Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 102 (2007) 582–592
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant capacity of coffees of several origins brewed following three different procedures

P. Parras, M. Martínez-Tomé, A.M. Jiménez, M.A. Murcia *

Department of Food Science, Veterinary Faculty, Campus of Espinardo, University of Murcia, Spain

Received 19 December 2005; received in revised form 8 May 2006; accepted 21 May 2006

Abstract

The antioxidant capacity of coffees (Arabica and Robusta) from 12 different points of origin (Uganda, Papua, Jamaica, Ethiopia, Kenya, Puerto Rico, "Caracolillo" Puerto Rico, Nicaragua, Colombia, Vietnam, Brazil and Guatemala) and two decaffeinated coffees from Colombia and Brazil prepared by three commonly used procedures (espresso, filter and Italian) were evaluated and compared with antioxidant standards and other phenolic compounds which have been described in coffee. All the coffees studied were very effective as scavengers of lipoperoxyl and OH· radicals. The results also showed that there are no significant differences ($p < 0.05$) between the three ways of brewing (espresso, filter and Italian). The H_2O_2 scavenging capacity was analysed in freshly made coffee and 6 h later, the antioxidant activity slightly increasing with time. The filtered coffee showed a greater capacity to react with H_2O_2 ($p < 0.05$) than the Italian and espresso coffees. All the coffee samples improved the oxidative stability of butter (Rancimat test), espresso and Italian coffee providing greater protection ($p < 0.05$) than the filtered beverages.

The coffee beverages from different origins did not show significant differences during 28 days of storage as regards the autoxidation of linoleic acid. However, filter brews produced stronger antioxidant activity ($p < 0.05$) during storage than espresso and Italian brewing techniques. The TEAC value provided a ranking in decreasing order of antioxidant capacity for samples at 6 min: Vietnam, Uganda, Nicaragua, Colombia, Brazil, "caracolillo", Puerto Rico, Guatemala, Kenya, Papua, decaffeinated Colombia, Ethiopia, Jamaica, and decaffeinated Brazil. In general, decaffeinated coffees (Colombia and Brazil) showed lower TEAC values than coffees with caffeine. Filter and Italian coffee analysed after 6 min exhibited higher TEAC value than espresso coffees.

All the coffees studied are good antioxidants regardless of their cost, origin and way in which they are brewed (espresso, filter or Italian), which is a point worth considering.

Keywords: Free radical; Antioxidant; Coffee; Coffee brewing; Procedures; Origins

1. Introduction

Coffee (the second most traded commodity after petroleum) is one of the most popular beverages in the world. Seventy five per cent of soft drinks consumed regularly are coffee (Rojo Camargo, Toledo, & Farah, 1999).

The coffee plant belongs to the genus *Coffea* of the family Rubiaceae. Of the many species, two are of major importance in the trade, *Coffea arabica* and *Coffea canephora* var. *robusta*. At present, coffees consisting of 100% Arabica beans are preferred by coffee drinkers since they are considered of better quality than Robusta coffees

Abbreviations: ABAP, azobis(2-amidinopropane)hydrochloride; ABTS, azinobis(3-ethylbenzo-thiazoline-6-sulfonic acid)diammonium salt; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DPPH, 2,2-diphenyl-2-picrylhydrazyl hydrate; EDTA, ethylenediamine-tetraacetic acid; MDA, malondialdehyde; MRP, Maillard reaction products; PBS, phosphate buffer solution; PF, protection factor; TBA, thiobarbituric acid; TEAC, Trolox equivalent antioxidant capacity; TRAP, total radical-trapping antioxidant potential; Trolox, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxilic acid.

* Corresponding author. Tel./fax: +34 968 364792.
E-mail address: mamurcia@um.es (M.A. Murcia).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.05.037

(González, Pablos, Martín, León-Camacho, & Valdenebro, 2001).

In green the coffee composition is dominated by carbohydrates, including polysaccharides (glucomannans, cellulose), disaccharides (sucrose) and monosaccharides (glucose, galactose, arabinose, fructose, manose, manitol, xylose and ribose) (Casella, Gatta, & Desimoni, 1998; Mazzafera, 1999; Hu, Zhou, Zhang, & Fang, 2001), lipids such as triglycerides (75%), sterols (stigmasterol, sitosterol), fatty acids (linoleic, linolenic, oleic, palmitic, stearic, araquidic, lignoceric and behenic), pentacyclic diterpens (methylcafestol, cafestol, kahweol) (Kurzrock & Speer, 2001), diterpenic alcohols, diterpenic and tripterpenic esters and ceramide. Tocopherols (α, β, γ) are also present together with the tocotrienols (González et al., 2001). The protein content includes free amino acid, mainly asparagine, glutamic acid, alanine, aspartic acid and lysine (Hu et al., 2001). Coffee contains several species of xanthines such as caffeine (Hostettmann, Marston, Ndjoko, & Wolfender, 2000), teobromine and theophylline (Nardini, Cirillo, & Sacaccini, 2002; Kiyohara et al., 1999). Other compounds include volatile aliphatic acids and non-volatile acids, trigonelline and nicotinic acid (Andrade, Leitao, Seabra, Oliveira, & Ferreira, 1998). Coffee beans also contain a variety of different elements, such as potassium, magnesium, calcium, phosphorus, sulphur, lead, chromium, zinc, copper, nickel and iron (Jaganyi & Madlala, 2000). Phenolic compounds, from 200 to 550 mg per cup in the coffee beverage have been detected (Kiyohara et al., 1999). Among these compounds are chlorogenic acids such as caffeic, ferulic, and *p*-coumaric acid, caffeoylquinic acid, with 5-*O*-caffeoyl-quinic acid being by far the most abundant, feruloyl quinic acid and di-caffeoyl-quinic conjugated with tyrosine, tryptophane or phenylalanine (cinnamoyl-amides depending on origins). Proanthocyanidins are also present in coffee. However, catechins are absent from coffee (Arts, van de Putte, & Hollman, 2000; Clifford & Knight, 2004).

The roasting process leads to profound changes in the chemical composition and biological activities of coffee as a result of the generation of compounds deriving from the Maillard reaction (Czerny, Mayer, & Grosch, 1999), and organic compounds resulting from pyrolysis (Daglia, Papetti, Gregotti, Bertè, & Gazzani, 2000). Sulphur compounds are changed by oxidation and thermal degradation (Kumazawa, Masuda, Nishimura, & Hiraishi, 1998). Vanillin increases strongly during the roasting process (Czerny & Grosch, 2000).

Coffee enhances gastric secretion and urine production (Rakicioglu, Pekcan, & Çevik, 1998), may decrease serum uric acid concentrations (Nardini et al., 2002), reduce the risk of developing gallstone disease (Leitzman et al., 1999), reduce asthma (Schwartz & Weiss, 1992), inhibit histamine and immediate type allergic reactions (Schenker, 2001) and decrease D-galactosamine induced liver injury (Sugiyama, He, Wada, & Saeki, 1999). Paper-filtered coffee does not elevate cholesterol since lipids content and diterpenes are negligible (Urgert & Katan, 1996).

Recently, diets rich in natural foods and food-derived components, such as antioxidative vitamins and phenolic phytochemicals, have received a great deal of attention because they are perceived as "safe" and "non-medicinal"; some are known to function as chemopreventive agents against oxidative damage, cerebrovascular disease, and aging (Lee et al., 2003). Related to this is the observation that 8-hydroxydeoxyguanosine, which is widely used as a biomarker of oxidative DNA, is decreased by coffee drinking in humans (Van Zeeland, de Groot, Hall, & Donato, 1999).

