Influence of Extraction Conditions on the Recovery of Phenolic Antioxidants from Spent Coffee Grounds

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ABSTRACT

The extraction of phenolic compounds from Spent Coffee Grounds (SCG), a waste produced in large amounts worldwide, was investigated. SCG were rich in fiber and protein and had a total phenolic content of 18.75±0.43 mg GAE per gram dry weight. Extraction experiments were carried out in batch mode, using ethanol–water mixtures as solvent. A two-level factorial design was used to study the effects of temperature (T = 20-60°C), extraction time (E = 30-80 min) and liquid-to-solid ratio (R = 10-50 mL g^{-1}) on the recovery of phenolic compounds. Under the best conditions (T = 60°C, E = 180 min, R = 50 mL g^{-1}), over 90% of the phenolics present in SCG were recovered. T and R were the most influential factors and both had a positive effect on the extraction efficiency. The antioxidant activity of the extracts, determined by the DPPH method, was highly correlated with the amount of phenolics in each sample, suggesting that these compounds are the main responsible for the antioxidant properties of the extracts. Overall, the results of this study provide clear evidence of the potential of SCG as a source of natural antioxidants and give useful directions on how to improve their recovery by proper selection of extraction conditions.

Keywords: Spent Coffee Grounds, Extraction, Phenolic Compounds, Antioxidant Activity, Influential Factor Analysis

1. INTRODUCTION

According to data from the International Coffee Organization (ICO, 2012), about 100 million bags of coffee, corresponding to over 7 million tons of green coffee beans, are produced annually in the world. *Coffea arabica* (Arabica) and *Coffea canephora* var. *robusta* (Robusta) are the two main species of the genus Coffea that are cultivated for commercial production. Arabica is considered to be superior to Robusta because of its milder and more flavorful taste (Alonso-Salces *et al*., 2009). It accounts for 70-75% of the world production and is mainly used for the preparation of espresso coffee, whereas Robusta is mostly used for the manufacturing of instant coffee (Wintgens, 2009).

The production of coffee beverages or instant coffee leads to the generation a solid residue known as Spent Coffee Grounds (SCG). SCG have no commercial value and are currently disposed of as a solid waste or, to a limited extent, used as fertilizers.

In recent years, the increasing demand for reducing the environmental impact of vegetable and food wastes has prompted the development of methods for their conversion into value-added products (Kosseva, 2009). The production of biofuels, such as ethanol and biodiesel (Singh *et al*., 2012), or the use as adsorbents for the removal of pollutants from wastewater (Lavecchia *et al*., 2010) are some of the proposed applications. Another promising, but still relatively unexplored, approach is the use of vegetable wastes as a raw material for the recovery of functional or healthpromoting compounds. Examples of this approach are the extraction of proanthocyanidins from grape seeds (Yilmaz *et al*., 2011) andthe recovery of

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lycopene from tomato processing waste (Zuorro and Lavecchia, 2010a; Zuorro *et al.*, 2011).

Coffee beans contain several classes of health-related chemicals, including phenolic compounds, melanoidins, diterpenes, xanthines and vitamin precursors (Mussatto *et al.*, 2011). Caffeine is the most studied coffee component because of its psychoactive and medicinal properties (Crozier *et al.*, 2012). Recently, however, coffee phenolics have attracted the greatest attention from the scientific and medical communities due to their strong antioxidant properties (Dai and Mumper, 2010). Their activity seems to be enhanced by the presenceof coffee melanoidins, which are thought to provide protection against free radical damage and reduce the risk of some degenerative diseases (Moreira *et al.*, 2012).

In a previous study (Zuorro and Lavecchia, 2012) we found that SCG collected from espresso machines or coffee capsules had a high content of total phenolics (about 20 mg GAE/g dry weight) and could be easily recovered by an aqueous extraction procedure. Chemical characterization of the extracts showed that chlorogenic acids and related compounds, particularly dichlorogenic acids and quinolactones, were the most abundant phenolic compounds (Panusa *et al.*, 2013). In view of these findings, the present study was undertaken to systematically investigate the influence of the main process parameters (solvent composition, temperature, extraction time and liquid-to-solid ratio) on the recovery of phenolic compounds. In addition, we were interested in understanding whether and how the extraction conditions could affect the antioxidant activity of the resulting extracts.

