JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Valorization of Cauliflower (*Brassica oleracea* L. var. *botrytis*) By-Products as a Source of Antioxidant Phenolics

RAFAEL LLORACH, JUAN CARLOS ESPÍN, FRANCISCO A. TOMÁS-BARBERÁN, AND FEDERICO FERRERES*

Research Group on Quality, Safety and Bioactivity of Plan Foods, Department of Food Science and Technology, CEBAS-CSIC, P. O. Box 4195, Murcia 30080, Spain

The present study reports the development of two extraction protocols, with potential industrial applicability, to valorize cauliflower (*Brassica oleracea* L. var. *botrytis*) byproducts as a source of antioxidant phenolics. In addition, the nonionic polystyrene resin Amberlite XAD-2 was used to obtain purified extracts. The extract yield, phenolic content, phenolic yield, and correlation between the antioxidant activity and the phenolic content were studied. The water and ethanol protocols yield a phenolic content of 33.8 mg/g freeze-dried extract and 62.1 mg/g freeze-dried extract, respectively. This percentage increased considerably when the extracts were purified using Amberlite XAD-2 yielding a phenolic content of 186 mg/g freeze-dried extract (water extract) and 311.1 mg/g freeze-dried extract (ethanol extract). Cauliflower byproduct extracts showed significant free radical scavenging activity (vs both DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals), ferric reducing ability (FRAP assay), and capacity to inhibit lipid peroxidation (ferric thiocyanate assay). In addition, the antioxidant activity was linearly correlated with the phenolics content. The results obtained indicate that the cauliflower byproducts are a cheap source of antioxidant phenolics very interesting from both the industrial point of view and the possible usefulness as ingredients to functionalize foodstuffs.

KEYWORDS: Cauliflower; *Brassica oleracea* var. *botrytis*; byproducts valorization; phenolics; antioxidant; ABTS; DPPH; FRAP; ferric thiocyanate; extraction protocol

INTRODUCTION

Cauliflower and broccoli are the main *Brassica* crops with a world production about 14 000 000 tons p.a. in which 71% of these products is produced by China and India (*1*). Spain is the fifth producer in the world with 374 000 tons (about 3% of world production). During the processing of these vegetables, an important amount of byproducts is produced. Regarding the byproduct proportion, leaves constitute about 50% of the total; the rest is mainly stem. These residues are responsible for important environmental problems in the industries (*2*) and diminishing their environmental impact has been the subject of an increasing concern in recent years.

Epidemiological studies have stressed the capacity of *Brassica* species to prevent cardiovascular diseases as well as to exert activity against some type of cancers (*3*). The substances that seem to be responsible for these properties are the glucosinolates and their derived products (*4*, *5*) as well as the flavonoids and other phenolic compounds (*6*, *7*). The role of flavonoids in the prevention of these diseases is mainly related to the prevention of the low-density lipoproteins oxidation (*8*, *9*) through a scavenging activity against peroxyl and hydroxyl radicals (*9*). The antioxidant activity of flavonoids, such as flavonols (present in *Brassica* species), has been reported to be greater than that of vitamins C and E (*10*).

In general, byproducts from handling and commercialization of vegetables have been traditionally valorized as animal feedstuff (*11*, *12*), fiber production (*13*−*15*), and fuel production (*16*). In the last years, a number of studies have proposed some vegetable byproducts as a source of natural antioxidants in order to valorize these wastes (*17*−*23*).

Research on *Brassica* vegetables has been focused on the edible parts. However, scarce information is available regarding their corresponding byproducts. The aim of this work is to propose a way to valorize cauliflower 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 activity and phenolic content will be studied.

MATERIAL AND METHODS

Reagents. Ammonium thiocyanate, 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$), chlorogenic acid (5-*O*-caffeoylquinic acid), ferrous chloride, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), linoleic acid, manganese dioxide (MnO_2), 3-*tert*-butyl-4-hydroxyanisole (BHA), ferric chloride, 2,4,6-tripyridyl-*s*-triazine (TPTZ), rutin, and

* To whom correspondence should be addressed. Fax: 34-968-396213. E-mail: federico@cebas.csic.es.

10.1021/jf021056a CCC: $25.00
Published on Web 03/15/2003

sinapic acid 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., U.S.A.) ultrapure water was used throughout this research.

Plant Material. The typical cauliflower byproducts (*Brassica oleracea* L. var. *botrytis*) mainly consist of leaves and, in less amount, stems. Both the basal and the middle part of the midrib were removed, since these parts were used to analyze its content in fiber. The byproduct was supplied by Agrosol Cooperative (Lorca, Murcia, Spain). After the extraction, fresh cauliflower byproducts were chopped with a sharp stainless steel knife in small pieces to improv the extraction.

Extraction Protocols. *Raw Extract.* Two different extracting solvents (ethanol and water) were used. One kilogram (fresh weight, fw) of cauliflower byproducts was extracted with boiling solvent (1:4 w/v) (ethanol or water) for 1 h. The plant material was then pressed, and the resultant liquids were pooled with either ethanol or water extracts. The extracts were cooled at room temperature and then filtered through filter paper Whatman No. 1 (Whatman, Maidstone, England). In the ethanol extract, the solvent was removed with a rotary evaporator and 200 mL of water was added. In 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. These freeze-dried extracts are hereafter termed CLW (cauliflower water extract) and CLEt (cauliflower ethanol extract).

