Lettuce and Chicory Byproducts as a Source of Antioxidant Phenolic Extracts

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A process to obtain enriched antioxidant phenolic extracts from lettuce (baby, romaine, and iceberg cultivars) and chichory byproducts as a way to valorize these byproducts was developed. Two extraction protocols using water and methanol as solvent were used. Amberlite XAD-2 nonionic polymeric resin was used to purify the extracts. The extraction yield, phenolic content, and phenolic yield were evaluated as well as the antioxidant capacity of the extracts (DPPH, ABTS, and FRAP assays). Baby and romaine lettuce byproducts showed the highest water extract yields [27 and 26 g of freeze-dried extracts/kg of byproduct fresh weight (fw), respectively], whereas baby and iceberg lettuce showed highest methanol extract yields (31 and 23 g of freeze-dried extracts/kg of byproduct fw, respectively). Methanol extraction yielded a raw extract with a high phenolic content, the baby and chicory extracts being the richest with ~50 mg of phenolics/g of freeze-dried extract. Regarding the purified extracts, water extraction yielded a higher phenolic content, baby and chicory being also the highest with mean values of ~190 and 300 mg of phenolics/g of freeze-dried extract, respectively. Both raw and purified extracts from baby and chicory showed the higher antioxidant contents (DPPH, ABTS, and FRAP assays). The antioxidant capacity was linearly correlated with the phenolic content. The results obtained indicate that lettuce byproducts could be, from the industrial point of view, an interesting and cheap source of antioxidant phenolic extracts to funcionalize foodstuffs.

KEYWORDS: Lettuce; chicory; byproduct valorization; phenolics; antioxidant; ABTS; DPPH; FRAP; extraction protocol

INTRODUCTION

About 18 million metric tons (Mt) of lettuce are produced over the world, Spain being the third largest producer with an estimated production of ~1 million Mt of lettuce (*1*). Within Spain, the region of Murcia is the largest producer, with an annual production of nearly 350000 Mt. Lettuce has two methods of commercialization, one as whole lettuce heads and the other as fresh-cut product. Nowadays, there has been a great development of the fresh-cut vegetable industry, fresh-cut lettuce being one of the most important products.

The packing houses dealing with vegetables produce large amounts of wastes and residues (leaves, stems, etc.). Sometimes these byproducts can reach 50% of the harvested material as in lettuce production. These residues are very perishable products; their management is not always easy, and they are responsible for environmental management problems in the industry. In addition, residues from the fresh-cut salad industry are nowadays an important problem from both environmental and hygienic points of view.

Different approaches have been taken for the valorization of the byproducts, including animal feedstuff (*2*), fiber production (*3*, *4*), and fuel production (*5*). In addition, recent studies have demonstrated that vegetable byproducts are an interesting and cheap source of health-promoting antioxidant polyphenols (*6*–*8*).

Epidemiological studies have suggested associations between the consumption of polyphenol-rich foods or beverages and prevention of some diseases (*9*). The role of polyphenols in the prevention of these diseases has been mainly attributed to the prevention of low-density lipoprotein oxidation (*9*, *10*) through a scavenging activity against peroxyl and hydroxyl radicals (*10*).

The polyphenolic content and composition of different lettuce and chicory varieties have been previously studied as well as the effect of variety, processing, and storage on this composition (*11*, *12*). These works have been focused mainly to improve the quality of the commercial lettuce products. Although different studies of lettuce have demonstrated that the external portions have higher contents of flavonoids than the internal portions, which are usually the edible portions (*13*), scarce information is available regarding their corresponding byproducts.

The aim of this work is to evaluate fresh-cut lettuce industry byproducts as a source of natural antioxidant polyphenols for their possible use as dietary or food antioxidants. To this purpose, the extract yield, phenolic yield, and correlation between antioxidant capacity and phenolic content were studied.

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MATERIALS AND METHODS

Reagents. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), chlorogenic acid (5-*O*-caffeoylquinic acid), manganese dioxide (MnO$_2$), ferric chloride, 2,4,6-tripyridyl-*s*-triazine (TPTZ), rutin, and apiin were purchased from Sigma (St. Louis, MO). All other reagents were of analytical grade and supplied by Merck (Darmstadt, Germany). Milli-Q system (Millipore Corp., Bedford, MA) ultrapure water was used throughout this research.

Plant Material. Byproducts from three different varieties of lettuce (*Lactuca sativa* L.) (romaine, iceberg, and baby) and one of sample of chicory (*Cichorium endivia* L.) (escarole) were used to evaluate their polyphenolic content as well as their antioxidant capacity. Both lettuce and chicory byproducts include mainly external leaves. The samples of romaine, iceberg, and chicory were supplied by KERNEL, S.A. (Los Alcazares, Murcia, Spain) and came from fresh-cut salad production. The samples of baby lettuce byproducts were supplied by Agrosol Cooperative (Lorca, Murcia, Spain) and came from the handling of fresh whole lettuce. Before the extraction, fresh lettuce and chicory byproducts were chopped with a sharp stainless steel knife in small pieces to improve the extraction.

Extraction Protocols. A recent method to obtain the polyphenol-enriched extracts from byproducts (*8*) has been used to obtain two different extracts (raw and purified).

Raw Extracts. One kilogram (fresh weight) of each byproduct sample was extracted by reflux with boiling solvent (1:3 w/v) (methanol or water) for 1 h. The plant material was then pressed, and the resultant liquids were pooled with either methanol or water extracts. The extracts were cooled at room temperature and then filtered through Whatman no. 1 filter paper (Maidstone, U.K.). In the methanol extract, the solvent was removed with a rotary evaporator and 200 mL of water was added. On the other hand, the raw water extract was concentrated with a rotary evaporator (40 °C) to facilitate its further freeze-drying process. Finally, the extracts were freeze-dried at −50 °C and stored.

