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Effects of Microwave Cooking Conditions on Bioactive Compounds Present in Broccoli Inflorescences

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Cooking as a domestic processing method has a great impact on food nutrients. Most *Brassica* (Brassicaceae, Cruciferae) vegetables are mainly consumed after being cooked, and cooking considerably affects their health-promoting compounds (specifically, glucosinolates, phenolic compounds, minerals, and vitamin C studied here). The microwave cooking process presents controversial results in the literature due to the different conditions that are employed (time, power, and added water). Therefore, the aim of this work was to study the influence of these conditions during microwave cooking on the human bioactive compounds of broccoli. The results show a general decrease in the levels of all the studied compounds except for mineral nutrients which were stable under all cooking conditions. Vitamin C showed the greatest losses mainly because of degradation and leaching, whereas losses for phenolic compounds and glucosinolates were mainly due to leaching into water. In general, the longest microwave cooking time and the higher volume of cooking water should be avoided to minimize losses of nutrients.

KEYWORDS: *Brassica oleracea*; microwave; domestic; cooking; glucosinolates; phenolic compounds; ascorbic acid; minerals

INTRODUCTION

There is strong epidemiological evidence that regular consumption of *Brassica* foods, including broccoli, is associated with a decreased risk for certain chemically induced cancers, most likely due to their glucosinolate content (*1*, *2*). Glucosinolates are sulfur-rich, anionic natural products (*3*), and numerous reviews have addressed their occurrence in vegetables, primarily the family Brassicaceae (syn. Cruciferae; including *Brassica* spp. and *Raphanus* spp.) (*4*, *5*). According to their structure, glucosinolates can be classified as aliphatic, aromatic, and indolic, and when the plant cell is damaged, they can be hydrolyzed to biologically active isothiocyanates by the plant enzyme myrosinase (*1*). Glucosinolates are also responsible for the characteristic flavor and odor of certain *Brassica* vegetables (*6*). On the other hand, using correlation and principal component analysis, Baik et al. (*7*) found little evidence that the glucosinolates were responsible for the distinctive flavor notes of broccoli.

In addition, several studies in the past few years have demonstrated that *Brassica* vegetables contain a wide range of natural antioxidants, such as vitamins and phenolic compounds (*8*–*10*). These dietary antioxidants, in addition to protecting against free radicals in the human body (*11*), are related to a reduced risk of some chronic diseases (*12*), cancers or heart diseases (*13*). Broccoli had strong antioxidant activity compared to other vegetables.

According to the American Food Standards Agency and the U.S. Department of Agriculture, broccoli is a good candidate as source of essential minerals for human consumption (*14*). Also, various mineral elements (calcium, zinc, iron, potassium, magnesium, etc.) are considered essential for humans and enter the food chain through plants (*15*). These elements have very varied functions, e.g., as electrolytes, enzymes, constituents, and/or building materials in bones and teeth. Nevertheless, human dietary nitrate and nitrite exposure should be controlled as they may be considered health risk factors (*16*).

Broccoli is mainly consumed cooked, and domestic handling and processing, including cooking processes, have been shown to considerably affect its health-promoting compounds (*17*–*20*). Zhang and Hamauzu (*21*) pointed out that both microwaving (600 W) and conventional cooking (10 g of broccoli floret with 200 mL of water for 30, 60, 90, 120, and 300 s) affected the antioxidant composition and in vitro activity of broccoli, but Turkmen et al. (*10*) established that after microwaving and conventional cooking, the total antioxidant activity remained unchanged in broccoli. A recent study (*22*) has found that the glucosinolate content of some *Brassica* vegetables, including broccoli, was maintained after steaming, microwave, and stir-

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fry cooking processes but was affected by the boiling cooking process due to the glucosinolates leaching into the cooking water. Cooking in water decreased significantly the content of polyphenols, glucosinolates, and vitamin C compounds in broccoli (*23*). Mineral nutrients in foods may also be affected by cooking methods, although it depends on the specific element (*24*), but microwaving broccoli may preserve a high percentage (*25*).

These controversies could be partially explained due to the different conditions of time, added water, or microwave power, making it difficult to understand the benefits or negative effects of microwave cooking. Because of this, the aim of this work was to study the effects of different combinations of cooking time, cooking water, and power settings in the microwave oven on the health-promoting compounds of this healthy food, including vitamin C, phenolic compounds, glucosinolates, and mineral nutrients.

MATERIALS AND METHODS

Broccoli. Fourty kilograms of fresh broccoli heads (*Brassica oleracea* L. var. italica cv. Nubia) was purchased from a local Agri-food Coop Enterprise (COATO, Totana, Murcia, Spain) at maturity or marketable stage (optimum quality heads 130 ± 10 mm in diameter, 250–280 g wet basis). For analytical purposes, inedible parts (leaves and stems) were removed and discarded. Edible parts (primary inflorescences) were washed thoroughly and cut into almost equal small pieces (~3 cm diameter and ~1 cm stalk; edible florets) with a sharp knife. All the pieces were well mixed, and three replicates of 150 g of fresh raw broccoli were randomly selected for each treatment.

Processing Treatments. Three replicates were retained as a raw control; the rest were cooked in a conventional microwave oven (Samsung, Cleveland, U.K.), using two different time periods (2.5 or 5 min) and water volumes (100 or 150 mL). Combinations of these parameters were considered as four different processing treatments: treatment 1, 5 min and 150 mL of water; treatment 2, 5 min and 100 mL of water; treatment 3, 2.5 min and 150 mL of water; and treatment 4, 2.5 min and 100 mL of water. For each treatment, three different powers (1000, 700, and 500 W) were applied.

Cooking condition limits were determined with a preliminary experiment by an informal tasting panel consisting of six trained people who decided the best conditions, among different options (*19, 20*), for microwaved broccoli regarding sensory parameters (sweetness, crispness, bitterness, juiciness, pungent, color, and broccoli flavor).

