Determination of extraction kinetics of bioactive compounds from spent coffee grounds (*Coffea arábica*)

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Abstract

The main objective of this work is to determine the extraction kinetics of bioactive compounds contained in spent coffee grounds (SCG) through conventional extraction (e.g. soxhlet extraction and ultrasonic assisted extraction). For this, it was analyzed the phenolic compounds and chlorogenic acid. The analyzed samples were taken at different time intervals during the extraction procedure and using ethanol 60%v/v as solvent. The importance of determining the extraction kinetics of a compound by implementing different extraction technologies was observed. At the same time, it was evidenced that the ultrasonic assisted extraction (UAE) presented the best concentrations for chlorogenic acid. Moreover, the UAE has a great advantage due to the low required time to extraction compared to soxhlet extraction.

Keywords: conventional extraction, extraction kinetics, spent coffee grounds, ultrasonic assisted extraction.

1. Introduction

According to The United States Department of Agriculture, coffee is one the most popular drinks in the world with an annual production of 156.6 million bags of 60 kilograms in 2016 [1]. Nevertheless, this product is responsible for the generation of large quantities of waste, as the coffee pot, grounds and mucilage [2]. Coffee grounds are the major residues of the soluble coffee industry, generating 6 million tons per year and causing economic and environmental issues when they are discharged to the surroundings or incinerated [3], [4]. For this reason, several authors have reported the use of the spent coffee grounds (SCG) to obtain different added-value products and thus, increasing the sustainability of the global coffee industry [5], [6]. Different studies have reported large quantities of polyphenolic components in the SCG with a high concentration of chlorogenic acid [2], [4]. There is several applications of these components in the food, pharmaceutics and cosmetic industry.

Polyphenolic compounds are part of secondary metabolites, being identified more than 8.000 compounds [7]. These components have great benefits for the human health, due to their anti-cancer, anti-diabetic, anti-microbial and anti-allergic potential [8], [9]. These compounds can be obtained through extraction methods. The most studied technologies are the solid-liquid and Soxhlet extraction. Nevertheless, faster extraction methods that

increase the selectivity with low operation costs are required. In this sense, non-conventional methods have begun to study as alternative extraction methods [10]. The ultrasonic assisted extraction (UAE) is a non-conventional technology with great potential for the extraction of polyphenolics compounds due to its simplicity, high-reproducibility, high-efficiency and low energy requirement [11]. UAE creates an acoustic cavitation effect in the dissolvent through an ultrasound wave. In addition, the ultrasound facilitates the diffusion of the dissolvent in the tissue of the residue, giving as a result, an increment in the contact area between the solid-liquid phases [13]. The improvement in the extraction obtained through ultrasonic is mainly attributed to the effect of acoustics cavitation produced in the dissolvent by the passage of an ultrasonic wave [14]. The ultrasonic method also generates a mechanic effect, allowing a better diffusion of the dissolvent in the tissue and raising the surface of contact between solid and liquid phases. As a result, the solute diffuses rapidly from the solid phase to the dissolvent. [12].

The determination of the extraction kinetic has high importance for the design of the used equipment for the extraction of polyphenolic compounds, allowing an optimization of the time, energy and cost of the process [15]. Additionally, the determination of the extraction kinetics provides a great knowledge about the extraction rate and analysis of the process rate through a kinetic study of the process. The aim of this work is to determine the kinetics of polyphenolics compounds through different extraction methods such as Soxhlet extraction and UAE, and to determinate the antioxidant capacity of SCG.

2. Methodology

2.1.Raw material and Reagents

As raw material was used spent coffee grounds. To develop the characterization it was employed sodium chlorite, sulfuric acid, sodium hydroxide, acetic acid 96% (MOL LABS), acetone (Panreac), ethanol 96% (Disproalquimicos) and distillated water. In the phenolic compounds determination was required sodium carbonate anhydrous (Panreac), gallic acid (Sigma-Aldrich), Folin-Ciocalteu reagent 2N (Sigma-Aldrich). For the chlorogenic acid determination was used the standard of this compound. Additional reagents like: methanol 99.8% (Panrec) and water were HPLC grade.