2. MATERIALS AND METHODS

2.1. Chemicals

Ethanol, hydrochloric acid, sodium acetate, sodium carbonate and aluminum chloride were purchased from Carlo Erba (Milano, Italy). Gallic acid (3,4,5trihydroxybenzoic acid), DPPH (2,2-diphenyl-1 picrylhydrazyl) and the Folin-Ciocalteu's phenol reagent were obtained from Sigma-Aldrich Co. (St. Louis, Mo, USA). All chemicals were of analytical grade, with purities greater than 99% and used without further purification.

2.2. Spent Coffee Grounds

SCG were collected on different days from coffee bars in the city of Rome. To prevent microbial growth, they were dried to 7-10% residual moisture and pooled together. Drying was carried out at 40°C for 12-15 h in an electric forced-air food dehydrator (Stöckli, Switzerland). The dried material was put in glass bottles and stored in the dark at room temperature until use.

SCG were analysed for moisture, fiber, protein, fat, sugar, wax and ash content using standard analytical methods. Prior to performing extraction, the phenolic content of the waste was also evaluated.

2.3. Analytical Methods

Moisture content was determined by an electronic moisture analyzer (model MAC 50/1, Radwag, Poland). To evaluate the initial phenolic content of coffee waste, a three-stage extraction procedure allowing an almost complete exhaustion of the solid was used (Zuorro and Lavecchia, 2012). Briefly, 0.2 g of SCG and appropriate amounts of solvent (20, 10 and 5 mL in and third stage, respectively) were poured into glass flasks thermostated at 60°C and stirred for 30 min. After this time, the liquid was recovered, filtered at 0.45 μm and assayed for total phenolics.

Total phenolics were determined by the Folin Ciocalteu's method. Five mL of 0.1 M HCl, 150 μL of Folin-Ciocalteu's reagent and 200 μL of the sample to be tested were poured into a graduated glass vial and an aqueous Na_2CO_3 solution (20% w/v) was added to a final volume of 10 mL. The vial was thoroughly shaken and kept at room temperature in the dark for 1 h. Then, the absorbance at 525 nm was measured with a colorimeter (HI83742, Hanna Instruments, Italy). The total phenolic content was expressed as gallic acid equivalents (mg GAE per g of dry solid), using a calibration curve obtained with gallic acid standards.

Antioxidant activity was evaluated by the DPPH assay. A DPPH stock solution at 1 g L^{-1} in methanol was first prepared and stored at -20°C. Just before performing the assay, a working DPPH solution at 0.05 g L^{-1} was made by diluting the stock solution with methanol. Fifty to 300 μL of the sample to be assayed were then poured into an optical glass cuvette and the working DPPH solution was added to a final volume of 4 mL. After 30-min incubation at room temperature in the dark, the absorbance at 517 nm was measured. The antioxidant activity was expressed as EC_{50} value, which represents the concentration (% v/v) of the sample in the cuvette necessary to decrease the initial DPPH concentration by 50%.

2.4. Extraction Procedure

Phenolic antioxidants were recovered from SCG by a batch extraction procedure using ethanol-water mixtures as extraction solvent. Appropriate amounts of SCG and the solvent were loaded into 50 or 100 mL screw-top pyrex flasks. The flasks were placed in a water bath thermostated at ±0.1°C and were magnetically stirred. At the desired time, a sample of the liquid was taken, passed through a 45-μm nylon filter and assayed for phenolic content and antioxidant activity.

A first series of experiments was performed to determine the optimal composition of the solvent mixture. They were carried out at 40°C with an extraction time of 105 min and a liquid-to-solid ratio of 30 mL g^{-1}. The mixture giving the highest yield was then used to assess the influence of the main process variables on the recovery of phenolic antioxidants.

To evaluate the amount of dry phenolic extracts obtainable from SCG, a mechanically stirred extractor with a working volume of 1.5 L was used. In these experiments, 20 g of SCG were contacted with 1 L of solvent at 60°C for 3 h. At the end of extraction, the solvent was evaporated in a Rotavapor R-215 (Büchi, Switzerland) and the solid residue analysed.

2.5. Influential Factor Analysis

A full-factorial design was used to evaluate the effects of the three main process parameters, Temperature (T), Extraction time (E) and liquid-to-solid Ratio (R), on the recovery of polyphenols from SCG. The levels of each factor were chosen so as to cover a range of values of practical interest (**Table 1**).

The test variables were coded to vary between -1 and +1 using the following Equations (1-3):

$$x_1 = \frac{T - 40}{20} \tag{1}$$

$$x_2 = \frac{E - 105}{75} \tag{2}$$

$$x_3 = \frac{R - 30}{20} \tag{3}$$

Four center-point ($x_1 = x_2 = x_3 = 0$) replicates were also added, for a total of $2^3 + 4 = 12$ runs (**Table 2**). The extraction yield of phenolic compounds (*y*) was used as the response variable. It was expressed as the percentage amount of extracted phenolics to the initial amount of phenolics in the waste.

