



Research Note

# Impact of the roasting degree of coffee on the in vitro radical scavenging capacity and content of acrylamide

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## Abstract

Due to the recognized toxicity of acrylamide, intensive efforts have been made to reduce the concentration of this undesired Maillard by-product in food. This work reports the results obtained from a series of experiments aimed at determining the concentration of acrylamide and the in vitro radical scavenging capacity in the same roasted and ground coffee samples, as it is well established that a significant part of the antioxidant activity in coffee is linked to the melanoidins, which are also considered as Maillard reaction products (MRPs). The radical scavenging capacity was measured using electroparamagnetic resonance (EPR). Coffee samples from the Robusta and Arabica varieties were roasted at 236 °C over different time periods to obtain very light, light, medium and dark roast. Color analyses were performed on all samples. Increasing the roasting degree led to a decrease in acrylamide concentration as well as radical scavenging capacity. The results of this work indicate that any mitigation efforts must also take into account the potential loss of desired food constituents and consequently changes to the risk/benefit characteristics of foods.

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**Keywords:** Acrylamide; Coffee; Antioxidants; Melanoidins; Maillard reaction

## 1. Introduction

Since the presence of acrylamide in heated starch-based foods was discovered in 2002, extensive efforts have been undertaken by public research institutions and the food and drink industry to investigate ways that may lead to a reduction of acrylamide during food processing (Stadler & Scholz, 2004; Taeymans et al., 2004). Relatively high levels of acrylamide have been found in potato and cereal products, bakery items and coffee. The high consumption of coffee in certain countries makes it a potentially significant source of dietary exposure to acrylamide. Coffee contributes 39% to the total acrylamide exposure in

Sweden (Svensson et al., 2003), and in Switzerland the intake of acrylamide from coffee was estimated to be about one-third of the total daily intake (Swiss Federal Office of Public Health, 2002). In this context, the concentration of acrylamide in roast and ground coffee samples have been reported to range from 45 to 539  $\mu\text{g k}^{-1}$  (Andrzejewski, Roach, Gay, & Musser, 2004; Delatour, Périsset, Goldmann, Riediker, & Stadler, 2004; Şenyuva & Gökmen, 2005). Nevertheless, statements on the concentration of acrylamide in coffee must be made with caution as independent reports have shown that acrylamide is not stable in coffee, i.e. its concentration decreases over time (Andrzejewski et al., 2004; Hönicke & Gatermann, 2005).

Coffee is also known to be a rich source of compounds with potent antioxidant activity. In a survey on dietary contribution of antioxidants from coffee, wine and vegetables performed among Norwegian adults, it was shown that coffee contributed to 64% of the total antioxidant intake, followed by fruits, berries, tea, wines,

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cereals and vegetables (Svilaas et al., 2004). Similar results were reported by (Pellegrini et al., 2003) when comparing the total antioxidant capacity of plant foods, beverages and oils consumed in Italy. In an “in-vivo” study performed with rats, it was observed that feeding rats with coffee brew resulted in an increase of the total antioxidant capacity of the plasma (Somoza et al., 2003). A major contributor to the antioxidant activity was identified as *N*-methylpyridinium, a recently discovered alkaloid that is present in roasted coffee at concentrations of up to 0.25% on a dry weight basis (Stadler et al., 2002). Moreover, coffee consumption has been associated with reduced incidences of several types of cancer (Leitzmann et al., 2002; Tavani & La Vecchia, 2000), Parkinson’s disease (Ascherio et al., 2001), liver cirrhosis (Tverdal & Skurtveit, 2003) and type 2 diabetes (Van Dam & Feskens, 2002). The antioxidant capacity of coffee has been attributed to its content in polyphenols and melanoidins (Anese & Nicoli, 2003; Borrelli, Visconti, Mennella, Anese, & Fogliano, 2002; Delgado-Andrade & Morales, 2005; Yen, Wang, Chang, & Duh, 2005). Acrylamide and melanoidins are both Maillard reaction products (MRPs) formed during the roasting of coffee, typically conducted at temperatures between 220 and 250 °C. Theoretically, any attempt to inhibit the Maillard reaction as a possible measure to minimize the formation of acrylamide would lead to a reduction of the antioxidant capacity of coffee, as it has been already observed in cookies (Summa et al., 2006). In this study, both acrylamide and the in vitro radical scavenging capacity were measured in the same coffee samples to establish whether these two important parameters are related to the degree of roast.

