 15′
Leaf firmness	Spaghuetti 5′	Spaghuetti 15′
Resistance to cutting	Asparagus tops	Asparagus stalks
Texture in mouth		
Moistness in mouth	Apple	Bean
Fibrosity in mouth	Asparagus tops	Asparagus stalks
Sharpness	Eggplant	Bean
Sticks to palate	Egg white	Pate
Flavor		
Bitter taste	Weak	Strong
Acidic taste	Weak	Strong
Sweet taste	Weak	Strong
Salty taste	Weak	Strong
Aftertaste persistence[2]	<10 s	60 s

[1] Pantone® COLOR SCALE

[2] Time in seconds

Fisher's protected least significant difference (LSD) at $P = 0.05$ [20]. Simple correlation coefficients ($P < 0.05$) among sensory traits were made in order to determine which traits better explains the sensory attributes of turnip tops and turnip greens. Total and individual glucosinolate content and total and individual phenolic compound content were quantified in the same set of varieties. Part of these results was published by Francisco et al. [21]. Therefore, simple correlations ($P < 0.05$) between these secondary metabolites and sensory characteristics were made in order to establish the relationships between them. All statistical analyses were made using SAS [22].

Results and discussion

Turnip greens

The combined analysis of variance showed significant differences for most traits (aroma intensity, stalk and leaf firmness, resistance to cutting, moistness and fibrosity in mouth, sharpness, sticks to palate, and bitter, acidic, sweet, and salty tastes) among environments (Table 3). Climatic

Table 3 Mean squares of the combined analysis of variance across four environments for sensory traits in the 12 *B. rapa* varieties (turnip greens and turnip tops) from northwestern Spain

	Turnip greens				Turnip tops			
	Environment (E)	Variety (V)	E × V	Error	Environment (E)	Variety (V)	E × V	Error
Aroma intensity	2.495*	1.723*	0.763	1.989	2.004	1.502	1.996	2.221
Leaf color	5.736	9.740*	4.285	3.188	2.913	11.946**	3.319	3.958
Leaf brightness	4.519	2.538	4.883**	2.309	29.387**	5.442	2.908	2.385
Stalk firmness	389.217**	3.728	6.084	4.110	391.556**	8.252	6.190	4.204
Leaf firmness	91.786**	8.501	11.693	8.709	111.577**	10.015*	4.351	4.677
Resistance to cutting	36.377**	7.668	7.813**	3.446	22.440**	18.265**	4.975	5.787
Moistness in mouth	14.005**	0.858	2.018	2.986	6.519*	4.300*	1.700	3.653
Fibrosity in mouth	12.411*	3.392	3.957	3.938	6.898	7.180*	2.488	4.489
Sharpness	25.343**	3.704	4.358*	2.519	20.719**	8.598**	1.844	2.802
Sticks to palate	11.091**	1.033	1.340	2.084	0.773	2.394	2.198	2.808
Bitter taste	16.088**	3.583	2.570	2.340	11.650**	5.183*	1.967	2.625
Acidic taste	14.925**	1.561	1.435	4.401	22.136**	2.597	2.682	5.915
Sweet taste	10.491**	0.957	1.183	3.543	13.486**	2.216	1.529	4.959
Salty taste	12.786**	4.705*	1.612	3.272	31.847**	2.519	1.291	4.183
Aftertaste	1.560	2.431	2.347	2.128	8.124*	3.334	2.566	3.117

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

conditions all along the crop cycle (between September 2006 and May 2008) were very different in each environment, the minimum temperatures and the precipitation between the years 2006 and 2008 being the main factors that considerably affected the sensory attributes, mainly for turnip greens (Fig. 1). Varieties were very similar for most traits and they only significantly differed for aroma intensity, leaf color, and salty taste. The analysis of variance for sensory traits showed a significant environment × variety interaction for leaf brightness, resistance to cutting, and sharpness (Table 3). For these three traits, individual analyses of variance were performed and varieties did not show significant differences among them. Regarding variety performance across environments, 'MBG-BRS0461'showed the highest aroma intensity and the lowest leaf color, and salted taste. By the other side, 'MBG-BRS0163' showed high salted taste, the lowest aroma intensity, and the highest leaf color (Table 4). A descriptive profile graphic of the 12 varieties in turnip greens and turnip tops is shown in Fig. 2.

Glucosinolates and phenolic compounds are secondary metabolites found in large quantities in *B. rapa* and responsible, among other roles, for the typical bitter taste and characteristic aroma of Brassica crops. The correlations between sensory traits with the content of aliphatic, indolic, aromatic, and total glucosinolate content as well as the correlations between sensory traits with the content of phenolic compounds are showed in Table 6. Data show that most correlations between glucosinolates and sensory traits were low and non significant. The most remarkable was the negative relationship between progoitrin with bitter and salty taste,

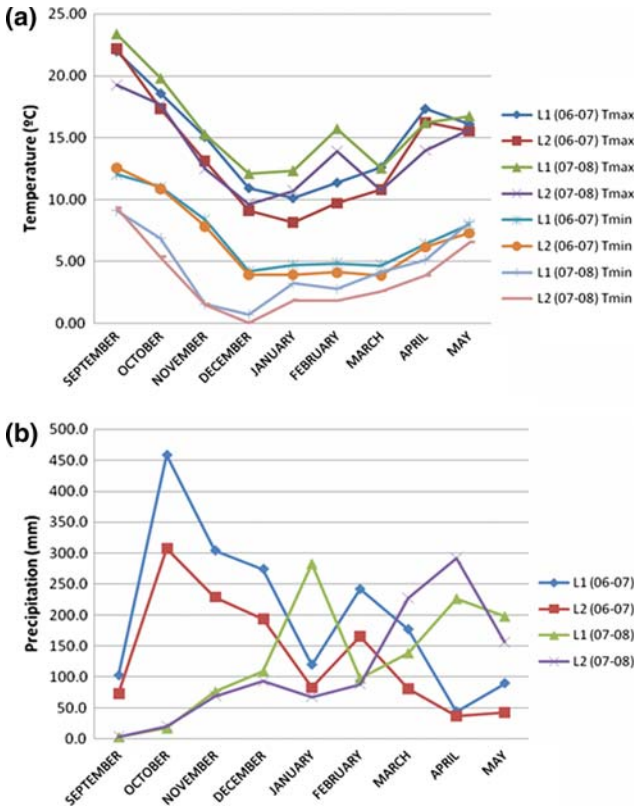

Fig. 1 Graphic representation of **a** minimum and maximum temperatures and **b** precipitations in two locations from 2006 to 2008

aftertaste presistance, and stickiness to palate (−0.69**, −0.60**, −0.74**, and −0.79**). Walters [23] found a very close relationship between bitter and sweet tastes,

Table 4 Mean of sensory traits for the 12 turnip green varieties evaluated in this study in two locations and 2 years in northwestern Spain

Variety	Aroma intensity	Leaf color	Leaf brightness	Stalk firmness	Leaf firmness	Resistance to cutting	Moistness in mouth	Fibrosity in mouth
MBG-BRS0082	5.64ab	6.04bc	5.57ab	5.07a	4.83ab	5.07bc	6.91a	5.74abc
MBG-BRS0143	5.14ab	6.48abc	5.62ab	4.81ab	5.87a	5.76ab	7.07a	6.04ab
MBG-BRS0163	4.85b	7.30a	5.39ab	1.44d	2.40c	4.29c	5.88b	4.77c
MBG-BRS0173	5.40ab	6.35abc	5.36ab	4.75ab	4.70ab	5.60ab	6.92a	5.82abc
MBG-BRS0184	5.19ab	5.87cd	5.39ab	5.33a	5.08ab	5.30bc	6.94a	5.61abc
MBG-BRS0197	5.14ab	6.15bc	4.47c	2.97c	3.90bc	5.15bc	6.47ab	5.13bc
MBG-BRS0401	4.96ab	6.90abc	5.69a	4.74ab	4.54ab	5.34bc	6.93a	6.10ab
MBG-BRS0433	5.26ab	6.43abc	5.62ab	3.81bc	3.98bc	5.98ab	6.84ab	5.95ab
MBG-BRS0451	5.61ab	7.05ab	5.54ab	5.24a	6.02a	6.49a	6.99a	6.01ab
MBG-BRS0461	5.73a	4.90d	5.51ab	5.17a	5.23ab	4.96bc	6.88ab	5.42abc
MBG-BRS0472	4.99ab	6.40abc	4.77bc	5.15a	4.90ab	5.24bc	6.77ab	6.22ab
MBG-BRS0550	5.22ab	6.84abc	5.32abc	5.26a	5.24ab	5.96ab	7.14a	6.32a

