Antiglycative and carbonyl trapping properties of the water soluble fraction of coffee silverskin

M. Mesías a, M. Navarro a, N. Martínez-Saez a, M. Ullate b, M.D. del Castillo b, F.J. Morales a

a Institute of Food Science, Technology and Nutrition, ICTAN-CSIC, 28040 Madrid, Spain
b Institute of Food Science Research (CIAL-CSIC-UAM), 28049 Madrid, Spain

ABSTRACT

Carbonyl stress and accumulation of advanced glycation end-products (AGEs) in human tissues are involved in diabetic complications, atherosclerosis, Alzheimer’s disease and aging. The objective of this study was to evaluate the in vitro protective effect of aqueous extracts of coffee silverskin (CS) in the formation of AGEs and trapping of carbonyl reactive species such as methylglyoxal (MGO). Aqueous extracts of CS from Arabica and Robusta coffee varieties were obtained under environment friendly extraction conditions. CS extracts were characterized by the analysis of dietary fiber, caffeine, chlorogenic acids (CGAs), total phenolic compounds, browning, melanoidins, and antioxidant capacity. CS extracts and CGA exhibited a dose-dependent anti-AGE capacity in the protein-glucose model system (37 °C/21 days) with an IC50 of 0.6 mg/mL and 0.4 mg/mL, respectively. Caffeine did not prevent AGE formation under the studied conditions. Regardless to protein–MGO assay (37 °C/14 days), the anti-AGE capacity of CS extracts and CGA was also dose-dependent with an IC50 of 1.3 mg/mL and 0.1 mg/mL, respectively. Caffeine weakly inhibited the reaction of protein and MGO. The MGO trapping capacity was established as a model for protection against carbonyl stress. Robusta CS was very effective for the direct trapping of MGO with an IC50 of 0.055 mg/mL as compared with Arabica CS (IC50 of 0.6 mg/mL). CGA and caffeine showed an IC50 for MGO trapping capacity of 0.14 mg/mL and N10 mg/mL, respectively. The highest CGA content in the Robusta CS extract could explain its higher MGO trapping activity as compared with the Arabica CS extract. The anti-AGE and MGO trapping capacities of CS may be associated to other chemical components besides CGA. In conclusion, aqueous CS extract may be considered as a natural source of inhibitors of in vitro formation of AGEs and carbonyl stress. The inhibitory effect of the coffee extracts may be associated to their carbonyl trapping capacity.

INTRODUCTION

AGEs (advanced glycation end-products) are the final products derived from the Maillard reaction or non-enzymatic glycation process produced in the human body. It is known that AGEs are involved in the development of several health disorders such as diabetes and its complications (Vlassara & Palace, 2002), atherosclerosis (Vlassara, 1996), Alzheimer’s disease and normal aging (Münch, Thome, Foley, Schinzel, & Riederer, 1997). In addition, the increase in reactive carbonyls in tissues is known as carbonyl stress which leads to directly increase chemical modification of proteins (glycation) and lipids (lipoxidation) in diabetes. Reactive carbonyl species generated from carbohydrate, lipid and amino acid metabolism such as methylglyoxal (MGO), glyoxal, glyoxalaldehyde, dehydroascorbate, 3-deoxyglucosone and malondialdehyde, are potent precursors of AGE formation and protein cross-linking (Thornalley, Langborg, & Minhas, 1999). MGO derived AGE structures, including CEL (N-epsilon-(carboxyethyl)-lysine) and MOLD (methylglyoxal-lysine dimer), are increased in diabetes (Baynes & Thorpe, 1999). Thus, preventing AGE formation/accumulation may control significantly the pathogenesis of diabetes complications.

