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ARTICLE

Effect of Different Cooking Methods on Color, Phytochemical Concentration, and Antioxidant Capacity of Raw and Frozen *Brassica* Vegetables

Nicoletta Pellegrini,*,† Emma Chiavaro,‡ Claudio Gardana,§ Teresa Mazzeo,† Daniele Contino,§ Monica Gallo,# Patrizia Riso,§ Vincenzo Fogliano,# and Marisa Porrini§

†Department of Public Health, University of Parma, via Volturno 39, 43125 Parma, Italy, ‡Department of Industrial Engineering, University of Parma, viale GP Usberti 181/A, 43124 Parma, Italy, §Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, sezione Nutrizione Umana Università degli Studi di Milano, via Celoria 2, 20133 Milan, Italy, and #Department of Food Science, University of Napoli "Federico II", Parco Gussone, 80055 Portici, Napoli, Italy

This study evaluated the effect of common cooking practices (i.e., boiling, microwaving, and basket and oven steaming) on the phytochemical content (carotenoids, chlorophylls, glucosinolates, polyphenols, and ascorbic acid), total antioxidant capacity (TAC), and color changes of three generally consumed *Brassica* vegetables analyzed fresh and frozen. Among cooking procedures, boiling determined an increase of fresh broccoli carotenoids and fresh Brussels sprout polyphenols, whereas a decrease of almost all other phytochemicals in fresh and frozen samples was observed. Steaming procedures determined a release of polyphenols in both fresh and frozen samples. Microwaving was the best cooking method for maintaining the color of both fresh and frozen vegetables and obtaining a good retention of glucosinolates. During all cooking procedures, ascorbic acid was lost in great amount from all vegetables. Chlorophylls were more stable in frozen samples than in fresh ones, even though steaming methods were able to better preserve these compounds in fresh samples than others cooking methods applied. The overall results of this study demonstrate that fresh *Brassica* vegetables retain phytochemicals and TAC better than frozen samples.

KEYWORDS: Broccoli; Brussels sprouts; cauliflower; color; steaming; boiling; microwaving

INTRODUCTION

Epidemiological evidence supports the strategy of increasing plant food consumption as a tool for primary prevention against chronic degenerative diseases (*1*). Consequently, consumers became aware of the need to consume a variety of fresh vegetables. However, the seasonal nature of vegetable production and the difficulties in purchasing fresh vegetables have favored the use of those commercialized frozen.

Among vegetables, *Brassica* ones belonging to the family of Brassicaceae, namely, broccoli, cabbage, cauliflower, Brussels sprouts, and kale, are a good source of phytochemicals, such as vitamins, carotenoids, and polyphenols (*2*). In addition, cruciferous vegetables provide a large group of glucosinolates, which possesses rather low antioxidant activity per se, but the products of hydrolysis can modulate functions related with the endogenous defense system and ultimately protect against cancer development (*3*).

Vegetables, especially those frozen, are commonly cooked before being consumed. It is known that cooking induces significant changes in chemical composition, reducing vitamin C and other thermolabile compounds that may undergo oxidative degradation or be leached into the water during home cooking and industrial processing (*4*). However, processing can also lead to disruption of the food matrix, increasing the bioaccessibility of many phytochemicals and thus improving the nutritional quality of vegetables.

The effect of domestic cooking on phytochemicals of vegetables, especially *Brassica* ones, has been extensively studied, even though the data are fragmentary and incomplete, and comparison among the studies is difficult. Sultana et al. (*5*) focused their investigation only on total antioxidant capacity (TAC) and total polyphenols in cauliflower and other vegetables cooked by boiling, frying, and microwaving, demonstrating that all of the cooking methods affected the antioxidant properties. Similarly, TAC values were the only analytical parameter evaluated by Wachtel-Galor et al. (*6*) for analyzing the effect of cooking (i.e., microwaving, boiling, and steaming) on *Brassica* vegetables. In evaluating the impact of different cooking methods (i.e., boiling, steaming, and microwaving) on fresh and frozen broccoli and red pepper, only two parameters, that is, β-carotene and α-tocopherol, were explored (*7*). In the same way, Howard et al. (*8*) analyzed the content of *trans*-β-carotene and ascorbic acid before and after microwaving broccoli, carrots, and green beans, demonstrating

*Corresponding author (telephone ++39 0521 903907; fax ++39 0521 903832; e-mail nicoletta.pellegrini@unipr.it).

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that these cooking procedures had minimal effect on the evaluated parameters. As *Brassica* vegetables are almost the unique source of glucosinolates in human diet, some authors have focused their attention solely on this class of phytochemicals when exploring the effect of domestic and technological processes on such vegetables (*9*−*11*). When more complete investigations analyzing different parameters were carried out, such studies were focused on a single vegetable. In fact, Roy et al. (*4*) analyzed total polyphenols and flavonoids and TAC values in steam-processed broccoli, Zuang and Hamauzu (*12*) evaluated total polyphenols, ascorbic acid, carotenoids, and TAC in boiled and microwaved broccoli, and Volden et al. (*13*) determined glucosinolates, total monomeric anthocyanins, total polyphenols, ascorbic acid, and TAC in blanched, boiled, and steamed cauliflower.

Such incomplete literature makes it difficult to have an overview of the effect of domestic cooking on all of the phytochemicals present in *Brassica* vegetables, in both fresh and frozen form. Thus, the aim of this study was to evaluate the effect of common cooking practices (i.e., boiling, microwaving, and steaming) on phytochemical content (carotenoids, phenol compounds, glucosinolates, chlorophylls, and ascorbic acid), total antioxidant capacities, and color changes of three generally consumed *Brassica* vegetables (broccoli, Brussels sprouts, and cauliflower) analyzed fresh and frozen.

MATERIALS AND METHODS

Chemicals. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-*s*-triazine (TPTZ), *β*-carotene, lutein, quercetin, rutin, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, kaempferol, morin, and 2,6-di-*tert*-butyl-*p*-cresol (BHT) were purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO); *R*-phycoerythrin (R-PE) was from Prozyme (San Leandro, CA); 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP) was from Waco Chemicals (Richmond, VA); sinigrin (allyl glucosinolate) was from Extrasynthese (Genay, France); L-ascorbic acid was from Merck (Darmstadt, Germany); and chlorophyll *a* and chlorophyll *b* were from DHI Laboratory (Hoersholm, Denmark).

All chemicals and solvents used were of HPLC grade and purchased from Carlo Erba (Milan, Italy) and from Merck. High-purity water was produced in the laboratory by using an Alpha-Q system (Millipore, Marlborough, MA).

Preparation of Vegetables. Freshly harvested and frozen broccoli (*Brassica oleracea* L. cv. *Italica*), Brussels sprouts (*Brassica oleracea* L. cv. *gemmifera*), and white cauliflower (*Brassica oleracea* L. cv. *botrytis cauliflora*) of a single batch were analyzed.

Fresh Brussels sprouts were purchased from a local market on the day of processing after road transport under refrigerated conditions within 24 h of harvest and stored at 7 °C for up to 1 day before they were taken.

Fresh broccoli and cauliflower were obtained by a local purchaser and analyzed within 3 days of harvest. Samples were stored at 4 °C before analysis.

Frozen vegetables were purchased from a local supermarket. All frozen samples were blanched before freezing as labeled. Information was obtained from manufacturers. All samples were purchased within 20 days of harvest.

Fresh broccoli and cauliflower were cleaned by removing of inedible parts and then chopped into homogeneous pieces, leaving a stem of 2.5 cm. Brussels sprouts were deprived of the external leaves. Frozen vegetables were not defrosted before cooking according to common habits. Frozen broccoli consisted of both stem and florets, whereas only florets of frozen cauliflower were present in the package.

To obtain more homogeneous samples, each vegetable was prepared in batches of 500 g. Each batch was then divided into five equal portions. One portion was retained raw; the others were cooked in triplicate with four different methods, as given below.

Cooking Treatments. Cooking conditions were optimized by preliminary experiments carried out for each vegetable in which samples were considered to be cooked according to the judgment of a large group of semitrained panelists already employed for estimating the cooking time on other vegetables (*14*, *15*). For all cooking treatments, the minimum cooking time to reach tenderness for an adequate palatability and taste, according the Italian eating habits, was used.

Boiling. Fresh or frozen *Brassica* vegetables were added to boiling tap water in a covered stainless steel pot (1:5, food/water) and cooked on a moderate flame. Cooking time was 8, 10, and 10 min for fresh broccoli, Brussels sprouts, and cauliflower, respectively. Frozen broccoli, Brussels sprouts, and cauliflower were cooked for 15, 7, and 9 min, respectively. Cooking time was measured starting from putting samples in the boiling water. For each cooking trial, 10 samples were boiled. Then, samples were drained off for 30 s.

Steaming. Two types of steaming equipment were employed: an air/steam impingement oven and a domestic cooker equipped with a mesh basket.

Air/steam oven treatments were carried out in a Combi-Steal SL oven (V-Zug, Zurich, Switzerland). Nine specimens of fresh or frozen *Brassica* vegetables were placed in the oven equilibrated to room temperature before each cooking trial. Eight samples were arranged in a circle, and one put at the center to ensure uniform heating conditions in all samples for each cooking trial. Cooking time was 13, 17, and 13 min for fresh broccoli, Brussels sprouts, and cauliflower, respectively. Frozen broccoli, Brussels sprouts, and cauliflower were all cooked for 12 min. Samples were put into the oven when a temperature of 100 °C was reached (displayed by the apparatus).

A single layer of nine specimens of fresh or frozen *Brassica* vegetables was steamed into a domestic closed vessel using a stainless steel steam basket suspended above a small amount of boiling water. Cooking time was 15, 18, and 11 min for fresh broccoli, Brussels sprouts, and cauliflower, respectively. Frozen broccoli, Brussels sprouts, and cauliflower were cooked for 14, 10, and 10 min, respectively. Cooking time was measured starting from the moment in which the sample was suspended above boiling water.

Microwaving. Microwave treatments were carried out in a domestic microwave oven (Samsung Electronics Co. Ltd., Paldal-Gu Suwon Kyungki-Do, Korea) without water. Ten specimens of fresh or frozen *Brassica* vegetables were exposed at a frequency of 2450 Hz at low power (300 W) on the rotating turntable plate of the oven. Cooking time was 30, 18, and 30 min for fresh broccoli, Brussels sprouts, and cauliflower, respectively. Frozen broccoli, Brussels sprouts, and cauliflower were cooked for 13, 6, and 20 min, respectively.

Dry Matter Determination. For the determination of moisture, 3−4 g of raw or cooked sample (as triplicate), homogenized using a high-speed blender under nitrogen, was dried in a convection oven at 105 °C for at least 16 h until a constant weight was reached.