Variety	Sharpness	Sticks to palate	Bitter taste	Acidic taste	Sweet taste	Salty taste	Aftertaste
MBG-BRS0082	4.77abc	3.43b	5.92 cd	3.15b	3.16a	3.58b	6.44c
MBG-BRS0143	5.17ab	3.69ab	6.21bcd	3.66ab	3.11a	4.26ab	6.88abc
MBG-BRS0163	4.73abc	4.46a	7.11a	4.42a	2.99a	5.08a	7.17abc
MBG-BRS0173	5.55a	4.07ab	6.85ab	3.92ab	2.92a	4.58ab	7.52a
MBG-BRS0184	5.03abc	3.31b	6.19bcd	3.59ab	2.86a	3.99b	6.85abc
MBG-BRS0197	5.29a	4.00ab	6.81abc	3.78ab	3.11a	5.20a	7.35ab
MBG-BRS0401	5.42a	4.01ab	6.49abcd	3.80ab	2.83a	4.58ab	6.86abc
MBG-BRS0433	5.44a	3.83ab	5.94cd	3.19b	3.20a	4.15ab	6.83abc
MBG-BRS0451	5.02abc	3.66ab	5.74d	3.41ab	3.38a	3.82b	6.61bc
MBG-BRS0461	4.25bc	3.74ab	6.35abc	3.65ab	3.03a	3.54b	6.70abc
MBG-BRS0472	4.88abc	3.87ab	6.18bcd	3.41ab	3.01a	4.48ab	6.62bc
MBG-BRS0550	4.18c	3.48b	5.79d	3.43ab	3.33a	4.37ab	6.45c

Means with the same letter in the same column are not significantly different at $P \leq 0.05$

which could be explained the negative values found in our work for bitter or salty taste. Progoitrin is not the main glucosinolate found in leaves of turnip greens, but it has been often shown to be related to bitterness and taste preference in Brussels sprouts [8, 12]. Progoitrin has been defined as a non-bitter glucosinolate. However, it can be degraded enzymatically by the enzyme thioglucosidase or by heat treatment with the extremely bitter compound goitrin [8].

The concentration of chlorogenic and sinapic acids affects considerably the sensory quality of food, since they contribute to enzymatic browning of food products, thus inducing their astringency and bitter taste [24]. Hydroxycinnamic acids are present in high amounts in turnip greens. However, as far as we know, information about the relationship between sensory traits characteristics of this crop such as bitterness, aroma, or taste with flavonoids and hydroxycinnamic acids is lacking. Thus, this work means a real improvement in the study of nutritional quality of this crop. Some sensory traits evaluated in turnip greens seem to have important relationships with some phenolic compounds. For instance, moistness had positive and high correlations with total phenolic compound content ($R = 0.75^{**}$) and with total hydroxycinnamic acids ($R = 0.74^{**}$), whereas stalk firmness had positive and high correlations with hydroxycinnamic acids ($R = 0.85^{**}$). Even if glucosinolates and flavonoids are not structural compounds of the plant, it seems that there is a kind of relationship among plant structure and these compounds, which could be related to the plant health status and the roles of flavonoids and glucosinolates such as providing protection against ultraviolet radiation, pathogens, and insect attack. All traits related to flavor (except sweetness) showed negative and significant correlation coefficients with total hydroxycinnamic acids and total phenolic compound content (ranging from $R = -0.58^{*}$ to $R = -0.82^{**}$). Total flavonoids only showed a significant and moderate relationship with acidic taste. Most of the literature related flavonoids with bitter, acidic or astringent tastes [25] but minor alterations in the flavonol structure can change their taste from bitter to sweet or the other way around [26].

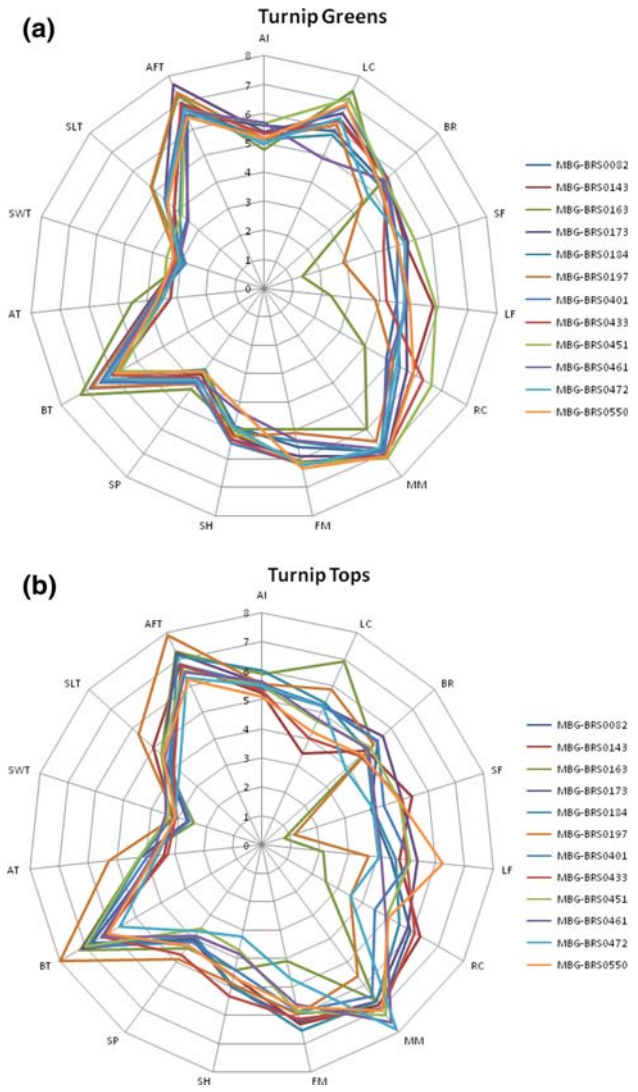

Fig. 2 Descriptive sensory analysis of 12 varieties in turnip greens **a** and turnip tops **b**. For abbreviations see Table 7

As summary, sensory traits evaluated in turnip greens seem to be more related to hydroxycinnamic acids and flavonoid compounds than to glucosinolates. Hydroxycinnamic acids and total phenolic compound content were positively related to firmness traits and negatively related to flavor traits.

Turnip tops

Similarly, to turnip greens, there were significant differences among environments regarding most traits, highlighting the importance of climatic conditions upon the sensorial quality of these crops. Thus, the choice of a particular variety in basis of its sensorial value should be done on many sites and years. The combined analysis of variance did not show any significant environment × variety interaction, which means the stability of different genotypes. Varieties were significantly different regarding color and firmness of leaves, moistness and fibrosity in mouth, sharpness, and bitter taste. Because of this variability, it would be possible to select in the future a particular variety according to consumer preferences. As well in turnip greens, 'MBG-BRS0163' displayed the highest leaf color. Besides, this variety had the lowest leaf firmness, fibrosity in mouth, stalk firmness, and resistance to cutting even though no differences for these last two traits were found among varieties. 'MBG-BRS0143' had the lowest leaf color and the highest fibrosity in mouth. With regard to the bitter taste, typical of this crop, the variety 'MBG-BRS0197' was the bitterest, whereas 'MBG-BRS0472' was the least bitter (Table 5). Although bitterness is usually considered as an unfavorable flavor trait, a certain degree of bitterness is appreciated by consumers because it is a typical characteristic of this vegetables.

Correlations between glucosinolates and phenolic compounds were also calculated for turnip tops (Table 6). In contrast to turnip greens, sensory traits evaluated in turnip tops seem to be more related to glucosinolates than to phenolic compounds. Total glucosinolate concentration in turnip tops (25.6 μm g^{-1}) was higher than in turnip greens (17.6 μm g^{-1}). This difference was due to aliphatic glucosinolates (20.6 and 12.8 μm g^{-1}, respectively), which can explain the higher importance of glucosinolates on the sensory traits of turnip tops. Similar to turnip greens, information about the relationship between sensory traits and glucosinolates, and flavonoids content on turnip tops is scarce. Our study proves that some traits defining the texture and taste were related to glucosinolate content. For example, leaf and stalk firmness and resistance to cutting had negative, significant and moderate to high correlations (ranging from $R = -0.58^*$ to $R = -0.88^{**}$) with indolic glucosinolate content and with the aromatic glucosinolate GST. On the other hand, bitter taste, acidic taste, and aftertaste showed moderate correlations (from $R = 0.61^*$ to $R = 0.74^{**}$) with indolic, aliphatic, aromatic, and total glucosinolate content. Regarding individual glucosinolate composition, gluconapin (the major glucosinolate in these crops) showed positive and significant correlations with aftertaste, moistness, acidic and bitter tastes. For sweet taste this correlation was negative ($R = -0.59^*$). In broccoli and cauliflower Brückner et al. [27] showed that sweetness was high and negatively related to the total glucosinolate content, which in turn coincided closely with bitter and pungent tastes.

