

OPTIMISATION OF ULTRASOUND AND MICROWAVE-ASSISTED EXTRACTION OF CAFFEYLQUINIC ACIDS AND CAFFEINE FROM COFFEE SILVERSKIN USING RESPONSE SURFACE METHODOLOGY

A. GUGLIELMETTI, V. D'IGNOTI, D. GHIRARDELLO, S. BELVISO and G. ZEPPA*
DISAFA, Department of Agricultural, Forestry and Food Sciences, Università degli Studi di Torino, L. go P.
Braccini 2, 10095 Grugliasco, Italy
*Corresponding author. Tel.: +39 0116708705; fax: +39 0116708549
E-mail address: giuseppe.zeppa@unito.it

ABSTRACT

Ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) of caffeoylquinic acids and caffeine from coffee silverskin (CS) at two particle size were investigated using response surface methodology and compared to a conventional solvent extraction (CSE). The impact of time and temperature on the extraction process was evaluated and extraction efficiency was optimised by measuring total phenolic content (TPC), radical scavenging capacity (RSC), caffeoylquinic acids (TotCQAs) and caffeine content. UAE allowed to obtain extracts with values of TPC, RSC and TotCQAs higher or similar to CSE; moreover UAE produced the highest caffeine content (14.24 g kg⁻¹ dw) with a significant reduction of extraction time.

Keywords: caffeine, coffee silverskin, microwave-assisted extraction (MAE), polyphenolic compounds; ultrasound-assisted extraction (UAE), response surface methodology (RSM)

1. INTRODUCTION

Coffee is the second largest traded commodity in the world after petroleum with a production of 8.8 million tons in 2015 (INTERNATIONAL COFFEE ORGANIZATION, 2015). Since approximately 90% of the coffee cherry is discarded during the conversion of the cherry into coffee brew (DEL CASTILLO *et al.*, 2016), million tons of by-products are generated. Coffee silverskin (CS) is a thin tegument of the outer layer of the two beans forming the green coffee seed obtained as a by-product of the roasting process. Since CS represents about 4.2 % (w/w) of coffee beans (DEL CASTILLO *et al.*, 2016), hundreds of thousands tons of waste are produced generating a disposal cost for industry. In the last years CS has attracted great attention as an abundant source of bioactive compounds such as caffeoylquinic acids and caffeine (BRESCIANI *et al.*, 2014). In particular caffeoylquinic acids are phenolic compounds that belong to the family of chlorogenic acids; different studies have evaluated antifungal, antibacterial, anti-inflammatory, antioxidant, anti-glycative, anti-carcinogenic and neuroprotective properties of these compounds (DEL CASTILLO *et al.*, 2016). Considering caffeine, a moderate amount of this alkaloid increases energy availability, cognitive performance and neuromuscular coordination (GLADE, 2010). In order to optimise the extraction of bioactive compounds from CS, in the last years several extraction methods with different yield, complexity and cost, such as Soxhlet extraction, solid-state fermentation (SSF), subcritical water and solid-liquid extraction were applied (MURTHY and NAIDU, 2010; MACHADO *et al.*, 2012; NARITA and INOUE, 2012; BALLESTEROS *et al.*, 2014; COSTA *et al.*, 2014).

Among the extraction techniques, ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) are considered efficient for extracting analytes reducing extraction time and energy consumption. Regarding UAE, the responsive of ultrasonic effect are the cavitation bubbles that grow during rarefaction phases and decrease in size during compression cycles; when the size of the bubbles reaches a critical point, they collapse during a compression cycle and release large amounts of energy that destroys the cell walls of the plant matrix producing the discharge of cell content into the medium (CHEMAT *et al.*, 2011). UAE applied on spent coffee ground (SCG), the solid waste obtained after coffee brewing and having similar composition to CS, has been reported to be an efficient method to improve the extraction of antioxidant compounds (SEVERINI *et al.*, 2016).

MAE is an efficient method that has garnered increasing interest in various fields mainly due to its particular heating mechanism and its moderate capital cost (CHAN *et al.*, 2011); microwaves can penetrate into certain materials and interact with the polar components of the matrix to generate heat. MAE has been found to be an optimal extraction technique to recover caffeine from a plant matrix such as tea (WANG *et al.*, 2011).