Color Analysis. Color determination was carried out using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D_{65}. Both raw and cooked samples were analyzed. The assessments were carried out at room temperature (25 °C) on preselected locations of broccoli and cauliflower florets and Brussels sprout surface. L^* (lightness, black = 0, white = 100), a^* (redness > 0, greenness < 0), b^* (yellowness, $b^* > 0$, blue < 0), C (chroma, 0 at the center of the color sphere), and hue° (hue angle, red = 0°, yellow = 90°, 180° = green, 270° = blue) were quantified on each sample using a 10 degree position of the standard observer (*16*). The individual differences in L^*, a^*, and b^* values of each cooking treatment with respect to the color of the raw samples were evaluated using ΔE calculation (*16*). A total of 15 determinations was performed on raw and cooked samples.

Determination of Phytochemical Compounds and Total Antioxidant Capacity. All samples were cooled with ice after cooking. For the analyses of phytochemical compounds, with the exception of ascorbic acid, the samples were freeze-dried utilizing a Brizzio-Basi instrument (Milan, Italy). Dried sample material was finely ground, kept in sealed bags, and stored at −20 °C until analysis. Determinations of ascorbic acid and TAC were performed within 24 h of cooking on samples cooled but not freeze-dried.

Carotenoids and Chlorophylls. Lyophilized samples (100 mg) were extracted at least four times (until colorless) with 5 mL of tetrahydrofuran in an ultrasonic bath, vortexed for 1 min, and centrifuged for 5 min at 1500*g*. The supernatants were combined, dried under nitrogen, and stored

at −80 °C until HPLC analysis. The residue was dissolved in 10 mL of a solution of methanol/tetrahydrofuran (95:5, v/v) before HPLC analysis.

Carotenoids and chlorophylls were evaluated using a HPLC system (Alliance and DAD model 2996, Waters, Milford, MA) equipped with a Vydac 201TP54 (4.6 × 250 mm) column. The elution was carried out by linear gradient using methanol (A) or THF (B) as eluent. The gradient was as follows: 0% B for 5 min, from 0 to 15% B in 15 min, and then 15% B for 10 min. The flow rate was 1.2 mL/min. Lutein and β-carotene were acquired at 445 nm, chlorophyll *a* was acquired at 663 nm, and chlorophyll *b* was acquired at 645 nm.

Polyphenols. One gram of lyophilized sample was extracted with 10 mL of 60% aqueous methanol solution containing 0.25 mg of morin as an internal standard, it was hydrolyzed by addition of 20 mM sodium diethyldithiocarbamate and 5 mL of 6 M HCl, and then it was refluxed at 90 °C for 2 h. A total of 20 μL of the extract was analyzed by HPLC as previously described (*14*).

Glucosinolates. About 200 mg of freeze-dried sample was sonicated with 4 mL of methanol for 10 min. The mixture was centrifuged at 1500*g* for 5 min, and the supernatant was transferred into a 10 mL flask, whereas the solid residue was extracted and treated as described above. The supernatants were mixed and the final volume adjusted to 10 mL by methanol. Before being injected in the chromatographic system, the extract was diluted with eluent and centrifuged at 4000*g* for 1 min.

The chromatographic system consisted of an Alliance model 2695 (Waters) coupled with a model 2996 DAD (Waters) and a triple-quadrupole mass spectrometer model Quattro Micro (Micromass, Beverly, MA). A 3 μm C_{18} Luna(2) column (150 × 2.1 mm, Phenomenex, Torrance, CA) was used for the separation, which was performed by means of a linear gradient elution using as eluents 0.1% HCOOH (A) and CH_3CN (B). The linear gradient was as follow: from 5 to 50% B in 30 min and returned to 5% B in 5 min. The flow rate was 250 μL/min, the column was maintained at 30 °C, and 5 μL was injected in the chromatographic system. The capillary voltage was set to 3.0 kV; the cone voltage and the collision energy were specific for each compound. The source temperature was 130 °C, the desolvating temperature was 350 °C, and argon was used at 2.0×10^{-3} mbar to improve fragmentation in the collision cell. Data were acquired by Masslinx 4.0 with Quan-Optimize option for fragmentation study. The MS/MS spectra of glucosinolates showed the presence of typical product ions with $(m/z)^-$ 97 Da corresponding to the sulfate moiety $(SO_3H)^-$. Neoglucobrassicin and 4-methoxyglucobrassicin showed typical UV spectra and identical parent (m/z 477) and product ions (m/z 97); thus, they were differentiated by comparison with reported elution sequence during RP-LC. Alkyl-glucosinolates such as glucoiberin, progoitrin, and sinigrin were not well separated in RP-LC due to their high polarity. On the other hand, successful separation of these compounds was achieved by LC-MS/MS with MRM detection; thus, the partial peak overlap does not affect the quantification of these compounds.

The following fragmentation transitions for the multiple reaction monitoring (MRM) were used: $(m/z)^-$ 358→97 (sinigrin), 388→97 (progoitrin), 422→97 (glucoiberin), 436→97 (glucoraphanin), 447→97 (glucobrassicin), 463→97 (4-hydroxyglucobrassicin), and 477→97 (4-methoxyglucobrassicin and neoglucobrassicin), with a dwell time of 0.2 s per transition. A calibration curve was obtained from sinigrin stock solutions prepared by dissolving 5 mg of standard powder in 50 mL of methanol. The working solutions were prepared in 0.1% HCOOH in the range of 0.5−10 μg/mL. Glucosinolates were assayed using the sinigrin calibration curve, and their amounts were normalized by the molecular mass ratios.

Ascorbic Acid. Ascorbic acid was extracted using the method proposed by Dürust et al. (*17*). Briefly, a homogenized portion of raw and cooked vegetables was added to an equivalent weight of oxalic acid solution (0.4%, w/v). The mixture was homogenized in a high-speed blender. A portion of the homogenized sample (~1 g) was subsequently diluted with an appropriate volume (according to ascorbic acid content expected) of oxalic acid solution, shaken, and centrifuged at 1000*g* for 5 min. All samples were immediately analyzed by HPLC as described by Gokmen et al. (*18*).

TAC Determination. Raw and cooked samples were extracted for the measurements of the TAC values as previously described by Miglio et al. (*14*). Food extracts were immediately analyzed in triplicate for their antioxidant capacity by three different TAC assays: Trolox equivalent antioxidant capacity (TEAC) assay (*19*), total radical-trapping antioxidant parameter (TRAP) assay (*20*), and ferric reducing antioxidant power (FRAP) assay (*21*). The TEAC and TRAP values were expressed as millimoles of Trolox per 100 g of sample. FRAP values were expressed as millimoles of Fe^{2+} equivalents per 100 g of sample.

Table 1. Color Indices of Raw and Cooked *Brassica* Vegetables[a]

	raw	boiled	microwaved	basket steamed	oven steamed
			Fresh Broccoli		
L^*	49.9 ± 2.0 a	38.3 ± 3.0 c	41.5 ± 2.4 b	38.8 ± 1.8 c	38.7 ± 2.1 c
a^*	−4.7 ± 1.0 c	−7.7 ± 1.7 d	−1.2 ± 1.1 a	−3.1 ± 1.4 b	−3.6 ± 0.6 b
b^*	8.0 ± 3.4 b	10.6 ± 2.7 a	9.0 ± 2.6 ab	8.8 ± 2.0 ab	7.7 ± 1.6 c
ΔE		12.8 ± 2.3 a	9.7 ± 2.0 b	11.5 ± 1.8 a	11.4 ± 2.0 a
C	9.4 ± 3.4 b	13.1 ± 3.1 a	9.2 ± 2.6 b	9.4 ± 2.3 b	8.5 ± 1.7 b
hue°	121.9 ± 4.7 b	126.2 ± 2.6 a	97.8 ± 6.7 e	108.7 ± 5.2 d	115.5 ± 2.3 c
			Frozen Broccoli		
L^*	40.0 ± 1.6 b	42.0 ± 2.0 a	36.1 ± 1.3 c	38.6 ± 1.4 b	38.8 ± 1.1 b
a^*	−8.4 ± 2.9 bc	−9.6 ± 1.2 c	−5.8 ± 0.8 a	−6.2 ± 1.1 a	−7.8 ± 1.0 b
b^*	13.7 ± 6.4 bc	19.2 ± 3.0 a	11.8 ± 1.8 c	14.3 ± 2.2 bc	16.1 ± 2.2 ab
ΔE		6.9 ± 4.0 a	5.4 ± 2.1 ab	4.4 ± 1.2 b	4.1 ± 1.3 b
C	16.1 ± 6.9 bc	21.2 ± 1.6 a	13.2 ± 2.4 c	15.6 ± 2.4 bc	18.0 ± 2.4 b
hue°	122.9 ± 4.5 a	117.1 ± 2.0 b	116.3 ± 1.7 b	111.9 ± 1.7 c	116.1 ± 1.6 b
			Fresh Brussels Sprouts		
L^*	60.8 ± 1.8 a	51.7 ± 1.5 b	58.6 ± 1.9 a	42.9 ± 1.7 d	47.9 ± 0.5 c
a^*	−10.5 ± 0.6 b	−9.5 ± 1.0 b	−9.9 ± 2.0 b	−1.9 ± 0.7 a	−2.2 ± 0.6 a
b^*	30.4 ± 1.6 b	33.7 ± 1.8 a	34.1 ± 3.5 a	23.7 ± 1.8 c	28.4 ± 1.5 b
ΔE		9.9 ± 1.0 c	5.9 ± 1.5 d	21.0 ± 2.0 a	15.5 ± 0.8 b
C	31.8 ± 2.3 b	35.0 ± 2.0 ab	35.7 ± 3.3 a	23.8 ± 1.8 d	28.5 ± 1.5 c
hue°	109.4 ± 0.8 a	106.0 ± 1.6 b	106.3 ± 2.9 b	94.6 ± 1.6 c	94.4 ± 1.2 c
			Frozen Brussels Sprouts		
L^*	39.0 ± 0.7 a	39.1 ± 0.8 a	37.9 ± 0.7 b	38.5 ± 0.8 ab	37.9 ± 0.8 b
a^*	−12.0 ± 1.1 c	−11.8 ± 1.0 c	−11.6 ± 0.8 bc	−7.4 ± 1.7 a	−8.0 ± 1.1 b
b^*	19.5 ± 1.9 a	17.5 ± 1.6 b	17.1 ± 1.2 bc	15.8 ± 1.5 c	16.2 ± 1.5 bc
ΔE		2.7 ± 1.4 b	2.9 ± 1.2 b	6.0 ± 1.8 a	3.5 ± 1.3 b
C	22.9 ± 1.8 a	21.2 ± 1.6 b	20.7 ± 1.1 b	17.5 ± 1.6 c	18.1 ± 1.4 c
hue°	121.7 ± 3.1 a	124.2 ± 2.6 a	124.2 ± 2.4 a	81.8 ± 5.2 b	116.4 ± 3.6 b
			Fresh Cauliflower		
L^*	79.5 ± 4.5 a	70.3 ± 3.9 b	79.3 ± 3.0 a	71.6 ± 3.9 b	62.8 ± 3.7 c
a^*	0.3 ± 0.3 a	−2.4 ± 0.4 c	−0.6 ± 0.5 b	−2.5 ± 0.5 c	−3.2 ± 0.6 d
b^*	17.2 ± 2.7 a	11.5 ± 2.1 c	16.9 ± 2.9 ab	14.4 ± 2.7 b	8.2 ± 2.3 d
ΔE		11.3 ± 3.9 b	4.0 ± 1.2 c	9.2 ± 4.1 b	19.4 ± 3.6 a
C	17.2 ± 2.7 a	11.7 ± 2.1 c	16.9 ± 2.9 ab	14.6 ± 2.6 b	8.8 ± 2.2 d
hue°	88.9 ± 1.0 d	102.0 ± 1.8 b	92.2 ± 1.9 c	100.4 ± 3.6 b	112.4 ± 5.7 a
			Frozen Cauliflower		
L^*	75.5 ± 1.1 a	71.9 ± 2.8 c	76.0 ± 1.4 a	74.3 ± 1.4 ab	72.4 ± 1.7 bc
a^*	−2.7 ± 0.3 b	−2.2 ± 0.3 a	−2.3 ± 0.3 a	−2.4 ± 0.1 ab	−2.7 ± 0.3 b
b^*	12.2 ± 2.2 ab	13.5 ± 2.8 a	12.2 ± 1.8 ab	11.7 ± 1.1 ab	10.2 ± 2.3 b
ΔE		5.0 ± 2.3 a	2.1 ± 1.1 b	2.7 ± 1.3 b	4.5 ± 1.8 a
C	12.5 ± 2.2 ab	13.7 ± 2.7 a	12.4 ± 1.7 ab	12.0 ± 1.1 ab	10.6 ± 2.2 b
hue°	102.9 ± 2.2 b	99.8 ± 2.8 c	100.8 ± 1.6 bc	101.8 ± 1.2 bc	105.7 ± 3.6 a