## 2. Materials and methods

### 2.1. Instrumental

#### 2.1.1. Determination of the radical scavenging capacity

An electro-paramagnetic resonance (EPR) bench-top spectrometer, of high sensitivity for spin resonance spectroscopy, MS 200 (Mini Scope 200- Magnettech) was used to determine the antioxidant activity of the coffee samples. The measurement conditions with the EPR system were: Magnetic field 3360 G; sweep time 50 s (period of time in seconds needed for 1 scan); modulation amplitude 460 mG; microwave power 13 dB (corresponds to ~5 mW) and gain 2 (for amplification of the recorded signal). The EPR spectrum of the Fremy’s salt (nitrosodisulfonate) radical was obtained after 20 min, by which time the reaction was complete. The velocity by which the signal decreases determines the antioxidant potential and this decrease in signal intensity provides a measure of the antioxidant capacity. A 1 mmol L<sup>-1</sup> (2.50 mg (10 mL)<sup>-1</sup> methanol) trolox solution in methanol:water (50%) was used for calibration purposes.

#### 2.1.2. Determination of acrylamide

The concentration of acrylamide in coffee was determined following a validated analytical method reported elsewhere (Delatour et al., 2004).

#### 2.1.3. Color measurements

Colorimetric measurements were carried out using a Chromameter CR-410 (Konica Minolta, NJ, USA) which relies on the *L\*a\*b* color space, devised in 1976 by the Commission Internationale de l’Eclairage (CIE), as a means of expressing color numerically. In this color space, *L\** indicates lightness and *a\** and *b\** are the chromaticity coordinates. After calibration of the colorimeter using the instrument’s reference white plate, color profiles of the samples were obtained by measuring the coffee powder directly in the 50 mm (diameter) cell, in duplicate.

### 2.2. Reagents

All reagents used were of analytical reagent grade or higher.

*Fremy’s salt solution* (nitrosodisulfonate) (Sigma-Aldrich, Bornem, Belgium): A solution of 2 mmol L<sup>-1</sup> of Fremy’s salt was prepared in phosphate buffer pH = 7.4.

*Trolox* (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) *solution* (Sigma-Aldrich): A solution of 1 mmol L<sup>-1</sup> (2.50 mg (10 mL)<sup>-1</sup> methanol) of trolox was prepared in methanol:water (50%). The trolox solution was diluted 1:2; 1:4; 1:8 with methanol:water (50%) to have a concentration range in the sample vial from 0.063 to 0.250 mmol L<sup>-1</sup> for the calibration curve.

About 0.1 mol L<sup>-1</sup> phosphate buffer pH 7.4 was prepared by mixing 405 mL of a solution 0.2 mol L<sup>-1</sup> Na<sub>2</sub>HPO<sub>4</sub> (Sigma-Aldrich) and 95 mL of a solution 0.2 mol L<sup>-1</sup> NaH<sub>2</sub>PO<sub>4</sub> (Sigma-Aldrich) and diluting up to a final volume of 1 L.

### 2.3. Sample preparation

Very light-, light-, medium- and dark-roasted coffees comprised of 100% Robusta and 100% Arabica beans were used in this study. The origin of the Robusta coffee was Togo whilst the Arabica coffee was obtained from Colombia. The coffee beans were roasted in batches of 300 g using a benchscale roaster (type Neuhaus RFB-L 50.384). Four batches were combined for every category analyzed. The beans were roasted at 236 °C over a period of 260–620 and 235–560 s, for Robusta and Arabica beans, respectively. The very light-, light-, medium- and dark-roasted coffee samples were characterized by reflectance measurement (color test Neuhaus) and expressed as arbitrary color units (CTN values) of 120 (very light roast, roast time 235–260 s), 100 (roast time 280–320 s), 80 (roast time 370–430 s) and 60 (very dark roast, roast time 560–620 s). Roasting losses of 10.03%, 11.22%, 12.27% and 15.07%, respectively for the Robusta coffee samples

and 12.73%, 13.7%, 14.73%, 17.67%, respectively for the Arabica samples, were obtained.

