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ARTICLE

Isoflavones in Coffee: Influence of Species, Roast Degree, and Brewing Method

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This paper reports the isoflavone contents of roasted coffee beans and brews, as influenced by coffee species, roast degree, and brewing procedure. Total isoflavone level is 6-fold higher in robusta coffees than in arabica ones, mainly due to formononetin. During roasting, the content of isoflavones decreases, whereas their extractability increases (especially for formononetin). Total isoflavones in espresso coffee (30 mL) varied from ~40 μg (100% arabica) to ~285 μg (100% robusta), with long espressos (70 mL) attaining more than double isoflavones of short ones (20 mL). Espressos (30 mL) prepared from commercial blends contained average amounts of 6, 17, and 78 μg of genistein, daidzein, and formononetin, respectively. Comparison of different brewing methods revealed that espresso contained more isoflavones (~170 μg/30 mL) than a cup of press-pot coffee (~130 μg/60 mL), less than a mocha coffee (~360 μg/60 mL), and amounts similar to those of a filtered coffee cup (~180 μg/120 mL).

KEYWORDS: Arabica; coffee; isoflavones; roast; robusta

INTRODUCTION

In the past few years, a refreshing positive connotation is being given to moderate coffee consumption, due to some potential health benefits. More than a stimulant, due to caffeine, a coffee brew is a combination of numerous bioactive compounds with biochemical and physiological actions (*1*).

Polyphenols have been a focus of special attention, mainly concerning their antioxidant and free radical scavenging properties (*2*). Chlorogenic acids and derivates are the most abundant polyphenols in coffee, especially robusta (up to 350 mg of chlorogenic acid per cup) (*3*). Although in minor amounts, other phenolic compounds were also described in coffee, namely, lignans and, more recently, isoflavones (*4*, *5*).

Isoflavones are estrogen-like molecules with a common 3-phenylchromen-4-one core structure, differing by substituents such as methoxy, hydroxy, and glucoside functions (*6*, *7*). Genistein (4′,5,7-trihydroxyisoflavone) and daidzein (4′,7-dihydroxyisoflavone) are the most plentiful and the best characterized compounds of this group (*8*) and are particularly abundant in soy and soy products (0.1−3.0 mg/g) (*9*). However, soy oils and soy lecithin, used in several foods, are devoid of isoflavones, due to their migration with the protein fraction of the soybean during its processing (*9*). Biochanin A (5,7-dihydroxy-4′-methoxyisoflavone) and formononetin (7-hydroxy-4′-methoxyisoflavone) are methylated precursors of genistein and daidzein, respectively, and exist in high amounts in red clover (5 mg/g) (*10*). Isoflavones are also present, in lower amounts, in alfalfa sprouts (43 μg/g), peanuts (1.5 μg/g), barley (0.2 μg/g), apple (0.1 μg/g), broccoli (0.1 μg/g), and cauliflower (0.1 μg/g), among others (*11*). For instant coffee powder, about 9 μg/g of total isoflavones is reported (*12*).

In plants, the 6″-*O*-malonyl-7-*O*-β-D-glucoside derivates are the prevailing forms. During food processing or sample analysis 6″-*O*-acetyl-7-*O*-β-D-glucosides are produced, along with 7-*O*-β-D-glucosides and free isoflavones. Therefore, in processed foods, these compounds are usually found as glucoside derivates and free aglycones (*6*).

When consumed by humans, isoflavones are hydrolyzed not only by gastric acid but also by bacterial glucosidases of intestinal microflora. Sugar moieties are cleaved and aglycones released, which can be absorbed intact by enterocytes or further biotransformed by bacteria to specific metabolites, including equol and *O*-desmethylangolensin (metabolites of daidzein), and the nonoestrogenic *p*-ethylphenol (metabolite of genistein) (*8*, *13*, *14*). Equol and *O*-desmethylangolensin are pharmacologically important metabolites, because they are more estrogenic than daidzein. Interestingly, there are substantial interindividual differences in daidzein metabolism: 80−90% of the human population produces *O*-desmethylangolensin, whereas only 30−50% produce equol (*15*). Moreover, as dietary isoflavones are predominantly metabolized by the gastrointestinal flora, the dietary intake, antibiotic use, bowel disease, gender, etc., are interfering factors in that metabolism (*16*).