Purified Extracts Using Amberlite XAD-2. A procedure to recovery flavonoids from the water solutions using the nonionic polystyrene resin (Amberlite XAD-2) has been used (*24*). This procedure can recover more than 95% of the phenolics present in aqueous solutions (*25*). New raw extracts were obtained as described above. The ethanol extract was added with the same volume of water and concentrated with a rotary evaporator (40 °C) until all of the ethanol 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, U.S.A.) (column of 50 cm × 4 cm) as described by Ferreres et al. (*24*). A 10 L volume of water was used to wash out the salts and sugars before the phenolics compounds were collected. Then, 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 eluates were freeze-dried at −50 °C. The final freeze-dried extracts are hereafter termed ACLW (Amberlite CLW) and ACLEt (Amberlite CLEt). The term "extract yield" (*21*) was defined as the amount of freeze-dried extract (g) obtained from 1 kg of fresh weight byproducts (extract (g)/kg fresh byproduct).

High-Performance Liquid Chromatography (HPLC) Analysis. Ten milligrams of each extract was dissolved in 1 mL of 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 pump model L-6200 (Merck Hitachi) and Shimadzu SPD-MSA photodiode array UV−vis detector. Separations were achieved on a Licrocart column (Merck) (RP-18, 25 cm × 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 min, 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 Tomás-Barberán et al. (*26*), sinapic acid derivatives as reported by Price et al. (*27*), and flavonoids as described by Wilson et al. (*28*). Caffeic acid derivatives were quantified by comparison with external standards as chlorogenic acid (5-*O*-caffeoylquinic acid), sinapic acid derivatives as sinapic acid, and the flavonols as rutin equivalent, respectively.

The term "phenolic yield" (*21*) was defined as the amount of total phenolic compounds (g) (caffeic acid derivatives, sinapic acid derivatives, and flavonoids) obtained from 1 kg of fw byproducts. The term "phenolic content" (*21*) was defined as the amount of total phenolic compounds (mg) obtained from 1 g of freeze-dried extract. The results presented are the mean of three experiments ± the standard error.

Antioxidant Activity. The free radical scavenging activity (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays), ferric reducing ability (ferric reducing antioxidant power (FRAP) assay), and capacity to inhibit lipid peroxidation (ferric thiocyanate (FTC) assay) of the extracts were compared to those of Trolox (DPPH$^{\bullet}$, ABTS$^{\bullet+}$, FRAP, and FTC assays) and BHA (FTC assay).

DPPH$^{\bullet}$ Assay. The free radical scavenging activity using the free radical DPPH$^{\bullet}$ (*29*) 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 (*30*). Cauliflower byproducts extracts (10 mg) were dissolved in 1 mL of MeOH:water (80:20 v/v). The reaction started by adding 20 μL of the corresponding sample to the cuvette containing 80 μM (methanol solution) (980 μL) of the free radical (DPPH$^{\bullet}$). The final volume of the assay was 1 mL. The reaction was followed with a UV-1603 Shimadzu spectrophotometer (Tokyo, Japan).

ABTS$^{\bullet+}$ 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 32 μM (water solution) (995 μL) of the free radical (ABTS$^{\bullet+}$). The radical was chemically generated with MnO_2 as described by Espín and Wichers (*31*). The experiments were always performed on freshly made up solutions. The final volume of the assay was 1 mL. The disappearance of ABTS$^{\bullet+}$ 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 Benzie and Strain (*32*) 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 ($FeCl_3$ $6H_2O$). This solution was used as a blank. A 950 μL volume of warmed (37 °C) FRAP solution was mixed with 50 μL of freshly disolved extract (10 mg/mL of water). The ferric reducing ability of byproducts 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 = (SD/mean) × 100) was always less than 10%. In addition, calibration curves were made for each assay using Trolox as the standard. The antioxidant activity (DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and FRAP assays) was expressed as Trolox equivalent antioxidant activity (TEAC) following the nomenclature of Rice-Evans and Miller (*33*).

The "antioxidant yield" (AY) (*21*) correlated the Trolox equivalent antioxidant capacity (g of TEAC) in 1 kg of fresh cauliflower byproducts taking into account the extract yield: AY = (g TEAC/g extract) × extract yield.

FTC Assay. The FTC method was carried out according to Larrosa et al. (*34*) to determine the inhibition of lipid peroxidation. The assay mixture consisted of linoleic acid (2.5%) in ethanol (0.25 mL); 50 mM sodium phosphate buffer, pH 7 (1.0 mL); ethanol (0.25 mL); milli-Q water (0.9 mL); and sample (0.1 mL) (cauliflower byproducts extracts and the standards Trolox and BHA) and 1.8 mM AAPH (25 μL) to accelerate the lipid oxidation. The incubation assay amounts (final incubation assay of 2.525 mL) were 1 mg of freeze-dried extract of cauliflower byproducts. Trolox and BHA assay concentrations were 150 and 25 μM, respectively. Linoleic acid peroxidation was determined by measuring hydroperoxide accumulation as the increase in absorbance at 500 nm in the above spectrophotometer. Peroxidation inhibition (%) was expressed as 100 − (A sample/A control × 100). The ratio A sample/A control was calculated after 10 h of reaction. Oxidation (100%) was taken as the maximum absorbance reached by control sample (without antioxidant) after 10 h of reaction. The FTC assay was repeated three times. The coefficient of variation was always <10%.