Until now, the main use for coffee use has been for its caffeine for the abatement of drowsiness and fatigue, and for improving most measures of cognitive performance, although, more recently, data on its antioxidant activity have increased interest in the product. This paper aims to evaluate the free radical scavenging activities and the antioxidant capacities of 14 coffees from different countries of origin (varying greatly in price) prepared in three commonly used ways (espresso, filter and Italian coffee).

2. Materials and methods

2.1. Materials

Fourteen commercially available coffees (Arabica and Robusta coffee beans from different origins, Table 1) were supplied by Productos Continental Cafés Salzillo, S.L., Murcia, Spain.

All the chemicals used were of chromatography grade quality and were purchased from Sigma Chemical Co. (Poole, Dorset). The widely used food antioxidants, α-tocopherol (E-307), BHA (E-320), BHT (E-321) and propyl gallate (E-310) (at the permitted concentration of 100 μg/g (FAO/WHO, 1999)) were analysed as antioxidant standards and other phenolic compounds (chlorogenic acid, caffeic acid and *p*-coumaric at 0.5 mM) described in the composition of coffee were used as active standards.

2.2. Samples preparation

The 14 varieties of coffee were obtained from every country by the manufacturing industry during one month. The coffee beans were roasted with a "ROURE K60", for 18–20 min at 190 ± 3 °C (Table 1) depending on the humidity and size of beans in Productos Continental Cafés Salzillo, S.L. and packed into 250 g portions.

The 14 coffee samples were finely ground in a coffee grinder Mod. Molino Negro 1CV Ref. 71.704^{R} (Italy) to a particle size of 0.30 mm. Each coffee sample was prepared in three different coffee makers in our laboratory, according Table 2 in a solid–liquid extraction with deionised water in a Brita filter® (Germany).

Filter coffee beverages were brewed using an electric drip filter coffee maker equipped with a paper filter Melitta (Melitta®, Lisbon, Portugal).

Table 1
Relation of commercially available Arabica and Robusta coffees from different origins indicating roasting temperature and length

Coffea arabica L.	Location geographic	Temperature[a] (°C)	Temperature[b] (°C)	Time[c] (min)
cv. Guatemala var. Maragogype	Antigua 1600/1700 m	165.0	215.0	19.0
cv. Nicaragua var. Maragogype	Masaya 1000/1300 m	170.1	215.1	19.1
cv. Colombia	Medellin 1000/1100 m	169.3	216.2	20.0
cv. Colombia (decaffeinated)	Medellin 1000/1100 m	167.1	210.1	19.3
cv. Ethiopia	Sidamo 1500/2000 m	157.2	215.2	20.0
cv. Kenya	Bungoma 1500/2100 m	169.3	216.2	20.1
cv. Jamaica	Blue Mountain 600/2000 m	170.0	217.3	22.1
cv. Puerto Rico	Yauco 200/1100 m	167.2	216.1	20.0
cv. Puerto Rico "Caracolillo"	Yauco 200/1100 m	170.1	215.2	20.2
cv. Brazil	Patrocinio. Cerrado 900/1200 m	170.0	214.1	19.0
cv. Brazil (decaffeinated)	Minas Gerais 900/1200 m	166.3	207.1	20.0
Coffea canephora L. var. *robusta*				
cv. Vietnam	Darlak 500/900 m	162.0	219.1	20.0
cv. Uganda	Bugisu 1300/1500 m	169.1	215.2	19.5
cv. Papua (Nueva Guinea)	Chimbu 1300/1800 m	160.1	214.1	19.6

[a] Coffee temperature before roasting.
[b] Coffee temperature after roasting.
[c] Roasting time.

Table 2
Preparation of coffees from different origins with three types of coffee maker

Manner of beverages coffee	Ground coffee (g)	Water (ml)	Coffee brew (ml)	Water (°C)	Coffee brew (°C)
Filter	7.0	70.0 ± 5.2	40.2 ± 5.0	90.2 ± 2.2	65.0 ± 5.1
Italian	7.0	55.1 ± 5.1	40.1 ± 5.0	100.1 ± 3.0	90.1 ± 5.1
Espresso	7.0[a]	45.1 ± 5.1[a]	40.1 ± 5.1	90.0 ± 2.1	80.0 ± 5.2

[a] The amount of ground coffee and water volume was established from the instructions provided for the espresso coffee maker. The volume of water used in the other two coffee makers (filter and Italian) was adjusted to obtain the same volume of beverage, using the same quantity of ground coffee.

- "Italian" coffee beverages were brewed by boiling water. The resulting steam passed through the ground coffee into the upper part of the device, where it remained until served. Mod. Mia Express® (Pezzeti, Italy).
- Espresso coffee beverages were made using a coffee maker (8–9 bar pressure) Mod. Seletron Plus® (La Spaziale) (Bologna, Italy).

Aliquots of 42 coffee samples were used for the different assays to determine free radical scavenger capacity and antioxidant activity.

3. Free radicals scavenging assays

3.1. $LOO^{\cdot}$ scavenging as inhibit of peroxidation

The ability of samples to inhibit lipid peroxidation at pH 7.4 was tested using ox-brain phospholipid liposomes, essentially as described in Martínez-Tomé et al. (2004). The experiments were conducted in a physiological saline buffer (PBS) (3.4 mM Na_2HPO_4–NaH_2PO_4 0.15 M NaCl), pH 7.4. In a final volume of 1 ml, the assay mixtures were made up with PBS, 0.5 mg/ml phospholipid liposomes, 100 μM $FeCl_3$, 100 μl of samples (or 100 μl of food common antioxidants dissolved in water), and 100 μM ascorbate (added last to start the reaction). BHT is not fully soluble in aqueous solution, and its emulsion is not homogeneous. In order to dissolve it, de-ionised water with a conductivity of not more than 4 μS/cm was used (Rosas-Romero et al., 1999). Incubations were at 37 °C for 60 min, at the end of which, 1 ml each of 1% (wt/vol) thiobarbituric acid (TBA) and 2.8% (wt/vol) trichloroacetic acid were added to each mixture. The solutions were heated in a water bath at 80 °C for 20 min to develop the malondialdehyde thiobarbituric adduct ($(TBA)_2$-MDA). The $(TBA)_2$-MDA chromogen was extracted into 2 ml of butan-1-ol and the extent of peroxidation was measured in the organic layer as absorbance at 532 nm.

3.2. Hydroxyl radical scavenging

In a final volume of 1.2 ml, the reaction mixtures contained the following reagents: 10 mM KH_2PO_4–KOH buffer (pH 7.4), 2.8 mM H_2O_2, 2.8 mM deoxyribose (where used), 50 μM $FeCl_3$ premixed with 100 μM EDTA before addition to the reaction mixture, and 100 μl of the tested samples (or 100 μl of food common antioxidants dissolved in water). Ascorbate (100 μM), where used, was added to start the reaction. The tubes were incubated at 37 °C for 1 h. The products of the hydroxyl radical ($OH^{\cdot}$) attack upon deoxyribose were measured as described in Murcia et al. (2002).

3.3. Scavenging of hydrogen peroxide

The samples (100 μl) (or 100 μl of common food antioxidants dissolved in water) to be tested with H_2O_2 were incubated with 0.84 mM H_2O_2 for 10 min at 25 °C. Aliquots of these compounds were then taken and assayed for remaining H_2O_2 by using the peroxidase system Murcia, Jiménez, and Martínez-Tomé (2001). The remaining $H_2O_2$was measured as the formation of a cromophore recorded at 436 nm in reaction mixtures containing, in a final volume of 1 ml, 0.150 M KH_2PO_4–KOH buffer, pH 7.4, 50 μl guaiacol solution (made by adding 100 μl of pure guaiacol to 100 mL water) and 10 μl of Sigma type IV horseradish peroxidase (5 mg/mL in the same phosphate buffer).

