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Investigations on the High Molecular Weight Foaming Fractions of Espresso Coffee

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The target of the present work was the chemical, technological, and sensorial characterization of the brown polymers (foaming fractions) of freshly prepared espresso coffee. The total foaming fraction (TFF) was precipitated with ammonium sulfate from the defatted freshly prepared beverage and then subfractionated by adding 2-propanol/water to give an insoluble fraction (foaming fraction A, FFA) and a soluble fraction (foaming fraction B, FFB). The former is almost colorless, has a higher molecular weight and a lower nitrogen content, and contains mostly polysaccharides, whereas the latter has a lower molecular weight and a higher protein/melanoidin content, which results in a darker color. FFB showed greater foaming capability, but FFA contributed to the stability of the foam. FFB was further fractionated with solid-phase extraction and characterized by different analytical methods (size exclusion chromatography, UV, HPLC-DAD, ^{1}H NMR). All of the melanoidin-rich fractions showed antioxidant properties with the 2,2-diphenyl-1-picrylhydrazyl hydrate method.

KEYWORDS: Coffee; espresso; browning; Maillard reaction; melanoidins; foamability; antioxidant activity; color

INTRODUCTION

Coffee, the second most widely consumed beverage worldwide after water, may be prepared via a variety of brewing techniques traditionally applied in different countries: decoction methods (boiled coffee, Turkish coffee, percolator coffee, and vacuum coffee), infusion methods (filter coffee and *Napoletana*), and the original Italian pressure methods (Moka and espresso) (*1*).

Espresso coffee has some distinctive sensorial properties (*2*), deriving from its peculiar brewing technique: a small amount of hot water is percolated in a very short time at high pressure through a layer of ground roasted coffee, the coffee cake, to produce very efficiently a concentrated brew, containing not only soluble solids but also lipophilic substances, which are lacking in all other coffee brews. Typical conditions for Italian espresso preparation are 6.5 ± 1.5 g for ground coffee portion, 90 ± 5 °C for water temperature, 9 ± 2 bar for inlet water pressure, and 30 ± 5 s for percolation time (*1*). Two features are unique to espresso: the foam (*crema*), constituted of a layer of small bubbles with a particular tiger-tail pattern, and the opaque appearance. In fact, espresso is an emulsion of microscopic oil droplets in an aqueous solution of sugars, acids, protein-like materials, and caffeine, with dispersed gas bubbles and solids (*3*). The emulsion preserves flavors, so that the espresso coffee taste lingers in the mouth for several minutes (*3*). Foam has little interest for taste, but its color, texture, and persistence are important for recognizing the quality of the espresso coffee brew (*2*).

In many foods foam formation is an important feature: most foams are formed and stabilized by an absorbed layer of macromolecules at the air−water interface (*4*). Very often these polymers are hydrophobic proteins, and any chemical or enzymatic modification of their structure may modify their foaming behavior.

During roasting, the structure of coffee proteins is drastically modified by the Maillard reaction (*5*, *6*), and new brown polymeric materials, the melanoidins, are formed (*7*−*9*). They have been demonstrated to contain about 8−9% proteins (N × 6.25), 30% carbohydrates (*10*), and 33−42% polyphenols (*9*, *11*). This suggests that coffee melanoidins may derive from a noncolored polymeric skeleton (proteins and/or polysaccharides), linked with chromophoric groups, probably deriving in part from the Maillard reaction and in part from the decomposition of chlorogenic acids.

Some features of the foaming fractions of roasted coffee have been already investigated in part by Petracco et al. (*4*), who have extracted the total foaming fractions (TFF) by boiling ground coffee with hot water and separated them by solvent separation into two subfractions, named foaming fractions A and B, respectively. The nitrogen content and the brown color clearly indicate that foaming fraction B contained at least some melanoidins (*4*).

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Because the distinctive features of espresso coffee clearly indicate that the solvent properties of water inside the espresso machine are very different from those of boiling water at room pressure, in this work the foaming fractions were directly separated from espresso coffee and not from a hot water extract of ground coffee, as was done previously (*4*). Several authors have attributed part of the antioxidant properties of coffee to melanoidins (*12*, *13*), so the foaming fractions were investigated in this paper also for their possible antioxidant activities. The study was focused on a commercial blend of *Coffea arabica*, because this species is considered to give the best, mildest espresso cup (*1*).

MATERIALS AND METHODS

Materials. Hexane, 2-propanol, and HPLC-grade methanol, acetonitrile, and formic acid were purchased from Baker (Deventer, The Netherlands). Unless otherwise stated, the water used in all procedures as well as that used as eluent in HPLC was produced with a Milli-Q water purification system (Millipore, Billerica, MA). 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH; 95%), glucose (96%), psicose (95%), rhamnose (99%), acetic acid (>99%), maltotriose (>95%), maltopentose (>95%), and dextran standards (>99%) were purchased from Sigma Aldrich (Milan, Italy); trifluoroacetic acid (TFA; >99%), 2,6-di-*tert*-butyl-4-methylphenol (BHT; >99%), ferulic acid (>99%), caffeic acid (>99%), chlorogenic acid (>97%), ammonium sulfate (>99%), and sodium chloride (>99%) were from Fluka (Milan, Italy); arabinose (>98%), galactose (>98%), maltose (>98%), mannose (>98%), and xylose (>98%) were from Carlo Erba (Rodano, Milan, Italy); 6′-α-D-galactosylmannotriose (GM3; >98%) was purchased from Megazyme (Bray, Co. Wicklow, Ireland). HPLC eluents and samples were filtered through 0.45 μm disposable nylon filters (Alltech, Milan, Italy).