To calculate the radical scavenging capacity of the samples, 2 g samples of homogenized coffee powder were dissolved in 20 mL of water (~40–50 °C), vortexed, and then shaken in a water bath (60 °C) for 15 min. Each sample was then centrifuged at 3500 rpm for 20 min (at 5 °C). The aqueous phase underneath each sample's fat layer was then transferred to a new vial. An aliquot of the aqueous phase was then taken from each sample vial and centrifuged again at 4000 rpm for 10 min at 5 °C. The resulting aqueous phase underneath the fat layer, obtained from the second centrifugation, was then filtered (via 0.45 µm nylon syringe top filter) before measurement by EPR. Prior to analysis, each sample was diluted 1:100 with water. A 100 µL aliquot of sample solution/or Trolox solution was mixed with 100 µL of Fremy's salt in a 1.5 mL Eppendorf tube and 50 µL (volume of a standard capillary) of this mixture was utilized for the analysis.

For the determination of acrylamide, the coffee samples were treated as described by Delatour et al. (2004).

Three independent analyses were performed on every sample for both the determination of acrylamide and the radical scavenging capacity.

### 3. Results and discussion

#### 3.1. Influence of the roasting degree on the concentration of acrylamide and the *in vitro* radical scavenging capacity

There are two coffee species of commercial importance, namely *Coffea arabica* (Arabica) and *Coffea anephora* (Robusta). Arabica accounts for some 64% while Robusta accounts for about 35% of the world's coffee production. Regarding the chemical composition, both species are characterized by different contents of minerals, volatile substances, chlorogenic acids and caffeine (Rubayiza & Meurens, 2005). Differences have also been found between the two species in the high-molecular-weight material extracted with hot water from green and roasted coffees as affected by the degree of roast (Nunes & Coimbra, 2002). The two species, Arabica and Robusta, were selected to perform the present study in order to check the influence of the coffee composition on the studied parameters.

Green coffee samples were not analyzed as coffee is usually not consumed without a certain degree of roasting.

It is well-known that during the typical temperatures of roasting, the loss of antioxidant activity due to the thermal degradation of natural antioxidants (mainly polyphenols such as chlorogenic acids), can be compensated or even enhanced by the formation of active MRPs such as melanoidins (López-Galilea, Andueza, di Leonardo, Paz de Peña, & Cid, 2004), which have been proven to be important contributors to the radical scavenging properties of coffee (Hofmann, Bors, & Stettmeier, 1999).

As depicted in Fig. 1, both the antioxidant activity as well as the concentration of acrylamide decrease in

Robusta and Arabica coffee beans upon darker roasting, i.e. lower CTN values. With the exception of the Robusta samples CTN 80 and CTN 100, the radical scavenging capacity and acrylamide content for the different degrees of roast were significantly different within a series when analyzed by one-way ANOVA ( $\alpha = 0.05$ ), reflecting a significant impact of thermal treatment upon these parameters. The acrylamide content of the Robusta coffee versus the Arabica variety were also observed to be significantly different when analyzed by one-way ANOVA ( $\alpha = 0.05$ ). It is, however, important to note that Robusta and Arabica coffees are botanically different species and differ in chemical composition. Thus, Robusta and Arabica coffees roasted to comparable CTN values are not exposed to the same thermal load, a major contributor that favors pyrolytic processes and consequently affords the differences observed in chemical parameters (and sensorial properties). Comparable results were also obtained for Robusta and Arabica coffee samples from different origins, namely Uganda and Honduras, respectively, analyzed prior to performing the study reported in this work.