3.4. Rancimat test for oxidative stability

Sample preparation for the Rancimat test consisted of macerating 25 g butter (free of added antioxidants or preservatives) with 20% of the tested samples (or 100 μg/g common food antioxidants) for 3 h at 38 °C before analysis. Samples were centrifuged for 45 min and 3 g were submitted to analysis.

The Rancimat method (Metrohm® model 743, Herisan, Switzerland) determines the induction period of samples in butter by measuring the increase in the volatile acidic by-products released from the oxidising oil at 110 °C and in an air flow of 20 l/h. The concentration of the degradation products, which are transferred into distilled water, is assessed by measuring the conductivity. Longer induction periods suggest stronger antioxidant activity of the added tested samples. The relative antioxidant activity is expressed by the protection factor (PF), which is calculated by dividing the induction period of butter containing the added of tested samples by the induction period of the control (butter alone) (Murcia et al., 2002).

3.5. Antioxidant activity assays

3.5.1. Determination of antioxidant activity in a linoleic acid system

To a solution of linoleic acid 10 ml (11.7 g/l in 99.8% ethanol) and phosphate buffer 10 ml (200 mM, pH 7.0), 5 ml of the tested sample (or 100 μg/g of the common food antioxidants dissolved in water) were added. The total volume was adjusted to 25 ml with de-ionised water. This solution mixture was incubated at 40 °C and the degree of oxidation was measured. For this, 10 ml of ethanol (75%), 0.2 ml of an aqueous solution of ammonium thiocyanate (30%), 0.2 ml sample (solution mixture) and 0.2 ml ferrous chloride solution (20 mM in 3.5% HCl) were stirred for 3 min. The absorption values of the mixtures measured at 500 nm were taken to reflect the peroxide content. The percent inhibition of linoleic acid peroxidation, 100 − (Abs increase of sample/Abs increase of control) × 100 was calculated to express antioxidative activity (Yen, Wu, & Duh, 1996).

3.5.2. Measurement of total antioxidant activity by TEAC assay

The $ABTS^{\bullet-}$ radical solution was generated from the following reagents: 2.5 mM ABAP, 20 mM $ABTS^{2-}$ stock solution in phosphate buffer solution (PBS) (containing 100 mM phosphate and 150 mM NaCl, pH 7.4). These were incubated at 60 °C for 12 min protected from light and stored at room temperature. Absorbance at 734 nm was measured to check $ABTS^{\bullet-}$ formation (the results must be between 0.35 and 0.45) (van den Berg, Haenen, Van den Berg, & Bast, 1999).

The antioxidant activity of the samples analysed (40 μl mixed with 1960 μl of the radical solution) was measured at 734 nm observed for 6 min. The decrease in absorption at 734 nm observed 6 min after addition of each compound was used to calculate the TEAC.

A calibration curve was prepared with different concentrations of Trolox (standard solution used to evaluate equivalent antioxidant capacity). By measuring the increase in absorption during 6 min (standard range of 0–10 μM), absorbance values were corrected for the solvent.

$$\Delta Abs_{Trolox} = Abs_{t=6\ min\ Trolox} - Abs_{t=6\ min\ solvent}$$

The regression coefficient (r.c.) was calculated from the calibration curve.

$$\Delta Abs_{Trolox} = r.c. \times [Trolox]$$

To establish the TEAC of commercial antioxidants or analysed samples, the increase in absorption was measured in the same way. The TEAC was calculated as follows:

$$TEAC_{sample} = \Delta Abs_{sample}/r.c.$$

The TEAC represents the concentration of a Trolox solution that has the same antioxidant capacity as the analysed sample.

3.5.3. Statistical analysis

All experiments were carried out in triplicate after preparing 10 coffee beverages for every origin. The results were analysed using the Statistical Package for Social Sciences Windows 9.0, and the analysis of variance (ANOVA) procedure. Fisher's least significant difference (LSD) multiple range tests was used to discriminate between means.

4. Results

4.1. Free radical scavenging assays

4.1.1. Antioxidant capacity expressed as scavenging of lipoperoxyl radical

This assay examines whether a substance inhibits the peroxidation of artificial lipid systems, such as brain phospholipid liposomes incubated with $FeCl_3$ and ascorbic acid, by scavenging lipoperoxyl radicals. A decrease in the absorption spectrum after the sample is added indicates that the sample scavenges $LOO^{\bullet}$ radical (Aruoma, Murcia, Butler, & Halliwell, 1993).

Table 3 shows the inhibition of lipid peroxidation in the presence of coffee beverages from different origins compared with the activity of standards (typical coffee compounds and common food additives). All the coffees studied, regardless of origin, were very effective scavengers of lipoperoxyl radicals, in the following decreasing order: Colombia (decaffeinated), Uganda, Papua, Jamaica, ''Caracolillo'', Ethiopia, Kenya, Puerto Rico, Nicaragua, Colombia, Brazil (decaffeinated), Vietnan, Brazil, Guatemala, with no significant differences between them ($p < 0.05$). Both decaffeinated coffees (Colombia and Brazil) exhibited higher scavenging capacity than the equivalent coffees containing caffeine from the same origin.

All the coffee samples analysed showed higher antioxidant activity than typical coffee compounds and common food additives, with significant differences ($p < 0.05$). Among coffee compounds, chlorogenic acid showed the most effective scavenging capacity of lipoperoxyl radical ($p < 0.05$) followed by *p*-coumaric and caffeic acid. Chlorogenic acid showed a higher antioxidant value than BHT and α-tocopherol and lower than BHA and propyl gallate. The results pointed to no significant differences ($p < 0.05$) between the three ways of brewing coffee (espresso, filter and Italian).

4.1.2. Antioxidant capacity expressed as scavenger of hydroxyl radical

Hydroxyl (OH·) radicals are extremely reactive and may be generated under physiological conditions in the human body, where they can react with non-selective compounds such as proteins, DNA, unsaturated fatty acids and almost every biological membrane. The deoxyribose assay is used to detect possible scavengers of OH· radicals, which are formed by a mixture of ascorbate and $FeCl_3$-EDTA. The products of the OH· radical attack upon deoxyribose were evaluated with thiobarbituric acid (Martínez-Tomé et al., 2001).

Table 4 shows the antioxidant activity of the different coffees expressed as the scavenging of OH· radical compared with standards (typical coffee compounds and com-

Table 3
Inhibition of peroxidation in the lipid system using ox brain phospholipids by coffees of different origin compared with the activity of standards (typical coffee compounds and common food additives)[a]