Isolation of the Total Foaming Fraction (TFF) from Espresso Coffee. Pure *arabica* coffee (Illy, Trieste, Italy) was ground just before use. Fresh espresso (6 cups = 118 mL) was prepared with a professional machine (ground coffee portion = 6.3 g; water temperature = 91 °C; inlet water pressure = 9.2 atm; percolation time = 30 s) and continuously extracted with hexane for 16 h, to eliminate all of the lipids, and then ammonium sulfate was added to saturation; the solution was kept at 4 °C overnight (*4*). The precipitate was separated by centrifugation at 12063*g* for 15 min at 4 °C, resuspended in water, ultrafiltered on Amicon membranes (cutoff limit = 3000 Da), and then washed with 1 M NaCl and water to eliminate all nonpolymeric materials. The retentate was freeze-dried to obtain 1.5 ± 0.4 g of fluffy brown material, TFF. Data refer to three independent samples.

Subfractionation of TFF. The protocol applied for the stepwise separation of TFF is shown in **Figure 1**. TFF (1 g) was suspended in water (50 mL) at room temperature under stirring, and then the mixture was sonicated for 20 min to achieve maximum solubilization. The solution was centrifuged for 10 min at 4 °C at 12063*g* to eliminate any solid residue, and then 125 mL of 2-propanol was added; the mixture was kept at 4 °C overnight (*4*). The solid precipitate was separated by centrifugation at 12063*g* for 20 min at 4 °C. The precipitate and the supernatant were freeze-dried to obtain foaming fraction A (FFA) (0.228 ± 0.05 g; 23% yield) and foaming fraction B (FFB) (0.416 ± 0.08 g; 42% yield), respectively. Results are the average of three independent experiments.

Fractionation of FFB by Solid-Phase Extraction (SPE). FFB was fractionated using a high-capacity C18 cartridge (Alltech, Milan, Italy), with a volume capacity of 15 mL. The stationary phase was conditioned by flushing 5 mL of methanol and then 5 mL of water at 5 mL/min. Thirty milligrams of foaming fraction, dissolved in water/methanol 70:30, was loaded, and the elution was carried out in sequence using water/methanol solutions in different ratios: 5 mL of water (FFB-1), 10 mL of 50% methanol (FFB-2), 7 mL of 80% methanol, and 5 mL of 100% methanol.

UV−Vis Spectrophotometric Analysis. These analyses were carried out using a spectrophotometer Lambda 40 Perkin-Elmer (Norwalk, CT);

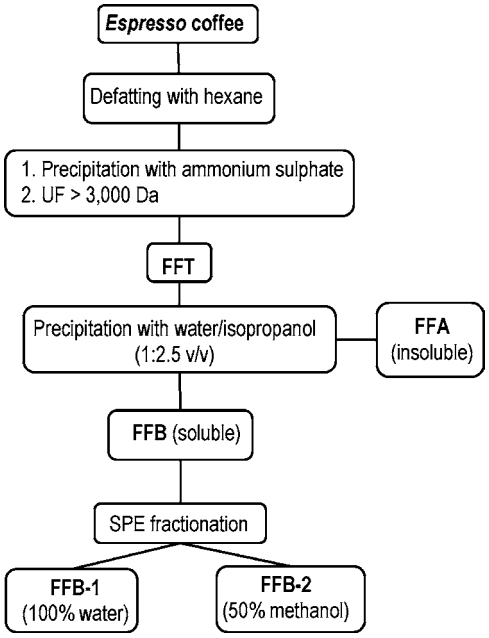

Figure 1. Analytical protocol developed for the separation and fractionation of the foaming fractions from espresso coffee brews.

the analytical conditions were as follows: scan speed, 240 nm/min; smooth, 0; slit, 2 nm; λ range, 200−600 nm.

Analysis by Size Exclusion Chromatography (SEC). An isocratic pump (Waters, Milford, MA) equipped with a Rheodyne injector (20 μL, loop) and a refractive index detector (RI) was used. The SEC separations were achieved on two columns with different molecular weight ranges: Ultrahydrogel 250 [7.8 × 300 mm, molecular weight (MW) range of 80000−1000 Da] and Ultrahydrogel 120 (7.8 × 300 mm, MW range of 5000−100 Da), both from Waters (Milford, MA). The analyses were carried out in isocratic conditions, using 1 M acetic acid in water as mobile phase (pH 4.5); the temperature was 30 °C, and the flow rates were 0.35 mL/min for Ultrahydrogel 250 and 0.5 mL/min for Ultrahydrogel 120. For the Ultrahydrogel 250 column the calibration curve to calculate the MW was performed with the following standards: maltose (MW, 360.3 Da), dextran 1 (MW, 4000−6000 Da), dextran 2 (MW, 10500 Da), and dextran 3 (MW, 68400 Da). For the calibration curve of Ultrahydrogel 120 the standards used were galactose (MW, 180 Da), maltose (MW, 360.3 Da), maltotriose (MW, 504.4 Da), 6′-α-D-galactosylmannotriose (GM3; MW, 666.6 Da), maltopentose (MW, 828.7 Da), maltoheptose (MW, 1153 Da), and dextran 1 (MW, 4000−6000 Da).

