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Antioxidant Properties of Ready-to-Drink Coffee Brews

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The influence of some technological variables on the changes of the antioxidant capacity of ready-to-drink coffee brews was investigated. Results showed that, depending on the roasting degree as well as on the packaging conditions adopted, redox reactions, which can take place during storage, are responsible for significant changes in the overall antioxidant capacity of the product. In particular, the redox potential of air-packaged coffee brews, obtained from light- and medium-roasted beans, showed maximum values after 2 days of storage, which corresponded to a minimum in the chain-breaking activity, while, in the case of the dark-roasted sample packaged under ordinary atmosphere, both the redox potential and the chain-breaking activity showed a maximum around 2−3 days of storage. In contrast, in the absence of oxygen, the coffee brews maintained the initial reducing properties over all the storage time, although the radical-scavenging activity values changed in a way very similar to that of the air-packaged sample. These results suggested that the changes in the antioxidant properties of the coffee brews may be attributed to a further development of the Maillard reaction during storage.

KEYWORDS: Chain-breaking activity; coffee brew; redox potential; storage; roasting degree

INTRODUCTION

The market for ready-to-drink coffee brews has greatly increased in recent years. In some countries, such as Japan, they encountered enormous success due to the lack of a traditional image of coffee as a freshly brewed beverage (*1*). In western countries they are presented as alternative products, able to accomplish functions different from and complementary to the traditional coffee brews. Although the sensorial properties of the ready-to-drink coffee brews are often lower than those of the freshly prepared ones, their high attribute of convenience favors their consumption in those cases in which the traditional product is not available. The recent introduction on the market of ready-to-drink coffee bottled in self-cooling or self-heating packaging has also contributed to spreading these beverages.

Ready-to-drink coffee brews generally undergo a rapid decay of their original sensorial properties. The mechanisms at the basis of this quality loss are still unknown, but they are generally accompanied by an increase in hydrogen ion concentration, which, in turn, affects the acid−base equilibria and the partition of volatile compounds in the vapor phase (*2*−*5*). In particular, a pH decrease has been recently attributed to hydrolysis of carbohydrates and/or melanoidins (*6*), which, although they are unavoidable, can be limited by proper technological intervention.

Experimental evidence has demonstrated that coffee beverages possess high antioxidant properties (*7*−*9*). In fact, although most of the naturally occurring phenolic compounds are lost during roasting of coffee beans (*10*), the overall antioxidant properties of coffee brews are maintained, or even enhanced, by the development of compounds with antioxidant properties, mainly represented by the Maillard reaction products (MRPs) (*8*, *11*). Nicoli et al. (*11*) showed that the oxygen-consuming and radical-scavenging properties reached a maximum for brews prepared from beans roasted at intermediate conditions, while further increases of the roasting degree caused a decrease in such properties. Steinhart et al. (*12*) and Del Castillo et al. (*13*) showed that the high-molecular-weight MRPs of coffee brews, obtained by gel filtration chromatography, had lower antioxidant activity than the low- and intermediate-molecular-weight ones over the range of roasting conditions examined.

To our knowledge, no data are available on the changes in the overall antioxidant properties of coffee brews during storage. However, the possibility to maintain the intrinsic antioxidant properties during all the technological steps which follow the roasting process represents a challenge in the production of coffee and coffee-based products with a high functional profile. This would allow us to estimate the role of antioxidant compounds in extending the shelf life of coffee products by slowing down undesirable oxidation reactions and possibly to predict their functionality as dietary products.