Extracts Purified Using Amberlite XAD-2. A procedure to recover flavonoids from the water solutions using the nonionic polystyrene resin (Amberlite XAD-2) has been used (*11*). This resin has been used recently to obtain polyphenolic-enriched extracts from byproducts (*8*). New raw extracts were obtained as described above. The methanol extract was added with the same volume of water and concentrated with a rotary evaporator (40 °C) until all of the methanol was evaporated and only the water remained. Afterward, the extracts were poured in a column previously packed with a nonionic resin Amberlite XAD- 2 (Supelco, Bellfonte, PA) (column of 50 × 4 cm) as described by Ferreres et al. (*12*). Water (10 L) was used to wash out the salts and sugars before collection of the phenolic compounds. The phenolic compounds were eluted with methanol, which was further removed with a rotary evaporator (40 °C). Afterward, 200 mL of water was added. These extracts were freeze-dried at −50 °C.

The term "extract yield" (*7*) was defined as the amount of freeze-dried extract (grams) obtained from 1 kg of fresh weight byproducts [extract (g)/(kg of fresh byproduct)].

HPLC Analysis. Ten milligrams of each extract was dissolved in 1 mL of distilled water and filtered through a 0.45 μm membrane filter Millex HV$_{13}$ (Millipore Corp). A 20 μL sample of each extract was analyzed using an HPLC system equipped with a model L-6200 pump (Merck Hitachi) and a Shimadzu SPD-MSA photodiode array UV−vis detector. Separations were achieved on a Licrocart column (Merck) (RP-18, 25 × 0.4 cm; 5 μm particle size). The mobile phase was water with 5% formic acid (v/v) (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 1 mL/min. The linear gradient started with 10% B in A to reach 20% B at 25 min, 50% B at 40, 50% B at 45 min, and 90% B at 60 min. Chromatograms were recorded at 335 nm.

Phenolic Compound Identification and Quantification. The identification of caffeic acid derivatives was carried out according to their UV spectra and retention times as previously reported by Tomas-Barberan et al. (*11*) and flavonoids as described by Dupont et al. (*13*).

Caffeic acid derivatives were quantified by comparison with external standards as chlorogenic acid (5-*O*-caffeoylquinic acid) and flavonoids as rutin.

The term "phenolic yield" (*7*) was defined as the amount of total phenolic compounds (milligrams) (caffeic acid derivatives, flavones, and flavonols) obtained from 1 kg of fresh weight byproducts. The term "phenolic content" (*7*) was defined as the amount of total phenolic compounds (milligrams) obtained from 1 g of freeze-dried extract. The results presented are the mean of three experiments. The standard deviation was always <10%.

Antioxidant Capacity. The free radical scavenging activities (DPPH• and ABTS•+ assays) as well as the ferric reducing ability (FRAP assay) were used to evaluate the antioxidant capacity of the extracts.

DPPH• Assay. The free radical scavenging activity using the free radical DPPH• (*14*) was evaluated by measuring the variation in absorbance at 515 nm after 1 h of reaction in parafilm-sealed glass cuvettes (to avoid methanol evaporation) at 25 °C (*15*). Lettuce and chicory byproduct extracts (10 mg) were dissolved in 1 mL of MeOH/water (80:20 v/v). The reaction was started by adding 20 μL of the corresponding sample to the cuvette containing 80 μM (methanol solution) (980 μL) of the free radical (DPPH•). The final volume of the assay was 1 mL. Reaction was followed with a UV-1603 Shimadzu spectrophotometer (Tokyo, Japan).

ABTS•+ Assay. The extracts (10 mg) were dissolved in 1 mL of Milli-Q water. The reaction started by adding 5 μL of the corresponding sample to the cuvette containing a 32 μM water solution (995 μL) of the free radical (ABTS•+). The radical was chemically generated with MnO$_2$ as described by Espín and Wichers (*16*). The experiments were always performed on freshly made up solutions. The final volume of the assay was 1 mL. The disappearance of ABTS•+ was determined by measuring the decrease in absorbance at 414 nm for 1 h at 25 °C in the above-described spectrophotometer.

FRAP Assay. The FRAP assay was performed according to the method of Benzie and Strain (*17*) with some modifications. The freshly made up FRAP solution contained 25 mL of 0.3 M acetate buffer (pH 3.6) plus 2.5 mL of 10 mM TPTZ solution in 40 mM HCl (previously prepared) and 2.5 mL of 20 mM ferric chloride (FeCl$_3$·6H$_2$O). This solution was used as blank. Nine hundred and fifty microliters of warmed (37 °C) FRAP solution was mixed with 50 μL of freshly dissolved extract (10 mg/mL of water). The ferric reducing ability of byproduct extracts was measured by monitoring the increase of absorbance at 593 nm for 45 min.

All of the antioxidant assays were repeated three times, and the coefficient of variation [CV = (standard deviation (SD)/mean) × 100] was always <10%. In addition, calibration curves were made for each assay using Trolox as standard. The antioxidant capacity (DPPH•, ABTS•+, FRAP assays) was expressed as Trolox equivalent antioxidant capacity (TEAC) following the nomenclature of Rice-Evans and Miller (*18*).