Samples were cooked in a microwave-safe polypropylene bowl (recycling PP 5) covered with a punched polyethylene transparent food-wrapping film in order to prevent water losses. All the cooked samples were flash-frozen with liquid N_2; after cooling to room temperature, the samples were freeze-dried, ground to fine powder, and kept at −80 °C for further analysis. Both edible parts and cooking water were saved for analysis.

Extraction and Determination of the Amounts of Vitamin C. Freeze-dried samples (0.2 g) were homogenized in a vortex stirrer for 20 s with 10 mL of extractant solution, consisting of MeOH and dH_2O (5:95), and 2.1% (v:v) dissolved citric acid, 0.05% (v:v) EDTA, and 0.01% (v:v) NaF. All reagents were of analytical grade. The homogenate was filtered through a four-layer cheesecloth, and the pH was adjusted to 2.2–2.4 by addition of 6 N HCl. The extract was centrifuged (3600*g* for 15 min at 4 °C), and the supernatant was recovered and filtered through a C_{18} Sep-Pack cartridge (Waters, Milford, MA) previously activated with 10 mL of methanol followed by 10 mL of deionized water (dH_2O), and then 10 mL of air. The collected extract was filtered through a 0.45 μm polyethersulfone filter (Millex-HV, Millipore, Bedford, MA). Ascorbic (AA) and dehydroascorbic (DHAA) acid contents were determined as described previously (*26*). HPLC analysis was achieved after derivatization of DHAA into the fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-*b*]quinoxaline-1-one (DFQ) with 1,2-phenylenediamine dihydrochloride (OPDA). Samples (20 μL) were analyzed with a Merck-Hitachi (Tokyo, Japan) HPLC system, equipped with an L-4000 UV detector and an L-6000 pump. Separations of DFQ and AA were achieved on a Kromasil 100 C-18 column (25 cm × 0.4 cm; 5 μm particle size; Tecnokroma, Barcelona, Spain). The mobile phase was methanol and water (5:95, v:v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate at pH 4.5. Solvents and reagents were of HPLC-gradient and analytical grade, respectively. The flow rate was 0.9 mL/min; the detector wavelength was initially set at 348 nm and, after elution of DFQ, was manually shifted to 261 nm for AA detection. The DHAA and AA contents were expressed as milligrams of DHAA and AA per 100 g of fresh weight separately (*26*).

Extraction and Determination of the Amounts of Phenolic Compounds. Freeze-dried powder samples (1 g) were homogenized three times with 25 mL of a 70% (v:v) MeOH/dH_2O mixture, and the homogenates were filtered through four-layer cheesecloth. All the samples were kept on ice. The homogenates were centrifuged (100*g* for 5 min at 4 °C), and the supernatants were concentrated in a rotary evaporator under vacuum at 30 °C to approximately 1 mL and diluted to 2 mL with dH_2O. The obtained extract was filtered through a 0.45 μm Millex-HV filter. The extracted samples (20 μL) were analyzed on a Merck-Hitachi liquid chromatograph equipped with a pump (model L-6200) and a UV–vis DAD (model L-7420). Separations were achieved on a LiChroCART column (Merck, Darmstadt, Germany) (ODS-18, 25 cm × 0.4 cm, 5 μm particle size). The mobile phase was a mixture of water and formic acid (95:5, v:v) (A) and methanol (B). The flow rate was 1 mL/min in a linear gradient starting with 10% B and reaching 15% B at 5 min, 30% B at 20 min, 50% B at 35 min, and 90% B at 40 min. Chromatograms were recorded at 280, 320, and 360 nm. Caffeoylquinic acid derivatives were quantified as chlorogenic acid (5-caffeoylquinic acid, Sigma, St. Louis, MO), flavonoids as quercetin 3-rutinoside (Sigma), and sinapic acid and ferulic derivatives as sinapic acid (Sigma). The total content of phenolic compounds was expressed as milligrams of chlorogenic and sinapic acid equivalent per 100 g of fresh weight.

Extraction and Determination of the Amounts of Glucosinolates. A modification of the multipurpose phytochemical method (*27*) was used for the extraction and analysis of glucosinolates, as reported by Martínez-Sánchez et al. (*28*). Freeze-dried samples (60 g) were placed with 1.5 mL of 70% MeOH, in a sonicator bath for 10 min to improve the homogenization. Then the mixture was extracted at 70 °C for 30 min, in a thermostated bath with occasional vortex shaking and further centrifugation (30 min at 17500*g* and 4 °C). Supernatants were collected, and methanol was completely removed using a rotary evaporator; the obtained dry material was redisolved in 1 mL of ultrapure water and filtered through 0.45 μm polyethersulfone filter (Millipore). Each sample (20 μL) was analyzed in a Waters HPLC system (Waters Cromatografía S.A., Barcelona, Spain) consisting of a W600E multisolvent delivery system, an in-line degasser, a W717plus Autosampler, and a W2996 photodiode array dectector at 227 nm, using a LiChrospher 100 RP18 column (25 cm × 0.4 cm, 5 μm particle size; Merck KGaA, Darmstadt, Germany) with a LiChroCART 4-4 guard column. The mobile phase was a mixture of water and formic acid (99:1, v:v) (A) and acetonitrile (B). Glucosinolates were eluted off the column in 35 min. The flow rate was 1 mL/min in a linear gradient starting with 1% B and reaching 20% B at 30 min and 1% B at 40 min. Samples were then identified using the previously described intact glucosinolate LC–MS method and quantified by HPLC–DAD using sinigrin (sinigrin monohydrate from horseradish, Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) as a standard (*29*). The glucosinolate content was expressed as milligrams of sinigrin equivalent per 100 g of fresh weight.

