TITLE: Influence of extraction process on antioxidant capacity of spent coffee.

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ABSTRACT

Spent coffee that is produced in tons by restaurants and cafeterias, and consumers at domestic levels, could be a good opportunity to have an important source of natural antioxidants. The main aim of this work was to study the influence of several process factors on the antioxidant capacity extraction from spent coffee. Total phenolic compounds, radical scavenging activity (ABTS and DPPH) and browned compounds (Abs 420nm) of spent coffee extracts obtained with continuous (soxhlet 1h and 3h) and discontinuous methods (solid-liquid extraction and filter coffeemaker), several solvents (water, ethanol, methanol and their mixtures), successive extractions, and water with different pHs (4.5, 7.0 and 9.5) were carried out. Spent coffee extracts with the highest antioxidant capacity were obtained after one extraction with neutral water (pH 7.0) in a filter coffeemaker (24 g spent coffee per 400mL water). Furthermore, spent coffee defatting and extracts lyophilization allowed us to obtain spent coffee extracts powder with high antioxidant capacity that can be used as an ingredient or additive in food industry with potential preservation and functional properties.

KEYWORDS: coffee, spent coffee, by-products, antioxidant, phenolic compounds, melanoidins.

1. INTRODUCTION

The preparation of a good cup of coffee requires several technological steps from the coffee fruit. During coffee processing and roasting, some residues are generated. Coffee pulp, and cherry and parchment husks are produced in plantations to obtain green coffee beans. In the roasting process, the teguments of green coffee beans, named silverskin, are removed. And during the manufacture of soluble and concentrated coffee, wastes are produced in coffee industry. These coffee residues are frequently used by the industry as animal feed and fertilizer, but during last few years other more friendly environment uses, such as biofuel production have been proposed (Saenger, Hartge, Werther, Ogada, & Siagi, 2001; Silva, Nebra, Machado, & Sanchez, 1998). Furthermore, during the last few years an increasing number of studies have shown the presence of phytochemicals related with health benefits in coffee residues (Borrelli, Esposito, Napolitano, Ritieni, & Fogliano, 2004; Ramalakshmi, Jagan Mohan Rao, Takano-Ishikawa, & Goto, 2009). In the case of silverskin, some authors have demonstrated the presence of high amounts of dietary fiber as well as antioxidant activity (Borrelli et al., 2004; Napolitano, Fogliano, Tafuri, & Ritieni, 2007). Also in the residues obtained during processing of soluble coffee, antioxidant properties, which may be attributed to phenolic and nonphenolic compounds, have been found (Pushpa & Madhava Naidu, 2010; Ramalakshmi et al., 2009; Yen, Wang, Chang, & Duh, 2005). Moreover, technological factors play an important role in antioxidants extraction during brewing process (Andueza, Vila, de Peña, & Cid, 2007; Pérez-Martínez, Caemmerer, De Peña, Cid, & Kroh, 2010) and, consequently, may influence on the presence of remained antioxidant compounds in spent coffee. For this reason, the results of the antioxidant activity reported for coffee residues from the soluble coffee industry can not be directly extrapolated to those spent coffee obtained with coffeemakers.

Spent coffee that is produced in tons by restaurants and cafeterias, and consumers at domestic levels, could be a good opportunity to have an important source of natural antioxidants, also from the economical point of view. However, studies about the health related phytochemicals, such as antioxidants of spent coffee obtained during brewing process have not been found. So that, previously it would be necessary to develop an easy, efficient, safe and cheap method to obtain spent coffee antioxidant extracts. In previous cited studies, the extraction of antioxidants from industry soluble coffee residues has been made for hours using continuous (soxhlet) and discontinuous methods, with different solvents, such as water, ethanol, methanol, n-hexane, isopropanol and their mixtures at different proportions. Moreover, the antioxidant activity has been also measured by different methods. For all these reasons, the main aim of this work was to study the influence of several process factors on the antioxidant capacity extraction from spent coffee in order to establish the most efficient procedure, i.e. for obtaining spent coffee extracts with the highest antioxidant capacity. Consequently, the antioxidant capacity of spent coffee extracts obtained with continuous and discontinuous methods, several solvents, successive extractions, and solvents with different pHs were compared and the most efficient extraction conditions have been selected. Moreover, the influence of spent coffee defatting and extracts lyophilisation on the antioxidant capacity have been also studied in order to obtain a new product that can be used as a natural antioxidant or ingredient with potential preservation or functional properties.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents. Methanol, ethanol and petroleum ether used were of analytical grade from Panreac (Barcelona, Spain). Folin-Ciocalteau reagent, sodium