[a] Values are expressed in colorimetric units and presented as mean ± SD (n = 15). Means in rows followed by different letters differed significantly ($p \leq 0.05$).

Statistical Analysis. Means and standard deviations (SD) of data were calculated with SPSS (version 16.0, SPSS Inc., Chicago, IL) statistical software. SPSS was used to perform one-way analysis of variance (ANOVA) with type of cooking as dependent factor. The least significant difference (LSD) test at a 95% confidence level ($p \leq 0.05$) was further used to identify differences among groups.

RESULTS AND DISCUSSION

In the following paragraphs the effects of cooking practices are separately listed for the three *Brassica* species investigated.

Broccoli. *Effect of Cooking on Color Parameters*. Color values are reported in **Table 1** for fresh and frozen broccoli. Cooking of

Table 2. Phytochemical Compounds of Raw and Cooked Fresh Broccoli[a]

	raw	boiled	microwaved	basket steamed	oven steamed
carotenoids (mg/100 g)					
lutein	8.4 ± 0.4 a	5.6 ± 1.1 b	5.5 ± 0.0 b	8.8 ± 1.3 a	10.5 ± 1.1 a
β-carotene	5.0 ± 0.4 a	4.1 ± 1.2 a	2.0 ± 0.2 b	4.8 ± 0.7 a	5.5 ± 0.6 a
total carotenoids	13.4 ± 0.8 ab	9.7 ± 2.3 bc	7.5 ± 0.2 c	13.7 ± 1.9 ab	16.0 ± 1.7 a
chlorophylls (mg/100 g)					
chlorophyll *a*	71.2 ± 1.9 a	ND[b]	14.5 ± 1.4 d	24.3 ± 3.6 c	38.0 ± 0.9 b
chlorophyll *a*'[c]	0.4 ± 0.4 c	ND	0.8 ± 0.1 c	3.6 ± 0.3 b	5.5 ± 0.4 a
chlorophyll *b*	29.9 ± 0.8 a	10.4 ± 2.5 cd	7.6 ± 0.7 d	12.9 ± 2.3 bc	16.9 ± 2.0 b
chlorophyll *b*'[d]	ND	ND	ND	2.2 ± 0.5	3.2 ± 0.9
pheophytin *a*[e]	ND	43.3 ± 4.6	2.9 ± 0.0	24.2 ± 1.0	20.5 ± 1.1
chlorophyll *a* derivative[f]	ND	7.4 ± 1.1	ND	6.0 ± 0.8	4.0 ± 0.2
total chlorophylls	101.7 ± 2.3 a	61.0 ± 7.3 b	25.8 ± 2.3 c	73.2 ± 8.3 b	88.1 ± 4.4 a
glucosinolates[g] (μg/g)					
glucoiberin	248.0 ± 16.5 b	242.2 ± 7.5 bc	219.4 ± 13.8 c	266.0 ± 2.5 b	329.6 ± 3.2 a
glucoraphanin	982.5 ± 7.5 b	1319.4 ± 45.4 a	908.7 ± 18.9 b	1263.5 ± 48.0 a	1315.2 ± 19.0 a
glucobrassicin	731.1 ± 28.7 d	589.3 ± 27.8 e	884.7 ± 43.0 c	1298.3 ± 13.2 a	1162.4 ± 5.0 b
4-hydroxyglucobrassicin	50.7 ± 2.5 c	90.7 ± 6.3 b	93.9 ± 12.0 b	89.8 ± 10.5 b	124.2 ± 5.3 a
neoglucobrassicin	261.7 ± 16.7 b	181.9 ± 9.3 c	329.4 ± 20.6 a	325.7 ± 13.5 a	341.6 ± 4.8 a
4-methoxyglucobrassicin	527.4 ± 30.1 b	245.5 ± 17.3 d	425.7 ± 17.2 c	595.8 ± 9.2 a	618.1 ± 15.4 a
total glucosinolates	2801.4 ± 97.0 b	2668.9 ± 113.5 b	2861.9 ± 125.5 b	3839.1 ± 96.9 a	3891.1 ± 46.4 a
ascorbic acid (mg/100 g)	802.0 ± 25.3 a	654.5 ± 53.8 b	13.3 ± 1.6 c	620.9 ± 51.4 b	643.5 ± 19.5 b
phenol compounds (mg/100 g)					
caffeic acid	6.6 ± 1.1 b	2.2 ± 0.4 c	2.9 ± 0.2 c	4.0 ± 0.1 c	9.1 ± 1.3 a
coumaric acid	11.2 ± 0.1 b	3.1 ± 0.0 c	1.5 ± 0.1 d	3.7 ± 0.1 c	12.5 ± 0.9 a
sinapic acid	27.3 ± 0.3 b	14.8 ± 0.0 c	13.6 ± 0.7 c	23.4 ± 0.5 b	38.7 ± 3.4 a
chlorogenic acid	20.2 ± 0.5 c	88.9 ± 2.8 b	5.7 ± 0.2 c	19.1 ± 0.7 c	135.2 ± 12.2 a
ferulic acid	4.3 ± 0.1 a	0.8 ± 0.0 b	1.2 ± 0.2 b	1.8 ± 0.3 b	4.6 ± 0.9 a
quercetin	23.5 ± 0.1 b	10.0 ± 0.1 cd	5.7 ± 0.3 d	12.2 ± 0.3 c	31.6 ± 4.1 a
kaempferol	18.4 ± 0.5 b	8.4 ± 0.5 d	6.0 ± 0.6 e	10.4 ± 0.3 c	31.6 ± 0.0 a
total phenol compounds	111.4 ± 0.8 b	128.2 ± 3.6 b	36.7 ± 1.5 d	74.6 ± 1.0 c	263.3 ± 20.1 a

[a] Values are presented as mean value ± SD ($n = 3$) and expressed on dry weight basis. Means in rows followed by different letters differed significantly ($p \leq 0.05$). All compounds were identified by pure standards, unless differently reported. [b] ND, not detected. [c] Tentatively identified as the C-10 epimeric isomer of chlorophyll *a* and quantified as chlorophyll *a*. [d] Tentatively identified as the C-10 epimeric isomer of chlorophyll *b* and quantified as chlorophyll *b*. [e] Tentatively identified as pheophytin *a* and quantified as chlorophyll *a*. [f] Tentatively identified as a derivative of chlorophyll *a* and quantified as chlorophyll *a*. [g] Quantified by sinigrin calibration curve and their amounts corrected by the molecular mass ratios. The identity of glucosinolates was preliminarily assessed by UV spectra and molecular weight comparison, and then the different compounds were dissociated by induced collision (CID-MS) and their identity established through characteristic product ions (see Materials and Methods for further details).

fresh broccoli induced a significant L^* decrease for boiled and both steamed products. Loss of greenness ($-a^*$ and $H°$) was also generally shown and more markedly in microwaved vegetables. On the contrary, boiled products became greener. Chroma (C) remained substantially unvaried except for boiled broccoli, which showed a significant increase of color saturation, probably as a consequence of greenness increase.

A more intense green color was previously observed for both stem and florets of broccoli after boiling (*14*). The color of green vegetables was mainly related to chlorophyll pigment content in plant material, and the decrease of greenness was generally associated with the formation of pheophytin by the exchange of Mg^{2+} by H^+ in the center of the porphyrin ring of the pigment (*22*). A higher content of pheophytin *a* (**Table 2**) was detected for boiled broccoli in comparison with the other cooked products, although greenness was found to increase, in agreement with previous findings (*22*). Thus, the greenness increase ($-a^*$) was probably related to an alteration of surface reflecting properties and depth of light penetration into tissues of boiled vegetables, caused by loss of air and other dissolved gases by cells and their replacement by cooking water and cell juices, as previously hypothesized (*14*, *22*). The formation of green-colored degradation products from chlorophylls *a* and *b* cannot be excluded, either (*22*).

Cooking of frozen products induced less color change than did cooking of fresh product, as shown by lower ΔE values. This could be related to the thermal inactivation of enzymes as well as to the deaeration of plant tissue and the consequent reduction of oxygen content induced by blanching. This hypothesized reduction might have limited degradation of chlorophylls during frozen storage, making the pigments more stable after cooking, as previously observed (*23*, *24*). However, $H°$ slightly decreased for all cooking treatments, shifting toward yellow values, probably as a consequence of the small increase of pheophytin content observed after all cooking treatments with the exception of microwaving (**Table 3**). The formation of pheophytin was previously related to the change of color of processed and stored vegetables from bright green to olive brown (*22*).

Effect of Cooking on Phytochemical Profile and Antioxidant Capacities. The effects of cooking on phytochemical compounds present in fresh and frozen broccoli are reported on a dry weight basis in **Tables 2** and **3**, respectively.