Extraction and Determination of the Amounts of Mineral Elements. The analysis of mineral nutrients was carried out after HNO_3/$HClO_4$ (2:1) acid digestion of the lyophilized material. Mineral elemental analysis was performed by optical inductively coupled plasma (ICP) spectrometry (ICP-OES spectrometer, IRIS Intrepid II XDL, Thermo Electron Corp., Franklin, MA), equipped with a 2000 W RF generator and full wavelength coverage. The mineral content was expressed as milligrams per 100 g of fresh weight.

Statistical Analysis. Data were statistically analyzed using SPSS 13.0, by analysis of variance (ANOVA), and by Tukey's test. For each

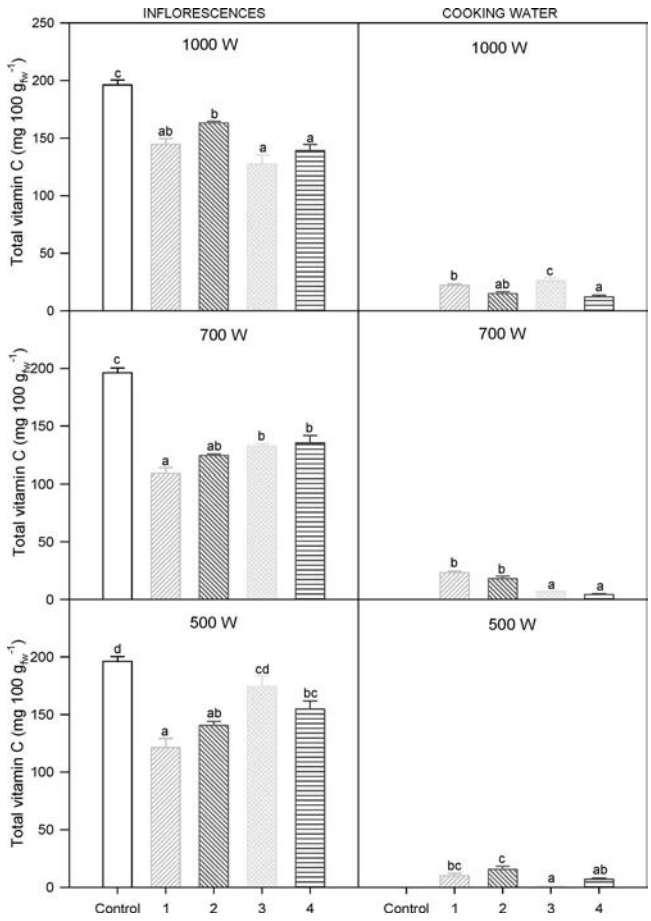

Figure 1. Total vitamin C content in inflorescences and remaining cooking water after cooking treatments described in Materials and Methods. Data are means ± the standard error ($n = 3$). Columns with the same letters are not significantly different ($p < 0.05$, Tukey test). Statistical analysis was done separately for different powers (1000, 700, and 500 W).

main factor, we used a unifactorial analysis of variance. Significant differences were determined at the $p < 0.05$ level.

RESULTS AND DISCUSSION

Effect on Vitamin C Concentration. The effect of microwave cooking treatments on vitamin C content in "Nubia" broccoli florets and the corresponding results in the cooking water are shown in **Figure 1**. Vitamin C in cooked broccoli showed a general decrease (20–40%), with respect to the raw uncooked control, and at least 10% of these losses could be associated to lixiviation processes, as this vitamin was found in the cooking water (**Figure 1**). Vallejo et al. (*19*) reported that microwave cooking was the most aggressive domestic processing for vitamin C content in broccoli florets, with respect to conventional boiling, steaming, or pressure cooking (*19*). In general, our results agree with those but showed greater losses of vitamin C than results recently reported (*30*). In our work, vitamin C was significantly affected by every factor involved separately: power, cooking time, and volume of cooking water (**Figure 1**). Nevertheless, the combination of power × time (**Table 1**) caused the greatest loss, followed by the combination of higher water volume and cooking time (treatment 1) at any given power set, whereas treatment 3 (2.5 min and 150 mL of water) at 500 W presented the highest level of retention in inflorescence (90%) and a reduced amount of vitamin C in the cooking water. We here confirmed that microwave cooking caused important losses of vitamin C in broccoli, as previously shown by Zhang and Hamauzu (*21*), using 600 W and 200 mL of boiled water, for cooking 10 g of broccoli florets (*21*). This fact has also been documented and mainly explained by the thermal and enzymatic reactions of degradation (*31*). Consequently, our results indicate that the combination of power and cooking time is also decisive for vitamin C loss.

Table 1. Analysis of Variance for the Total Vitamin C in Inflorescences and Remaining Cooking Water

	total vitamin C in inflorescences	total vitamin C in cooking water
power	*a*	*a*
time	*b*	*a*
cooking water	*c*	*b*
power × time	*a*	*a*
power × water	ns[d]	*a*
time × water	*b*	ns[d]
power × time × water	ns[d]	ns[d]

[a] Where $0.001 > p$. [b] Where $0.01 > p > 0.001$. [c] Where $0.05 > p > 0.01$. [d] Not significant.

Effect on Concentrations of Phenolic Compounds. At 1000 W, the total content of phenolic compounds in Nubia broccoli (caffeoylquinic derivatives and ferulic and sinapic derivatives) decreased in all the treatments, while increasing phenolic levels were found in the cooking water, treatment 1 being the highest in all the power sets (**Figure 2**). The response in edible florets was somehow different for the power set at 700 W, where we could see a significant decrease in total phenolics in edible florets (cooking with the higher volume of water), whereas the presence of the phenolic compounds in the cooking water decreased at lower cooking times, and also the volume of cooking water. At 500 W, there was a general decrease with cooking process in the edible parts, as at 1000 W, except for treatment 3, which did not show any significant loss in the cooked florets. From these results, the microwave cooking using short cooking times and no-contact-with-cooking-water combinations were better in terms of favoring the retention of phytochemicals in broccoli (*20*). Here we also demonstrate that phenolic compounds are very sensitive to heat treatments, even with short cooking periods (*32*). We could also observe that phenolic compounds were significantly affected by the cooking time, and the power × water, time × water, and power × time × water interactions (**Table 2**). Evidence regarding this point is somehow controversial, since previous findings showed that microwave cooking increased the phenolic content of foods, mainly because of the effect of the high-power treatments (1000 W for 1.5 min) on the levels of free flavonols (*10*). The levels of retention and losses with the different microwave cooking treatments that we employed here (**Figure 2**) could be more related to the pass or transfer of phenolic compounds into the cooking water, marked by the corresponding decrease in phenolic content with the different power sets.

