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In vitro antioxidant activity of coffees brewed using different procedures (Italian, espresso and filter)

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Abstract

The possible effects of different preparation methods (Italian or Mocha, Filter and Espresso) on the antioxidant activities of brewed coffee were assessed using two methods: Ferric reducing power (FRAP) and scavenging capacity (ABTS). In addition, the polyphenol content was estimated. The order of ferric reducing ability per gram of dry matter (dm) of the different brewed coffees tested, in terms of the coffee-making procedure used, was freeze-dried > filter ≈ espresso ≈ Italian. The order of ferric reducing ability per serving was filter > espresso > freeze-dried ≈ Italian. In the case of scavenging activity the order was similar to that described for the FRAP assay. There was a high correlation between the estimated polyphenol contents and the FRAP, or the ABTS values (r: 0.98, $P < 0.01$; r: 0.99, $P < 0.01$, respectively). In the case of FRAP and ABTS assays; a serving of filtered coffee was equivalent to 2653 ± 297 and 1295 ± 262 μg trolox, respectively. In the USA and Northern Europe, the pot containing the coffee is usually kept hot (85 °C) for several hours. We found that antioxidant activity increased significantly (by 34%) after four hours of heating. The cause of this increase would seem to be the formation of Maillard products, due to the heat process. These compounds also appear to be responsible for the fact that antioxidant capacity was higher in dark-roast than in other brewed coffees tested. Antioxidant activity decreased when milk was added to the espresso coffee.

Keywords: Coffees; Processing; Total phenolics; Antioxidant activity; Trolox equivalent antioxidant capacity; Radical scavenging; Reductive power

1. Introduction

Coffee is a drink prepared by extraction, in boiling water, of the soluble material from roasted grounds that come from a tropical bush named "*cafeto*" (Desroisier, 1987). The generic name covers over sixty different species, only three of which, *Coffea arabica* L., *Coffea robusta* L. and *Coffea iberica* L., have commercial value (Montero, Marqués, & Muniz, 1999). The first, which originated in Ethiopia, is the most widespread and is the source of 80% of world coffee production (de la Mota, 1991; Desroisier, 1987).

Regarding physiological and metabolic activities, coffee is known for its tonifying qualities, which activate the nervous system, enhance perception and reduce fatigue. Most of these activities are associated with caffeine. Some authors (Corti et al., 2002; Klag, Wang, Meoni, Bracanti, & Ford, 2003) suggest that these activities could be associated with compounds other than caffeine that raise blood pressure and activate the sympathetic nervous system.

Studies on the association between coffee consumption and degenerative diseases have produced contradictory results. Checkoway, Powers, Smith-Weller, Franklin, and Swanson (2002) and Mikuls et al. (2002) found no relationship between them, and Varnam, Jane, and Sutherland (1997) found no significant association with the incidence of cancer. As regards cardiovascular diseases, some authors (Aro, Tuomilehro, & Pietinen, 1987; Bak & Grobee, 1989; D'Amicis, Scaccini, & Bernini, 1996; Van Rooij et al., 1995; Zock, Katan, & Harryran, 2002) have shown that coffee, when boiled as opposed to being filtered, raises the total cholesterol level by 10%, with consequent harm to human health, and they have shown that the diterpenic compounds

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cafestol and kaweol are responsible for this effect on plasma.

It is known that coffee has antioxidant properties (Castillo, Ames, & Gordon, 2002; Natella, Nardini, Granneti, Dattilo, & Scaccini, 2002; Pojjana, Ames, & del Castillo, 2002; Yanagimoto, Lee, Ochi, & Shibamoto, 2002). These properties have a role in the prevention of diabetes, arteriosclerosis, neurodigestive diseases and cancer, which will depend partly on the lack of oxidant balance in the body (McBrian & Slater, 1982; Meyer, Heinonen, & Frankel, 1998). Sources of oxidant activity in coffee are the phenolic compounds (Lajolo, Saura-Calixto, Penna, & Wenzel, 2001), specifically chlorogenic acids (Olthof, Zock, & Katn, 2001), concentrations of which range from 6% to 10% of the dry weight of the grounds. The antioxidant capacity of caffeine has been assessed (Devasagayam, Kamat, Mohan, & Kesavan, 1996; Shi, Dalal, & Join, 1991), and it has been shown to have a major radioprotective effect on tissues (George, Hebbor, Kale, & Kesavan, 1999). Lee (2000) suggests that the source of caffeine's antioxidant capacity is its degradation into the metabolites methylxanthine and methylurgic acid.

