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Isolation of polyphenols from spent coffee grounds and silverskin by mild hydrothermal pretreatment

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ABSTRACT

In this study, a new method for isolation of polyphenols (PP) from spent coffee grounds (SCG) and coffee silverskin (CS) is described. The method consisted of a mild hydrothermal pretreatment at 120°C, for 20 min, using a liquid-to-solid ratio of 20 mL/g. PP (determined as gallic acid equivalents, GAE) were the most abundant components in the extracts produced by this method, corresponding to 32.92 mg_{GAE}/g_{SCG} and 19.17 mg_{GAE}/g_{CS}, among which flavonoids corresponded to 8.29 and 2.73 mg quercetin equivalents/g of SCG and CS, respectively. Both extracts presented antioxidant activity but the results were higher for SCG extract, probably due to the highest content of PP present. Negligible effects (less than 1% solubilization) were caused by the hydrothermal pretreatment on cellulose, hemicellulose, and protein fractions of these materials. Some mineral elements were present in the extracts, with potassium being the most abundant. Hydrothermal pretreatment under mild conditions was demonstrated to be an efficient method to recover antioxidant PP from coffee residues.

KEYWORDS

Antioxidant activity; coffee silverskin; mild hydrothermal pretreatment; polyphenols; spent coffee grounds

Introduction

Coffee is one of the most consumed beverages in the world and is also the second largest traded commodity worldwide after petroleum. As a consequence of this big market, millions of tons of residues are generated every year during the coffee beans processing. Spent coffee grounds (SCG), for example, are obtained in high amounts during the process for instant coffee elaboration, and are also obtained in large amounts in restaurants, bars, and cafeterias, while coffee silverskin (CS) is the main residue obtained during the beans roasting step. Most of these residues have still no special use, being mostly discharged into the environment or burned. Nevertheless, interest in reusing these residues has increased in the last years, mainly in the last 5 years, when the number of studies published on the topics "spent coffee grounds" and "coffee silverskin" increased significantly by approximately fourfold. Such interest has been motivated by environmental concerns and also because these residues contain in their composition several components that can be of value for application in food, cosmetic, and pharmaceutical areas.[1,2]

The presence of polyphenols (PP) in SCG and CS has been reported in several studies, and some technologies have also been proposed for their recovery from these residues.[3-7] Among such technologies, the use of organic solvents is usually proposed due to their high extraction capacity. However, the use of organic solvents generates a toxic effluent that must be treated prior to discharge to the environment. As an alternative to avoid this problem, this study evaluated the possibility of recovering antioxidant PP by hydrothermal pretreatment of SCG and CS. By using this strategy, the residues are submitted to a reaction using only water as extraction solvent under temperatures higher than those usually employed for extraction with organic solvents, and the use of these toxic chemicals is avoided. This pretreatment technology can be used to extract different valuable compounds from biomass wastes and is considered a very attractive technology because it can provide high extraction efficiency and does not require the use of toxic chemicals for extraction.[8] As a consequence, the liquid streams neutralization and/or treatment are also not required because no acids or organic substances are added to the reaction.

Several applications have been described for the PP in the food industry, including as antimicrobial agents and to prevent food deterioration. Nevertheless, most of the attention that has been paid to these compounds is for application in the pharmaceutical area, due to the numerous benefits that they can promote for human health, benefits that are mainly related to their antioxidant effect. Antioxidant PP may act against a number of diseases, such as atherosclerosis, certain cancers, and neurodegenerative diseases, as well as against aging processes.[2,9-11] Due to these important applications, it has been strongly desired to establish economic and environmentally friendly processes to obtain PP.

In the present study, SCG and CS were submitted to hydrothermal pretreatment under mild reaction conditions and the produced extracts were chemically characterized to determine the contents of PP, flavonoids, sugars, proteins, and minerals. The antioxidant activity of the extracts was also determined. The results were compared with other studies reported in

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Table 1. Chemical composition of the extracts produced by mild hydrothermal pretreatment of spent coffee grounds (SCG) and coffee silverskin (CS).