Effect on Glucosinolate Concentration. The total content of intact glucosinolates in Nubia broccoli florets was significantly affected by the cooking parameters [power, time, and added water (**Figure 3**)] and the combination of the three parameters [power × time × water (**Table 3**)]. The results of treatment 1 highlighted a general decrease in the level of glucosinolates compared with the uncooked control. The levels of retention would depend on the different cooking factors, accounting at least for the transfer to the cooking water, as well as the effects on the thermal degradation during the cooking process. Vallejo et al. (*19*) previously found important losses (74%) in total glucosinolate content using analogous conditions (microwave set at 1000 W, 5 min, and 150 mL of added water),

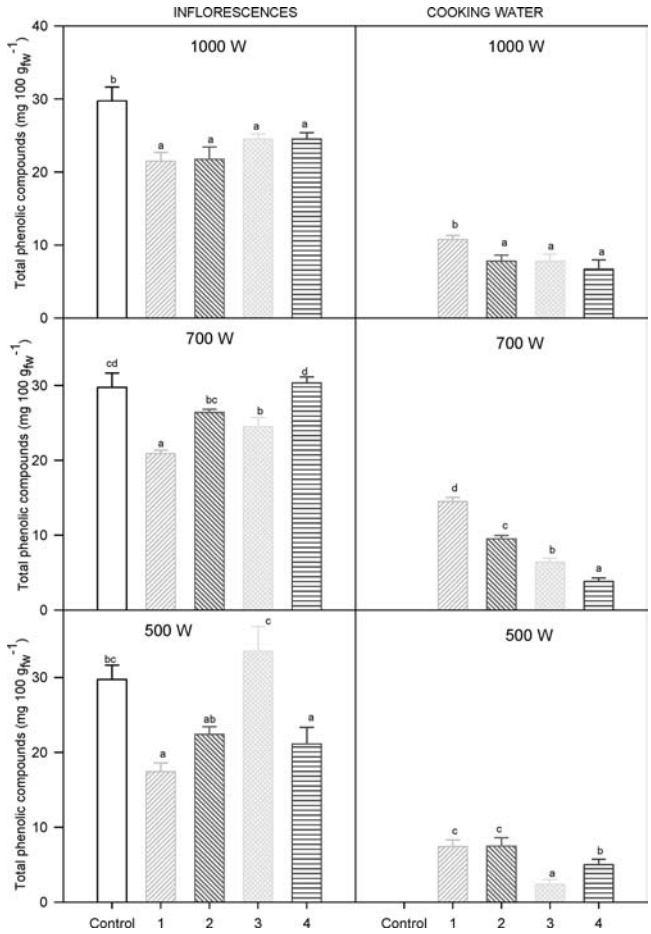

Figure 2. Total content of phenolic compounds in inflorescences and remaining cooking water after cooking treatments described in Materials and Methods. Data are means ± the standard error ($n = 3$). Columns with the same letters are not significantly different ($p < 0.05$; Tukey test). Statistical analysis was done separately for different powers (1000, 700, and 500 W).

Table 2. Analysis of Variance for the Total Phenolic Compounds in Inflorescences and Remaing Cooking Water

	total phenolic compounds in inflorescences	total phenolic compounds in cooking water
power	ns[a]	*c*
time	*b*	*c*
cooking water	ns[a]	*b*
power × time	ns[a]	*b*
Power × water	*b*	*b*
time × water	*d*	*d*
power × time × water	*b*	ns[a]

[a] Not significant. [b] Where $0.01 > p > 0.001$. [c] Where $0.001 > p$. [d] Where $0.05 > p > 0.01$.

and the high rate of water evaporation which contained leached compounds from the florets could explain those dramatic losses; however, in our case, there was an only 18% loss (*19*). It is remarkable that the only difference was the plastic recipient (bowl) used for cooking. In this sense, our results agreed with Verkerk and Dekker (*33*) who microwaved cabbages, using a similar approach, and found not significant losses in the total content of glucosinolates. This high level of retention in the edible part probably reflected the absence of leaching into the cooking water (*33*). These authors also explained the increase in the level of glucosinolates with microwave cooking as a result of the increased extractability of these bioactive phytochemicals