The possible effects of different preparation methods on the antioxidant properties of related beverages, such as green and black tea, have also been assessed (Langley-Evans, 2000). However, there has been no study concerning the antioxidant properties of brewed coffee on the basis of the different preparation procedures.

Coffee was once thought to be the first antioxidant source in beverages in the Spanish diet (Pulido, Hernández, & Saura-Calixto, 2003), and it seems that processing affects the coffee's physiological properties. In light of that, the object of this study was to assess antioxidant activity in coffee on a multifunctional basis, in vitro (Frankel & Meyer, 2000).

2. Materials and methods

2.1. Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of vitamin E, was from Aldrich Co (St Louis, MO). 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) was from Fluka Chemicals (Madrid, Spain). ABTS 2,2′-azinobis-(3-ethyl benzothiazoline-6-sulphonic acid) was purchased from Fluka Chemicals, Steinheim, Germany Sigma Chemical Co. (Madrid, Spain). All reagents used were of analytical grade.

The water used was double-distilled (18.2 meqΩ/cm) using a Milli Q System (Millipore Ibérica, Spain).

2.2. Materials

Several varieties of ground Columbian coffee were selected (*C. arabica* L.) by La Mexicana S.A. Madrid (Spain): Medium roasted coffee (*natural*), dark-roasted coffee (roasted with sugar, *torrefacto*), a 70%/30% mixture of medium-roasted and dark-roasted coffee, freeze-dried coffee, and with the exception of dark-roasted coffee, their decaffeinated counterparts. These samples are described in Table 1.

Table 1
Description of tested samples

Type of coffee	Samples	Denomination
Italian or Mocca pot	Medium roasted coffee	IT1
	Mixed[a]	IT2
	Medium roasted coffee decaffeined	IT3
	Mixed decaffeinated	IT4
	Dark-roasted coffee	IT5
Espresso or food service industry	Medium roasted coffee	EX1
	Mixed[a]	EX2
	Medium roasted coffee decaffeined	EX3
	Mixed decaffeinated[a]	EX4
	Dark-roasted coffee	EX5
Filter electric coffee	Medium roasted coffee	F1
	Mixed[a]	F2
	Medium roasted coffee decaffeined	F3
	Mixed decaffeinated[a]	F4
	Dark-roasted coffee	F5
Freeze-dried	Whole	FD1
	Decaffeinated	FD2

[a] Mixed: 70% Medium roasted coffee and 30% Dark-roasted coffee.

Coffees were ground to one of three granule sizes (0.55, 0.43, 0.67 mm mesh) depending on the coffee-making procedure used (Italian or Mocha pot, espresso or food service industry, or electric filter coffee-maker).

2.3. Methods

2.3.1. Extraction systems

The most common means of extracting coffee in households, hotels and restaurants is based on hot water or vapour. Several methods were tested:

2.3.1.1. Italian or mocca coffee pot. The coffee pot is made of metal and consists of an upper, a middle and lower parts; the upper and lower parts are screwed together. The lower part contains the water and has a safety valve to allow vapour to escape if the passage to the upper part is blocked. The middle part contains the coffee and is inserted into the lower part. When the coffee pot is heated on the hob, the air remaining in the lower part expands and pushes the water. The water, at a temperature of 93 °C, flows up a tube and through the middle part where the coffee is. The beverage is gathered in the upper part. Extraction time is short (about one minute).

2.3.1.2. Filter or drop electric coffee-maker. This consists of a structure incorporating a water tank with a resistor for heating and a hot-plate, a filter-holder and a jug. To make coffee, the tank is filled with water; a filter containing coffee is placed in the filter-holder; this is placed atop the jug, which is placed on the hot-plate. When the apparatus is switched on, the water is heated and percolates through the solid coffee. The beverage is collected in the jug below. Extraction takes 7–8 min, at 90 °C. Once extraction is complete, the coffee in the jug is kept at 83 °C on the hot-plate.