The inhibition of AGE formation might follow several mechanisms involving, e.g., aldose reductase, antioxidant activity, reactive dicarbonyl trapping, sugar autoxidation inhibition and amino group binding, where the antiglycative activity of phytochemicals has been usually linked to oxidative reactions (Bousová et al., 2005). The inhibition of AGE formation by some synthetic compounds such as aminoguanidine (AG) has been well documented. However, this compound has been associated with several adverse effects in in vivo studies (Thornalley, 2003; Williams, 2004) since it is a highly reactive nucleophilic reagent that reacts with many biological molecules (pyridoxal phosphate, pyruvate, glucose, malondialdehyde, and others). Hence, the search for natural products which can inhibit AGE formation has recently been an objective of worldwide research (Peng, Cheng, et al., 2008; Povichit, Phrutivorapongkul, Suttajit, Chaiyasut, & Leelapornpisid, 2010; Wang, Sun, Cao, & Tian, 2009).
Coffee consumption has been associated with reduction in chronic disease risk such as type 2 diabetes (Van Dam & Hu, 2005). Coffee has been suggested as a potential natural source of inhibitors of AGEs. Verzelloni, Taglizucchi, Rio, Calani, and Conte (2011) stated that coffee melanoids inhibit the formation of AGEs by (i) acting as a radical scavenger and an Fe-chelator in the post-Amadori phase of the glycation reaction and (ii) inhibiting dicarbonyl reactive compound formation during glucose autoxidation. However, only chlorogenic acid (CGA) effectively inhibits protein glycation and dicarbonyl compound formation.

CS is the tegument of green coffee beans (outer layer) and is the major by-product of the roasting procedure (Napolitano, Fogliano, Tafuri, & Ritieni, 2007). CS is characterized by the presence of high amounts of dietary fiber and antioxidant activity (Borrelli, Esposito, Napolitano, Ritieni, & Fogliano, 2004; Napolitano et al., 2007), and contains several bioactive compounds with potential application in food and health (Pourfarzad, Mahdavian-Mehr, & Sedaghat, 2013). Indeed, CS has been proposed as a functional ingredient due to its health promoting properties (Esquivel & Jiménez, 2012). The use of aqueous extracts of coffee silverskin (CS) as health promoter has been recently proposed (del Castillo et al., 2013; Martinez-Saez et al., 2014). Among other bioactive compounds, the extract contains CGA and its health benefits have been in part associated to the presence of this bioactive phytochemical. No studies on the effect of the aqueous CS extract as a natural source of scavengers of dicarboxyls and its anti-AGE properties have been reported which are of great interest and it is the goal of the present investigation.

MATERIALS AND METHODS

Materials and reagents
Coffee silverskin from Arabica (Coffea arabica) and Robusta (Coffea canephora) varieties were provided by Fortaleza S.A. (Spain). According to the manufacturer the weight portion of the coffee silverskin represents 0.5% of the green beans and 0.6% of the roasted beans.

Bovine serum albumin (BSA), 40% methylglyoxal solution (MGO), sodium azide, aminoguanidine (AG), 5-methylquinoxaline (5-MQ), ophenyldiamine (OPD), 2,2-diphenyl-1-picrylhydrazyl (DPPH), fluorescein disodium, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), potassium persulfate, ammonium bicarbonate, caffeine (CFF) and chlorogenic acid (CGA) standards were purchased from Sigma (St. Louis, MO, USA). Folin–Ciocalteu reagent, iron(III) chloride, sodium phosphate monobasic, sodium bicarbonate, hydrogen peroxide, sodium chloride, chloroform, and hydrochloric acid were obtained from Panreac (Madrid, Spain). 2,2'-Azobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tri(2-pyridyl)-striazine (TPTZ), 2,2-azobis (methylpropionamide) dihidro (AAPH) and piridoxamine were purchased from Fluka Chemical (Madrid, Spain). Fiber kit was from Megazyme International Ireland Ltd. Methanol, acetonitrile, glacial acetic acid, potassium hexacyanoferrate and zinc sulfate were purchased from Merck (Darmstadt, Germany). Milli-Q water used was produced using an Elix3 Millipore water purification system coupled to a Milli-Q module (model Advantage10) (Millipore, Molsheim, France). All other chemicals and reagents were of analytical grade.