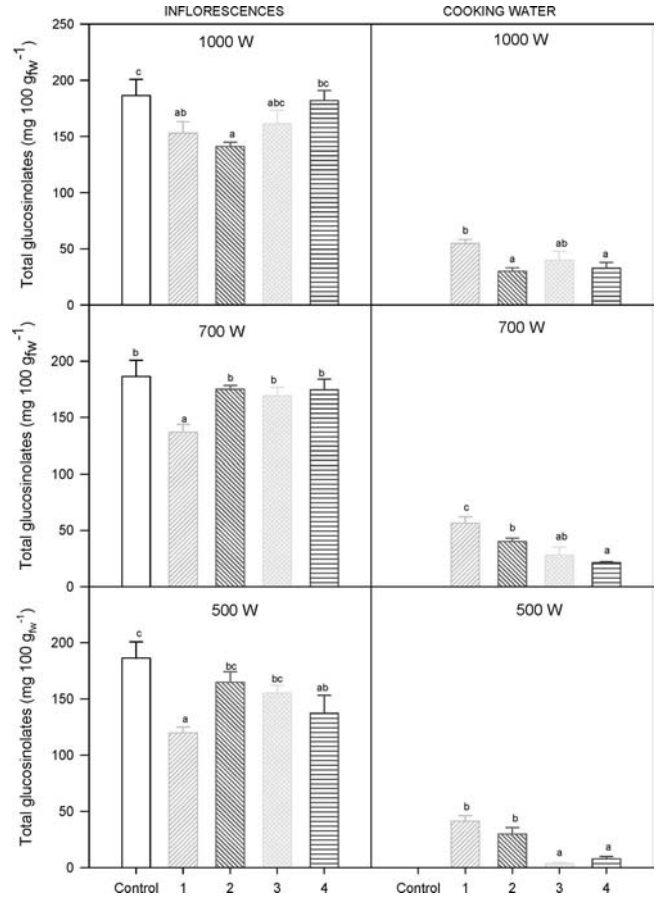

Figure 3. Total glucosinolate content in inflorescences and remaining cooking water after cooking treatments described in Materials and Methods. Data are means ± the standard error ($n = 3$). Columns with the same letters are not significantly different ($p < 0.05$; Tukey test). Statistical analysis was done separately for different powers (1000, 700, and 500 W).

Table 3. Analysis of Variance for the Total Glucosinolates in Inflorescences and Remainig Cooking Water

	total glucosinolates in inflorescences	total glucosinolates in cooking water
power	*a*	*b*
time	*c*	*b*
cooking water	*a*	*c*
power × time	ns[d]	*c*
power × water	ns[d]	ns[d]
time × water	ns[d]	*a*
power × time × water	*c*	ns[d]

[a] Where $0.05 > p > 0.01$. [b] Where $0.001 > p$. [c] Where $0.01 > p > 0.001$. [d] Not significant.

after an intense heat treatment, which is similar to what was also found with carotenoids (*34*). Therefore, different processes and factors can determine the fate of glucosinolates during microwave cooking of broccoli. The membrane of the plant cell vacuoles keeps the myrosinase isolated from the intact glucosinolates (*35*). During the processing for cooking (steaming or microwaving), many factors (i.e., cutting, long cooking time, microwave radiation, etc.) induce plant injury, and glucosinolates are released from vacuoles and hydrolyzed by plant myrosinase (*33*). Under our cooking conditions, more than 2 min of microwave cooking at high power sets would prevent the intact myrosinase from hydrolyzing glucosinolates, because these are denaturalizing conditions for this enzyme (*33*). Then, the glucosinolate contents were strongly retained in the cooked

Table 4. Individual Glucosinolate Content in Inflorescences (milligrams per 100 g wet basis) after Cooking Treatments Described in Materials and Methods[a]

		glucoraphanin	gluconapin	4-OH-glucobrassicin	glucobrassicin	gluconasturtiin	4-MeO-glucobrassicin	neoglucobrassicin
	control	44.8 ± 6.0 a	10.5 ± 0.7 a	13.3 ± 0.6 b	57.4 ± 2.1 b	17.8 ± 1.7 b	12.0 ± 0.7 a	30.5 ± 7.9 a
1000 W	1	39.0 ± 3.8 a	10.9 ± 0.5 a	9.9 ± 0.3 ab	44.7 ± 1.8 a	12.2 ± 0.4 a	8.8 ± 0.7 a	27.5 ± 4.1 a
	2	37.3 ± 3.7 a	11.4 ± 0.5 a	9.1 ± 0.2 a	42.8 ± 1.3 a	11.8 ± 0.5 a	8.3 ± 0.5 a	20.5 ± 3.1 a
	3	42.8 ± 4.2 a	11.5 ± 0.9 a	10.2 ± 1.5 ab	49.0 ± 2.7 ab	13.4 ± 1.1 ab	8.7 ± 1.3 a	25.7 ± 2.0 a
	4	49.8 ± 0.7 a	11.3 ± 1.1 a	13.3 ± 0.2 b	52.0 ± 4.1 ab	14.7 ± 0.9 ab	11.2 ± 1.0 a	29.7 ± 3.4 a
	control	44.8 ± 6.0 a	10.5 ± 0.7 a	13.3 ± 0.6 a	57.4 ± 2.1 b	17.8 ± 1.7 b	12.0 ± 0.7 b	30.5 ± 7.9 a
700 W	1	37.4 ± 1.6 a	11.5 ± 0.2 a	12.0 ± 0.4 a	39.0 ± 1.9 a	11.1 ± 0.2 a	8.7 ± 0.7 a	17.2 ± 3.2 a
	2	47.3 ± 0.4 a	11.6 ± 0.5 a	14.1 ± 0.2 a	52.1 ± 2.2 b	13.5 ± 0.1 ab	11.4 ± 0.1 ab	24.9 ± 1.7 a
	3	45.8 ± 2.9 a	12.3 ± 0.8 a	14.1 ± 0.4 a	49.0 ± 1.7 b	12.6 ± 0.6 a	10.4 ± 0.2 ab	24.9 ± 3.7 a
	4	48.5 ± 2.8 a	11.5 ± 0.7 a	12.4 ± 0.7 a	50.6 ± 2.6 b	14.1 ± 1.1 ab	10.9 ± 1.2 ab	26.5 ± 1.9 a
	control	44.8 ± 6.0 a	10.5 ± 0.7 a	13.3 ± 0.6 b	57.4 ± 2.1 b	17.8 ± 1.7 b	12.0 ± 0.7 b	30.5 ± 7.9 b
500 W	1	33.6 ± 0.8 a	9.2 ± 0.4 a	9.0 ± 0.5 a	38.5 ± 2.2 a	11.8 ± 0.9 a	6.8 ± 0.5 a	10.9 ± 0.7 a
	2	46.8 ± 1.3 a	12.5 ± 1.4 a	11.0 ± 0.4 ab	50.5 ± 3.7 ab	15.5 ± 0.3 ab	9.7 ± 0.7 ab	18.8 ± 2.7 ab
	3	41.5 ± 2.1 a	14.8 ± 2.0 a	13.4 ± 0.3 b	44.1 ± 2.7 ab	15.1 ± 0.4 ab	11.3 ± 0.7 b	15.3 ± 1.2 ab
	4	38.3 ± 4.5 a	9.7 ± 1.3 a	11.5 ± 1.6 ab	39.9 ± 4.5 a	12.6 ± 1.1 a	9.0 ± 1.1 ab	16.4 ± 2.3 ab

[a] Data are means ± the standard error ($n = 3$). Treatments with the same letters are not significantly different ($p < 0.05$; Tukey test). Statistical analysis was done separately for different powers (1000, 700, and 500 W).