2.2.Procedure

2.2.1. Characterization of raw material

The physicochemical characterization of raw material was performed in triplicate. The moisture, extractives (NREL/TP-510-42619), ash (NREL/TP-510-42622), holocellulose (ASTM D1104), cellulose (T203 os-74 ASTM 1695-77) and lignin (T222) contents were determined according to international norms and methods [13][14][15].

2.3.2. Solvent extraction

The sample was disposed in a recipient with ethanol 60%v/v in a 20:1 solvent-solid ratio (%v/w). The procedure was carried out during 8 hours at constant temperature *(25 °C ± 2 °C).* Then, the liquid fraction was separated by filtration.

2.3.3. Soxhlet extraction

SCG (10 g) were disposed in a porous recipient (thimble) and placed in a Soxhlet extractor together with 250 ml of ethanol 60%v/v at constant reflux, allowing to flow through the thimble during 6 hours and filtering the bioactive compounds.

2.3.4. Ultrasonic assisted extraction

The sample was placed in a container with ethanol 60%v/v in the ultrasonic processor UP50H (Hielscher Ultrasound Technology). A solvent/solid ratio of 20:1 was used with a constant temperature (50 °C ± 2 °C) during 60 minutes. Ultrasonic power and frequency were set at 750 W and 20 kWh, respectively [19]. Finally, the liquid was separated by vacuum filtration and centrifugation (6000 rpm during 10 min) for the removal of suspended particles. The obtained extracts were disposed in a dark place with temperatures between 0 – 4°C.

2.4 Data Analysis

The concentration of polyphenolics compounds for each extraction process was determined by the approximation of the obtained results to regression models. The results were analyzed by the software CurveExpert Professional. In the software, different approximation methods were analyzed considering a confidence interval of 95% ($P < 0.05$) and obtaining three predictive models for the concentration of total polyphenolics compounds and chlorogenic acid. In each case, the generated influence in the use of different technologies was analyzed. In this case, three technologies were analyzed: Solvent extraction, Soxhlet extraction and Ultrasonic assisted extraction (UAE).

2.5 Estimation of Total Phenolic content (TPC)

The Folin-Ciocalteu method detects phenolic groups found in the extracts in the absence of light due to the reagents photosensibility. For this method, a calibration curve was prepared at different dilutions of the stock solution. 100 µL of the obtained extract was mixed with 1600 µL of Folin-Ciocalteu reagent 1N, shaking the formed solution and allowing to stand for 5 minutes. 200 µL of carbonate 20% (w/v) were added. The solution was mixed and allowed to stand in a dark place for 2 hours. The samples were submitted to a spectrophotometer and measured at 765 nm [21].

2.8 High Performance Liquid Chromatography

The HPLC system consist in a LC-2010A HT (SHIMADZU), with a liquid chromatograph, an UV-visible detector, a quaternary pump, a vacuum-degasifier and an automatic sampler. The chromatographic separation was performed in a column C18 with a size of 150 mm x 4.6 mm and a particle size of 5 µm. The chlorogenic acid separation required an elution gradient with acetic acid 0.5% (A) and methanol (B), a temperature of 25°C, a flux of 0.7 $mLmin^{-1}$, a wavelength of 310 nm for chlorogenic acid. The chlorogenic acid presented an elution profile in which the dissolvent B started with a concentration of 10% during 15 min and then, the concentration increased to 30% in 25 min [25].

Results and discussion

The physicochemical composition of the evaluated residue (Table 1), was performed by triplicate, in which it was evidenced a high extractives content, showing its high potential for the studied case. In addition, the holocellulose (cellulose and hemicellulose) content represented more than 50% of the total dry weight of SCG, showing a high potential to obtain sugars and added-value products such as ethanol, lactic acid, xylitol, among others [16][17][18][19]. The SCG composition have a prominent lignin content providing, in the same way, a potential for some process such as energy generation and production of artificial vanillin [20][21].