carbonate, sodium bicarbonate, lactic acid and sodium hydroxide were also obtained from Panreac (Barcelona, Spain). Gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), 2,2'-Azinobis (3-ethylbenzothiazonile-6-sulfonic acid) diammonium salt (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH˙) were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2 Spent Coffee preparation. Guatemala Arabica roasted coffee was provided by a local factory. Coffee beans were ground for 20 s using a grinder (model Moulinex super junior "s"). Filter coffee brew was prepared from 24 g of ground roasted coffee for a volume of 400 mL of water, using a filter coffeemaker (model AVANTIS 70 Inox, Ufesa, Spain). Extraction took approximately 6 min at 90ºC. Ground roasted coffee after brewing, namely spent coffee, was dried to a constant weight for 2 hours at 102 ±3 ºC in an oven JP SELECTA (Barcelona, Spain).

Spent coffee was defatted with Petroleum Ether (1:11, w/v) at 60 ºC for 3 h in a Soxhlet extraction system Extraction Unit B-811 Standard BÜCHI (Flawil, Switzerland).

2.3 Spent Coffee Extracts Preparation. Three procedures were used to prepare spent coffee extracts: solid-liquid extraction, filter coffeemaker and Soxhlet extractor. Spent coffee extracts were prepared from 24 g of spent coffee for a volume of 400 mL in all cases. For solid-liquid extraction, spent coffee was mixed with water at 80 ºC for 10 min, cooled in an ice bath for 10 min and filtered through Whatman No. 1 filter paper. For extraction with filter coffeemaker, spent coffee was extracted with water in a filter coffeemaker (model AVANTIS 70 Inox, Ufesa, Spain) for approximately 6 min at 90 ºC. And for Soxhlet extraction, spent coffee was extracted with water at 100ºC, boiling for 15 min followed by reflux for 45 min (1 h of total extraction time) and for 165 min (3 h) in a SOXTEST SX-6 MP, Raypa (Terrassa, Spain).

For the selection of solvent experiment, 400 mL of water, four different water:ethanol mixtures (80:20, 60:40, 40:60, 20:80), pure ethanol, two different water:methanol mixtures (30:70, 70:30), and pure methanol were used to extract 24 g of spent coffee by means of a filter coffeemaker (model AVANTIS 70 Inox, Ufesa, Spain) for approximately 6 min at 90ºC.

For successive extractions study, 24 g of spent coffee was extracted five times with 400 mL of water each time in a filter coffeemaker (model AVANTIS 70 Inox, Ufesa, Spain) for approximately 6 min at 90ºC.

For the extraction with aqueous solutions at different pH, 400 mL of an acid solution (pH 4.5), water (pH 7), and alkaline solution (pH 9.5) were used to extract 24 g of spent coffee by means of a filter coffeemaker (model AVANTIS 70 Inox, Ufesa, Spain) for approximately 6 min at 90ºC. Acid solution (pH 4.5) was prepared with 36.03 mg of lactic acid and 6.99 mg Sodium Hydroxide. Alkaline solution (pH 9.5) was prepared with 1.68 g Sodium Bicarbonate and 3.77 g of Sodium Carbonate.

For the last experiment, aqueous extracts from undefatted and defatted spent coffee were lyophilized using a CRYODOS Telstar (Terrassa, Spain).

2.4 Total Phenolic Compounds. Total phenolic compounds were measured using the Folin-Ciocalteau reagent according to the Singleton´s method (Singleton & Rossi, 1965) and then calculated using Gallic Acid (GA) as standard. For every spent coffee extracts, 3:10 dilutions with demineralized water were prepared. A volume of 500 µL of Folin-Ciocalteau reagent was added to a mixture of 100 µL of the extract sample and 7.9 mL of demineralized water. After a 2 min delay, 1.5 mL of a 7.5% sodium carbonate solution was added. Next, the sample was incubated in darkness at room temperature for 90 min. The absorbance of the sample was measured at 765 nm in a spectrophotometer Lambda 25 UV/VIS (Perkin Elmer Instruments, Madrid, Spain). Gallic Acid was used

as reference, and the results were expressed as milligrams of GA per gram of spent coffee dry matter (mg GA / g dm)

