JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effects of Different Cooking Methods on Antioxidant Profile, Antioxidant Capacity, and Physical Characteristics of Artichoke

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In this study, the effects of three common cooking practices (i.e., boiling, steaming, and frying) on the artichoke phenolic compounds pattern were evaluated by LC-MS/MS analysis. The variation of carotenoids, antioxidant capacity, and artichoke physical properties after cooking was also investigated. The major phenolic compounds present in the raw sample were 5-*O*-caffeoylquinic and 1,5-di-*O*-caffeoylquinic acids; after cooking treatments, an increase of the overall caffeoylquinic acids concentration due to the formation of different dicaffeoylquinic acid isomers was observed. Steamed and fried samples showed similar patterns of dicaffeoylquinic concentrations, which were higher with respect to the boiled samples. On the other hand, all cooking practices, particularly frying, decreased flavonoid concentration. The antioxidant capacity of cooked artichokes, measured by three different assays, enormously increased after cooking, particularly after steaming (up to 15-fold) and boiling (up to 8-fold). The observed cooking effect on the artichoke antioxidant profile is probably due to matrix softening and increased extractability of compounds, but the increase of antioxidant capacity is much higher than the increase of antioxidant concentration. These results suggest that some common cooking treatments can be used to enhance the nutritional value of vegetables, increasing bioaccessibility of health-promoting constituents.

KEYWORDS: Artichoke; antioxidant activity; cooking; flavonoids; carotenoids

INTRODUCTION

The edible flower of artichoke *Cynara scolymus* L. is a typical vegetable consumed in Mediterranean countries. It is known that it has a marked health protective capacity: in vivo and in vitro studies have shown its hepatoprotective functions and the inhibition of cholesterol biosynthesis in hepatocytes (*1, 2*).

Artichokes are characterized by a complex antioxidant profile: caffeoylquinic acids are the major phenolic compounds reported in artichoke heads (*3*), but many other bioactive constituents such as apigenin and luteolin glycosides as well as hydrolyzable and condensed tannins have also been described (*4*). Recently, a MS/MS study by Schütz and co-workers (*5*) clearly showed that 1,5-di-*O*-caffeoylquinic acid is the main dicaffeoylquinic acid and that 5-*O*-caffeoylquinic acid is the major monocaffeoylquinic acid, whereas apigenin 7-*O*-glucuronide is the major flavonoid.

Data of vegetable composition are usually determined on raw material; however, for nutritional purposes it should be considered that most of them are cooked in different ways before consumption. These cooking processes would bring about a number of changes in the physical characteristics and chemical composition of vegetables (*6*−*8*).

Sahlin et al. (*9*) showed that boiling and baking had a small effect on the ascorbic acid, total phenolic, lycopene, and antioxidant activity of tomatoes, whereas frying significantly reduced ascorbic acid, total phenolic, and lycopene. Zhang and Hamauzu (*7*) demonstrated that cooking affected the antioxidant compound contents of broccoli. Ismail and co-workers (*10*) showed that heating decreased the total phenolic content in some vegetables such as kale, spinach, cabbage, swamp cabbage, and shallots. Recently, we have reported that cooking practices differently affect the phytochemical profile of courgettes, carrots, and broccoli (*8*).

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10.1021/jf800408w CCC: $40.75 © 2008 American Chemical Society
Published on Web 08/30/2008

Important parameters in the cooking quality of vegetables, which may strongly influence consumer preferences, are texture and color. Cooked vegetables have less brilliant color with respect to fresh ones (*11*), whereas texture changes, due to the membrane disruption and associated loss of turgor, are often desired in many vegetable-based recipes (*12*).

No literature data are available on the cooking effect on polyphenol compounds of artichoke, especially about the behavior of caffeoylquinic acid isomers.

Therefore, the present study was undertaken to investigate the effects of different cooking methods (boiling, steaming, and frying) on artichoke, evaluating the ratio of caffeoylquinic acid isomers and monitoring the change of flavonoid and phenol contents. Carotenoids, antioxidant capacity, and physical characteristic changes after cooking were also investigated.

MATERIALS AND METHODS

Materials. All reagents and solvents of HPLC grade were purchased from Merck (Darmstadt, Germany). 5-*O*-Caffeoylquinic acid (chlorogenic acid), luteolin 7-*O*-glucoside, β-carotene, and lutein standards were from Sigma (Milano, Italy), 1,3-Di-*O*-caffeoylquinic acid (cynarin) standard was from Carl Roth GmbH & Co. (Karlsruhe, Germany), and narirutin and naringenin 7-*O*-glucoside were from Indofine (Hillsborough, NJ).

Preparation of Vegetables. Freshly harvested artichokes (*C. scolymus* L.) were purchased from a local market on the day of processing and kept at room temperature for about 2 h before treatment. Information on the pretreatment history of the artichokes was obtained by purchaser. Artichokes were road transported under refrigerated conditions within 24 h from harvesting and stored at 7 °C for up to 1 day before they were taken. Edible parts of artichokes were quartered and cooked in three different methods in triplicate, as given below.

Cooking Treatments. Three of the most common cooking methods, that is, boiling, steaming, and frying, were used. Cooking conditions were optimized by preliminary experiments carried out for each vegetable. For all cooking treatments, the minimum cooking time to reach a similar tenderness for an adequate palatability and taste, according to Italian eating habits, was used.