The lutein and β-carotene values of fresh raw broccoli were in the range reported by Gliszczynska-Swiglo et al. (*25*), but lower than those found in other studies (*14*). Total carotenoids of frozen broccoli were slightly lower that previous values (*26*).

The effect of cooking methods on fresh and frozen broccoli was different: in the case of fresh broccoli, steaming methods did not affect the content of carotenoids, whereas microwaving determined a significant decrease of both lutein and β-carotene (35 and 60%, respectively) and boiling a significant decrease of lutein (33%). These results do not confirm those of previous reports (*14*, *25*) in which a significant increase of carotenoids was observed following cooking methods (i.e., steaming and boiling), but they are in agreement with those of other authors that showed slight decreases of these compounds after thermal treatments (*12*, *26*). In the case of

Table 3. Phytochemical Compounds of Raw and Cooked Frozen Broccoli[a]

	raw	boiled	microwaved	basket steamed	oven steamed
carotenoids (mg/100 g)					
lutein	12.3 ± 0.4 a	8.8 ± 0.2 b	6.0 ± 0.5 cd	6.9 ± 0.2 c	5.8 ± 0.5 d
β-carotene	9.4 ± 0.3 a	6.5 ± 0.6 b	3.8 ± 0.5 c	3.8 ± 0.2 c	3.7 ± 0.1 c
total carotenoids	21.8 ± 0.3 a	15.3 ± 0.4 b	9.8 ± 1.0 c	10.7 ± 0.4 c	9.6 ± 0.5 c
chlorophylls (mg/100 g)					
chlorophyll *a*	54.8 ± 6.8 a	40.6 ± 2.2 b	39.0 ± 5.0 b	30.8 ± 1.1 bc	24.8 ± 2.7 c
chlorophyll *a*'[b]	6.4 ± 1.2 a	4.5 ± 0.3 b	4.3 ± 0.6 b	4.1 ± 0.2 b	3.4 ± 0.3 b
chlorophyll *b*	16.9 ± 2.7 a	20.0 ± 0.2 a	11.7 ± 1.3 b	12.0 ± 1.0 b	9.9 ± 0.7 b
chlorophyll *b*'[c]	4.5 ± 0.8 a	4.7 ± 0.3 a	2.6 ± 0.6 b	2.3 ± 0.6 b	1.6 ± 0.1 b
pheophytin *a*[d]	3.1 ± 0.4 c	9.0 ± 0.4 b	2.9 ± 0.3 c	11.6 ± 0.3 a	9.5 ± 1.3 a
total chlorophylls	85.7 ± 10.3 a	78.6 ± 2.3 a	60.5 ± 7.8 b	60.8 ± 2.0 b	49.2 ± 2.3 b
glucosinolates[e] (μg/g)					
glucoiberin	143.3 ± 11.9 a	79.8 ± 3.9 c	124.1 ± 6.2 ab	122.0 ± 7.4 b	120.6 ± 7.1 b
glucoraphanin	760.8 ± 35.2 a	449.2 ± 3.9 d	516.1 ± 20.4 c	717.1 ± 6.4 a	615.3 ± 26.0 b
glucobrassicin	764.1 ± 31.9 a	170.1 ± 7.6 c	532.8 ± 17.2 b	718.7 ± 5.1 a	512.5 ± 27.5 b
4-hydroxyglucobrassicin	72.2 ± 6.8 a	0.0 ± 0.0 c	47.0 ± 1.0 b	45.2 ± 3.0 b	42.5 ± 0.8 b
neoglucobrassicin	194.1 ± 13.1 a	50.3 ± 4.3 c	181.7 ± 15.8 a	138.3 ± 7.3 b	135.4 ± 0.9 b
4-methoxyglucobrassicin	285.6 ± 25.5 a	48.6 ± 5.9 c	225.1 ± 12.6 b	225.0 ± 5.1 b	206.7 ± 4.6 b
total glucosinolates	2220.2 ± 124.3 a	798.0 ± 25.5 d	1629.8 ± 73.1 c	1966.6 ± 21.4 b	1633.0 ± 55.9 c
ascorbic acid (mg/100 g)	917.2 ± 19.4 a	376.1 ± 17.9 e	733.6 ± 37.0 b	545.5 ± 21.1 d	642.8 ± 20.6 c
phenol compounds (mg/100 g)					
caffeic acid	7.0 ± 0.5 b	1.8 ± 0.1 e	5.3 ± 0.1 c	8.1 ± 0.4 a	3.1 ± 0.0 d
coumaric acid	9.4 ± 0.2 a	3.0 ± 0.0 c	7.1 ± 0.0 b	9.0 ± 1.0 a	6.0 ± 0.1 b
sinapic acid	35.6 ± 0.6 b	12.2 ± 0.6 d	17.8 ± 0.2 c	39.5 ± 1.3 a	10.6 ± 0.4 d
chlorogenic acid	9.3 ± 1.1 c	12.8 ± 3.0 bc	19.6 ± 0.0 b	54.8 ± 5.8 a	19.9 ± 4.9 b
ferulic acid	2.3 ± 0.3 b	0.9 ± 0.0 c	1.2 ± 0.0 c	3.4 ± 0.0 a	1.0 ± 0.0 c
quercetin	17.9 ± 0.2 b	6.5 ± 0.4 e	13.3 ± 0.0 c	20.7 ± 1.0 a	10.7 ± 0.0 d
kaempferol	4.2 ± 0.2 d	1.5 ± 0.2 e	8.2 ± 0.0 a	5.2 ± 0.1 c	7.3 ± 0.0 b
total phenol compounds	85.7 ± 1.5 b	38.7 ± 3.7 e	72.4 ± 0.4 c	140.7 ± 7.8 a	58.6 ± 4.3 d

[a] Values are presented as mean value ± SD ($n = 3$) and expressed on dry weight basis. Means in rows followed by different letters differed significantly ($p \leq 0.05$). All compounds were identified by pure standards, unless differently reported. [b] Tentatively identified as the C-10 epimeric isomer of chlorophyll *a* and quantified as chlorophyll *a*. [c] Tentatively identified as the C-10 epimeric isomer of chlorophyll *b* and quantified as chlorophyll *b*. [d] Tentatively identified as pheophytin *a* and quantified as chlorophyll *a*. [e] Quantified by sinigrin calibration curve and their amounts corrected by the molecular mass ratios. The identity of glucosinolates was preliminarily assessed by UV spectra and molecular weight comparison, and then the different compounds were dissociated by induced collision (CID-MS) and their identity established through characteristic product ions (see Materials and Methods for further details).

frozen broccoli, all carotenoids were negatively affected by all of the cooking methods applied, as previously observed by Bernhardt and Schlich (*7*) measuring *all-trans*-β-carotene of frozen broccoli. This trend in frozen broccoli could be related to the blanching step leading to a softening of the vegetable matrix; further process (i.e., cooking) leads to a loss of these compounds.

The total chlorophyll content of raw fresh broccoli, lower than that observed by Turkmen et al. (*22*) but close to that reported by Kmiecik et al. (*27*), was significantly decreased by all of the cooking methods applied except for oven steaming. In agreement with Turkmen et al. (*22*), chlorophyll *a* was more sensitive to cooking than chlorophyll *b* and pheophytin *a* was found in the highest amount by boiling, followed by steaming methods and microwaving. Uncooked frozen broccoli had a high amount of the C-10 epimers of chlorophylls *a* and *b*, which are formed during processing. Boiling determined no changes in total chlorophylls, whereas other cooking methods decreased significantly these compounds, mainly due to the degradation of chlorophylls *a* and *b*. Moreover, the formation of pheophytin *a* during processing followed the same pattern seen for fresh broccoli.

The total glucosinolate content of fresh raw broccoli, presented in **Table 2**, was in the reported range of broccoli cultivars (*28*), and the predominance of glucoraphanin and glucobrassicin was consistent with the findings of Rungapamestry et al. (*9*).

The total content of glucosinolates of fresh broccoli significantly increased by both steaming methods, as already shown (*14*, *25*), whereas it was not modified by boiling, as the main compound (i.e., glucoraphanin) had a slight increase and indolic compound (brassicin groups) slightly decreased. However, the modification of glucosinolates concentration upon cooking was not significantly different between aliphatic and indolic compounds. With regard to boiling, different effects on fresh broccoli glucosinolates have been reported in the literature: some authors showed a reduction (*10*, *11*, *14*, *25*), whereas, in agreement with the results of this study, Rungapamestry et al. (*9*) demonstrated a retention of these compounds. In the case of microwaving, there was no change of total glucosinolates, but only slight changes in single glucosinolate content. This retention is probably justified by the lack of water in our microwave cooking, confirming that the great loss of these compounds is due to high cooking water evaporation containing leached compounds, as suggested by Vallejo et al. (*29*).

On the contrary, in frozen broccoli, boiling, as well as other cooking methods, led to a decrease in glucosinolate amount, confirming a previous observation (*9*). This different behavior of frozen vegetable should be once more related to the previous process (i.e., blanching and freezing storage) softening the vegetable matrix and causing losses of these compounds from the vegetable matrix of frozen materials rather than those of fresh.

Fresh raw broccoli contained 802 mg/100 g of dry weight (82.6 mg/100 g of fresh weight) of ascorbic acid (**Table 2**), a value in agreement with previous literature (*25*). All cooking methods significantly affect the content of ascorbic acid. In the case of boiling and both steam-cooking treatments, losses of about 20% were observed, in agreement with Miglio et al. (*14*). As already reported (*29*), microwave cooking caused the almost complete loss of ascorbic acid. However, such loss was greater than that

observed by Vallejo et al. (*29*), probably due to the different cooking conditions applied by us (lower power for longer time).

In frozen broccoli (**Table 3**), the content of ascorbic acid was significantly reduced by all of the cooking processing; however, in contrast with fresh broccoli, microwaved frozen broccoli retained the most of ascorbic acid content, whereas boiling determined the greatest loss.