2.3.1.3. Espresso or food service industry coffee maker. An espresso coffee machine incorporates the following basic systems: *Boiler*: This generates hot water and steam for extraction of the coffee. The internal temperature is in the range 114–121 °C. *Extraction port*: This is where the filter-holder is inserted. The filter-holder is an accessory containing a filter, which is filled with ground coffee. The water used to extract the coffee is heated in the boiler. *Filter-holder*: This is the part that holds the portion of ground or compressed coffee. The force required to compress the coffee is 20 kg. To penetrate through the compacted grounds in the filter-holder, the hot water must be delivered at the right pressure (8–9 bars) and temperature (90–96 °C).

In the case of the Italian coffee pot, to produce six cups, an average of 29.25 g of grounds was used in an average initial water volume of 330 ml. This gives a dosage of 0.08 g of coffee/ml of initial water. The mass of solubilized coffee was 6.875 and the final volume of water was 289 ml, giving an output of 0.023 g coffee/ml of final water.

In the filter coffee-maker, two cups were produced with an average of 21.8 g in an average of 266 ml of initial water. This gives an input ratio of 0.081 g coffee/ml of initial water. The mass of solubilized coffee was 5.6 g and the final volume of water was 222 ml, giving an output of 0.025 g coffee/ml of final water.

In the case of the espresso machine, an average of 2.82 g coffee was used in an average of 90 ml water to produce two cups of coffee. This gives an output of 0.031 g coffee/ml of water.

The coffees made by these procedures were used to evaluate the polyphenol content and the antioxidant activity.

2.3.2. Extraction of phenols

One gram of ground coffee sample was placed in a test tube; 40 ml methanol/water (50:50) plus HCl were added to obtain a final pH 2.0. The tube was thoroughly shaken at room temperature for 1 h. The tube was centrifuged at 2500*g* for 10 min and the supernatant was recovered. 40 ml of acetone/water (70:30) were added to the residue, and shaking and centrifugation were repeated. Both extracts were mixed. Extracts or brewed coffee were produced in triplicate and used to estimate the total phenolics (TP) content and the antioxidant capacity. TP of the extracts were determined photometrically according to the Folin–Ciocalteu procedure (Montreau, 1972) using gallic acid as standard (concentration range 5–25 mg/100 ml), and expressing the results as gallic acid equivalents (GAE) per gramme of dry coffee. Extracts were used to estimate the polyphenol content in the coffee ground, whereas the polyphenol content was also estimated in the brewed coffees.

2.3.3. Antioxidant activity

Antioxidant activities of the brewed coffees were estimated by (a) the $ABTS^{+\cdot}$ test, free radical scavenging of $ABTS^{+\cdot}$ radical cation and (b) the FRAP test, ferric reducing power. In (a) ($ABTS^{+\cdot}$ test). the antioxidant capacity was estimated in terms of radical scavenging activity, following the procedure described elsewhere (Jiménez-Escrig, Dragsted, Daneshvar, Pulido, & Saura-Calixb, 2003). Briefly, ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{+\cdot}$ solution (two days stable) was diluted with 5 mM phosphate buffered saline (pH 7.4) to an absorbance of 0.70 ± 0.02 at 730 nm. After addition of 10 μl of sample or trolox standard to 4 ml of diluted $ABTS^{+\cdot}$ solution, absorbance readings were taken every 20 s, using a Beckman DU-640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA). The reaction was monitored during 6 min. In the presence of antioxidant inhibition, absorbance vs. time was plotted and the area below the curve (0–6 min) was calculated. Ethanol solutions of known trolox concentrations, a water-soluble analogue of vitamin E were used for calibration.

In (b) (FRAP test) the reduction power of coffees was estimated according to the procedure described by Benzie and Strain (1996) with some modifications introduced in our laboratory (Pulido, Bravo, & Saura-Calixto, 2000). Briefly, 900 μl of FRAP reagent, freshly prepared and warmed at 37 °C, were mixed with 90 μl distilled water and either 30 μl of test sample or standard or appropriate reagent blank. The FRAP reagent contained 2.5 ml of a 10 mM TPTZ solution in 40 mM HCl, plus 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$, plus 25 ml 0.3 mM acetate buffer pH 3.6. Readings at the absorption maximum (595 nm) were taken every 15 s, using a Beckman DU-640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA) equipped with a thermostatit autocell-holder. Temperature was maintained at 37 °C. The readings at 30 min were selected for calculation of FRAP values. Methanolic solutions of known trolox concentrations were used for calibration.