Boiling. Vegetable material was added to boiling tap water in a covered stainless steel pot (food/water, 1:5) and cooked on a moderate flame for 15 min. For each cooking trial, 10 samples were boiled. Then, samples were drained off for 30 s.

Steaming. Steaming treatments were carried out in a Combi-Steal SL oven (V-Zug, Zurich, Switzerland). Nine specimens were placed in the oven equilibrated to room temperature before each cooking trial. Eight samples were arranged in a circle and one was put at the center to ensure uniform heating conditions in all samples for each cooking trial. The samples were cooked under atmospheric pressure for 22 min.

Frying. Vegetable was added to 2.2 L of peanut oil in a domestic deep-fryer (De Longhi, Italy) set at 170 °C and fried for 5 min. Ten samples were fried for each cooking trial. At the end of each trial, samples were drained off and dabbed with blotting paper to allow absorption of excess oil.

After all cooking experiments, samples were cooled rapidly on ice for antioxidant analyses. The texture analyses were performed on cooked samples at 50 °C, referred to as the temperature of consumption, whereas color analyses were performed at room temperature (25 °C). Both temperatures were controlled by inserting a thermocouple (K-type; Ni/Al−Ni/Cr) connected to a multimeter acquisition system (Keithley Instruments Inc., Cleveland, OH) to the thermal center of one sample for each cooking trial.

Physical Analysis. The texture of the raw and cooked samples was analyzed by shear force test using a TA.XT2 Texture Analyzer equipped with a 25 kg load cell (Stable Micro Systems, Goldalming, U.K.), and the parameters were quantified using the application software provided (Texture Expert for Windows, version 1.22).

Shear force analysis was performed using a Warner-Bratzler blade (3 mm thick), which cut artichoke quarters between the stem and the bracts at a constant speed of 60 mm min^{-1} and which were pushed through the slot (4 mm wide). The maximum force (N) required to shear the sample was measured. Softening percentage was calculated as

$$\text{softening percentage } (\%) = \left[1 - \frac{\text{shear force of cooked sample (N)}}{\text{shear force of raw sample (N)}}\right] \times 100$$

A total of 15 determinations was performed for each cooking treatment.

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 on the bracts and stems of artichoke quarters, separately. 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 angle (red = 0°, yellow = 90°, 180° = green, 270° = blue) were quantified on each samples using a 10° position of the standard observer.

A total of 15 determinations was performed for each cooking treatment.

Determination of Antioxidant Compounds and Total Antioxidant Capacity (TAC). The analyses of TAC were performed on fresh samples within 24 h of cooking.

Conversely, for the analyses of antioxidant compounds, the samples were freeze-dried utilizing a Brizzio-Basi instrument (Milan, Italy). The freeze-dried sample material (DM) was finely ground, kept in sealed bags, and stored at −20 °C.

Determination of Total Phenols Content. The amount of phenolic compounds is given as gallic acid equivalents and determined according to the Folin−Ciocalteu method (*13*). Briefly, 2.5 mL of Folin−Ciocalteu reagent, diluted 10-fold in water, was added to the different artichoke extracts. The mixture was incubated for 2 min at room temperature, and 2 mL of sodium carbonate was added. The mixture was incubated for 15 min at 50 °C and finally cooled in a water−ice bath. The specific absorbance at 760 nm was immediately measured.

LC-MS/MS Analysis of Phenolic Compounds. Three grams of freeze-dried material was extracted with 30 mL of methanol/water (70:30, v/v) by sonication at room temperature for 30 min. The mixtures were centrifuged at 2800*g* for 10 min at room temperature, filtered through a 0.45 μm Whatman filter paper (Whatman International Ltd., Maidstone, U.K.), and then used for LC analysis.

Chromatographic separation was performed using an HPLC apparatus equipped with two Micropumps series 200 (Perkin-Elmer, Shellton, CT), a UV−vis series 200 (Perkin-Elmer) detector set at 330 and 280 nm, and a Prodigy ODS3 100 Å column (250 × 4.6 mm, particle size = 5 μm) (Phenomenex, Torrance, CA). The eluents were (A) 0.2% formic acid in water and (B) acetonitrile/methanol (60:40 v/v). The gradient program was as follows: 20−30% B (6 min), 30−40% B (10 min), 40−50% B (8 min), 50−90% B (8 min), 90−90% B (3 min), 90−20% B (3 min), at a constant flow of 0.8 mL/min. The LC flow was split, and 0.2 mL/min was sent to the mass spectrometer. Injection volume was 20 μL.

MS and MS/MS analyses were performed on an API 3000 triple-quadrupole mass spectrometer (Applied Biosystems, Canada) equipped with a TurboIonspray source working in the negative ion mode. The analyses were performed using the following settings: drying gas (air) was heated to 400 °C, capillary voltage (IS) was set at 4000 V, nebulizer gas (air) 12 (arbitrary units), curtain gas (N_2) 14 (arbitrary units), collision gas (N_2) 4 (arbitrary units). The declustering potential (DP), focus potential (FP), and collision energy (CE) were optimized using 5-*O*-caffeoylquinic acid standard. It was infused directly into the mass spectrometer (10 μg/mL) at a constant flow rate of 5 μL/min using a model 11 syringe pump (Harvard Apparatus, Holliston, MA).