Table 1. Physicochemical composition of SCG.

	This work*	**Reference** [6]	**Reference**[2]
Extractives	25.64 ± 0.27	ND	---
Holocellulose	50.32 ± 2.03	---	---
Cellulose	21.48 ± 0.06	8.6 (Glucose)	12.40 ± 0.70 (Glucose)
Hemicellulose	28.84 ± 2.09	36.7	39.10 ± 1.90
Lignin	19.83 ± 0.76	ND	23.90 ± 1.70
Ash	0.94 ± 0.05	1.6	1.30± 0.10

*All the percentages are expressed by weight.

The extraction of polyphenolic compounds presents different yields depending of the used technology. In this sense, technologies such as solvent extraction, Soxhlet extraction and UAE are some of the available alternatives. **Figure 1** shows the total concentration of polyphenolics compounds as function of time. In the characterization of polyphenolic compounds by Folin-Ciocalteu method [21] [33], a calibration curve was done by triplicate, obtaining the tendency of the concentration of polyphenolics compounds using the gallic acid molecule. In the obtained results from the analyzed samples, it can be observed that UAE presents a less operation time and thus, provides the best yields in comparison with the other analyzed technologies. This technology can achieve a concentration of polyphenolic compounds of 650.45 mg/L in 60 min whereas the solvent and Soxhlet extraction present a concentration of 392.50 mg/L in 480 min and 559.09 mg/L in 290 min, respectively. It is noteworthy that the extraction has a direct influence in the required extraction time as evidenced in the UAE.

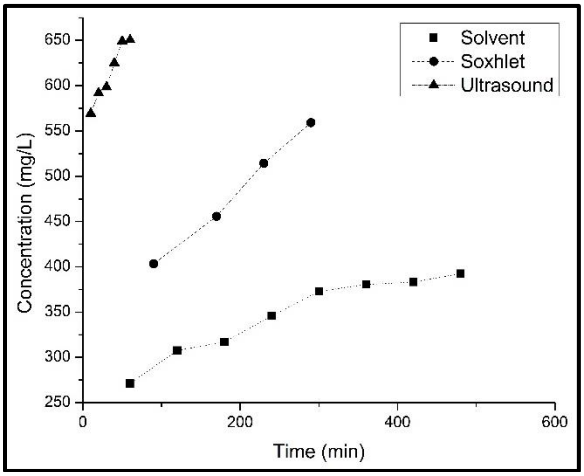

Figure 1. Polyphenolics compounds concentration vs time.

With the aim to analyze the yields that can be obtained, it is necessary to determine the extraction kinetics. **Equations 1-9** allow the determination of the content of total phenolics compounds, where "TPC" is the Total Polyphenol Content given in mg/L and "x" the time of the extraction process in minutes. Additionally, the ANOVA analysis was performed with the aim to validate the results from these equations. **Equations 1-3** present the Gaussian, Richards and Reciprocal Quadratic models for the solvent extraction, respectively. **Equations 4-6** show the Weibull, Richards and Exponential models for the Soxhlet extraction, respectively. **Equations 7-9** present the Richards, Weibull and Gaussian models for UAE, respectively. **Figure 2** illustrates the obtained adjustment for each model and implemented technology for the total polyphenolics compounds. Equations 1, 4 and 7 present the best adjustment with a regression coefficient of 0.9842, 0.9999 y 0.9825, respectively.