Evaluation of foamability and Foam Persistence. Each foaming fraction (40 mg) was dissolved in 10 mL of water in a 25 mL plugged graduated glass cylinder. The cylinder was wrist shaken for 10 s, and after 5 min, both total and liquid volumes were measured (*4*). The foaming capacity was quantified by measuring the foam volume after 5 min. To evaluate the foam persistence, foam volumes were evaluated also after 10, 15, and 20 min. Each experiment was repeated three times.

Color Dilution Analysis (CDA). To evaluate the contribution of each fraction to browning, color dilution factors were measured according to the method proposed by Hofmann (*14*). The freeze-dried material (10 mg) was dissolved in 1 mL of water and diluted serially 1:2 by volume until the color difference between the sample and two blanks of water could be visually detected in a triangle test. Color dilution factors (CD_{total}) corresponded to the final dilution factors.

Sensory Evaluation. A nonprofessional panel carried out the informal sensory evaluation. The samples were prepared by dissolving 5 mg of each foaming fraction in 1 mL of water. A small amount of each sample was sipped, kept in the mouth for a few seconds, and then expectorated. Aroma, taste quality, and flavor persistence were recorded. At least five untrained persons participated in each sensory evaluation.

Antioxidant Activity. The antioxidant properties were evaluated using a stable, blue 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•),

Table 1. Chemical and Technological Characterization of the Foaming Fractions from Espresso Coffee: Elemental Analysis, CD Factors (CD_{total}) (*14*), Foamability (*4*), and Sensory Analysis

	elemental analysis			technological properties		sensory properties[a]		
fraction	% C	% H	% N	CD_{total}	foam[b] (mL)	bitter taste	coffee aroma	taste persistence
TFF	42.9	5.5	2.9	32000	1.5 ± 0.1	+	+	+
FFA	45.7	5.6	2.0	8000	1.0 ± 0.1	–	–	–
FFB	45.0	5.1	4.1	128000	2.0 ± 0.1	+++	++	++

[a] –, not perceived; +, weak; ++, medium; +++, strong. [b] Foaming values are presented as the mean ± SD ($n = 3$).

according to the method proposed by Brand-Williams et al. (*15*). The antioxidant activity was calculated by measuring the decrease of the absorbance at 515 nm, due to the transformation of the radical into the reduced colorless form. One hundred microliters of a 1 mg/mL solution of each foaming fraction in water/methanol (60:40) was added to a methanol solution of DPPH$^{\bullet}$ (2.9 mL, 6×10^{-5} M). As the molecular weights of the foaming fractions were unknown, to compare the antioxidant activity, all samples had the same concentration (33 μg/mL; **Table 4**). The bleaching rate was measured at 515 nm on a Lambda 40 spectrophotometer (Perkin-Elmer, Norwalk, CT) at 25 °C for 30 min, to reach the steady state.

The antioxidant activity was expressed as "chain-breaking activity" ($-Abs^{-3}\ min^{-1}\ mg_{dm}^{-1}$) according to eq 1 (*16*)

$$Abs_t^{-3} - Abs_0^{-3} = -3kt \quad (1)$$

where Abs_0 is the absorbance at the initial time, Abs_t is the absorbance at time t, and k is the DPPH$^{\bullet}$ bleaching rate. Each determination was repeated at least three times.

^{1}H NMR. The analyses were conducted on a Bruker AMX-600 (600.1 MHz), using D_2O as solvent and tetramethylsilane (TMS) as internal standard. Chemical shift values were expressed in parts per million (δ).

Hydrolysis of FFA and FFB and Analysis of Their Carbohydrate Content. Each fraction (10 mg) was hydrolyzed with 2 M TFA (1 mL) for 1 h at 110 °C (*17*); the solution was evaporated under vacuum, and the elimination of water was completed with azeotropic distillation with methanol (3 × 0.5 mL). The solid residue was dissolved in water (concentration = 10 mg/mL) and then analyzed by HPLC. The HPLC analyses were performed on a carbohydrate column (4.6 × 250 mm, Waters, Milford, MA), using a pump (Waters) equipped with a Rheodyne injector (20 μL, loop) and a refractive index detector (RI). The analyses were carried out in isocratic conditions, using acetonitrile/water 80:20, the flow rate was 0.8 mL/min, and the temperature was 30 °C (*18*). The peak attribution was achieved by comparison with the retention times of the commercial standards of arabinose, galactose, glucose, maltose, mannose, psicose, rhamnose, and xylose (*10*).