The "antioxidant yield" (AY) (*7*) correlated the Trolox equivalent antioxidant capacity (grams of TEAC) in 1 kg of fresh cauliflower byproducts taking into account the "extract yield": AY = [(g of TEAC/g of extract) × extract yield].

Graphs and Data Analysis. Plots, fittings, and statistical analysis were carried out by using the Sigma Plot 6.0 program (SPSS Science, Chicago, IL). Statistical significance was set at $P < 0.01$.

RESULTS AND DISCUSSION

Extract Yield. The "extract yield" is an interesting index to evaluate the possible use of these methods with regard to possible industrial use. Lettuce and chicory residues showed different behaviors depending on the studied sample (**Table 1**). The water extraction protocol of romaine and chicory byproducts had extract yields 1.96 and 1.2 times higher than those extracted with methanol. These results are in agreement with those reported for artichoke (*7*) and cauliflower byproducts (*8*). With regard to the baby and iceberg lettuce byproducts, no significant differences were observed between the extraction methods (**Table 1**).

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Table 1. Yields and Phenolic Acid and Flavonoid Contents of Lettuce Byproducts

| | | phenolic compounds | | | | | | | | | |
| | | caffeic acid derivatives | | flavones | | flavonols | | total flavonoids | | total phenolics | |
	extract yield[a]	phenolic content[b]	phenolic yield[c]	phenolic content[b]	phenolic yield[c]	phenolic content[b]	phenolic yield[c]	phenolic content[b]	phenolic yield[c]	phenolic content[b]	phenolic yield[c]
baby[d]											
BAW	27.2	40.00	1088.00	0.20	5.80	5.80	157.7	6.00	163.2	46.00	1251.20
BAM	31	39.20	1215.20	0.70	21.70	10.33	320.23	11.03	341.93	50.23	1557.13
ABAW	0.7	ND[e]	ND	5.02	3.54	181.40	127.00	186.42	130.44	186.42	130.54
ABAM	1	ND	ND	6.20	6.20	146.00	146.00	152.20	152.20	152.20	152.20
romaine											
ROW	25.6	19.37	496.00	1.81	46.33	3.29	84.22	5.10	130.56	24.47	626.43
ROM	13	17.00	221.00	2.34	30.42	6.55	85.15	8.89	115.57	25.89	336.57
AROW	0.8	2.40	1.93	14.06	11.24	19.20	15.36	33.26	26.60	35.66	28.50
AROM	1	1.00	1.00	10.20	10.20	16.30	16.30	19.78	19.78	27.50	27.50
iceberg											
ICW	21	10.05	211.05	0.34	7.14	1.04	21.84	1.38	29.00	11.43	240.03
ICM	23	4.70	108.10	0.40	9.20	1.06	24.38	1.46	33.58	6.16	141.68
AICW	0.4	58.70	23.48	13.17	5.26	45.16	18.06	58.33	23.32	117.03	46.81
AICM	0.6	17.36	10.42	15.27	9.26	79.20	47.52	94.47	56.68	111.83	67.1
chicory											
CHW	18	23.36	420.50	ND	ND	19.24	346.32	19.24	346.32	42.60	766.80
CHM	14.8	28.07	415.43	ND	ND	27.50	407.00	27.50	407.00	55.57	822.43
ACHW	0.8	23.90	19.12	ND	ND	284.90	227.93	284.90	227.93	308.80	247.04
ACHM	0.8	6.29	5.03	ND	ND	187.77	150.22	187.77	150.22	194.06	155.25

[a] Freeze-dried extract, g/kg of byproduct fw. [b] Total phenolic compounds, mg/g of freeze-dried extract. [c] Total phenolic compounds, mg/kg of byproducts fw. [d] BA, baby; RO, romaine; IC, iceberg; CH, chicory; A, Amberlite; W, water extracted; M, methanol extracted. [e] Not detected. Standard deviation was always <10%.

The extract yield from raw extracts was considerably higher than those obtained from purified extracts with a range from 52.5-fold (iceberg byproducts water extract vs purified iceberg byproducts water extract) to 13-fold higher (romaine byproduct methanol extract vs purified romaine byproduct methanol extract). The methanol method yielded a higher extract yield than the water extraction method; in chicory byproducts did both methods show the same extract yield.

Different types of Amberlite XAD have been used to purify extracts from byproducts (*8*, *19*). The extract yield of lettuce and chicory byproducts purified extracts were lower than those previously obtained from cauliflower byproducts using Amberlite XAD-2 (*8*) and similar to those obtained from apple pomace using Amberlite XAD-16 HP (*19*).

Phenolic Compound Identification and Quantification. The HPLC analyses of lettuce byproducts revealed the presence of both hydroxycinnamic acids and flavonoids. The HPLC profiles are shown in **Figure 1**.

With regard to the raw extracts, all samples showed very similar hydroxycinnamic profiles from a qualitative point of view. Concerning the hydroxycinnamic acids, both caffeoylquinic and caffeoyltartaric acid derivatives have been identified. The main hydroxycinamic acid derivative identified was dicaffeoyltartaric acid (chicoric acid) (peak 7, **Figure 1**) followed by chlorogenic acid (5-*O*-caffeoylquinic acid) especially in the methanol extracts (peak 4). This compound undergoes isomerization in warm aqueous media as previously reported (*8*, *20*), leading to changes in the extract composition (**Figure 2**). This process could explain the presence of peaks 1 and 3, possibly neochlorogenic acid (3-*O*-caffeoylquinic acid) and cryptochlorogenic acid (4-*O*-caffeoylquinic acid), respectively. These compounds have been previously reported in both iceberg and romaine lettuce (*11*). In addition, different isomers of isochlorogenic acid (3,5-*O*-dicaffoylquinic acid) were identified (peaks 6 and 9).