Table 5. Individual Glucosinolates in Remaining Cooking Water (milligrams per 100 g) after Cooking Treatments Described in Materials and Methods[a]

		glucoraphanin	gluconapin	4-OH-glucobrassicin	glucobrassicin	gluconasturtiin	4-MeO-glucobrassicin	neoglucobrassicin
1000 W	1	14.1 ± 1.4 a	3.8 ± 0.2 a	3.1 ± 0.1 a	15.9 ± 0.8 b	4.0 ± 0.1 b	3.6 ± 0.3 a	10.4 ± 1.5 a
	2	8.1 ± 1.4 a	1.9 ± 0.1 a	1.9 ± 0.2 a	9.2 ± 0.3 a	1.8 ± 0.0 ab	2.1 ± 0.1 a	5.0 ± 1.6 a
	3	10.7 ± 1.6 a	2.3 ± 1.2 a	2.2 ± 1.1 a	13.6 ± 1.0 b	1.9 ± 0.9 ab	2.0 ± 1.0 a	7.2 ± 1.6 a
	4	9.3 ± 2.0 a	2.2 ± 0.1 a	2.4 ± 0.3 a	9.5 ± 1.2 a	1.3 ± 0.7 a	2.2 ± 0.3 a	5.9 ± 1.4 a
700 W	1	14.9 ± 1.0 c	4.2 ± 0.1 b	4.4 ± 0.2 b	17.1 ± 0.9 b	3.5 ± 0.1 a	4.3 ± 0.2 b	7.7 ± 4.1 a
	2	10.4 ± 1.1 bc	2.4 ± 0.2 ab	2.6 ± 0.1 ab	10.8 ± 0.1 a	2.7 ± 0.5 a	2.9 ± 0.1 ab	8.0 ± 1.4 a
	3	7.3 ± 0.6 ab	1.8 ± 0.9 a	1.8 ± 0.9 a	9.2 ± 1.7 a	1.8 ± 0.9 a	1.8 ± 0.9 a	4.3 ± 1.6 a
	4	3.7 ± 1.8 a	1.7 ± 0.2 a	2.0 ± 0.1 a	6.9 ± 1.2 a	2.2 ± 0.3 a	1.7 ± 0.2 a	3.3 ± 0.5 a
500 W	1	10.5 ± 1.9 a	3.3 ± 0.4 b	3.8 ± 0.3 a	13.0 ± 1.3 b	4.2 ± 0.5 c	2.9 ± 0.4 b	3.5 ± 0.5 b
	2	6.4 ± 3.3 a	1.7 ± 0.9 ab	1.6 ± 0.8 a	11.2 ± 0.2 b	3.5 ± 0.1 c	1.5 ± 0.8 ab	3.8 ± 0.3 b
	3	1.4 ± 0.7 a	0.0 ± 0.0 a	0.8 ± 0.8 a	1.7 ± 0.8 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	4	2.4 ± 1.0 a	0.0 ± 0.0 a	1.8 ± 0.3 a	0.0 ± 0.0 a	1.8 ± 0.3 b	1.8 ± 0.4 ab	0.0 ± 0.0 a

[a] Data are means ± the standard error ($n = 3$). Treatments with the same letters are not significantly different ($p < 0.05$; Tukey test). Statistical analysis was done separately for different powers (1000, 700, and 500 W).

florets. Treatment 1 (5 min and 150 mL of added water) seemed to be the worst condition, producing a higher rate of thermal degradation than enzymatic hydrolysis, keeping in mind the fraction of the glucosinolates that probably were leached into the cooking water.

When we examined the composition of individual intact glucosinolates in Nubia broccoli florets and the glucosinolates released to the cooking water (**Tables 4** and **5**), we reported the quantified glucosinolates being intact glucosinolates normally present in broccoli cultivars as previously described by HPLC–DAD and HPLC–MS/MS methodologies (*29*). There we could find additional small entity peaks by HPLC–DAD, so we focused our discussion on the major glucosinolates present in our samples. There were remarkable differences in the behavior of individual glucosinolates for the different treatments, with respect to the total glucosinolate content. We found in the relative composition of the raw/uncooked broccoli aliphatic glucoraphanin (24% of total glucosinolates) and indole glucobrassicin (30%) as the main glucosinolates in Nubia broccoli florets, knowing that qualitatively differences between broccoli samples could be expected (*1*). We found an analogous response to the treatments of the individual glucosinolates and the total content, with the greater percentages of losses appearing at 500 W (treatment 1), except for that of gluconasturtiin (**Table 4**). For glucoraphanin and neoglucobrassicin, the results were also analogous to the total content of glucosinolates. Aliphatic glucoraphanin and gluconapin displayed higher levels of retention in general. It was established that indole glucosinolates present thermolabilities higher than those of aliphatic glucosinolates in broccoli (*19*), in red cabbage (*36*), and in white cabbage (*37*), but they proposed that the greater losses of indole than aliphatic glucosinolates were not a result of the higher thermolability of those compounds, but of their more effective diffusion into the cooking water. In our case, as presented in **Table 5**, glucosinolates were all leached into the cooking water, and the corresponding redistribution was similar to that of the glucosinolates in the florets.