Added to reaction mixtures	% Inhibition	Added to reaction mixtures	% Inhibition
Guatemala		*Brazil*	
Filter coffee	73.1 ± 4	Filter coffee	76.0 ± 1
Italian coffee	77.2 ± 3	Italian coffee	77.1 ± 1
Espresso coffee	78.1 ± 3	Espresso coffee	77.0 ± 1
Nicaragua		*Brazil (decaffeinated)*	
Filter coffee	76.0 ± 2	Filter coffee	80.0 ± 3
Italian coffee	78.0 ± 3	Italian coffee	74.2 ± 4
Espresso coffee	79.1 ± 3	Espresso coffee	78.1 ± 3
Colombia		*''Caracolillo'' Puerto Rico*	
Filter coffee	74.2 ± 4	Filter coffee	79.1 ± 2
Italian coffee	79.2 ± 3	Italian coffee	79.1 ± 1
Espresso coffee	80.1 ± 4	Espresso coffee	77.1 ± 2
Colombia (decaffeinated)		*Puerto Rico*	
Filter coffee	85.0 ± 3	Filter coffee	77.2 ± 2
Italian coffee	81.1 ± 4	Italian coffee	79.2 ± 2
Espresso coffee	78.2 ± 4	Espresso coffee	78.0 ± 1
Vietnam		*Kenya*	
Filter coffee	78.0 ± 1	Filter coffee	78.0 ± 1
Italian coffee	77.2 ± 2	Italian coffee	78.1 ± 1
Espresso coffee	76.4 ± 2	Espresso coffee	79.3 ± 1
Papua		*Jamaica*	
Filter coffee	81.3 ± 2	Filter coffee	79.4 ± 2
Italian coffee	81.2 ± 4	Italian coffee	80.2 ± 1
Espresso coffee	77.1 ± 3	Espresso coffee	78.1 ± 1
Ethiopia		*Uganda*	
Filter coffee	77.0 ± 3	Filter coffee	82.1 ± 2
Italian coffee	80.1 ± 2	Italian coffee	82.0 ± 1
Espresso coffee	78.2 ± 2	Espresso coffee	79.1 ± 3
Standards			
Propyl gallate	51.0 ± 2	Chlorogenic acid	45.1 ± 2
α-Tocopherol	15.3 ± 1	Caffeic acid	18.1 ± 1
BHT	22.3 ± 2	*p*-Coumaric	20.4 ± 1
BHA	71.4 ± 1		

[a] Statistical differences were analysed by ANOVA ($p < 0.05$).

Table 4
Deoxyribose damage by OH· radical in the presence of coffees of different origin compared with the activity of standards (typical coffee compounds and common food additives)[a]

Added to reaction mixtures	Damage to deoxyribose			Added to reaction mixtures	Damage to deoxyribose		
	RM + DR[b]	% Inhibition	Without Asc[c]		RM + DR[b]	% Inhibition	Without Asc[c]
None (control)	1.000 ± 0.01	–	0.191				
Guatemala				*Brazil*			
Filter coffee	0.575 ± 0.03	42.0	0.480	Filter coffee	0.519 ± 0.02	48.1	0.446
Italian coffee	0.517 ± 0.02	48.0	0.403	Italian coffee	0.538 ± 0.02	46.2	0.389
Espresso coffee	0.535 ± 0.01	46.1	0.417	Espresso coffee	0.550 ± 0.03	44.9	0.357
Nicaragua				*Brazil (decaffeinated)*			
Filter coffee	0.643 ± 0.05	41.7	0.490	Filter coffee	0.625 ± 0.01	37.5	0.492
Italian coffee	0.488 ± 0.01	51.1	0.413	Italian coffee	0.552 ± 0.02	44.8	0.361
Espresso coffee	0.516 ± 0.02	48.2	0.348	Espresso coffee	0.621 ± 0.01	37.9	0.412
Colombia				*''Caracolillo'' Puerto Rico*			
Filter coffee	0.499 ± 0.02	50.0	0.443	Filter coffee	0.633 ± 0.02	36.7	0.456
Italian coffee	0.509 ± 0.03	49.1	0.474	Italian coffee	0.586 ± 0.01	41.4	0.389
Espresso coffee	0.439 ± 0.04	56.0	0.474	Espresso coffee	0.581 ± 0.01	41.8	0.342
Colombia (decaffeinated)				*Puerto Rico*			
Filter coffee	0.694 ± 0.05	39.6	0.592	Filter coffee	0.501 ± 0.03	49.9	0.419
Italian coffee	0.531 ± 0.02	47.0	0.508	Italian coffee	0.446 ± 0.02	55.4	0.396
Espresso coffee	0.512 ± 0.03	48.8	0.547	Espresso coffee	0.461 ± 0.01	53.9	0.326
Vietnam				*Kenya*			
Filter coffee	0.532 ± 0.03	46.8	0.483	Filter coffee	0.641 ± 0.03	35.9	0.523
Italian coffee	0.441 ± 0.02	55.8	0.484	Italian coffee	0.614 ± 0.02	38.6	0.446
Espresso coffee	0.417 ± 0.01	51.3	0.473	Espresso coffee	0.582 ± 0.01	41.8	0.422
Papua				*Jamaica*			
Filter coffee	0.557 ± 0.04	44.3	0.579	Filter coffee	0.620 ± 0.03	37.9	0.463
Italian coffee	0.481 ± 0.02	51.8	0.386	Italian coffee	0.580 ± 0.02	41.9	0.420
Espresso coffee	0.474 ± 0.01	52.6	0.371	Espresso coffee	0.561 ± 0.02	43.9	0.380
Ethiopia				*Uganda*			
Filter coffee	0.547 ± 0.03	45.3	0.541	Filter coffee	0.496 ± 0.02	50.4	0.535
Italian coffee	0.455 ± 0.01	54.5	0.413	Italian coffee	0.468 ± 0.01	53.2	0.364
Espresso coffee	0.474 ± 0.01	52.6	0.445	Espresso coffee	0.459 ± 0.02	54.1	0.369
Standards							
Propyl gallate	1.312 ± 0.01	–	0.525	Chlorogenic acid	1.059 ± 0.01	–	0.452
α-Tocopherol	0.967 ± 0.03	3.2	0.178	Caffeic acid	1.136 ± 0.02	–	0.630
BHT	0.910 ± 0.02	8.9	0.149	*p*-Coumaric	0.708 ± 0.01	29.2	0.290
BHA	0.746 ± 0.05	25.4	0.415				

(–) No % inhibition detected.
[a] Statistical differences were analysed by ANOVA ($p < 0.05$).
[b] RM, reaction mixtures; DR, deoxyribose; ASC, ascorbate.
[c] When deoxyribose was omitted, values ranged from 0.001 to 0.006 absorbance units.

mon food additives). All the coffee samples reduced deoxyribose damage (expressed as inhibition percentages). The coffee samples in this assay acted as secondary antioxidants, because, when ascorbate was omitted, the level of pink chromogen exceeded that of the control. Coffee beverages probably react with ascorbate to decrease the amount of OH· generated (Murcia et al., 2002).

The different coffees could be classified according to their antioxidant capacity in decreasing order as follows: Puerto Rico = Uganda = Colombia = Vietnam ⩾ Ethiopia ⩾ Papua ⩾ Nicaragua ⩾ Brazil ⩾ Guatemala ⩾ Colombia (decaffeinated) ⩾ Jamaica ⩾ Brazil (decaffeinated) = "Caracolillo" = Kenya ($p < 0.05$).

All the coffee samples exhibited better antioxidant activity in this assay than the standards analysed. Among the typical coffee compounds, only *p*-coumaric was able to reduce deoxyribose damage better than BHA and BHT, acting, like coffee, as secondary antioxidant in its reaction with ascorbate. However, chlorogenic acid and caffeic acid showed a prooxidant capacity similar to propyl gallate in the face of this radical (Murcia et al., 2001).

The three types of coffee beverage (espresso, filter and Italian) analysed produce good antioxidant activity and there were no significant differences ($p < 0.05$) between them.

4.1.3. Antioxidant activity expressed as hydrogen peroxide scavenging

H_2O_2 may be generated in vivo by several oxidase enzymes or by activated phagocytes in the killing of several

bacterial and fungal strains. There is increasing evidence that H_2O_2, either directly or indirectly via its reduction product, $OH^{\cdot}$, may act as a messenger molecule in the synthesis and activation of several inflammatory mediators. When the samples scavenge the hydrogen peroxide, by using the peroxidase test, there is a decrease in the absorption spectrum measured at 436 nm (Martínez-Tomé et al., 2004).