The flavonoid profile of lettuce byproducts was composed by flavones (luteolin derivatives) and flavonols (quercetin derivatives), showing minor differences between lettuce samples,

whereas the chicory byproducts were composed only by kaempferol derivatives.

Luteolin 7-*O*-glucuronide (peak 10) was identified, and this compound has been previously identified in some lettuce cultivars (*13*). Regarding the quercetin derivatives quercetin 3-*O*-glucuronide (peak 11), quercetin 3-*O*-glucoside (peak 12), and quercetin 3-*O*-(6-*O*-malonyl)glucoside (peak 13) have been identified. In addition, in the baby byproduct extracts two other quercetin derivatives have been identified as quercetin 7-*O*-glucuronide-3-*O*-(6′′-malonyglucoside) and quercetin 7-*O*-glucoside-3-*O*-(6′′-malonylglucoside). This last compound has been previously identified in the red lettuce cv. Lollo Rosso (*12*). With regard to chicory byproducts the HPLC of raw extracts showed a kaempferol 3-*O*-glucoside as the main flavonol (peak 14), and this compound has already been reported in chicory (*13*).

Phenolic compounds were quantified in the different extracts (**Table 1**). The methanol raw extracts show a higher total phenolic content than the water raw extracts with the only exception of iceberg raw water extract, which shows a higher phenolic content than the corresponding methanol raw extract (**Table 1**). Chicory byproducts showed a 1.3-fold higher phenolic content when the extraction was carried out with the methanol protocol. With regard to iceberg lettuce byproducts, the water raw extract showed a phenolic content 1.8 times higher than methanol raw extracts (**Table 1**). The phenolic content increased in the purified extracts mainly in the iceberg lettuce byproducts, reaching an 18.5-fold increase when the purified methanol extracts were compared to the raw methanol extracts and a 10.6-fold increase when the purified water extracts were compared to the raw water extracts (**Table 1**). Otherwise, romaine lettuce byproducts showed the lowest values with only 1.4- and 1.1-fold increases when the purified methanol extracts were compared to the raw methanol extracts and the purified water extracts were compared to the raw water extracts, respectively.

Hydroxycinnamic acid derivatives are the main compounds present in the lettuce byproduct extracts with an overall
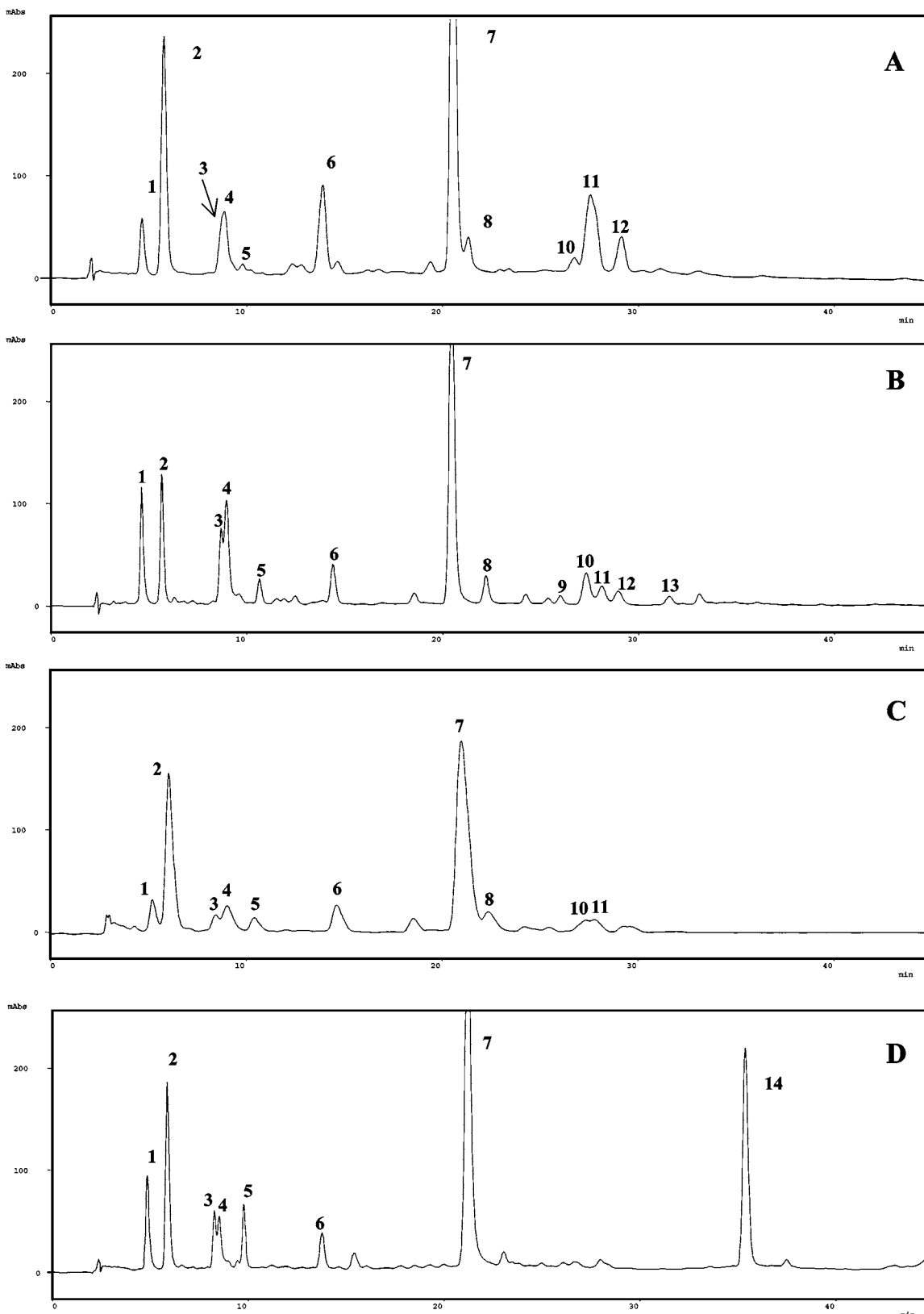
Figure 1. HPLC profiles of lettuce and chicory byproduct water raw extracts: **(A)** baby; **(B)** romaine; **(C)** iceberg; **(D)** escarole. Peak identifications: (1) neochlorogenic acid; (2) caffeoyltartaric acid; (3) crytochlorogenic acid; (4) chlorogenic acid; (5) caffeic acid derivative; (6) isochlorogenic acid; (7) chicoric acid; (8) caffeic acid derivative; (9) isochlorogenic acid; (10) luteolin 7-*O*-glucuronide; (11) quercetin 3-*O*-glucuronide; (12) quercetin 3-*O*-glucoside; (13) quercetin 3-*O*-(6-*O*-malonyl)glucoside; (14) kaempferol 3-*O*-glucoside. Chromatograms were recorded at 335 nm.