Analysis of FFB-1 by RP-HPLC. HPLC analyses were conducted with an HP-1050 quaternary pump (Hewlett-Packard, Palo Alto, CA) fitted with a Rheodyne injector (20 μL, loop) and equipped with an HP-1050 photodiode array detector (HPLC-DAD). Data were processed with an HP ChemStation (Agilent Technologies, Palo Alto, CA). Chromatographic separation was achieved on a Lichrospher 100 RP-18 column (250 × 4 mm, 5 μm, Merck, Darmstadt, Germany). The HPLC analysis of FFB-1 was carried out using a linear gradient from 5:95 acetonitrile/water at pH 2.5 for H_3PO_4 to 50:50 acetonitrile/water at pH 2.5 for H_3PO_4, over 50 min, and then 10 min isocratic; the flow rate was 0.7 mL/min. Chromatograms were recorded at 254, 270, 280, and 329 nm.

RESULTS

Separation and Characterization of the Foaming Fractions. The separation of the foaming fractions from espresso coffee was carried out according to the method proposed by Petracco et al. (*4*) with some small modifications (**Figure 1**). After the espresso had been defatted with hexane, the TFF was precipitated by adding ammonium sulfate and then separated by selective precipitation with water/2-propanol, giving an insoluble light brown solid (FFA) and a dark brown solution, which was freeze-dried to give a dark solid (FFB).

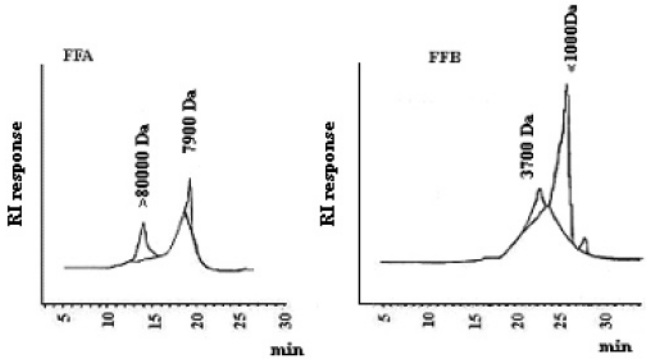

Figure 2. SEC analyses of FFA and FFB on Ultrahydrogel 250 (7.8 × 300 mm, MW range of 80000–1000 Da). The analyses were carried out in isocratic conditions, using 1 M acetic acid in water as mobile phase (pH 4.5) with RI detection at 30 °C and flow rate of 0.35 mL/min.

The elemental analyses of TFF, FFA, and FFB are reported in **Table 1**. TFF contains 42.9% carbon, 5.5% hydrogen, 2.9% nitrogen, and 48.7% oxygen, in reasonable agreement with the results of Petracco et al. (*4*). The high content of oxygen suggests that polysaccharides should be the main components in these materials, whereas nitrogen indicates the presence of some proteins or Maillard reaction protein degradation products (melanoidins). During this separation procedure, most of the polysaccharides precipitated selectively in the 2-propanol-insoluble fraction FFA, which had a lower nitrogen content, whereas proteins/melanoidins remained mostly in FFB, the nitrogen content of which was ~2-fold higher. The presence of melanoidins was confirmed by the brown color of FFB; however, the content of polysaccharides was rather high, with an oxygen percentage of 45.8%.

The fractions were then submitted to analytical SEC (**Figure 2**). FFA contained two peaks, one with MW >80000 Da and the other with MW of ~7900 Da. FFB, by comparison, shows a very broad peak with maximum at ~3700 Da and a sharp one with MW <1000 Da, indicating the presence of abundant nonpolymeric materials.

To characterize the polysaccharide content of the two foaming fractions, they were hydrolyzed and analyzed on a carbohydrate-dedicated column (*18*). Hydrolysis of the carbohydrates of FFA gave three main peaks, which were identified as mannose (79.2% of total peak area), glucose (12.4%), and galactose (8.4%), whereas hydrolysis of the carbohydrates of FFB gave 48.5% arabinose and 5.5% galactose (two other peaks remained unidentified, not belonging to usual sugars).

To better evaluate the contribution of each fraction to the overall color of espresso coffee, their color dilution factors (CD_{total}) (**Table 1**) were estimated according to the method proposed by Hofmann (*14*). FFB had a more intense color than FFA, probably related to the presence of melanoidins (*19*).

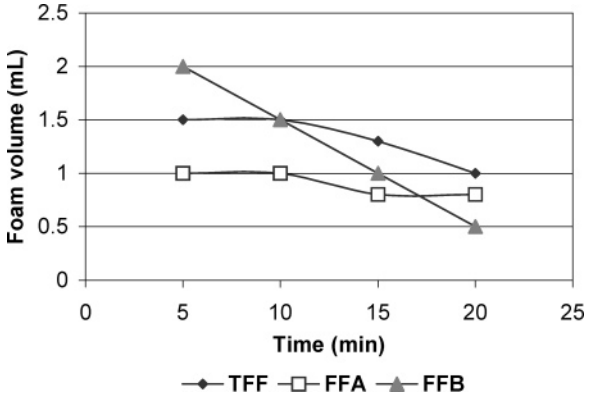

Figure 3. Foam persistence of TFF, FFA, and FFB. Foam volumes were evaluated at 5, 10, 15, and 20 min. Each experiment was repeated three times.

Table 2. Fractionation of FFB by SPE on a High-Capacity C18 Cartridge[a]

loaded (mg)	FFB-1 (mg)	FFB-2 (mg)	FFB-3 (mg)	FFB-4 (mg)	total recovery (mg)
30	11	7.2	1.7	0.7	20.6
brown	colorless	brown	brown	brown	

[a]The fractions were eluted in sequence with different water/methanol mixtures: FFB-1 with 100% water (5 mL), FFB-2 with 50% methanol (10 mL), FFB-3 with 80% methanol (7 mL); FFB-4 with 100% methanol (5 mL).

