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Acrylamide in espresso coffee: Influence of species, roast degree and brew length

Rita C. Alves *,[1], C. Soares [1], Susana Casal, J.O. Fernandes, M. Beatriz P.P. Oliveira

REQUIMTE/Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha 164, 4099-030 Porto, Portugal

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ABSTRACT

Espresso coffees were analysed for acrylamide contents by matrix solid-phase dispersion and GC–MS. The influence of coffee species, roast degree, and brew length were ascertained. Mean acrylamide contents of medium roasted espressos (30 mL) were 1.16 ± 0.25 and 2.31 ± 0.43 μg for pure arabica and robusta samples, respectively. Espressos prepared from commercial blends contained an average acrylamide level of 1.26 ± 0.28 μg. A 25% decrease was observed when comparing espressos prepared with medium and dark roasted coffee. The extraction efficacy of acrylamide for standard espressos of 30 mL was near 80%, being only affected by brew volume, with long espressos (70 mL) containing practically all acrylamide of the coffee cake (99%), almost double that of short ones (20 mL). When compared with other common coffee beverages, espresso acrylamide concentration (μg/L) was higher. However, due to the small volume per cup, it may contribute less to acrylamide ingestion.

1. Introduction

Acrylamide (2-propenamide), labelled by the International Agency for Research on Cancer (IARC, 1994) as probably carcinogenic to humans (Group 2A), is presently a focus of worldwide concern, especially since the announcement, in 2002, of its widespread occurrence in carbohydrate-rich cooked foods by the Swedish National Food Administration (SNFA, 2002). Several scientific initiatives have been launched in order to fully understand its chemistry and toxicology, focusing chiefly on its formation mechanism and possible human consequences.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2005) highlighted the importance of acrylamide occurrence data in foods consumed in developing countries, as a valuable tool in conducting intake assessments and mitigation approaches to reduce human exposure (a high priority for governments and industries) (Arisseto & Toledo, 2006).

A high relevance is being given to coffee, as an important dietary source of acrylamide, mainly in the Nordic European countries where it may contribute up to one third of total dietary intake (Dybing & Sanner, 2003; Guenther, Anklam, Wenzl, & Stadler, 2007; Svensson et al., 2003). Among other possible reaction pathways, the Maillard reaction represents the main route for acrylamide formation in coffee, being initiated by the condensation of asparagine and reducing carbohydrates or reactive carbonyls, when the beans are subjected to the high roasting temperature (Guenther et al., 2007). Acrylamide formation starts rapidly at the beginning of the roasting process and it decreases shortly after reaching a maximum level, probably due to physical and chemical losses (Bagdonaite, Derler, & Murkovic, 2008; Guenther et al., 2007; Lantz et al., 2006; Senyuva & Gökmen, 2005; Taeymans et al., 2004). Therefore, the degree of roasting will be a key factor in acrylamide content, with light roasted coffee attaining significantly higher amounts when compared with dark roasted counterparts (Bagdonaite et al., 2008; Guenther et al., 2007; Lantz et al., 2006; Senyuva & Gökmen, 2005; Taeymans et al., 2004). Moreover, when comparing the two coffee species of higher economical importance, namely *Coffea arabica* and *Coffea canephora* (also known as arabica and robusta coffees, respectively) increased levels of acrylamide are described for the latter (Bagdonaite et al., 2008; Guenther et al., 2007; Lantz et al., 2006; Summa, de la Calle, Brohee, Stadler, & Anklam, 2007). As a result, the reported levels for roasted coffee beans vary widely, usually within the range of 35–540 μg/kg of coffee (Aguas, Fitzhenry, Giannikopoulos, & Varelis, 2006; Andrzejewski, Roach, Gay, & Musser, 2004; Delatour, Périsset, Goldmann, Riediker, & Stadler, 2004; Friedman, 2003; Guenther et al., 2007; Hoenicke & Gatermann, 2005; Lantz et al., 2006; Murkovic, 2004; Roach, Andrzejewski, Gay, Nortrup, & Musser, 2003; Senyuva & Gökmen, 2005; Summa et al., 2007).

While the majority of published studies have focused on the assessment of acrylamide content in coffee beans, a research priority is to investigate the amount of it effectively ingested by consumers through coffee brews. Acrylamide is highly soluble in water and, thus, easily transferred from the coffee powder to the beverage (Andrzejewski et al., 2004). However, the chemical composition of coffee brew is highly dependent on several factors,

* Corresponding author. Fax: +351 222 003 977.
E-mail address: rita.c.alves@gmail.com (R.C. Alves).
[1] Both authors contributed equally to this project.