	SCG	CS
Polyphenols (mg_{GAE}/g dry matter)	32.92 ± 2.37	19.17 ± 1.06
Flavonoids (mg_{QE}/g dry matter)	8.29 ± 0.67	2.73 ± 0.76
Sugars (mg/g dry matter)	0.93 ± 0.17	2.42 ± 0.52
Protein (mg_{BSA}/g dry matter)	15.24 ± 4.09	3.75 ± 0.65
Minerals (mg/g dry matter)		
Potassium	9.84 ± 0.28	17.84 ± 0.16
Phosphorous	1.48 ± 0.05	1.00 ± 0.01
Magnesium	0.74 ± 0.04	1.39 ± 0.01
Sulfur	0.40 ± 0.02	0.90 ± 0.01
Calcium	0.30 ± 0.01	0.82 ± 0.01

Note. GAE = gallic acid equivalents; QE = quercetin equivalents; BSA = bovine serum albumin.

the literature for PP recovery from SCG and CS by using different extraction methods.

Experimental

Raw materials

SCG and CS samples were provided by NovaDelta Comércio e Indústria de Cafés, S.A. (Campo Maior, Portugal). In order to avoid deterioration and allow safe storage, samples were dried in an oven at approximately 60°C until constant weight. The final moisture content of the samples was determined by using a moisture analyzer, model MAC 50/1/NH (Radwag, Poland), and corresponded to 6.8% and 5.4% (w/w) for SCG and CS, respectively. After being dried, the samples were sieved through a 500-μm mesh screen and stored in closed recipients, which were maintained at room temperature. Only particles ≤500 μm were used in the experiments.

Hydrothermal pretreatment

For the hydrothermal pretreatment, the coffee residues were mixed with distilled water in a liquid-to-solid ratio of 20 mL/g. The moisture content of the samples was considered in the material balances. Then the mixtures were introduced in an autoclave and were heated at 120°C during 20 min (time zero was considered as the beginning of the isothermal stage). After this time, the produced extracts were separated by centrifugation (2500 × g, 10 min), using a Scanspeed 416 centrifuge (Labogene, Denmark), followed by filtration in number 1 filter paper (Whatman), and were stored at −20°C until analyses. The volume of extract recovered after each extraction was measured and used for calculation. The hydrothermal assays were carried out in quintuplicate.

Analytical determinations

PP was determined by the Folin–Ciocalteu colorimetric methods adapted for use in a 96-well microplate.[12] The total PP content was expressed as milligram gallic acid equivalent (GAE) per dry weight of material (mg_{GAE}/g). Flavonoids were quantified by colorimetric assay[13] and expressed as milligrams quercetin equivalent (QE) per dry weight of material (mg_{QE}/g). Total sugar content was determined by the anthrone method.[14] Protein was estimated by the Bradford assay adapted for use in a 96-well microplate.[12] The protein content was expressed as milligrams bovine serum albumin (BSA) per dry weight of material (mg_{BSA}/g). Mineral elements were determined by inductively coupled plasma–atomic emission spectrometry (ICP-AES) as described by Meneses et al.[12]

Table 2. Polyphenols recovery from spent coffee grounds (SCG) and coffee silverskin (CS) by different extraction methods and conditions.

Extraction method	Conditions	Polyphenols recovered (mg_{GAE}/g_{SCG})*	Reference
Spent coffee grounds			
Soxhlet	Water, 50 mL/3 g SCG, 100°C, 1 hr	10.2	Bravo et al.[6]
	Water, 50 mL/3 g SCG, 100°C, 3 hr	13.6	
Solid-liquid extraction	Methanol 60%, 40 mL/g SCG, 60–65°C, 90 min	16.0	Mussatto et al.[3]
	Methanol 50%, 25 mL/g SCG, 60–65°C, 90 min	18.0	
	Water, 40 mL/g SCG, 60–65°C, 90 min	7.4	
	Ethanol 60%, 50 mL/g SCG, 60°C, 30 min	28.26	Panusa et al.[18]
	Water, 50 mL/g SCG, 60°C, 30 min	19.62	
	Water, 50 mL/3 g SCG, 80°C, 10 min	17.4	Bravo et al.[6]
Solid-state cultivation of microorganisms	*Penicillium purpurogenum*, 5×10^5 spores/g SCG, 70% moisture, 30°C, 6 days	7.02	Machado et al.[19]
	Neurospora crassa, 5×10^5 spores/g SCG, 70% moisture, 30°C, 6 days	6.50	
Mild hydrothermal pretreatment	Water, 20 mL/g SCG, 120°C, 20 min	32.92	Present study
Coffee silverskin			
Soxhlet	Isopropanol 60%, 10 mL/g CS, 27°C	13.2	Murthy and Naidu[5]
Solid-liquid extraction	Ethanol 60%, 35 mL/g CS, 60–65°C, 30 min	13	Ballesteros et al.[17]
	Water, 50 mL/g CS, 25°C, 1 hr	6	Narita and Inouye[20]
	Water, 50 mL/g CS, 80°C, 1 hr	7	
	0.1 *M* HCl, 50 mL/g CS, 25°C, 1 hr	5	
	0.1 *M* HCl, 50 mL/g CS, 80°C, 1 hr	7	
	0.1 *M* NaOH, 50 mL/g CS, 25°C, 1 hr	5	
	0.1 *M* NaOH, 50 mL/g CS, 80°C, 1 hr	8	
Subcritical water extraction	Water, 50 mL/g CS, 180°C, 10 min	22	Narita and Inouye[20]
Solid-state cultivation of microorganisms	*Penicillium purpurogenum*, 5×10^5 spores/g CS, 70% moisture, 30°C, 6 days	3.47	Machado et al.[19]
	Neurospora crassa, 5×10^5 spores/g CS, 70% moisture, 30°C, 6 days	2.92	
Mild hydrothermal pretreatment	Water, 20 mL/g CS, 120°C, 20 min	19.17	Present study