With regard to polyphenol content in uncooked broccoli, the most abundant phenolic acid was sinapic acid, in accordance with Mattila and Hellstron (*30*), followed by chlorogenic and coumaric acids, whereas quercetin was the major flavonoid. As far as fresh broccoli, results showed large differences among the four cooking treatments on flavonoid and phenolic acid contents (**Table 3**). Boiling had a detrimental effect on flavonoid content, determining about 50% loss, even though this was lower than that previously observed in broccoli boiled for 15 min (*31*). Similarly, phenolic acids decreased, with the exception of chlorogenic acid for which a 4 times increase was observed. Oven steaming determined an increase of all the compounds, confirming that this processing is the best for preserving the polyphenol contents in fresh broccoli, probably owing to the inactivation of oxidative enzymes and/or to the nondirect contact with water, which prevents their solubilization (*14*, *32*). Surprisingly, the basket steaming procedure determined significant losses of both flavonoids and phenolic acids. Phytochemical degradation was also detected when broccoli was microwaved, namely, high losses of flavonoids (between 67 and 75%) and coumaric (87%) and chlorogenic and ferulic acids (72%). These results confirm those reported by Vallejo et al. (*36*), even though broccoli was microwaved without water in the present study. Concerning frozen broccoli polyphenols (**Table 3**), cooking processing exhibited a different influence with respect to that shown for fresh ones. Boiling had a significant negative effect on both flavonoids and phenolic acids, determining a 54% loss of total polyphenols, similar to that observed by Gebczynski and Lisiewska (*26*) in the case of blanched frozen broccoli boiled for 4 min. In microwaved frozen broccoli, the retention of these compounds was higher than in the fresh ones, being also a double increase of chlorogenic acid and kaempferol. The different cooking time of frozen vegetables compared to fresh ones, being shorter in the case of microwaving and longer in that of boiling, could explain the different effects on polyphenols content. For steaming, cooking times were similar for frozen and fresh broccoli, but surprisingly different effects were observed: in the case of frozen broccoli, basket steaming determined a significant increase of all the phenolic compounds, whereas oven steaming had a detrimental effect on phenolic acids, especially on sinapic acid and quercetin.

The TAC of fresh and frozen broccoli cooked in different conditions is shown in **Table 4**. For fresh broccoli, boiling and oven steaming generally led to an increase of TAC, confirming previous reports (*6*, *14*, *25*). Microwaving had always a detrimental effect, in disagreement with other authors (*6*), probably because the microwave conditions here applied are more severe than in the previous papers. Finally, the effect of basket steaming strongly depends on TAC assay. The different results obtained applying the three TAC assays could be due to their different abilities to measure the different phytochemicals. The TEAC assay measures the ability of antioxidants to quench a radical cation (ABTS) in both lipophilic and hydrophilic environments, whereas TRAP and FRAP assays evaluate the chain-breaking antioxidant potential and the reducing power of the sample, respectively, mainly in a hydrophilic environment (*33*). Thus, the increase of carotenoids, besides that of polyphenols, found in oven-steamed fresh broccoli was better caught by the TEAC assay than by the other antioxidant assays.

Table 4. TEAC, TRAP, and FRAP Values of Raw and Cooked *Brassica* Vegetables[a]

	raw	boiled	microwaved	basket steamed	oven steamed
			Fresh Broccoli		
TEAC	2.0 ± 0.1 c	2.7 ± 0.1 b	1.6 ± 0.1 d	2.7 ± 0.1 b	3.3 ± 0.1 a
FRAP	5.7 ± 0.2 c	8.8 ± 0.3 a	5.3 ± 0.1 c	5.3 ± 0.2 c	6.8 ± 0.2 b
TRAP	3.2 ± 0.2 b	4.5 ± 0.0 a	2.7 ± 0.1 c	2.2 ± 0.0 d	3.3 ± 0.1 b
			Frozen Broccoli		
TEAC	2.5 ± 0.1 a	1.7 ± 0.2 b	2.2 ± 0.3 ab	1.7 ± 0.1 b	1.8 ± 0.3 b
FRAP	10.7 ± 0.4 a	6.0 ± 0.5 c	9.2 ± 0.7 ab	8.9 ± 1.0 b	7.9 ± 0.5 b
TRAP	4.0 ± 0.1 a	3.3 ± 0.1 b	2.7 ± 0.2 c	1.3 ± 0.0 e	2.3 ± 0.0 d
			Fresh Brussels Sprouts		
TEAC	1.3 ± 0.0 cd	3.1 ± 0.2 a	1.3 ± 0.1 d	1.8 ± 0.1 b	1.5 ± 0.0 c
FRAP	3.2 ± 0.0 c	6.2 ± 0.1 a	3.0 ± 0.1 c	5.6 ± 0.3 b	6.4 ± 0.4 a
TRAP	1.3 ± 0.1 c	2.3 ± 0.2 a	1.5 ± 0.1 c	2.0 ± 0.2 b	1.4 ± 0.1 c
			Frozen Brussels Sprouts		
TEAC	2.2 ± 0.2 a	1.9 ± 0.1 ab	1.3 ± 0.0 cd	1.6 ± 0.2 bc	1.1 ± 0.0 d
FRAP	9.4 ± 0.6 a	6.0 ± 0.1 c	9.2 ± 0.6 a	10.0 ± 0.7 a	7.5 ± 0.4 b
TRAP	3.1 ± 0.2 a	1.7 ± 0.1 d	2.6 ± 0.1 b	3.4 ± 0.1 a	2.3 ± 0.1 c
			Fresh Cauliflower		
TEAC	1.9 ± 0.1 c	2.3 ± 0.1 b	0.7 ± 0.0 d	3.1 ± 0.1 a	2.9 ± 0.1 a
FRAP	3.4 ± 0.1 d	6.0 ± 0.5 c	2.0 ± 0.0 e	7.4 ± 0.1 b	8.6 ± 0.5 a
TRAP	2.1 ± 0.0 d	2.3 ± 0.1 c	0.8 ± 0.0 e	3.1 ± 0.0 a	2.9 ± 0.1 b
			Frozen Cauliflower		
TEAC	1.2 ± 0.1 ab	0.9 ± 0.0 c	1.2 ± 0.1 b	1.5 ± 0.1 a	1.1 ± 0.1 bc
FRAP	6.5 ± 0.4 a	2.9 ± 0.2 b	6.1 ± 0.5 a	6.3 ± 0.5 a	5.5 ± 0.3 a
TRAP	1.5 ± 0.1 b	0.4 ± 0.0 d	1.9 ± 0.1 a	1.2 ± 0.1 c	1.9 ± 0.1 a

[a] Values are presented as mean value ± SD (n = 3) and expressed as mmol/100 g of dry weight. Means in rows followed by different letters differed significantly ($p \leq 0.05$).

With regard to frozen broccoli, the effect of cooking procedures was extremely clear: all of the cooking methods applied determined losses of TAC values, independent of assay, with the exception of microwaving, which generally determined no loss of TAC values. Such a general detrimental effect of cooking on the nutritional quality of frozen broccoli suggests that the disruption of the cell membrane, due to the blanching and subsequent frozen storage of these vegetables, even though not affecting homogeneously all antioxidant compounds, determines a severe reduction of antioxidant capacity during cooking.

Brussels Sprouts. *Effect of Cooking on Color Parameters.* Color parameters for fresh and frozen Brussels sprouts and their changes after cooking are summarized in **Table 1**. Among cooking cycles, both steaming treatments induced more marked changes. Brussels sprouts cooked under these procedures became less bright (L^*), green ($-a^*$, $H°$), and yellow (b^*), resulting in a marked loss of color saturation (C). On the contrary, color changes were less evident for boiled and microwaved Brussels sprouts. Both of these cooked vegetables showed similar greenness ($-a^*$) as fresh products, and this is probably related to the change of reflecting properties of the vegetable surfaces and/or the formation of green-colored degradation products, as described above for broccoli. Preservation or increase of green intensity values ($-a^*$) was previously observed by Olivera et al. (*34*) on Brussels sprout heads after boiling and/or microwaving blanching treatments in comparison with fresh products, although times of these treatments were significantly shorter than those employed in the present study.

Table 5. Phytochemical Compounds of Raw and Cooked Fresh Brussels Sprouts[a]

	raw	boiled	microwaved	basket steamed	oven steamed
carotenoids (mg/100 g)					
lutein	0.9 ± 0.1 b	2.1 ± 0.1 a	1.1 ± 0.1 b	0.9 ± 0.1 b	0.8 ± 0.0 b
β-carotene	1.7 ± 0.1 a	1.5 ± 0.1 a	1.0 ± 0.1 b	0.7 ± 0.2 bc	0.5 ± 0.0 c
total carotenoids	2.6 ± 0.2 b	3.6 ± 0.1 a	2.1 ± 0.2 c	1.6 ± 0.3 cd	1.3 ± 0.1 d
chlorophylls (mg/100 g)					
chlorophyll *a*	2.4 ± 0.3 a	ND[b]	1.8 ± 0.4 a	1.4 ± 0.2 ab	1.1 ± 0.1 b
pheophytin *a*[c]	ND	1.4 ± 0.2	ND	0.6 ± 0.1	0.3 ± 0.0
chlorophyll *a* derivative[d]	ND	0.5 ± 0.0	ND	0.6 ± 0.0	0.5 ± 0.0
total chlorophylls	2.4 ± 0.3 a	1.9 ± 0.2 b	1.8 ± 0.4 b	2.6 ± 0.2 a	2.0 ± 0.1 b
glucosinolates[e] (μg/g)					
glucoiberin	615.2 ± 15.8 ab	635.1 ± 4.0 a	570.7 ± 25.1 c	589.2 ± 2.0 bc	649.1 ± 9.4 a
glucoraphanin	209.3 ± 16.9 a	205.8 ± 10.8 a	144.3 ± 11.7 b	80.4 ± 8.5 c	124.9 ± 10.4 b
glucobrassicin	816.4 ± 13.5 b	766.8 ± 48.1 b	1083.7 ± 51.9 a	864.1 ± 42.4 b	1135.9 ± 52.6 a
4-hydroxyglucobrassicin	74.6 ± 5.0 b	91.7 ± 14.4 ab	122.7 ± 10.0 a	93.4 ± 14.8 ab	123.2 ± 12.1 a
neoglucobrassicin	85.1 ± 15.5 d	171.7 ± 18.2 c	244.3 ± 11.7 a	186.2 ± 12.7 bc	219.9 ± 17.3 ab
total glucosinolates	1800.6 ± 66.9 b	1871.0 ± 87.6 b	2165.7 ± 110.5 a	1813.3 ± 34.0 b	2253.1 ± 101.7 a
ascorbic acid (mg/100 g)	1096.9 ± 18.1 a	607.7 ± 36.6 c	385.1 ± 5.1 e	533.8 ± 36.9 d	837.0 ± 4.2 b
phenol compounds (mg/100 g)					
caffeic acid	8.5 ± 1.2 a	5.8 ± 0.6 b	9.9 ± 0.4 a	5.3 ± 0.0 b	1.6 ± 0.6 c
coumaric acid	5.1 ± 0.3 cd	5.4 ± 0.1 c	6.9 ± 0.2 b	18.1 ± 0.2 a	4.5 ± 0.4 d
sinapic acid	18.2 ± 1.3 c	38.3 ± 0.9 a	32.7 ± 0.9 b	30.7 ± 0.7 b	3.6 ± 0.2 d
chlorogenic acid	11.2 ± 0.3 d	29.3 ± 0.6 c	67.0 ± 2.4 a	38.3 ± 6.0 b	14.6 ± 0.3 d
ferulic acid	1.3 ± 0.1 c	1.9 ± 0.0 c	2.3 ± 0.1 c	8.0 ± 0.1 a	4.7 ± 0.1 b
quercetin	12.9 ± 0.3 d	23.3 ± 0.3 c	25.5 ± 1.0 b	25.0 ± 0.4 b	36.4 ± 0.1 a
kaempferol	5.6 ± 0.0 b	7.9 ± 0.3 a	5.0 ± 0.1 b	7.0 ± 0.5 a	4.7 ± 0.6 b
luteolin	2.3 ± 0.5 b	1.1 ± 0.1 c	3.6 ± 0.3 a	2.6 ± 0.1 b	0.4 ± 0.0 d
naringenin	25.5 ± 0.6 a	21.2 ± 0.8 b	19.7 ± 1.9 b	7.0 ± 0.5 c	4.3 ± 0.5 d
total phenol compounds	90.7 ± 3.5 d	134.3 ± 3.7 c	172.4 ± 6.6 a	142.0 ± 5.4 b	74.9 ± 0.2 e

[a] Values are presented as mean value ± SD ($n = 3$) and expressed on dry weight basis. Means in rows followed by different letters differed significantly ($p \leq 0.05$). All compounds were identified by pure standards, unless differently reported. [b] ND, not detected. [c] Tentatively identified as pheophytin *a* and quantified as chlorophyll *a*. [d] Tentatively identified as a derivative of chlorophyll *a* and quantified as chlorophyll *a*. [e] Quantified by sinigrin calibration curve and their amounts corrected by the molecular mass ratios. The identity of glucosinolates was preliminarily assessed by UV spectra and molecular weight comparison, and then the different compounds were dissociated by induced collision (CID-MS) and their identity established through characteristic product ions (see Materials and Methods for further details).