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including the amount of arabica and robusta used to prepare the blend, their degree of roasting, as well as the coffee/water ratio used, which depends on cultural and personal preferences (Alves, Casal, & Oliveira, 2007). Some studies have reported acrylamide levels in common coffee beverages (as plunger pot and filtered coffee) ranging between 2 and 25 μg/L (Andrzejewski et al., 2004; Dybing et al., 2005; Granby & Fagt, 2004; Pérez & Osterman-Golkar, 2003; Svensson et al., 2003).

Among all coffee brews, espresso is highly appreciated in Portugal, and its consumption is increasing worldwide. This brew is prepared by a special brewing technique in which a limited amount (20–50 mL) of hot water under high pressure (9 ± 2 atm, 90 ± 5 °C) is percolated in a very short time (30 ± 5 s) through a ground coffee cake (6.5 ± 1.5 g). The result is a concentrated and intensely flavoured brew covered by a dense foam layer, which should be tasted at the exact moment of extraction (Alves et al., 2007).

Lantz et al. (2006) reported that espresso coffee brewing incompletely extracts acrylamide from ground coffee, unlike other coffee brews (as plunger pot or filtered coffee) due to the short contact time with water. Therefore, it is of interest to study the technological parameters affecting acrylamide extraction into the espresso brew. Our group has already reported some preliminary data on acrylamide levels of standard espresso coffee (0.32–1.46 μg/30 mL or 10.7–48.7 μg/L) (Soares, Cunha, & Fernandes, 2006). The aim of the present work was to focus exclusively on this peculiar beverage, ascertaining the factors implicated in the acrylamide extraction, contributing to a better knowledge of the exposure levels of espresso consumers. The influence of coffee species and their degree of roasting, together with the extractability achieved by different percolation periods, are detailed. The acrylamide levels were also evaluated in several commercial samples, decaffeinated and servings included, and the values compared with those described for other common coffee beverages.

2. Materials and methods

2.1. Chemicals and reagents

Acrylamide was obtained from Aldrich (Steinheim, Germany). The internal standard $^{13}C_3$-acrylamide was purchased from Cambridge Isotope Laboratories (Andover, MA, USA) as a 1 mg/mL solution in methanol.

Preparative C_{18} sorbent (125 Å, 55–105 μm) was from Waters (Milford, MA, USA). The ISOLUTE C_{18}/Multimode layered solid-phase extraction (SPE) columns were from Biotage (2 g/15 mL, Uppsala, Sweden). The HPLC water was purified with a "Seral" system (SeralPur Pro 90 CN).

Potassium bromide (IR spectroscopy grade) and bromine (analytical grade) were from Merck (Darmstadt, Germany). Hydrobromic acid (48%) and sodium thiosulphate (1 mol/L) were from Riedel-de Häen (Seelze, Germany). A saturated bromine–water solution was prepared by adding bromine (~3 mL) to 200 mL of water until precipitation became visible (Fernandes & Soares, 2007).

Sodium chloride (analytical grade) was from J.T. Baker (Deventer, the Netherlands). *n*-Hexane, ethyl acetate (pesticide residue analysis grade), methanol and acetonitrile (ultrapure grade) were all from Fluka (Madrid, Spain). All other chemicals were of analytical grade.

2.2. Standards

A stock solution of acrylamide (2 g/L) was prepared by dissolving it in acetonitrile and then serially diluted to prepare working standard solutions. A working 10 mg/L solution of the internal standard was also prepared in acetonitrile. All stock and working solutions were stored at 4 °C.

2.3. Coffee samples

Commercial caffeinated ($n = 14$) and decaffeinated ($n = 6$) roasted beans, as well as servings ($n = 7$), were obtained in local supermarkets and cafeterias, and some supplied by a Portuguese industrial importer and roaster of coffee.

Green samples of *C. arabica* ($n = 8$) and *C. canephora* var. *robusta* ($n = 8$), from different geographical origins, were kindly supplied by the same industry. The arabica samples were from Hawaii, Costa Rica, Jamaica, Colombia, Ethiopia, Honduras ($n = 2$) and Brazil, and robusta samples were from India ($n = 2$), Uganda ($n = 2$), Cameroon ($n = 2$), Ivory Coast and Indonesia. All robusta samples were dry processed. Samples of arabica coffees were wet processed, except the one from Brazil, which was dry processed. Samples were individually roasted in a Probat L12 coffee roaster from Probat-Werke according to a standard method (210 °C, 10 min), usually practiced by the local industrial roaster. Additionally, four green coffee samples, two arabicas (Honduras and Brazil) and two robustas (Uganda and Ivory Coast), were also subjected to three different lengths of heat exposure (8–11 min, 210 °C, Probat Pré 1Z 2000, from Probat-Werke), in order to achieve three final roasting degrees (light, medium and dark), not exceeding the range of commercial espresso roasts usually practiced in Portugal. The roasting degree was determined by photometric analysis with infrared radiation Colorimeter Colorette 3 from Probat-Werke and also by the organic roast loss (ORL) evaluation in dry weight (% ORL dw) (Clarke, 1989; Illy & Viani, 2005). Sample moisture to calculate ORL was determined by drying at 103 ± 2 °C until a constant weight was reached. All samples were stored at 4 °C before analysis.