2.5 Antioxidant Capacity by ABTS Assay. The ABTS antioxidant capacity was performed according to the method of Re et al. (1999). The radicals $ABTS^{\cdot+}$ were generated by the addition of 0.36 mM potassium persulfate to a 0.9 mM ABTS solution prepared in phosphate buffered saline (PBS) (pH 7.4), and the $ABTS^{\cdot+}$ solution was stored in darkness for 12 h. The $ABTS^{\cdot+}$ solution was adjusted with PBS to an absorbance of 0.700 (± 0.020) at 734 nm in a 3 mL capacity cuvette (1 cm length) at 25ºC (Lambda 25 UV-VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain). An aliquot of 100 µL of each spent coffee extract sample diluted with demineralized water (3:100) was added to 2 mL of $ABTS^{\cdot+}$ solution. The absorbance was measured spectrophotometrically at 734 nm after exactly 18 min. Calibration was performed with Trolox solution (a water-soluble vitamin E analogue), and the antioxidant capacity was expressed as micromoles of Trolox per gram of spent coffee dry matter (µmol Trolox / g dm).

2.6 Antioxidant Capacity by DPPH Assay. The antioxidant capacity was also measured using 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) decolorization assay (Brand-Williams, Cuvelier, & Berset, 1995). A 6.1 x 10^{-5} M. $DPPH^{\cdot}$ methanolic solution was prepared immediately before use. The $DPPH^{\cdot}$ solution was adjusted with methanol to an absorbance of 0.700 (± 0.020) at 515 nm in a 3 mL capacity cuvette (1 cm length) at 25 ºC (Lambda 25 UV-VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain). Spent coffee extracts were diluted 3:10 in demineralized water prior to analysis. Samples (50 µL) were added to 1.95 mL of the $DPPH^{\cdot}$ solution. After mixing, the absorbance was measured at 515 nm after exactly 1 min and then every minute for 18 min. Calibration was performed with Trolox solution (a water-soluble vitamin E

analogue). The antioxidant capacity was expressed as micromoles of Trolox per gram of spent coffee dry matter (μmol Trolox / g dm).

2.7 Browned Compounds (Abs 420 nm). Fifty microliters of spent coffee extracts was diluted up to 2 mL with demineralized water. Browned Compounds were measured by the absorbance of samples at 420 nm, after exactly 2 minutes in a 3 mL capacity cuvette (1 cm length) with a Lambda 25 UV-vis spectrophotometer (Perkin-Elmer Instruments, Madrid, Spain) connected to a thermostatically controlled chamber (25 ºC) and equipped with UV WinLab software (Perkin Elmer). This measurement was employed as a convenient index of the development of caramelization and Maillard reactions (MRs) (Meydav, Saguy & Kopelman, 1977).

2.8 Statistical analysis. Each parameter was analyzed in triplicate. Results are shown as means ±standard deviations. A one-way ANOVA was applied for each parameter in each study. A T-Tukey test was applied as a test a posteriori with a level of significance of 95%. All statistical analyses were performed using the SPSS v.15.0 software package.

3. RESULTS AND DISCUSSION

3.1 Influence of extraction system. Three different extraction methods were selected to prepare spent coffee extracts: solid-liquid extraction, filter coffeemaker and soxhlet extraction system. The first, solid-liquid extraction, is frequently used by several authors to obtain concentrated coffee extracts (Anese & Nicoli, 2003; Budryn et al., 2009; del Castillo, Ames, & Gordon, 2002; López-Galilea, Andueza, di Leonardo, de Peña, & Cid, 2006). The filter coffeemaker is the most commonly used brewing procedure and one of the most efficient method to extract the antioxidant activity of ground roasted coffee (Parras, Martínez-Tomé, Jiménez, & Murcia, 2007; Pérez-

Martínez et al., 2010; Sánchez-González, Jiménez-Escrig, & Saura-Calixto, 2005). Soxhlet system is frequently used as a continuous method to extract fat or lipophilic compounds, including antioxidants, with organic solvents in several food systems (Chung, Ji, Canning, Sun, & Zhou, 2010; Yu, Haley, Perret, Harris, & Wilson, 2002), but rarely used to prepare extracts from coffee (Ramalakshmi, Rahath Kubra, & Jagan Mohan Rao, 2008). In this work, for soxhlet extraction technique two extraction times (1 and 3 hours) were applied in order to assure a high antioxidant activity extraction and to know the efficiency of this extraction method. In all extraction methods, spent coffee obtained by filter coffeemaker from Guatemala Arabica coffee was extracted with water at identical proportion of spent coffee:water (1:17) to reduce the variability induced by the coffee/water ratio (Andueza, Maeztu, Pascual, Ibanez, de Peña, & Cid, 2003; López-Galilea, de Peña, & Cid, 2007).