Information-dependent acquisition (IDA) (*14*) was used to identify the caffeoylquinic acids and the flavonoids. IDA was carried out in the range of m/z 50−1100 with a cycle time of 0.5 s and a step size of m/z 0.2; collision energy was set at 50 V. Identified compounds were then analyzed in multiple reaction monitoring (MRM). Monocaffeoylquinic acids were quantified as chlorogenic acid, and dicaffeoylquinic acids were quantified as cynarin.

Table 1. Physical Properties of Raw and Cooked Artichokes[a]

	raw	boiled	steamed	fried
		Texture[a]		
shear force (N)	98.2 ± 4.9	7.6 ± 0.9 b	9.2 ± 0.5 b	12.8 ± 1.1 a
softening (%)		92.3 ± 1.0 a	90.6 ± 0.9 a	87.0 ± 1.1 b
		Color[a]		
bracts				
*L**	41.2 ± 2.0 c	46.7 ± 3.0 ab	46.3 ± 2.3 b	50.8 ± 4.5 a
*a**	7.1 ± 1.8 a	−1.7 ± 1.7 c	2.5 ± 0.6 b	8.8 ± 4.1 a
*b**	2.3 ± 1.0 c	16.0 ± 3.5 a	11.4 ± 1.8 b	19.9 ± 4.4 a
C	7.6 ± 1.5 c	14.2 ± 2.7 b	11.7 ± 1.8 b	22.1 ± 4.5 a
hue angle	80.2 ± 2.1 b	98.6 ± 4.2 a	77.5 ± 3.2 b	67.5 ± 5.4 b
stems				
*L**	57.3 ± 4.9 a	51.2 ± 2.1 b	45.4 ± 2.6 c	51.6 ± 2.0 b
*a**	−1.8 ± 1.7 b	−1.7 ± 1.3 b	0.6 ± 0.9 a	1.7 ± 1.7 a
*b**	29.7 ± 3.5 a	24.1 ± 2.5 b	17.8 ± 2.8 c	24.1 ± 4.1 b
C	29.8 ± 3.5 a	24.2 ± 2.5 b	17.8 ± 2.8 c	24.2 ± 4.1 b
hue angle	93.7 ± 3.4 a	94.1 ± 3.1 a	88.0 ± 3.0 ab	86.4 ± 2.0 b

[a] Values are presented as mean ± SD ($n = 15$). The same letter within each row does not significantly differ ($p \leq 0.05$). Statistical significance of raw sample was not considered.

For calibration curves chlorogenic acid, cynarin, and luteolin 7-*O*-glucoside standards were diluted with methanol (1 mg/mL) and were analyzed in the linearity range of 10−500 μg/mL.

Carotenoids Determination. The determination of carotenoids was carried out by HPLC analysis as previously described by Leonardi et al. (*15*). Briefly, 0.1 g of lyophilized sample was extracted with tetrahydrofuran containing 0.01% BHT as antioxidant agent, dried under nitrogen flow in dark tubes, dissolved in dichloromethane, and analyzed using an HPLC (Shimadzu LC10, Japan) with diode array detector and a Prodigy column (5 μm, 250 × 4.6 mm; Phenomenex). The carotenoids were eluted with a flow of 0.8 mL/min following this linear gradient: starting condition, 82% A and 18% B; at 20 min, 76% A and 24% B; at 30 min, 58% A and 42% B; at 40 min, 40% A and 60% B; 45 min, 82% A and 18% B. Phase A was a mixture of acetonitrile, *n*-hexane, methanol, and dichloromethane (2:1:1:1, v/v), whereas phase B was acetonitrile. β-Carotene, phytoene, and phytofluene were quantified by calibration curves built with β-carotene pure standard. Lutein was quantified by a calibration curve built with lutein pure standard. Quantification was achieved extracting from the diode array data the chromatograms recorded at 450 nm for α- and β-carotene and lutein, at 350 nm for phytofluene, and at 290 nm for phytoene. A fixed concentration of ethyl-*apo*-8′-carotenal (10 μg/mL) was used as internal standard with a calculated recovery of 98%.

TAC Determination. The TAC values were determined as previously described (*16*). Briefly, raw and cooked samples were homogenized under nitrogen flow in a high-speed blender (Brawn Multimix MX32), and a weighed amount (∼1 g) was extracted with 4 mL of water under agitation for 15 min at room temperature and centrifuged at 1000*g* for 10 min; the supernatant was collected. The extraction was repeated with 2 mL of water, and the two supernatants were combined. The pulp residue was re-extracted by the addition of 4 mL of acetone under agitation for 15 min at room temperature and centrifuged at 1000*g* for 10 min, and the supernatant was collected. The extraction was repeated with 2 mL of acetone, and the two supernatants were combined. Water and acetone extracts of all samples were diluted in the corresponding solvent (depending on their activity) and analyzed separately in triplicate for their antioxidant capacity by three different TAC assays: Trolox equivalent antioxidant capacity (TEAC) assay (*17*), total radical-trapping antioxidant parameter (TRAP) assay (*18*), and ferric reducing antioxidant power (FRAP) assay (*19*). The TAC values were obtained by summing the antioxidant capacity of two extracts (i.e., water and acetone extracts). The TEAC and TRAP values were expressed as millimoles of Trolox per 100 g of dry weight. FRAP values were expressed as millimoles of Fe^{2+} equivalents per 100 g of dry weight.

Statistical Analysis. Means and standard deviations (SD) of data were calculated with SPSS (version 14.0, SPSS Inc., Chicago, IL) statistical software. SPSS was used to perform one-way analysis of variance (ANOVA) and least significant difference test (LSD) at a 95% confidence level ($p \leq 0.05$) to identify differences among groups.