Due to their weak estrogenic (*17*) and antioxidant activity (in vitro and in vivo) (*13*, *18*, *19*), these compounds seem to offer some protection against hormone-related cancers such as

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prostate (*20*), breast (*21*), and bowel (*22*), cardiovascular diseases, osteoporosis, and menopause symptoms (*23*, *24*). Some studies showed that coffee has estrogenic properties (*25*, *26*) and isoflavones might, in part, contribute to that effect.

Few reports exist on these compounds in coffee (*4*, *5*, *12*). Their contents in beverages and instant coffee have been determined by gas and liquid chromatography coupled to mass spectrometry (*4*, *5*, *12*), but no information is given about the brewing method, and practically no data were found concerning coffee species. The effect of roast degree, as far as we know, was not evaluated.

Therefore, the aim of this work was to ascertain the influence of three imperative factors in coffee brew composition regarding daidzein, genistein, and formononetin levels: coffee species (arabica and robusta), roast degree, and brewing procedure. Moreover, brews prepared from commercial samples were also analyzed, and daily isoflavone intake of the Portuguese population through espresso coffee (EC) was estimated.

EXPERIMENTAL PROCEDURES

Chemicals and Solutions. Isoflavone standards daidzein (≥98%), genistein (≥98%), and formononetin (≥99%) were obtained from Sigma (St. Louis, MO). Stock solutions (1 g/L) were prepared in H_2O/methanol (25:75, v/v) and stored at −20 °C in amber glass vials. The effective concentrations were verified by absorbance readings according to the method of Alves et al. (*27*). The internal standard, 2′-methoxyflavone, was obtained from Sigma-Aldrich (St. Louis, MO). A 0.1 g/L working solution was prepared in methanol and also stored at −20 °C in an amber glass vial.

Acetonitrile and methanol (both of HPLC grade) and formic acid (98−100%) were all from Merck (Darmstadt, Germany), and HPLC water was purified with a "Seral" system (SeralPur Pro 90 CN). Hydrochloric acid 32% was from Panreac (Barcelona, Spain). The antioxidant butylated hydroxytoluene (BHT) was obtained from Aldrich (Madrid, Spain), and a 1% solution was prepared in methanol.

Coffee brews were prepared with deionized water (Amberlite MD20).

Samples. Different samples of regular (n = 10) and decaffeinated (n = 3) commercial coffee blends (roasted beans), as well as servings (commercial unidoses of ground coffee) (n = 6), were obtained in local supermarkets.

Adittionally, green bean samples of *Coffea arabica* (Brazil and Honduras) and *Coffea canephora* var. *robusta* (Uganda and Ivory Coast) were kindly supplied by BICAFÉ. These samples were used to evaluate the influence of both species and roast degree in coffee isoflavone content. They were subjected to three different periods of heat exposure (8−11 min, 210 °C, Probat Pré 1Z 2000, from Probat-Werke) to achieve three final roasting degrees (light, medium, and dark), not exceeding the range of commercial espresso roasts usually practiced in Portugal.

Beans were mechanically powdered (Krups 408-75 coffee grinder) to pass through a 0.75 mm sieve. Roasting degree was determined by photometric analysis with infrared radiation (Colorimeter Colorette 3 from Probat-Werke) and also by organic roast loss (%ORL) (*28*, *29*). Sample moisture to calculate ORL was determined by drying at 103 ± 2 °C until a constant weight was reached.

Brews Preparation. Standard ECs (6.5 g of ground coffee per 30 mL of water) were prepared in an HL3854/A Espresso Professional (Philips, The Netherlands). For extractability evaluation, espressos with different volumes were prepared, ranging from 20 mL, a typical "ristretto" or "Italian", to 70 mL, the longest EC (*30*).