The aim of the present investigation was to study the influence of the roasting degree and packaging atmosphere on the changes of the overall antioxidant capacity of ready-to-drink coffee brews, which were prepared from light-, medium-, and dark-roasted beans, obtained from a same starting blend. The antioxidant capacity was evaluated by means of chain-breaking activity and redox potential measurements. Among the great number of methodologies available for assessing the chain-breaking activity of a compound or a mixture of compounds (*14*−*20*), the DPPH$^{\bullet}$ method (*15*) was used. This methodology

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Table 1. Weight Loss during Roasting, Moisture, and *L**, *a**, and *b** Hunter Scale Parameters of Light-, Medium-, and Dark-Roasted Coffee Powders

degree of roasting	weight loss (%)	moisture[a] (%)	color parameters[a]		
			*L**	*a**	*b**
light	14.5	2.47 ± 0.17	31.3 ± 0.79	10.8 ± 0.39	10.4 ± 0.99
medium	16.2	2.43 ± 0.20	26.0 ± 0.71	8.8 ± 0.18	5.8 ± 0.40
dark	18.9	2.43 ± 0.05	24.3 ± 0.42	6.5 ± 0.11	1.1 ± 0.20

[a] Moisture and color values are presented as the mean ± SD ($n = 3$).

allows us to assess the antioxidant properties of a pool of antioxidants, such as those present in coffee beverages, which in the presence of the reference radical generate complex multistep reactions (*21*). Chain-breaking activity and redox potential measurements give conceptually different information: the former is a kinetic measure, the latter a thermodynamic one. Hence, while the assessment of the chain-breaking activity allows us to estimate the quenching rate of the coffee compounds that are most reactive toward a reference radical, the redox potential gives an indication of the effective oxidation/reduction efficiency of all the antioxidants present, including the "slow" ones, which cannot be detected by the kinetic method (*22*). These compounds are expected to play an important role in determining and maintaining the antioxidant properties of ready-to-drink coffee brews during storage.

MATERIALS AND METHODS

Sample Preparation. Light-, medium-, and dark-roasted beans were supplied by the Nestlè Research Centre of Lausanne. They were obtained from the same coffee blend. Weight loss during roasting, moisture, and *L**, *a**, and *b** Hunter values of the light-, medium-, and dark-roasted coffees are shown in **Table 1**.

Coffee brews were prepared by solid−liquid extraction with deionized water at 100 °C for 10 min. The ratio between coffee, previously ground using a domestic grinder, and water was 1:10 (w/w). After filtration, samples were rapidly cooled in cold running water and then filtered through Whatman No. 4 filter paper. The beverages (10 mL) were bottled in 20-mL capacity vials in the presence of ordinary atmosphere or nitrogen. A ratio of 1:1 between liquid and headspace volumes was used in order to avoid oxygen being the limiting factor. Nitrogen was flushed into the vials for 3 min. The vials were then hermetically sealed with butyl septa and metallic caps and stored at 30 °C for 17 days.

Weight Loss. Weight loss during roasting was determined from the difference in weight of coffee beans.

Color Analysis. Color analyses were carried out on coffee beverages using a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-200 measuring head. The instrument was standardized against a white tile before measurements. Color was expressed in *L**, *a**, and *b** Hunter scale parameters, and *a** and *b** values were used to compute hue angle ($\tan^{-1}$ *b**/*a**) (*23*).

pH Measurement. The pH was measured at 25 °C using a pH meter (Crison, model 2002, Alella, Spain) equipped with a combination of glass electrodes and a temperature probe.

Redox Potential Determination. Measurements were made using a platinum indicating electrode and a silver/silver chloride reference electrode, connected to a voltmeter (Hanna Instruments, model 8417, Milano, Italy), according to the methodology proposed by Manzocco et al. (*21*). Calibration was performed against a redox standard solution (Reagecon, Shannon, Co. Clare, Ireland) having a redox potential value of 220 mV at 25 °C. Electrodes were placed in a 50-mL three-neck flask containing a 15-mL volume of each sample. Prior to analysis, oxygen was removed from the system by continuous nitrogen flushing for 10 min. The redox potential was recorded for at least 20 min at 25 °C, until a stable potential was arbitrarily defined as a change of less than 2 mV in a 3-min period.