Table 2. LC-MS/MS Characteristics of Phenolic Compounds in Artichokes

peak	compound	retention time (min)	$[M - H]^-$ *m/z*	MS^2 ions *m/z*
1	3-*O*-caffeoylquinic acid	7.5	353	MS^2 [353]: 191 (100), 179 (49), 135 (13), 173 (3)
2	5-*O*-caffeoylquinic acid	9.6	353	MS^2 [353]: 191 (100), 179 (3)
3	4-*O*-caffeoylquinic acid	9.9	353	MS^2 [353]: 173 (100), 179 (53), 191 (16), 135 (12)
4	1,3-di-*O*-caffeoylquinic acid	12.2	515	MS^2 [515]: 353 (100), 179 (55), 335 (17), 191 (51)
5	luteolin 7-*O*-rutinoside	16.5	593	MS^2 [593]: 285
6	luteolin 7-*O*-glucoside	18.04	447	MS^2 [447]: 285
7	luteolin 7-*O*-glucuronide	18.2	461	MS^2 [461]: 285
8	dicaffeoylquinic acid	18.9	515	MS^2 [515]: 353 (100), 203 (20), 299 (10), 255 (7)
9	3,4-di-*O*-caffeoylquinic acid	19.02	515	MS^2 [515]: 353 (100), 173 (26), 179 (30), 335 (17)
10	3,5-di-*O*-caffeoylquinic acid	19.08	515	MS^2 [515]: 353 (100), 191 (14), 179 (6)
11	narirutin	19.47	579	MS^2 [579]: 271
12	apigenin 7-*O*-rutinoside	19.8	577	MS^2 [577]: 269
13	1,5-di-*O*-caffeoylquinic acid	19.9	515	MS^2 [515]: 353 (100), 191 (87), 335 (11)
14	naringenin 7-*O*-glucoside	21.6	433	MS^2 [433]: 271
15	apigenin 7-*O*-glucoside	21.8	431	MS^2 [431]: 269
16	4,5-di-*O*-caffeoylquinic acid	21.9	515	MS^2 [515]: 353 (100), 173 (44), 179 (37), 203 (6), 299 (3)
17	apigenin 7-*O*-glucuronide	22.6	445	MS^2 [445]: 269, 175

RESULTS AND DISCUSSION

Effect of Cooking on Physical Characteristics. The physical properties of artichokes are reported in **Table 1**. Raw artichokes showed a shear force value of 98.2 9 ± 4.9 N. Cooking significantly varied the shear force, lowering it especially in boiled and steamed products. Thus, softening was significantly higher for both boiled and steamed artichokes in comparison with fried products.

The color of bracts showed increases of *L**, *b**, and *C* values after cooking. *a** values became significantly lower for both boiled and steamed bracts, whereas they remained unchanged in fried samples. Stem color was characterized by a decrease of *L**, *b**, and *C* values, more markedly in steamed samples. It is noteworthy that boiled bracts showed a marked increase in greenness (*a** decrease, hue angle shift) in comparison with the other two cooking treatments. Thus, cooking treatments generally seem to have no detrimental effects on the color of artichokes, especially on bracts. In agreement, Ihl and co-workers (*20*) reported a better preservation of the original color (*a** more negative, *b** more positive) of boiling water-blanched artichokes in comparison with steam blanched. The authors related this observation to lower chlorophyllous pigment degradation and/or a different pattern of chlorophyll conversion in boiled samples. Moreover, the rapid expulsion of intercellular air and other dissolved gases by cells in boiled samples and their replacement by cooking water and cell juices may have influenced surface reflectance and depth of light penetration into tissues, as observed for other green vegetables, causing a more consistent color retention after boiling (*21, 22*).

LC-MS/MS Identification and Quantification of Phenolic Compounds. Flavonoid and caffeoylquinic acid isomers identified in artichoke extracts, their retention times, pseudomolecular