percentage between 85.6% (raw water extracts) and 72.5% (raw methanol extracts), whereas in the case of the chicory byproducts these proportions decrease to 54.8 and 50.51%, respectively. With regard to the purified extracts, this percentage decreases

considerably, even reaching 0% in the case of the baby lettuce byproduct extracts. However, iceberg lettuce extracts show a rare behavior with high values reaching 50% in the case of the water extract and 30% in the methanol extract. This resin is
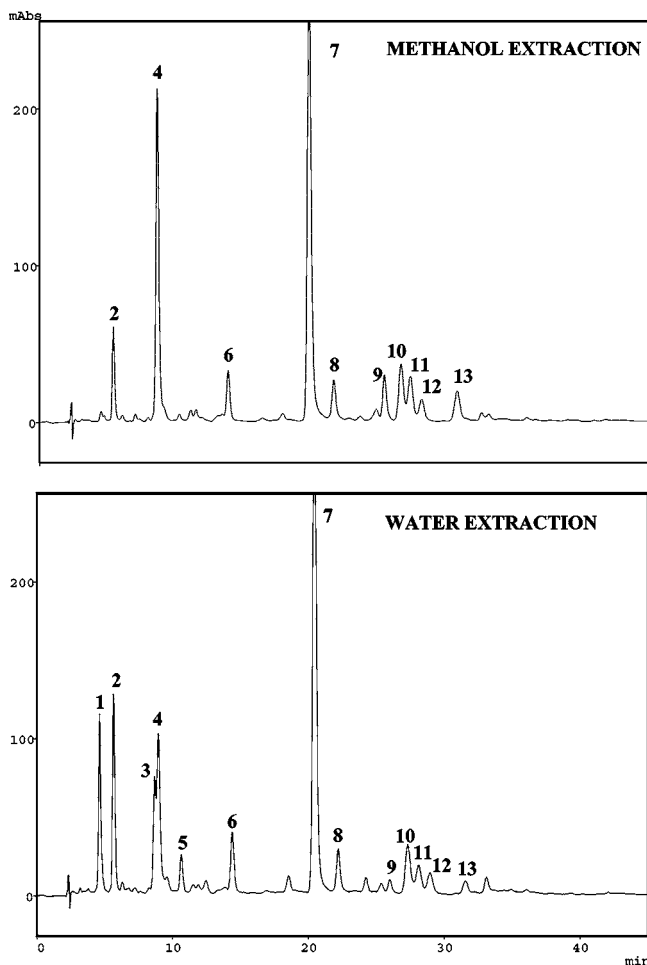
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Figure 2. Comparison of romaine byproduct water and methanol extracts. For peak assignments, see **Figure 1**. Chromatograms were recorded at 335 nm.

normally used to adsorb lipophilic compounds from aqueous solutions, as, for example, flavonoids; nevertheless, the specific sample conditions such as pH and matrix complex could affect the adsorption of other compounds such as phenolic acids (*19*). In this context, Schieber et al. (*19*) obtained 12% of phenolic acids in purified Amberlite XAD-16 HP extracts from apple pomace. This percentage is quite higher than that of our purified extracts, and only those extracted from iceberg byproducts were higher than those obtained from apple pomace.

Other byproducts have been considered to be a good source of phenolics compounds (*7, 8, 21, 22*). The lettuce and chicory byproducts contain important amounts of phenolic compounds with an overall phenolic yield of ∼8 g/kg of dry weight; baby lettuce and chicory byproducts being those that present higher phenolic yields (15 and 8 g/kg of dw, respectively). This overall phenolic yield (8 g/kg of dw) is ∼2-fold lower than those obtained for both artichoke (18 g/kg of dw) (*7*) and cauliflower byproducts (17 g/kg of dw) (*8*). Otherwise, this is quite larger than that reported from grape marc (1 g/kg of dw) (*21*) and similar to the apple pomace (7.24 g/kg of dw) (*22*).