Table 2. Chain-Breaking Activity and Redox Potential Values of Nonroasted and Freshly-Made Light-, Medium-, and Dark-Roasted Coffee Brews

degree of roasting	chain-breaking activity[a] ($-Abs^{-3}/min/mg_{d.m.}$)	redox potential[a] (mV)
nonroasted	0.321 ± 0.011 a	+109 ± 5.6 a
light	0.273 ± 0.038 a	+45.4 ± 2.3 b
medium	0.284 ± 0.022 a	−1.7 ± 0.1 c
dark	0.390 ± 0.035 b	−35 ± 1.8 d

[a] Chain-breaking activity and redox potential values are presented as the mean ± SD ($n = 3$). Means in the same column with different letters are significantly different ($P < 0.05$).

Chain-Breaking Activity Determination. The chain-breaking activity of coffee brews was measured by means of the DPPH• (2,2-diphenyl-1-picrylhydrazyl) method (*15*). A volume of 1.9 mL of 6.0×10^{-5} M DPPH• (Sigma-Aldrich, Steinheim, Germany) methanol solution was used. The reaction was started by the addition of 100 μL of sample, previously diluted 1:200 (v/v) with distilled water. The bleaching rate was followed at 515 nm (Beckman DU 640, Beckman Instruments, Inc., Fullerton, CA) at 25 °C for at least 20 min.

The reaction rates were calculated using eq 1 (*21*):

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

where k is the DPPH• bleaching rate, Abs_0 is the initial absorbance value, and Abs is the absorbance at increasing time t. The antioxidant activity was expressed as $-Abs^{-3}$/min/mg of dry matter.

Statistical Analysis. The results reported here are the average of at least three measurements, and the coefficients of variation, expressed as the percentage ratio between the standard deviation (SD) and the mean values, were lower than 0.2% for pH, 6% for redox, and 15% for color and chain-breaking activity. One-way analysis of variance was determined using the Tukey−Krammer test (*24*). Differences between means were considered to be significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

The chain-breaking activity and the redox potential values of the freshly prepared light-, medium-, and dark-roasted coffee brews as well as of the nonroasted one are shown in **Table 2**. The roasting process seems to more strongly affect the redox potential than the chain-breaking activity. In fact, while the high values of the chain-breaking activity only slightly increased as the roasting degree increased, the redox potential decreased from +109 to −34 mV, indicating a considerable gain in reducing properties. As already pointed out, though the naturally occurring phenols, such as caffeic acid and chlorogenic acid, are the principal antioxidants of nonroasted coffee (*25*), the antioxidant properties of the roasted coffee brews are mainly attributed to the Maillard reaction products (MRPs), which are formed during the heating process (*11*). In particular, these brown polymers are known to possess strong reducing power, which increases with increasing intensity of the heat treatment (*26*, *27*). It must be pointed out that the remaining phenolic compounds and/or their degradation products may exhibit antioxidant activity as well (*28*). The results reported in **Table 2** suggest that the formation of the heat-induced antioxidants would balance the thermal loss of naturally occurring phenolic compounds. However, the redox potential values indicate that the antioxidant efficiency of the former is higher than that of the natural ones.

Figure 1 shows the changes in the chain-breaking activity and redox potential of the light-, medium-, and dark-roasted coffee brews during storage at 30 °C under ordinary atmosphere. The redox potential values of these samples greatly increased