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

RESULTS AND DISCUSSION

Extract Yield and Phenolic Content of Cauliflower Byproducts. There was a significant difference in the extract yield

Table 1. Yield and Phenolic Content of Extracts from Cauliflower by-products

		phenolic content				
		caffeic acid derivatives	sinapic acid derivatives	total lavonoids	total	extract yield[a]
CLW						53.3
	phenolic content[b]	1.4 ± 0.10	0.46 ± 0.020	32.0 ± 0.20	33.8 ± 0.32	
	phenolic yield[c]	0.07 ± 0.01	0.024 ± 0.010	1.7 ± 0.11	1.8 ± 0.12	
CLEt						25.6
	phenolic content[b]	2.1 ± 0.10	1.45 ± 0.200	58.7 ± 0.20	62.1 ± 0.30	
	phenolic yield[c]	0.05 ± 0.01	0.037 ± 0.010	1.5 ± 0.01	1.6 ± 0.01	
ACLW						6.4
	phenolic content[b]	N.D.[d]	1.74 ± 0.270	185.0 ± 5.80	186.8 ± 6.10	
	phenolic yield[c]	N.D.	0.011 ± 0.001	1.2 ± 0.10	1.2 ± 0.10	
ACLEt						3.6
	phenolic content[b]	N.D.	6.10 ± 0.014	305.0 ± 0.28	311.1 ± 5.80	
	phenolic yield[c]	N.D.	0.022 ± 0.001	1.1 ± 0.01	1.1 ± 0.01	

[a] Freeze-dried extract g/kg byproducts f.w. [b] Total phenolic compounds mg/g freeze-dried extract. [c] Total phenolic compounds g/kg byproducts f.w.

obtained by the different extraction protocols (**Table 1**). The water extraction protocol yielded an extract yield value 2-fold higher (around 53 g/kg fresh weight) than the ethanol protocol (around 26 g/kg fw). This could be due to the extraction of salts and other water soluble compounds that were not extracted with ethanol.

The nonionic resin Amberlite XAD-2 has been previously used to recover flavonoids from plant extracts with the removal of other water soluble compounds (*24*). The extract yield obtained after Amberlite purification was around 8-folds lower than that of raw extracts (**Table 1**), which results in phenolic-enriched extracts (**Table 1**). After Amberlite XAD-2 purification, the water protocol also rendered the double amount of extract (6.4 g/kg fw) with respect to the ethanol protocol (3.2 g/kg fw).

The HPLC analysis of cauliflower byproduct extracts revealed the presence of both flavonoids and hydroxycinamic acids (caffeic acid and sinapic acid). The HPLC profiles of both ethanol and water extracts are shown in **Figure 1A**−**D**. Different combinations of flavonols such as kaempferol and quercetin with sinapic acid and glucose were the main phenolics compounds present in both ethanol and water extracts. The main compounds identified (**Figure 1A**−**D**) were kaempferol-3-*O*-sophoroside-7-*O*-glucoside (**3**) and its sinapoyl derivative kaempferol-3-*O*-(sinapoylsophoroside)-7-*O*-glucoside (**4**). These compounds have been previously identified in related species such as cabbage (*Brassica oleracea* L.) (*35*) and oilseed rape (*Brassica napus* L.) (*28*). Another minor identified kaempferol derivative was kaempferol-3-*O*-sophoroside (**5**) (**Figure 1A**−**D**), which was also described in leaves of *Brassica napus* (*28*). In addition, the quercetin derivative quercetin-3-*O*-sophoroside-7-*O*-glucoside (**2**) (**Figure 1A**−**D**) was identified. Regarding sinapic acid derivatives, the compounds 1,2-disinapoylgentiobioside (**6**) and 1,2,2′-trisinapoylgentiobiose were identified mainly in ethanol extracts (**7**) (**Figure 1A**,**C**). Furthermore, neochlorogenic acid (3-*O*-caffeoylquinic acid) (**1**) was the main identified caffeic acid derivative in raw extracts (**Figure 1A**,**B**), but these compounds were not retained by Amberlite XAD-2 due to its polarity (**Figure 1C**,**D**). Other minor phenolic compounds were also detected but not identified (**Figure 1A**−**D**).

Total phenolic compounds were quantified (see Materials and Methods) in the different extracts (**Table 1**). These results showed that the phenolic content of water extract was 33.8 mg/g and the ethanol extract contained 62.1 mg/g, whereas this content increased considerably in the extracts purified by Amberlite XAD-2 with 186.8 mg/g (water extract) and 311.1 mg/g (ethanol extract). In both methods, the ethanol extracts presented approximately double the phenolic content than the water extracts. However, taking into account the different extract yields, the phenolic content extracted from fresh byproducts (phenolic yield) was very similar (**Table 1**). This meant that both water and ethanol extracts showed approximately the same efficiency.

The edible part of cauliflower is rather poor in phenolic compounds and only hydroxycinnamic acid such as caffeic, sinapic, and ferulic acids was identified and quantified. The overall concentration of these compounds (0.18 g/kg fw) (*36*) was 2-fold higher than that found in the cauliflower byproducts (0.094 g/kg fw, **Table 1**). However, the flavonoids concentration in cauliflower byproducts was much higher than that found in the edible parts where only trace amounts were detected (*37*). Furthermore, the cauliflower byproducts presented 3-fold higher flavonols content than other *Brassica* species (*37*).