*GAE = gallic acid equivalents.

The antioxidant activity of the extracts was determined by two methods: the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity (DPPH), and the ferric reducing antioxidant power (FRAP) assay, both adapted for use in a 96-well microplate.[15] The DPPH values were expressed as percent inhibition, and the FRAP values were expressed as millimoles of ferrous equivalent per gram of dry weight material (mmol Fe(II)/g).

Results and discussion

The contents of PP, flavonoids, sugars, protein, and minerals in the extracts obtained by mild hydrothermal pretreatment of SCG and CS are shown in Table 1. It is evident from these results that the conditions used for hydrothermal pretreatment were more suitable to extract PP than other components from the coffee residues structure, since PP were the most abundant components in both SCG and CS extracts. Negligible effects (less than 1% solubilization) were caused by the hydrothermal pretreatment on cellulose and hemicellulose fractions of these materials, since the sugars derived from these fractions were present in small amounts in the extracts (less than 2.5 mg/g). Sugars (in the form of cellulose and hemicellulose) in SCG and CS correspond to approximately 500 and 400 mg/g dry matter, respectively.[7] Such negligible effect on sugars recovery was expected since the hydrothermal pretreatment was performed under mild conditions (120°C). Sugars extraction by this process usually occurs under more elevated temperatures, between 150°C and 230°C.[16]

Protein was also present in both the extracts (Table 1). However, taking into account that the protein content in SCG and CS corresponds to 174 mg/g and 187 mg/g, respectively,[7] it can be concluded that the mild hydrothermal pretreatment caused also little effect on the protein fraction of these materials, promoting solubilization of less than 10% of the total present in their composition. Among the minerals, it must be highlighted that there is an elevated concentration of potassium in the extracts, which is justifiable since potassium is the most abundant mineral element in SCG and CS.[7]

In terms of PP recovery, which was the objective of this study, the extracts contained 32.92 mg_{GAE}/g_{SCG} and 19.17 mg_{GAE}/g_{CS}, among which flavonoids corresponded to 8.29 and 2.73 mg_{QE}/g of SCG and CS, respectively (Table 1). These results are higher than the amounts recovered in our previous studies using organic solvents as extraction agent,[3,17] and can also be compared well to other methods reported in the literature for PP recovery from these residues. Table 2 summarizes different methods and conditions that have already been reported for PP recovery from SCG and CS. It is worth noting that solid–liquid extraction with water under low temperatures (around 60–65°C) has been demonstrated as being not efficient for PP extraction from SCG and CS. However, the use of water under higher temperature (120°C) as proposed in the present study was quite efficient for this purpose, revealing the importance of setting the temperature to improve the extraction results.

Many possible applications have been described for the PP in the industrial sector, especially in the pharmaceutical field, due to the numerous benefits that these compounds can promote in human health, benefits that are related to their antioxidant potential. In the present study, the antioxidant activity of the hydrothermal extracts produced from SCG and CS was determined by two different methods (DPPH and FRAP) in order to have a better conclusion about the antioxidant potential of these extracts, as each method is based on a different principle of reaction. The DPPH method is based on the ability of DPPH radical to react with hydrogen donor species such as phenols and flavonoids present in the extract material; in the FRAP method, the antioxidant activity is determined based on the ability to reduce Fe^{3+} to Fe^{2+}.