Equipments
Synergy™ HT-multimode microplate reader with an automatic reagent dispense and temperature control from Biotek Instruments (VT, USA), capillary electrophoresis apparatus Agilent G1600A (Agilent, Madrid, Spain) and HPLC Shimadzu (Kyoto, Japan) equipped with a LC-20AD pump, a SIL-10ADvp autosampler, a CTO-10ASVP oven, and a DAD (SPD-M20A).

Preparation of soluble extracts from coffee silverskin
Arabica and Robusta CS extracts were prepared by aqueous extraction according to the procedure patented by del Castillo et al. (2013b). Briefly, 50 mL of boiling water was added to 2.5 g of coffee silverskin. The mixture was stirred at 250 rpm for 10 min, filtered by Whatman paper no. 4 and the filtrate was freeze dried. The powdered extracts were stored in dark and dry place until analysis.

Determination of total, soluble and insoluble fiber
Total, soluble and insoluble dietary fibers of CS extracts were determined by an enzymatic–gravimetric method based on the AOAC methods 991.43 and 985.29 (Lee, Prosky, & De Vries, 1992; Prosky, Asp,
and employing a commercial kit. All measurements were performed in triplicate. Results were expressed as mg fiber/g CS extracts.

**Determination of melanoidins**
Melanoidin content of CS extracts (1 mg/mL) was determined according to Silván, Morales, and Saura-Calixto (2010). Extracts were then subjected to ultrafiltration using a Microcon YM-10 regenerated cellulose 10 kDa (Millipore, Bedford, MA) at 12000 g for 10 min. Melanoidin content was measured spectrophotometrically at 405 nm. Results were expressed as mg/g CS extracts.

**Determination of CGA and caffeine**
The procedure for the determination of CGA and caffeine was performed according to del Castillo, Ames, and Gordon (2002). The separation was carried out in a capillary electrophoresis system provided with an ultraviolet visible detection system. Calibration curves of caffeine and CGA were constructed. The analyses were performed in triplicate. Results were expressed as mg/g CS extracts.

**Browning determination**
Browning was measured at 405 nm using a microplate reader. CS extracts were dissolved in water at 10 mg/mL in order to obtain an absorbance reading less than 1.5 arbitrary units. All the measurements were made in triplicate. Results were expressed as absorbance units/g CS extracts.

**Determination of total phenolic content**
Total phenolic content (TPC) was determined by the Folin–Ciocalteu method as described by Contini, Baccelloni, Massantini, and Anelli (2008). CGA calibration curve was used for quantification. All measurements were performed in triplicate. Results were expressed as mg CGA equivalent/g CS extract.

**ABTS+ assay**
ABTS+ assay estimated in terms of radical scavenging activity was employed for determining the antioxidant capacity of the extracts as described by Mesías, Navarro, Gökmen, and Morales (2013). Absorbance reading was taken using a microplate reader. Aqueous solutions of Trolox at various concentrations were used for calibration. All measurements were performed in triplicate. Results were expressed as μmol Trolox equivalent antioxidant capacity (TEAC)/g CS extract.

**ORAC assay**
ROO• scavenging activity was measured by monitoring the fluorescence decay as result of ROO-induced oxidation of fluorescein, known as the oxygen radical absorbance capacity (ORAC) assay as described by Dávalos, Gómez-Cordovés, and Bartolomé (2004). ORAC values were expressed as Trolox equivalents by using the standard curve calculated for each assay and the final results were expressed in μmol equivalents of Trolox/g CS extract. All measurements were performed in triplicate.

**FRAP assay**
Ferric reducing antioxidant power (FRAP) was determined as described by Morales, Martin, Açar, Arribas-Lorenzo, and Gökmen (2009). Aqueous solutions of Trolox at various concentrations were used for calibration. All measurements were performed in triplicate. Results were expressed as μmol TEAC/g CS extract.

**DPPH assay**
DPPH radical-scavenging activity was determined as described by Morales et al. (2009). Aqueous solutions of Trolox at various concentrations were used for calibration. All measurements were performed in triplicate. Results were expressed as μmol TEAC/g CS extract.