Hydroxycinnamic acids and flavonoids had insignificant relationship with the sensory traits evaluated in turnip tops. Therefore, its worth pointing out that flavonoids kaempferol-3-O-(caffeoyl)sophoroside-7-O-glucoside and quercetin-3-O-(caffeoyl)sophoroside-7-O-glucoside displayed correlations highest than $R = 0.60$ for leaf and stalk firmness (once

Table 5 Mean of sensory traits for the 12 turnip tops varieties evaluated in this study in two locations and 2 years in northwestern Spain

Variety	Aroma intensity	Leaf color	Leaf brightness	Stalk firmness	Leaf firmness	Resistance to cutting	Moistness in mouth	Fibrosity in mouth
MBG-BRS0082	5.37a	5.26bc	5.31ab	3.96d	4.47bc	5.88ab	6.92ab	5.91ab
MBG-BRS0143	5.26a	3.47d	4.87abc	5.63ab	4.83bc	6.29a	6.86ab	6.31a
MBG-BRS0163	5.89a	6.90a	5.11abc	0.80e	2.11d	2.52e	6.58abc	4.09c
MBG-BRS0173	5.53a	5.22bc	5.60a	5.05abcd	5.36ab	5.75ab	6.67abc	6.22a
MBG-BRS0184	6.00a	5.35bc	4.82abc	4.00d	4.62bc	5.45abc	6.70abc	6.51a
MBG-BRS0197	5.57a	5.86ab	5.18abc	1.18e	3.68c	3.51de	5.61c	5.96ab
MBG-BRS0401	5.52a	5.27bc	5.34ab	4.39bcd	5.11ab	4.45bcd	6.45bc	5.86ab
MBG-BRS0433	5.30a	4.14cd	4.60bc	5.23abcd	5.11ab	6.07a	7.08ab	6.05ab
MBG-BRS0451	5.54a	4.56cd	5.33ab	5.80a	5.48ab	4.89abcd	7.32ab	5.81ab
MBG-BRS0461	5.65a	4.75bc	5.00abc	4.27cd	4.22bc	4.95abcd	7.55ab	5.64ab
MBG-BRS0472	5.55a	5.02bc	4.33c	5.32abc	5.05b	4.09cd	7.70a	4.75bc
MBG-BRS0550	5.07a	4.21cd	4.70abc	5.46abc	6.48a	5.03abcd	7.08ab	5.57ab

Variety	Sharpness	Sticks to palate	Bitter taste	Acidic taste	Sweet taste	Salty taste	Aftertaste
MBG-BRS0082	4.93a	4.08ab	6.73bc	3.61b	3.11a	4.17bc	6.72b
MBG-BRS0143	4.58ab	4.24ab	6.21bcd	3.38b	3.20a	5.00ab	6.82ab
MBG-BRS0163	4.40ab	4.38ab	7.23ab	4.28ab	2.48a	4.66abc	7.29ab
MBG-BRS0173	5.00a	3.98ab	7.10ab	4.19ab	2.68a	4.55abc	7.23ab
MBG-BRS0184	4.97a	3.90ab	6.87bc	4.19ab	3.19a	4.24bc	7.10ab
MBG-BRS0197	4.88a	4.85a	8.00a	5.28a	3.15a	5.71a	7.92a
MBG-BRS0401	4.40ab	4.11ab	6.98abc	4.00ab	2.63a	4.32bc	6.78b
MBG-BRS0433	5.27a	4.65ab	6.40bcd	3.18b	3.01a	3.96bc	6.80ab
MBG-BRS0451	3.82bc	3.68b	6.95bc	3.93ab	3.28a	4.34bc	6.59b
MBG-BRS0461	3.81bc	3.82ab	6.34bcd	3.74ab	3.43a	4.46abc	6.54b
MBG-BRS0472	3.10c	4.09ab	5.61d	3.38b	2.84a	3.48c	6.29b
MBG-BRS0550	4.47ab	4.44ab	5.98cd	3.54b	3.04a	4.39bc	6.19b

Means with the same letter in the same column are not significantly different at $P \leq 0.05$

again negatives) and for taste traits (acidic, salty, bitter, and aftertaste persistence). The highest coefficient correlation was found between salty taste and quercetin-3-O-(caffeoyl) sophoroside-7-O-glucoside ($R = 0.82$). The highest difference between turnip greens and turnip tops was found in the hydroxycinnamic acid content. Turnip greens had 27 μm g^{-1} of hydroxycinnamic acids concentration and turnip tops 19.3 μm g^{-1}, which can partially explain the minor importance of these compounds on the flavor of turnip tops.

In summary, indolic and aromatic glucosinolates seem to be more related to traits indicative of texture while all glucosinolate types (indolic, aliphatic and aromatic) seem to affect considerably flavor traits, mainly bitterness, acidic taste, and aftertaste.

Selection of sensorial traits

Simple correlation coefficients among all sensory traits were calculated to determine which trait gives a better measure of sensorial value in turnip greens and turnip tops (Table 7). Two attributes related to product appearance, i.e., aroma intensity and leaf brightness, and one trait related to preference, i.e., sharpness, were not correlated with any other trait. Therefore, these traits have not been useful to describe the flavor attributes of turnip tops and turnip greens but, depending on the consumer preferences, may play a main role in the evaluation of the products.

The highest correlation (0.94**) was found between two traits related to hand texture, leaf firmness, and stalk firmness. As it was previously explained, leaves and shoots are the plant parts consumed regarding turnip greens and turnip tops, respectively. Thus, leaf firmness would be associated with turnip greens, whereas stalk firmness would be associated with turnip tops. Regarding correlation coefficients altogether, three groups of relationships among the sensory traits evaluated could be differentiated. First, relations between traits linked to texture in hand (leaf firmness, stalk firmness, resistance to cutting) and texture in mouth as

Table 6 Simple correlations among sensory traits and glucosinolates and phenolic compounds content

	Glucosinolates				Phenolic compounds		
	Aliphatic	Indolic	Aromatic	Total	Flavonoids	Hydroxycinnamic acids	Total
Turnip greens							
Aroma intensity	0.22	−0.11	0.03	0.17	0.16	0.57	0.50
Leaf color	0.18	0.20	0.17	0.23	0.03	−0.38	−0.28
Leaf brightness	0.32	−0.20	0.09	0.27	−0.24	0.27	0.11
Stalk firmness	0.51	−0.49	−0.02	0.41	0.46	0.85**	0.82
Leaf firmness	0.43	−0.28	0.07	0.35	0.57	0.73	0.77
Resistance to cutting	0.44	0.02	0.28	0.42	0.54	0.42	0.51
Moistness in mouth	0.51	−0.33	0.09	0.43	0.51	0.74**	0.75**
Fibrosity in mouth	0.32	−0.30	0.05	0.25	0.57	0.55	0.63*
Sharpness	0.22	−0.34	−0.15	0.25	−0.11	−0.28	−0.27
Sticks to palate	−0.03	−0.04	−0.51	0.07	−0.54	−0.72**	−0.75**
Bitter taste	0.01	0.01	−0.55	0.10	−0.58	−0.76**	−0.79**
Acidic taste	0.11	0.26	−0.21	0.19	−0.64*	−0.76**	−0.82**
Sweet taste	0.10	0.37	0.42	0.11	0.54	0.36	0.47
Salty taste	−0.05	0.20	−0.28	0.05	−0.16	−0.79**	−0.67*
Aftertaste	0.41	0.02	−0.44	0.50	−0.42	−0.58*	−0.61*
Turnip tops							
Aroma intensity	0.31	0.36	0.35	0.37	0.26	0.47	0.41
Leaf color	0.55	0.76**	0.73**	0.61*	0.27	0.43	0.39
Leaf brightness	0.55	0.33	0.42	0.55	0.11	0.35	0.27
Stalk firmness	−0.38	−0.88**	−0.76**	−0.46	−0.23	−0.36	−0.33
Leaf firmness	−0.05	−0.58*	−0.65*	−0.17	−0.30	−0.50	−0.47
Resistance to cutting	−0.31	−0.70**	−0.68*	−0.39	−0.19	−0.10	−0.15
Moistness in mouth	−0.60*	−0.59*	−0.58	−0.62*	0.01	−0.11	−0.06
Fibrosity in mouth	0.01	−0.48	−0.45	−0.07	−0.30	−0.30	−0.33
Sharpness	0.32	0.13	−0.05	0.26	−0.19	0.21	0.04
Sticks to palate	0.27	0.44	0.30	0.27	−0.29	−0.01	−0.14
Bitter taste	0.65*	0.68*	0.67*	0.70*	0.07	0.22	0.17
Acidic taste	0.69*	0.74**	0.70*	0.73**	0.09	0.05	0.07
Sweet taste	−0.54	−0.40	−0.55	−0.58*	−0.13	−0.38	−0.30
Salty taste	0.36	0.57	0.53	0.40	0.17	0.06	0.12
Aftertaste	0.61*	0.61	0.70*	0.67*	0.04	0.27	0.19