With regard to the amount of acrylamide measured in the samples, these are in good agreement with data reported in the literature (Stadler & Scholz, 2004; Taeymans et al., 2004), i.e. light-roasted coffees may contain relatively higher amounts of acrylamide than very dark-roasted beans. This is due to the fact that acrylamide is formed at the beginning of the roasting step, declining then steeply towards the end of the roasting cycle due to higher rates of elimination (through physical and chemical losses) versus formation (Stadler & Scholz, 2004).

The evident reduction of radical scavenging capacity with increasing degree of roast is in good agreement with the results reported by Del Castillo, Ames, & Gordon (2002), who observed a higher antioxidant activity for light- and medium-roasted coffees than for green or dark coffee, using the ABTS<sup>+</sup> [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] method. Also, Borrelli et al. (2002) observed that the free radical scavenging properties of melanoidins determined by ABTS<sup>+</sup> and DMPD<sup>+</sup> (1,1-diphenyl-2-picrylhydrazyl) decreased as the intensity of roasting increased. The same authors also found that the ability to prevent linoleic acid peroxidation was higher in dark-roasted compared to light- and medium-roasted coffees and suggested that melanoidin polarity and solubility play a major role in affecting the surface tension of linoleic acid-containing micelles dispersed in the water phase, influencing linoleic acid oxidation. This theory may also explain why Delgado-Andrade and Morales (2005) failed to observe any significant differences amongst different roasting degrees for melanoidins and pure melanoidins (i.e. obtained after removal of low-molecular-weight and non-covalently bound compounds), based on the antiperoxide radical activity measured with linoleic acid.

In this work, the antioxidant activity of the coffee samples was evaluated by measuring the capacity of the