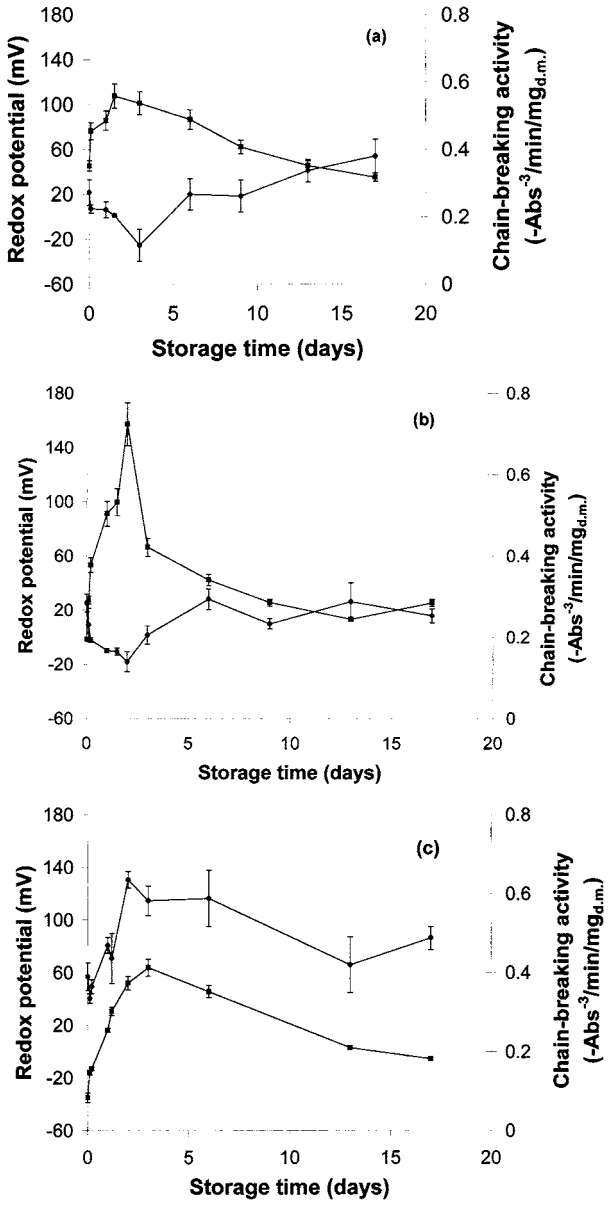

Figure 1. Changes in chain-breaking activity (●, right *y*-axis) and redox potential (■, left *y*-axis) values of (a) light-, (b) medium-, and (c) dark-roasted coffee brews during storage at 30 °C in ordinary atmosphere.

in the first few days. After a prolonged storage time, a significant decrease in the redox potential was observed. These data indicate that, according to the Nernst equation, in the early days of storage, compounds having a high standard redox potential (E °) and/or the oxidized forms of the redox couples prevailed. In contrast, for longer storage times, coffee components evolved in such a way that they exhibited progressively lower E ° values and/or were mainly present in their reduced form. In other words, at the beginning of the storage, the brews lost a portion of their initial reservoir of antioxidants, which, however, progressively increased with aging.

The maximum in redox potential, observed for both the light- and medium-roasted samples, corresponded to a minimum in the chain-breaking activity values (**Figure 1a**,**b**). In contrast, in the case of the dark-roasted sample, the radical-scavenging properties significantly increased in the first 2 days of storage, while after a prolonged storage time, no further increase was observed (**Figure 1c**). Besides, it must be pointed out that the dark-roasted sample always had higher reducing and chain-breaking properties than the light- and medium-roasted ones at the corresponding storage times.