2.4. Brews preparation

All coffee beans were mechanically powdered to pass through a 0.75 mm sieve in the integrated grinder of a HL3854 *Espresso Professional* (Philips, The Netherlands). Espresso coffees (ECs) of 30 mL were prepared with deionized water (Amberlite MD 20) in the same espresso machine, using 6.5 g of ground coffee, exactly weighted. To evaluate extraction efficiency, an arabica (Honduras) and a robusta (Ivory Coast) sample were individually extracted (6.5 g) with different volumes of water ranging from 20 mL, a typical "ristretto" or "Italian", to 70 mL, the longest EC usually consumed in Portugal. ECs from paper coated servings were prepared in the same machine, by changing the filter chamber to one adapted to servings. ECs from aluminium coated servings were extracted in a Krups XN2105 (Germany).

2.5. Samples analysis

2.5.1. Matrix solid-phase dispersion

Acrylamide content and extractability were analysed using a previously optimised and validated methodology based on matrix solid-phase dispersion (Soares, Alves, Casal, Fernandes, & Oliveira, submitted for publication). Briefly, a 2.5 mL aliquot of beverage (or 0.5 g of ground coffee) was spiked with the internal standard (25 μL) and dispersed with C_{18} sorbent (previously conditioned with methanol and water). The mixture was then transferred to a preconditioned ISOLUTE C_{18}/Multimode SPE column and carefully compressed with a frit on the top. A Visiprep SPE Vacuum Manifold 57030-U (Supelco, Bellefonte, PA, USA) was used to manipulate the cartridges and the acrylamide elution was achieved with 4 + 4 mL of water, allowing soaking steps of 5 min. The sorbents dryness

was carefully avoided during the entire procedure. All samples were analysed in duplicate.

2.5.2. Bromination

The bromination step was performed as described elsewhere (Soares et al., 2006). Briefly, calcinated potassium bromide (1 g), hydrobromic acid (~150 μL) and saturated bromine solution (~2 mL) were added to the collected extract. The mixture was kept on ice, in the dark, at least for 1 h. Sodium thiosulphate (~150 μL) was used to neutralise the excess of bromine. After adding NaCl (~4 g), a double liquid–liquid extraction with ethyl acetate/*n*-hexane 4:1 (v/v) (10 + 5 mL) was performed. The collected organic extract was dried with anhydrous Na_2SO_4, centrifuged, concentrated (N_2, 60 °C) to about 0.5 mL and injected in the gas chromatograph.

2.5.3. Gas chromatographic–mass spectrometric analysis

The chromatographic analysis was carried out in a gas chromatograph (Agilent GC-6890N) equipped with a split–splitless injector and an automatic sampler (Agilent 7683B Series) coupled to a mass selective detector (Agilent MSD-5975N, Agilent, Palo Alto, CA, USA), according to Soares et al. (2006). The chromatographic separation was achieved on a capillary column MDN-12 (30 m × 0.25 μm, 0.25 mm i.d.) from Supelco (Bellefonte, PA, USA) and helium (1 mL/min, constant flow) was used as carrier gas. The sample injection volume was 1 μL (splitless, pulsed pressure 32 ψ, 60 s, 280 °C). The oven temperature was initially programmed at 85 °C for 1 min, increasing at 15 °C/min to 280 °C (10 min hold), and the transfer line set at 280 °C. The mass selective detector was set in the selected ion monitoring mode (SIM), selecting three characteristic fragments of each derivatized acrylamide (2,3-dibromopropionamide: *m*/*z* 106, 150, and 152) and derivatized internal standard (2,3-$^{13}C_3$-dibromopropionamide: *m*/*z* 110, 153, and 155). Peak identification was accomplished by retention time and comparison with standards. Quantification was performed on the basis of the internal standard using ions *m*/*z* 150 and 155, for 2,3-DBPA and 2,3-$^{13}C_3$-DMPA, respectively.