All cooking treatments induced less change on frozen products than for fresh samples, as previously observed for broccoli. Among cooking treatments, both steaming procedures, and the basket one in particular, induced more marked changes, making vegetables less green ($-a^*$, $H°$) and significantly lowering color saturation (C). A significant decrease of total chlorophylls was observed after basket steaming (see **Table 6**), and this may be related to the formation of further products by chlorophyll degradation not determined in the present study, which were reported to affect the color of processed vegetables (*35*).

Effect of Cooking on Phytochemical Profile and Antioxidant Capacities. The effects of cooking on phytochemical compounds present in fresh and frozen Brussels sprouts are reported on a dry weight basis in **Tables 5** and **6**, respectively.

Fresh and frozen uncooked Brussels sprouts analyzed had an amount of total carotenoids lower than the range reported in the literature (*36*), likely due to a difference in the cultivar. Boiling of fresh Brussels sprouts led to a significant increase of total carotenoids, mainly due to lutein release. This is in agreement with that previously reported (*14*, *25*), but not with the trend observed for broccoli. Other cooking procedures did not significantly affect the content of lutein of this vegetable, whereas the β-carotene significantly decreased. In the same way, carotenoid content was retained in frozen Brussels sprouts, independent of cooking method applied. This behavior was once more different from that observed in the processing of frozen broccoli.

In the analyzed raw fresh Brussels sprouts, the only chlorophyll detected was chlorophyll *a* (**Table 5**), which was present in very low concentration (0.36 mg/100 g of fresh weight) with respect to literature data (*23*, *37*). On the contrary, the pattern and the content of chlorophylls of frozen Brussels sprouts were more similar to those previously reported (*23*, *37*). As already suggested (*35*, *37*), the cooking treatments led to a reduction of total chlorophyll content as well as the formation of degradation products in fresh samples. In fact, in each treatment, with the exception of microwaving, there was the formation of pheophytin *a* and chlorophyll *a* derivative, the latter compound being also found in fresh cooked broccoli. This compound, characterized by a long retention time under chromatographic conditions adopted in the present study, could be a "pyro" derivative formed from the corresponding pheophytins by the loss of the C-10 carbomethoxy group [$-CO_2CH_3$], as suggested by Schwartz et al. (*35*) in cooked spinach. With regard to the cooking effect on the chlorophyll content of frozen Brussels sprouts, none of the cooking treatments determined changes in total content, with the exception of basket steamed vegetables, but affected the single chlorophyll. Pheophytin *a* followed the same behavior of broccoli, being more formed during boiling, whereas oven steaming preserves chlorophylls *a* and *b* better than other methods and the content of chlorophyll *a*′ was significantly retained by all of the cooking procedures.

Fresh Brussels sprouts exhibited a content of glucosinolates lower than the range reported in the literature (*28*) (**Table 5**). With regard to the single glucosinolates, glucobrassicin was the most predominant compound (123.3 μg/g of fresh weight and 816 μg/g on a dry weight basis) followed by glucoiberin (92.9 μg/g of fresh weight and 615.2 μg/g on a dry weight basis). In frozen Brussels sprouts, the total content of glucosinolates was similar to that previously reported (*11*), even though a different glucosinolate profile was described, glucoiberin being the major compound. Similar to fresh broccoli, no changes of total glucosinolates were observed in boiled fresh Brussels sprouts upon thermal treatment,

Table 6. Phytochemical Compounds of Raw and Cooked Frozen Brussels Sprouts[a]

	raw	boiled	microwaved	basket steamed	oven steamed
carotenoids (mg/100 g)					
lutein	1.6 ± 0.1 a	1.7 ± 0.2 a	1.6 ± 0.0 a	1.7 ± 0.1 a	1.4 ± 0.1 a
β-carotene	1.1 ± 0.0 b	1.0 ± 0.1 b	1.3 ± 0.1 a	1.0 ± 0.0 b	1.1 ± 0.0 b
total carotenoids	2.7 ± 0.1 a	2.7 ± 0.3 a	2.9 ± 0.1 a	2.7 ± 0.2 a	2.5 ± 0.1 a
chlorophylls (mg/100 g)					
chlorophyll *a*	8.0 ± 1.1 a	3.5 ± 1.3 c	5.4 ± 0.3 bc	5.4 ± 0.6 bc	7.2 ± 0.2 ab
chlorophyll *a*'[b]	2.0 ± 0.2 a	1.9 ± 1.2 a	2.0 ± 0.4 a	1.2 ± 0.3 a	1.5 ± 0.1 a
chlorophyll *b*	3.0 ± 0.9 ab	0.9 ± 0.2 c	3.9 ± 0.9 a	1.7 ± 0.6 bc	3.2 ± 0.3 ab
chlorophyll *b*'[c]	ND[d]	1.2 ± 0.2	0.8 ± 0.3	ND	0.9 ± 0.2
pheophytin *a*	3.6 ± 0.4 b	7.6 ± 0.6 a	2.9 ± 0.4 c	2.3 ± 0.1 d	2.8 ± 0.2 c
total chlorophylls	16.7 ± 1.9 a	15.2 ± 0.6 a	15.1 ± 1.0 a	10.6 ± 1.3 b	15.5 ± 0.2 a
glucosinolates[e] (μg/g)					
glucoiberin	1199.2 ± 32.2 a	594.7 ± 17.7 e	757.8 ± 30.3 d	1077.4 ± 27.3 b	984.6 ± 22.0 c
glucoraphanin	209.6 ± 8.8 a	100.7 ± 5.1 d	154.3 ± 10.6 c	175.0 ± 6.9 bc	192.9 ± 9.6 ab
glucobrassicin	931.4 ± 26.2 a	669.6 ± 42.8 c	928.7 ± 63.6 a	786.5 ± 31.9 b	928.7 ± 15.9 a
4-hydroxyglucobrassicin	98.4 ± 3.7 ab	85.1 ± 4.7 b	103.8 ± 8.2 a	92.0 ± 3.9 ab	91.2 ± 5.4 ab
neoglucobrassicin	180.8 ± 17.0 a	103.9 ± 5.2 c	175.6 ± 19.1 a	121.7 ± 5.8 bc	152.3 ± 10.1 ab
total glucosinolates	2619.4 ± 87.9 a	1554.0 ± 75.5 d	2120.2 ± 71.1 c	2252.7 ± 50.5 bc	2349.7 ± 43.8 b
ascorbic acid (mg/100 g)	1324.9 ± 10.4 a	380.1 ± 6.4 d	650.9 ± 70.4 c	1207.8 ± 7.5 b	1168.1 ± 17.7 b
phenol compounds (mg/100 g)					
caffeic acid	9.3 ± 0.0 b	8.0 ± 0.6 c	19.5 ± 0.0 a	6.6 ± 0.0 d	7.9 ± 0.1 c
coumaric acid	4.2 ± 1.1 b	5.5 ± 1.3 b	12.1 ± 0.5 a	5.0 ± 0.1 b	5.4 ± 0.2 b
sinapic acid	35.8 ± 0.1 b	29.2 ± 0.9 c	58.8 ± 1.1 a	28.4 ± 0.1 c	27.8 ± 0.9 c
chlorogenic acid	4.8 ± 0.2 c	27.6 ± 0.8 b	58.0 ± 15.4 a	53.3 ± 0.2 a	48.2 ± 0.9 a
ferulic acid	1.4 ± 0.1 a	0.4 ± 0.1 c	1.6 ± 0.2 a	0.5 ± 0.0 c	0.8 ± 0.0 b
quercetin	25.0 ± 0.5 b	20.9 ± 0.8 c	47.2 ± 0.3 a	21.4 ± 0.1 c	21.0 ± 0.7 c
kaempferol	4.5 ± 0.1 c	4.8 ± 0.1 c	7.6 ± 0.2 a	4.7 ± 0.0 c	5.6 ± 0.0 b
luteolin	2.0 ± 0.5 b	3.5 ± 0.3 b	7.0 ± 2.2 a	4.2 ± 0.1 b	2.8 ± 0.2 b
naringenin	17.0 ± 0.2 a	6.7 ± 0.4 c	15.0 ± 3.7 a	8.7 ± 1.2 bc	13.4 ± 0.4 ab
total phenol compounds	103.9 ± 0.1 c	106.7 ± 5.2 c	226.8 ± 19.5 a	132.8 ± 1.6 b	132.9 ± 3.1 b

[a] Values are presented as mean value ± SD ($n = 3$) and expressed on dry weight basis. Means in rows followed by different letters differed significantly ($p \leq 0.05$). All compounds were identified by pure standards, unless differently reported. [b] Tentatively identified as the C-10 epimeric isomer of chlorophyll *a* and quantified as chlorophyll *a*. [c] Tentatively identified as the C-10 epimeric isomer of chlorophyll *b* and quantified as chlorophyll *b*. [d] ND, not detected. [e] Quantified by sinigrin calibration curve and their amounts corrected by the molecular mass ratios. The identity of glucosinolates was preliminarily assessed by UV spectra and molecular weight comparison, and then the different compounds were dissociated by induced collision (CID-MS) and their identity established through characteristic product ions (see Materials and Methods for further details).