**In vitro glycation assay with BSA–glucose**
BSA–glucose (BSA–Glc) assay was based on Peng, Cheng, et al. (2008). Briefly, BSA (35 mg/mL) and glucose (175 mg/mL) were dissolved in phosphate buffer (0.1 M, pH 7.4). BSA solution also contained 0.1 g/mL sodium azide. BSA solution (200 μL) was incubated with glucose solution (400 μL) at 37 °C for 21 days in the absence or the presence (100 μL) of soluble CS extracts, caffeine, or CGA (concentration of the stock
solutions at 1, 5, and 10 mg/mL). In parallel, CS extracts and standards were incubated at 37 °C for 21 days in order to measure their intrinsic fluorescence. The final concentration of each reactant in the reaction medium was: 10 mg/mL for BSA, 100 mg/mL for glucose, 0.6 mg/mL for AG, and 0.14, 0.71 or 1.42 mg/mL for the different solutions of caffeine, CGA, and CS extracts. The IC50 values (concentration in mg/mL required to inhibit glycation by 50%) were calculated from the dose–response curves using Microsoft-Excel computer software.

In vitro glycation assay with BSA–MGO

BSA–MGO assay was performed according to Lunceford and Gugliucci (2005) with minor modifications. Briefly, BSA (35 mg/mL) and MGO (0.4 mg/mL) solutions were prepared in phosphate buffer (0.1 M, pH 7.4). Two hundred microliters of BSA solution, containing 0.1 g/mL sodium azide, was incubated with 400 µL of MGO solution. The incubation was carried out at 37 °C for 14 days in the absence or the presence (100 µL) of soluble CS extracts, caffeine, or CGA (concentration of the stock solutions at 1, 5, and 10 mg/mL). In parallel, CS extracts and standards were incubated at 37 °C for 14 days in order to measure their intrinsic fluorescence. The final concentration of each reactant in the reaction medium was 10 mg/mL for BSA, 0.23 mg/mL for MGO, 0.6 mg/mL for AG and 0.14, 0.71 and 1.42 mg/mL for the different solutions caffeine, CGA and CS extracts. The IC50 values were calculated from the dose–response curves using Microsoft-Excel computer software.

AGE fluorescence measurement

The measurement of fluorescent intensity of total AGEs and the intrinsic fluorescence of the CS extracts and standards after incubation were performed using a microplate spectrophotometer. The presence of total AGEs was characterized by a typical fluorescence with excitation and emission maxima at 360 and 420 nm, for BSA–Glc assay and 340 and 420 nm, for BSA–MGO assay. Percentage inhibition of AGE formation by each sample was calculated using the following equation; % inhibition = \( [1 - (\text{fluorescence of the solution with inhibitors} - \text{intrinsic fluorescence of the samples}) / \text{fluorescence of the solution without inhibitors}) ] \times 100%.

Evaluation of direct MGO trapping capacity

Direct MGO trapping capacity was as described by Peng, Zheng, et al. (2008) with some modifications (Mesías et al., 2013). Pyridoxamine (PM) was used as the positive control. The final concentration of each reactant in the reaction medium was 0.04 mg/mL for MGO, 0.1 mg/mL for PM and a range of 0.001–0.5 mg/mL for soluble CS extracts, caffeine and CGA. Samples were incubated at 37 °C for 168 h, after that 200 µL of OPD was added. The unreacted MGO was quantified by HPLC (Shimadzu, Kyoto, JP) on the basis of the amount of the derivatized product, 2-methylquinoxaline (2-MQ). The chromatographic separation was carried out on a Mediterranean-Sea-ODS2 column (150 × 3 mm, 5 µm, Tecknokroma, Barcelona, Spain). The amounts of unreacted MGO in the samples could be determined on the basis of the ratios of peak area of 2-MQ and 5-MQ. Percentage decrease in MGO was calculated using the following equation: MGO decrease % = \( [((\text{amounts of MGO in control} - \text{amounts of MGO in sample or PM solution}) / \text{amounts of MGO in control})] \times 100%\). The IC50 values of samples were calculated from the dose–response curves using Microsoft-Excel computer software.