2.6. Statistical analysis

Data were recorded as mean ± standard deviation and analysed by the one-way ANOVA and Student's *t*-tests. All analyses were carried out with Microsoft Excel statistical software (Microsoft Office Excel 2003, Microsoft Corp., Redmond, WA).

3. Results and discussion

3.1. Variability within commercial samples

The acrylamide contents of caffeinated ECs (30 mL), prepared from commercial coffee blends, were highly variable, as can be observed in Table 1. The results are in accordance with those previously reported for standard espressos: 10.7–48.7 μg/L (Soares et al., 2006).

Table 1
Acrylamide contents of espresso coffees prepared from commercially available samples (caffeinated and decaffeinated).[a]

Commercial samples	*n*	μg/L			μg/EC (30 mL)		
		Mean	Min.	Max.	Mean	Min.	Max.
Caffeinated	14	41.9 a	27.4	58.5	1.26 a	0.82	1.76
Decaffeinated	6	33.2 a	24.8	49.5	1.00 a	0.74	1.49
Servings*	7	42.3 a	33.4	55.3	1.27 a	1.00	1.66

EC, espresso coffee; S.D., standard deviation; *, caffeinated samples.
[a] Data followed by the same letters within each column are not significantly different according to ANOVA ($p > 0.05$).

Although average levels obtained for decaffeinated ECs were lower, when compared with caffeinated samples (Table 1), the differences between both groups were not statistically significant ($p > 0.05$), suggesting that decaffeination process does not significantly affect acrylamide precursors in green coffee beans.

Servings are individual doses of ground coffee (about 6–7 g) coated with a paper layer, commercially available and produced to make an EC in a rapid, simple, and clean way, in adapted machines. Recently, new coffee servings have emerged which are coated with an aluminium layer instead of paper. Also, no significant differences ($p > 0.05$) were found when comparing the espressos prepared from servings with regular and decaffeinated ECs (Table 1). The great variability found in samples tested (Table 1), with some espressos containing twice the acrylamide amount than others, can be justified by consideration of several factors. According to some researchers, the acrylamide content of roasted coffee beans differs mainly with the coffee species (Bagdonaite et al., 2008; Lantz et al., 2006), degree of roasting (Bagdonaite et al., 2008; Lantz et al., 2006; Summa et al., 2007), and storage conditions (Andrzejewski et al., 2004; Delatour et al., 2004; Hoenicke & Gatermann, 2005; Lantz et al., 2006).

3.2. Influence of coffee species: arabica and robusta

Table 2 shows the influence of each coffee species (arabica and robusta) on the acrylamide content of EC. Although the samples were medium roasted by a standard procedure, the organic roast loss was different within each species, as a consequence of the beans intrinsic characteristics.

Significantly higher amounts ($p < 0.001$) of acrylamide were found in robusta samples, with levels per cup (30 mL) ranging between 1.71 and 2.92 μg. For arabica coffee, the levels were half lower, varying from 0.87 and 1.52 μg/EC.

These results are in agreement with those described for coffee beans (Bagdonaite et al., 2008; Lantz et al., 2006). Lantz et al. (2006) reported average levels of 378 and 251 μg/kg, for robusta and arabica medium roasted coffees, respectively. This difference seems to be associated with an increased content of asparagine amount in robusta raw beans (Bagdonaite et al., 2008; Lantz et al., 2006), in comparison with arabica beans.

Arabica and robusta coffees, the two main species used to prepare brews, have different chemical and sensory properties. The quality of the beverage is usually dependent on the proportion of both in the blend, arabica being considered a higher value product. In Portugal, the majority of the commercially available coffee brands are mixtures of both arabica and robusta, with the latter usually at no more than 30% of the blend. However, some blends containing higher amounts of robusta can also be found in the market. The addition of this coffee species to the blend aims to increase the body and improve the espresso foam, together with some economical saving, since robusta price is lower.

Results shown in Table 2 are, therefore, indicative of the minimum and maximum levels of acrylamide that can be found in EC prepared with medium roasted coffee beans.

Although subjected to a different postharvest treatment, no significant ($p > 0.05$) differences were found between the dry-processed sample from Brazil and other wet-processed arabicas.