Sensory evaluation was performed to assess whether these fractions contribute to flavor and taste persistence. The test was performed informally by an untrained panel, taking into consideration the intensity and persistence of the bitter taste and coffee-like aroma (**Table 1**). All panelists agreed that FFB had an intense coffee aroma and persistent bitter taste, whereas FFA was practically tasteless.

The foaming properties of the three fractions were measured by taking into account two different parameters (*4*): the foamability or foam capacity (i.e., the ease and extent of foam formation) and the efficiency in foam stabilization (i.e., the rate of loss of foam structure once formed). FFB was more efficient in the production of the foam (**Table 1**); however, this foam was not very stable, whereas the foam produced by FFA was smaller, but much more persistent (**Figure 3**).

Fractionation of FFB and Characterization. Because FFB has a larger foaming capacity and antioxidant activity (see below), it was decided to separate it further. Different techniques were investigated, and the most efficient was SPE on C18 cartridges. Fractionation was achieved by eluting with methanol/water mixtures in different ratios, collecting in total four fractions (**Table 2**). Because the two most lipophilic fractions, FFB-3 and FFB-4, were very tiny, they were not analyzed in detail.

The most hydrophilic fraction, FFB-1, was colorless and in SEC showed two peaks, the former at ~5200 Da and the latter with MW <1000 Da. The UV spectrum had two maxima at 290 and 314 nm, resembling ferulic acid, and this fraction was submitted to RP-HPLC with diode array detection. It was possible to confirm the presence of ferulic acid, caffeic acid, and caffeoylquinic acid by comparing the retention times and UV spectra with those of authentic standards (**Figure 4**).

Other information was obtained by the ^{1}H NMR spectrum in D_2O (**Figure 5a**). Although very complex, it showed the signals of the aromatic hydrogens at 6.5 and 7.7 ppm, typical of phenolic compounds. In addition, it indicated the presence of carbohydrates: a weak group of signals between 5.0 and 5.5 ppm, typical of the anomeric hydrogens, and a complex multiplet between 3.5 and 4.5 ppm, corresponding to the C−H of the sugar backbone.

Fraction FFB-2, eluted with 50% methanol, was dark brown and appeared to be relatively homogeneous in SEC, because it contained only an intense peak at ~56000 Da. The UV spectrum showed two maxima at 280 and 325 nm and some absorbance between 400 and 500 nm. The ^{1}H NMR (**Figure 5b**) was very complex; however, there was clear indication of the presence of some cinnamic derivatives (C−H between 6.3 and 7.5 ppm) and polysaccharides (anomeric C−H in the range of 5.0−5.5 ppm and a multiplet between 3.5 and 4.5 ppm, corresponding to the C−H of the sugar backbone). In addition, there was also a complex broad tangle of aliphatic C−H, which may indicate the presence of proteins, possibly in part modified by the Maillard reaction. The very low resolution of the area between 6 and 8 ppm may be related to the presence of the aromatic signals of the melanoidins (*19*).

Table 3 reports the foaming properties of these two fractions: FFB-1 showed no foamability, whereas FFB-2 was very effective either in foam formation or in stabilization.

Antioxidant Activity. Recent literature (*12*, *13*, *16*, *20*) indicates that roasted coffee has a strong antioxidant activity, probably due to the presence of Maillard reaction products formed during roasting. That prompted us to measure the antioxidant capacity of our polymeric fractions, with the DPPH method proposed by Brand-Williams et al. (*15*). The method consists of monitoring the blanching of this stable radical at 515 nm and the "chain-breaking activity", which represents the rate of radical scavenging, to be calculated.

The results of these experiments are shown in **Table 4** in comparison with the antioxidant capacity of BHT, used as a standard. Most of the fractions isolated in this study, TFF included, have antioxidant activity. The antioxidant capacity of FFB, expressed as chain-breaking activity, is much larger than that of FFA. This is not surprising considering the structural considerations already discussed above: this activity may be due either to nitrogen-containing moieties or to polyphenol residues included in the polymers (*12*, *13*), but not to polysaccharides.

During the fractionation of FFB, very hydrophilic compounds, with scarce antioxidant capacity, were eluted with water from the SPE in fraction FFB-1. HPLC analysis showed that this fraction still contained some free polyphenols, too tiny to produce a detectable antioxidant activity, whereas ^{1}H NMR analysis revealed that the major components were polysaccharides.

Slightly more hydrophobic compounds are eluted in FFB-2. At least some of them have an exceptional antioxidant capacity, because the chain-breaking activity is 160 OD^{-3} min^{-1} mg^{-1}, a value almost 10 times higher than that of BHT.

DISCUSSION

Roasting produces drastic modifications of the chemical structure of most of the components of green coffee: proteins are strongly degraded and chemically modified by pyrolytic reactions, whereas polysaccharides are in part depolymerized and degraded and give condensation complexes with proteins, protein fragments, and other breakdown products (*2*). All of these processes reduce very much the possibility of separating homogeneous materials from roasted seeds and make a complete chemical characterization very difficult.