Commercial blend servings (individual doses of ground coffee coated with paper) were extracted in the same espresso machine by changing the filter chamber. Mocha, filter, and press-pot coffees were prepared as previously described (*31*).

Isoflavone Extraction. Isoflavone aglycones were extracted, in triplicate, using a previously developed and validated method for isoflavone analysis in coffee, based on acid hydrolysis (*27*). Briefly, for ground coffee, 200 mg was spiked with the internal standard (150 μL), the antioxidant (50 μL), methanol (2 mL), and 3.4 N hydrochloric acid (2 mL). For coffee brews, 1.7 mL was spiked with the internal standard (150 μL), the antioxidant (50 μL), 1.7 mL of methanol, and 670 μL of 10.2 N hydrochloric acid. Hydrolyses were performed at 75 °C for 150 min, under reflux. The final pH was adjusted with sodium hydroxide, and the extract was preserved at 4 °C for chromatographic analysis on the same day. Each chromatographic injection was performed in duplicate.

HPLC Equipment. The chromatographic analysis was performed in a HPLC integrated system (Jasco, Japan) equipped with an AS-950 automated injector (20 μL loop), two Jasco PU-2080 Plus HPLC pumps, and an MD-2010 Plus multiwavelength diode array detector. Aglycones were separated in a Mediterranea Sea18 column (5 μm, 15 cm × 4.6 mm i.d.) from Teknokroma (Barcelona, Spain), with a gradient solvent system of formic acid 0.1% and acetonitrile (*27*).

Analytes were monitored at 254 nm, and quantification was performed on the basis of the internal standard method. Chromatographic data were analyzed using Borwin-PDA Controller Software (JMBS Developments, Le Fontanil, France).

Confirmation of isoflavone identities was performed by comparing retention times and coelution with authentic standards and by UV absorption spectral analysis (**Figure 1**). Correlation coefficients of calibration curves were >0.999. The detection and quantification limits were, respectively, 4.7 and 14.1 ng/mL for daidzein, 4.5 and 13.7 ng/mL for genistein, and 8.2 and 25 ng/mL for formononetin. The linearity ranges were 14−3000 ng/mL for daidzein, 14−3000 ng/mL for genistein, and 25−5000 ng/mL for formononetin. Good intra- and interday precisions (<7%) and accuracies (recoveries of 95% for ground coffee and 92% for espressos) were achieved (*27*).

Statistical Analysis. Data are reported as mean ± standard deviation and analyzed by one-way ANOVA, and Student's t tests were used to discriminate between any two groups under consideration. Data treatment was carried out with Microsoft Excel statistical software (Microsoft Office Excel 2003, Microsoft Corp., Redmond, WA).

RESULTS AND DISCUSSION

Variability within Commercial Samples. EC is the most popular coffee brew in Portugal due to its peculiar characteristics: presence of foam, concentrated texture, and strong flavor.

Daidzein, genistein, and formononetin contents in ECs prepared from commercial coffee blends, both caffeinated (regular and from servings) and decaffeinated, are presented in **Table 1**. A large variability in the compounds' contents was observed within all groups. Mean total isoflavones (μg) per brew (30 mL) were 100 (range = 48−244), 69 (53−100), and 88 (45−123), for regular, decaffeinated, and ECs from servings, respectively. Despite this variation, the compound profile was very similar for all brews, with formononetin present in higher levels, followed by daidzein and genistein, in this order.

Comparison of the individual compounds among the three groups (**Table 1**) revealed no significant differences (p > 0.05) found. Although the average level for formononetin in decaffeinated ECs was approximately half that of caffeinated samples (regular and servings), the difference is not statistically significant, due to the high range of variation within each group, together with the small number of decaffeinated samples analyzed. On this basis, a study with a higher number of samples seems to be needed before conclusions about the influence of the decaffeination process can be drawn.