Table 1. Factors and levels of the experimental design

Factor	Units	Level -1	Level 0	Level +1
Temperature (T)	°C	20	40	60
Extraction time (E)	min	30	105	180
Liquid-to-solid ratio (R)	mL g^{-1}	10	30	50

Table 2. Experimental design layout

Std order	x_1	x_2	x_3	y
1	-1	-1	-1	46.70
2	+1	-1	-1	62.89
3	-1	+1	-1	48.69
4	+1	+1	-1	64.65
5	-1	-1	+1	58.20
6	+1	-1	+1	86.53
7	-1	+1	+1	65.53
8	+1	+1	+1	90.35
9	0	0	0	71.87
10	0	0	0	72.39
11	0	0	0	73.04
12	0	0	0	74.45

To minimize the effect of variability in the response due to extraneous factors, the experiments were performed in a random order.

The analysis of results was carried out using the statistical software Minitab$^®$ (Minitab, 2009).

3. RESULTS

3.1. Characterization of SCG

The results of the physicochemical characterization of SCG are presented in **Table 3**. As can be seen, the waste contained significant amounts of fiber (17.8%, on a dry weight basis) and protein (10.6%), while fats (4.4%) and sugars (<1%) were comparatively lower. Waxes and ash were in the range of values found for similar plant waste materials (Zuorro and Lavecchia, 2010b).

The initial moisture content of SCG was 48.2±2.2 (% w/w) and the total phenolic content, expressed as gallic acid equivalents per gram dry weight, was 18.75±0.43 mg g^{-1}.

3.2. Phenolic Extraction and Influential Factor Analysis

Experiments aimed at determining the optimal composition of the ethanol-water mixture gave the results displayed in **Fig. 1**. The solvent composition had a strong influence on the extraction yield, which was maximum in mixtures containing 60% (v/v) ethanol. We also found that the position of the maximum was not appreciably affected by the extraction temperature and the liquid-to-solid ratio. Accordingly, the 60:40 ethanol water mixture was used in all subsequent experiments.
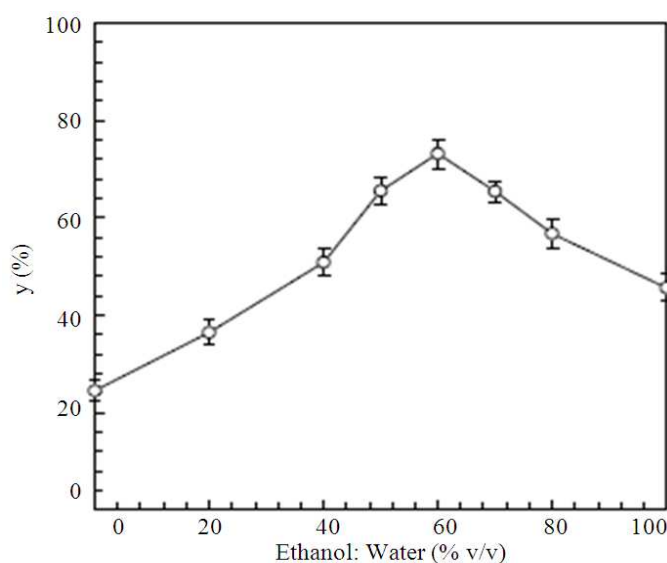
Fig. 1. Effect of solvent composition on the phenolic extraction yield (y)

Table 3. Physicochemical properties of SCG

Parameter	Method	Value	Unit
Crude fiber	ISTISAN 96/34 68	17.8±0.8	% w/w
Protein	ISTISAN 96/34 13	10.7±0.4	% w/w
Fats	ISTISAN 96/34 39	4.4±0.2	% w/w
Sugars	ISTISAN 96/34 45	<1	% w/w
Total waxes	GU CE L161 2007	115±10	mg kg^{-1}
Ash	ISTISAN 96/34 77	2.28±0.03	% w/w
Ash alkalinity	DM 03/02/89 II.14	293±10	mL HCl

The results of the experimental design are shown in **Table 2**. The extraction yields were between 46.7 and 90.35%, corresponding to, respectively, 8.76 and 16.94 mg GAE per g of dry SCG. The maximum yield was achieved at 60 °C with an extraction time of 180 min and a liquid-to-solid ratio of 50 mL g^{-1}.