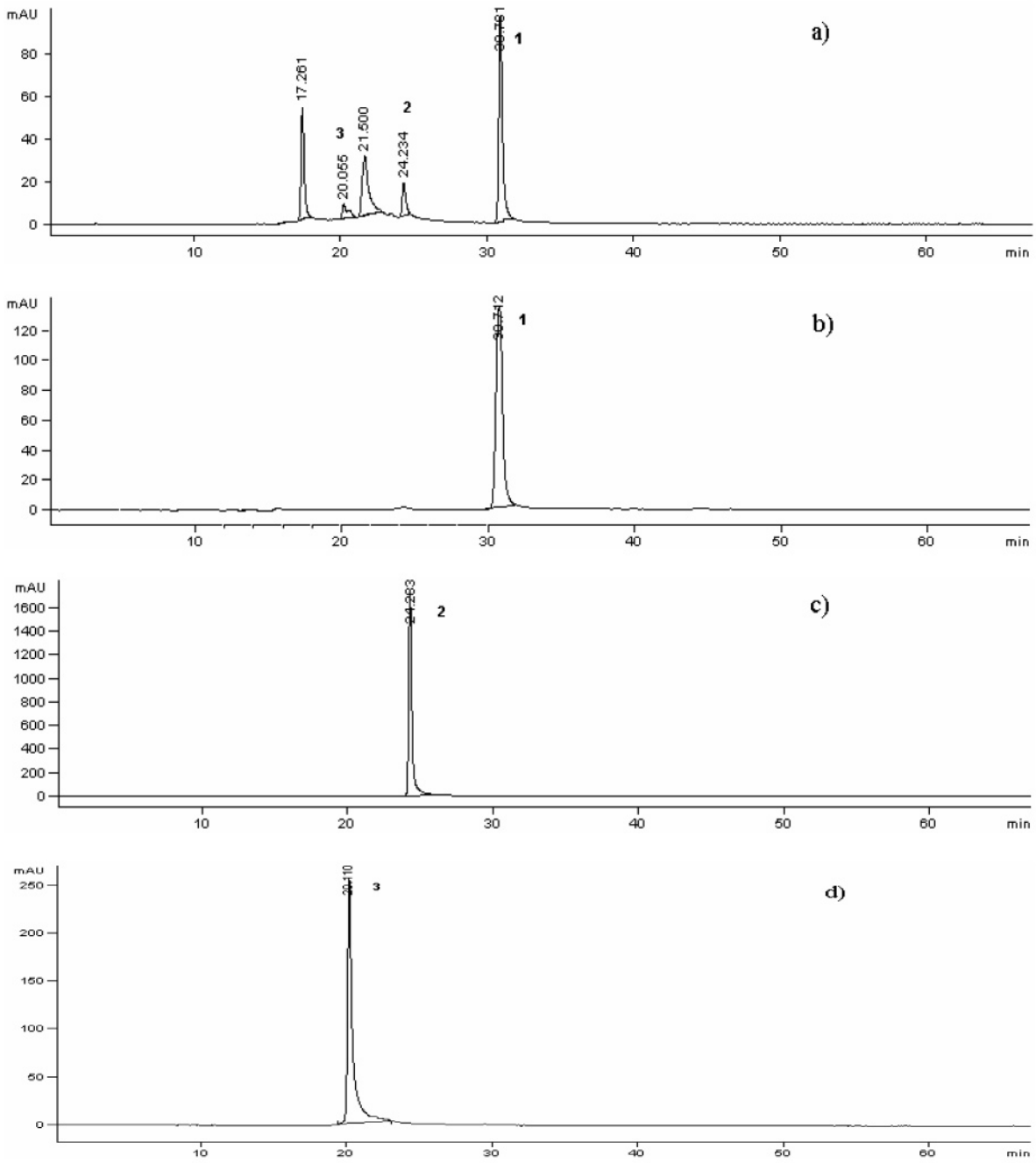

Figure 4. RP-HPLC analyses at 329 nm of FFB-1 fraction (**a**), ferulic acid (**b**), caffeic acid (**c**), and chlorogenic acid (**d**). Analyses were performed on a 250 × 4.6 mm Lichrospher 100 RP-18 column using a linear gradient from 5:95 acetonitrile/water at pH 2.5 for H_3PO_4 to 50:50 acetonitrile/water at pH 2.5 for H_3PO_4, over 50 min, and then 10 min isocratic at a flow rate of 0.7 mL/min. Peak identification: 1, ferulic acid; 2, caffeic acid; 3, chlorogenic acid.

The only possibility of acquiring knowledge in this field is the application of multistep procedures for the purification of fractions with selected technological or biological properties. Considering this background, the method developed in the present investigation was very successful, because it permitted the isolation of some polymeric fractions from espresso coffee characterized by different chemical data and technological properties (color, flavor, taste, foamability, foam persistence, and antioxidant activity).