Coffees such as Ethiopia (64.92%), Kenya (63.46%), Jamaica (60.58%), Colombia (58.60%), Vietnam (58.34%) and Papua (55.12%) exhibited higher percentages of H_2O_2 scavenging than the rest of the coffees and standards analysed.

The reaction of H_2O_2 with coffee beverages and standards is measured after 2 min and 6 h. The results show that antioxidant activity is slightly higher in all the samples analysed after 6 h than after 2 min, such as Ethiopia coffee produces percentages of inhibition of 64.92% and 58.60%, respectively.

As regards the standards, the results show that propyl gallate (30.21%) reacts with H_2O_2 better than typical coffee compounds ($p < 0.05$), which are, in decreasing order, caffeic acid (28.61%), chlorogenic acid (25.30%) and *p*-coumaric acid (8.13%). According to Murcia et al. (2001), the other common food additives do not scavenge H_2O_2.

The three different procedures (filter, Italian and espresso) were analysed and compared. The results for freshly made filter coffee (58.60%) analysed after 2 min showed a better capacity to react with H_2O_2 ($p < 0.05$) than the Italian (37.97%) and espresso coffee beverages (35.52%). When the assay was repeated after 6 h the results with the same beverage were, in decreasing order, filter (64.92%) > Italian (45.32%) > espresso (40.30%).

4.1.4. Oxidative stability in foods elaborated with butter

The Rancimat test, "accelerated oxidation", is used to obtain information on whether a food (rich in oils or fats) resists heating at high temperature. Because different substances can accelerate and/or inhibit the formation of hydroperoxides, it is possible to evaluate the protection they offer. The time required for the formation of a sufficient concentration of initiating radicals is greater when samples with antioxidant activity are added, delaying the onset of the propagation phase of the radical chain reaction (Martínez-Tomé et al., 2001).

Results show the effect of coffees from different origins on the oxidative stability of butter expressed as protection factor in the following order of stability: Vietnam ⩾ Colombia (decaffeinated) ⩾ Colombia ⩾ "Caracolillo" = Jamaic ⩾ Nicaragua = Guatemala = Kenya = Puerto Rico ⩾ Brazil (decaffeinated) ⩾ Ethiopia = Papua ⩾ Uganda ⩾ Brazil ($p < 0.05$).

The espresso coffee shows PF values between 1.48 and 3.33, and Italian coffee between 1.20 and 3.14. The espresso and Italian coffee beverages offered greater protection to butter ($p < 0.05$) than the filter beverages (PF between 2.21 and 0.83). As regards the standards, caffeic acid (PF = 2.78) provided better protection ($p < 0.05$) than the other two typical coffee compounds analysed. Among common food additives propyl gallate exhibits the best protection factor (6.48) followed by α-tocopherol (PF = 3.17), BHA (PF = 2.40) and BHT (PF = 1.40) (Murcia et al., 2001).

4.2. Antioxidant activity assays

4.2.1. Total antioxidant activity evaluation during storage

The linoleic acid system, which is used to determine antioxidant activity during storage at unfavourable temperatures (40 °C), measures the inhibition of linoleic acid autoxidation (Murcia et al., 2001).

Fig. 1 shows the absorbance values obtained for the autoxidation of linoleic acid during 28 days of storage in the presence of coffee beverages from different origins and prepared with different types of coffee maker, compared with standards. The statistical data show no significant differences between the results obtained with the 14 different coffees analysed. However, filter brews showed stronger antioxidant activity ($p < 0.05$) than the espresso and Italian coffee beverages.

According to the statistical analyses, the differences could be divided into five groups ($p < 0.05$). The first group includes common food additives with high antioxidant activity, such as propyl gallate (97% inhibition on the 28th day of storage) and BHT (96%). A second group includes BHA and filter coffee (from different origins), which exhibited good levels of antioxidant activity after 28 days of storage, with 84% and 79% inhibition, respectively. A third group includes Italian and espresso coffee (from different origins) with inhibition percentages of around 65%. A fourth group is constituted by α-tocopherol, with medium levels of antioxidant activity after 28 days of storage (27% inhibition). A fifth group includes substances with no antioxidant activity, such as caffeic acid, *p*-coumaric acid and chlorogenic acid, which showed absorbances similar to that of the control (Fig. 1).

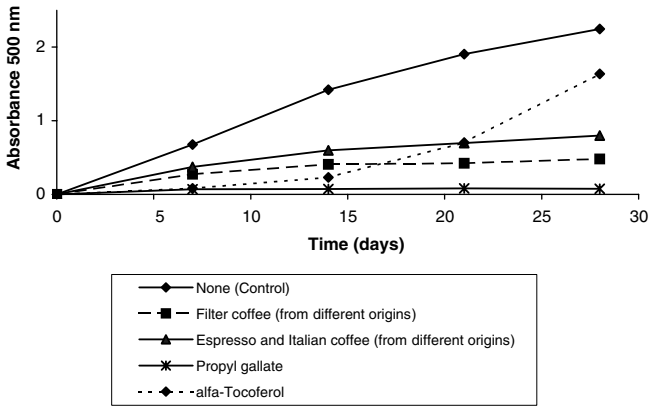

Fig. 1. Evolution of the absorbance at 500 nm for the oxidation of linoleic acid in the presence of coffees from different origin and coffees prepared with different types of coffee maker compared with the activity of standards (typical coffee compounds and common food additives) during 28 days of storage.

4.2.2. Total antioxidant activity evaluation as TEAC

Foods are high in antioxidant structures, such as vitamins and phenolic compounds and flavonoids, which may even have an additive or synergistic effect. The scavenging capacity of ABTS radical anions generated from ABAP and $ABTS^{2-}$, compared with that of Trolox (water-soluble analogue of vitamin E) is called TEAC (Trolox equivalent antioxidant capacity). A TEAC value can be assigned to all compounds able to scavenge the $ABTS^{\bullet-}$ by comparing their scavenging capacity to that of Trolox. Quantitative evaluation of the antioxidant capacity using TEAC can be used to provide a ranking order of antioxidants (Murcia et al., 2001).

Table 5 shows TEAC value at 6 min and 24 h obtained for coffees from different origins compared with standards. The results are Vietnam, Uganda, Nicaragua, Colombia, Brazil, "caracolillo", Puerto Rico, Guatemala, Kenya, Papua, decaffeinated Colombia, Ethiopia, Jamaica, and decaffeinated Brazil, in decreasing order for samples at 6 min. Decaffeinated coffees (Colombia and Brazil) show lower TEAC values than their equivalents with caffeine.

The TEAC values (24 h) shown by the coffee beverages were, in decreasing order, Vietnam, Nicaragua, Brazil, Guatemala, "caracolillo", Colombia, Puerto Rico, Uganda, decaffeinated Brazil, decaffeinated Colombia, Ethiopia, Jamaica, Kenya, and Papua. Decaffeinated Colombia and Brazil were slightly less effective $ABTS^{\bullet-}$ scavengers than that their equivalents containing caffeine.