$$TPC = 390.74 * \exp\left(-\frac{(x - 478.66)^2}{2 * 494.07^2}\right) \qquad Eq.1$$

$$TPC = \frac{390.05}{(1 + \exp(6.56 - 0.02 * x))^{\frac{1}{15.22}}} \qquad Eq.2$$

$$TPC = \frac{1}{4.00 * 10^{-3} + 6.30 * 10^{-6} * x + 6.92 * 10^{-9} * x^2} \qquad Eq.3$$

$$TPC = 582.55 - 189.54 * \exp(-5.20 * 10^{-8} * x^{3.09}) \qquad Eq.4$$

$$TPC = \frac{559.31}{(1 + \exp(83.89 - 0.29 * x))^{\frac{1}{171.23}}} \qquad Eq.5$$

$$TPC = 346.80 * \exp(1.66 * 10^{-3} * x) \qquad Eq.6$$

$$TPC = \frac{650.45}{(1 + \exp(292.44 - 5.61 * x))^{\frac{1}{1{,}761.17}}} \qquad Eq.7$$

$$TPC = 657.59 - 86.90 * \exp(-6.42 * 10^{-5} * x^{2.62}) \qquad Eq.8$$

$$TPC = 702.73 * \exp\left(-\frac{(x - 128.40)^2}{2 * 182.02^2}\right) \qquad Eq.9$$