Statistical analysis

Statistical analyses were performed using the Statgraphics Centurion XV statistical program (Herndon, VA). Data were expressed as the mean value ± SD. Analysis of variance (ANOVA) and the Duncan test were applied to determine differences between means. Differences were considered to be significant at p < 0.05.

RESULTS AND DISCUSSION

Table 1 summarizes the content in dietary fiber (total, soluble and insoluble), melanoidins, chlorogenic acids, caffeine, total phenolic, antioxidant and browning of water soluble CS extracts. TPC was of 31.0 and 35.4 mg equivalents CGA/g for Arabica and Robusta CS extracts, respectively. CS has previously been considered a good source of phenolic compounds (Bresciani, Calani, Bruni, Brighenti, & Del Rio, in press). CGA content was significantly higher in Robusta CS extract (68.52 mg/g) than in Arabica CS extract (11.18 mg/g). Results of CGA are in line with those reported by Narita and Inouye (2012) and del Castillo et al. (2013). In a similar way, caffeine content was also higher in Robusta CS extract (33.98 mg/g) in comparison with Arabic
CS extracts (30.26 mg/g). For browning determination and melanoidin content, moreover, these differences were significant. These results agree with those from Martinez-Saez et al. (2014) who described that levels of CGA, caffeine concentration, melanoidins and browning, expressed as color, were significantly greater (p < 0.05) in beverages made with Robusta coffee silverskin extract than Arabica.

The antioxidant capacity (radical scavenging, and hydrogen and electron donating capacities) of the CS extracts was assessed by ABTS, ORAC, DPPH and FRAP assays, showing results of 85.20, 1194, 829.8 and 219.9 μmol TEAC/g sample in the case of Arabica CS extract and 225.8, 1513, 640.1 and 231.3 μmol TEAC/g sample for Robusta CS extract. Arabica CS extract showed higher reducing power than Robusta CS extract. In contrast, Robusta CS extract showed significantly higher scavenging properties against the ABTS radical than Arabica CS extract. The results agree with those described by Napolitano et al. (2007), del Castillo et al. (2013) and Martinez-Saez et al. (2014), who found, a higher proportion of extractable antioxidants in aqueous solution for the samples of Robusta silverskin, in comparison with Arabica. The differences found in the total antioxidant capacity values in ACS and RCS extracts may be related to those detected by analyzing their components, melanoidins and CGA since, as it has been suggested, these compounds contribute to the antioxidant properties of coffee silverskin.

Fig. 1 shows the effect of CS extracts, caffeine, and chlorogenic acid on the formation of fluorescent AGES in glycation model systems composed by BSA and glucose treated at 37 °C for 21 days. Caffeine did not affect the formation of AGES under our particular conditions. CGA showed antiglycative capacity being its IC50 value of 0.4 mg/mL. Kim et al. (2011) already described the antiglycative capacity of CGA in the formation of fluorescent AGES and crosslinking of collagen. Arabica and Robusta CS extracts showed similar and dose-dependent rates of AGE inhibition with an IC50 of 0.6 mg/mL for both extracts. The only significant differences (p < 0.05) were found at the concentration of 0.71 mg/mL where ACS showed a significantly higher antiglycative activity than RCS.

Fig. 2 represents the antiglycative capacity of the Arabica and Robusta CS extracts, caffeine, and chlorogenic acid in glycoxidation model systems (BSA–MGO) at 37 °C for 14 days. MGO was reported as a potent agent for AGE generation and modified proteins irreversibly by targeting the side chains of arginine at a much faster rate than reducing sugars (Oya et al., 1999). Caffeine (1.4 mg/mL) exerted a weak antiglycative capacity corresponding to 10% of the inhibition of the formation of fluorescent AGES. The antiglycative capacity of CGA was notably higher in the BSA–MGO assay (IC50 of 0.1 mg/mL) as compared with the BSA–glucose assay. However, the antiglycative capacity of Arabica and Robusta CS extracts was lower (IC50 of 1.3 mg/mL) as compared with the BSA–glucose assay. Again, significant differences between both CS extracts were not observed, and they behaved in a dose-dependent manner.