Table 7. Phytochemical Compounds of Raw and Cooked Fresh Cauliflower[a]

	raw	boiled	microwaved	basket steamed	oven steamed
glucosinolates[b] (μg/g)					
glucoiberin	460.5 ± 18.5 a	120.3 ± 9.5 d	248.7 ± 17.7 c	251.6 ± 3.2 c	343.3 ± 6.9 b
glucoraphanin	39.1 ± 2.0	ND[c]	ND	ND	ND
glucobrassicin	880.4 ± 74.8 bc	747.7 ± 17.5 c	746.2 ± 34.0 c	925.4 ± 25.8 b	1295.1 ± 74.0 a
4-hydroxyglucobrassicin	49.5 ± 9.0 ab	34.3 ± 5.0 b	38.9 ± 7.5 ab	44.0 ± 2.6 ab	54.7 ± 5.6 a
neoglucobrassicin	67.8 ± 10.1 b	97.8 ± 11.0 ab	105.4 ± 16.9 ab	104.4 ± 10.5 ab	126.6 ± 19.8 a
methoxybrassicin	239.6 ± 30.9 a	85.9 ± 9.7 b	117.8 ± 15.1 b	216.4 ± 14.1 a	230.0 ± 20.4 a
total glucosinolates	1737.0 ± 145.0 b	1086.0 ± 24.0 c	1257 ± 91.0 c	1542.0 ± 28.0 b	2050.0 ± 113.0 a
ascorbic acid (mg/100 g)	1194.5 ± 13.8 a	691.9 ± 19.5 c	62.9 ± 8.1 e	809.5 ± 43.5 b	586.5 ± 45.0 d
phenol compounds (mg/100 g)					
caffeic acid	16.5 ± 4.3 a	0.8 ± 0.1 c	8.2 ± 1.3 b	6.7 ± 0.9 b	11.7 ± 1.0 ab
coumaric acid	5.9 ± 1.3 ab	6.7 ± 0.0 a	4.5 ± 0.0 b	4.9 ± 0.4 b	4.9 ± 0.1 b
sinapic acid	4.7 ± 0.4 bc	4.0 ± 0.1 c	5.4 ± 0.1 a	5.3 ± 0.3 ab	4.9 ± 0.0 ab
chlorogenic acid	21.1 ± 1.7 a	12.6 ± 0.1 c	11.0 ± 0.3 cd	9.5 ± 0.2 d	16.5 ± 0.3 b
ferulic acid	1.5 ± 0.5 a	0.8 ± 0.0 b	1.1 ± 0.0 ab	0.7 ± 0.0 b	0.9 ± 0.0 b
quercetin	3.6 ± 0.6 ab	3.4 ± 0.7 ab	3.6 ± 0.0 ab	4.4 ± 0.1 a	3.2 ± 0.0 b
kaempferol	4.7 ± 0.2 ab	2.7 ± 0.0 c	6.3 ± 0.2 a	4.6 ± 1.4 ab	3.5 ± 0.1 bc
luteolin	2.0 ± 0.4 b	1.7 ± 0.1 b	2.1 ± 0.1 b	4.2 ± 0.9 a	3.8 ± 0.0 a
total phenol compounds	59.9 ± 9.5 a	32.7 ± 0.8 c	42.2 ± 2.0 bc	40.4 ± 0.6 bc	49.3 ± 0.5 ab

[a] Values are presented as mean value ± SD ($n = 3$) and expressed on dry weight basis. Means in rows followed by different letters differed significantly ($p \leq 0.05$). All compounds were identified by pure standards, unless differently reported. [b] Quantified by sinigrin calibration curve and their amounts corrected by the molecular mass ratios. The identity of glucosinolates was preliminarily assessed by UV spectra and molecular weight comparison, and then the different compounds were dissociated by induced collision (CID-MS) and their identity established through characteristic product ions (see Materials and Methods for further details). [c] ND, not detected.

whereas Song et al. (*10*) found a significant decrease applying a longer cooking time (boiling for up to 30 min). Other cooking procedures (e.g., oven steaming and microwaving) led to an about 20% increase of total glucosinolates, confirming the positive effect of steaming (*14*, *25*) and microwaving without adding water (*38*). As already shown in the case of frozen broccoli and in accordance with previous studies (*9*, *11*), frozen Brussels sprouts lost total glucosinolates upon cooking with a reduction

Table 8. Phytochemical Compounds of Raw and Cooked Frozen Cauliflower[a]

	raw	boiled	microwaved	basket steamed	oven steamed
glucosinolates[b] (μg/g)					
glucoiberin	428.7 ± 11.2 b	311.0 ± 4.4 d	455.9 ± 12.3 a	382.4 ± 3.7 c	456.7 ± 2.0 a
glucoraphanin	18.9 ± 4.2 abc	14.8 ± 0.8 c	23.4 ± 1.6 a	17.3 ± 1.1 bc	21.4 ± 1.8 ab
glucobrassicin	496.5 ± 11.1 b	294.0 ± 9.6 c	564.1 ± 8.6 a	509.0 ± 19.5 b	479.6 ± 27.6 b
4-hydroxyglucobrassicin	31.6 ± 5.0 ab	14.9 ± 2.9 c	40.0 ± 1.9 a	33.0 ± 3.4 ab	27.8 ± 1.9 b
neoglucobrassicin	96.1 ± 15.1 ab	51.2 ± 8.7 c	126.2 ± 21.6 a	117.9 ± 12.4 a	67.3 ± 11.6 bc
methoxybrassicin	147.0 ± 11.7 ab	64.8 ± 9.0 c	176.9 ± 11.4 a	128.7 ± 18.4 b	132.3 ± 15.6 b
total glucosinolates	1218.7 ± 58.5 b	750.7 ± 35.5 c	1386.3 ± 57.5 a	1188.0 ± 10.0 b	1185.3 ± 56.5 b
ascorbic acid (mg/100 g)	748.8 ± 82.2 a	359.7 ± 15.2 c	472.9 ± 29.6 bc	552.9 ± 17.8 b	459.7 ± 62.3 bc
phenol compounds (mg/100 g)					
caffeic acid	16.3 ± 0.3 a	4.5 ± 0.6 e	10.8 ± 0.8 c	14.0 ± 0.8 b	6.7 ± 0.2 d
coumaric acid	3.9 ± 0.1 b	3.4 ± 0.0 b	4.1 ± 0.9 b	8.7 ± 1.2 a	3.5 ± 0.3 b
sinapic acid	4.3 ± 0.2 b	2.6 ± 0.0 c	4.5 ± 0.5 b	6.2 ± 0.3 a	2.7 ± 0.7 c
chlorogenic acid	1.9 ± 0.3 b	4.0 ± 1.0 b	7.4 ± 2.0 a	1.2 ± 0.1 c	1.7 ± 0.2 b
ferulic acid	2.4 ± 0.2 a	0.5 ± 0.0 c	1.9 ± 0.4 ab	1.7 ± 0.1 b	0.7 ± 0.1 c
quercetin	4.1 ± 0.1 a	1.5 ± 0.0 b	4.1 ± 0.4 a	4.4 ± 0.4 a	2.2 ± 0.0 b
kaempferol	6.9 ± 1.0 ab	4.9 ± 0.2 c	6.4 ± 0.9 bc	8.3 ± 0.6 a	5.9 ± 0.0 bc
luteolin	5.2 ± 1.4 ab	1.9 ± 0.1 c	3.7 ± 0.3 b	6.1 ± 0.2 a	4.3 ± 0.1 b
total phenol compounds	45.1 ± 0.1 ab	23.3 ± 0.5 c	43.1 ± 2.8 b	50.5 ± 3.8 a	27.7 ± 0.9 c

[a] Values are presented as mean value ± SD ($n = 3$) and expressed on dry weight basis. Means in rows followed by different letters differed significantly ($p \leq 0.05$). All compounds were identified by pure standards, unless differently reported. [b] Quantified by sinigrin calibration curve and their amounts corrected by the molecular mass ratios. The identity of glucosinolates was preliminarily assessed by UV spectra and molecular weight comparison, and then the different compounds were dissociated by induced collision (CID-MS) and their identity established through characteristic product ions (see Materials and Methods for further details).

between 10 and 40% from all of the treatments applied, thus confirming the deleterious influence of freezing procedures on these compounds observed in broccoli.

The content of ascorbic acid in uncooked fresh Brussels sprouts (165 mg/100 g of fresh weight and 1096 mg/100 g on a dry weight basis) was in agreement with literature data (*36*). The influence of cooking procedures on ascorbic acid of fresh and frozen Brussels sprouts was quite similar to that described in broccoli. Microwaving was the worst method for ascorbic acid preservation in fresh Brussels sprouts, probably because of the long cooking time applied in the present study (*12*), followed by basket steaming, boiling, and oven steaming. In frozen Brussels sprouts, boiling determined the greatest loss of this compound, whereas microwaving retained better ascorbic acid than in fresh samples.

Uncooked fresh and frozen Brussels sprouts contained a similar pattern of phenolic compounds (**Tables 5** and **6**), sinapic acid being the most abundant cinnamoyl acid and naringenin and quercetin the major flavonoids in fresh and frozen, respectively. On the fresh weight basis, Brussels sprouts have a similar content of single flavonoids with respect to Brussels sprouts analyzed in the USDA data set (*39*). Cooking procedures brought about general increases of total polyphenols in both fresh and frozen Brussels sprouts. Surprisingly, oven steaming is the most disadvantageous method for cooking fresh Brussels sprouts, determining a significant and strong decrease of a few cinnamoyl acids (e.g., sinapic and caffeic acids) and flavonoids, mostly naringenin. This behavior was not consistent with previous data (*6*, *14*, *15*) and with those obtained for fresh broccoli, although it should be considered that for broccoli the cooking time was shorter.

Freezing procedures together with the subsequent thermal treatments could have determined the polyphenol release and in turn the increase of the chemical extraction of these compounds. This could explain the general polyphenol increases in cooked frozen Brussels sprouts, as already shown for blanched, frozen, and stored Brussels sprouts (*34*). Among single polyphenols, a significant increase of chlorogenic acid upon all thermal treatments was observed probably due to the ability of cooking processes to disrupt the (covalently or not) interaction of this acid with the polysaccharide moiety of fiber, as already shown in cooked artichoke (*15*).

In fresh Brussels sprouts, boiling and steaming by basket led to a significant increase of TAC values (**Table 4**), whereas oven steamed and microwaved samples exhibited no significant changes in TAC. This marked effect of boiling is likely partly related to the observed increase of carotenoids and the increase of some polyphenols (e.g., quercetin). The increases of TAC values observed in boiled and steamed Brussels sprouts are whatever supported by earlier studies on TAC values of different vegetables (*12*, *14*, *15*). In the case of frozen samples, there was a different behavior related to TAC assay, even though a general decrease was found to be partly due to the ascorbic acid decrease.