For the caffeic acid derivatives, it has been reported that the lettuce content ranged from 182 to 381 mg/kg of fw in cultivars grown in the field and from 40 to 108 mg/kg of fw in greenhouse-grown plants (*23*), whereas lettuce (including chicory) byproducts showed an overall amount of 550 mg/kg of fw. These differences could be due to the fact that the byproducts are mainly composed by external portions, and these are more exposed to the UV radiation and accumulate more

polyphenols than the internal parts. Other factors that could affect the polyphenol composition are the agronomic practices, differences between varieties, etc.

Dupont et al. (*13*) quantified flavonoids in iceberg lettuce and escarole chicory, obtaining values of around 0.3 and 110 mg/kg of fw, respectively. These contents are quite lower than those obtained in these lettuce and chicory byproducts (**Table 1**). However, the flavonoid content of red lettuce cv. Lollo Rosso (*12*) was quite higher than those found in the present work in the green lettuce byproducts.

Hollman and Arts (*24*) reviewed the dietary intake of flavonols and reported that the main foods providing flavonols are tea (36 mg/L), onion (347 mg/kg of fw), apples (36 mg/kg of fw), and red wine (8.3 mg/L). Lettuce (including chicory) byproducts with a mean value of ∼200 mg/kg of fw would be an important source of these compounds.

Antioxidant Capacity. Three in vitro antioxidant assays were approached as a routine way to assess the potential antioxidant capacity of extracts from lettuce and chicory byproducts. Further extrapolation to in vivo systems requires further research (bioavailability, structure−activity relationship, etc.) far from the aim of the present study.

Free Radical Scavenging Capacity (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ Assays). The DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays were carried out in different solutions, methanol and water, respectively (see Materials and Methods). Therefore, both DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays are useful to evaluate the free radical scavenging of water- and non-water-soluble compounds.

The extracts for lettuce and chicory byproducts showed a high capacity for scavenging both DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ (**Tables 2 and 3**). The results obtained with the DPPH$^{\bullet}$ assay showed that the raw water extracts have higher antioxidant capacity (1.2-fold) than the methanol extracts. Baby lettuce and chicory byproduct extracts were those showing the highest activity, followed by romaine and finally iceberg extracts. Similar results were obtained using the ABTS$^{\bullet+}$ assay.

With regard to the purified extracts, water extractions gave a 1.25-fold higher antioxidant content (DPPH$^{\bullet}$ assay) than the methanol extractions. Both water and methanol extracts of chicory showed the highest antioxidant content followed by baby lettuce water extract (2-fold lower water extract and 1.45-fold lower methanol extract), iceberg lettuce, and finally romaine lettuce extract. The results obtained with the ABTS$^{\bullet+}$ assay were similar to those obtained with the DPPH$^{\bullet}$ assay. The chicory extracts showed the highest antioxidant content followed by baby lettuce extracts (1.4-fold lower, water extracts), although the antioxidant content of the methanol extract was very similar to that of the baby lettuce methanol extract. The romaine lettuce extracts were those with the lowest antioxidant contents of 80 and 61 mg of TEAC/g of freeze-dried extract, water and methanol extracts, respectively.

Artichoke and cauliflower byproducts have been proposed as important sources of antioxidant phenolics. Thus, the mean value of the DPPH assay of artichoke (*7*) was quite higher than both lettuce and chicory byproduct raw extracts, although 2-fold lower than that of lettuce purified extracts and similar to those obtained from chicory purified extracts. However, the mean value of cauliflower byproduct raw extracts was very similar to that of lettuce byproduct raw extracts and 1.2 lower than chicory byproduct raw extracts. The purified extracts of both lettuce and chicory byproducts showed values 2.6- and 6-fold higher than those obtained by purified extracts from cauliflower byproducts (*8*).

Table 2. Free Radical Scavenging Activity (DPPH• and ABTS•+ Assays) and FRAP Values of Raw Lettuce Byproduct Extracts

	DPPH		ABTS		FRAP	
	water	MEOH	water	MEOH	water	MEOH
baby						
antioxidant yield[a]	1.05 ± 0.04	0.93 ± 0.07	1.30 ± 0.06	1.38 ± 0.10	1.51 ± 0.02	1.40 ± 0.10
antioxidant content[b]	38.8 ± 1.80	30.3 ± 2.30	48.2 ± 2.40	44.5 ± 3.50	55.6 ± 1.00	45.2 ± 3.00
romaine						
antioxidant yield[a]	0.79 ± 0.05	0.31 ± 0.02	0.90 ± 0.03	0.42 ± 0.02	0.95 ± 0.02	0.87 ± 0.03
antioxidant content[b]	31.2 ± 2.00	24.3 ± 1.40	35.3 ± 1.30	32.6 ± 1.90	37.1 ± 0.90	34.1 ± 1.10
iceberg						
antioxidant yield[a]	0.51 ± 0.01	0.43 ± 0.01	0.53 ± 0.01	0.39 ± 0.01	0.62 ± 0.04	0.40 ± 0.01
antioxidant content[b]	24.3 ± 0.30	18.9 ± 0.50	25.5 ± 0.70	17.1 ± 0.40	29.8 ± 2.00	18.6 ± 0.40
chicory						
antioxidant yield[a]	0.66 ± 0.02	0.53 ± 0.02	0.82 ± 0.03	0.62 ± 0.02	0.84 ± 0.03	0.76 ± 0.02
antioxidant content[b]	37.1 ± 1.00	36.0 ± 1.30	45.6 ± 2.09	42.9 ± 1.73	46.7 ± 2.50	42.7 ± 1.40

[a] Expressed as g of TEAC/kg of fresh byproducts. [b] Expressed as mg of TEAC/g of freeze-dried extract.

