Comparison of the Antioxidant Activity of Commonly Consumed Polyphenolic Beverages (Coffee, Cocoa, and Tea) Prepared per Cup Serving

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In this study, the in vitro low-density lipoprotein oxidation model was used to assess the relative antioxidant activity of the polyphenolic beverages tea, coffee, and cocoa on a cup-serving basis. The beverages were prepared as 0.7−2.5% soluble coffee and 1.5−3.5% cocoa; teas (green, black, or herbal) were prepared as one tea bag infused over 5 min in 220 mL of hot water. Under these standard cup serving conditions, the antioxidant activity as determined by the lag time was in the range of 292−948 min for coffee, 217−444 min for cocoa, 186−338 min for green tea, 67−277 min for black tea, and 6−78 min for herbal tea. Addition of milk did not alter the antioxidant activity. The influence of coffee bean source and degree of roasting was further investigated. Green coffee beans of Robusta coffee exhibited a 2-fold higher antioxidant activity than Arabica coffee, but after roasting this difference was no longer significant. In conclusion, these commonly consumed beverages have a significant antioxidant activity, the highest being soluble coffee on a cup-serving basis.

Keywords: *LDL oxidation; antioxidants; polyphenols; beverages; coffee; cocoa; teas*

INTRODUCTION

Flavonoids are secondary plant metabolites occurring widely in plant foods such as fruits, vegetables, cereals, and beverages (*1*−*3*). Common foods contain a variety of flavonoids and phenolic compounds in amounts ranging from traces to several grams per kilogram of fresh weight (*3*). Several commonly consumed beverages are rich in flavonoids and phenolic acids, for example, 200−550 mg of polyphenols/cup of coffee, 150−200 mg/cup of teas, and 200−800 mg/glass of wine (*3*, *4*). These beverages make a significant contribution to the polyphenol intake of the diet.

Flavonoids and other plant phenolics have a significant antioxidant activity with respect to vitamins C and E (*5*, *6*). The antioxidant activity of this diverse group of compounds depends on the individual structure and number of hydroxyl groups.

Green tea contains flavanoid derivatives of catechins in significant amounts, which are rapidly extracted in hot water infusions. During the manufacture of black tea, enzyme-catalyzed oxidation of the catechins leads to the formation of catechin quinones, which subsequently react to form more complex structures such as theaflavins and thearubigins. The antioxidant activity of tea and its active components (catechins) has been extensively studied in vitro (*7*−*9*) and in vivo (*10*−*14*).

Unfermented cocoa beans are rich in polyphenols, which comprise 12−18% of the whole beans' dry weight. The polyphenols present in cocoa are catechins, procyanidins, and anthocyanidins (*15*), the latter being responsible for the purple color of the unfermented cocoa beans. Upon fermentation and drying, the flavonoids undergo a variety of reactions including oxidation and polymerization to procure the tannins. Cocoa polyphenols exhibit antioxidant properties in vitro (*16*−*20*).

Green coffee contains a large amount and variety of polyphenols exemplified by chlorogenic acid, caffeic acid, ferulic acid, and *p*-coumaric acid. Roasting markedly affects the composition of the coffee polyphenols through Maillard reactions and confers to coffee its pleasant taste and aroma. In addition, carbohydrate caramelization and pyrolysis of organic compounds occur (*21*, *22*). On the basis of 10 g of coffee per cup of brew, a cup contains 15−325 mg of chlorogenic acids with an average of 200 mg/cup for American coffee. Antioxidant activity of ferulic acid and caffeic acid has been demonstrated in vitro (*23*−*25*) and in vivo (*26*).

The aim of the present study is to evaluate the antioxidant activity of the commonly consumed polyphenolic beverages. We have compared the antioxidant activities of coffee, cocoa, and tea per serving using the in vitro low-density lipoprotein (LDL) oxidation model. The effect of milk proteins has been also considered as it has been debated that milk proteins inhibit the antioxidant activity of teas (*10*, *27*). Furthermore, in the case of coffee, the bean source (Arabica versus Robusta) and the impact of the degree of roasting on the antioxidant activity of the beverage are also investigated.