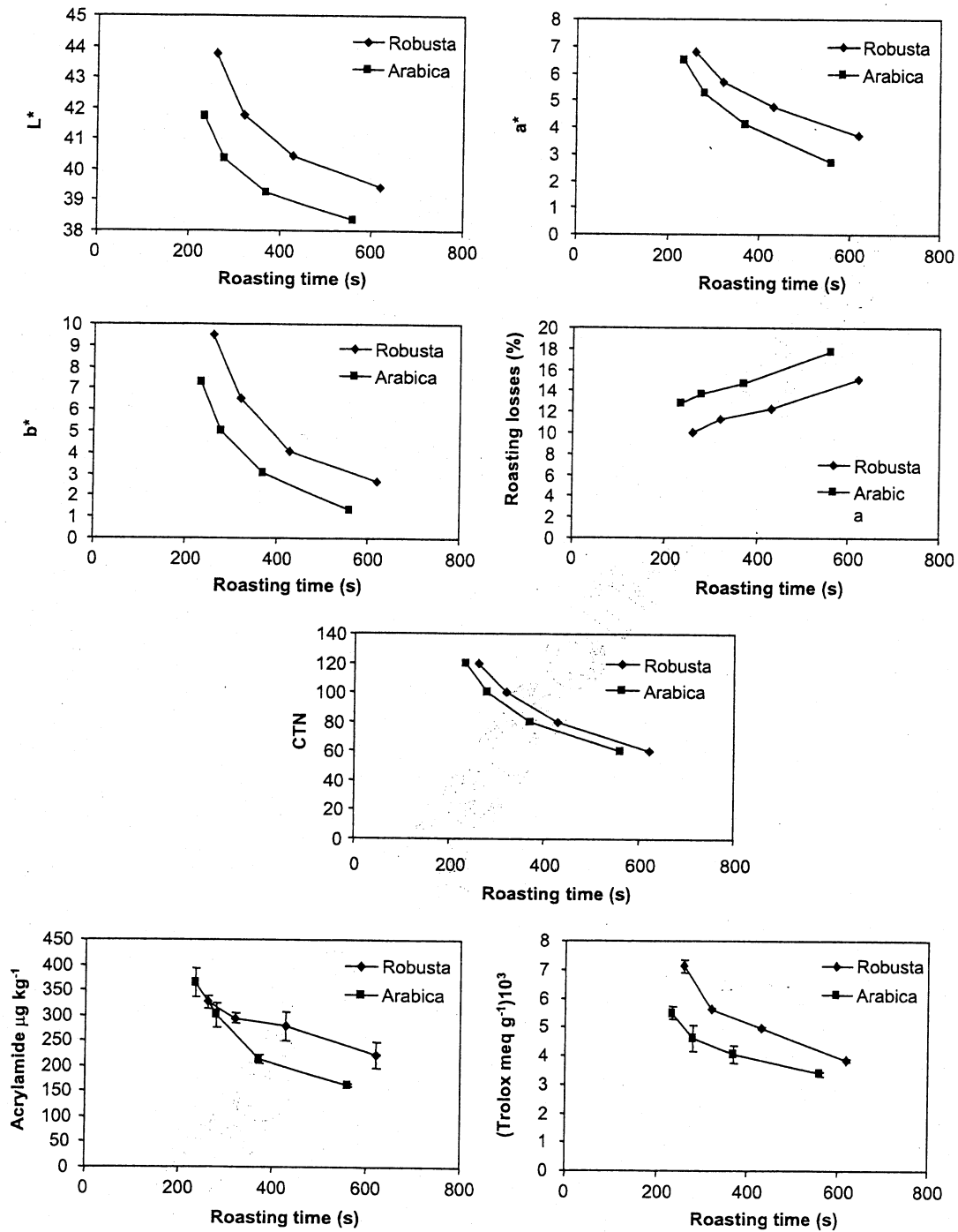


Fig. 1. Changes in coffee beans during hot air roasting at 236 °C.

sample to scavenge radicals using EPR. Thereby, free radicals were directly measured avoiding the use of reference colored radical cations or other references, such as linoleic acid, which could undergo different mechanisms of reaction and could then possibly lead to results that are difficult to compare. The use of EPR to determine the antioxidant activity of coffee has already been reported in the literature (Cämmerer, Adlouni, & Kroh, 2003).

### 3.2. Correlation between color, acrylamide and the radical scavenging capacity

The roasting degree is usually measured by the reflectance of light and can be described in terms of lightness ( $L^*$ ). This parameter is a suitable indicator of the thermal load corroborated by a good correlation of  $L^*$  versus  $C^*$  (chromaticity),  $C^* = (a^{*2} + b^{*2})^{1/2}$ ,  $r^2 = 0.9610$ ,

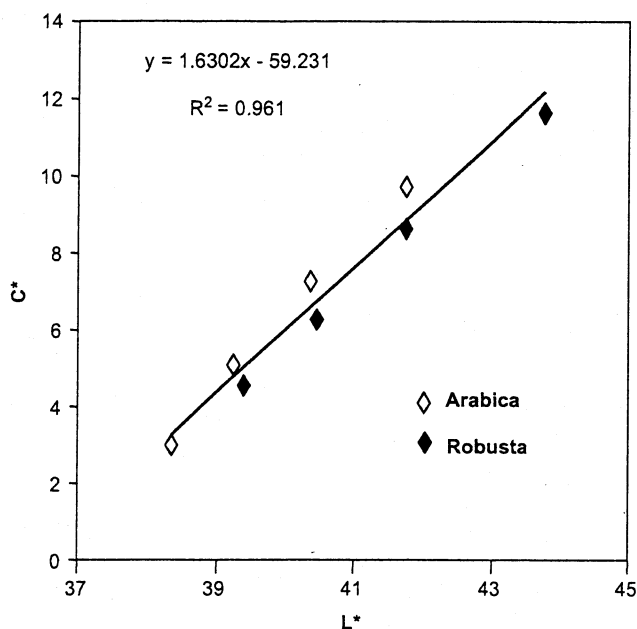


Fig. 2. Relation between  $L^*$  and  $C^*$  in the Arabica and Robusta coffee samples roasted at 236°C.