As already refereed, other authors described the presence of isoflavones in coffee brews, although no information is given about coffee/water ratio or brewing procedure. Mazur et al. (*5*) analyzed six coffee brews: one contained daidzein (10−66 μg/100 g), two contained genistein (15−29 μg/100 g), and three contained formononetin (72−78 μg/100 g of coffee brew). Thompson et al. (*4*) reported smaller levels of 0.2, 0.3, and 0.5 μg per cup of coffee brew (287 mL) for genistein, daidzein, and formononetin, respectively.

With the expectation that not only the brewing procedure but also the proportion of coffee species (arabica/robusta) in the blend and roast degree could influence the final content of

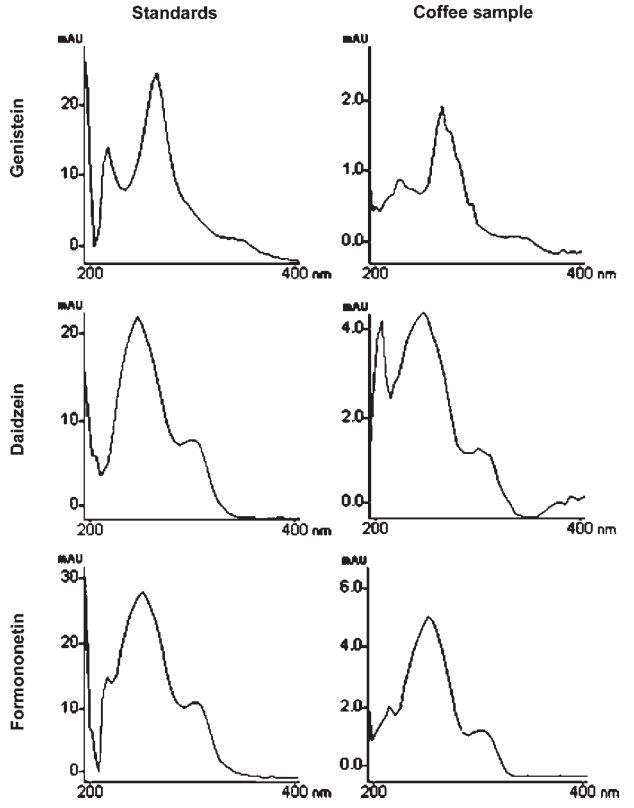

Figure 1. UV spectra of genistein (λ_{max} (nm) 259, 329 sh), daidzein (λ_{max} (nm) 247, 299 sh), and formononetin (λ_{max} (nm) 250, 303 sh) in a standard mixture (~3 μg/mL) and a coffee sample, both subjected to the same extraction procedure.

Table 1. Isoflavone Contents (Micrograms per 30 mL) in Espresso Coffees Prepared from Commercial Blends[a]

		genistein			daidzein			formononetin		
espresso	*n*	mean	min	max	mean	min	max	mean	min	max
regular[b]	10	5.6 a	0.8	16.2	17.2 a	11.2	26.1	77.5 a	34.1	214.5
decaffeinated	3	6.1 a	2.0	13.9	23.5 a	18.0	32.3	39.6 a	31.0	53.7
servings[b]	6	4.9 a	2.7	7.4	19.1 a	13.4	25.0	64.1 a	25.2	91.9

[a] Data followed by similar letters within each column are not significantly different according to ANOVA, at $p > 0.05$. [b] Caffeinated samples.

isoflavones in beverage, as already shown for other compounds (*31*, *32*), a detailed study about this subject was performed.

Influence of Coffee Species and Roast Degree. Two samples of arabica coffee and two of robusta, all from different geographical origins, were roasted at three different degrees to embrace the range of coffee roasts that can be found in the market. Ground roasted coffees as well as ECs prepared from each sample were analyzed, and compound extractability was also evaluated. The results are depicted in **Table 2**.