To evaluate the contribution of the three main factors Temperature (T), Extraction time (E), liquid-to-solid Ratio (R) and their interactions to the extraction efficiency, the experimental data were correlated by the following Equation 4:

$$y = \alpha_0 + \sum_{i=1}^{3} \alpha_i x_i + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \alpha_{ij} x_i x_j + \alpha_{123} x_1 x_2 x_3 \tag{4}$$

where, a_i are the coefficients associated with the main effects, a_{ij} are those related to the binary interactions, a_{123} is the quaternary interaction coefficient, y is the response variable and the x's are the coded variables.

The polynomial model described by Equation 4 contains eight unknown coefficients, representing the contribution of each factor, alone or in combination, to the extraction yield. They were determined using the data of runs 1-8 in **Table 2**. Their values are summarized in **Table 4**. To assess the statistical significance of these coefficients we followed the procedure described in a previous paper (Zuorro *et al.*, 2013). In particular, the standard deviation of the experimental response was first estimated from the central points of the factorial design (runs 9-12 in **Table 2**). Then, the 95% confidence interval of each coefficient was determined by the Student's t-test and the coefficients with confidence intervals not spanning zero were considered statistically significant (p<0.05).

An examination of the results reported in **Table 4** reveals that five out of the eight coefficients were statistically significant. In addition to the intercept, a_0, they included the coefficients associated with the three main factors and the interaction between T and R.

The positive sign of T, R and E indicates that they had a positive effect on the extraction yield, with T and R exerting a stronger influence. The value and sign of the interaction coefficient suggest that an increase in temperature has a more pronounced effect on the recovery of phenolics at higher liquid-to-solid ratios. These considerations are well illustrated by the Pareto chart in **Fig. 2**, where the significant coefficients are marked with an asterisk.
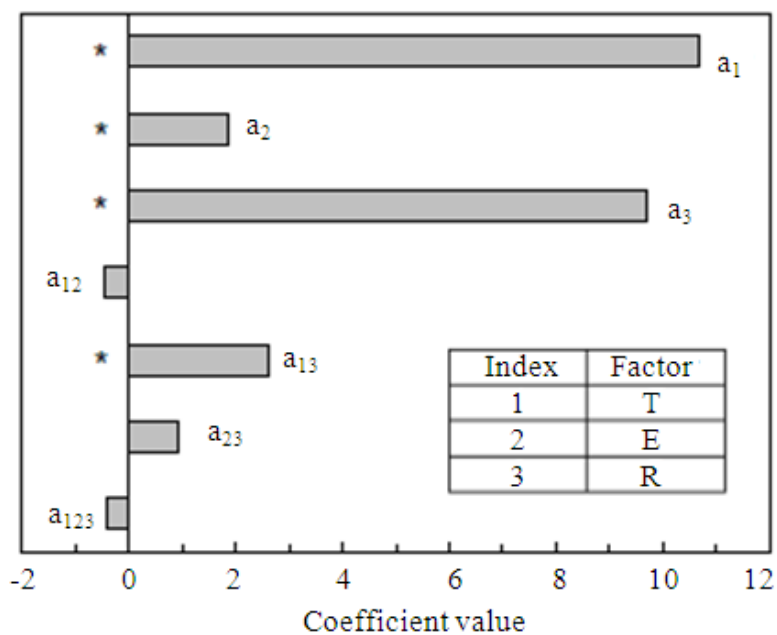
Fig. 2. Pareto chart showing the effects of the main factors and their interactions on the phenolic extraction yield. Significant coefficients are marked with an asterisk
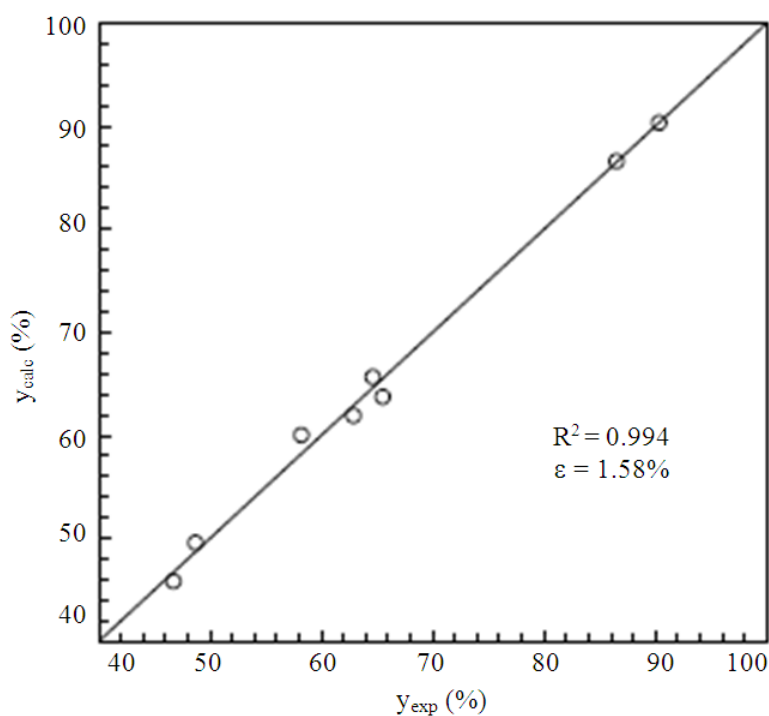