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

fibrosity. Coefficients among these traits were higher than $R = 0.86$ and the highest value ($R = 0.92$**) was found between leaf firmness and fibrosity. This suggests that fibrosity of plant samples detected by panelist increased with firmer leaves . The second type of remarkable correlations was found between flavor traits (bitter, salty, and acidic tastes) and aftertaste persistence. It is well known that a flavor more intense remains more time after eating, i.e., it is more persistent. In this case, the highest correlation was found between aftertaste persistence and bitterness ($R = 0.91$**). Finally, the third kind of relationship was found between flavor traits (bitter, salty, acidic and aftertaste and acidic tastes) with moistness in mouth. Among these, the highest coefficient value was found between bitterness and moistness ($R = -0.89$**). Coefficients were always negative suggesting that as moistness in mouth increases, scale values for bitter, acidic, and salty tastes (but not for sweet taste) decrease. In addition, a significant and high correlation was also found among salty, acidic and bitter tastes. A possible explanation is that flavor is probably very complex and difficult to evaluate objectively. The separate identification of each flavor trait is difficult because flavors are usually mixed, and they are often misunderstood.

Table 7 Simple correlations among sensory traits on 12 *B. rapa* varieties grown in four environments in northwestern Spain

	LC	BR	SF	LF	RC	MM	FM	SH	SP	BT	AT	SWT	SLT	AFT
AI	−0.21	0.37	0.05	−0.01	0.03	0.15	−0.14	−0.17	−0.44	0.17	0.16	0.21	−0.37	0.09
LC		−0.03	−0.65*	−0.62*	−0.68*	−0.67*	−0.62*	0.15	0.51	0.61*	0.58*	−0.63*	0.49	0.43
BR			0.27	0.20	0.37	0.11	0.24	0.33	−0.21	0.16	−0.02	−0.25	−0.24	0.06
SF				0.94**	0.86**	0.90**	0.87**	−0.12	−0.74**	−0.74**	−0.68*	0.37	−0.64*	−0.59*
LF					0.87**	0.76**	0.92**	−0.10	−0.71*	−0.67*	−0.53	0.51	−0.44	−0.53
RC						0.68*	0.91**	0.26	−0.54	−0.60*	−0.66*	0.48	−0.47	−0.37
MM							0.60*	−0.43	−0.77**	−0.89**	−0.81**	0.49	−0.85**	−0.82**
FM								0.25	−0.53	−0.56	−0.54	0.37	−0.35	−0.35
SH									0.38	0.39	0.09	−0.34	0.31	0.58*
SP										0.66*	0.47	−0.48	0.76**	0.66*
BT											0.89**	−0.57	0.75**	0.91**
AT												−0.438	0.77**	0.77**
SWT													−0.32	−0.50
SLT														0.77**

AI Aroma intensity, *LC* leaf color, *BR* leaf brightness, *SF* stalk firmness, *LF* leaf firmness, *RC* resistance to cutting, *MM* moistness in mouth, *FM* fibrosity in mouth, *SH* sharpness, *SP* sticks to palate, *BT* bitter taste, *AT* acidic taste, *SWT* sweet taste, *SLT* salty taste, *AFT* aftertaste

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

Conclusions

Brassica rapa varieties from northwestern Spain showed differences in their sensory profiles. This variability could be used to select the best variety for sensorial characteristics according to vegetable market and consumer preferences. Secondary metabolites as glucosinolates and phenolic compounds, which play a crucial role in fruit and vegetable quality, were responsible of texture and flavor, depending on the organ evaluated. So, glucosinolates in turnip tops and hydroxycinnamic acids and flavonoids in turnip greens were found to be related to some sensory traits responsible for taste and firmness. Regarding phenolic compounds, correlations with structural and flavor traits were found, thus indicating the relationship of these compounds with the cell wall integrity as well as characteristic taste. Hydroxycinnamic acids play a very important role in the life of the cell wall. They are principal components governing cell wall integrity, shape, and defense against pathogenic access [28]. Glucosinolates, especially progoitrin (in turnip greens) and gluconapin (in turnip tops), showed high correlation with flavor traits as bitter taste and aftertaste persistence. Hence, these compounds may be mainly responsible for the mostly disliked bitter and pungent taste in these vegetables, leading to low consumer acceptability of some Brassica crops [10, 15, 25]. Some sensory traits evaluated in this study, mainly those related to texture as leaf and stalk firmness and resistance to cutting and those related to taste as bitter, salty and aftertaste could be used as important parameters for measuring the sensorial value of turnip tops and turnip greens. This first study has allowed us not only to define the sensory attributes of these crops but also to select those traits that would be good candidates for a rapid screening of material. This invaluable information would help us to improve the quality and potential health value of turnip greens and turnip tops.

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CAPÍTULO VIII

Discusión

8. DISCUSIÓN

El crecimiento y desarrollo de las plantas depende del genotipo y de diversos factores abióticos y bióticos (Boyer, 1982). Los factores abióticos incluyen las condiciones ambientales y los factores bióticos incluyen daños ocasionados por diferentes patógenos (plagas y enfermedades). Cada cultivo tiene además diferentes requisitos medioambientales. Para lograr el máximo potencial de rendimiento, un cultivo debe crecer en un entorno particular que cumpla estos requisitos. Así, unas condiciones ambientales desfavorables pueden producir un estrés en las plantas reduciendo su rendimiento (Diepenbrock, 2000). Por lo tanto, uno de los objetivos primordiales de los programas de mejora es obtener cultivos de alto rendimiento y una amplia gama de adaptabilidad a condiciones edáficas y climáticas.

El estudio de diferentes caracteres agronómicos en 12 variedades de *B. rapa* cultivadas en varias localidades a lo largo de tres años permitirá escoger las variedades más adecuadas para la producción en fresco de nabizas y de grelos. Las variedades más aptas para la producción de nabizas deberán tener un crecimiento rápido y vigoroso de las hojas lo cual conlleva una elevada producción en fresco, mientras que un número abundante de tallos secundarios, una floración precoz y una elevada producción son características deseables para la producción de grelos (Monteiro y Dias, 1996; Padilla y otros, 2005). Para la producción de grelos, desde el punto de vista del agricultor-productor, el período de floración es un carácter de gran importancia ya que define la precocidad del material. Las variedades tardías retrasan la producción de grelo mientras que las variedades precoces permiten ofrecer grelos en el mercado antes de las fechas habituales. Lo ideal sería identificar variedades productivas con distinta precocidad para así obtener grelos en fechas escalonadas en el mercado durante un mayor periodo de tiempo. Cabe destacar que un período corto de producción de grelo se asocia a una mayor sincronía de floración entre las plantas de la misma variedad, lo que permitirá una cosecha uniforme, mientras que largos períodos de producción se asocian a una baja sincronía con lo cual será necesario realizar cosechas sucesivas en el tiempo y se considera generalmente una desventaja para esa variedad. El carácter vigor temprano es importante en nabizas ya que se refiere a la capacidad de una variedad de competir con las malas hierbas en los primeros estadios después del trasplante. En base

a todas estas características agronómicas, las variedades más destacadas *a priori* para la producción en fresco de nabizas serían MBG-BRS163, MBG-BRS0184 y MBG-BRS0451 mientras que dos variedades, MBG-BRS0143 y MBG-BRS0472, serían las más destacadas para la producción de grelos. Por otro lado, las variedades MBG-BRS0550 y MBG-BRS0082 compartieron características idóneas para la producción de ambos cultivos. Cabe destacar que estas dos últimas variedades junto con MBG-BRS0184 y MBG-BRS0433 son variedades muy tardías, con fechas de recolección de grelos fuera de la época habitual para este tipo de hortalizas. Este hecho hace que la producción de estas variedades sea muy dependiente de las condiciones ambientales. Por lo tanto, y aunque en algunos años y localidades estas cuatro variedades fueron las mejores para la producción de grelos, en otros años se perdió la cosecha. Estas variedades no serían pues deseables para la producción de este cultivo y, debido a ello, se eliminaron de los posteriores análisis centrados en el rendimiento de grelo que serán discutidos a continuación.