Mineral Content. Mineral nutrients in microwaved broccoli behaved in a manner similar to that of the previously reported data in primary and secondary inflorescences (*38*). Even if general upkeep with different microwaving conditions was observed (**Tables 6** and **7**), there was high level of retention of the different minerals (60–100%).

To analyze the density of nitrate in the edible broccoli, we could not observe any remarkable difference between treatments for the nitrate content in the inflorescences or the cooking water. Nitrate levels detected were low, in contrast with those of other vegetables such as spinach (282 mg per 100 g fresh weight), parsley (120.4 mg per 100 g fresh weight), or dill (224.3 mg per 100 g fresh weight) (*39*), and the level of the concentration presented could not be considered as a health risk factor.

The highest levels of retention (90–100%) of calcium, magnesium, sodium, and manganese were obtained at 700 W [when using 2.5 min and 150 mL of added water (**Table 6**)]. On the other hand, for iron and phosphorus, the 700 W power with the 5 min and 100 mL water combination was the best. At 500 W, zinc exhibited its highest level of retention in treatment 3 and potassium in treatment 4. Only copper contents showed higher levels of retention at 1000 W in treatment 1. Previous works revealed that different cooking methods, including

Table 6. Mineral Content in Inflorescence (milligrams per 100 g wet basis) after Cooking Treatments Described in Materials and Methods[a]

		Ca	K	Mg	Na	Cu	Fe	Mn	Zn	NO_3^-
	control	40.43 ± 1.62 b	399.95 ± 8.92 b	27.33 ± 0.90 c	34.87 ± 0.58 b	0.14 ± 0.00 a	10.55 ± 0.47 a	0.35 ± 0.03 b	0.57 ± 0.07 b	12.17 ± 1.65 a
1000 W	1	30.22 ± 2.34 a	296.90 ± 2.30 a	17.71 ± 0.78 a	22.75 ± 0.73 a	0.16 ± 0.03 a	7.86 ± 1.38 a	0.24 ± 0.01 a	0.35 ± 0.04 a	15.70 ± 1.98 a
	2	33.35 ± 2.30 ab	337.32 ± 25.27 ab	21.34 ± 2.02 ab	26.24 ± 2.37 a	0.12 ± 0.00 a	8.48 ± 0.44 a	0.27 ± 0.01 a	0.46 ± 0.02 ab	10.29 ± 1.21 a
	3	34.28 ± 1.13 ab	342.01 ± 3.16 ab	22.18 ± 0.95 abc	29.12 ± 1.93 ab	0.13 ± 0.01 a	9.09 ± 0.80 a	0.27 ± 0.01 a	0.42 ± 0.03 ab	10.22 ± 1.42 a
	4	38.30 ± 0.99 ab	373.65 ± 14.39 b	23.93 ± 0.81 bc	34.27 ± 2.07 b	0.14 ± 0.01 a	9.06 ± 0.18 a	0.29 ± 0.01 ab	0.40 ± 0.04 ab	16.00 ± 0.59 a
	control	40.43 ± 1.62 a	399.95 ± 8.92 b	27.33 ± 0.90 b	34.87 ± 0.58 a	0.14 ± 0.00 ab	10.55 ± 0.47 a	0.35 ± 0.03 a	0.57 ± 0.07 a	12.17 ± 1.65 ab
700 W	1	38.20 ± 0.29 a	317.88 ± 8.79 a	21.88 ± 0.61 a	25.84 ± 0.57 a	0.13 ± 0.01 a	9.77 ± 0.48 a	0.27 ± 0.03 a	0.46 ± 0.06 a	12.85 ± 0.95 ab
	2	38.34 ± 0.97 a	353.11 ± 5.60 ab	25.71 ± 0.68 b	35.00 ± 1.12 a	0.16 ± 0.01 b	12.56 ± 0.47 b	0.33 ± 0.01 a	0.52 ± 0.00 a	13.97 ± 2.79 b
	3	42.32 ± 1.66 a	365.10 ± 26.28 ab	27.14 ± 0.68 b	37.62 ± 4.33 a	0.15 ± 0.00 ab	11.44 ± 0.34 ab	0.34 ± 0.01 a	0.46 ± 0.03 a	10.67 ± 0.56 ab
	4	38.80 ± 2.19 a	375.62 ± 6.45 ab	25.91 ± 0.54 b	32.61 ± 4.16 a	0.14 ± 0.01 ab	10.58 ± 0.25 a	0.33 ± 0.01 a	0.46 ± 0.03 a	5.81 ± 1.02 a
	control	40.43 ± 1.62 b	399.95 ± 8.92 b	27.33 ± 0.90 b	34.87 ± 0.58 c	0.14 ± 0.00 a	10.55 ± 0.47 a	0.35 ± 0.03 b	0.57 ± 0.07 a	12.17 ± 1.65 b
500 W	1	31.66 ± 0.56 a	344.57 ± 12.13 ab	22.87 ± 0.05 a	21.33 ± 1.05 a	0.12 ± 0.00 a	10.01 ± 0.25 a	0.27 ± 0.00 a	0.50 ± 0.02 a	3.56 ± 0.53 a
	2	31.51 ± 0.65 a	298.69 ± 19.86 a	22.82 ± 0.58 a	20.77 ± 0.31 a	0.13 ± 0.01 a	10.03 ± 0.41 a	0.27 ± 0.01 a	0.49 ± 0.03 a	9.35 ± 0.97 b
	3	32.46 ± 1.60 a	346.37 ± 40.13 ab	24.14 ± 0.25 a	22.66 ± 0.59 a	0.13 ± 0.00 a	9.91 ± 0.19 a	0.29 ± 0.01 ab	0.53 ± 0.01 a	9.68 ± 1.24 b
	4	34.69 ± 1.00 a	376.15 ± 11.27 ab	24.73 ± 0.32 a	27.18 ± 0.70 b	0.13 ± 0.00 a	10.68 ± 0.17 a	0.29 ± 0.00 ab	0.45 ± 0.01 a	12.76 ± 1.29 b

[a] Data are means ± the standard error ($n = 3$). Treatments with the same letters are not significantly different ($p < 0.05$; Tukey test). Statistical analysis was done separately for different powers (1000, 700, and 500 W).