FFA. All of the data collected (pale color, chemical analyses, and scarce antioxidant activity) indicate that FFA contains mostly polysaccharides, with the following composition: 79% mannose, 12% glucose, and 8% galactose. It is interesting to compare these values with the corresponding ones obtained by Petracco et al. (*4*), who extracted ground coffee with boiling water. On the whole, most of the characteristics of both fractions are in reasonable agreement; however, the sugar compositions are different. In fact, the FFA separated by Petracco and coworkers (*4*) contained 80% mannans (containing small amounts of galactose and arabinose) and ~20% of arabinogalactan, but did not contain glucose. On the contrary, arabinose was not detected by us in the FFA from espresso coffee, although this sugar is well represented in FFB.

Owing to its efficiency in foam stabilization, FFA may have an important role in the persistence of espresso foam, although it does not contribute very much to the foamability of the beverage. Because of their hydrophilic character, most polysaccharides have low surface activity at the air−water interface

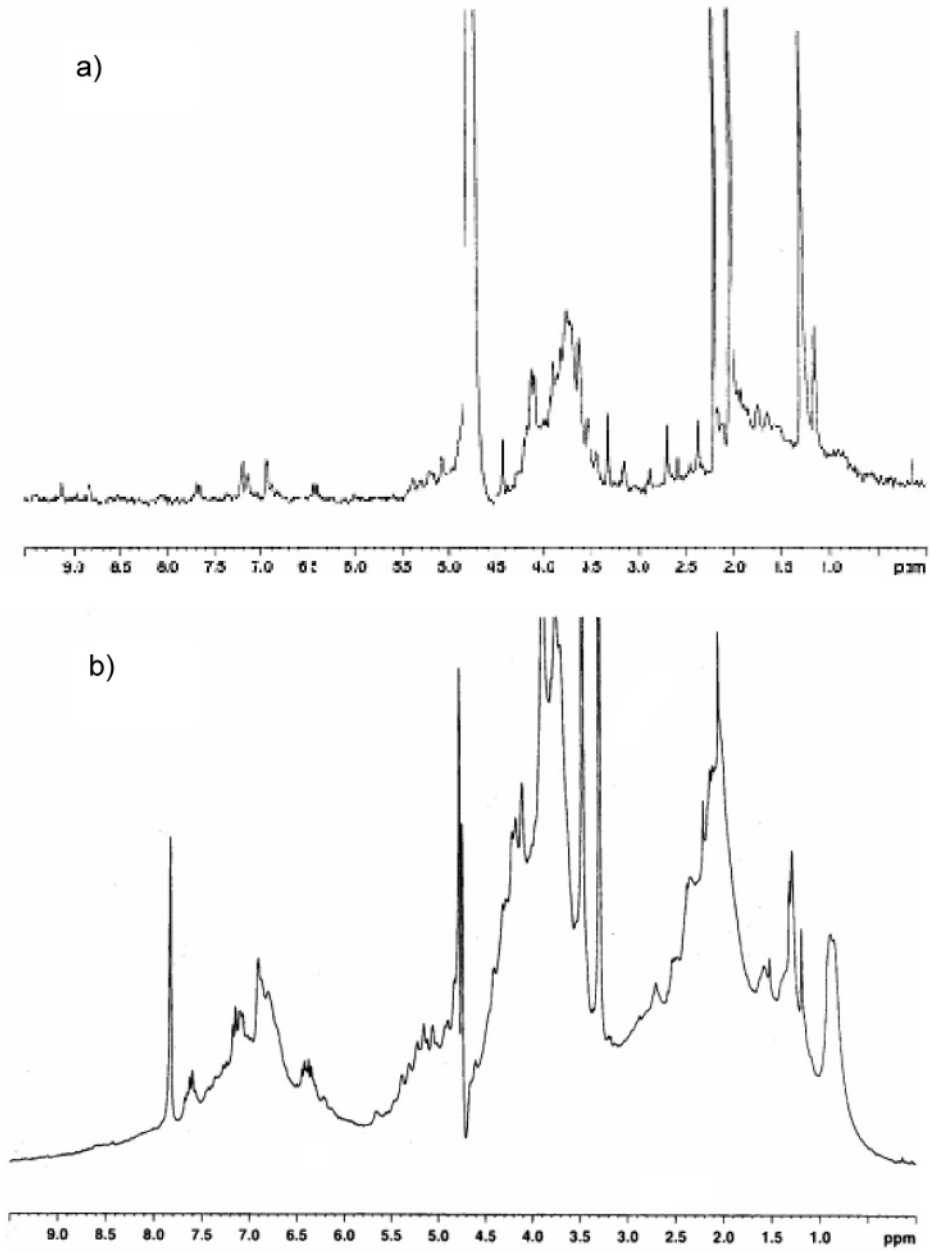

Figure 5. ^{1}H NMR of the foaming fractions FFB-1 (**a**) and FFB-2 (**b**) registered at 600 MHz (Bruker AMX-600) in D_2O, using TMS as internal standard. Chemical shift values were expressed in parts per million (δ).

Table 3. Foaming Properties of the Two Subfractions FFB-1 and FFB-2, Detected in 10 mL of a 4 mg/mL Solution[a]

	foam volume (mL)			
fraction	5 min	10 min	15 min	20 min
FFB-1	0 ± 0.1	0 ± 0.1	0 ± 0.1	0 ± 0.1
FFB-2	2.0 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1

[a] Foamability was measured 5 min after shaking and foam persistence after 10, 15, and 20 min.