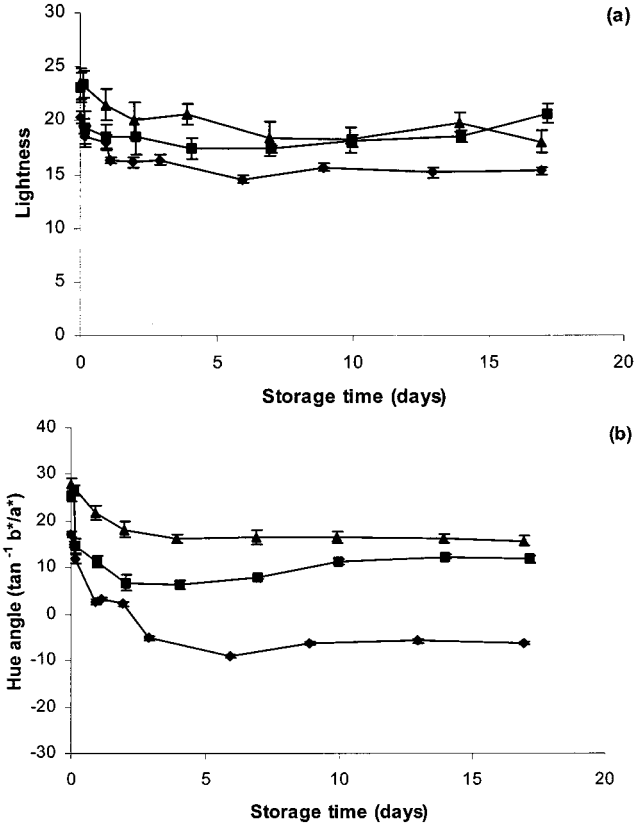

Figure 2. Changes in lightness (a) and hue angle (b) values of light- (▲), medium- (■), and dark-roasted (◆) coffee brews during storage at 30 °C in ordinary atmosphere.

It is well known that reactive radicals, exerting pro-oxidant activity, are formed in the early steps of the Maillard reaction, which develops when the roasting process is applied (*18*, *29*, *30*). The intensity and the duration of the heat treatment may influence the formation of such pro-oxidant compounds (*11*). In particular, it is likely that when low-temperature heating is applied, the reaction steps that contribute to the formation of pro-oxidant substances last longer than in the case of high-temperature treatments. During storage, free radical reactions may occur in coffee beverages, leading to the formation of nonradical forms as well as to the generation of other radical species that are chemically different from those initially present (*31*). By means of EPR measurements, it was demonstrated that the decomposition of intermediate radical cations, formed in the very early stages of the Maillard reaction in foods subjected to intense heating, was accompanied by color development (*29*, *32*). Development of browning is, in turn, associated with an increase in the antioxidant properties (*33*). **Figure 2** shows the changes in lightness and hue angle values of the light-, medium-, and dark-roasted coffee brews during storage at 30 °C in ordinary atmosphere. All the examined samples showed in the first days of storage a significant decrease in both color parameters, that is, a further increase in brown color. The higher the roasting degree, the higher the browning development. These results are consistent with those reported by Groetzbach et al. (*34*), who found that the color changes of coffee brews during warm storage increased as the oxygen concentration, either dissolved in the solution or present in the headspace, was increased. Therefore, these results suggest that the Maillard reaction may further develop during storage, leading to the

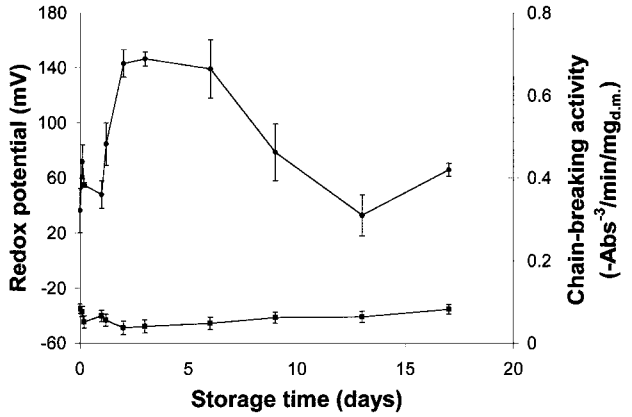

Figure 3. Changes in the chain-breaking activity (●, right *y*-axis) and redox potential (■, left *y*-axis) values of the dark-roasted coffee brew during storage at 30 °C in nitrogen atmosphere.

formation of new products. Hence, the initial reduction in the overall antioxidant properties of the light- and medium-roasted coffees can be attributed to the prevalence of pro-oxidant species. However, the gain in both radical-scavenging and reducing activity during storage is likely attributable to the formation of MRPs with antioxidant properties from the radical precursors. In the case of the dark-roasted coffee sample, due to the higher intensity of the roasting process, no correspondence between the evolution of the chain-breaking activity and the redox potential was observed, suggesting that the relationship between these two parameters is more complex than that expected and that probably they are indicators of different phenomena.