Of the standards, propyl gallate exhibited a higher TEAC value (6 min) than the rest of the common food additives and typical coffee compounds. Chlorogenic acid and caffeic acid also showed better results than *p*-coumaric,

Table 5
Scavenging of ABTS radical anions by coffees of different origin compared with the activity of standards (typical coffee compounds and common food additives)[a]

Samples	TEAC[b]		Samples	TEAC[b]	
	Time: 6 min	24 h		Time: 6 min	24 h
Guatemala			*Brazil*		
Filter coffee	10.32 ± 0.04	12.92 ± 0.02	Filter coffee	10.63 ± 0.07	13.54 ± 0.07
Italian coffee	10.21 ± 0.05	12.46 ± 0.04	Italian coffee	10.91 ± 0.04	12.02 ± 0.01
Espresso coffee	8.67 ± 0.01	11.11 ± 0.07	Espresso coffee	9.38 ± 0.02	11.24 ± 0.07
Nicaragua			*Brazil (decaffeinated)*		
Filter coffee	12.29 ± 0.07	13.46 ± 0.07	Filter coffee	7.75 ± 0.07	12.95 ± 0.07
Italian coffee	11.51 ± 0.06	12.42 ± 0.01	Italian coffee	9.68 ± 0.05	11.78 ± 0.05
Espresso coffee	8.74 ± 0.02	11.23 ± 0.03	Espresso coffee	7.80 ± 0.01	10.59 ± 0.01
Colombia			*"Caracolillo" Puerto Rico*		
Filter coffee	12.13 ± 0.04	13.40 ± 0.03	Filter coffee	10.91 ± 0.06	13.33 ± 0.04
Italian coffee	10.34 ± 0.01	11.63 ± 0.02	Italian coffee	11.00 ± 0.01	12.08 ± 0.05
Espresso coffee	8.60 ± 0.03	10.99 ± 0.06	Espresso coffee	8.46 ± 0.03	10.84 ± 0.01
Colombia (decaffeinated)			*Puerto Rico*		
Filter coffee	8.06 ± 0.07	12.91 ± 0.07	Filter coffee	9.54 ± 0.07	13.27 ± 0.07
Italian coffee	10.85 ± 0.03	11.95 ± 0.05	Italian coffee	10.66 ± 0.02	11.67 ± 0.02
Espresso coffee	8.54 ± 0.06	10.32 ± 0.02	Espresso coffee	9.20 ± 0.04	11.04 ± 0.04
Vietnam			*Kenya*		
Filter coffee	11.90 ± 0.03	13.41 ± 0.01	Filter coffee	10.69 ± 0.07	13.11 ± 0.07
Italian coffee	12.82 ± 0.05	12.32 ± 0.05	Italian coffee	10.28 ± 0.02	11.39 ± 0.02
Espresso coffee	11.42 ± 0.07	11.23 ± 0.01	Espresso coffee	7.36 ± 0.04	9.96 ± 0.04
Papua			*Jamaica*		
Filter coffee	10.07 ± 0.07	13.10 ± 0.06	Filter coffee	8.82 ± 0.07	12.93 ± 0.05
Italian coffee	10.42 ± 0.01	11.76 ± 0.01	Italian coffee	9.51 ± 0.05	11.51 ± 0.01
Espresso coffee	7.31 ± 0.07	9.46 ± 0.07	Espresso coffee	6.97 ± 0.02	10.42 ± 0.06
Ethiopia			*Uganda*		
Filter coffee	9.80 ± 0.07	13.07 ± 0.02	Filter coffee	10.95 ± 0.07	13.38 ± 0.05
Italian coffee	8.77 ± 0.04	11.21 ± 0.04	Italian coffee	11.85 ± 0.05	11.75 ± 0.03
Espresso coffee	8.23 ± 0.02	10.76 ± 0.07	Espresso coffee	10.51 ± 0.04	10.84 ± 0.05
Standards					
Propyl gallate	17.20 ± 0.01	17.44 ± 0.01	Chlorogenic acid	14.80 ± 0.01	>19
α-Tocopherol	1.10 ± 0.04	2.30 ± 0.04	Caffeic acid	12.40 ± 0.02	>19
BHT	0.26 ± 0.02	0.72 ± 0.02	*p*-Coumaric	2.70 ± 0.01	>19
BHA	0.44 ± 0.04	1.41 ± 0.04			

[a] Statistical differences were analysed by ANOVA ($p < 0.05$).

[b] TEAC is the micromolar concentration of a Trolox solution having the antioxidant capacity equivalent to the dilution of the substance under investigation.

α-tocopherol, BHT and BHA. However, all the typical coffee compounds analysed after 24 h showed higher TEAC values than all the coffee brews and common food additives analysed, perhaps because they show slow antioxidant behaviour.

Table 5 also shows that coffees made with filter and Italian coffee makers and analysed after 6 min exhibited higher TEAC value than espresso coffees ($p < 0.05$). When coffee samples were analysed after 24 h, the differences between the three type of coffee beverage had increased ($p < 0.05$), being in decreasing order filter >Italian > espresso.

Good TEAC values were also shown by Steinhart, Luger, and Piost (2001), with important variations when the incubation time increased. The difference between the antioxidant capacity at the two times was due to the different sizes of the complex molecules. The bigger the molecule, the lower the antioxidant capacity because of the steric hindrance of complex molecules (Pellegrini et al., 2003).

5. Discussion

Coffee was better than cereal in inhibiting lipid peroxidation in the DPPH assay and decreased diene conjugates (Krings & Berger, 2001). It is more efficient than cocoa or black tea in delaying LDL oxidation (Illy & Viani, 1995). Caffeine has a significant ability to scavenge highly reactive free radicals as $LOO^{\cdot}$ when rat liver microsomes are used, protecting crucial biological molecules against these species. This ability is rather similar to that of the well established biological antioxidant, glutathione, and significantly much higher than that of ascorbic acid. The reaction of oxygen with electrons and hydrogen atoms results in the formation of very potent oxidising intermediates, such as H_2O_2. The removal of electrons by caffeine in competition with oxygen spares cells from excessive oxidative damage (Devasagayam & Kesavan, 1996). Using the TRAP assay, it also showed pronounced peroxyl radical scavenging and a capacity to neutralise $OH^{\cdot}$ (Natella, Nardini, Giannetti, Dattilo, & Scaccini, 2002).

The volatile heterocyclics of coffee possess antioxidant activity in the aldehyde-$OH^{\cdot}$ system. This activity is not as strong as BHT, however, because a tremendous number of these compounds are present in coffee, their activity perhaps being comparable to those of known antioxidants (Fuster, Mitchell, Ochi, & Shibamoto, 2000). The heterocyclic compounds in the water insoluble coffee fraction revealed inhibitory activity over 40 days (Yanagimoto, Ochi, Lee, & Shibamoto, 2004).

Similar data to our results were previously observed in a Rancimat test (Aeschbach & Rossi, 1995) and in the linoleate system at 40 °C, where trigonelline was suggested as the cause (51). Coffee brews have a high antioxidant efficiency corresponding to about 30 mg of Trolox per g of dry matter (Schwarz et al., 2001). In the B-carotene-linoleic acid system and using TEAC, Pellegrini et al. (2003) established a good position for coffee, followed by citrus, among other beverages, the activity decreasing by 25–30% in decaffeinated espresso coffee. However, our results for decaffeinated coffee agree with those of Richelle, Tavazzi, and Offord (2001) the antioxidant activity being similar to that of caffeinated coffee. The main reason in the variation for specially treated coffee is the loss of chlorogenic acid, because about 10–20% of antioxidant activity is lost during steam treatment (Steinhart et al., 2001).

The substituted phenols are more hydrophobic than their non-substituted counterparts, which may enhance access to the detergent-emulsified lipids to counteract intermediate $LOO^{\cdot}$ (Stadler, Markovic, & Turesky, 1995), and are better than α-tocopherol and ascorbic acid acting as an H-atom donor terminating the chain radical reaction (Ohnishi, Morishita, Toda, Yase, & Kido, 1998).