MATERIALS AND METHODS

Preparation of Beverages. Commercially available beverages were prepared as follows: cocoa drink was reconstituted from a 97% cocoa powder in hot water to a final concentration of 1.5−3.5% cocoa; teas (black, green, or herbal) were prepared with one tea bag infused over 5 min in 220 mL of hot water. Codes A−I correspond to different commercial brands of teas. Soluble coffee was prepared at 0.7−2.5% by dilution of coffee powder in hot water.

To investigate the effect of coffee bean source and degree of roasting, noncommercially available coffee samples were prepared as follows: For the two types of coffee bean, Arabica (Colombia) and Robusta (Indonesia), green coffee beans were ground and prepared as a brew [60 g of ground coffee bean

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per liter using a paper filter (Melitta)]. For the effect of roasting, green coffee beans were roasted, in a pilot plant, at different levels and characterized by a color test Neuhaus (CTN) value of 110, 85, or 60, corresponding to light, medium, and high roasting, respectively. A final solution of roasted coffee beans was prepared as 1.7%.

To evaluate the effect of milk proteins, beverages were also prepared with milk as instructed for the consumer, that is, soluble coffee and black tea with 10% milk and cocoa beverage with 66% milk.

LDL Oxidation Assay. Blood from healthy volunteers was collected in ice-cooled tubes containing EDTA (1 mg/mL) and centrifuged immediately at low speed (3000*g*). The resulting plasma was pooled, and sucrose was added to reach a final concentration of 0.6% (*28*). Plasma was aliquoted and stored at −80 °C. LDL was isolated by discontinuous density gradient ultracentrifugation (*29*). Before oxidation experiments, LDL was desalted with prepackaged columns (Econo-Pac 10 DG column, Bio-Rad, Richmond, CA) filled with gel P6. The total cholesterol of the salt-free LDL sample was determined with the CHOD-PAP enzymatic test kit (Boehringer, Mannhein, Germany), and the concentration was adjusted with PBS (pH 7.4) to 80 μg of cholesterol/mL. LDL samples (1 mL) were incubated with 1 μL of beverages at 37 °C for 2 min prior to the initiation of oxidation with 1.7 mM copper sulfate or with 1.25 mM azobis(2-amidinopropane) dihydrochloride (AAPH) (Polysciences). Immediately after the addition of the pro-oxidant agent, the measurement of the conjugated dienes was monitored at 234 nm at 5-min intervals for a period of 5 h. The kinetics of LDL oxidation are characterized by three parameters: (a) the lag time or the time during which antioxidants are consumed; (b) the rate of oxidation; and (c) the maximum production of conjugated dienes. The increase of lag time before the onset of lipid peroxidation in the LDL particle was taken as a measure of the antioxidant activity (*30*, *31*). A control LDL was run with each set of experiments, and the coefficient of variation of the lag time was 7%. Results were presented as the mean of three to five individual experiment ± standard deviation.

RESULTS

In the presence of antioxidants, LDL oxidation is delayed as characterized by the increase of lag time, whereas the rate of oxidation as well as the maximum production of conjugated dienes remains constant. This inhibition of LDL oxidation is dose-dependent. Different pro-oxidant agents can initiate the LDL oxidation and have different actions with antioxidants, for example, scavenging versus chelation. We have compared the oxidation of LDL induced either by cupric ions or by AAPH. Cupric ions require small concentrations of lipid peroxides in LDL and bind to specific sites on the apolipoprotein B-100. On the other hand, AAPH is a water-soluble thermolabile initiator, which generates free radicals at a constant rate over a few hours. In this study, the kinetics of resistance of LDL against oxidation were similar with both pro-oxidant agents at any concentration of the polyphenolic beverages. For the sake of brevity, only the kinetic data from the cupric ion experiments are reported in the figures.