Table 4. Comparison of the Chain-Breaking Activity of Foaming Fractions from Espresso Coffee and of the Subfractions of FFB, with That of BHT, a Commercial Antioxidant

fraction	concn (μg/mL)	chain-breaking activity ($-OD^{-3}\ min^{-1}\ mg^{-1}$)
TFF	33	4.20
FFA	33	0.82
FFB	33	12.0
FFB-1	33	0.92
FFB-2	33	160
BHT	33	19

and do not form absorbed layers in food colloids containing proteins and low molecular weight surfactants (*21*). Nevertheless, polysaccharides generally confer colloid stability through their thickening behavior and/or through interaction with absorbed proteins to form protein−polysaccharide complexes (*4*, *21*), in agreement with their chemical composition. Moreover, FFA does not contribute to the retention of coffee flavor and contributes only marginally to the antioxidant activity of roasted coffee.

FFB. The higher solubility in 2-propanol points out that FFB is more hydrophobic than FFA. The higher nitrogen content and the ^{1}H NMR spectrum clearly indicate that it contains some protein-like material, probably denatured or degraded proteins or proteins modified by the Maillard reaction, melanoidins. This

fraction contributes very much to the formation of the foam on the top of espresso and to coffee flavor retention and has antioxidant properties.

The separation of FFB into four subfractions with different hydrophobicities was achieved by SPE on C18 cartridges. FFB-1, the least hydrophobic fraction, contained polysaccharides and some free polyphenols, such as ferulic acid, caffeic acid, and caffeoylquinic acid. Evidently, when ammonium sulfate was added to defatted espresso, these compounds were retained by the polymers.

Most of the melanoidins were recovered in FFB-2, which is probably the main fraction responsible for the efficacy of FFB as foaming agent and antioxidant.

In a recent work (*22*), the foamability and the efficiency of foam stabilization of espresso coffee have been determined as a function of the degree of roasting, along with other chemical parameters, such as the total solids, the pH value, and fat, protein, and carbohydrate percentages. Principal component analysis indicated a correlation between foamability and protein content and between efficiency of foam stabilization and the content of high molecular weight polysaccharides (or, better, complexes between polysaccharide, protein, and phenolic compounds caused by the roasting process, probably products of Maillard reaction) (*23*).

In many foods the formation of foams is an important phenomenon. As the presence of a stable head of foam is a major factor in assessing beer quality, beer froth is one of the most investigated food liquid foams. It has been found that it is due to and stabilized by the proteinaceous materials derived from proteins that have undergone extensive proteolysis and heat denaturation during the brewing process (*24*). Foam-active protein−polysaccharide complexes (*25*, *26*) and a high molecular weight surface-active fraction, with the characteristics of a melanoidin, have been separated and characterized from beer (*27*). Beer foam stability is due to the presence of hydrophobic proteins (*28*) and polypeptides and melanoidins (*29*). These results strongly suggest a possible similarity between beer and espresso coffee foams, as far as the chemical nature of the surface-active compounds is concerned.

Antioxidant Activity of FFB. Although polyphenols are certainly very important antioxidant components of coffee, the evidence that during coffee roasting the antioxidant activity increases to the medium-dark roasted stage (*13*) demonstrates that also melanoidins contribute to the overall antioxidant properties of coffee (*30*). In fact, although some of the antioxidant components of the green seed, such as chlorogenic acids and other phenolic compounds, are destroyed during coffee roasting, the overall antioxidant activity is preserved owing to the formation of melanoidins (*12*).

The stepwise procedure presented here may be considered successful because it permitted the isolation of fractions with increasing antioxidant activity from espresso. As they have increasing nitrogen content, our data confirm the importance of melanoidins in determining the antioxidant capacity of coffee. Another important result of this work is the demonstration that two completely different features, such as foamability and antioxidant capacity, are related to the same fractions of espresso coffee.

Assessing the health consequences of this antioxidant activity is a very difficult task. As a starting point, many authors (*31*−*33*) have pointed out that by analyzing the antioxidant activity of food components by means of different assays, different results are obtained. In particular, chain-breaking activity and redox potential measurements give conceptually different information, because the former is a kinetic measure, whereas the latter is a thermodynamic one. In addition, the rate of intestinal absorption of melanoidins has been estimated to be very low (*34*).

However, research in this field continues, and recent in vivo studies have indicated that moderate coffee consumption may increase plasma glutathione concentration (*35*) and plasma antioxidant capacity (*36*). Moreover, *N*-methylpyridinium isolated from coffee brew is a chemopreventive compound (*37*), and pronyl-L-lysine, which derives from the Maillard reaction, is able to modulate the phase II enzyme glutathione *S*-transferase in vivo (*38*).

ABBREVIATIONS USED

TFF, total foaming fraction; FFA, foaming fraction A; FFB, foaming fraction B; CD, color dilution; DAD, diode array detector; HPLC, high-performance liquid chromatography; MW, molecular weight; NMR, nuclear magnetic resonance; RI, refractive index; SEC, size exclusion chromatography; SPE, solid-phase extraction; BHT, 2,6-di-*tert*-butyl-4-methylphenol; DPPH, 2,2-diphenyl-1-picrylhydrazyl hydrate; TFA, trifluoroacetic acid; GM3, 6′-α-D-galactosylmannotriose.

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