Table 7. Mineral Content in Remaining Cooking Water (milligrams per 100 g) after Cooking Treatments Described in Materials and Methods[a]

		Ca	K	Mg	Na	Cu	Fe	Mn	Zn	NO_3^-
1000 W	1	4.48 ± 0.20 b	0.00 ± 0.00 a	4.12 ± 0.06 c	4.51 ± 0.25 c	0.01 ± 0.00 b	0.06 ± 0.00 b	0.03 ± 0.00 b	0.06 ± 0.00 b	0.46 ± 0.02 a
	2	2.25 ± 0.19 a	0.00 ± 0.00 a	2.21 ± 0.19 ab	2.28 ± 0.36 a	0.01 ± 0.00 a	0.03 ± 0.00 a	0.02 ± 0.00 a	0.04 ± 0.01 ab	0.93 ± 0.12 ab
	3	3.96 ± 0.23 b	70.35 ± 10.00 c	2.76 ± 0.15 b	3.91 ± 0.30 bc	0.01 ± 0.00 b	0.03 ± 0.00 a	0.02 ± 0.00 ab	0.05 ± 0.00 ab	1.20 ± 0.17 b
	4	2.70 ± 0.15 a	36.89 ± 0.00 b	2.02 ± 0.18 a	2.88 ± 0.35 ab	0.00 ± 0.00 a	0.03 ± 0.00 a	0.02 ± 0.00 a	0.03 ± 0.01 a	0.53 ± 0.07 a
700 W	1	4.40 ± 0.13 c	0.00 ± 0.00 a	3.87 ± 0.08 c	5.30 ± 0.41 c	0.01 ± 0.00 c	0.05 ± 0.00 c	0.03 ± 0.00 c	0.08 ± 0.01 b	0.82 ± 0.09 a
	2	2.35 ± 0.07 ab	0.00 ± 0.00 a	2.57 ± 0.10 b	3.58 ± 0.16 b	0.01 ± 0.00 b	0.03 ± 0.00 b	0.02 ± 0.00 b	0.04 ± 0.00 a	0.64 ± 0.15 a
	3	2.81 ± 0.26 b	36.13 ± 6.02 b	1.66 ± 0.24 a	3.14 ± 0.49 ab	0.01 ± 0.00 ab	0.02 ± 0.00 a	0.01 ± 0.00 ab	0.04 ± 0.01 a	0.76 ± 0.04 a
	4	1.78 ± 0.23 a	31.54 ± 6.08 b	1.31 ± 0.21 a	1.94 ± 0.30 a	0.00 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.02 ± 0.00 a	2.32 ± 0.42 b
500 W	1	2.91 ± 0.27 b	54.62 ± 0.28 c	2.55 ± 0.35 b	2.71 ± 0.29 b	0.01 ± 0.00 c	0.03 ± 0.00 b	0.02 ± 0.00 b	0.06 ± 0.01 b	0.87 ± 0.05 c
	2	2.22 ± 0.09 ab	0.00 ± 0.00 a	2.47 ± 0.13 b	2.13 ± 0.14 ab	0.01 ± 0.00 bc	0.03 ± 0.00 b	0.02 ± 0.00 b	0.06 ± 0.01 b	0.59 ± 0.05 b
	3	1.85 ± 0.13 a	16.25 ± 3.86 ab	0.85 ± 0.17 a	1.32 ± 0.23 a	0.00 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.03 ± 0.01 a	0.35 ± 0.04 a
	4	1.84 ± 0.06 a	32.29 ± 6.74 b	1.24 ± 0.15 a	1.74 ± 0.05 a	0.00 ± 0.00 ab	0.01 ± 0.00 a	0.01 ± 0.00 ab	0.03 ± 0.01 a	0.53 ± 0.03 ab

[a] Data are means ± the standard error ($n = 3$). Treatments with the same letters are not significantly different ($p < 0.05$; Tukey test). Statistical analysis was done separately for different powers (1000, 700, and 500 W).

microwave cooking, did not significantly affect the mineral content in broccoli from different origins and conditions, and the level of retention of minerals was always high for almost all the analysed mineral elements (C. López-Berenguer et al.. unpublished results). Some researchers assured that vegetables cooked by microwave techniques retained percentages of phytochemicals as well as minerals (magnesium and calcium) higher than the levels of those cooked by conventional methods (*25*).

Common Brassicas consumed in Brazil [butterhead lettuce, rucola, watercress, kale, chicory, cabbage, Chinese cabbage, and the spinach substitute (*Tetragonia expansa*)], conventionally cooked for 3 min without addition of water, exhibited small losses, on the same order of magnitude as the analytical error between replicates (*40*). Then, we could conclude that, with respect to the mineral elements, of interest for human nutrition, the losses during cooking could be rejected when the cooking time is brief and no water is added. On the other hand, it must be considered that thermal treatment would cause denaturation and precipitation of metalloproteins and decomposition of original metallobiomolecules, but the extent of element species alteration varied with the nature of the element and the stability of the element species (*24*).

In general, vitamin C seemed to be the bioactive phytochemical most affected by the microwave cooking of broccoli florets. On the other hand, the most stable phytonutrients were the different mineral nutrients determined. In general, the losses during the cooking treatments of the human health-related compounds (phenolics, glucosinolates, and minerals) were mainly due to leaching into the cooking water. Therefore, we could conclude that losses of phytochemicals would be prevented with shorter cooking times and avoiding cooking with water, even if this last treatment could make the texture unacceptable for some consumers.

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