3.3. Influence of degree of roasting

When coffee beans are subjected to the high temperatures of roasting, innumerous chemical reactions occur as well as physical modifications that might influence the extraction of some compounds to the brew (Illy & Viani, 2005). In order to observe the

Table 2
Acrylamide contents in espresso coffees prepared with arabica or robusta medium roasted beans.[a]

Coffee species and geographical origin	% ORL	Color	μg/L	μg/EC(30 mL)
			Mean ± S.D.	Mean ± S.D.
Arabica				
Hawaii	5	132	29.1 ± 0.8	0.87 ± 0.02
Costa Rica	11	127	38.0 ± 0.3	1.14 ± 0.01
Jamaica	11	126	33.3 ± 0.2	1.00 ± 0.01
Colombia	7	122	32.7 ± 0.2	0.98 ± 0.01
Ethiopia	12	128	44.6 ± 1.5	1.34 ± 0.04
Honduras (1)	9	123	49.4 ± 0.5	1.48 ± 0.01
Honduras (2)	9	130	50.8 ± 1.2	1.52 ± 0.04
Brazil	10	138	32.8 ± 0.3	0.99 ± 0.01
Total mean (n = 8)	9	128	38.8 ± 8.4 a	1.16 ± 0.25 a
Robusta				
India (1)	10	118	56.9 ± 1.4	1.71 ± 0.04
India (2)	11	133	84.1 ± 3.4	2.52 ± 0.10
Uganda (1)	9	122	75.5 ± 0.8	2.27 ± 0.02
Uganda (2)	11	122	73.5 ± 1.4	2.20 ± 0.04
Cameroon (1)	16	124	80.5 ± 1.5	2.41 ± 0.05
Cameroon (2)	12	132	89.4 ± 3.7	2.68 ± 0.11
Ivory Coast	10	131	97.3 ± 3.9	2.92 ± 0.12
Indonesia	9	125	58.0 ± 0.2	1.74 ± 0.01
Total mean (n = 8)	11	126	76.9 ± 14.2 b	2.31 ± 0.43 b

ORL, organic roast loss; EC, espresso coffee; S.D., standard deviation.
[a] Data followed by different letters within each column are significantly different according to ANOVA at $p < 0.001$.

influence of the degree of roasting on the acrylamide content and extractability, four green coffee samples (two arabicas, Brazil and Honduras, and two robustas, Uganda and Ivory Coast) were roasted at three different roasting degrees as described in the experimental section. Organic roast loss and colours achieved are summarised in Table 3, together with the results for acrylamide. The total acrylamide content was calculated for 6.5 g of ground coffee (cake weight) and the acrylamide extractability obtained by the following formula: espresso content/cake content × 100.

In a general way, acrylamide levels in ground coffee (both arabica and robusta) significantly decreased ($p < 0.05$) with increased roasting period, for each individual sample. In fact, very high levels of acrylamide were detected in the lightest roasted coffee samples, with maximum of 1240 and 2190 μg/kg, for arabica and robusta, respectively (Table 3). Also Taeymans et al. (2004) reported that levels of about 2000 μg/kg were observed at the early stages in the process of coffee beans roasting. Moreover, the amount present in dark roasts (Table 3) corresponds only to 15% and 23% of that present in light roasts, for arabica and robusta, respectively. Therefore, considering all the samples together, mean loss of about 80% occurred from the light roast to the dark one. Taeymans et al. (2004), based on experiments with isotope-labelled acrylamide, reported that more than 95% of the total acrylamide generated by roasting is further degraded during the process and is no longer found in the final product. Comparing the two coffee species analysed (Table 3), the average acrylamide contents of the compound were always significantly higher ($p < 0.05$) for robusta ground coffee, in all roasting stages.

The results obtained for espresso coffees follow a similar profile (Table 3): significant decreases ($p < 0.05$) of acrylamide were observed during roasting; pure robusta espressos contained approximately double amounts of acrylamide of arabica, for all degrees of roasting; and mean decreases of 30% and 20%, for arabica and robusta, respectively, were found when comparing medium roasted ECs with dark roasted counterparts. Thus, ECs prepared from dark roasted commercial blends might have about 25% less acrylamide than medium roasted brews.

Concerning the extraction efficacy of acrylamide, no significant differences ($p > 0.05$) were found between different degrees of roasting in each sample analysed. Also, no differences ($p > 0.05$) existed when arabica and robusta groups were compared. Therefore, although coffee species and degree of roasting influence the acrylamide content of the brew they do not affect acrylamide extractability (mean extraction of 80%, in a standard Portuguese espresso of 30 mL).

3.4. Influence of EC volume

The mean EC volume, usually consumed in Portugal, is around 30–40 mL. However, it may vary from a "ristretto" (20 mL or less) to a "lungo" (50 mL or more), according to the consumer's preference. Espresso percolation was described by Lantz et al. (2006) as the only brewing procedure that incompletely extracted acrylamide from ground coffee when compared with other coffee brews, due to the short contact time between coffee and water. In order to study the influence of the water volume on the amount of acrylamide of espresso, two coffee samples (one arabica and one robusta) were used to prepare ECs of different lengths (20, 30, 50 and 70 mL). The results are shown in Fig. 1.