AGE formation can be suppressed by inhibitors at the post-Amadori stage which can scavenge carbonyl precursors. As a consequence, the direct MGO-trapping capacity of Arabica and Robusta CS extracts was determined. Fig. 3 shows the MGO trapping ability of the CS extracts at 168 h in a range of concentrations from 0.001 to 0.5 mg/mL. Pyridoxamine (0.1 mg/mL) was used as control since it reacts with methylglyoxal to form stable adducts (Nagaraj et al., 2002). IC50 value for PM was 0.006 mg/mL. Caffeine, chlorogenic acid, and the Arabica and Robusta CS extracts trapped MGO in a dose-dependent manner. The lowest effectiveness was shown by the caffeine whereas only 20% of the initial MGO was decreased in the presence of 0.5 mg/mL caffeine. MGO was effectively quenched by CGA with an IC50 of 0.14 mg/mL. Arabica and Robusta CS extracts showed significant differences in their ability to trap MGO with an IC50 of 0.055 and 0.65 mg/mL for Robusta and Arabica CS extracts, respectively. Robusta CS extract was nearly 10-fold more active against MGO than Arabica CS extract.

To get more insight into the MGO trapping capacity of the extracts, kinetic evaluation for MGO-trapping capacity for 168 h at a concentration of 0.1 mg/mL was performed and compared with that of the PM (Fig. 4). PM and Robusta CS extract already trapped 50% of the initial MGO in the systemat 27 and 66 h, respectively. In contrast, the Arabica CS extract only reached to trap around 14% of the MGO at 168 h, which showed a significant lower MGO-trapping capacity of this extract. The MGO-trapping capacity of the Arabica and Robusta CS extracts increased continuously during the time of incubation at 37 °C, following the same trend as to that of PM.
In the present study the antiglycative capacity measured as the inhibition of the formation of fluorescent AGEs, and mitigation of carbonyl radical stress (in terms of MGO-trapping capacity) of Arabica and Robusta CS aqueous extracts have been demonstrated. These results are in agreement with Verzelloni, Pellacani, et al. (2011) and Verzelloni, Tagliazucchi, et al. (2011) who reported that coffee contains molecules with in vitro antiglycative capacity. Our investigation demonstrates that antiglycative properties are also maintained in coffee by-products which may be related to phenolic compounds naturally present in coffee beans (Napolitano et al., 2007) and melanoidins formed through Maillard reaction during roasting (Delgado-Andrade & Morales, 2005). According to Borrelli et al. (2004) antioxidant compounds present in coffee can form covalent links with carbohydrates resulting in a fiber–antioxidant complex. Specifically the phenolic compounds from CS, mainly chlorogenic acid, may react with polysaccharide components forming melanoidins, which exerts the antioxidant activity (Borrelli, Visconti, Mennella, Anese, & Fogliano, 2002). According to Verzelloni, Pellacani, et al. (2011) and Verzelloni, Tagliazucchi, et al. (2011) the high molecular weight compound fraction of coffee has high antioxidant, chelating and antiglycative activities. Chlorogenic acid and fiber contents were significantly higher in Robusta CS extract as compared with Arabica CS extract.

Antiglycative effects of phenolic compounds have been previously reported. These compounds can exert their inhibition through their antioxidant properties, scavenging of free radicals, and quenching of carbonyl radical species (Delgado-Andrade & Morales, 2005; Kim & Kim, 2003; Sang et al., 2007; Wu & Yen, 2005). Wu, Hsieh, Wang, and Chen (2009) evaluated the ability of phenolic acids to inhibit glycation, concluding that these compounds were effective in the prevention of glucose-mediated protein modification, which are considered potent inhibitors of both AGE formation and the subsequent crosslinking of proteins. Among the phenolic acids studied by these authors, chlorogenic acid was considered to be one of the major antiglycative compounds, being in line with our results. In a similar way, Gugliucci, Bastos, Schulze, and Souza (2009) reported for yerba mate water extract containing chlorogenic acid and caffeic acid a high percentage of AGE fluorescence inhibition.