A clear decrease in the isoflavone levels of beans was observed during the roast, for each individual sample. Independent of the roast degree, robusta coffees always contained superior amounts of isoflavones. The main distinction between both species was formonotenin (approximately 6-fold higher in robusta coffees, for all roasts). Although robusta samples also showed, in a general way, superior amounts of daidzein and genistein than arabica ones, this difference was not so pronounced as in the case of formononetin. In fact, on the basis of average values, robustas contained 2-fold higher daidzein contents than arabicas, for all roasts. Curiously, the light-roasted Brazilian beans showed slightly superior amounts of daidzein than the light-roasted sample from Uganda. Robusta samples also contained double amounts of genistein, for light and medium roasts, but similar mean values were found for both dark-roasted species.

With regard to total isoflavones (μg) in roasted coffee (g), average amounts in arabica samples were 36 ± 0, 23 ± 3, and 20 ± 4, for light, medium, and dark roast degrees, respectively. For robusta ones, 114 ± 16, 89 ± 11, and 77 ± 12 were found, for the same roasting stages. Mean isoflavone loss from light to dark roasts was about 30−40%.

For brews, in a general way, robusta ECs contained about 5-fold higher levels of total isoflavones than the arabica ones (**Table 2**). Average amounts of total isoflavones in arabica ECs were 50 ± 3, 46 ± 8, and 47 ± 17 μg/cup, for light, medium, and dark roasts, respectively. In 100% robusta ECs, 189 ± 62, 239 ± 67, and 229 ± 37 μg/cup were found, for the same roast degrees. Thus, isoflavone degradation during the roast was not as visible through the brews as it was in beans: practically no variation in total content was observed during the roast in arabica ECs, whereas in robusta ones an increase was detected for medium and dark roasts.

When the four samples were analyzed individually (**Table 2**), a general increase of formononetin levels in medium-dark-roasted ECs was noted. For daidzein, a general decrease was observed, the sample from Uganda being an exception with higher contents in medium- and dark-roasted ECs. For genistein, the behavior was variable, with decrease, increase, or maintenance of brew contents. These different behaviors seem to result from the combination of two factors, namely, the degradation profile in beans and the extractability variation during the roast, both characteristics of each compound and sample. In a general way, the compounds' extractability significantly increased ($p < 0.05$) from light- to medium-dark-roasted degrees. Even when dark ECs presented lower amounts of daidzein or genistein, in comparison with light ones (due to a decrease in roasted beans), the extraction rate of these compounds to the brew augmented. This increase is, however, less marked to daidzein and genistein, in comparison with formononetin. An exception was the Ivory Coast sample, for which no significant differences ($p < 0.05$) in daidzein extractability were detected during the roast. When all of the samples are considered together, mean extraction percentages of genistein, daidzein, and formononetin were, respectively, 23 ± 3, 18 ± 2, and 26 ± 10% for light-roasted coffees; 30 ± 3, 24 ± 5, and 40 ± 10% for medium-roasted ones; and 37 ± 9, 25 ± 4, and 48 ± 9% for dark roasts. The results also show that although the extractability of the individual isoflavones varied with the sample, in a general way, formononetin is more extensively extracted than other isoflavones, especially at dark roast degrees, followed by genistein and daidzein, in this order.

This fact is curious, because formononetin (a methylated derivate) is less polar than the other two isoflavones. However, as already referred to, in plants, isoflavones are usually bound to other molecules that modify their solubility properties. Aglycones are hydrophobic compounds, but conjugation to glucose residues increases their solubility (while acetylation or malonylation of the glucones reduces it) (*9*, *33*). Previous studies in our laboratory indicated that, in roasted coffee, isoflavones under the free form are practically nonexistent, a hydrolytic step being necessary to quantify aglycones (*27*). If isoflavones occur predominantly as malonyl glucoside derivates in green coffee, as described for other plants (*34*), cleavage of chemical bindings will certainly occur during the high temperatures of roast, producing acetyl glucosides and glucosides conjugates, these last being more polar substances, which seems to be a warrantable explanation for