The arabica cake under test (6.5 g) contained 1.82 ± 0.03 μg of acrylamide, while the robusta contained 3.67 ± 0.03 μg. The behaviour of acrylamide extraction was very similar in both coffee species, showing that an increase in the water volume that percolates through the coffee cake is responsible for a higher extraction of the compound. The extraction percentage variation according to the brew volume was also very similar: from 59% to

Table 3
Acrylamide contents and extractability in espresso coffees at different roast degrees.[a].

Sample	Roast degree	ORL (%)	Color	Ground coffee (μg/kg) Mean ± S.D.	μg/L Mean ± S.D.	μg/EC (30 mL) Mean ± S.D.	Extraction (%) Mean ± S.D.
Arabica							
Brazil	Light	7	200	782.78 ± 33.71 a	135.6 ± 5.7 a	4.07 ± 0.17 a	79.9 ± 0.1 a
	Medium	10	138	183.37 ± 8.35 b	32.8 ± 0.3 b	0.99 ± 0.01 b	82.7 ± 2.9 a
	Dark	13	119	131.79 ± 5.75 c	22.8 ± 1.3 c	0.68 ± 0.04 c	79.7 ± 1.1 a
Honduras	Light	6	200	1243.14 ± 67.34 a	216.0 ± 11.0 a	6.48 ± 0.33 a	80.2 ± 0.3 a
	Medium	9	130	279.26 ± 4.09 b	50.8 ± 1.2 b	1.52 ± 0.04 b	84.0 ± 3.3 a
	Dark	12	112	185.54 ± 1.24 c	33.8 ± 0.5 c	1.01 ± 0.01 c	84.1 ± 1.7 a
Robusta							
Uganda	Light	7	200	1384.50 ± 69.02 a	236.6 ± 0.5 a	7.10 ± 0.01 a	79.0 ± 3.8 a
	Medium	11	122	454.85 ± 2.67 b	73.5 ± 1.4 b	2.20 ± 0.04 b	74.6 ± 1.0 a
	Dark	13	97	383.46 ± 18.93 c	68.5 ± 2.1 b	2.05 ± 0.06 b	82.6 ± 6.5 a
Ivory Coast	Light	6	200	2191.18 ± 20.37 a	390.0 ± 16.6 a	11.70 ± 0.50 a	82.1 ± 4.3 a
	Medium	10	131	564.06 ± 25.05 b	97.3 ± 3.9 b	2.92 ± 0.12 b	79.6 ± 0.3 a
	Dark	13	110	441.91 ± 6.44 c	72.8 ± 1.7 c	2.18 ± 0.05 c	76.0 ± 0.6 a

ORL, organic roast loss; EC, espresso coffee; S.D., standard deviation.
[a] Data followed by different letters within each column, for each geographical origin, are significantly different according to Student's t-tests at $p < 0.05$.

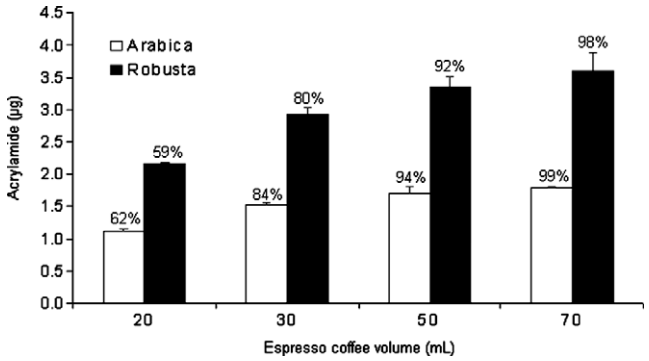

Fig. 1. Influence of volume on espresso coffee acrylamide content. Percentages on each bar represent acrylamide extractability.

98%, for robusta, and from 62% to 99%, for arabica. Therefore, a "lungo" EC practically contains all the acrylamide initially present in coffee cake (almost the double than a "ristretto"). Although the final content of acrylamide increases with volume (Fig. 1), the brew concentration (in ng/mL) simultaneously decreases, as expected, due to a reduction in the coffee/water ratio: from 108.2 ± 1.5 to 50.1 ± 3.9 µg/L, for robusta ECs, and from 56.3 ± 1.3 to 24.2 ± 0.3 µg/L, for arabica.

