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Seasonal isothiocyanates variation and market availability of Brassicaceae species consumed in Mendoza

Recomendaciones de consumo para aprovechar los fitoquímicos bioactivos presentes en *Brassicaceae* a lo largo del año en Argentina

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Abstract

Along with the recommendation of a healthy diet, it is suggested to increase the consumption of fruits and vegetables. Among the latter, Brassicaceae species are preferred, because they show many phytochemicals mainly belonging to the isothiocyanates (ITCs) family compounds, with proven activities related to the prevention of chronic diseases and cancer. A survey about seasonal availability and phytochemical levels of Brassicaceae species in the total of vegetables marketed in the province of Mendoza (located in the centre west of Argentina) was done. Results throw that Brassicaceae vegetables are an important part of Mendoza vegetable market reaching up to 23% of the vegetables commercialized. Regarding ITCs content, watercress and rocket were the vegetables with the highest ITCs levels, being, therefore, the most promising vegetables studied herein by their potential functional activities. Finally, high levels of variation (up to 10 times) on ITCs content along the year in a single species were found. These facts should be considered when designing Brassicaceae species phytochemical characterization assays to achieve more reliable results. This work represents the first report of Brassicaceae availability and seasonal phytochemical variability in local conditions.

Keywords
Cruciferae • isothiocyanates • vegetable availability • phytochemical seasonal variability

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Resumen

El consumo de frutas y hortalizas es recomendado para mantener una dieta saludable. Dentro de las hortalizas, las especies de la familia Brassicaceae se destacan por poseer grandes contenidos de diversos fitoquímicos que han demostrado su acción en la prevención de enfermedades crónicas y cáncer. En el presente trabajo, se realizó un relevamiento sobre disponibilidad estacional y niveles de fitoquímicos de especies de Brassicaceae considerando el total de hortalizas comercializadas en la provincia de Mendoza (ubicada en centro oeste de Argentina). Los resultados demostraron que los vegetales de la familia Brassicaceae contribuyen de modo importante en el mercado representando hasta un 23% de las hortalizas comercializadas. Respecto del contenido de ITCs (isotiocianatos), los mayores niveles fueron encontrados en berro y rúcula y como consecuencia de ello representan los vegetales más prometedores por sus potenciales propiedades funcionales. Además, se pudo observar que la variabilidad en los niveles de ITCs a lo largo del año en una misma especie, puede ser muy amplia (hasta 10 veces). Por esta razón es que podemos recomendar tener en cuanta la fluctuación estacional al evaluar niveles de fitoquímicos en especies de esta familia. Cabe destacar, que este trabajo representa el primer reporte de la disponibilidad de hortalizas de la familia Brassicaceae y la variabilidad fitoquímica estacional en las condiciones locales.

Palabras clave
Crucíferas • isotiocianatos • disponibilidad • variación estacional de fitoquímicos

Introduction

Brassicaceae family contains more than 350 genera and 3,000 species that are distributed worldwide (5). Despite the great diversity among the Brassicaceae family members, only a few genera are consumed, mainly those belonging to the *Brassica* genus. These vegetables have been consumed for their distinctive flavor and for their health enhancing properties. Consumption of Brassicaceae vegetables has been associated with a reduced risk of cardiovascular disease and different kinds of cancer (4). These beneficial properties have been attributed to a group of sulphur compounds, the isothiocyanates (ITCs), which possesses a characteristic biting taste and pungent odour. ITCs are produced when plant tissue is damaged, allowing the hydrolysis of glucosinolates (GLS), catalysed by the action of myrosinase enzyme (thioglucoside glucohydrolase, EC 3.2.1.147). Although ITCs are the primary reaction products, depending on the medium conditions such as pH, availability of ferrous ions and activity level of specific co-factors as the epithiospecifier protein; other several breakdown products could be formed including nitriles, thiocyanates, epithionitriles and oxazolidine-2-thione (7).

Brassicaceae vegetables consumption can be highly variable across different cultures, ethnic groups and countries. Americans consume broccoli, cauliflower, and cabbage primarily; the residents of Japan prefers daikon, Chinese people consume mainly cabbage, wasabi, watercress, and oriental mustards; and those residing in the United Kingdom prefer cabbage, sauerkraut, and brussels sprouts (14). Although, these habits could be influenced by the Brassicaceae vegetables availability in each region; nowadays, the globalized market contributes getting access to species from the different geographic origin (11).