The antioxidant capacity of the different spent coffee extracts analyzed by colorimetric assays (Folin-Ciocalteau, ABTS and DPPH) and the amount of browned compounds (Abs 420 nm) are shown in Table 1. Total phenolic compounds of spent coffee extracts were in the range of 10.20-17.44 mg GA per gram dm. With the exception of solid-liquid extraction, which showed significantly ($p<0.05$) higher amount of phenolic compounds, similar results were reported by other authors (Pushpa & Madhava Naidu, 2010; Ramalakshmi et al., 2009). The total phenolic compounds amount of spent coffee extract obtained by solid-liquid extraction, was followed by the extracts obtained using filter coffeemaker and soxhlet during 3 h with no significant differences ($p>0.05$) between them. Solid-liquid was also the most efficient in browned compounds extraction measured by the absorbance at 420nm. Browned compounds include those originated from Maillard reactions, such as melanoidins, during coffee roasting process. These compounds might contribute to the highest total phenolic compounds result

because it has been demonstrated that Folin-Ciocalteau assay evaluates not only the phenolic compounds but also the reducing or antioxidant capacity of other nonphenolic chemical compounds, such as Maillard reaction products (Pérez-Martínez et al., 2010). Browned compounds were in the range of 0.090-0.160 Abs 420 nm showing 2 to 3-fold lower values than filter coffee brews (López-Galilea et al., 2007).

The antioxidant capacity was also measured by chain-breaking activity that allows evaluation of the quenching rate of coffee compounds toward two reference radicals 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). The decay of the radicals caused by the presence of antioxidants in a sample is monitored by the decolourization at 734 nm and 515 nm, respectively, in a spectrophotometer and compared to those of a Trolox solution. The results of ABTS assay were in the range of 92.98-140.30 μmol Trolox per gram dm and those of the DPPH assay were in the range of 38.65-82.40 μmol Trolox per gram dm. In both parameters, spent coffee extracts obtained with filter coffeemaker showed the highest values of radical scavenging activity followed by those prepared with the solid-liquid extraction system, and the soxhlet extractor at 3h and at 1h. Although, these results were 2.5 to 3.8-fold lower than those of filter coffee brews obtained with the same coffee/water ratio (Pérez-Martínez et al., 2010), they showed that free radical scavenging compounds still remain in spent coffee and they may be extracted.

In conclusion, the use of a filter coffeemaker can be proposed as the most efficient method to extract the antioxidants of spent coffee because it showed the highest values in both radical scavenging assays, and the second highest in total phenolic compounds. Furthermore, the extraction time with the filter coffeemaker was the shortest, approximately 6 min, in comparison to 10 min for the solid-liquid system and 1h and 3h for the Soxhlet extractor. Consequently, the filter coffeemaker was selected as the most

efficient and fast extraction method for obtaining spent coffee extracts in subsequent experiments.

3.2 Influence of solvents. The antioxidant capacity of spent coffee extracts prepared with different solvents (water, ethanol, methanol and their mixtures) was analyzed by colorimetric assays (Folin-Ciocalteau, ABTS and DPPH) (Table 2). Polar solvents were chosen because other authors (Ramalakshmi et al., 2008) found that phenolic compounds are extracted in increasing amounts with solvent polarity in green coffee beans extracts.

As shown in Table 2, the results were in the range of 2.65-17.48 mg GA, 41.87-152.64 μmol Trolox and 5.02-73.85 μmol Trolox per gram dm, for total phenolic compounds, ABTS and DPPH, respectively. The spent coffee extract obtained with pure ethanol showed significantly ($p<0.05$) lower results of antioxidant activity in comparison with pure water and water:ethanol mixtures. This could be because ethanol precipitates high molecular weight melanoidins (Bekedam, Schols, Van Boekel, & Smit, 2006) and then these compounds, together with the phenolics binding by them, could not be extracted. Thus, it could be said that water is necessary to extract more phenolic and nonphenolic antioxidants from spent coffee. The highest values of antioxidant capacity measured by total phenolic compounds and ABTS were found in the spent coffee extract obtained with 40:60 water:ethanol and 80:20 water:ethanol mixtures, whereas for DPPH the highest results were found in the extracts with pure water. In Table 2, it can be also observed that methanol decreased the extraction of radical scavenging compounds from spent coffee in comparison with pure water spent coffee extracts, showing that water is crucial for antioxidants extraction. Also Bekedam, Roos, Schols, Van Boekel, & Smit (2008a) observed that water, in methanol:water mixtures, is necessary to extract low molecular weight melanoidins. These melanoidins

have higher phenolic compounds linked to their core than high molecular weight melanoidins showing a higher contribution to the antioxidant capacity of coffee extracts (Bekedam, Schols, Van Boekel, & Smit, 2008b; Delgado-Andrade, Rufían-Henares, & Morales, 2005).