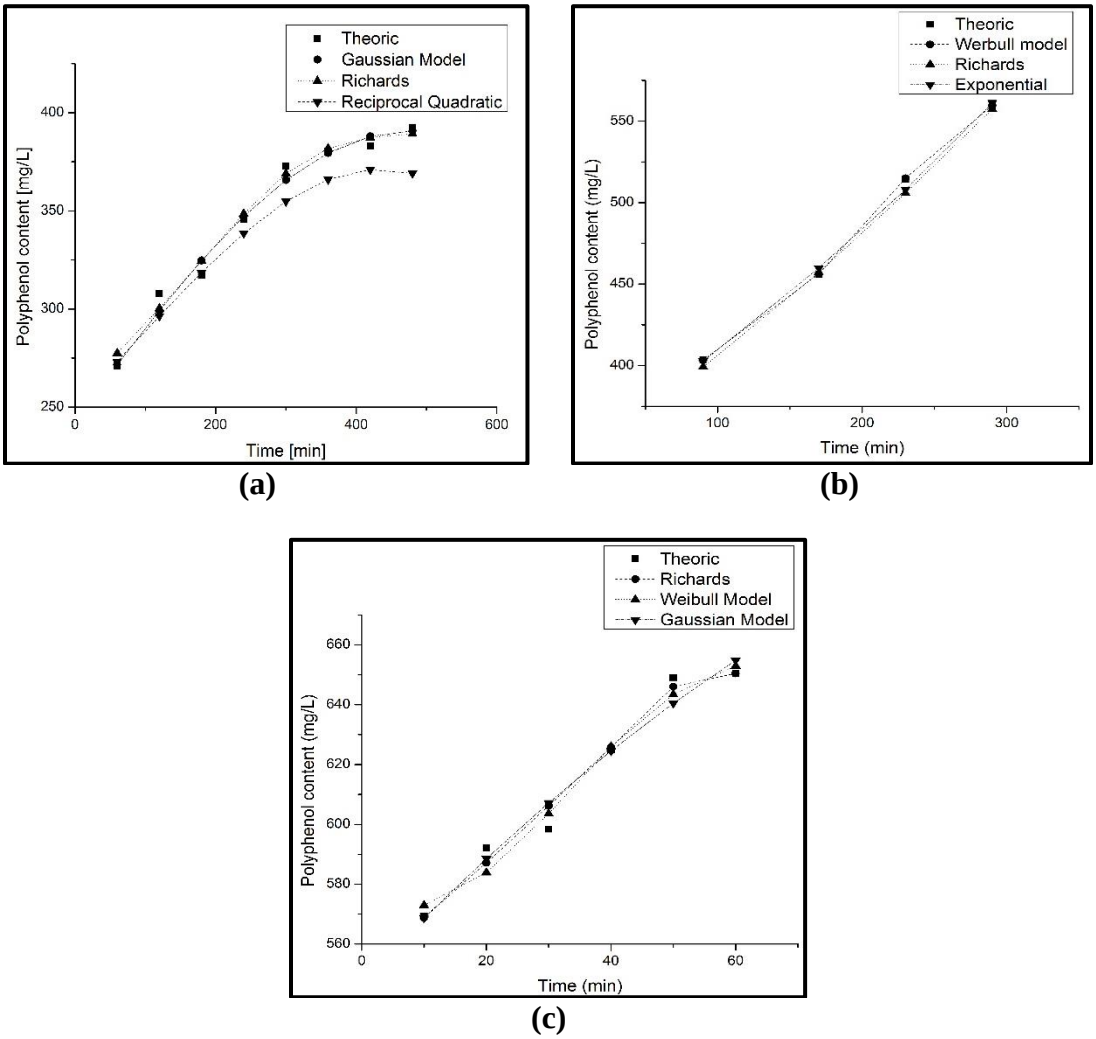

Figure 2. Model adjustment for the extraction of total polyphenolics compounds. **(a)** Solvent extraction. **(b)** Soxhlet extraction. **(c)** UAE

Among the phenolic compounds, it can be found the chlorogenic acid that can be extracted through the three mentioned technologies, showing best yields in the UAE. While the solvent extraction and Soxhlet extraction present similar values (24.87 y 25.22 mg/L,

respectively), a chlorogenic acid concentration of 46.53 mg/L through UAE can be obtained. This can be observed in Figures 3a, 3b and 3c, where "CAC" is the content of chlorogenic acid and "x" denotes time. In the same way, the predictive models with better adjustment were presented. For the solvent extraction, the better adjustment were Steinhart-Hart, Exponential Association 3 and Rational (**Equations 10 – 12**). For the Solvent extraction were the Weibull, Bleasdale and Steinhart-Hart models (**Equations 13 – 15**). For UAE, the better models were Heat Capacity, Shifted Power and Modified Hoerl (**Equations 16 – 18**), where the equations with better adjustment were **Equations 10, 13 y 16** with regression coefficients of 0.9975, 0.9973 y 0.9990, respectively.