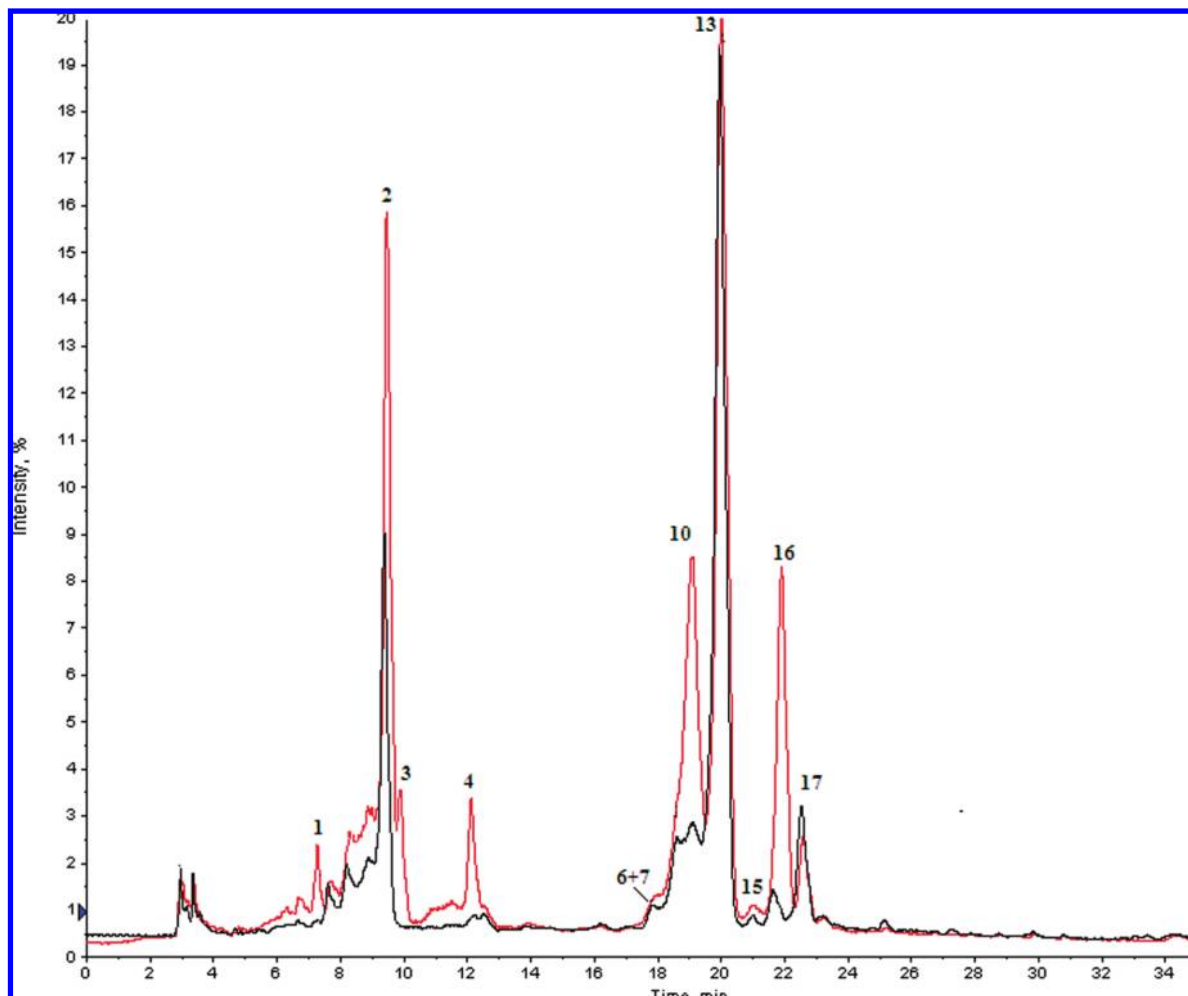

Figure 1. UV chromatogram at 330 nm of polyphenol extracts from raw artichoke (black line) and steamed artichoke (red line). Peak numbers are as reported in **Table 2**.

ions in negative mode $[M - H]^-$, and MS/MS fragmentations patterns are reported in **Table 2**.

The UV chromatogram of a steamed artichoke extract recorded at 330 nm is shown in **Figure 1** together with the highlighted assignment of the main UV-detectable phenolic compounds, performed by MS/MS. Chlorogenic acid (5-*O*-caffeoylquinic acid) and cynarin (1,3-di-*O*-caffeoylquinic acid) standards were used to quantify monocaffeoylquinic acids and dicaffeoylquinic acids, respectively. Calibration curves were built by acquiring chromatograms at 330 nm in the linearity range of 10−500 μg/mL.

Among flavonoids, only UV detectable peaks such as apigenin 7-*O*-glucoside and apigenin 7-*O*-glucuronide were quantified. In this case the quantification was performed using luteolin 7-*O*-glucoside as standard, and calibration curves were built at 280 nm in the linearity range of 10−500 μg/mL.

The identification of 5-*O*-caffeoylquinic acid, 1,3-di-*O*-caffeoylquinic acid, luteolin 7-*O*-glucoside, naringenin 7-*O*-glucoside, and narirutin in artichoke extracts was based on the comparison of their pseudomolecular ion in negative mode, fragmentation pattern, UV spectrum, and elution time with the standards. As no standards were available for the other artichoke phenolic compounds, their tentative identification was achieved by IDA analyses. This acquisition method generates a survey scan, single MS spectra with molecular mass information, product ion spectra (MS^2), and extracted ion fragments (XICs) (*23, 24*). The results obtained with this procedure on the flavonoids moiety of artichoke are shown in **Figure 2**.

Therefore, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, 1,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, luteolin 7-*O*-glucuronide, luteolin 7-*O*-rutinoside, apigenin 7-*O*-glucoside, apigenin 7-*O*-glucuronide, and apigenin 7-*O*-rutinoside were tentatively identified primarily by means of their parent ion, by comparison of their fragmentation patterns, UV spectrum, and sequence of chromatographic elution analyzed in comparison with available literature data. The pattern of artichoke phenolic compounds found in this work substantially confirms that previously found by other authors (*5, 25*), so the MS/MS procedure setup can be adopted to verify the effect of cooking practice on the profile of artichoke phenolic compounds.

The concentration of polyphenolic compounds varies considerably with variety and maturity stage of artichokes (*26*). Our data are in accordance with results reported by Romani et al. (*27*) and Schütz et al. (*5*).

Literature data show a total content of dicaffeoylquinic acids ranging from 9500 to 25000 mg/kg of dry weight, whereas monocaffeoylquinic acid concentrations vary from 1500 to 3500 mg/kg of dry matter (*5, 26, 27*).