All these results agree with those observed by other authors who found the highest antioxidant yields in extracts obtained with water from roasted coffee residues (Yen et al., 2005) or with the highest amount of water in alcoholic mixtures from green coffee (Madhava Naidu, Sulochanamma, Sampathu, & Srinivas, 2008). Moreover, both chlorogenic acids and melanoidins were found to be in lower amounts in ethanolic extracts than in water extracts from green and roasted coffee showing that these antioxidants were better soluble in water (Budryn et al., 2009).

In conclusion, water was selected as the solvent to be used in subsequent studies mainly because its high efficiency to extract antioxidants. In addition, water extracts had higher yields (90% v/v) than ethanolic and methanolic extracts (65% v/v). Moreover, the use of water is more convenient than alcohols from safety and toxicological points of view, both in the laboratory and in the food industry in order to obtain antioxidant spent coffee extracts and to use them as a food natural additive or ingredient.

3.3 Influence of successive extractions. After analyzing and checking the antioxidant capacity of the first extraction of spent coffee, the next step in this study was to prove if remaining by-products still had antioxidants that may be extracted in significant amounts. For this study, spent coffee (24g) was extracted five times with water (400 mL) in a filter coffeemaker for approximately 6 min at 90ºC. In each extract, the antioxidant capacity measured by colorimetric assays (Folin-Ciocalteau, ABTS and DPPH), and browned compounds (Abs 420nm) were analyzed. Figure 1 and Figure 2 show the results of total phenolic (Figure 1a) and browned compounds (Figure 1b), and

the radical scavenging antioxidant capacity by ABTS (Figure 2a) and DPPH (Figure 2b) assays in the five spent coffee water extracts. In all parameters, the results of the first extraction were considerably higher than the others. Moreover, after the second water extraction of spent coffee only traces values of antioxidant capacity and browned compounds were observed. In fact, phenolic compounds in the second water extraction only accounts for 15% of those found in the first spent coffee extraction. Budryn et al. (2009) also observed that chlorogenic acids were in very low amounts in ethanolic extracts from spent coffee, maybe because spent coffee was previously washed three times with water, but also because ethanol is not a good solvent to extract antioxidants as it was discussed before. In ABTS and DPPH assays, the first extraction showed values of 140.30 and 82.40μmol Trolox per gram dm, whereas the antioxidant capacity of the next extracts was less than 14% and 11%, respectively. However, browned compounds of the second water extraction of spent coffee were found in greater proportion (33%) in comparison to the other parameters. This could be due both (1) because contribution of the roasting-induced antioxidant to the overall antioxidant capacity of coffee extracts is rather limited (Bekedam et al., 2008a; Pérez-Martínez et al., 2010) and (2) because melanoidins with higher antioxidant activity or with higher phenolics binding capacity, like low molecular weight melanidins(Bekedam et al., 2008b; Delgado-Andrade et al., 2005), were extracted better in the previous water extracts (coffee brew and first spent coffee extraction). As far as our knowledge, there are not any other works on coffee that obtain successive extractions and study them individually. In other papers, only one extract was obtained (Ramalakshmi et al., 2009; Yen et al., 2005), or all extracts were merged into one for analysis (Madhava Naidu et al., 2008).

According to all these results, only the first extraction was selected as the most efficient to extract the antioxidants from spent coffee. Moreover, it should be taking into account that the addition of the second or further water extracts to the first one would dilute the antioxidants. In this case, to obtain a powder that could be used as food ingredient or additive, more intensive conditions of dehydratation should be needed. Therefore, the next experiments were performed only with the first water extraction.

3.4 Influence of water pH. Acid water is normally used to extract the phenolic acids in coffee in order to be analyzed (Maeztu, Andueza, Ibañez, de Peña, Bello, & Cid, 2001; Pérez-Martínez, Sopelana, de Peña, & Cid, 2008). For this reason, the use of an acid water pH could be proposed as a good strategy to obtain spent coffee extracts with higher antioxidant capacity. So that, at the present study, the influence of the water pH on the antioxidant capacity of spent coffee water extracts has been studied. Three spent coffee extracts with acid water (pH 4.5), neutral water (pH 7.0) and alkaline water (pH 9.5) were obtained following the same procedure described before. The pH selection was based on the values established by the Council Directive 98/83/EC for water for human consumption that must be from 6.5 to 9.5. However, for still water put into bottles or containers, the minimum value may be reduced to 4.5 pH units (The Council of the European Union, 1998).