As mentioned above, the present investigation was carried out using aqueous extracts in order to perform a cost-effective and environmentally friendly procedure, as well as being more interesting from safety and toxicological points of view for their industrial application. Several authors have evaluated the influence of the extraction process on the antioxidant capacity, concluding that water is highly efficient at extracting antioxidants. In this sense, Budryn et al. (2009) reported that both chlorogenic acids and melanoidins were found in higher amounts in aqueous extracts than in ethanolic extracts from green and roasted coffee, showing that these antioxidants were more soluble in water. In a similar way, Yen, Wang, Chang, and Duh (2005) indicated that the highest antioxidant yields in extracts were obtained with water from roasted coffee residues. Furthermore, Bravo, Monente, Juániz, De Peña, and Cid (2013) stated that water is necessary to extract more phenolic and nonphenolic antioxidants from spent coffee. Consequently, it can be supposed that the aqueous CS extracts contain the majority of the antioxidant compounds present in the CS including chlorogenic acid and, hence, it might justify the high antiglycative activity of the extracts.

Regarding caffeine, it has been shown that this compound has a low in vitro antioxidant activity (Somoza et al., 2003; Yen et al., 2005). In addition, caffeine has displayed a low inhibitory effect on AGE formation in in vitro assay systems (Nakagawa, Yokozawa, Tarasawa, Shu, & Juneja, 2002). These findings are in accordance with those found in the present study, since caffeine practically did not exhibit any antiglycative effect in the assays of BSA–glucose and BSA–MGO and a low MGO trapping capacity in comparison with that found for CS extracts. However, reactive oxygen species scavenging has been recently proposed for caffeine (Leon-Carmona & Golano, 2011).

Concerning the MGO-trapping capacity, Robusta CS extract trapped MGO more rapidly and efficiently as compared with Arabica CS extract. It might be indicated that the different compositions of the extracts influenced their MGO-trapping ability but not significantly the antiglycative capacity in the protein assays. Although FRAP and DPPH assays did not show large differences among Arabica CS and Robusta CS extracts, ABTS and ORAC exhibited a higher antioxidant capacity in the Robusta variety. In addition, despite no differences were observed in total phenolic content of the two studied samples, CGA content and specifically 3-CGA, 4-CGA and 5-CGA contents were also higher in the Robusta specie (8.2, 9.1 and 26.3
mg/g versus 1.5, 1.8 and 6.4 mg/g in Arabica CS), which could explain the major antiglycative capacity of this extract. On the other hand, it should be taken into account that the presence of several AGE inhibitors in an extract may have synergistic effects (Chompoo, Upadhyay, Kishimoto, Makise, & Tawata, 2011). During coffee roasting, a part of CGA is incorporated into the melanoidins (Moreira, Nunes, Domingues, & Coimbra, 2012) and the new structures may contribute to the overall antiglycative capacity of the CS extracts. Specifically, according to Rice-Evans, Miller, and Paganga (1996), phenolic compounds may have synergistic or antagonistic effects among them or with other constituents of an extract. Therefore, it can be supposed that chlorogenic acid together with other phenolic compounds, and CGA–melanoidins rich might contribute overall to the antiglycative activity of the extracts.

In spite of the antiglycative properties of the aqueous extract of coffee silverskin demonstrated in the in vitro assays in the present study, it cannot be assured that this effect also occur in vivo experiments. According to Verzelloni, Pellacani, et al. (2011) and Verzelloni, Tagliazucchi, et al. (2011), after ingestion by humans polyphenolic compounds can be metabolized generating different metabolites in the colon, which can or not exert inhibitory activity against AGE formation. These observations may be taken into account for future research.