Aunque el rendimiento es el resultado de los efectos del genotipo (G), del ambiente (E) y de la interacción genotipo × ambiente (GE), solamente el efecto principal del genotipo y de la interacción GE son de relevancia en la evaluación de los genotipos (Yan y otros, 2000). Diferentes métodos multiplicativos, como por ejemplo el análisis AMMI (Additive Main Effects and Multiplicative Interaction) o el análisis SREG (Sites Regresion Method) han sido descritos con la idea de obtener una información estadística más completa sobre el comportamiento de los genotipos de la que tradicionalmente ofrecen los ANOVA (Crossa y Cornelius, 1997). Estos análisis consisten esencialmente en combinar las técnicas del análisis de varianza y el análisis de componentes principales en un sólo modelo, siendo útiles para estimar patrones de respuesta de los ambientes y de los genotipos y permiten un claro entendimiento de la interacción GE, pero además, el análisis SREG tiene otras ventajas adicionales. El modelo SREG incluye G+GE en el termino bilineal y proporciona un análisis gráfico de fácil interpretación del comportamiento de los genotipos más el efecto de la interacción GE, denominado biplot GGE (Yan y otros, 2000). Así, es posible identificar los genotipos de mayor producción en cada ambiente, pero además, es posible caracterizar aquellos genotipos que exhiben mayor estabilidad.

El análisis SREG sobre la producción en fresco de nabizas y grelos ha puesto de manifiesto que este carácter está muy influenciado por el G, el cual es responsable de más del 64% de la varianza del término G+GE. Para los dos cultivos, los genotipos más productivos lo fueron para casi todos los ambientes, por tanto la interacción GE fue no cruzada. El GGE biplot mostró que las variedades más productivas para nabizas son MBG-BRS0550, MBG-BRS0082 y MBG-BRS0184. En una evaluación previa llevada a cabo por Padilla y otros (2005), MBG-BRS0082 y MBG-BRS0184 habían sido también variedades apropiadas para la producción de nabizas. Las variedades más idóneas para la producción de grelos fueron MBG-BRS0143 y MBG-BRS0472. Además, esta última variedad fue muy estable ya que presentó altas producciones en todos los ambientes donde se llevó a cabo la evaluación. De todos los ambientes estudiados, Salcedo 2008 y Oroso 2008 fueron los más productivos para nabizas y grelos, respectivamente. Los ambientes más estables fueron Guitiriz 2008 y Salcedo 2008 para los dos cultivos. Por tanto, existe variabilidad genotípica y ambiental en la producción de ambos cultivos, hecho que hace posible seleccionar variedades en función de su uso y elegir aquellos ambientes más idóneos para su producción. Además, las diferencias encontradas en el ciclo de cada variedad facilitará la producción escalonada de grelos aumentando su oferta en el mercado y/o en empresas conserveras.

El consumo de vegetales del género *Brassica* se ha relacionado inversamente con el riesgo de padecer cierto tipo de enfermedades relacionadas con procesos oxidativos como son algunos tipos de cáncer, enfermedades neurodegenerativas y cardiovasculares. Esta asociación se ha atribuido a la presencia de una gran variedad de nutrientes como vitaminas, minerales y proteínas (Liu, 2004). Estos vegetales contienen además grandes cantidades de compuestos fenólicos y son fuentes únicas de compuestos azufrados denominados glucosinolatos. Estos fitoquímicos ejercen su efecto sobre una variedad de funciones fisiológicas incluyendo la actividad antioxidante, la regulación enzimática y el control de la apoptosis y el ciclo celular, funciones que actúan todas ellas en beneficio de la salud humana (Holst y Williamson, 2004; Cartea y Velasco, 2008; Traka y Mithen, 2008). Este hecho ha conferido a las brásicas la categoría de "alimentos funcionales" (Fahey y Kensler, 2007).

Los cultivos del género *Brassica*, especialmente los de la especie *B. oleracea*, han sido ampliamente investigados en relación a su perfil y contenido en glucosinolatos (Kushad y otros, 1999; Ciska y otros, 2000; Padilla y otros, 2007; Cartea y otros, 2008) y compuestos fenólicos (LLorach y otros, 2003; Vallejo y otros, 2004; Ferreres y otros, 2005; 2008; Fernandes y otros, 2007; Sousa y otros, 2008). Estos compuestos han sido estudiados de forma independiente mediante el uso de diferentes métodos de extracción e identificación para glucosinolatos y fenoles. En el presente trabajo y, por primera vez en un cultivo del género *Brassica*, se han identificado y caracterizado de forma simultánea diferentes glucosinolatos intactos y compuestos fenólicos mediante el método de cromatografía líquida con detector de diodos acoplado a un detector de masas (LC / UV-PAD / ESI-MSn). De este modo, doce glucosinolatos intactos, pertenecientes a las tres clases (alifáticos, indólicos y aromáticos) y más de 30 compuestos fenólicos (flavonoides y ácidos hidroxicinámicos) se identificaron en nabizas y grelos.

Ambas partes de la planta compartieron el mismo perfil de compuestos bioactivos si bien difirieron en su concentración. Esta diferente concentración de glucosinolatos entre órganos de la planta ha sido descrita anteriormente por otros autores. Brown y otros (2003) encontraron que en *Arabidopsis,* las semillas contienen mayor concentración de glucosinolatos seguida de las inflorescencias, silicuas, hojas y raíces. En el presente estudio, se ha encontrado que el contenido total en glucosinolatos fue mayor en grelos que en nabizas, resultados que concuerdan con Bellostas y otros (2007) los cuales encontraron que en *B. rapa* el contenido en glucosinolatos aumenta en los estados reproductivos. Los glucosinolatos alifáticos fueron predominantes, llegando a representar más del 70% y el 80% del contenido total en glucosinolatos en nabizas y grelos, respectivamente. Al igual que otros cultivos de la misma especie, la gluconapina fue el glucosinolato mayoritario seguido por la glucobrasicanapina (Rosa, 1997; Padilla y otros, 2007; Yang y Quirós, 2010). Respecto a los glucosinolatos indólicos, para este grupo de compuestos no se encontraron diferencias entre las dos partes de la planta evaluadas (nabizas y grelos).

Al contrario de lo que ocurría con los glucosinolatos, el contenido total en compuestos fenólicos fue mayor en nabizas que en grelos. Los ácidos hidroxicinámicos

fueron los compuestos fenólicos mayoritarios en todas las variedades de *B. rapa* evaluadas, siendo el ácido sinápico el principal compuesto en ambos órganos. También cabe destacar la presencia de compuestos derivados de los ácido sinápico unidos a glucosas (gentobiósidos), especialmente en grelos, proporcionando un valor nutricional añadido a este cultivo debido a que estos compuestos son muy eficaces en la prevención del daño lipídico causado por radicales libres de oxígeno (Plumb y otros, 1997).

Al igual que otros cultivos de la misma especie, la mayoría de flavonoides encontrados en nabizas y grelos fueron derivados del kaempferol (Romani y otros, 2006; Ferreres y otros, 2008), pero además, y a diferencia de los cultivos de la especie *B. oleracea* también se encontraron derivados de isorhamnetina, siendo isorhamnetina-3,7-di-*O*-glucósido uno de los flavonoides mayoritarios. Este diferente patrón fenólico podría servir como un marcador bioquímico de estos cultivos, tal y como han señalado Romani y otros (2006).

Debido a que la concentración final de glucosinolatos y fenoles depende, además del genotipo, de diferentes factores ambientales, se llevó a cabo el estudio del efecto del genotipo (G) y del ambiente (E) en los niveles de estos compuestos bioactivos. El análisis de regresión SREG ha sido descrito como un modelo adecuado para estudiar la influencia del G, el E y su correspondiente interacción (GE) sobre un carácter determinado (Yan y otros, 2000). Aunque este método ha sido utilizado mayoritariamente para conocer la estabilidad del carácter rendimiento en un conjunto de ambientes diversos, hoy en día se está aplicando en el estudio de la estabilidad de otros caracteres de interés en la mejora genética en diferentes cultivos. Entre ellos cabe citar el estudio de la relación entre hospedador y patógeno (Yan y Falk, 2002), interacciones entre QTLs y ambiente (Yan y Tinker, 2005) y el estudio de correlaciones entre un gen y el ambiente (Yan y Rajcan, 2002; Lee y otros, 2003).