Other byproducts have been considered as a good source of phenolics compounds (*2*, *20*, *38*). In this way, cauliflower byproducts contain an interesting amount of phenolic compounds, with an overall phenolic yield around 17 g/kg dw (cauliflower byproducts contained approximately a 90% water content). This level is quite higher than that reported in grape marc (1 g/kg dw) (*2*), approximately 2-fold higher than the apple pomace (7.24 g/kg dw) (*38*), and 2-fold lower than the grape pomace (40 g/kg dw) (*20*).

Antioxidant Activity. Four in vitro antioxidant assays were approached as a routine way to assess the potential antioxidant capacity of extracts from cauliflower byproducts. Further extrapolation to in vivo systems requires other more deep 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 nonwater soluble compounds.

The extracts from cauliflower byproducts showed a good scavenging activity against both DPPH$^\bullet$ and ABTS$^{\bullet+}$ radicals (**Tables 2** and **3**). The free radical scavenging capacity of CLW and CLEt extracts was 2.3- and 2.2-folds higher, respectively, against ABTS$^{\bullet+}$ than that against DPPH$^\bullet$. Regarding the Amberlite XAD-2 purified extracts, the activity of ACLW and ACLEt extracts was 5- and 5.3-fold higher against ABTS$^{\bullet+}$ than against DPPH$^\bullet$.

Antiradical activity against both DPPH$^\bullet$ and ABTS$^{\bullet+}$ was linearly correlated with the total phenolics amount (**Figure 2**).