We compared the antioxidant activity of one cup of coffee to that of one cup of tea (green teas, black teas, or herbal teas) and to that of one cup of cocoa, prepared according to the manufacturer's instructions. Taking into account that the preference for a beverage as well as the method of preparation varies markedly from one country to another, we prepared each beverage with a range of concentrations to cover the different habits of consumers. Coffees were prepared with 0.7−2.5% soluble coffee in 220 mL of hot water, covering a large range of consumer habits. The dosage of 0.7−1% corresponds to the habits of U.S. consumers, whereas 1.7% corresponds to the dosage of Swiss consumers and 2.0% to French consumers (Nestlé, consumer information). The antioxidant activity of a one cup serving of coffee was related to its concentration in the beverage (Figure 1). Coffee exhibited a high antioxidant activity as characterized by an increase in the lag time from 292 ± 27 to 948 ± 89 min for 0.7−2.5% coffee. Cocoa beverages were also prepared at different concentrations (1.5−3.5% cocoa), and the antioxidant activity was again dose-dependent, ranging from 217 ± 10 to 444 ± 40 min (Figure 2). The antioxidant activity of green teas varied between 186 ± 2 and 338 ± 6 min for different brands (Table 1). Similarly, the antioxidant activity of black tea varied according to the brand and amount of tea included in the tea bag (1.5 or 3 g) and ranged from 67 ± 17 to 277 ± 35 min (Table 1). Herbal teas also exhibited an antioxidant activity but with a lower order of magnitude, ranging from 6 ± 2 to 78 ± 1 min (Table 1).

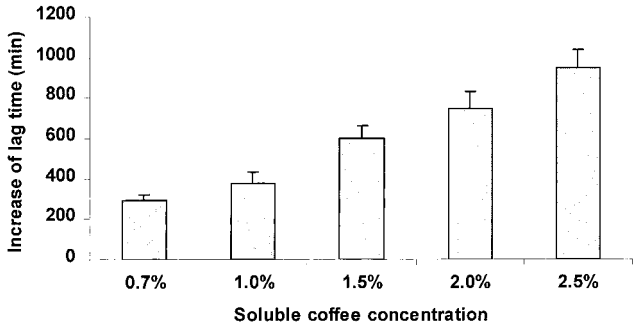

Figure 1. Antioxidant activity of soluble coffee prepared per serving as usually consumed in different countries. A cup of coffee was prepared with increasing amounts of soluble coffee in 220 mL of hot water to achieve concentrations of 0.7−2.5%. One microliter of beverage was incubated with 80 μg of LDL cholesterol, and LDL oxidation was started with copper ions (as described under Materials and Methods). The antioxidant activity of the beverage is represented by the increase of the lag time (lag time of LDL in the presence of coffee beverage with respect to the lag time of control LDL). Results are expressed as mean (of three to five individual experiments) ± SD.

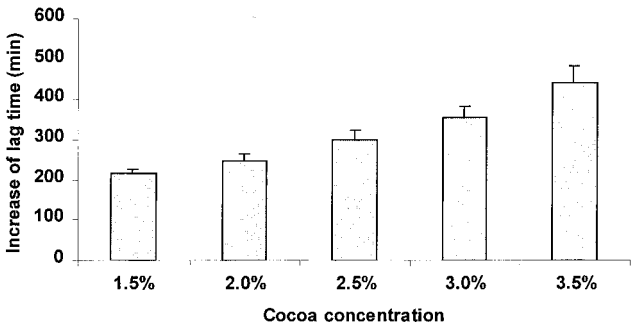

Figure 2. Antioxidant activity of cocoa prepared per serving as usually consumed in different countries. A cup of cocoa was prepared with increasing amounts of cocoa powder (97% cocoa solid) to achieve concentrations of 1.5−3.5% in 220 mL of hot water. The antioxidant activity was determined as described for Figure 1.