CONCLUSIONS

The antiglycative capacity of coffee silverskin (CS) extracts, obtained from Arabica and Robusta varieties, was evaluated using different in vitro models. Both Arabica and Robusta CS extracts exhibited an anti-AGE capacity in BSA–glucose and BSA–MGO assays at concentrations ranging from 0.1 to 1.5 mg/mL. No significant differences among the Arabica and Robusta CS extracts were observed. On the other hand, the direct MGO-trapping assay showed that the CS extracts trapped MGO in a dose-dependent manner, but the Robusta CS extract showed a higher trapping capacity (IC50=0.55 mg/mL) in comparison with Arabica CS extract. Chlorogenic acid content in samples did not explain the antiglycative properties of the CS extracts, although the MGO trapping capacity was greatly related to the CGA content. The anti-AGE capacity of the samples might be related to the antioxidiant capacity of the CS, explained by the phenolic compound content, especially chlorogenic acid, but also by the presence of high molecular weight polymers (melanoidins) formed through Maillard reaction during the roasting of coffee. The synergistic contribution of other constituents of the CS extracts to the antiglycative and carbonyl trapping properties in vitro should not be discarded. Further studies should be performed for evaluating the bioactivity of the extracts in vivo.

Acknowledgments

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References


FIGURES AND TABLES

Table 1. Characterization of Arabica coffee silverskin (ACS) and Robusta coffee silverskin (RCS) extracts.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ACS extract</th>
<th>RCS extract</th>
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<tr>
<td>Fiber</td>
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<tr>
<td>Total (mg/g)</td>
<td>286.89 ± 19.15 a</td>
<td>362.18 ± 13.74 b</td>
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<td>Soluble (mg/g)</td>
<td>240.15 ± 19.5a</td>
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<td>Insoluble (mg/g)</td>
<td>46.75 ± 0.34a</td>
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<td>Melanoidins (mg/g)</td>
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<td>239.46 ± 8.49b</td>
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<td>829.8 ± 38.16a</td>
<td>640.1 ± 39.78b</td>
</tr>
<tr>
<td>DPPH (μmol TEAC/g)</td>
<td>219.9 ± 4.34a</td>
<td>231.3 ± 4.73b</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD for n = 4. Different letters mean significant differences.

Figure 1. Antiglycative capacity of Arabica and Robusta coffee silverskin extracts (ACS, RCS), caffeine (CFF) and chlorogenic acid (CGA) in the BSA-glucose assay. Concentrations assayed were 0.14, 0.71 and 1.42 mg/mL. Results are expressed as mean ± SD for n = 4. Aminoguanidine (AG) (0.6 mg/mL) showed an antiglycative activity of 91.2%. Different letters mean significant differences among the ACS and RCS extracts for a same concentration.

[Graph showing antiglycative activity of different concentrations and letters indicating significant differences]
Figure 2. Antiglycative capacity of Arabica and Robusta coffee silverskin extracts (ACS, RCS), caffeine (CFF), and chlorogenic acid (CGA) in the BSA–MGO assay. Concentrations assayed were 0.14, 0.71 and 1.42 mg/mL. Results are expressed as mean ± SD for n = 4. Aminoguanidine (AG) (0.6 mg/mL) showed an antiglycative activity of 99.2%.

Figure 3. Methylglyoxal trapping capacity of Arabica and Robusta coffee silverskin extracts (ACS, RCS), pyridoxamine (PM), chlorogenic acid (CGA), and caffeine (CFF) at 168 h. Results are expressed as mean ± SD for n=4. PM (0.1 mg/mL) showed a MGO trapping capacity of 99.6%. ACS; RCS; PM; caffeine; CGA.
Figure 4. Time-course MGO trapping capacity of the Arabica and Robusta coffee silverskin extracts (ACS, RCS). Results are expressed as mean ± SD for n = 4. Pyridoxamine (PM) (0.1 mg/mL) showed a MGO trapping capacity of 99.6%. ▲ PM; ➔ ACS; ➖ RCS.