Effect of Cooking on Polyphenols Profile. Data about the effect of the various cooking practices on the concentration of antioxidant polyphenol compounds of artichokes are reported in **Table 3**.

In the raw artichokes the 5-*O*-caffeoylquinic acid and 1,5-di-*O*-caffeoylquinic acid had the highest concentrations (3050 and 3180 mg/kg on a dry matter basis, respectively). Among

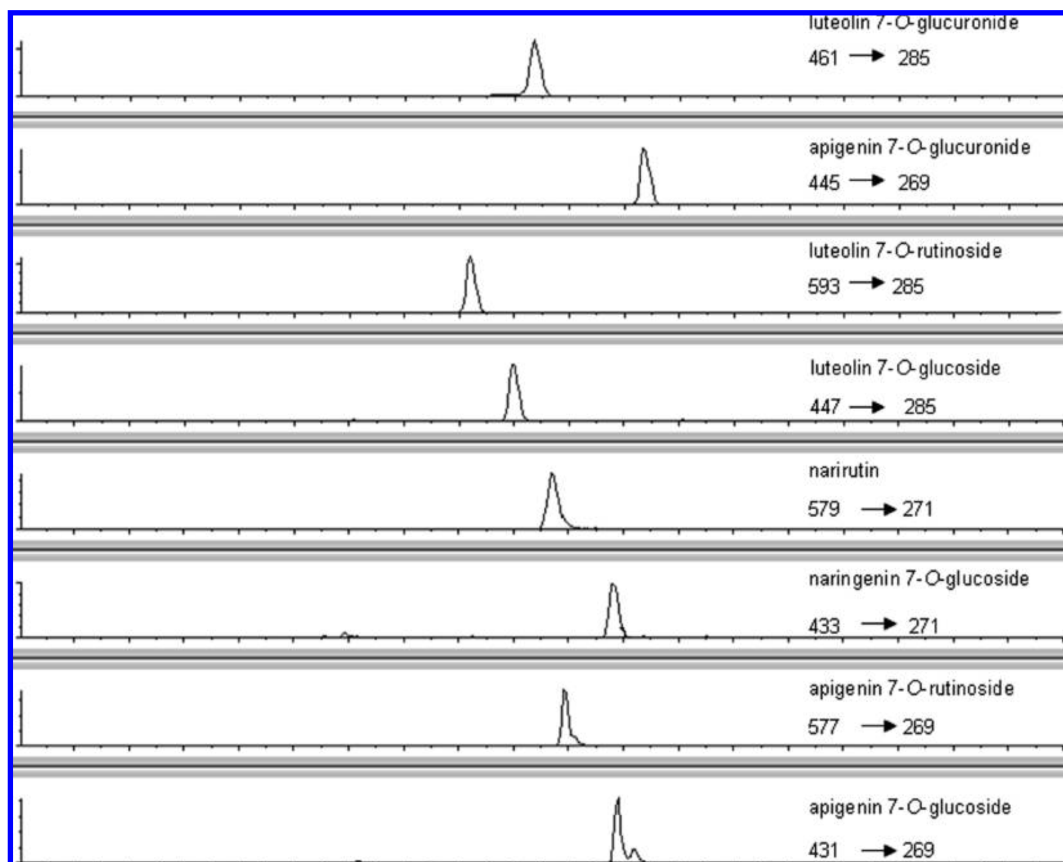

Figure 2. Extraction ion chromatograms (XICs) of the main flavonoids present in the polyphenol extracts of artichoke.

Table 3. Polyphenol Content in Raw and Cooked Artichokes[a]

phenol	raw (mg/kg of DM)	boiled (mg/kg of DM)	steamed (mg/kg of DM)	fried (mg/kg of DM)
3-*O*-caffeoylquinic acid	ND[b]	768 ± 3.54 a	265 ± 1.77 b	133 ± 1.20 c
5-*O*-caffeoylquinic acid	3050 ± 11.53 c	4040 ± 3.75 b	6560 ± 21.21 a	6150 ± 7.07 a
4-*O*-caffeoylquinic acid	203 ± 3.4 b	768 ± 2.33 a	265 ± 4.88 b	136 ± 2.19 c
1,3-di-*O*-caffeoylquinic acid	99 ± 2.33 d	777 ± 9.89 a	303 ± 1.20 b	226 ± 3.96 c
3,4-di-*O*-caffeoylquinic acid	NQ[c]	NQ	NQ	NQ
3,5-di-*O*-caffeoylquinic acid	158 ± 1.48 a	1560 ± 9.19 b	1480 ± 7.3 b	584 ± 5.37 c
1,5-di-*O*-caffeoylquinic acid	3180 ± 13.43 a	2390 ± 14.85 b	3380 ± 36.77 a	3570 ± 30.40 a
4,5-di-*O*-caffeoylquinic acid	188 ± 0.85 b	1140 ± 0.0 a	1090 ± 26.16 a	947 ± 10.68 a
total caffeoylquinic acids	**6878**	**11443**	**13343**	**11746**
apigenin 7-*O*-glucoside	104 ± 0.83 a	74 ± 0.35 b	76 ± 1.27 b	39 ± 0.37 c
apigenin 7-*O*-glucuronide	1790 ± 15.56 a	1140 ± 1.13 c	1370 ± 1.41 b	737 ± 7.07 d
total apigenin derivatives	**1894**	**1214**	**1446**	**776**

[a] Values are presented as mean value ± SD ($n = 3$). The same letters within each row do not significantly differ ($p \leq 0.05$). [b] ND, not detected. [c] NQ, not quantifiable.

flavonoids, apigenin 7-*O*-glucuronide and apigenin 7-*O*-glucoside were the predominant peaks, with concentrations of 1790 and 104 mg/kg on a dry matter basis, respectively.