Although Richelle, Tavazzi and Offord, in 2001, observed that the antioxidant activity of Robusta was double that of Arabica, both two species of coffee showed similar antihydroxyl radical activity of around 48% with no differences between the samples (Daglia et al., 2004). There are several antioxidant compounds in coffee each with different polar and hydrophilic features. The difference in the levels of reducing substances observed by some authors between Arabica and Robusta are probably due to their different polyphenol compound content and the different rates at which these are lost during roasting and the successive formation of other antioxidant compounds, such as MRP or pyrolysis products (Daglia et al., 2000). The inhibitory activity from linoleic acid increased in the order caffeic acid < 5-caffeoylquinic acid < caffeoyltryptophan < DL-α-tocopherol (Ohnishi et al., 1998). As a consequence, roasted coffee shows an increase in antioxidant activity of about 25%. Although one of the main active compounds, chlorogenic acid, decreases, one possible explanation might also be the incorporation of fragments of these decomposed compounds in the melanoidin structure (Steinhart et al., 2001).

Melanoidins, produced among MRP, are one of the major components of coffee beverages, greatly contributing to the whole coffee antiradical activity through different mechanisms, e.g., chain breaking, oxygen scavenging or metal chelating (Illy & Viani, 1995).

There have been several studies on the health-promoting effects of bioactive chlorogenic acid and its absence in the body after drinking coffee. However, doubts exist about the bioavailability of chlorogenic acid (Sánchez-González, Jiménez-Escrig, & Saura-Calixto, 2005). This compound, which is esterified with sugars and lipids, undergoes hydrolysis in the gastrointestinal tract by cytosolic estearases (which is poorly absorbed in the small intestine and 33% in the gut) in the gut mucose or by the gut microflora. The caffeic acid released is then absorbed and enters the vascular system (Kiyohara et al., 1999). It has been found in plasma and liver (Gonthier, Verny, Besson, Rémésy, & Scalbert, 2003).

Similar to our findings, Sánchez-González et al. (2005) observed a high correlation between the polyphenol

content and the FRAP values in different types of coffee (filter > espresso > Italian).

In conclusion, this paper is based on "in vitro" results using lower amounts of coffee than normally consumed daily by the average consumer, although there are still losses by bioavailability. Because of the number of factors that may influence the antioxidant activity of the compounds and of the foods/beverages, these activities should be assessed in conditions as close as possible to those which occur in real life. For this reason the beverages were elaborated in three commonly used ways.

Since there are differences in the free radical-gene rating system, molecular target, end point, kinetic, biological matrix, residence in lipo and hydrophilic compartment and physiological relevance, the influence of all these parameters cannot be evaluated using only one assay protocol (Pulido, Hernández-García, & Saura-Calixto, 2003).

Accurate data on population-wide intakes of phenolic and antioxidant compounds are not available (Karakaya, Ei, & Tas, 2001), although it is known that an intake of 96 g of coffee beverage is sufficient to provide the RDA of vitamin C and E (Pulido et al., 2003); a consumer intake of 4–5 cups of coffee/day has been estimated to cover around 64% of the total dietary antioxidant capacity (Svilaas et al., 2004). Coffee, then should be considered another food to be taken into account in nutritional and epidemiological studies (Saura-Calixto & Goñi, 2006).

All the coffees studied are good antioxidants, the high or low price according to origin and the way in which it is brewed (espresso, filter or Italian) having no bearing on the antioxidant capacity, which is a point worth considering.

Acknowledgements

This work was funded by *Consejería de Ciencia, Tecnología, Industria y Comercio* project from *Región de Murcia* (Spain), Ref. No. 30476 and a research contract with Productos Continental Cafés Salzillo, S.L., Murcia (Spain).

References

Aeschbach, R., & Rossi, P. (1995). Novel approach for antioxidant extraction. *21st world congress of the international society for fat research* (Vol. 2). Bridgwater, England: Barnes & Associates.

Andrade, P. B., Leitao, R., Seabra, R. M., Oliveira, M. B., & Ferreira (1998). 3,4-Dimethoxycinnamic acid levels as a tool for differentiation of *Coffea canephora* var. *robusta* and *Coffea arabica*. *Food Chemistry, 61*, 511–514.

Arts, I. C. W., van de Putte, B., & Hollman, P. C. H. (2000). Catechin contents of food commonly consumed in the Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. *Journal of Agricultural and Food Chemistry, 48*, 1752–1757.

Aruoma, O. I., Murcia, A., Butler, J., & Halliwell, B. (1993). Evaluation of the antioxidant and prooxidant actions of gallic acid and its derivatives. *Journal of Agricultural and Food Chemistry, 41*, 1880–1885.

Casella, I. G., Gatta, M., & Desimoni, E. (1998). Applications of a copper-modified gold electrode for amperometric detection of polar aliphatic compounds by anion-exchange chromatography. *Journal of Chromatography A, 814*, 63–70.

Clifford, M. N., & Knight, S. (2004). The cinnamoyl-amino acid conjugates of green robusta coffee beans. *Food Chemistry, 87*, 457–463.

Czerny, M., & Grosch, W. (2000). Potent odorants of raw arabica coffee. Their changes during roasting. *Journal of Agricultural and Food Chemistry, 48*, 868–872.

Czerny, M., Mayer, F., & Grosch, W. (1999). Sensory study on the character impact odorants of roasted arabica coffee. *Journal of Agricultural and Food Chemistry, 47*, 695–699.

Daglia, M., Papetti, A., Gregotti, C., Bertè, F., & Gazzani, G. (2000). In vitro antioxidant and ex vivo protective activities of green and roasted coffee. *Journal of Agricultural and Food Chemistry, 48*, 1449–1454.

Daglia, M., Racchi, M., Papetti, A., Lanni, C., Govoni, S., & Gazzani, G. (2004). In vitro and ex vivo antihydroxyl radical activity of green and roasted coffee. *Journal of Agricultural and Food Chemistry, 52*, 1700–1704.

Devasagayam, T. P. A., & Kesavan, P. C. (1996). Radioprotective and antioxidant action of caffeine: mechanistic considerations. *Indian Journal of Experimental Biology, 34*, 291–297.

FAO/WHO. Codex Alimentarius. Rome. Italy; 1999.

Fuster, M. D., Mitchell, A. E., Ochi, H., & Shibamoto, T. (2000). Antioxidative activities of heterocyclic compounds formed in brewed coffee. *Journal of Agricultural and Food Chemistry, 48*, 5600–5603.

Gonthier, M. P., Verny, M. A., Besson, C., Rémésy, C., & Scalbert, A. (2003). Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. *Journal of Nutrition, 133*, 1859.

González, A. G., Pablos, F., Martín, M. J., León-Camacho, M., & Valdenebro, M. S. (2001). HPLC analysis of tocopherols and triglycerides in coffee and their use as authentication parameters. *Food Chemistry, 73*, 93–101.

Hostettmann, K., Marston, A., Ndjoko, K., & Wolfender, J. L. (2000). The potential of African plants as a source of drugs. *Current Organic Chemistry, 4*, 973–1010.

Hu, Q., Zhou, T., Zhang, L., & Fang, Y. (2001). Study of the separation and determination of monosaccharides in soluble coffee by capillary zone electrophoresis with electrochemical detection. *Analyst, 126*, 298–301.

Illy, A., & Viani, R. (1995). The plant. In *Espresso coffee: The chemistry of quality* (pp. 9–38). London, UK: Academic Press.

Jaganyi, D., & Madlala, S. P. (2000). Kinetics of coffee infusion: a comparative study on the extraction kinetics of mineral ions and caffeine from several types of medium roasted coffees. *Journal of the Science Food and Agriculture, 80*, 85–90.