Table 3. Free Radical Scavenging Activity (DPPH• and ABTS•+Assays) and FRAP Values of Purified Lettuce Byproduct Extracts

	DPPH		ABTS		FRAP	
	water	MEOH	water	MEOH	water	MEOH
baby						
antioxidant yield[a]	0.11 ± 0.004	0.13 ± 0.010	0.14 ± 0.005	0.18 ± 0.011	0.12 ± 0.002	0.15 ± 0.008
antioxidant content[b]	162.7 ± 5.90	137.6 ± 8.3	196.8 ± 7.9	178.8 ± 11.7	179.16 ± 2.3	149.85 ± 8.4
romaine						
antioxidant yield[a]	0.05 ± 0.002	0.05 ± 0.003	0.06 ± 0.002	0.06 ± 0.003	0.06 ± 0.002	0.06 ± 0.003
antioxidant content[b]	60.43 ± 2.6	50.02 ± 3.4	80.5 ± 2.9	61.11 ± 3.4	71.61 ± 1.9	57.43 ± 3.7
iceberg						
antioxidant yield[a]	0.05 ± 0.004	0.07 ± 0.005	0.06 ± 0.004	0.07 ± 0.005	0.05 ± 0.002	0.06 ± 0.004
antioxidant content[b]	130.72 ± 10.2	118.45 ± 9.3	150.05 ± 10.2	125.52 ± 8.4	134.17 ± 5.7	99.98 ± 7.6
chicory						
antioxidant yield[a]	0.24 ± 0.010	0.16 ± 0.01	0.22 ± 0.012	0.15 ± 0.010	0.25 ± 0.016	0.17 ± 0.008
antioxidant content[b]	308.00 ± 20.4	200.36 ± 14.5	280.26 ± 15.4	185.16 ± 13.7	311.98 ± 20.6	210.86 ± 10.34

[a] Expressed as g of TEAC/kg of fresh byproducts. [b] Expressed as mg of TEAC/g of freeze-dried extract.

For the ABTS•+ assay, the mean value of lettuce and chicory raw extracts was lower than those of both artichoke and cauliflower byproduct raw extracts. Nevertheless, purified extracts from chicory byproducts showed a higher antioxidant content than cauliflower byproduct purified extracts.

FRAP Assay. The capacity to reduce TPTZ−Fe^{3+} complex to TPTZ−Fe^{2+} of lettuce and chicory byproducts for both raw and purified extracts varied markedly (**Tables 2** and **3**). The results showed that water extraction provides the extracts (both raw and purified) with a higher antioxidant content than those obtained with methanol extraction, with mean values around 1.2- and 1.3-fold higher when compared the raw and purified extracts, respectively.

For the raw extracts, those obtained from baby lettuce were the highest, with a values of ∼50 mg of TEAC/g of freeze-dried extract followed by chicory and romaine, iceberg being the lowest with values of ∼20 mg of TEAC/g of freeze-dried extract (**Table 2**). In this way, purified extracts from chicory byproducts were the highest, followed by baby and iceberg, romaine being the lowest with a values between 71 mg of TEAC/g of freeze-dried extract (water extract) and 57 mg of TEAC/g of freeze-dried extract (methanol extract) (**Table 3**).

The FRAP assay has been used as a tool to evaluate the antioxidant capacity of cauliflower byproducts (*8*). In this context, both baby lettuce and chicory byproducts provide the raw and purified extracts that showed higher antioxidant contents than cauliflower byproducts. However, the Romaine and iceberg byproducts provide extracts with lower levels of antioxidant content.

Ou et al. (*25*) recently evaluated the antioxidant capacity as FRAP values of 927 freeze-dried sample vegetables including white cabbages, broccoli, carrots, white and purple onions, and spinach as well as red and green peppers. Compared with these results, lettuce (including chicory) byproducts show a value between those of broccoli and tomato, being higher than those of the white and purple onions and lower than those of the spinach and peppers.

Correlation between Phenolic Content and Antioxidant Capacity. As a general rule the antioxidant capacity has been positively correlated with phenolic content. The different phenolic contents of both water and methanol extracts were positively correlated with the antioxidant capacity (**Figures 3** and **4**). With regard to raw extracts the R^2 values of the water extracts were higher than those of the methanol extracts. The values were 0.98 (water extracts, DPPH assay) and 0.93 (methanol extracts, DPPH assay), 0.99 (water extracts, ABTS assay) and 0.96 (methanol extracts, DPPH assay), and 0.93 (water extracts, FRAP assay) and 0.92 (methanol extracts, FRAP assay). The water purified extracts showed also high values of R^2. Thus, the values were 0.97 (water extracts, DPPH assay) and 0.96 (methanol extracts, DPPH assay), 0.99 (water extracts, ABTS assay) and 0.95 (methanol extracts, ABTS assay), and 0.98 (water extracts, FRAP assay) and 0.93 (methanol extracts, FRAP assay). These values are similar to those obtained by Kang and Saltveit (*26*) for two different types of lettuce.

It is of note that some antioxidant capacity was detected in the absence of phenolics (**Figures 3** and **4**). In the raw extracts ∼20 mg of TEAC as well as ∼28 mg of TEAC in the purified extracts was due to other nonphenolic compounds. Previous reports indicated that substances such as soluble fiber (*27*) as well as sesquiterpene lactones such as lactucin and their derivatives (*28*) could be responsible for such activity.