3.5. Comparison of EC with other coffee brews

The highly water-soluble acrylamide is easily extracted from the ground coffee to the liquid phase of the beverage (Andrzejewski et al., 2004). Some studies reporting acrylamide levels in common coffee beverages (as plunger pot and filtered coffee) have already been published, reporting values between 2 and 25 µg/L. The acrylamide concentrations reported in this study, for standard espressos (30 mL), are higher than those reported by other authors for other coffee brews. Indeed, considering all caffeinated samples presented in Table 1 ($n = 21$) the mean acrylamide concentration was 40 ± 9 µg/L. Moreover, acrylamide levels of medium roasted ECs could vary between 38 ± 8 and 77 ± 14 µg/L, when robusta percentage in blend ranges from 0% to 100%, respectively. These high concentrations (compared with other beverages) depend essentially on the coffee/water ratio used to prepare the brew, factor that varies with consumers' preferences and geographical habits: 20 g/L in USA (Andrzejewski et al., 2004) and 40 g/L in Northern Europe (Granby & Fagt, 2004), while in Portuguese espressos may range from 325 ("ristretto") to 93 g/L ("lungo") (Alves et al., 2007).

Considering the final acrylamide content per cup, it will obviously depend on the ingested amount of beverage. While EC is, generally, a very short beverage, higher volumes per cup of other coffee beverages are usually consumed. For example, a cup of filter coffee may achieve 200 mL, because it is considered a light brew (Alves et al., 2007). Therefore, according to the concentrations reported by other authors, one can estimate that a cup of 200 mL may contain up to 5 µg of acrylamide. This value was never achieved in our study, not even in the longest pure robusta ECs, because although very concentrated, EC is a very small beverage. Thus, the acrylamide intake through espresso brews will mainly depend on the consumption habits, considering type, strength and volume of beverage, and intake frequency, factors that are influenced by cultural and personal preferences of consumers.

4. Conclusions

Acrylamide intake through espresso coffee brew is mainly dependent on the type of coffee used to prepare the blend (arabica or robusta) and their degree of roasting, with the lowest amounts found in dark arabica roasted samples. The acrylamide extraction efficiency for standard espressos approached 80% and this value was only affected by brew volume increment: "lungo" ECs (70 mL) practically contained all the acrylamide initially present in coffee cake (almost the double than a "ristretto" of 20 mL). When compared with other common coffee beverages, EC is a very concentrated brew. However, its acrylamide content per cup may be lower, due to its small volume.

With the results obtained from commercial ECs (30 mL) it is possible to estimate that a moderate espresso consumer (3 to 5 doses per day) will ingest about 4–6 µg of acrylamide per day via this beverage. There are very limited processes available to reduce acrylamide level without affecting the quality of the brew, especially in relation to its sensory properties. A complementary option to reduce the amount of acrylamide ingested trough EC is to select commercial blends with higher arabica percentages and darker degrees of roasting and, simultaneously, prefer shorter brews instead of long ones, but this will obviously depend on the consumers' preferences.

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References

Aguas, P. C., Fitzhenry, M. J., Giannikopoulos, G., & Varelis, P. (2006). Analysis of acrylamide in coffee and cocoa by isotope dilution liquid chromatography–tandem mass spectrometry. *Analytical and Bioanalytical Chemistry, 385*, 1526–1531.

Alves, R. C., Casal, S., & Oliveira, B. P. P. (2007). Factors influencing the norharman and harman contents in espresso coffee. *Journal of Agricultural and Food Chemistry, 55*, 1832–1838.

Andrzejewski, D., Roach, J. A., Gay, M. L., & Musser, S. M. (2004). Analysis of coffee for the presence of acrylamide by LC–MS/MS. *Journal of Agricultural and Food Chemistry, 52*, 1996–2002.

Arisseto, A. P., & Toledo, M. C. F. (2006). Acrylamide in foods: A review. *Brazilian Journal of Food Technology, 9*, 123–134.

Bagdonaite, K., Derler, K., & Murkovic, M. (2008). Determination of acrylamide during roasting of coffee. *Journal of Agricultural and Food Chemistry, 56*, 6081–6086.

Clarke, R. J. (1989). Roasting and grinding. In R. J. Clarke & R. Macrae (Eds.). *Coffee: Technology* (Vol. 2, pp. 73–107). Great Yarmouth, UK: Elsevier Applied Science.

Delatour, A., Périsset, T., Goldmann, T., Riediker, S., & Stadler, R. H. (2004). Improved sample preparation to determine acrylamide in difficult matrixes such as chocolate powder, cocoa, and coffee by liquid chromatography tandem mass spectroscopy. *Journal of Agricultural and Food Chemistry, 52*, 4625–4631.