Fig. 3. Comparison between experimental and calculated (by Equation 5) extraction yields
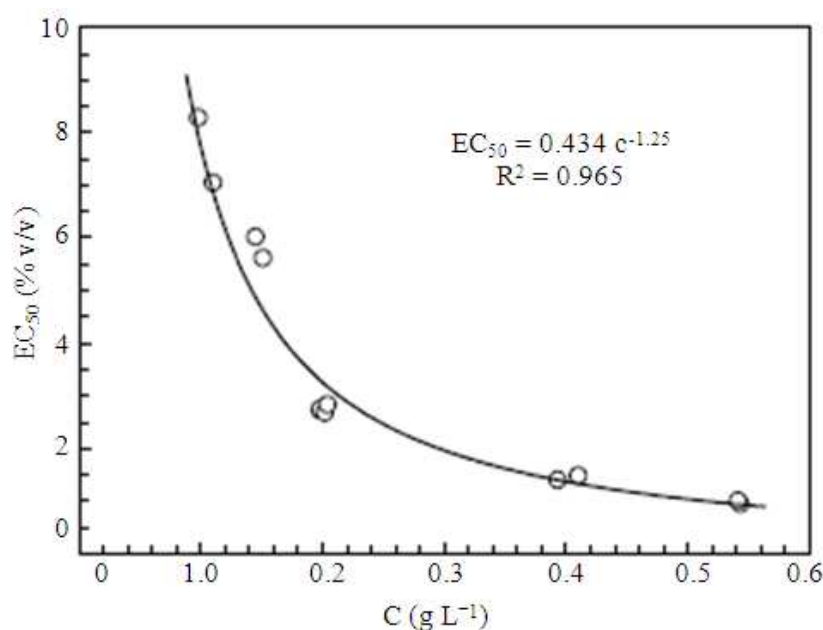
$$EC_{50} = 0.434 \, c^{-1.25}$$
$$R^2 = 0.965$$

Fig. 4. Observed dependence of antioxidant activity, expressed as EC_{50} values, on the total phenolic content (*c*) of the extracts

Table 4. Values and *t*-statistics for the coefficients in Equation 4. Significant values (p<0.05) are represented in bold

Coefficient	Effect	Value	*t-value*
a_0	-	65.443	165.835
a_1	T	10.663	27.019
a_2	E	1.863	4.720
a_3	R	9.710	24.606
a_{12}	T-E	-0.467	1.185
a_{13}	T-R	2.625	6.652
a_{23}	E-R	0.925	2.344
a_{123}	T-E-R	-0.410	1.039

By removing the non-significant terms from the full polynomial model, the following simplified expression was derived:

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{13} x_1 x_3 \qquad (5)$$

The five coefficients in Equation (5) were estimated from the experimental data by least-squares regression analysis. A very good agreement was found between experimental and calculated yields (**Fig. 3**), with an average error of 1.58%.

Finally, from the experiments performed in the mechanically-stirred extractor we determined the amount of dry phenolic extract obtainable from SCG (A) and its phenolic titer (TI), expressed in terms of gallic acid equivalents. Their values were: A = 173±10 mg g^{-1} (on a dry weight basis) and TI = 15±2 (% w/w).

3.3. Antioxidant Activity of the Phenolic Extracts

All the extracts obtained from SCG exhibited high antioxidant activity, with EC_{50} values ranging from 0.86 to 7.53 (% v/v). It should be remembered that the lower the EC_{50}, the higher the antioxidant activity.