Table 2. Isoflavone Contents in Coffee and Espresso Coffees at Different Roast Degrees[a]

				ground coffee (μg/6.5 g)			espresso coffee (μg/30 mL)			% of extraction		
sample	RD	ORL	color	genistein	daidzein	formononetin	genistein	daidzein	formononetin	genistein	daidzein	formononetin
arabica												
Brazil	L	7	200	15.3 ± 0.6 a	139.8 ± 7.7 a	77.2 ± 3.3 a	3.4 ± 0.2 ab	28.3 ± 0.42 a	15.5 ± 0.5 ab	22 ± 2 b	20 ± 0 b	20 ± 2 c
	M	10	138	13.0 ± 0.9 b	71.1 ± 3.0 b	52.9 ± 1.9 b	3.5 ± 0.0 a	22.3 ± 0.7 b	14.2 ± 0.2 b	27 ± 2 ab	31 ± 1 a	27 ± 1 b
	D	13	119	11.1 ± 0.3 c	48.6 ± 1.1 c	52.3 ± 2.8 b	3.1 ± 0.0 b	13.9 ± 0.9 c	18.2 ± 0.6 a	28 ± 1 a	29 ± 1 a	35 ± 2 a
Honduras	L	6	200	6.5 ± 0.2 a	92.3 ± 2.2 a	134.1 ± 5.0 a	1.3 ± 0.1 b	14.4 ± 0.1 a	36.4 ± 0.3 b	20 ± 0 b	16 ± 1 b	27 ± 1 c
	M	9	130	6.1 ± 0.3 a	62.6 ± 3.3 b	93.6 ± 3.0 b	1.9 ± 0.1 a	13.0 ± 0.2 b	37.0 ± 1.6 b	31 ± 1 a	21 ± 1 a	40 ± 2 b
	D	12	112	5.3 ± 0.2 b	57.3 ± 2.1 b	83.4 ± 2.6 c	1.7 ± 0.0 a	11.2 ± 0.6 b	46.2 ± 1.2 a	33 ± 2 a	20 ± 0 a	55 ± 0 a
robusta												
Uganda	L	7	200	17.9 ± 0.8 a	129.4 ± 4.1 a	520.1 ± 9.6 a	4.9 ± 0.1 ab	21.5 ± 0.7 c	206.8 ± 3.5 c	27 ± 1 c	17 ± 0 c	40 ± 0 b
	M	11	122	13.5 ± 0.6 b	121.5 ± 2.1 ab	497.5 ± 12.6 a	4.6 ± 0.1 b	26.4 ± 0.4 b	254.9 ± 7.0 a	34 ± 2 b	22 ± 0 b	51 ± 3 a
	D	13	97	10.6 ± 0.1c	116 ± 1.8 b	431.7 ± 12.9 b	5.2 ± 0.2 a	30.1 ± 1.0 a	219.2 ± 2.0 b	49 ± 1 a	26 ± 1 a	51 ± 2 a
Ivory Coast	L	6	200	33.3 ± 1.6 a	202.9 ± 8.3 a	579.3 ± 17.4 a	7.3 ± 0.1 a	39.1 ± 0.3 a	99.1 ± 0.8 c	22 ± 1 c	19 ± 1 a	17 ± 1 c
	M	10	131	26.7 ± 1.5 b	152.1 ± 5.6 b	348.0 ± 14.2 b	8.1 ± 0.2 a	32.8 ± 1.0 b	150.7 ± 3.5 b	30 ± 0 b	22 ± 1 a	43 ± 1 b
	D	13	110	9.2 ± 0.5 c	104.0 ± 2.9 c	334.0 ± 16.2 b	3.4 ± 0.1 b	25.3 ± 1.2 c	173.9 ± 7.2 a	37 ± 3 a	24 ± 2 a	52 ± 3 a

[a] Data are presented as mean ± standard deviation. Data followed by different letters within each column, for each geographical origin, are significantly different according to Student's t test at $p < 0.05$. RD, roast degree; L, light; M, medium; D, dark. ORL, organic roast loss.

the extractability increase of isoflavones during roast. Moreover, isoflavones also participate in Maillard reactions, for example, by reacting with amino acids and proteins (*35*). Many other unknown combinations might also occur during the chemical complexity of roasting, and the possible binding of isoflavones to more polar molecules should also be considered.