Table 3 shows the results of the antioxidant capacity (total phenolic compounds, ABTS and DPPH tests) and browned compounds (Abs 420nm) in spent coffee extracts obtained with different water pH. The increase of the pH in water used to obtain spent coffee extracts increased antioxidant capacity and browned compounds. In fact, spent coffee extract obtained with alkaline water (pH 9.5) had the highest antioxidant capacity followed by samples extracted with neutral (pH 7.0) and acid water (pH 4.5) in Folin-Ciocalteau and ABTS assays. However, spent coffee extract obtained with neutral water

(pH 7.0) was the most antioxidant extract in the DPPH assay. The absorbance at 420 nm of spent coffee extracts obtained with alkaline water (pH 9.5) was extremely high giving a very dark brown colour to the extract, and showing higher extraction of Maillard reaction products, such as melanoidins. Moreover, the unpleasant aroma of this extract clearly indicates that there were some chemical reactions that induced the formation or extraction of chemical compounds uncommonly present in coffee brews and extracts. These results disagree with the findings in previous studies, which showed that acid pH (below 5) extracted the highest amounts of phenolic antioxidants in green tea (Zimmermann & Gleichenhagen, 2011). Nevertheless, in other study developed by Pérez-Martínez et al. (2010), an increase in the antioxidant capacity of coffee brew was found when there was a limited increase in the pH (5.2 to 5.4) due to the addition of pH-regulator agents to extent coffee brew self-life. These discrepancies might be explained by the fact that the antioxidant capacity of coffee, and consequently spent coffee, is due to both phenolics and Maillard reaction products. For that reason, even acid water may facilitate phenolics extraction, the extraction of browning compounds (Maillard reaction products and others) seems to be increased with higher pH. In conclusion, the water pH selected for extraction of antioxidants from spent coffee was neutral (7.0).

3.5 Influence of defatting and lyophilization. Defatting process with organic solvents is normally used to remove fatty compounds in coffee before extraction of antioxidants or other coffee compounds, such as melanoidins, (Bekedam et al., 2008a; Nunes & Coimbra, 2007; Ramalakshmi et al., 2009; Rufian-Henares & Morales, 2008). However, this process might influence on the antioxidant capacity of remained coffee samples because some organic antioxidants can be also removed. On the other hand, defatting process can also prevent fat rancidity and radicals formation during long storage of spent coffee extracts. The lyophilization not only avoids the microorganism growth

extending self-life, but also facilitates the handling of spent coffee extracts in powder for further applications, for instance as an ingredient or an additive in food industry. However, as far as our knowledge, the influence of both processes (defatting and lyofilization) on the antioxidant capacity of spent coffee extract has not been studied before.

In this work, the last step for selection and optimization of the most efficient extraction method of antioxidants from spent coffee was to study the influence of defatting process and lyophilization on the antioxidant capacity of spent coffee water extracts. Three different extracts were prepared: defatted with petroleum ether using a Soxhlet extractor for 3h, lyophilized, and defatted and lyophilized spent coffee water extracts. The total phenolic compounds, ABTS, DPPH and browned compounds were analyzed in these extracts and the results were compared to a control spent coffee extract prepared as described before. The results of Table 4 show that the antioxidant capacity of spent coffee extracts was not negatively affected with deffating and lyophilization. In fact, a significantly ($p<0.05$) increase in all the parameters was observed when spent coffee was defatted and the extracts were lyophilized. Thus, both processes can be proposed as good strategies to preserve the antioxidant capacity of spent coffee water extracts. Focused on radical scavenging activity of spent coffee extracts, it could be said that defatting process influenced more than lyophilization. Actually, in DPPH assay only significant differences ($p<0.05$) were found between defatted and undefatted samples, and also results in ABTS for defatted extracts were higher than those of the undefatted ones. Thus, the removal of fat could facilitate the water soluble antioxidants extraction.