The antioxidant results were expressed as μmol equivalents of trolox per g of coffee (dry matter).

2.3.4. Dry matter

Dry matter (dm) content of the coffee samples (extracts and beverages) was obtained in an oven at 105 °C overnight.

2.3.5. Solubilization

The percentage of solubilization was calculated using the following expression:

$$\text{Solubilization } (\%) = 100 * ((\text{M-RM})/\text{M})$$

M = Amount of coffee loaded in the coffee-maker, expressed as dry matter; RM = Remaining matter on the coffee-maker, after the production of brewed coffee, expressed as dry matter. RM of freeze-dried coffee = 0.

2.3.6. Statistical analysis

Results are expressed as mean values ± standard deviation. Means of six measurements were compared, using a significance level of $P < 0.05$, by one way analysis of variance (ANOVA). The computer system used was Statgraphic Computer System, version 5.1.

3. Results and discussion

3.1. Antioxidant activity measurement

The generation of radical oxidative species involves either radical processes or different potential redox systems. The soluble properties of antioxidant compounds determine their effective antioxidant activities in either aqueous or lipid systems (Frankel & Meyer, 2000). Therefore, two aqueous-based models were chosen to assess the antioxidant activity of the coffees, one measuring radical-scavenging activities, and the other measuring total reductive power.

3.2. Polyphenol content

The range of phenolic values estimated in organic extraction of the different ground coffees was from 3.9 to 6.91 g/100 g dm, not including the two freeze-dried samples (Table 2). This content was lower in the case of brewed coffees made by the various procedures, ranging from 1.87 to 3.74 g/100 g dm (Table 2). The solubilization percentages of the three types of coffee prepared in Italian, espresso and filter coffee-makers were 23.5% ± 1.61, 20.6% ± 1.30 and 26.11% ± 1.19, respectively. Polyphenol content was highest in filter-brewed coffee.

3.3. Antioxidant activity

A useful way of viewing the interactions among various antioxidants is to take into account oxidation–reduction potentials (E^0) (Olson, 1996). The antioxidant potentials of the brewed coffees were estimated from their power to reduce the TPTZ–Fe(III) complex to TPTZ–Fe(II) complex. In terms of coffee-making procedures, the order of activity in the different brewed coffees tested was freeze-dried > filter ≈ espresso ≈ Italian (Table 3). There was a high correlation between the estimated polyphenol contents and the FRAP values (r: 0.989, $P < 0.01$) for all the types of coffee tested. The antioxidant activities of brewed coffee were also measured in terms of radical scavenging power, according to the ABTS method. In this case, the profile was similar to that of ferric reducing capacity. In terms of coffee-making procedures, the order of activity in the different brewed coffees tested was freeze-dried > filter ≈ Italian > espresso (Table 3). As in the previous method, there was a high correlation between the estimated polyphenol content of the brewed coffees and the radical scavenging activity (r: 0.99, $P < 0.01$). It would therefore appear that the level of antioxidant activities measured by in vitro testing methods is determined mainly by the polyphenol components of the brewed coffees.

If we consider the different types of coffees in terms of the roasting process, in general the dark-roast samples exhibited better antioxidant action than the medium-roast samples (Table 3). This could be due to enhancement of Maillard reaction (MR) products during roasting, due to the presence of added sugars. As

Table 2
Total polyphenols in coffee organic-acid extracts and brewed coffees (expressed as g gallic acid eq./100 g dry matter)