Plotting the EC_{50} values of the extracts against the corresponding phenolic content (c) gave the results shown in **Fig. 4**. It can be seen that the two quantities were highly correlated, leading to the conclusion that phenolic compounds are the main responsible for theantioxidant activity of the extracts.

4. DISCUSSION

While the interest for the use of SCG as a source of health-promoting compounds has rapidly grown in the last few years, little attention has so far been given to the technological aspects of the recovery process (Mussatto *et al.*, 2011; Zuorro and Lavecchia, 2012). In this study we have investigated the influence of the main process parameters (temperature, extraction time and liquid-to-solid ratio) on the extraction of phenolic antioxidants from SCG using aqueous ethanol as the solvent. The waste

was collected from coffee bars in the city of Rome and used without any special treatments except drying.

The first point to note is the high phenolic content of the SCG examined: 18.75 mg GAE per gram dry weight. This value is in line with those reported in the few published studies on SCG (Ramalakshmi *et al*., 2009; Zuorro and Lavecchia, 2011; Murthy and Naidu, 2012; Bravo *et al*., 2013). A comparison with the values found for other agro-industrial wastes, such as grape pomace (Louli *et al*., 2004), carrot peel waste (Chantaro *et al*., 2008), kiwi and apple peel wastes (Wijngaard *et al*., 2009), shows that SCG have a higher amount of phenolic compounds. This provides further support to the idea of using SCG as a source of natural antioxidants.

As is evident from **Fig. 1**, the composition of the solvent had a marked effect on the extraction yield and the use of the 60:40 ethanol-water mixture gave better results compared to pure ethanol or pure water. Similar results were obtained in studies on the extraction of phenolics from different materials, such as peanut skins (Nepote *et al*., 2005), olive leaves (Mylonaki *et al*., 2008) and the byproducts of kiwifruit juicing (Sun-Waterhouse *et al*., 2009). One explanation may lie in the fact that phenolic compounds exhibit a wide range of solubilities in polar solvents and are often more soluble in solvents less polar than water (Liu *et al*., 2000). We can therefore assume that the 60:40 ethanol–water mixture allows higher amounts of phenolic compounds to be dissolved. Nevertheless, other solvent-related effects could also be involved, such as a weakening of the solute-matrix interactions or the improved swelling of the plant matrix (Lavecchia and Zuorro, 2008). Swelling results from the adsorption of solvent molecules on the hydroxyl and carboxyl groups of cellulose fibers. Following adsorption, the distances between the fibers increase, causing the expansion of the material and an increase of surface area. This favors solvent penetration and the release of soluble compounds into the liquid. It is known that swelling inducers are characterized by small molar volume, high hydrogen bonding capability and large basicity, as is the case of ethanol (El Seoud, 2009).

By varying the process conditions, significant changes were observed in both the extraction yield and the antioxidant activity of the extracts. The highest recovery (y = 90.35%) was achieved at T = 60°C, E = 180 min and R = 50 mL g^{-1}. Another point to be emphasized is the significant contribution of temperature and liquid-to-solid ratio to the extraction yield of phenolics. By contrast, the effect of extraction time was less pronounced. A possible explanation is that most of the phenolic compounds contained in SCG are extracted at short times, so that a prolonged extraction has only limited effect on their recovery. From a practical point of view, this means that the use of longer times to get a quantitative extraction is neither technically appropriate nor economically justified. The positive effects of temperature and liquid-to-solid ratio can be explained by considering that an increase in temperature favors the release of phenolic compounds from SCG and that higher liquid-to-solid ratios enhance the mass-transfer of the dissolved substances from the solid to the solvent (Cussler, 2009).

The simplified model described by Equation 5 accounts for all of the above-mentioned effects and can therefore be used for a preliminary assessment of the influence of process variables on the recovery of phenolics from SCG.

Plotting the EC$_{50}$ values of the extracts against the corresponding phenolic content (c) gave the results shown in **Fig. 4**. It can be seen that the two quantities were highly correlated, leading to the conclusion that phenolic compounds are the main responsible for the antioxidant activity of the extracts.

5. CONCLUSION

This study demonstrates that phenolic-rich extracts can be obtained from SCG using a simple and environmentally friendly solvent-extraction procedure. The exploitation of SCG for these purposes would not only lead to the production of new products of potential interest to the cosmetic, pharmaceutical and food industries but also contribute to reducing the amount of coffee waste to be disposed of.

We have also shown that the recovery of phenolic compounds from SCG can be significantly improved by proper selection of extraction conditions. In this regard, the influential factor analysis we performed provides useful directions for future research and practical applications.

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