These beverages are often consumed with milk; it therefore seems important to evaluate also the antioxidant activity of these beverages in the presence of milk. Interestingly, the antioxidant activities of coffee, cocoa, and black tea were not affected by the presence of milk (Figure 3).

We further investigated the antioxidant activity of coffee in relation to the coffee bean source and the

Table 1. Antioxidant Activity of Green, Black, and Herbal Teas Prepared per Seving As Usually Consumed in Different Countries[a]

	increase of lag time (min)
green teas	
A	338 ± 6
B	186 ± 2
C	250 ± 27
D	217 ± 21
black teas	
E	89 ± 21
F	67 ± 17
G	127 ± 4
H	256 ± 29
I	277 ± 35
herbal teas	
chamomile	6 ± 2
lime flower	49 ± 16
verveine	56 ± 11
mint	75 ± 8
rosehip	78 ± 1

[a] Teas were prepared with one tea bag infused over 5 min in 220 mL of hot water. Codes A−I correspond to different commercial brands of teas.

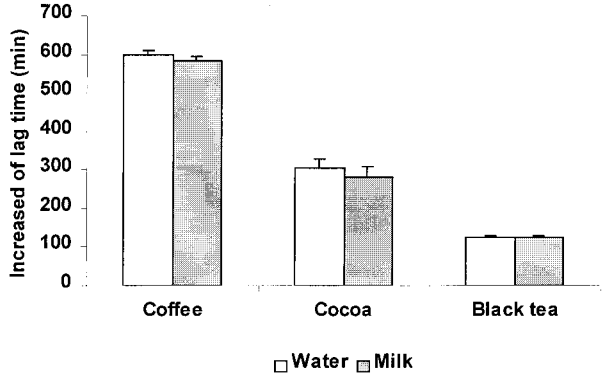

Figure 3. Effect of milk on the antioxidant activity of coffee, cocoa, and black tea. Coffee was prepared as 1.7% soluble coffee, cocoa as 3.3%, and black tea as one tea bag per 220 mL of hot water cup size. To evaluate the effect of milk proteins, beverages were also prepared with milk as instructed for the consumer, that is, soluble coffee and black tea with 10% milk and cocoa beverage with 66% milk. The antioxidant activity was determined as described for Figure 1.

degree of roasting, using noncommercially available coffee preparations. Two main sources of coffee bean are generally employed: Robusta and Arabica. Although green coffees are never consumed in their native state, we extracted polyphenols from green coffee beans, in a similar manner as for roasted coffee beans, to evaluate the antioxidant activity of coffee before and after various degrees of roasting. Robusta coffee exhibited a 2-fold higher antioxidant activity than Arabica coffee (643 ± 69 versus 366 ± 74 min, respectively) (Table 2). Green coffee beans were roasted to different levels in a pilot plant and the antioxidant activities of the soluble coffees compared. Light roasting (CTN 110) profoundly decreased the antioxidant activity of coffee; however, this reduction was more marked for the Robusta coffee than for the Arabica coffee, leading to a similar final antioxidant activity after roasting (294 ± 41 versus 284 ± 80 min for Robusta and Arabica, respectively). The increase of coffee roasting level from CTN 110 to CTN 60 slightly reduced the antioxidant activity of both types of coffee (Table 2). It is well-known that the composition of polyphenols is greatly modified by this temperature treatment. At any roasting level, the antioxidant activities of Robusta and Arabica coffees were quite similar.

Table 2. Effect of Degree of Roasting of Arabica and Robusta Coffee Beans on Antioxidant Activity[a]

	Arabica	Robusta
ground green coffee	366 ± 74	643 ± 68
roasted coffee		
CTN 110	284 ± 80	294 ± 41
CTN 85	206 ± 30	190 ± 39
CTN 60	168 ± 23	134 ± 34

[a] A brew with ground green coffee beans from both origins was prepared as 60 g/L. Both coffee beans were roasted to different degrees to have values of the color test Neuhaus (CTN) of 110, 85, and 60, corresponding to low, medium, and high roasting, respectively. Coffee beverages were prepared as 1.7%.