Samples	Coffee organic-acid extracts	Brewed coffee
IT1	3.9 ± 0.2	3.2 ± 0.1
IT2	3.7 ± 0.2	3.3 ± 0.1
IT3	3.9 ± 0.2	3.2 ± 0.2
IT4	3.7 ± 0.5	2.3 ± 0.1
IT5	4.0 ± 0.1	3.7 ± 0.1
Mean	3.9 ± 0.12a	3.2 ± 0.28a
EX1	6.1 ± 0.5	1.8 ± 0.1
EX2	6.9 ± 0.2	2.3 ± 0.1
EX3	6.8 ± 0.3	2.0 ± 0.1
EX4	6.9 ± 0.2	2.1 ± 0.1
EX5	6.7 ± 0.5	2.2 ± 0.1
Mean	6.7 ± 0.16b	2.1 ± 0.22b
F1	4.2 ± 0.2	2.9 ± 0.1
F2	4.2 ± 0.2	2.5 ± 0.1
F3	4.2 ± 0.3	2.6 ± 0.1
F4	4.0 ± 0.3	2.7 ± 0.1
F5	4.0 ± 0.8	2.6 ± 0.1
Mean	4.1 ± 0.20a	2.7 ± 0.22a
FD1	10.6 ± 0.5	12.1 ± 0.3
FD2	11.0 ± 0.8	12.1 ± 0.1
Mean	10.8 ± 0.94c	12.1 ± 0.32c

Each value is mean ± SD ($n = 6$).
IT: Italian, EX: Espresso, F: Filter, FD: Freeze-dried.
For nomenclature of each coffee see Table 1.
Different letters in the same column imply significant difference.

Table 3
Total antioxidant capacity by FRAP and ABTS methods of brewed coffee (expressed as μmol trolox eq./g dry matter)

Samples	FRAP	ABTS
IT1	173 ± 9	103 ± 12
IT2	269 ± 21	126 ± 2
IT3	244 ± 13	111 ± 9
IT4	116 ± 5	113 ± 3
IT5	193 ± 10	136 ± 6
Mean	199 ± 28a	117 ± 17a
EX1	148 ± 8	80 ± 4
EX2	178 ± 28	73 ± 4
EX3	162 ± 10	75 ± 3
EX4	163 ± 7	85 ± 2
EX5	160 ± 13	76 ± 5
Mean	162 ± 35a	77 ± 8b
F1	240 ± 4	97 ± 6
F2	244 ± 15	111 ± 3
F3	242 ± 15	115 ± 13
F4	236 ± 12	118 ± 6
F5	221 ± 2	143 ± 9
Mean	236 ± 25a	116 ± 18a
FD1	1008 ± 156	437 ± 15
FD2	1007 ± 56	450 ± 14
Mean	1007 ± 165c	443 ± 21c

FRAP = Ferric reducing power.
ABTS = Free radical scavenging of $ABTS^{+\cdot}$ radical cation Each value is the mean ± SD ($n = 6$).
IT: Italian, EX: Espresso, F: Filter, FD: Freeze-dried.
For nomenclature of each coffee see Table 1.
Different letters in the same column imply significant difference.

Table 4
Polyphenol content, and total antioxidant capacities by FRAP and ABTS methods, of brewed coffees (expressed as g galic acid eq./serving, and μg trolox eq./serving, respectively)

Samples	PP	FRAP	ABTS
IT1	0.14 ± 0.0039	807 ± 20	479 ± 13
IT2	0.16 ± 0.0093	1310 ± 13	613 ± 6
IT3	0.17 ± 0.0054	1319 ± 41	599 ± 18
IT4	0.11 ± 0.0092	561 ± 46	546 ± 45
IT5	0.18 ± 0.0090	954 ± 46	673 ± 33
Mean	0.15 ± 0.028a	990 ± 66a	582 ± 73ab
EX1	0.17 ± 0.074	1401 ± 58	756 ± 31
EX2	0.20 ± 0.085	1554 ± 65	636 ± 34
EX3	0.22 ± 0.0013	1776 ± 103	826 ± 48
EX4	0.20 ± 0.009	1593 ± 73	829 ± 29
EX5	0.21 ± 0.006	1520 ± 60	722 ± 23
Mean	0.20 ± 0.019b	1253 ± 136b	753 ± 80b
F1	0.30 ± 0.015	2554 ± 130	968 ± 65
F2	0.27 ± 0.014	2686 ± 140	1217 ± 63
F3	0.30 ± 0.009	2801 ± 93	1337 ± 44
F4	0.29 ± 0.018	2527 ± 155	1261 ± 100
F5	0.31 ± 0.016	2609 ± 138	1695 ± 52
Mean	0.29 ± 0.015c	2635 ± 297c	1295 ± 262c
FD1	0.12 ± 0.0001	1012 ± 151	453 ± 48
FD2	0.12 ± 0.0001	1015 ± 96	454 ± 29
Mean	0.12 ± 0.0001a	1015 ± 178a	454 ± 56a