El E afectó de forma significativa a la concentración final de glucosinolatos, tanto de indólicos como de aromáticos, especialmente para la concentración de indólicos totales en grelos, donde el E explicó el 71% de la variación total, mientras que el G explicó el 5% y la interacción GE el 23%. Si nos centramos en el término G + GE, para los glucosinolatos alifáticos el G fue responsable de más del 60% de la varianza de

dicho término, mientras que para los glucosinolatos indólicos, el G explicó menos del 45%. Algunos estudios han comparado el efecto del genotipo frente a la contribución ambiental en la concentración final de glucosinolatos pero la mayoría de ellos se centran en los glucosinolatos mayoritarios en el brécol y determinan este efecto a partir del análisis de varianza sin hacer un estudio detallado de la interacción GE ni de la estabilidad de los genotipos y ambientes. De acuerdo con nuestros resultados, estudios previos han encontrado que los glucosinolatos indólicos son más sensibles a los efectos ambientales (Kushad y otros, 1999; Brown y otros, 2002; Kim y otros, 2002; Velasco y otros, 2007), aunque cierta parte de la variación también se debe a factores genéticos Rucker y Röbbelen, 1994). Por el contrario, la variación en glucosinolatos alifáticos se debe principalmente a factores genéticos (Raybould y Moyes, 2001; Brown y otros, 2002). Rucker y Röbbelen (1994) ya habían descrito una alta heredabilidad (h^2= 0.87) para este tipo de glucosinolatos y Toroser y otros (1995) encontraron entre dos y cinco genes independientes que parecen controlar el 71% de la variación de la concentración de glucosinolatos alifáticos en *B. napus*.

En lo referente a la variación de los fenoles, varios estudios, revisados por Parr y Bolwell (2000), han demostrado que la composición de fenoles en la planta puede variar debido tanto a estreses bióticos como abióticos. Otros estudios han encontrado que los factores genéticos pueden tener además efectos importantes en el contenido en estos metabolitos (Tomás-Barberán y Espín, 2001). Sin embargo, se sabe muy poco sobre la influencia de cada uno por separado o de la interacción de ambos. En el presente trabajo se ha profundizado en este tema, encontrando que en nabizas sólo el E influyó significativamente en la concentración final de flavonoides y ácidos hidroxicinámicos. En grelos, los dos tipos de compuestos fenólicos estuvieron afectados en gran medida por el efecto de la interacción cruzada de GE (hasta el 80% de la varianza del término G + GE). De este modo, en cada ambiente, diferentes genotipos presentaron las concentraciones más altas de estos compuestos. Este hecho refleja su baja heredabilidad y complicaría la selección de variedades con alto contenido en compuestos fenólicos en un programa de mejora.

Para establecer qué parámetros climatológicos tienen una mayor importancia en la concentración final de glucosinolatos y fenoles se llevó a cabo un análisis de

correlaciones. La temperatura fue un parámetro importante en el contenido final de dichos compuestos. Así, a medida que se incrementa el número de días con temperaturas por debajo de los 0 °C los glucosinolatos alifáticos disminuyen, mientras que cuando la temperatura se mantiene superior a los 20 °C, este tipo de glucosinolatos aumenta. Por otro lado, el contenido en glucosinolatos indólicos estuvo correlacionado de forma negativa con la precipitación. Otros autores han descrito anteriormente que las bajas temperaturas y las altas precipitaciones disminuyen la concentración en glucosinolatos totales (Rosa y otros, 1997; Ciska y otros, 2000; Velasco y otros, 2007). Con respecto al contenido en fenoles totales e individuales, en nabizas no se hallaron correlaciones significativas entre el contenido de estos compuestos y los diferentes factores climáticos. Por el contrario, en el caso de los grelos se encontró una relación positiva entre el número de días con temperaturas inferiores a 10 °C y 0 °C y el contenido total en fenoles, ácidos hidroxicinámicos y flavonoides. El grelo es un cultivo típico de invierno y, por tanto, gran parte de su ciclo tiene lugar a temperaturas medias por debajo de 10 °C y temperaturas mínimas por debajo de 0 °C. Las bajas temperaturas pueden producir acumulación de compuestos fenólicos en la planta (Stefanowska y otros, 2002). Estos compuestos intervienen, entre otras funciones, en procesos defensivos frente a patógenos, predadores o radiación ultravioleta. Es posible, además, que ejerzan un papel de defensa frente a condiciones meteorológicas adversas.

La gran variabilidad intervarietal observada permitió identificar variedades con el mayor contenido en glucosinolatos y fenoles y determinar la estabilidad de dichas variedades en los ambientes evaluados. Las variedades MBG-BRS0163, MBG-BRS0197 y MBG-BRS0173 fueron las variedades más prometedoras para futuros programas de mejora centrados en la selección por altos niveles de glucosinolatos. A pesar de que las variedades no mostraron diferencias significativas para el contenido en compuestos fenólicos en nabizas, sí difirieron en el estado de grelo, siendo MBG-BRS0143 y MBG-BRS0401 las más destacadas por su alto contenido en flavonoides.

Tanto las nabizas como los grelos, especialmente las primeras, mostraron altos contenidos de Ca, K, Mg y P, con respecto a los valores descritos en otros cultivos del mismo género como el brécol, las coles de Bruselas, la col rizada o la col china (Fahey, 2003). El Ca es un mineral esencial para la salud humana, participando en las funciones

biológicas de varios tejidos. Según Lucarini y otros (1999), el Ca presente en las brásicas muestra una excelente biodisponibilidad, debido a los bajos niveles de ácidos oxálico y fítico. Otros elementos considerados micronutrientes esenciales como el Fe, Cu, Mn, Zn y B también se encontraron en cantidades apreciables. Por otro lado, las concentraciones de aniones tóxicos en las partes comestibles de *B. rapa* fueron muy bajas y, en especial, el contenido en NO_3^-. Aunque este anión está relacionado con la inducción de efectos nocivos para la salud humana, los valores encontrados en grelos y nabizas fueron inferiores a los descritos para otros cultivos del género. Por lo tanto, nabizas y grelos son una buena fuente de componentes beneficiosos, hecho que hace que estos cultivos puedan ser consumidos regularmente como parte de una dieta rica en frutas y verduras.

Las nabizas y los grelos se consumen después de un proceso de cocinado. Por ello, resulta de vital importancia evaluar la degradación de estos compuestos bioactivos tras diferentes técnicas de cocinado y poder así conocer cuál es la ingesta real de dichos compuestos por parte del consumidor. Se estudió el efecto de tres métodos de cocción diferentes: cocinado al vapor, hervido tradicional y cocinado en olla a presión en más de 20 compuestos bioactivos incluyendo glucosinolatos, fenoles y vitamina C. El contenido en glucosinolatos y fenoles se redujo considerablemente después del cocinado y dichas pérdidas fueron similares en grelos y nabizas, excepto en el cocinado al vapor. El hervido tradicional y el cocinado en olla a presión presentaron pérdidas similares, cercanas al 64% para el contenido en glucosinolatos totales y 75% para el contenido en fenoles totales. En general, se ha demostrado que los métodos convencionales de cocción, tales como hervir, cocer al vapor, a presión y microondas reducen el contenido de glucosinolatos entre un 30 y un 60%, dependiendo del método, la intensidad y el tipo de compuesto (Rodrigues y Rosa, 1999; Verkerk y otros, 2001; Rangkadilok y otros, 2002; Verkerk y Dekker, 2004). Estas pérdidas son debidas principalmente a la rotura celular que sufre el material vegetal tras el tratamiento térmico, hecho que pone en contacto los glucosinolatos y la mirosinasa, enzima encargada de mediar la hidrólisis de los glucosinolatos en sus productos de degradación. En el caso de los compuestos fenólicos, estas pérdidas pueden alcanzar el 90% (Czarniecka-Skubina, 2002; Vallejo y otros, 2003c; Zhang y Hamauzu, 2004). De

acuerdo con otros trabajos previos (Vallejo y otros, 2002; Volden y otros, 2008), el cocinado al vapor fue el método con el que se obtuvo la mayor retención de compuestos bioactivos. En nabizas cocinadas al vapor las pérdidas de glucosinolatos y fenoles fueron del 9% y 15%, respectivamente. En grelos estas pérdidas fueron mayores aunque no superaron el 20% del contenido total en glucosinolatos y el 35% de fenoles.