Karakaya, S., Ei, S. N., & Tas, A. A. (2001). Antioxidant activity of some foods containing phenolic compounds. *International Journal of Food Sciences and Nutrition, 52*, 501–508.

Kiyohara, C., Kono, S., Honjo, S., Todoroki, I., Sakurai, Y., Nishiwaki, M., et al. (1999). Inverse association between coffee drinking and serum uric acid concentrations in middle-aged Japanese males. *British Journal of Nutrition, 82*, 125–130.

Krings, U., & Berger, R. G. (2001). Antioxidant activity of some roasted foods. *Food Chemistry, 72*, 223–229.

Kumazawa, K., Masuda, H., Nishimura, O., & Hiraishi, S. (1998). Change in flavor of coffee drink during heating. *Nippon Shokuhin Kagaku Kogaku Kaishi, 45*, 108–113.

Kurzrock, T., & Speer, K. (2001). Diterpenes and diterpene esters in coffee. *Food Review International, 17*, 433–450.

Lee, D. H., Jacobs, D. R., Gross, M., Kiefe, C. I., Roseman, J., Lewis, C. E., et al. (2003). Gamma glutamyltransferase is a predictor of incident diabetes and hypertension: the CARDIA study. *Clinical Chemistry, 49*, 1358–1366.

Leitzman, M. F., Wilett, W. C., Rimm, E. B., Stampher, M. J., Spiegelman, D., Colditz, G. A., et al. (1999). A prospective study of coffee consumption and the risk of symptomatic gallstone disease in men. *The Journal of the American Medical Association, 281*, 2106–2112.

Martínez-Tomé, M., Jiménez, A. M., Ruggieri, S., Frega, N., Strabbioli, R., & Murcia, M. A. (2001). Antioxidant properties of Mediterranean spices compared with common food additives. *Journal of Food Protection, 64*, 1412–1419.

Martínez-Tomé, M., Murcia, M. A., Frega, N., Ruggieri, S., Jiménez, A. M., Roses, F., et al. (2004). Evaluation of antioxidant capacity of cereal brans. *Journal of Agricultural and Food Chemistry, 52*, 4690–4699.

Mazzafera, P. (1999). Chemical composition of defective coffee beans. *Food Chemistry, 64*, 547–554.

Murcia, M. A., Jiménez, A. M., & Martínez-Tomé, M. (2001). Evaluation of the antioxidant properties of Mediterranean and Tropical fruits compared with common food additives. *Journal of Food Protection, 65*, 2037–2046.

Murcia, M. A., Martínez-Tomé, M., Jiménez, A. M., Vera, A., Honrubia, M., & Parras, P. (2002). Antioxidant activity of edible fungi (truffles and mushrooms): losses during industrial processing. *Journal of Food Protection, 65*, 1614–1622.

Nardini, M., Cirillo, E., & Sacaccini, C. (2002). Absorption of phenolic acids in humans after coffee consumption. *Journal of Agricultural and Food Chemistry, 50*, 5735–5741.

Natella, F., Nardini, M., Giannetti, I., Dattilo, C., & Scaccini, C. (2002). Coffee drinking influences plasma antioxidant capacity in humans. *Journal of Agricultural and Food Chemistry, 50*, 6211–6216.

Ohnishi, M., Morishita, H., Toda, S., Yase, Y., & Kido, R. (1998). Inhibition in vitro linoleic acid peroxidation and haemolysis by caffeoyltryptophan. *Phytochemistry, 47*, 1215–1218.

Pellegrini, N., Serafini, M., Colombi, B., Del Río, D., Salvatore, S., Bianchi, M., et al. (2003). Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *Journal of Nutrition, 133*, 2812–2819.

Pulido, R., Hernández-García, M., & Saura-Calixto, F. (2003). Contribution of beverages to the intake of lipophilic and hydrophilic antioxidants in the Spanish diet. *European Journal of Clinical Nutrition, 57*, 1275–1282.

Rakicioglu, N., Pekcan, G., & Çevik, A. (1998). The effect of coffee and caffeine consumption on serum lipids in rats. *International Journal of Food Science and Nutrition, 49*, 441–445.

Richelle, M., Tavazzi, I., & Offord, E. (2001). Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving. *Journal of Agricultural and Food Chemistry, 49*, 3438–3442.

Rojo Camargo, M., Toledo, C. F., & Farah, G. (1999). Caffeine daily intake from dietary sources in Brazil. *Food Additives Contaminants, 16*, 79–87.

Rosas-Romero, A. J., Rojano, B., Hernández, C. A., Martínez Manchado, C., Silva, J., & Herrera, J. C. (1999). A novel approach to quantitative structure-property relationships in antioxidants. *Ciencia, 7*, 78–87.

Sánchez-González, I., Jiménez-Escrig, A., & Saura-Calixto, F. (2005). In vitro antioxidant activity of coffees brewed using different procedures (Italian, espresso and filter). *Food Chemistry, 90*, 133–139.

Saura-Calixto, F., & Goñi, I. (2006). Antioxidant capacity of the Spanish Mediterranean diet. *Food Chemistry, 94*, 442–447.

Schenker, S. (2001). Coffee drinking: grounds for concern? British Nutrition Foundation. *Nutrition Bulletin, 26*, 5–6.

Schwartz, J., & Weiss, S. T. (1992). Caffeine intake and asthma symptoms. *Ann Epidemiology, 2*, 627–635.

Schwarz, K., Bertelsen, G., Nissen, L. R., Gardner, P. T., Heinonen, M. I., Hopia, A., et al. (2001). Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. *European Food Research and Technology, 212*, 319–328.

Stadler, R. H., Markovic, J., & Turesky, R. (1995). Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. *Biology Trace Element Research, 47*, 299–305.

Steinhart, H., Luger, A., Piost, J. (2001). Antioxidative effect of coffee melanoidins. In: Colloque Scientifique International sur le Café 19th ed; (pp. 67–74). ASIC.

Sugiyama, K., He, P., Wada, S., & Saeki, S. (1999). Teas and other beverages suppress D-galactosamine-induced liver injury in rats. *Journal of Nutrition, 129*, 1361–1367.

Svilaas, A., Sakhi, A. K., Andersen, L. F., Svilaas, T., Ström, E. C., Jacobs, D. R., et al. (2004). Intakes of antioxidants in coffee, wine, and vegetables are correlated with plasma carotenoids in humans. *Journal of Nutrition, 134*, 562–567.

Urgert, R., & Katan, M. B. (1996). The cholesterol-raising factor from coffee beans. *Journal of the Royal Society of Medicine, 89*, 618–621.

van den Berg, R., Haenen, G. R. M. M., Van den Berg, H., & Bast, A. (1999). Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chemistry, 66*, 511–517.

Van Zeeland, A. A., de Groot, A. J. L., Hall, J., & Donato, F. (1999). 8-Hydroxydeoxyguanosine in DNA from leukocytes of healthy adults: relationship with cigarette smoking, environmental tobacco smoke, alcohol and coffee consumption. *Mutation Research, 439*, 249–257.

Yanagimoto, K., Ochi, H., Lee, K. G., & Shibamoto, T. (2004). Antioxidative activities of fractions obtained from brewed coffee. *Journal of Agricultural and Food Chemistry, 52*, 592–596.

Yen, G. C., Wu, S. C., & Duh, P. D. (1996). Extraction and Identification of antioxidant components from the leaves of mulberry (*Morus alba* L.). *Journal of Agricultural and Food Chemistry, 44*, 1687–1690.