Literature data reporting the effect of cooking on vegetable polyphenols content are scarce and often limited to only the total phenolic compounds concentration, measured by Folin−Ciocalteu method. In these studies, mainly carried out on other vegetables, a reduction of phenolic compounds and of water-soluble metabolites after cooking methods was reported: Zhang and Hamauzu (*7*) observed a loss of phenolic compounds for boiled and microwaved broccoli, and similar results were reported by Sahlin et al. (*9*) for fried and baked tomatoes and by Ismail et al. (*10*) for blanched and boiled spinach. Using the same cooking methods, we have already reported a general decrease of polyphenols, mainly phenolic acids, in all of the analyzed vegetables and using all of the cooking methods, despite a general increase of TAC (*8*). On the other hand, Turkmen et al. (*11*) reported that cooking had no deleterious effect on total antioxidant activity and total phenolic compounds content in squash, peas, and leek.

More recently, Takenaka et al. (*28*) found an increase of 3-caffeoylquinic acid, 4-caffeoylquinic acid, 3,4-dicaffeoylquinic acid. and 4,5-dicaffeoylquinic acid in boiled potatoes.

In the present study, a significant increase of total caffeoylquinic acid (calculated as the sum of the concentrations of the single compounds quantified by MS/MS) was observed for boiling, steaming, and frying procedures. Increases of 66, 94, and 71% with respect to the content of uncooked artichokes were shown for boiling, steaming, and frying, respectively. This increase was mainly due to the increase of 5-*O*-caffeoylquinic and 1,5-di-*O*-caffeoylquinic acid, particularly in steamed and fried samples.

Looking at the data shown in **Table 3**, it is clear that the effect is the result of both isomerization and hydrolysis events,

Table 4. Carotenoid Content in Raw and Cooked Artichokes[a]

carotenoid	raw (mg/ 100 g of DM)	boiled (mg/ 100 g of DM)	steamed (mg/ 100 g of DM)	fried (mg/ 100 g of DM)
lutein	1.0 ± 0.1 d	8.7 ± 0.0 a	6.9 ± 0.0 b	4.0 ± 0.0 c
β-carotene	0.27 ± 0.0 c	1.2 ± 0.0 a	1.2 ± 0.1 a	1.0 ± 0.0 b
phytoene	0.3 ± 0.0 d	0.7 ± 0.0 b	0.6 ± 0.0 c	0.8 ± 0.0 a
phytofluene	ND[b]	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0
total carotenoids	1.6	11.2	9.4	6.5

[a] Values are presented as mean value ± SD ($n = 3$). The same letters within each row do not significantly differ ($p \leq 0.05$). [b] ND, not detected.

leading to a substantial redistribution of phenolic acids concentrations due to a massive transesterification phenomenon occurring during processing. This is particularly evident for 3,5- and 4,5-di-*O*-caffeoylquinic acids, which have very low concentrations in the raw product and are extensively formed during processing.

Several studies on *Cynara cardunculus* species reported that in the raw material only 5-*O*-caffeoylquinic and 1,5-di-*O*-caffeoylquinic acids were found and, according to different studies, small amounts of other caffeoylquinic isomers could be generated during solvent extraction (*5, 29, 30*). According to Slanina et al. (*29*) the enhancement of caffeoylquinic acids isomers content during heat treatment was caused by intramolecular transesterification of 5-*O*-caffeoylquinic and 1,5-di-*O*-caffeoylquinic acids promoted by the high temperatures. Our results confirm that cooking practices caused a marked intramolecular transesterification of caffeoylquinic acid.

Higher polyphenol content in blanched artichokes compared with raw samples was also observed by Llorach et al. (*31*). The authors explained this finding with the action of the polyphenol oxidase (PPO)-catalyzed reactions. PPO, which has very high activity in artichoke, could be responsible for the loss of phenolic compounds in raw artichoke, whereas, in the case of blanched artichoke, the use of boiling water could involve PPO inactivation with the subsequent preservation of phenolic compounds. Accordingly, Schütz et al. (*5*) reported higher phenolic compound concentrations in artichoke obtained after water blanching compared with fresh samples.

For flavonoids, processing had a general negative effect on apigenin 7-*O*-glucoside and apigenin 7-*O*-glucuronide. As shown in **Table 3**, the total decreases of apigenin derivatives were 36% after boiling, 24% after steaming, and 59% after frying. In this case the thermal degradation of flavonoids seems to overcome the increased bioaccessibility caused by matrix softening effect.

Effect of Cooking on Artichoke Carotenoid Profile. Data reported in **Table 4** show that the concentration of all carotenoids increased after cooking, particularly after boiling. As already observed for carrots by Miglio et al. (*8*), small amounts of the carotene precursors (i.e., phytoene and phytofluene) become detectable during all cooking treatments. The effect of thermal treatments on carotenoid concentration was largely expected: many literature data reported an increase of carotenoid concentration in processed tomato products (*32*) as well as in cooked spinach (*33*). The increase is due to the thermal disruption of the non-covalent association between carotenoids and proteins present in the cell chloroplasts with the consequent better extractability of free carotenoids.