Los glucosinolatos alifáticos generalmente se han caracterizado por ser más estables que los indólicos después de un proceso de cocinado (Ciska y Kozlowska, 2001; Vallejo y otros, 2002). Sin embargo, en este trabajo se han encontrado reducciones similares en el contenido total de alifáticos e indólicos, si bien los porcentajes de pérdida variaron para cada uno de los glucosinolatos individuales. Después del hervido tradicional y del cocinado en olla a presión, las mayores reducciones se encontraron en 4-hidroxiglucobrasicina, glucobrasicina y gluconasturtina, glucosinolatos que presentaron pérdidas cercanas al 100%. Por el contrario, después del cocinado al vapor se encontró un incremento en los valores de glucosinolatos indólicos. El incremento de los niveles de glucosinolatos después del cocinado al vapor ha sido descrito previamente por Gliszynska-Swiglo y otros (2006) y puede deberse al incremento de la disponibilidad de este tipo de compuestos después del cocinado.

Hasta el momento todos los estudios sobre el efecto del cocinado en fenoles se habían centrado en el contenido total de flavonoides en brécol. Por lo tanto, y en este trabajo, se aportan nuevos datos sobre la degradación de flavonoides y ácidos hidroxicinámicos individuales en otros cultivos. Los niveles más altos de retención se encontraron para los flavonoides desacilados: kaempferol-3,7-di-*O*-glucósido e isorhamnetina-3,7-di-*O*-glucósido, por lo que es posible que durante el cocinado los flavonoides pierdan los ácidos y pasen a su forma desacilada. En cuanto a los ácidos hidroxicinámicos, las pérdidas después del cocinado al vapor fueron escasas, mientras que en olla a presión fueron cercanas al 100%.

La vitamina C sufrió una drástica pérdida durante el manejo de las muestras y después del cocinado se perdió completamente. Esto indica que no sólo el proceso de cocción, sino también la manipulación afecta a la retención de ácido ascórbico en los tejidos, debido a su alto grado de solubilidad en agua y a su poca estabilidad.

El análisis nutricional del agua de cocción demostró que una gran parte de los compuestos bioactivos que se pierden en la parte vegetal pasan al agua. Cabe destacar que después del cocinado al vapor, la suma del contenido en glucosinolatos de la parte comestible junto con los del agua de cocinado no fueron significativamente diferentes del contenido que presentaba la porción cruda. Por el contrario, tras los otros dos métodos de cocción, se recuperó menos del 70% del contenido total en glucosinolatos que presentaba la porción cruda. Como se ha comentado con anterioridad, estas pérdidas son debidas principalmente a la rotura celular que sufre el material vegetal tras el tratamiento térmico y la consiguiente degradación de los glucosinolatos por la acción de la mirosinasa. Con respecto a los fenoles, se recuperó el 100% del contenido de estos compuestos tras la suma del agua de cocción y la parte cocinada. Además, la concentración de los flavonoides desacilados fue mayor en esta suma que la presentada en crudo. Por tanto, el uso del agua de cocinado para la preparación de sopas o salsas aumentaría la ingesta de estos compuestos antioxidantes.

El análisis sensorial de nabizas y grelos demostró diferencias entre variedades para los perfiles sensoriales. En nabizas, la variedad MBG-BRS0461 obtuvo el valor más alto de intensidad de aroma y los más bajos en color de hoja y sabor salado. Por otro lado, MBG-BRS0163 fue la variedad con el sabor más salado, poca intensidad de aroma y destacó por el color de la hoja. Para grelos, la variedad MBG-BRS0143 obtuvo los valores más altos para fibrosidad en boca. Con respecto al sabor amargo típico de los grelos la variedad MBG-BRS0197 fue la que destacó por su amargor, mientras que MBG-BRS0472 fue la menos amarga. A pesar de que el amargor está considerado un sabor no deseado en los alimentos, las nabizas y los grelos se caracterizan por tener un cierto grado de amargor y esta característica es apreciada por los consumidores. Gracias a la variabilidad intervarietal observada para determinados atributos sensoriales, sería posible seleccionar en un futuro una variedad particular de acuerdo a las preferencias de los consumidores. Además, la mayoría de los caracteres evaluados mostraron diferencias entre localidades, lo que pondría de manifiesto que las condiciones climáticas en las cuales se desarrolla la planta tendrían influencia en las características sensoriales de estos cultivos.

Por otro lado, se realizaron correlaciones entre los descriptores para determinar qué caracteres describen mejor las cualidades sensoriales de grelos y nabizas y se encontró que algunos de ellos, principalmente los caracteres relacionados con la textura como la resitencia de la hoja y del tallo y resistencia al corte y aquellos relacionados con el sabor, como el sabor amargo, salado o duración del retrogusto juegan un papel importante en la evaluación sensorial de nabizas y grelos y podrían ser utilizados en la optimización de un panel de cata de grelo. Sería interesante, por último, realizar una cata de aceptación-preferencia para tratar de correlacionar estos descriptores objetivos con las preferencias del mercado, lo que ayudaría a seleccionar variedades en función de unos pocos descriptores.

Tal y como se ha comentado anteriormente, los compuestos fenólicos y glucosinolatos además de influir en la calidad nutritiva de las brásicas, también se han relacionado con sus atributos sensoriales (Fenwick y otros, 1983a; van Doorm y otros, 1998; Engel y otros, 2002). En este trabajo, se encontró una relación entre las variedades con mayor concentración en glucosinolatos y el sabor. Así, por ejemplo las variedades MBG-BRS0197 y MBG-BRS0163, con alto contenido en glucosinolatos, fueron las variedades con el sabor más amargo y salado, respectivamente. La progoitrina (en nabizas) y la gluconapina (en grelos) se correlacionaron con el sabor amargo y la persistencia en boca. Por lo tanto, estos compuestos pueden ser los principales responsables del sabor amargo y picante en estos vegetales. Otros autores (Schonhof y otros, 2004; Padilla y otros, 2007) han descrito que el amargor está influenciado por la gluconapina aunque pueden existir otros compuestos que ayuden a conferir este carácter.

En resumen, se puede concluir que las nabizas y los grelos son una buena fuente de compuestos beneficiosos para la dieta. Si bien los grelos contienen altas concentraciones de glucosinolatos y derivados sinápicos, las nabizas son ricas en compuestos fenólicos totales además de poseer cantidades ligeramente mayores de minerales y vitamina C que los grelos. Después de estudiar el efecto de varios tipos de cocinado en ambas partes de la planta, se recomendaría el consumo de nabizas al vapor ya que es el órgano y el método que conserva mejor los compuestos que le confieren las propiedades beneficiosas.

La variabilidad morfo-agronómica, nutricional y organoléptica que presentaron las variedades de *B. rapa* estudiadas, hace posible la selección de aquellas más productivas en función de su uso y con mejor valor nutricional. En general, las variedades con alto contenido en glucosinolatos y fenoles no destacaron por su rendimiento en fresco, a excepción de MBG-BRS0143 destacada para la producción de grelos y con alto contenido en flavonoides y MBG-BRS0184 destacada para la producción de nabiza y alto contenido en glucosinolatos y fenoles.

El efecto importante debido al genotipo y la interacción no cruzada GE en la producción en fresco de nabizas y grelos así como en el contenido en glucosinolatos alifáticos favorecería la selección de variedades enfocadas a la mejora de las características agronómicas y nutritivas de las variedades actualmente cultivadas. Sin embargo, el contenido en glucosinolatos indólicos y fenoles presentó una gran influencia ambiental y una interacción cruzada GE. Este hecho dificultaría la selección de variedades en base al contenido en estos metabolitos. Por tanto, en el futuro y, con el fin de obtener variedades de nabizas y grelos con altos contenidos en compuestos bioactivos que aporten un valor nutricional añadido conviene realizar un estudio más detallado de los factores bióticos y abióticos que pueden modificar el contenido de estos compuestos, así como un estudio de la expresión de los genes implicados en su síntesis.

CAPÍTULO VIII

Conclusiones

9. CONCLUSIONES

1. Las variedades de *B. rapa* evaluadas mostraron una gran diversidad intervarietal lo que permitió escoger aquellas variedades más apropiadas para la producción en fresco de nabiza y grelo. En concreto, las variedades MBG-BRS0550, MBG-BRS0082 y MBG-BRS0184 fueron las más adecuadas para la producción de nabizas y las variedades MBG-BRS0143 y MBG-BRS0472 para la producción de grelos. El estudio del efecto ambiental sobre el rendimiento y la estabilidad de los genotipos determinó que el ambiente Salcedo 2008 y el genotipo MBG-BRS0472 son los más productivos y estables. Para la producción en fresco de nabizas y de grelos, el porcentaje de variación debido al genotipo fue mayor que el debido a la interacción genotipo × ambiente. Este hecho facilitará la obtención de variedades más productivas y mejor adaptadas a las condiciones ambientales.