An important role in the extraction of caffeoylquinic acids and caffeine from a plant matrix is represented by the particle size of the sample (PINELO *et al.*, 2007; ASTILL *et al.*, 2001). In literature no studies are reported on the use of ultrasounds and microwaves for the extraction of bioactive compounds from CS and on the influence of CS particle size on the extraction process.

Response surface methodology (RSM) is a multivariate statistic technique used to optimise extraction processes of bioactive compounds from plant matrices; in particular different studies reported the application of RSM to optimise the extraction of caffeine and chlorogenic acid from a plant matrix (D'ARCHIVIO *et al.*, 2016; BAE *et al.*, 2015).

This study aimed to optimise UAE and MAE of the three most abundant caffeoylquinic acids and caffeine from CS using RSM; this statistical approach was used to evaluate the impact of time and temperature on total phenolic content, radical scavenging capacity, caffeoylquinic acids and caffeine content of the extracts. Coffee silverskin was used at 80-

and 250 μm particle size in order to test a possible effect of the grain size on the extraction process. Finally extraction efficiency of UAE and MAE methods was compared to a conventional solvent extraction (CSE).

2. MATERIALS AND METHODS

2.1. Plant material and chemical reagents

A CS blend produced by roasting Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*) coffee beans was provided by Torrefazione della Piazza (Sant'Antonino di Susa, Turin, Italy).

Caffeine, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, ethanol, methanol, formic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Folin-Ciocalteu reagent and sodium carbonate were purchased from Sigma-Aldrich (Milano, Italy). Ultrapure water was produced using a Milli-Q System (Millipore, Milan, Italy).

2.2. Sample preparation

CS was ground to obtain a powder with 80- and 250- μm particle size using an ultracentrifugal mill Retsch ZM 200 (Retsch GmbH, Haan, Germany) and stored in vacuum bags at +4°C until use. The moisture content of CS (6.9 \pm 0.3%) was determined using an electronic moisture balance (Eurotherm, Gibertini Elettronica, Milan, Italy) with 5 g of sample in order to express the results on dry weight basis (dw).

2.3. Extraction procedure

According to the optimization study performed on CS by BALLESTEROS *et al.* (2014), 60% (v/v) ethanol and solvent/solid ratio of 35 mL g⁻¹ were used as fixed parameters for UAE, MAE and CSE. After the extractions, extracts were cooled in an ice bath to stop the extraction process and centrifuged at 16,800 g for 10 min; supernatants were then filtered (0.45 μm) and immediately analysed.

2.3.1. Ultrasound-assisted extraction (UAE)

UAE was carried out using an ultrasonic bath (Sonica® 3300EP S3; Soltec, Milan, Italy) at a 40 kHz frequency with 300 W of power. The flasks containing 1 g of CS and 35 mL of 60% (v/v) ethanol were immersed into the ultrasonic bath, where the water level was fixed at 2 cm above the liquid surface in the flask. For each experimental condition of the Central Composite Design (CCD) reported in Table 1, water bath temperature was set and maintained constant (± 1 °C) using an external chiller.

2.3.2. Microwave-assisted extraction (MAE)

MAE was carried out using a Start D microwave digestion system (Milestone, Bergamo, Italy) with an SK Rotor equipped with digestion vessels composed of high-purity PTFE. CS (1 g) and 35 mL of 60% (v/v) ethanol were put into the digestion vessels and a power of 280 W was then applied. For each experimental condition of CCD (Table 1), temperature was measured using the internal temperature probe of the apparatus.

Table 1. CCD experimental matrix.

| Run | Extraction time (coded value) (x_1) | Extraction temperature (coded value) (x_2) | Extraction time (real value) (X_1 , min) | Extraction temperature (real value) (X_2 , °C) |
|------|--|---|--|--|
| 1 | -1 | -1 | 19 | 37 |
| 2 | 1 | -1 | 41 | 37 |
| 3 | 1 | 1 | 41 | 73 |
| 4 | -1 | 1 | 19 | 73 |
| 5 | -1.4141 | 0 | 15 | 55 |
| 6 | 1.4141 | 0 | 45 | 55 |
| 7 | 0 | -1.4141 | 30 | 30 |
| 8 | 0 | 1.4141 | 30 | 80 |
| 9-13 | 0 | 0 | 30 | 55 |

2.3.3. Conventional solvent extraction (CSE)

CSE was carried out mixing 1 g of CS and 35 mL of 60% (v/v) ethanol into a 40 mL amber vial inserted into a Digital Pulse Mixer (Glas-Col, U.S.A.); stirring was carried out at 200 rpm, 70% duty cycle and 70 pulses per minute.