FRAP = Ferric reducing power.
ABTS = Free radical scavenging of $ABTS^{+\cdot}$ radical cation.
Each value is the mean ± SD ($n = 6$).
IT: Italian, EX: Espresso, F: Filter.
FD = Freeze-Dried.
For nomenclature of each coffee see Table 1.
Different letters in the same column imply significant difference.

regards the antioxidant capacity of MR-derived compounds, melanoidins are polymeric compounds formed in the last stage of the MR (Martins, Jongen, & van Bekel, 2001). They are abundant in coffee and present antioxidant activity (Morales & Babbel, 2002; Nicoli & Lerici, 1997), although little is known of their intestinal absorption, transit on metabolic biotransformation (Faist & Erbersdobler, 2001).

The potential antioxidant activity (FRAP test) in ground coffees was estimated on the basis of the polyphenol content obtained by organic acid extraction. Taking into account the relation ship (value in brackets), between these activities and those obtained for the brewed coffees, the order of the power reduction of the different brewed coffee tested was: Espresso (3.34 ± 0.31) > filter (1.63 ± 0.19) ≈ Italian (1.37 ± 0.27) > freeze-dried (0.88 ± 0.03). The order was similar in the case of ABTS testing. This implies that, apart from the freeze-dried samples, the antioxidant capacity of the ground coffee was preserved most effectively by the Italian coffee-maker.

There was generally no difference between the antioxidant activity of brewed coffees as a whole and equivalent decaffeinated coffees (Table 3). Although the antioxidant activity of caffeine is relatively high in in vitro testing (Devasagayam et al., 1996; Shi et al., 1991), it does not seem to be so effective in the coffee matrix.

In terms of servings of the different coffees tested, the order of antioxidant activity in the brewed coffees, as tested by the FRAP method, was: Filter > espresso > freeze-dried ≈ Italian. The order was similar in the case of ABTS testing (Table 4).

Consumption of filter coffee is high in the USA and northern Europe (Montero et al., 1999). In these countries, it is usual for the pot containing the coffee to be left on the heat source (85 °C) for several hours. We tested the antioxidant capacity of coffee exposed to heat at this temperature, in order to assess the destruction or formation of compounds with antioxidant activity. Antioxidant capacity, as measured by FRAP, tended to decrease significantly in the first 60 min and to increase thereafter. We found that antioxidant activity increased significantly (by $34\% \pm 4.1$) after 4 h of heating. These results were obtained taking into account evaporation losses (15.62%, after 4 h). The apparent cause of this increase is heat-induced formation of Maillard products. It should be noted, however, that denaturation commences when coffee is heated for over 1 h (Forum café, 2003).

We tested the antioxidant activity of coffee with milk in the case of espresso coffee. When milk was added to the coffee, antioxidant activity decreased. As compared to coffee on its own, mixing with 17 ml significantly reduced antioxidant activity by 62% (± 3.9), and mixing with 100 ml of milk significantly reduced it by 95% (± 7.1). In other words, the reduction of antioxidant activity was proportional to the amount of milk added.

The protective effects of coffee in humans require further research. It should be remembered that in vitro activities are simply indicators of potential biological activities, reflecting only theoretical bioavailability. Polyphenols from coffee are partially bioavailable, and consequently their in vivo activity could be significant. The bioavailability of chlorogenic acids is problematical, given existing doubts as to the presence of esterase enzymes responsible for intestinal hydrolysis (Andreason & Garcia Conesa, 2001; Keiko, Katsunari, & Terao, 2000; Nardini, Cirillo, & Sacaccini, 2002; Plumb, Couteau, & Faulds, 2001). To assess the efficiency of these compounds in the human body, we therefore need to know more about how they are metabolised.

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