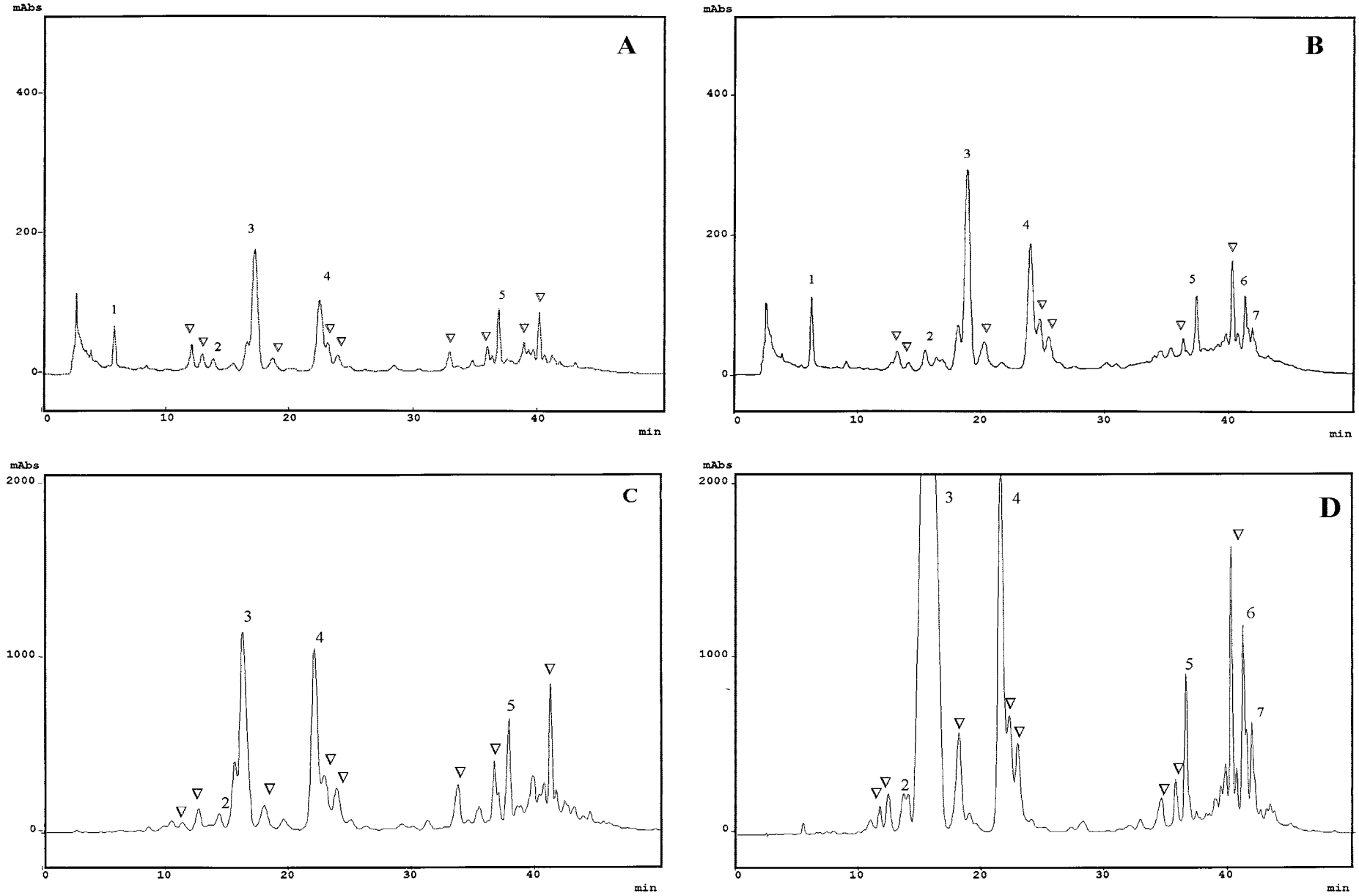

Figure 1. HPLC profiles of cauliflower byproduct extracts. (**A**) Raw water extract, (**B**) raw ethanol extract, (**C**) purified water extract, and (**D**) purified ethanol extract. Peak identifications: (1) neochlorogenic acid; (2) quercetin-3-*O*-sophoroside-7-*O*-glucoside; (3) kaempferol-3-*O*-sophoroside-7-*O*-glucoside; (4) kaempferol-3-*O*-(2″-sinapoylsophoroside)-7-*O*-glucoside; (5) kaempferol-3-*O*-sophoroside; (6) 1,2-disinapoylgentiobiose; (7) 1,2,2′-trisinapoylgentiobiose; (▽) unidentified flavonoids.

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

	DPPH•		ABTS•+		FRAP	
	CLW	CLEt	CLW	CLEt	CLW	CLEt
antioxidant yield (g TEAC/kg fresh byproducts)	1.40	0.94	3.20	2.05	1.55	1.30
mg TEAC/ g freeze-dried extract	26.30	36.30	60.07	80.10	29.19	51.02

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

	DPPH•		ABTS•+		FRAP	
	ACLW	ACLEt	ACLW	ACLEt	ACLW	ACLEt
antioxidant yield (g TEAC/kg fresh byproducts)	0.23	0.16	1.15	0.86	0.85	0.60
mg TEAC/ g freeze-dried extract	36.30	45.05	180.20	240.30	133.70	163.70

This linear dependence was statistically significant for both DPPH• and ABTS•+ ($R = 0.99$, $P < 0.01$; $R = 0.99$, $P < 0.01$). The highest antiradical activity against both DPPH• and ABTS•+ was found in the purified ethanol extract (ACLEt).

FRAP Assay. The cauliflower byproducts showed a relevant antioxidant capacity, estimated from their ability to reduce the TPTZ−Fe^{III} complex to TPTZ−Fe^{II} (**Tables 2** and **3**). It is difficult to compare these results with other byproducts because this assay has not been previously approached to evaluate their antioxidant activity. The mean FRAP values of cauliflower byproducts were 1.4-fold lower than cauliflower edible portions (*39*, *40*) and similar than those of broccoli and white cabbage (*39*). On the other hand, it is well-known that tea (*41*) and red wine (*42*) have a strong capacity to reduce Fe^{III} to Fe^{II}. In this way, 16 g (dw) of cauliflower byproducts can provide the same antioxidant capacity than one cup of tea of normal strength (1−2%) or one glass of red wine. In addition, FRAP values were also linearly correlated with total phenolics content (**Figure 2**) ($R = 0.96$, $P < 0.01$).

Inhibition of Linoleic Acid Oxidation. Extracts from cauliflower byproducts showed a good capacity to inhibit linoleic acid peroxidation when the FTC assay was performed (**Figure 3**). AClEt showed the highest activity to inhibit lipid peroxidation under these assay conditions. The activity of 1 mg of AClEt (31% after 10 h of reaction) was equivalent to that of 150 μM Trolox and 1.4-fold higher than that of 25 μM BHA. However, the capacity to inhibit lipid peroxidation decreased to 11 and 6% when ACLW and CLEt were assayed, respectively, reaching 0% in CLW after 8 h of reaction (**Figure 3**). The capacity to inhibit linoleic acid peroxidation (%) was also linearly correlated with total phenolics ($R = 0.97$, $P < 0.01$) (**Figure 4**).

On the other hand, the cauliflower byproducts showed lower FTC values than artichoke byproducts, which showed between 80 and 38% of capacity to inhibit linoleic acid peroxidation (*21*) in the same assay conditions.

It is of note that some antioxidant activity (DPPH•, ABTS•+, and FRAP assays) was detected in the absence of phenolics (intercept with the ordinate axe; **Figure 2A−C**). This meant that 20−48 mg of TEAC, depending on the assay method, was due to other nonphenolic compounds. Although the present study did not investigate which nonphenolic compounds were involved in this antioxidant activity, previous reports indicated that substances such as soluble fiber (*44*), glucosinolates, and their breakdown products (*44*) could be responsible for such activity. However, this antioxidant activity due to nonphenolic compounds was not detected in the FTC assay when cauliflower byproduct extracts were assayed. In fact, according to **Figure 4**, it seems that lower values of phenolic content than 33 mg/g are not able to prevent lipid peroxidation.