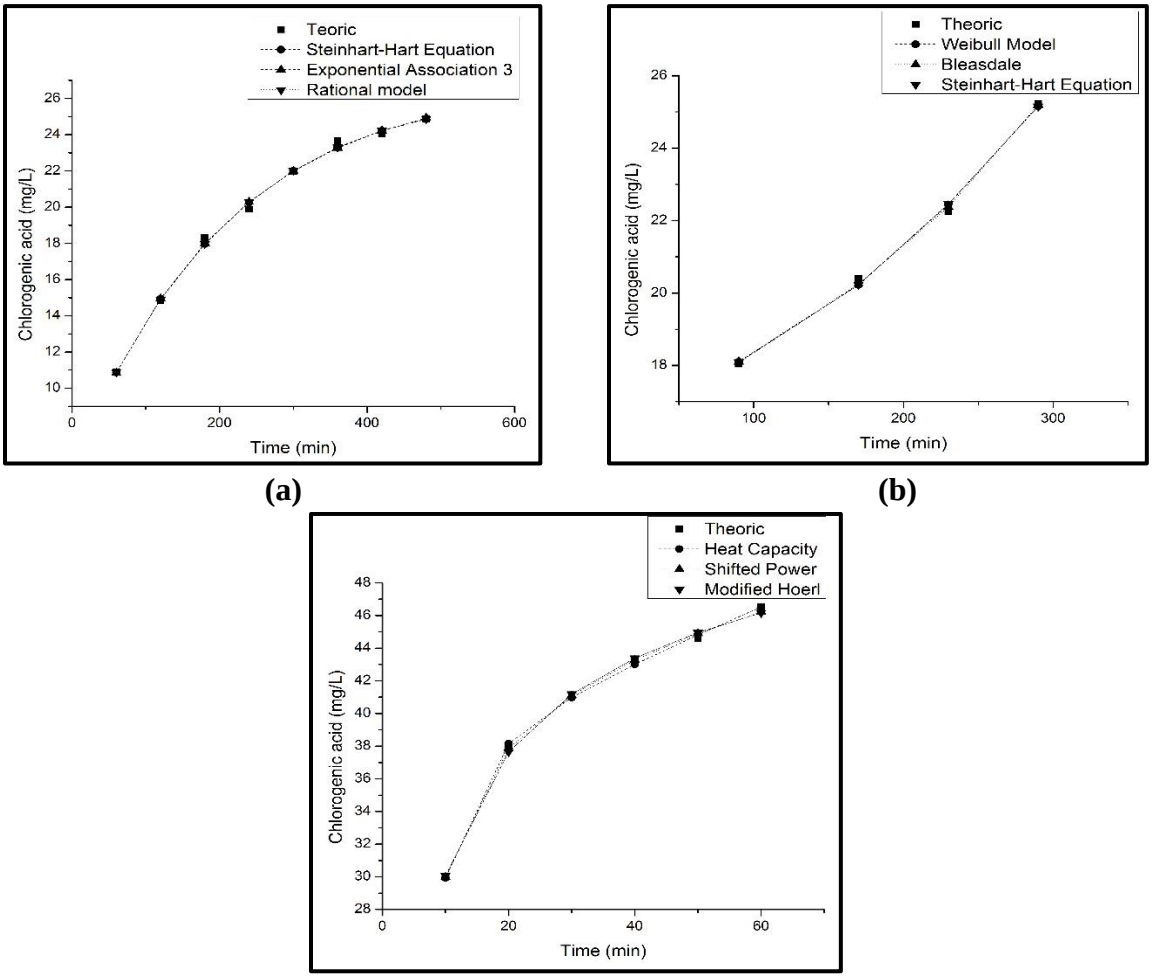

Figure 3. Model adjustment for the extraction of chlorogenic acid. **(a)** Solvent extraction. **(b)** Soxhlet extraction. **(c)** UAE

$$CAC = \frac{1}{0.33 - 6.80 * 10^{-2} * Ln(x) + 5.38 * 10^{-4} * Ln(x)^3} \qquad Eq.10$$

$$CAC = 21.65 * (1.25 - \exp(-4.86 * 10^{-3} * x) \qquad Eq.11$$

$$CAC = \frac{5.61 + 0.11 * x}{1 + 2.13 * 10^{-3} * x + 1.59 * 10^{-6} * x^2} \quad Eq.12$$

$$CAC = 243.85 - 226.74 * \exp(-1.33 * 10^{-6} * x^{1.80}) \quad Eq.13$$

$$CAC = (1.13 * 10^{-2} - 1.97 * 10^{-5} * x)^{-\frac{1}{1.60}} \quad Eq.14$$

$$CAC = \frac{1}{-1.37 * 10^{-2} + 2.54 * 10^{-2} * Ln(x) - 4.96 * 10^{-4} * Ln(x)^3} \quad Eq.15$$

$$CAC = 37.15 + 0.16 * x - \frac{882.04}{x^2} \quad Eq.16$$

$$CAC = 26.83 * (x - 7.76)^{0.14} \quad Eq.17$$

$$CAC = 34.57 * (3.54 * 10^{-2})^{\frac{1}{x}} * x^{8.42 * 10^{-2}} \quad Eq.18$$

3. Conclusions

The ultrasonic assisted extraction method, with ethanol 60%v/v as dissolvent, proved to be an effective alternative method for the extraction of polyphenolic compounds, resulting in concentrations of phenolic compounds. In addition, spent coffee grounds presented a high concentration of chlorogenic acid making these coffee residues an attractive raw material for the food and pharmaceutical industry.

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