Intake data on phytonutrients from fruits and vegetables is limited (12). Therefore, knowing ITCs levels in different Brassicaceae species along the year is important for predicting health enhancing effects derived from these species, while achieving specific consumption recommendations. In this sense, Jiao *et al.* (1998) analysed total ITCs content in nine Brassicaceae species commonly consumed in Asia (including species scarcely consumed in America such as choi sum, kai choi and bok choi among others). On the other hand, Tang *et al.* (2013) also measure ITCs content in *Brassica* vegetables but in those consumed in the United States (including broccoli, cabbage, cauliflower, Brussel sprouts, kale and collar green among others). To our knowledge, this is the first report of ITCs concentration data of Brassicaceae vegetables commonly consumed and cultivated in Central and South America. Moreover, there is no local data on the seasonal variation of these compounds in the different species.

The work was focusing on evaluate the availability of Brassicaceae vegetables in Mendoza, considering seasonal variability; and determination of total isothiocyanates levels content of all species funded in each season. Finally, consumption recommendation considering the phytochemicals role in cancer chemoprevention for the species studied was proposed.

Materials and methods

Sampling and sample conditioning

A survey of all vegetables marketed in the Cooperative Market of Godoy Cruz, Mendoza, was made (considering Brassicaceae species and non-Brassicaceae species) in order to define the representativeness of this botanical family in that market. Besides, the availability of Brassicaceae species during each season of the year was registered.

A total of 160 samples of nine commonly consumed Brassicaceae vegetables, including broccoli, cabbage, Brussels sprouts, radish, mustard green, cauliflower, rocket, and watercress, were purchased from local stores located in that market. One Kilogram of each species was purchased in five different stores. Later, a single batch of one kilogram was formed and processed in the laboratory. This process was repeated in four different sampling dates (July, October of 2016 and January and April of 2017).

A subsample of each homogenous batch was analysed in triplicate. The edible part of such vegetables (table 1) was conditioned by properly cleaning. Phytochemicals extraction and moisture content determination were made on the same day of purchase. For moisture assays, the samples were processed, weighted (3 g of each vegetable) and dried in a convection oven (DALVO, Santa Fe, Argentina) at 100 ± 10°C until constant weight was reached.

Phytochemical extraction

The extraction was carried out following an optimized technique previously reported by our group (6). Ten g of fresh vegetable was placed in a blender with 50 mL of water and homogenized for 9 min (Blender, 600 W, 60 Hz, model HR2030/10, Phillips, Buenos Aires, Argentina), then, the obtained juice was sonicated in an ultrasound bath for 5 min in a 100 mL glass beaker (US-bath, 40 kHz and 600 W, model tb 04, Testlab, Buenos Aires, Argentina).

Total ITCs content

Hydrolysis of GLS to ITCs was carried out by stirring an aliquot of 5 mL homogenate in a glass vial for two hours in a water bath at 37°C (2). The cyclocondensation reaction was carried out according to Tang *et al.* (2013). An aliquot of 250 µL of the hydrolysed homogenate was mixed with 250 µL of 100 mmol L^{-1} potassium phosphate buffer (pH 8.5) and 500 µL of 10 mmol L^{-1} benzene-1,2-dithiol solution in 2-propanol in a 4 mL vial and kept at 65°C for 2 hours.

Table 1. Availability of Brassicaceae vegetables in Mendoza, Argentine and edible parts used for analysis.

Tabla 1. Disponibilidad de las especies de Brassicaceae en Mendoza, Argentina y descripción de las partes comestibles analizadas.