The observation, in **Table 2**, that in light-roasted coffees the extractability of the three isoflavones is not as different as it is in dark-roasted ones leads to the inference that their degradation pathways during roasting are different. In the final stage of roast, formononetin should be linked to more polar groups, whereas daidzein is probably conjugated to less polar substances because it presents the lowest extraction rates.

Influence of EC Volume. A standard Portuguese espresso is usually prepared with 6.5 g per 30−40 mL. Nevertheless, the final volume is variable according to the consumers' preferences. A "short" EC (~20 mL) is more appreciated by those who enjoy a more concentrated brew, whereas a "long" one (~70 mL) is considered, by the generality of Portuguese consumers, as a "weaker" brew, due to the higher water/coffee ratio. However, as we reported in other studies (*31*, *32*) a longer percolation may allow a better extraction of some compounds from ground coffee to beverage. The influence of volume in the isoflavone content of EC was studied, and results are shown in **Figure 1**.

A significant increase ($p < 0.05$) of isoflavones in EC was observed with volume extent. Extraction percentages varied in a similar way for both arabica and robusta coffees. Averages ranged from 19 ± 5, 16 ± 1, and 31 ± 4% in 20 mL ECs to 61 ± 3, 50 ± 5, and 72 ± 1% in 70 mL brews, for genistein, daidzein, and formononetin, respectively. In the same perspective, total isoflavones varied from 38 to 101 μg in the arabica sample and from 145 to 347 μg in the robusta one. Thus, a long espresso contains more than double the isoflavones of a short one.

Influence of Brewing Method. All over the world, coffee beverages are prepared according to several methods. The coffee/water ratio used is also variable, depending primarily on the consumers' liking. To study the influence of brewing method, the same commercial sample was used to prepare four kinds of brew, namely, espresso, mocha, press-pot, and filter, based on references for both preparation procedure and coffee/water amount (*31*). Results are described in **Table 3**.

The presented values are reported as isoflavone concentration (μg/L) and as isoflavone amount (μg) per cup of brew, calculated for the mean volumes generally consumed (*36*). It can be observed that not only the coffee/water ratio but also the brewing method significantly influences the isoflavones content of the beverage.

Lower water/coffee ratios result in more concentrated brews, as expected. However, although the mocha ratio was almost half that of standard EC, that beverage contained higher amounts of genistein and daidzein (per mL) and similar levels of formononetin. This is probably due to the higher percolation extent that, as already observed in **Figure 2**, improves the compounds' extractability. From a comparison of press-pot with filter coffee (same water/coffee ratios) a clear difference can be observed in the results, with the filtered brew presenting the lowest concentrations. Two hypotheses can justify this difference. On the one hand, the press-pot procedure allows a more effective contact between ground coffee and water. On the other hand, studies reported that lipids and hydrophobic compounds are mainly retained in the spent coffee, practically not crossing the paper filter (*37*). It was already discussed in this paper that the polarity of isoflavones during roasting might vary according to the chemical structures that are linked to them. In a specific roast degree, as in this case, isoflavones might be present under different forms, and a possible interference of paper filter in their extractability should also be considered.

By comparison of the different brews (**Table 3**), and consideration of the mean volumes generally consumed (*36*), it can be observed that a cup of espresso contains more total isoflavones (~170 μg/30 mL) than a cup of press-pot coffee (~130 μg/60 mL), less than a mocha coffee (~360 μg/60 mL), and amounts similar to those of a filter coffee cup (~180 μg/120 mL).