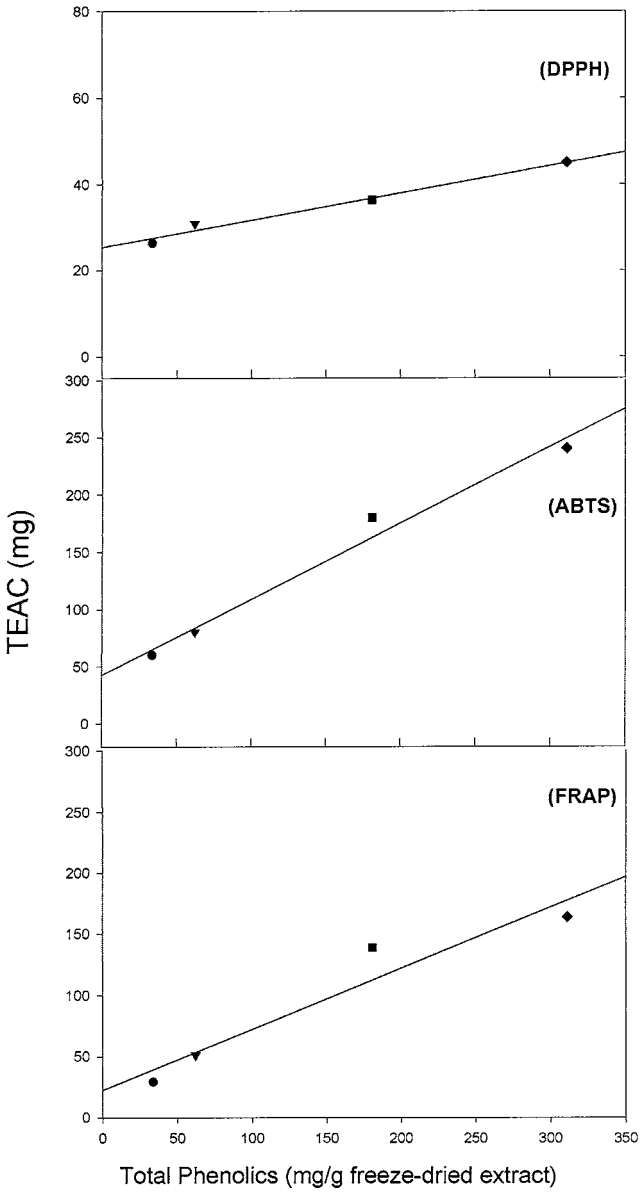

Figure 2. Dependence of antioxidant activity (TEAC) (DPPH, ABTS, and FRAP assays) on phenolic content of cauliflower byproducts extracts. (●) CLW; (▼) CLEt; (■) ACLW; (◆) ACLEt.

To consider a possible industrial application to valorize the cauliflower byproducts, the ACLEt seems to be the best extract due its phenolic content. However, from an industrial point of view, it is necessary to evaluate the total cost of their production. Regarding this cost of production, the main factors to be taken in account are the price of fresh byproducts, as well as the extraction and purification protocols (*34*). The cost of fresh byproducts is currently negligible (information provided by the industry). The factors involved in the extraction protocol are mainly related to the difficulty of the manipulation and the price

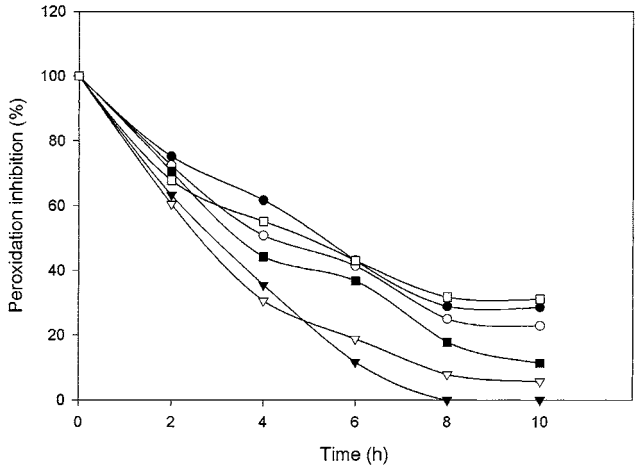

Figure 3. Inhibition of linoleic peroxidation (FTC method). The mean of three separate experiments is shown. The coefficient of variation was always <10%. (▼) CLW; (▽) CLEt; (■) ACLW; (●) ACLEt; (□) trolox; (○) BHA.

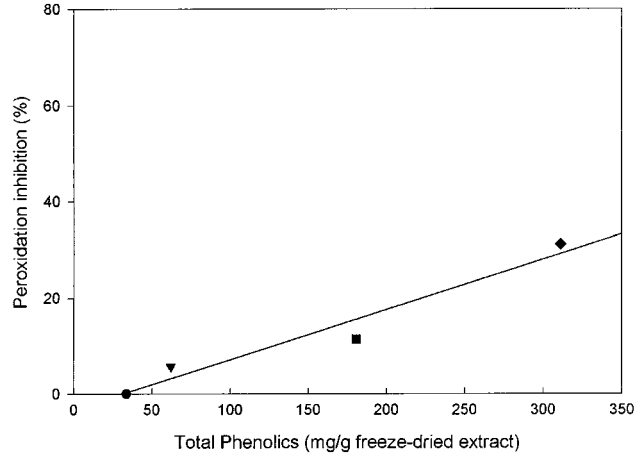

Figure 4. Dependence of peroxidation inhibition (percent) (FTC assay) on phenolic content of cauliflower byproducts extracts. (●) CLW; (▼) CLEt; (■) ACLW; (◆) ACLEt.

of the extraction solvents. According to the European law, ethanol is a product that needs special storage and very careful manipulation since it is a toxic and highly flammable product, and obviously, ethanol is quite more expensive than water. Regarding the purification protocol, the cost of the resin is the most important factor to be taken in account. However, further studies related to its reuse and cleaning would contribute to minimize the cost. In this context, the water extract was revealed to be more feasible since it is cheaper and nontoxic or pollutant.