To study the changes in the overall antioxidant properties in the absence of oxygen, the coffee brews were stored at 30 °C, also under nitrogen atmosphere. **Figure 3** shows the changes in the chain-breaking activity and redox potential of the dark-roasted coffee brew during storage. Although the radical-scavenging activity values changed in a way very similar to that of the air-packaged sample, no changes in the reducing properties were detected, indicating that, in the absence of oxygen, the coffee brew maintained the initial reducing properties over all the storage time. Similar results were obtained for the light- and medium-roasted coffee brews (data not shown). These results suggest that the changes in the chain-breaking activity may not be attributable to redox reactions. It is likely that the latter are due to non-oxidative polymerization reactions of melanoidins or melanoidin precursors. These results are consistent with the fact that the generation of radicals is greatly influenced by the presence of oxygen (*31*). Therefore, under nitrogen atmosphere storage conditions, no evolution of free radicals into new compounds seems to be possible. Results on the changes in redox potential and radical-scavenging activity of coffee samples stored under nitrogen would confirm that these two parameters are indices of different events, not correlated with each other. In addition, neither lightness nor hue angle values changed during storage of the coffee brews in nitrogen atmosphere (data not shown). These data are in agreement with those of Groetzbach et al. (*34*), who found that the addition of a reducing substance to the coffee beverages greatly decreased color changes.

Finally, as the acceptability of coffee brews is greatly affected by the pH decrease during storage, the influence of air and nitrogen atmospheres on this parameter was studied. **Table 3** shows the zero-order kinetic constants of the changes of hydrogen ion concentration ($[H^+]$) of the light-, medium-, and dark-roasted coffee brews, bottled in air or nitrogen. The kinetic constants were calculated from the slope of the linear regression analysis of $[H^+]$ as a function of storage time. The rate of pH decrease was not affected by the presence of oxygen, in agreement with previous observations (*35*), and over a period of 17 days all the samples reached the pH threshold for coffee acceptance of 4.8 (data not shown) (*36*). These results indicate that the decrease in pH may not be attributed to oxidation reactions. Thus, to reduce or inhibit pH changes, other technological "devices" should be applied.

Table 3. Rate Constants (*k*) of Changes of Hydrogen Ion Concentration ($[H^+]$) of Light-, Medium-, and Dark-Roasted Coffee Brews, Bottled in Air or Nitrogen

degree of roasting	atmosphere	*k* ($[H^+]$/day) (R^2)[a]
light	air	4×10^{-7} a (0.95)
	nitrogen	4×10^{-7} a (0.97)
medium	air	5×10^{-7} a (0.95)
	nitrogen	5×10^{-7} a (0.96)
dark	air	6×10^{-7} a (0.95)
	nitrogen	5×10^{-7} a (0.96)

[a] The kinetic constants were calculated from the slope of the linear regression analysis of $[H^+]$ as a function of storage time. Values in the same column with different letters are significantly different ($P < 0.05$).

From a practical standpoint, the results reported here suggest that the higher the intensity of the roasting process, the higher the overall antioxidant properties of coffee. Although the changes in the radical-scavenging properties during storage were not affected by the presence of oxygen, packaging of the ready-to-drink coffee brews under reducing conditions could represent an efficient way of keeping the redox potential—hence the reservoir of antioxidants which is dispatched during storage—low. Packaging under nitrogen also allowed the original color of coffee brews to be maintained over all the storage time examined but was ineffective in inhibiting or slowing down the pH decrease.

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