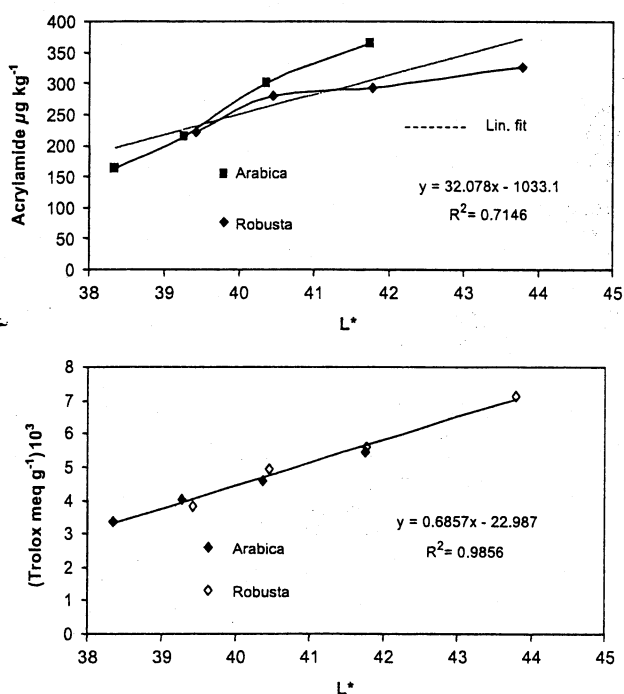


Fig. 3. Changes in acrylamide content and radical scavenging capacity in roasted Arabica and Robusta beans depending on lightness  $L^*$ .

$n = 8$ , Fig. 2 (Schenker, 2000) and enables a better comparison of botanically and chemically distinct species such as Arabica and Robusta coffee beans. In agreement with published data (Nicoli, Anese, Manzocco, & Lerici, 1997), the results for both Arabica and Robusta obtained

in this study show a tendency towards a decrease of  $L^*$ ,  $a^*$  and  $b^*$  (CIE) with increased roasting (Fig. 2, only data for  $L^*$  shown). In fact, the radical scavenging capacity is a linear function of  $L^*$  ( $r^2 = 0.9856$ ). This correlation follows the same pattern for the Arabica and the Robusta coffees, suggesting that the chemical composition of the green beans seems only of minor influence with regard to free radical scavenging capacity.

Plotting the acrylamide concentration as a function of  $L^*$  for Arabica and Robusta shows a different trend for the two coffee species (Fig. 3). They clearly follow a linear function albeit with a different rate of “loss” of acrylamide upon darker roasting. Particularly for Arabica, these indicate that only small changes in the processing conditions may have a large impact on the acrylamide amount in the final product.

A similar study was performed by Şenyuva and Gökmen (2005) who observed a non-linear logarithmic correlation between acrylamide concentration and  $a^*$ , and an exponential decrease of  $L^*$  and  $b^*$  with the roasting time. No further comparison between those results and the results obtained in the present work could be made as the experimental conditions clearly differ.

#### 4. Conclusions

More intense roasting, i.e. greater thermal load, of coffee beans has been considered as a way to decrease the concentration of acrylamide in coffee, albeit with a major impact on the organoleptic properties and consequently acceptability of the product. However, the results obtained in this study show that a reduction in the concentration of acrylamide with darker degrees of roasting is accompanied by a reduction of the radical scavenging capacity of coffee (within the same coffee species). Furthermore, temperature, time and the speed at which coffee is roasted have an important impact on the organoleptic properties of coffee and under extreme conditions could generate other “undesirable” compounds.

Similar results to those discussed here for coffee have been reported for cookies (Summa et al., 2006), in which also a direct correlation between acrylamide concentration and antioxidant activity has been found.

This and other recent studies emphasize the importance of considering the impact of mitigation on other constituents of the food that are generally considered as beneficial (risk–benefit), which is particularly relevant in foods such as coffee that have been reported to contribute significantly to the total antioxidant load in our daily diet.

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