4. CONCLUSIONS

In conclusion, the results achieved in this study indicate that filter coffeemaker is the most efficient extraction system for obtaining spent coffee extracts with high antioxidant capacity. Among different solvent mixtures (water, ethanol and methanol), water is showed as the most convenient solvent in order to obtain high antioxidant capacity extracts from spent coffee. Moreover, only the first extraction with water is enough to extract a significantly high amount of antioxidants from spent coffee. The variation of the extraction water pH showed that alkaline water (pH 9.5) extracted the highest antioxidant capacity, nevertheless the changes of the organoleptic characteristics lead to the selection of water with neutral pH (7.0) as the best option. Finally, the application of defatting and lyophilization processes had the ability to increase the antioxidant capacity of spent coffee extracts, being an added value to the samples preservation and allowed us to obtain spent coffee extracts powder that can be used as an ingredient or additive in food industry with potential preservation and functional properties.

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Table 1. Antioxidant Capacity of spent coffee water extracts obtained by different extraction systems.

	Total phenolic compounds (mg GA / g dm)	ABTS (µmol Trolox/ g dm)	DPPH (µmol Trolox/ g dm)	Browned compounds (Abs 420nm)
Solid-liquid extraction	17.44 ± 0.26^{c}	128.33 ± 0.80^{c}	66.38 ± 0.57^{c}	0.160 ± 0.000^{d}
Filter coffeemaker	13.94 ± 0.88^{b}	140.30 ± 2.80^{d}	82.40 ± 2.86^{d}	0.100 ± 0.002^{b}
Soxhlet 1 h	10.20 ± 0.01^{a}	92.98 ± 2.45^{a}	38.65 ± 1.21^{a}	0.110 ± 0.000^{c}
Soxhlet 3 h	13.58 ± 0.23^{b}	110.35 ± 1.40^{b}	58.00 ± 0.43^{b}	0.090 ± 0.000^{a}

All values are shown as means ± standard deviations (n=3). In each column, different superscripts indicate significant differences ($p<0.05$) among extraction system procedures.

Table 2. Antioxidant Capacity of spent coffee extracts obtained with different solvents in filter coffeemaker.

Solvent	Ratio of Solvent (%)	Total phenolic compounds (mg GA / g dm)	ABTS (µmol Trolox/ g dm)	DPPH (µmol Trolox/ g dm)
Water	100	13.94 ± 0.88^{d}	140.30 ± 2.80^{e}	82.40 ± 2.86^{g}
Water:Ethanol	80:20	17.01 ± 0.09^{fg}	152.64 ± 3.58^{f}	61.99 ± 2.51^{d}
	60:40	13.48 ± 0.31^{d}	124.47 ± 2.12^{d}	69.08 ± 1.44^{ef}
	40:60	17.48 ± 0.21^{g}	151.72 ± 2.07^{f}	73.85 ± 0.77^{f}
	20:80	14.91 ± 0.67^{e}	135.19 ± 4.05^{e}	46.83 ± 1.78^{c}
	0:100	2.65 ± 0.10^{a}	15.31 ± 0.31^{a}	5.02 ± 0.11^{a}
Water:Methanol	70:30	16.03 ± 0.16^{f}	133.45 ± 1.03^{de}	67.32 ± 1.45^{de}
	30:70	12.20 ± 0.26^{c}	111.20 ± 0.48^{c}	35.45 ± 1.61^{b}
	0:100	7.37 ± 0.66^{b}	41.87 ± 8.82^{b}	29.70 ± 5.03^{b}

All values are shown as means ± standard deviations (n=3). In each column, different superscripts indicate significant differences ($p<0.05$) among spent coffee extracts.

Table 3. Antioxidant Capacity of spent coffee extracts obtained with different water pH in filter coffeemaker.

	Total phenolic compounds (mg GA / g dm)	ABTS (µmol Trolox/ g dm)	DPPH (µmol Trolox/ g dm)	Browned compounds (Abs 420nm)
pH 4.5	8.17 ± 0.07[a]	81.97 ± 0.63[a]	37.14 ± 2.06[a]	0.061 ± 0.001[a]
pH 7.0	13.94 ± 0.88[b]	140.30 ± 2.80[b]	82.40 ± 2.86[c]	0.100 ± 0.002[b]
pH 9.5	19.01 ± 0.04[c]	190.00 ± 4.05[c]	64.97 ± 3.85[b]	0.582 ± 0.003[c]

All values are shown as means ± standard deviations (n=3). In each column, different superscripts indicate significant differences ($p<0.05$) among spent coffee extracts.

Table 4. Antioxidant Capacity of spent coffee water extracts applying defatting and lyophilization processes.