Cauliflower. *Effect of Cooking on Color Parameters*. Color indices of fresh and frozen cauliflower are shown in **Table 1**. Boiling and steaming induced significant changes of color in fresh product that became less bright (L^* decrease) and more green ($-a^*$ increase). This increase in greenness may be responsible for the observed darkening (L^* decrease). In addition, b^* significantly decreased for these cooked products, leading to a significant shift of $H°$ toward green and loss of color saturation (C). Surprisingly, cooking of fresh cauliflower by microwave treatment did not alter color parameters except for a slight increase of greenness ($-a^*$) and $H°$, as a consequence.

None of the cooking treatments markedly altered the color of frozen vegetables as also shown by lower ΔE values in comparison with fresh products. This is probably related to the stabilizing effect of blanching that has partially prevented discoloration of this type of *Brassica* vegetable after cooking. However, among treatments, boiling and oven steaming induced some changes of color parameters on frozen cauliflower as L^* was significantly reduced and the hue angle shifted in both cases.

Effect of Cooking on Phytochemical Profile and Antioxidant Capacities. The effects of cooking on phytochemical compounds present in fresh and frozen cauliflower are reported on a dry weight basis in **Tables 7** and **8**, respectively. The content of carotenoids and chlorophylls was not measured as white cauliflower contains a negligible amount of such compounds (*2*).

Cauliflower was a poor source of glucosinolates compared to the other *Brassica* vegetables analyzed. The total glucosinolate content of fresh cauliflower was in the range of earlier studies (*10*, *28*), glucobrassicin and glucoiberin being the

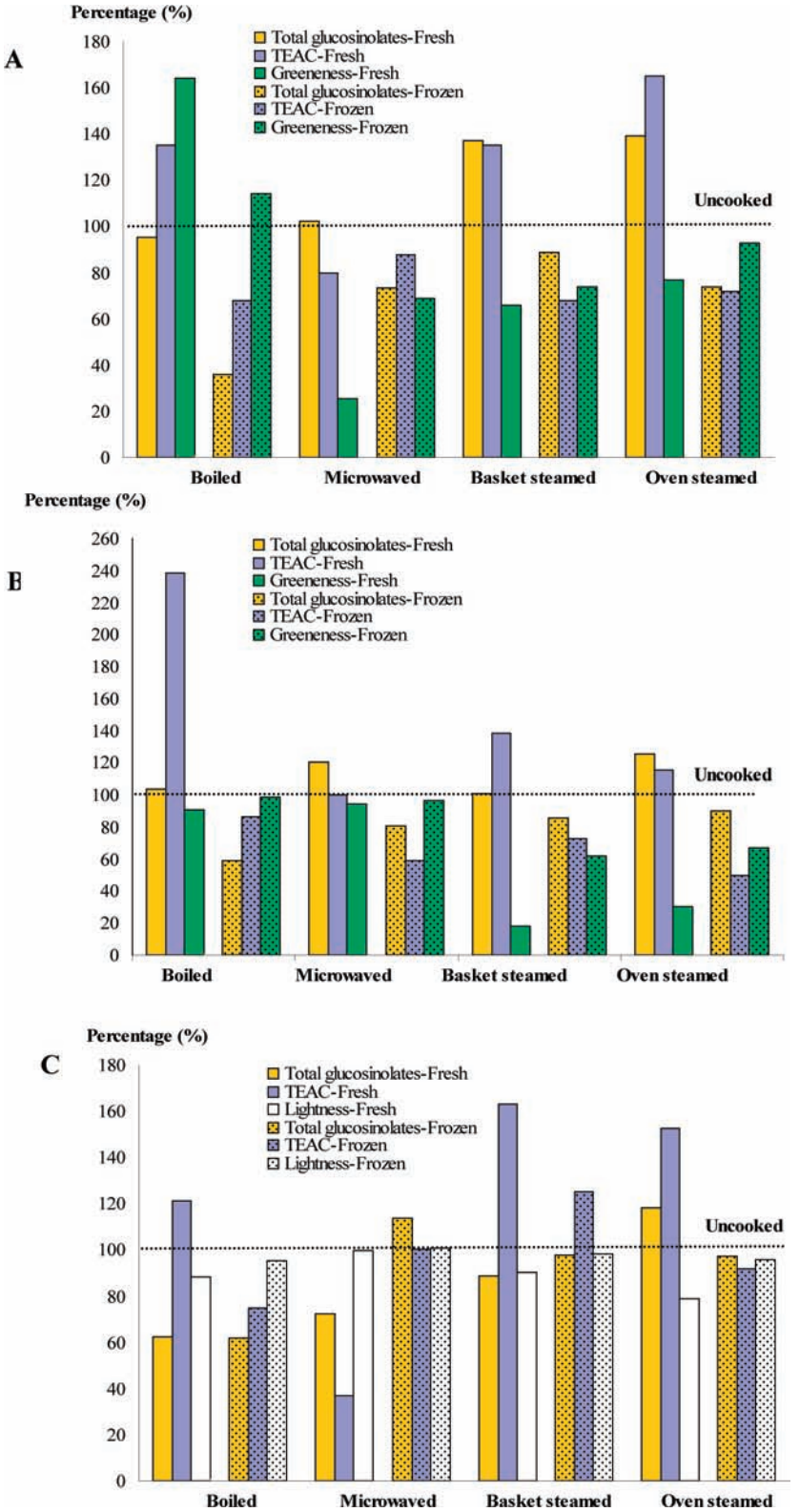

Figure 1. Percentage values of total glucosinolates, TEAC, and color index of fresh and frozen samples for the different cooking treatments: (**A**) broccoli; (**B**) Brussels sprouts; (**C**) cauliflower. Uncooked values were considered as equal to 100%.

major compounds. Frozen cauliflower contained lower total glucosinolates than previously reported (*11*), probably due to a different cultivar analyzed.

Cooking procedures determined different changes on glucosinolate content in fresh cauliflower. Boiled and microwaved cauliflower lost 37 and 25% of total glucosinolates, respectively, whereas both steaming methods had a positive effect, increasing or retaining the content of total glucosinolates, as already observed in fresh broccoli and Brussels sprouts. The loss of glucosinolates during boiling has been already shown by various authors on different *Brassica* vegetables (*10*, *11*, *14*). However, the negative effect of microwaving gave the opposite features observed in fresh broccoli and Brussels sprouts. This might suggest that the same cooking procedure can determine different

effects on phytochemical content depending on the vegetable analyzed. This was confirmed also by the results on frozen cooked cauliflower: contrary to that observed for other frozen vegetables analyzed, cooking procedures had not always a detrimental effect on total glucosinolates. In fact, both steaming procedures determined no changes in total glucosinolates, whereas microwaving led to a significant increase, resulting in a general increase of all compounds. Boiled frozen cauliflower was the only frozen cooked sample in which a significant reduction of total glucosinolates was found.

Fresh cauliflower was the richest source of ascorbic acid (81.5 mg/100 g of fresh weight and 1194.5 mg/100 g on a dry weight basis) relative to the other fresh vegetables analyzed, in agreement with earlier data (*2*) (**Table 7**). All cooking treatments significantly affected the content of ascorbic acid in both fresh and frozen cauliflower, as previously observed in other studies (*13*, *14*). Microwaving was the most deleterious method for retaining ascorbic acid in fresh vegetables, as already found for broccoli and Brussels sprouts analyzed.

The total content of polyphenols measured in fresh cauliflower (**Table 7**) (4.1 mg/100 g of fresh weight and 59.9 mg/100 g on a dry weight basis) was much lower than that reported in other studies (*13*). This can be related to both the high variability of polyphenols in *Brassica* vegetables and an overestimation in the studies in which total polyphenols were determined by Folin−Ciocalteu assay. The latter hypothesis is strengthened by the fact that the content of single flavonoids (i.e., quercetin, kaempferol, and luteolin) is in agreement with that reported in USDA flavonoids data set (*39*).

With regard to the effect of cooking on polyphenol content in fresh cauliflower, all of the treatments applied had a detrimental effect with the exception of oven steaming, which did not affect the content of these compounds. These results are similar to those reported by previous studies on the same vegetable, in which boiling and microwaving had a negative effect (*5*, *13*), whereas steaming retained polyphenolic content (*13*). For frozen cauliflower, a different influence of thermal processing was observed: boiled and oven steamed frozen cauliflower showed lower content of polyphenols than uncooked ones, whereas no changes were observed in the polyphenol content of microwaved and basket steamed cauliflower.

The TAC values of fresh cauliflower agreed with those in the Italian TAC database (*33*) (**Table 4**). For fresh cauliflower, TAC values were highest in steamed > boiled > microwaved, regardless of the TAC assay applied, in agreement with Wachtel-Galor et al. (*6*), who reported the same TAC ranking order for cooked cauliflower. As already shown for fresh broccoli and Brussels sprouts, microwaving was the most deleterious method for retaining the antioxidant capacity of fresh vegetables. For frozen cauliflower, only boiling caused a great and significant loss of TAC values, related to the significant decreases of ascorbic acid and polyphenols, whereas other cooking treatments had no significant effect on this parameter, probably because of a great retention of phytochemical compounds.

In conclusion, the present study demonstrates that during domestic cooking methods fresh *Brassica* vegetables retain phytochemicals and TAC better than frozen samples. This behavior is more evident for broccoli than for other vegetables analyzed, probably because of the different structural matrix of cell walls in this vegetable. On the contrary, frozen vegetables maintain better the color characteristics, which importantly affect consumer choice. These changes are summarized in **Figure 1**, in which the percentage value of total glucosinolates, TAC expressed as TEAC, and color index are shown for broccoli (**A**), Brussels sprouts (**B**), and cauliflower (**C**).

To preserve or even enhance the nutritional quality of all *Brassica* vegetables, steam cooking is the best procedure on all fresh types. For frozen ones, the choice is more dependent on vegetable and on the effect that freezing procedures have on the phytochemical behavior during cooking. Microwave cooking is the best cooking method to maintain the color of both fresh and frozen vegetables, and it also determined a good retention of glucosinolates, of which these vegetables are important and unique sources in the human diet. Finally, ascorbic acid is lost in great amount during all cooking procedures in all *Brassica* species analyzed.

All in all, the concept that cooked vegetables have lower nutritional values than uncooked ones is only true if ascorbic acid is used as a marker of nutritional quality. These results demonstrate that some domestic cooking procedures increased the bioaccessibility of polyphenols and carotenoids, highlighting the positive role of cooking on the nutritional qualities of vegetables.

ABBREVIATIONS USED

DAD, diode array detector; TAC, total antioxidant capacity; FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity; TRAP, total radical-trapping antioxidant parameter; USDA, United States Department of Agriculture; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic) acid; TPTZ, 2,4,6-tripyridyl-*s*-triazine; R-PE, *R*-phycoerythrin; ABAP, 2,2′-azobis(2-amidinopropane) dihydrochloride; ND, not detected; BHT, 2,6-di-*tert*-butyl-*p*-cresol .

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