Scientific name	Trivial name	Edible parts	Seasons availability*
Brassica juncea var. crispifolia	Mustard green	Leaves	1, 2, 3, 4
Brassica oleracea var. botrytis	Cauliflower	Inflorescences	1, 2, 4
Brassica oleracea var. capitata	Red cabbage	Leaves	1, 2, 3, 4
Brassica oleracea var. capitata	White cabbage	Leaves	1, 2, 3, 4
Brassica oleracea var. gemmifera	Brussels sprouts	Leaves	1, 4
Brassica oleracea var. italica	Broccoli	Inflorescences	1, 2, 3, 4
Eruca sativa	Rocket	Leaves	1, 2, 3, 4
Nasturtium officinale	Watercress	Leaves and stalks	1, 2, 3, 4
Raphanus sativus	Radish	Roots	1, 2, 4

* 1= winter; 2= spring; 3= summer and 4= autumn

* 1 = invierno; 2 = primavera; 3 = verano y 4 = otoño

Then, the mixture was centrifuged at 14000 rpm (15339 *g*) for 5 min and filtered with a 0.22 µm filter membrane before injection into the HPLC system. A liquid chromatograph Konik KNK-500 series, coupled to a UV/Vis detector (Konik, Barcelona, Spain) was used with a Waters C_{18} HPLC column (150 x 4.6 mm, I.D. 5 µm particle size) (Milford, Massachusetts, USA). Data obtained was processed by EZ Chrom Chromatography Data System Version 6.8 software. HPLC-UV conditions were chosen according to a previous report (6) with slight modifications: isocratic elution using as mobile phase 80:20 (v/v) MeOH-water solution at 1 mL min^{-1} and the detection wavelength was fixed at 365 nm (13). Peak identification was carried out by comparing retention times of the synthesized standard compound (1,3-benzodithiole-2-thione) according to Kristensen *et al.* (2007). Levels of total ITCs were quantified by a standard calibration curve and expressed in µmol per 100 grams of dry matter (µmol % g DW). The assays were carried out in triplicate.

Statistical analysis

All data were expressed as the mean ± standard deviation (SD). The data were analyzed by ANOVA using INFOSTAT software. Tukey's test compared the mean of each treatment group. p-values < 0.05 were considered significant.

Results and Discussion

Table 1 (page 405), shows the results of the market survey on the availability of the Brassicaceae family for each season. It was possible to find between six and nine different species in each season. Cauliflower and radishes were not available in the summertime, as well as Brussels sprouts, which were also not available in spring.

The survey considering more than 40 different vegetables offered in the Cooperative Market reveals an important commercial supply of Brassicaceae vegetables throughout the year, representing a 15 - 23 % of the total of marketed species considering number of different species.

Moisture contents of these vegetables revealed significant differences ($p< 0.05$) among the seasons ranging from 83 to 95 %. This analysis allows us to express the evaluated phytochemical levels in dry weight (DW) and avoid water content influence.

The study throughout the year implied 160 samples for the analysis of total ITCs. The results obtained were analysed by ANOVA, revealing significant differences ($p< 0.05$) for the same vegetable among seasons (table 2, page 407). Overall ITCs levels ranged from 21.5 to 1465.4 µmol % g DW in white cabbage in winter and rocket in autumn, respectively. The highest ITCs levels were found in rocket despite the season of the year (average value= 1233.9 µmol % g DW) and the lowest ITCs levels were found in cauliflower (average value= 33.4 µmol % g DW). It is also interesting to consider that ITCs fluctuations along the year could reach up to 10 times, which should be taken into account when characterizing these species in a single season or at a specific date of the year.

Winter and autumn were the seasons in which the greatest variety of Brassicaceae species could be found. Seven of the nine evaluated species evidenced significantly higher levels of total ITCs in autumn (table 2 and table 3, page 407). These findings are in concordance with the results reported by Rosa and Rodrigues (2010) for broccoli crops, which conclude that total or individual GLS (ITCs precursors) concentrations were higher from September to December (autumn in the North Hemisphere). The authors found lower biomass production in this season, but the level of organosulfur compounds was higher than for the other seasons.

Rockets and watercress were the vegetables that evidenced the highest ITCs levels throughout the year (table 2, page 407). These results are consistent with a previous report (8) which found that watercress evidenced the highest ITCs levels, up to 144.6 µmol % g, while regarding rockets, levels similar to those previously reported were measured (15).

Table 3 (page 407) shows the richest ITCs species along the year and allows inferring the ideal season for each species consumption: excluding rocket and watercress, in summertime the highest levels of ITCs were found in broccoli, red and white cabbage. In spring, the outstanding plants concerning ITCs were cauliflower, mustard green and radish; and finally, in autumn, the significant levels were found in broccoli and radish.

Table 2. Total ITCs concentration of Brassicaceae vegetables throughout the year.

Tabla 2. Concentración total de ITCs en hortalizas de la familia Brassicaceae en diferentes estaciones del año.