2. Respecto al perfil nutricional, las nabizas y los grelos se caracterizaron por ser una buena fuente de minerales, fibra, proteína y vitamina C. Además, mostraron altas concentraciones en glucosinolatos y fenoles, dos tipos de compuestos bioactivos relacionados con la salud humana. Las dos partes de la planta compartieron el mismo perfil metabólico aunque difirieron en su concentración. El contenido en glucosinolatos fue mayor en grelos que en nabizas, siendo la gluconapina el principal glucosinolato. Por otro lado, el contenido total en compuestos fenólicos fue mayor en nabizas que en grelos. El ácido sinápico y los flavonoides derivados del kaempferol fueron los fenoles mayoritarios. Por primera vez, glucosinolatos y fenoles se identificaron de forma simultánea en cultivos del género *Brassica*. Este método de determinación simultánea podría llegar a convertirse en un método de referencia para evaluar la calidad nutricional de diferentes cultivos de este género.

3. El contenido en compuestos bioactivos se vio significativamente afectado por el genotipo, el ambiente y la interacción genotipo × ambiente. Los glucosinolatos alifáticos tienen un fuerte control de genotipo, lo cual facilitaría la selección de variedades ricas en este tipo de glucosinolatos. Por el contrario, el ambiente y la interacción genotipo × ambiente fueron los principales factores que influyeron en el contenido final de glucosinolatos indólicos y de compuestos fenólicos. El

contenido en glucosinolatos totales disminuyó a bajas temperaturas y alta precipitación. Por el contrario, el contenido en fenoles totales aumentó con las bajas temperaturas. En general, la gran influencia que el ambiente y la interacción genotipo × ambiente ejercen sobre estos metabolitos dificulta la selección de variedades con altos contenidos en compuestos bioactivos.

4. El contenido en glucosinolatos, fenoles y vitamina C presentes en las partes comestibles de *B. rapa* se redujo considerablemente después del cocinado. De los métodos de cocción estudiados, el cocinado al vapor fue el método con el que se obtuvo la mayor retención de estos compuestos, aunque las tasas de degradación tras el cocinado variaron para cada uno de los compuestos individuales. Por otro lado, en el agua de cocción se encontraron gran parte de los compuestos bioactivos que se pierden en la parte vegetal, por lo que, el uso de este agua para la realización de sopas o salsas aumentaría la ingesta de estos compuestos beneficiosos.

5. Algunos caracteres sensoriales, principalmente los relacionados con la textura, podrían ser utilizados como parámetros importantes en la caracterización sensorial de estos cultivos y serían además buenos candidatos para una rápida evaluación del material. Los glucosinolatos, especialmente la progoitrina (en grelos) y la gluconapina (en nabizas), estuvieron correlacionados con los descriptores del sabor, como el sabor picante y la persistencia en boca. Por tanto, estos compuestos podrían ser los responsables del sabor amargo y picante característico de estos vegetales. Las variedades estudiadas mostraron diferencias en su perfil sensorial y esta variabilidad podría ser de utilidad para seleccionar la variedad con mejores características sensoriales de acuerdo a las exigencias del mercado y las preferencias de los consumidores.

9. CONCLUSIONS

1. The *B. rapa* varieties evaluated showed a wide range of intervarietal diversity to determine the most appropriate for either turnip greens or turnip tops fresh production. The varieties MBG-BRS0550, MBG-BRS0082 and MBG-BRS0184 had good agronomic performance as turnip greens whereas the most suitable varieties for turnip tops production were MBG-BRS0472 and MBG-BRS0143. The study of the environmental effect on crop production and the stability of the genotypes determined that, the environment Salcedo 2008 and the variety MBG-BRS0472 were the most productive and stable. For turnip greens and tops fresh production, the percentage of variation due to the genotype was larger than that due to genotype × environment interaction. This fact will favors the development of more productive varieties adapted to environmental conditions.

2. The nutritional profile of *B. rapa* crops has been exhaustively assessed. Turnip greens and turnip tops were found to be a good source of minerals, fiber, protein and vitamin C. Besides, they were rich in glucosinolates and phenolic compounds, two bioactive compounds related to human health. Both plant organs shared the same metabolic profile, but differed in their concentration. The glucosinolate content was higher in turnip tops than in turnip greens, being gluconapin the main glucosinolate. On the other hand, the total content of phenolic compounds was higher in turnip greens than in turnip tops. Flavonoid kaempferol derivatives, sinapic acid and their derivatives were the major phenols. For first time, glucosinolates and phenolic compounds were simultaneously identified in *Brassica* genus by using a multipurpose method which could become a reference method to evaluate the nutritional quality of different *Brassica* crops.

3. The content of the bioactive compounds evaluated was significantly affected by the genotype, the environment and the genotype × environment interaction. Aliphatic glucosinolates were clearly regulated by the genotype, which would facilitate the selection of varieties rich in this kind of glucosinolates. In contrast, the effects of environment and genotype × environment interaction on the content of indolic

glucosinolates and phenolics compounds appeared as the main effects of variation. Cool temperatures and abundant rainfall seem to have lower total glucosinolate content. On the contrary, total phenolics increased with lower temperatures. In general, the great influence that the environment and the interaction genotype × environment have on these metabolites makes more difficult the selection of varieties with high content of bioactive compounds.

4. The total content of glucosinolates, phenols and vitamin C present in the edible parts of *B. rapa* were significantly affected by the cooking procedure and the loss rate varied among individual compounds. Steaming was the method that better preserved glucosinolates and phenolic compounds. On the other hand, in the cooking water were found much of bioactive compounds that are lost at the edible part. Therefore, the use of this water for soups or gravies increase the intake of these health-promoting compounds.

5. Some sensory traits, mainly those related to texture, could be used as important parameters in the sensorial characterization of these crops and would be good candidates for a rapid screening of material. Glucosinolates, especially progoitrin (in turnip greens) and gluconapin (in turnip tops), showed high correlation with flavor traits as bitter taste and aftertaste persistence. Hence, these compounds may be responsible for the bitter and pungent taste of these vegetables. Varieties showed differences in their sensory profiles. This variability could be used to select the best variety for sensorial characteristics according to vegetable market and consumer preferences.

ANEXO

Descripción de las revistas científicas

Los resultados obtenidos en esta tesis se han incluido en cinco artículos, tres de ellos se encuentran publicados, uno aceptado y otro bajo revisión en revistas científicas de carácter internacional incluidas en el SCI (Science Citation Index). A continuación se detallan dichos artículos y el factor de impacto de las revistas científicas.

Artículos publicados:

Francisco M, Moreno D.A, Cartea M.E, Ferreres F, García-Viguera C y Velasco P. 2009. Simultaneous identification of glucosinolates and phenolic compounds in a representative collection of vegetable *Brassica rapa*. *Journal of Chromatography A*. 1216:661-6619.

Francisco M, Velasco P, Moreno D.A, García-Viguera C y Cartea M.E. 2010. Cooking methods of *Brassica rapa* affect the preservation of glucosinolates, phenolics and vitamin C. *Food Research International*. 43:1455-1463.

Francisco M, Velasco P, Romero A, Vázquez L y Cartea M.E. 2009. Sensory quality of turnip greens and turnip tops grown in northwestern Spain. *European Food Research and Technology*. 230:281-290.

Artículo aceptado:

Francisco M, Velasco P, Lema M y Cartea ME. 2010. Environmental influence on agronomic and nutritional value of *Brassica rapa*. Agronomy Journal.

Artículo enviado:

Francisco M, Cartea ME, Soengas P y Velasco P. 2010. Effect of genotype and environmental conditions on health-promoting compounds in *Brassica rapa*. Journal of Agricultural and Food Chemistry.

Factor de impacto de las revistas:

Journal of Chromatography A es una de las revistas científicas de referencia en investigación sobre química analítica, con un enfoque en las técnicas y métodos utilizados para la separación e identificación de compuestos. El factor de impacto de esta revista en 2009 fue de 4,101.

Food Research International se centra en la publicación de trabajos de investigación enfocados en la ciencia y tecnología de los alimentos. El factor de impacto de esta revista en 2009 fue de 2,414.

La revista *European Food Research and Technology* publica documentos científicos de calidad enfocados en las metodologías de análisis sensorial y física de los alimentos. El factor de impacto de esta revista en 2009 fue de 1,370.

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