Both extracts showed antioxidant activity by the two evaluated methods (Figure 1). However, the results obtained for SCG extract were higher than those observed for the CS extract, which is probably related to the presence of more PP in SCG extract (Table 1). As a whole, both extracts presented significant values of antioxidant activity, values that were higher than those reported for extracts produced from other agro-industrial residues such as brewer's spent grains, for example.[12] Taking into account the numerous benefits for the health related to the ingestion of antioxidant polyphenols, the present results suggest that PP recovered by

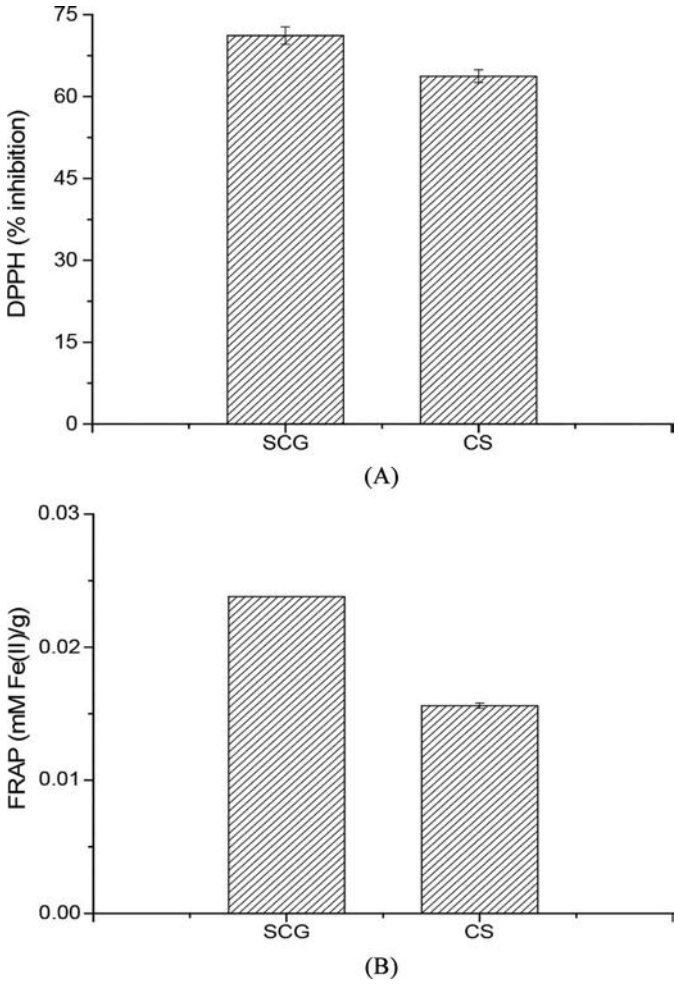

Figure 1. Antioxidant potential (determined by the 2,2-diphenyl-1-picrylhydrazyl [DPPH] free radical and the ferric reducing antioxidant power [FRAP] assay) of the extracts produced by mild hydrothermal pretreatment (120°C, 20 min, liquid-to-solid ratio of 20 mL/g) of spent coffee grounds (SCG) and coffee silverskin (CS).

mild hydrothermal pretreatment of SCG and CS could find numerous applications in the food and pharmaceutical areas.

Conclusions

Hydrothermal pretreatment under mild conditions was demonstrated to be an efficient method to recover polyphenols from spent coffee grounds and coffee silverskin. The extracts produced by this method presented antioxidant activity and could be then of interest for application in the food, cosmetic, and pharmaceutical areas. Further studies using different combinations of temperature, reaction time, and liquid-to-solid ratio can be useful in order to verify whether the extraction results can be still improved. Mild hydrothermal pretreatment is also an attractive process from an environmental point of view, since residues containing no chemicals are generated.

It is also worth mentioning that as this method causes negligible effects on cellulose and hemicellulose structures, the solid residue obtained after mild hydrothermal pretreatment is rich in sugars that can be further recovered by applying another pretreatment method. In this sense, the use of a mild hydrothermal pretreatment as an initial step for biomass fractionation can be considered an interesting alternative to give additional value to biomass residues, since it is selective for polyphenols recovery and contributes to a maximum valorization of the biomass wastes in a biorefinery context.

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