In summary, the isoflavone content of EC is highly variable. It depends essentially on the coffee species used for brew preparation and their roast degrees (robusta coffee contains about 6-fold higher amounts than arabicas). During the roast, genistein, daidzein, and formononetin levels significantly decreased in beans. Simultaneously, the compounds' extractability increased, especially that of formononetin. The volume of the brew was also an important factor to consider, as a long espresso (70 mL) doubled the isoflavones of a short one (20 mL). The amount of isoflavones in a 30 mL EC might vary from ~40 μg (100% arabica) to ~240 μg (100% robusta) and, according to the average volumes consumed, a cup of EC contains more total isoflavones than a cup of press-pot coffee, less than a mocha coffee, and contents similar to a filter coffee cup.

Table 3. Isoflavones in Different Coffee Brews[a]

type of brew[b]		genistein	daidzein	formononetin
espresso (6.5/30)	μg/L	230 ± 10 b	683 ± 36 b	4594 ± 25 a
mocha (25/200)	μg/L	252 ± 11 a	937 ± 32 a	4775 ± 45 a
press-pot (13.7/250)	μg/L	79 ± 19 c	459 ± 12 c	1644 ± 21 b
filter (13.7/250)	μg/L	nd[c]	158 ± 10 d	1356 ± 13 c
espresso (6.5/30)	μg/30 mL	6.9 ± 0.1 f	20.5 ± 0.3 g	137.8 ± 6.2 f
mocha (25/200)	μg/60 mL	15.1 ± 0.3 e	56.2 ± 3.1 e	286.5 ± 11.7 e
press-pot (13.7/250)	μg/60 mL	4.7 ± 0.1 g	27.5 ± 0.2 f	98.6 ± 0.4 g
filter (13.7/250)	μg/120 mL	nd	18.9 ± 0.6 g	162.6 ± 8.2 f

[a] Data are reported as mean ± standard deviation. Data followed by different letters within each column are significantly different according to Student's *t* test at $p < 0.05$. [b] Coffee (g)/water (mL) ratio is given in parentheses. [c] nd, value below the quantification limit.

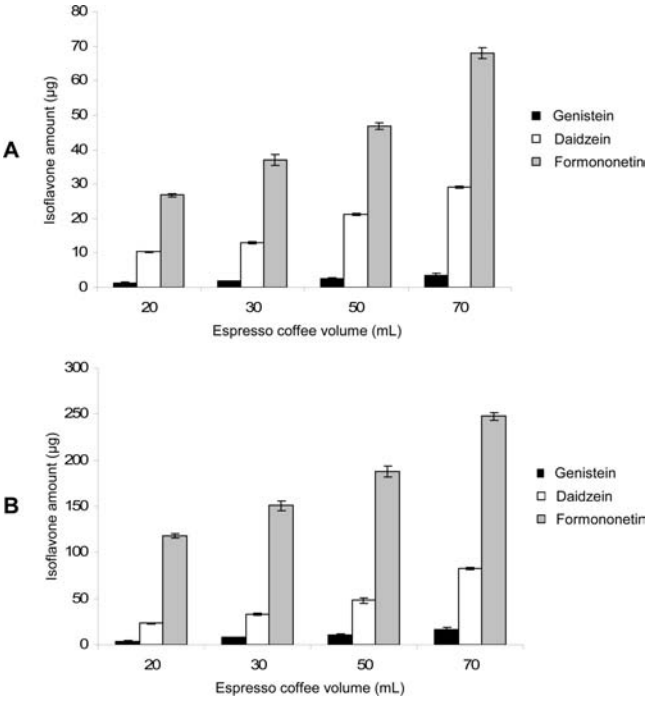

Figure 2. Influence of brew volume on genistein, daidzein, and formononetin contents of ECs: 100% arabica (**A**) and 100% robusta (**B**).

A blend of 70 arabica/30 robusta (usual in Portugal) and a moderate consumption of 3−5 cups/day could contribute to an isoflavone ingestion of 300−500 μg/day. However, this value can be increased by preferring long brews (70 mL) prepared from blends with higher percentages of medium-dark-roasted robusta coffee.

ACKNOWLEDGMENT

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