	Total phenolic compounds (mg GA / g dm)	ABTS (µmol Trolox/ g dm)	DPPH (µmol Trolox/ g dm)	Browned compounds (Abs 420nm)
Control	13.94 ± 0.88[a]	140.31 ± 2.80[a]	82.40 ± 5.19[a]	0.100 ± 0.002[a]
Defatted	23.43 ± 0.06[b]	218.38 ± 0.55[c]	110.33 ± 1.97[b]	0.156 ± 0.003[c]
Lyophilized	23.74 ± 0.05[b]	205.06 ± 6.32[b]	88.55 ± 5.52[a]	0.139 ± 0.002[b]
Defatted-Lyophilized	24.60 ± 0.18[b]	215.12 ± 2.18[c]	112.06 ± 2.21[b]	0.132 ± 0.006[b]

All values are shown as means ± standard deviations (n=3). In each column, different superscripts indicate significant differences ($p<0.05$) among spent coffee extracts.

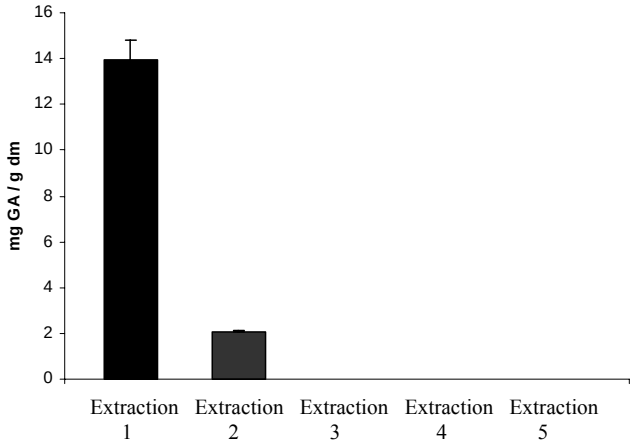
FIGURE CAPTIONS

Figure 1. Total phenolic compounds (a) and browned compounds (b) extracted from successive water extractions of spent coffee in filter coffeemaker.

Figure 2. ABTS antioxidant capacity (a) and DPPH antioxidant capacity (b) extracted from successive water extractions of spent coffee in filter coffeemaker.

Figure 1. Total phenolic compounds (a) and browned compounds (b) extracted from successive water extractions of spent coffee in filter coffeemaker.

a) Total phenolic compounds



b) Browned compounds

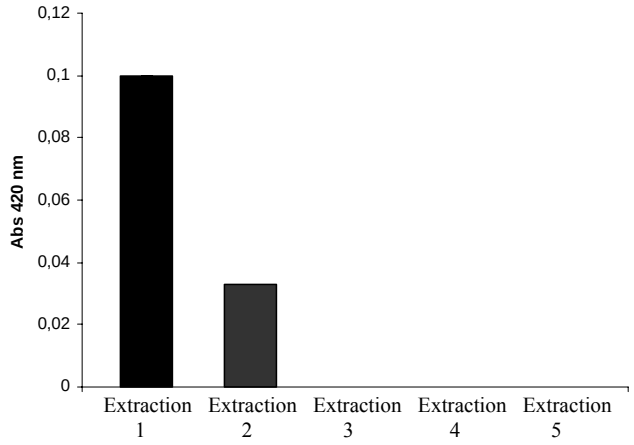
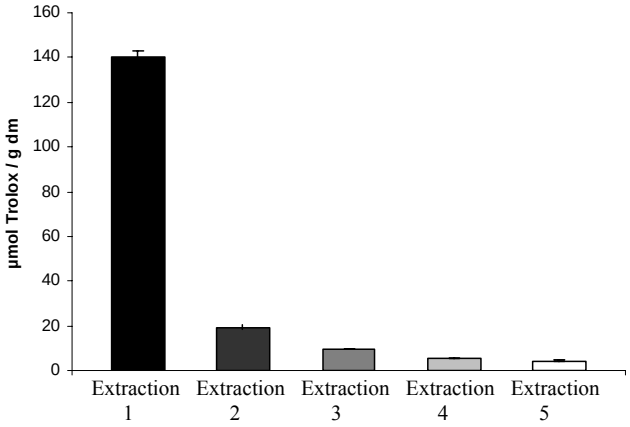


Figure 2. ABTS antioxidant capacity (a) and DPPH antioxidant capacity (b) extracted from successive water extractions of spent coffee in filter coffeemaker.

a) ABTS antioxidant capacity



b) DPPH antioxidant capacity