Brassicaceae VEGETABLES	[Total ITCs] µmol % g DW*				
	Winter	Spring	Summer	Autumn	Mean value (variation§)
Broccoli	65.0 ± 7.9^{a}	129.2 ± 14.0^{b}	177.4 ± 2.4^{c}	307.0 ± 11.3^{d}	169.6 (4.7)
Brussels Spouts	23.5 ± 1.7^{a}	--	--	63.9 ± 2.7^{b}	43.7 (2.7)
Cauliflower	21.7 ± 3.0^{a}	52.1 ± 1.2^{b}	--	26.4 ± 1.3^{a}	33.4 (2.4)
Mustard green	50.1 ± 4.0^{a}	71.4 ± 1.1^{b}	57.9 ± 4.2^{a}	77.0 ± 2.2^{c}	64.1 (1.5)
Radish	104.0 ± 11.1^{a}	134.0 ± 15.9^{b}	--	145.9 ± 0.4^{b}	127.9 (1.4)
Red Cabbage	25.5 ± 7.5^{a}	40.6 ± 4.6^{a}	257.1 ± 20.2^{b}	76.5 ± 0.2^{a}	99.9 (10)
Rocket	1044.2 ± 51.0^{a}	1294.3 ± 93.1^{b}	1132.0 ± 9.0^{a}	1465.4 ± 17.1^{c}	1233.9 (1.4)
Watercress	700.4 ± 28.1^{b}	803.0 ± 52.9^{c}	537.9 ± 2.1^{a}	906.3 ± 48.0^{d}	736.9 (1.7)
White Cabbage	21.5 ± 4.4^{a}	23.5 ± 3.5^{ab}	76.0 ± 7.0^{c}	32.5 ± 1.9^{b}	38.37 (3.6)

* Results expressed as mean ± SD; 95% confidence interval; µmol % g DW. Values followed by a different superscript lowercase letter are significantly different ($p<0.05$) on ITCs concentration (in ascending order), for each vegetable between seasons. (--) The vegetable was not available on that season. §Variation level calculated as: maximum value/minimum value

* Resultados expresados como media ± DE; Intervalo de confianza del 95%; µmol% g peso seco. Los valores seguidos por una letra minúscula de superíndice diferente son significativamente diferentes ($p <0,05$) en concentración de ITCs (en orden ascendente), para cada vegetal entre estaciones. (--) El vegetal no estaba disponible en esa temporada. §Valor de variación calculado como: valor máximo/valor mínimo

Table 3. Seasons of the year with the highest ITCs concentration for each Brassicaceae species.

Tabla 3. Estaciones del año con la concentración de ITCs más alta para cada especie de Brassicaceae.

Brassicaceae VEGETABLES	Season of the year			
	Winter	Summer	Autumn	Spring
Broccoli		**	*	
Brussels Spouts	*		**	
Cauliflower			*	**
Mustard green			*	**
Radish			**	*
Red Cabbage		**	*	
Rocket			**	*
Watercress			**	*
White Cabbage		**	*	

Where ** Shows the highest ITCs concentration and * The second largest ITCs concentration.

Donde ** Indica la concentración máxima de ITCs y * Indica la segunda concentración mayor.

Our current findings expand the previously reported (1, 3, 8, 13) because here the samples were taken from all the seasons and all the available Brassicaceae species were considered, including vegetables of global interest, usually less studied than *Brassica* genus. Consequently, taking into account that autumn was the season with both, highest species availability and ITCs levels, this season is recommended for consuming these vegetables, for the maximum phytochemical's achievement.

Conclusions

The present work demonstrates that Brassicaceae vegetables are an important part of Argentinean diet reaching up to 23% of the vegetables commercialized, considering total number of different species. In addition, it is interesting to take into account that due to the great diversity of species that are offered, there is availability of at least some of these species throughout the year. Regarding ITCs content, watercress and rocket were the vegetables with the highest ITCs levels, being the most promising vegetables of this family, given their potential functional activities. Finally, the variation level of ITCs content

along the year in a single species resulted to be remarkable, reaching up to ten times. This should be considered when designing Brassicaceae species characterization studies, in order to achieve more reliable comparisons among results. These findings will contribute to complete a comprehensive database that will be available for consumers and the scientific community.

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