Usually, Robusta and Arabica coffee beans are blended to produce coffees with different tastes. The antioxidant activities of these blended coffees varied minimally (data not shown). Interestingly, decaffeinated coffees exhibited antioxidant activities similar to those of the caffeinated coffees (data not shown).

DISCUSSION

This study investigates the relative antioxidant capacities of commonly consumed polyphenolic-rich beverages on a cup-serving basis. One factor, which influences greatly the antioxidant content of these beverages, is the method of preparation of a one-cup serving. For example, one cup of coffee is prepared as 0.7% soluble coffee by U.S. consumers, as 1.7% by Swiss consumers, and as 2.0% by French consumers. This will lead to a considerable difference in the antioxidant loads ingested by the different consumers. This parameter must be taken into account when the daily consumption of polyphenols of different populations are assessed (*3*, *4*, *9*). Under these standard cup-serving conditions, the antioxidant activities as determined by the lag time were in the range of 292−948 min for coffee, 217−444 min for cocoa, 186−338 min for green tea, 67−277 min for black tea, and 6−78 min for herbal tea.

The protection of LDL against oxidation is not due to a single polyphenolic compound but is the result of the action of several polyphenolic constituents. The physical and chemical composition properties of individual phenolic antioxidants strongly affect their antioxidant activities (*32*, *33*). In addition, these molecules could have a synergistic or antagonist effect when present in complex mixtures. Indeed, the polyphenol composition of the beverages varies greatly, consisting of mainly epicatechins in green teas; epicatechins and tannins in black teas; catechins, procyanidins, and anthocyanins in cocoa; and chlorogenic acids, caffeic acid, and melanoidins in coffee.

In the case of coffee, Robusta exhibits a higher antioxidant activity than Arabica, which could be due to the higher amount of chlorogenic acid. Following a light roasting, the antioxidant activities of both coffees decrease markedly. This could be explained by the loss of polyphenolic compounds and to the successive formation of other antioxidant compounds such as Maillard reaction products or pyrolysis products (less active) when more severe thermal conditions are applied.

As these polyphenolic-rich beverages are often consumed with milk, we also evaluated the effect of milk on the antioxidant activity of these beverages. We found that the antioxidant activities of coffee, cocoa, and black

tea were not affected by the presence of milk. Recently, it has been shown that the presence of milk does not inhibit the increased plasma antioxidant activity following tea consumption in humans (*27*).

An understanding of the protective role of dietary antioxidants in vivo requires a better characterization of the polyphenol composition of the antioxidant matrix as well as quantitative data on their absorption, their tissue distribution, their metabolism, and their biological actions (*34*, *35*). Indeed, after consumption, polyphenols have to cross the intestinal wall but must also resist further catabolism. The metabolism of polyphenols involves two important organs: the liver, where biotransformation enzymes convert them or their metabolites into conjugated forms such as glucuronides or sulfates, and the colon, where microorganisms degrade unabsorbed ones. At the present time only little information is available on the absorption of the vast diversity of polyphenols present in these beverages. Epicatechin is similarly absorbed from tea and cocoa (*18*, *20*) but to a lower extent than quercetin (*33*) or isoflavones (*36*). For coffee, caffeic acid is significantly absorbed in rats (*26*). These results provide only a small picture of the polyphenol absorption because the vast majority of the compounds are still unidentified.

In conclusion, coffee, cocoa, and tea beverages contain polyphenols with high antioxidant activities. This must be taken into consideration when the daily ingested dose of polyphenols is estimated. Further studies will be required to understand which are the most relevant polyphenols in vivo.

ABBREVIATIONS USED

AAPH, azobis(2-amidinopropane) dihydrochloride; CTN, color test Neuhaus; EDTA, ethylenediaminetetraacetic acid; LDL, low-density lipoproteins; PBS, phosphate buffer.

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