Antioxidant Polyphenols from Lettuce Byproducts

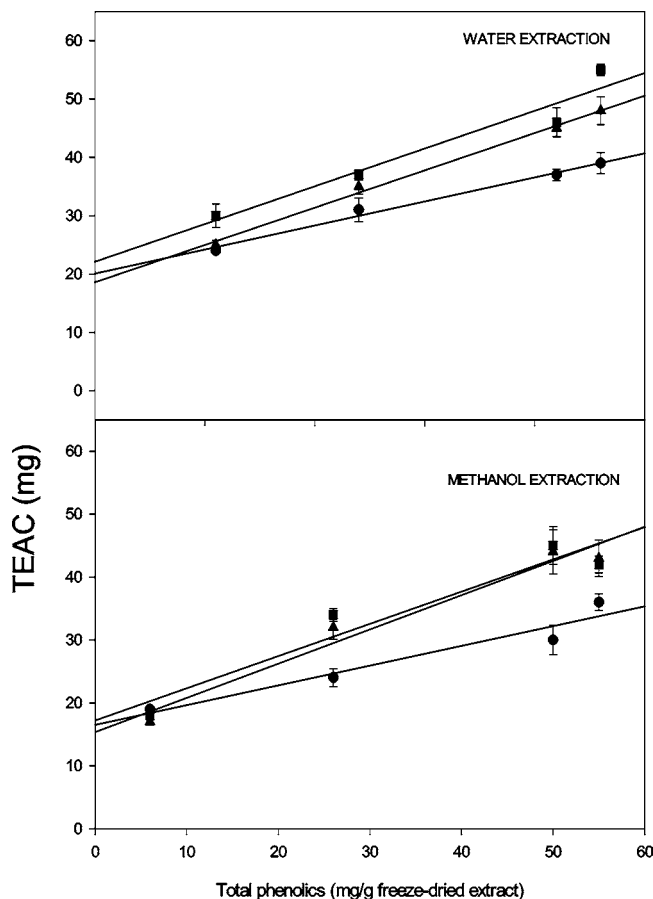*J. Agric. Food Chem.*, Vol. 52, No. 16, 2004 **5115**

Figure 3. Dependence of antioxidant activity (TEAC) (DPPH•, ABTS, and FRAP assays) on phenolic content of lettuce byproduct raw extracts: (●) DPPH values; (▲) ABTS values; (■) FRAP values. Data were taken from **Tables 1–3**. Vertical bars indicate standard deviations.

Figure 4. Dependence of antioxidant activity (TEAC) (DPPH•, ABTS, and FRAP assays) on phenolic content of lettuce byproduct purified extracts: (●) DPPH values; (▲) ABTS values; (■) FRAP values. Data were taken from **Tables 1–3**. Vertical bars indicate standard deviations.

From the industrial point of view both water and methanol protocols are easily scalable at industrial production level; nevertheless, it is necessary to take into account some factors. These factors are the price and availability of byproducts and the extraction and purification methods. Nowadays the price of these byproducts is negligible and it is possible to obtain it during the whole year (information provide by the industries). With regard to the extraction methods, the price of solvent and special management must be considered. Thus, the water extraction seems to be better than the methanol extraction protocol as the water is a nonpolluting solvent and it does not need special management, that is, special tanks for storage, and obviously it is quite cheaper than methanol. About the purification, the high price of the resin is another important factor; however, further studies related to its reuse and cleaning would contribute to minimize the cost.

The results obtained indicate that lettuce and chicory byproducts are an interesting and cheap source of antioxidant phenolics, especially when the huge amount of byproducts that are produced by both the fresh and fresh-cut industries is considered. These byproducts could provide extracts with antioxidant phenolics that could be used as natural antioxidants or to funcionalize foods as has been recently reported by Larrosa et al. (*29*). Obviously, before these byproducts are incorporated as dietary complements or as natural food antioxidants, it is necessary to carry out further studies about their toxicity (i.e., possible residual presence of pesticides), in vivo activity, and bioavailability.
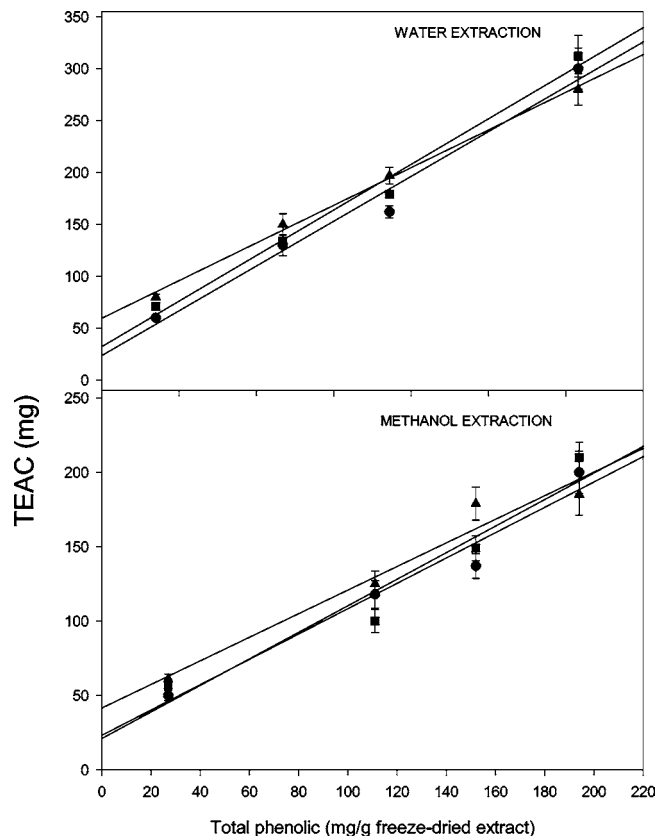
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