In summary, this study has shown that cauliflower byproducts are a good and cheap source of antioxidant phenolics, which could be industrially exploited. In addition, resins such as Amberlite XAD-2 could be used to obtain purified (phenolics-enriched) extracts. Obviously, before incorporating cauliflower byproducts as a dietary complement or as a natural food antioxidant, it is necessary to carry out further studies about their toxicity (i.e., possible residual presence of pesticides), in vivo activity, and bioavailability.

LITERATURE CITED

(1) F. A. O. *F. A. O. Quarterly Bulletin of Statistics*; F. A. O.: Rome, Italy, 1999; p 78.

(2) Bonilla, F.; Mayen, M.; Merida, J.; Medina, M. Extraction of phenolic compounds from red grape marc for use as food lipid antioxidants. *Food Chem.* **1999**, *66*, 209−215.

(3) Beecher, C. W. W. Cancer preventive properties of varieties of *Brassica oleracea*: a review. *Am. J. Clin. Nutr.* **1994**, *59*, 1166−1170.

(4) Fhaey, J.; Stephenson, K. Cancer chemoprotective effects of cruciferous vegetables. *HortScience* **1999**, *34*, 1159−1163.

(5) Mithen, R.; Dekker, M.; Verkerk, R., Rabot, S.; Johnson, I. The nutritional significance, bysynthesis and bioavailability of glucosinolates in human foods. *J. Sci. Food Agric.* **2000**, *80*, 967−984.

(6) Hertog, M. G. L.; Hollman, P. C.; Van de Putte, B. Content of potentially anticarcinogenc flavonoids of tea infusions, wines and fruit juices. *J. Agric. Food Chem.* **1993**, *41*, 1242−1246.

(7) Hollman, P. C.; Hertog, M. G. L.; Katan, M. B. Role of dietary flavonoids in protection against cancer and coronary heart disease. *Biochem. Soc. Trans.* **1996**, *24*, 785−789.

(8) Rankin, S.; de Whaley, C.; Hoult, J.; Jessup, W.; Wilkins, G.; Gollard, J.; Leake, D. The modification of low-density lipoprotein by the flavonoids myricetin and gossypetin. *Biochem. Pharmacol.* **1993**, *45*, 67−75.

(9) Cao, G.; Sofic, E.; Prior, P. L. Antioxidant and proxidant behavior of flavonoids: Structureactiviy relationships. *Free Radical Biol. Med.* **1997**, *22*, 749−760.

(10) Rice-Evans, C. A.; Miller, N. J.; Paganda, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

(11) Martínez Teruel, A.; Sánchez, J.; Megías, M. D.; Barrera, J. A.; Yáñez, A.; Ruiperez, F. Using of forages and byproducts in dairy cows farms of Murcia region. *Arch. Zootec.* **1998**, *47*, 33−42.

(12) Iñiguez, G.; Lange, S.; Rowell, R. Utilization of byproducts from the tequila industry: part 1: Agave bagasse as raw material for animal feeding and fiberboard production. *Bioresour. Technol.* **2001**, *77*, 25−32.

(13) Braddock, R. J.; Graulimch, T. R. Composition of fiber from citrus peel membranes, juice vessels and seeds. *Lebensm. Wiss. Technol.* **1981**, *14*, 229.

(14) Femenia, A.; Robertson, A.; Waldron, K.; Selvendran, R. Cauliflower (*Brassica oleracea* L.), Globe artichoke (*Cynara scolymus*) and Cichory Witloof (*Cichorium intybus*) processing byproducts as sources of dietary fibre. *J. Sci. Food Agric.* **1981**, *77*, 511−518.

(15) Marconi, E.; Graziano, M.; Cubadda, R. Composition and utilization of barley pearling byproducts for making functional pastas rich in dietary fiber and beta-glucans. *Cereal Chem.* **2000**, *77*, 133−139.

(16) Hang, Y. D. Production of fuels and chemicals from apple pomace. *Food Technol.* **1987**, *41*, 115−117.

(17) Rodríguez de Sotillo, D.; Hadley, M.; Holm, E. T. Potato peel waste, stability and antioxidant activity of a freeze-dried extract. *J. Food Sci.* **1994**, *59*, 1031−1033.

(18) Azizah, A. H.; Ruslawati, N. M.; Swee, T. Extraction and characterization of antioxidant form cocoa byproducts. *Food Chem.* **1999**, *64*, 199−202.

(19) Visioli, F.; Romani, A.; Mulinacci, N.; Zarini, S.; Conte, D.; Vincieri, F. F.; Galli, C. Antioxidant and other biological activities of olive mill wastewaters. *J. Agric. Food Chem.* **1999**, *47*, 3397−3401.

(20) Lu, Y.; Foo, L. Y. The polyphenol constituents of grape pomace. *Food Chem.* **1999**, *65*, 1−8.

(21) Llorach, R.; Espín J. C.; Tomás-Barberán, F. A.; Ferreres, F. Artichoke (*Cynara scolymus* L.) byproducts as a potential source of health-promoting antioxidant phenolics. *J. Agric. Food Chem.* **2002**, *50*, 3458−3464.

(22) Moure, A.; Cruz, M. J.; Franco, D.; Domínguez, J. M.; Sineiro, J.; Domínguez, H.; Núñez, M. J.; Parajó, J. C. Natural antioxidants from residual sources. *Food Chem.* **2001**, *72*, 145−171.