Dybing, E., Farmer, P. B., Andersen, M., Fennell, T. R., Lalljie, S. P., Müller, D. J., et al. (2005). Human exposure and internal dose assessments of acrylamide in food. *Food and Chemical Toxicology, 43*, 365–410.

Dybing, E., & Sanner, T. (2003). Risk assessment of acrylamide in foods. *Toxicological Sciences, 75*, 7–15.

Fernandes, J., & Soares, C. (2007). Application of matrix solid-phase dispersion in the determination of acrylamide in potato chips. *Journal of Chromatography A, 1175*, 1–6.

Friedman, M. (2003). Chemistry, biochemistry, and safety of acrylamide. *A review. Journal of Agricultural and Food Chemistry, 51*, 4504–4526.

Granby, K., & Fagt, S. (2004). Analysis of acrylamide in coffee and dietary exposure to acrylamide from coffee. *Analytica Chimica Acta, 520*, 177–182.

Guenther, H., Anklam, E., Wenzl, T., & Stadler, R. H. (2007). Acrylamide in coffee: review of progress in analysis, formation and level reduction. *Food Additives and Contaminants, 24*, 60–70.

Hoenicke, K., & Gatermann, R. (2005). Studies on the stability of acrylamide in food during storage. *Journal of AOAC International, 88*, 268–273.

International Agency for Research on Cancer (IARC) (1994). *Some industrial chemicals* (Vol. 60, pp. 389–441). Lyon: IARC monographs on the evaluation of carcinogenic risk to humans.

Illy, A., & Viani, R. (2005). *Espresso coffee: The science of quality*. London, UK: Academic Press.

Joint FAO/WHO Experts Committee on Food Additives (JECFA) (2005). Summary and conclusions of the sixty-fourth meeting of the Joint FAO/WHO Experts Committee on Food Additives (JECFA), Rome, 8–17 February; JECFA/64/SC.

Lantz, I., Ternité, R., Wilkens, J., Hoenicke, K., Guenther, H., & van der Stegen, G. H. (2006). Studies on acrylamide levels in roasting, storage and brewing of coffee. *Molecular Nutrition and Food Research, 50*, 1039–1046.

Murkovic, M. (2004). Acrylamide in Austrian foods. *Journal of Biochemical and Biophysical Methods, 61*, 161–167.

Pérez, H. L., & Osterman-Golkar, S. (2003). A sensitive gas chromatographic–tandem mass spectrometric method for detection of alkylating agents in water: Application to acrylamide in drinking water, coffee and snuff. *Analyst, 128*, 1033–1036.

Roach, J. A., Andrzejewski, D., Gay, M. L., Nortrup, D., & Musser, S. M. (2003). Rugged LC–MS/MS survey analysis for acrylamide in foods. *Journal of Agricultural and Food Chemistry, 51*, 7547–7554.

Senyuva, H. Z., & Gökmen, V. (2005). Study of acrylamide in coffee using an improved liquid chromatography mass spectrometry method: Investigation of colour changes and acrylamide formation in coffee during roasting. *Food Additives and Contaminants, 22*, 214–220.

Swedish National Food Administration (2002). Information about acrylamide in food, 24 April 2002. <http://www.slv.se>.

Soares, C., Cunha, S., & Fernandes, J. (2006). Determination of acrylamide in coffee and coffee products by GC–MS using an improved SPE clean-up. *Food Additives and Contaminants, 23*, 1276–1282.

Soares, C., Alves, R. C., Casal, S., Fernandes, J. O., & Oliveira, B. P. P. (submitted for publication). Validation of a matrix solid-phase dispersion method to determine acrylamide in coffee and coffee surrogates. *Food Additives and Contaminants*.

Summa, C. A., de la Calle, B., Brohee, M., Stadler, R. H., & Anklam, E. (2007). Impact of the roasting degree of coffee on the in vitro radical scavenging capacity and content of acrylamide. *LWT – Food Science and Technology, 40*, 1849–1854.

Svensson, K., Abramsson, L., Becker, W., Glynn, A., Hellenäs, K.-E., Lind, Y., et al. (2003). Dietary intake of acrylamide in Sweden. *Food and Chemical Toxicology, 41*, 1581–1586.

Taeymans, D., Wood, J., Ashby, P., Blank, I., Studer, A., Stadler, R. H., et al. (2004). A review of acrylamide: An industry perspective on research, analysis, formation, and control. *Critical Reviews in Food Science and Nutrition, 44*, 323–347.