The higher concentrations of carotenoids and polyphenols present in the cooked artichokes could be related to the increase of L^*, b^*, and C observed in the bracts and in the loss of vivid color (C decrease) found in the stem. This different behavior could be attributed to the different distributiond of antioxidant compounds in the stem and bracts and also to the different PPO activities, which cause the biosynthesis of melanin polymers.

Effect of Cooking on Artichoke TAC. The TAC values of raw artichokes are slightly lower, with the exception of the value measured by TEAC assay, than those reported in the TAC database (*16*), probably because a different variety was used.

As shown in **Table 5**, all of the cooking methods used determined a strong and significant increase of TAC values with respect to raw sample independent of the assay used to assess the TAC. Among the cooking methods, steaming was the method that determined the highest TAC increases (up to 1018 and up to 1423% in the case of TEAC and TRAP assays, respectively).

TAC increases of 5−15-fold, depending on the antioxidant assay and the cooking practice used, are partially explained by the observed increases of antioxidants. In fact, caffeoylquinic and dicaffeoylquinic acids, the major antioxidants in the artichokes, increased about twice. Differences of polyphenol content and TAC values cannot justify the different increases observed. Thus, it is worth noting that the TAC was obtained by summing the value obtained for water and acetone extracts, according to the previously used procedure (*16*). However, the concentrations of total phenolic compounds were similar in the extracts obtained using both extraction procedures (namely, for HPLC analysis and determination of antioxidant capacity), without any significant difference. Accordingly, the TAC values were the same whether the extraction was performed using the procedure previously used for MS/MS analysis.

However, the intramolecular transesterification of 5-*O*-caffeoylquinic and 1,5-di-*O*-caffeoylquinic acids promoted by the high temperatures could have determined an increased antioxidant activity of such neo-formed compounds with respect to their parent compounds. It was already observed in the cases of procyanidins that the spatial arrangement of the phenolic group in polyphenols can deeply affect the antioxidant activity of the molecules, and a paper dealing with the antioxidant activity of bamboo caffeoylquinic acids reported that differently substituted compounds had significantly different antioxidant activities as measured by DPPH assay (*34*). Moreover, cooking might have promoted a polymerization of polyphenols, leading to the formation of compounds that are not detected by MS/MS having very high antioxidant activity. A similar feature was

Table 5. Trolox Equivalent Antioxidant Capacity (TEAC), Total Radical-Trapping Antioxidant Parameter (TRAP), and Ferric Reducing Antioxidant Power (FRAP) Values of Raw and Cooked Artichokes[a]

artichoke	TEAC (mmol of Trolox/100 g of DM)	FRAP (mmol of Fe^{2}/100 g of DM)	TRAP (mmol of Trolox/100 g of DM)
raw	2.07 ± 0.10 c	5.69 ± 0.11 c	3.82 ± 0.26 c
boiled	15.89 ± 0.62 b (668%)	52.42 ± 2.68 b (822%)	20.31 ± 0.17 b (431%)
steamed	23.14 ± 1.59 a (1018%)	70.50 ± 5.00 a (1139%)	58.18 ± 2.05 a (1423%)
fried	13.40 ± 1.25 b (548%)	43.04 ± 5.45 b (657%)	19.52 ± 1.81 b (411%)

[a] All values were obtained by summing the antioxidant capacity of two extracts (i.e., water and acetone extracts) analyzed separately and are presented as mean value ± SD ($n = 3$). The percent variation with respect to raw vegetable due to the cooking method is given in parentheses. The same letters within each column do not significantly differ ($p \leq 0.05$).

already observed in catechin-rich foods such as cocoa (*35*) and apple juice (*36*), for which it was shown that procyanidins with polymerization degrees of up to 6−9 have an exceptionally high antioxidant activity.

The contribution of carotenoids to the TAC increases cannot be neglected, even if the concentration of these compounds in artichoke is much lower than that of polyphenols. Therefore, although carotenoids increased 4−7-fold in the cooked artichokes, their contribution to the overall TAC increase is probably not much relevant. Finally, it is worth remembering that the measure of the TAC of complex mixtures, being the result of the simultaneous action of different antioxidant compounds, does not give necessarily a linear response with the increase of the antioxidant concentration.

In conclusion, the present results demonstrated that common cooking methods applied to artichoke have increased the nutritional quality of this vegetable with no detrimental effects on their physical properties. Marked increases of caffeoylquinic acids and carotenoids concentrations, particularly upon steaming and boiling, were accompanied by a moderate decrease of flavonoid concentration, which became severe upon frying. This effect was probably ascribable to both matrix softening, as observed by texture analysis, and increased extractability upon cooking, and it was accompanied by the conversion of caffeoylquinic acids into other isomers, which were detected after thermal treatments.

However, these neo-formed compounds cannot explain completely the enormous increase of the antioxidant capacity observed in cooked artichokes, which could be also attributed to the conversion of polyphenols into, not yet identified, very active antioxidant species that synergistically determined a very high antioxidant capacity.

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Received for review February 9, 2008. Revised manuscript received June 20, 2008. Accepted July 30, 2008. This work is supported by Laboratorio Regionale per la Sicurezza e la Qualità degli Alimenti (SIQUAL) [Project 9, Programma Regionale per la Ricerca Industriale, l'Innovazione e il Trasferimento Tecnologico (PRRIITT)].

JF800408W