(23) Lu, Y.; Foo, L. Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chem.* **2000**, *68*, 81−85.
(24) Ferreres, F.; Gil, M. I.; Castañer, M.; Tomás-Barberán, F A. Phenolic metabolites in red pigmented lettuce (*Lactuca sativa*). Changes with minimal processing and cold storage. *J. Agric. Food Chem.* **1997**, *45*, 4249−4254.
(25) Tomás-Barberán, F. A.; Blazquez, A.; García-Viguera, C.; Ferreres, F.; Tomas-Lorente, F. A comparative study of different Amberlite XAD resins in flavonoid analysis. *Phytochem. Anal.* **1992**, *3*, 178−181.
(26) Tomás-Barberán, F. A.; Loaiza-Velarde, J.; Bonfanti, A.; Saltveit, M. E. Early wound and ethylene induced changes in phenylpropanoid metabolism in harvested lettuce. *J. Am. Soc. Hortic. Sci.* **1999**, *122*, 399−404.
(27) Price, K. R.; Casuscelli, F.; Colquhoun, I. J.; Rhodes, M. J. C. Hydroycinnamic acid esters from broccoli florets. *Phytochemistry* **1997**, *45*, 1683−1687.
(28) Wilson, K. E.; Wilson, M. I.; Greenberg, B. M. Identification of the flavonoid glycosides that accumulate in *Brassica napus* L. cv. Topas specifically in response to ultraviolet B radiation. *Photochem. Photobiol.* **1998**, *67*, 547−553.
(29) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* **1995**, *28*, 25−30.
(30) Espín, J. C.; Soler-Rivas, C.; Wichers, H. J. Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. *J. Agric. Food Chem.* **2000**, *48*, 648−656.
(31) Espín, J. C.; Wichers, H. J. Study of the oxidation of resveratrol catalyzed by polyphenol oxidase. Effect of polyphenol oxidase, laccase and peroxidase on the antioxidant capacity of resveratrol. *J. Food Biochem.* **2000**, *24*, 225−250.
(32) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.
(33) Rice-Evans, C. A.; Miller, N. J. Total antioxidant status in plasma and bloody fluids. *Methods Enzymol.* **1994**, *234*, 279−293.
(34) Larrosa, M.; Llorach, R.; Espín, J. C.; Tomás-Barberán, F. A. Increase of antioxidant activity of tomato juice upon functionalisation with vegetable byproducts extracts. *Lebensm. Wiss. Technol.* **2002**, *35*, 532−542.
(35) Nielsen, J. K.; Olsen, C. E.; Marianne, K. P. Acylated flavonol glucosides from cabbage leaves. *Phytochemistry* **1993**, *34*, 539−544.
(36) Tomás-Barberán, F. A.; Ferreres, F.; Gil, M. I. Antioxidant phenolic metabolites from fruit and vegetables and changes during postharvest storage and processing. In *Bioactive and Natural Products (Part D)*; Rahman, A., Ed.; Elsevier Science: Amsterdam, The Netherlands, 2000; pp 739−795.
(37) Hollman, P. C. H.; Arts, I. C. W. Flavonols, flavones and flavanols-nature, occurrence and dietary burden. *J. Sci. Food Agric.* **2000**, *80*, 1081−1093.
(38) Lu, Y.; Foo, L. Identification and quantification of mayor polyphenols in apple pomace. *Food Chem.* **1997**, *59*, 187−194.
(39) Ou, B.; Huang, D.; Wooddill, M. H.; Flanagan, J. A.; Deemer, E. K. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and Ferric reducing antioxidant power (FRAP) assays: A comparative study. *J. Agric. Food Chem.* **2002**, *50*, 3122−3128.
(40) Szeto, Y. T.; Tomlinson, B.; Benzie, I. F. F. Total antioxidant and ascorbic acid content of fresh fruits and vegetable implications for dietary planning and food preservation. *Br. J. Nutr.* **2002**, *87*, 55−59.
(41) Benzie, I. F. F.; Szeto, Y. T. Total antioxidant capacity of teas by the ferric reducing antioxidant power assay. *J. Agric. Food Chem.* **1999**, *47*, 633−636.
(42) Gil, M. I.; Tomás-Barberán, F. A.; Hess-Pierce, B.; Holcroft, D. M.; Kader, A. A. Antioxidant activity of Pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem.* **2000**, *48*, 4581−4589.
(43) Larrauri, J. A.; Goñi, I.; Martín-Carrón, N.; Rupérez, P.; Saura-Calixto, F. Measurement of health-promoting properties in fruit dietary fibres: antioxidant capacity, fermentability and glucose retardation index. *J. Sci. Food Agric.* **1996**, *71*, 515−519.
(44) Plumb, G. W.; Lambert, N.; Chambers, S. J.; Wanigatunga, S.; Heaney, R. K.; Plumb, J. A.; Aruoma, O. I.; Halliwell, B.; Miller, N. J.; Williamson, G. Are whole extracts and purified glucosinolates from cruciferous vegetables antioxidants? *Free Radical Res.* **1996**, *25*, 75−86.

Received for review October 18, 2002. Revised manuscript received January 21, 2003. Accepted January 27, 2003. This work has been partially supported by a grant from Spanish CICYT (Grant 1FD97-1809). R.LL. is holder of a contract from the same grant.

JF021056A