2.4. Total phenolic content (TPC)

Total phenolic content (TPC) was measured following Folin-Ciocalteu's method (SINGLETON and ROSSI, 1965) with modifications as described by ZHOU and YU (2006). Briefly, 0.05 mL of phenolic extract was mixed with 0.250 mL of Folin-Ciocalteu's reagent and 3 mL of ultrapure water. The mixture was incubated at room temperature for 3 minutes; then 0.75 mL of 20% (w/v) sodium carbonate was added and the obtained mixture was incubated in the dark at room temperature for 2 h. A mixture of the solvent and reagents was used as blank. The specific absorbance at 765 nm was measured using a UV-visible spectrophotometer (UV-1800 PharmaSpec, Shimadzu, Milan, Italy). Gallic acid was used to construct the calibration curve (linearity range 0-500 mg/L, $R^2=0.998$). TPC was expressed as g gallic acid equivalents (GAE) kg^{-1} of CS on dry weight basis (dw).

2.5. Radical scavenging capacity (RSC)

Radical scavenging capacity (RSC) of the extracts was determined according to GADOW *et al.* (1997). Phenolic extract (75 μ L) was mixed with 3 mL of 6.1×10^{-5} M DPPH \cdot solution in methanol and incubated for 1 h at room temperature in the dark (SHARMA and BHAT, 2009). Discolouration of the purple DPPH \cdot solution was measured at 515 nm. Methanol was used as a control, and a methanol solution of DPPH was used as a blank. The inhibition percentage (IP) of the DPPH \cdot by the phenolic extract was calculated using the following equation:

2.6. HPLC-PDA analysis

The three caffeoylquinic acids considered (3-O-caffeoylquinic acid, 3-CQA; 4-O-caffeoylquinic acid, 4-CQA; 5-O-caffeoylquinic acid, 5-CQA) and caffeine were quantified using an HPLC-PDA Thermo-Finnigan Spectra System (Thermo-Finnigan, Waltham, USA) equipped with a Finnigan Surveyor PDA Plus detector. ChromQuest software (version

5.0) was used for instrument control and data processing. Compounds were separated using a Kinetex Phenyl-Hexyl C18 column 5 μm , 150 \times 4.6 mm (Phenomenex, Castel Maggiore, Italy). Flow rate was set at 1.0 mL/min, temperature at 35°C and injection volume at 10 μL . A gradient elution with 0.1% formic acid (A) and methanol (B) as the solvents was applied as follows: 0.0–3.0 min, 10–30% of B; 3.0– 8.0 min, 30–35% of B; 8.0–11.0 min, 35–40% of B; 11.0–30.0 min, 40–80% of B; 30.0–32.0 min, 80–10% of B. PDA spectra were recorded using a full scan modality over the wavelength (λ) range 200 to 400 nm, and data were quantified using the external standard method with six-point calibration curves. In particular, the caffeoylquinic acids were quantified at 325 nm while caffeine at 273 nm with the respective standards ($R^2 = 0.9987$, LOD = 0.20 $\mu\text{g}/\text{mL}$, LOQ = 0.62 $\mu\text{g}/\text{mL}$ for 3-*O*-caffeoylquinic acid, $R^2 = 0.9893$, LOD = 0.25 $\mu\text{g}/\text{mL}$, LOQ = 0.66 $\mu\text{g}/\text{mL}$ for 4-*O*-caffeoylquinic acid, $R^2 = 0.9993$ for 5-*O*-caffeoylquinic acid, LOD = 0.16 $\mu\text{g}/\text{mL}$, LOQ = 0.50 $\mu\text{g}/\text{mL}$ and $R^2 = 0.9837$, LOD = 0.014 $\mu\text{g}/\text{mL}$, LOQ = 0.046 $\mu\text{g}/\text{mL}$ for caffeine). Data were expressed as g kg^{-1} of CS on dw.

2.7. Experimental design and statistical analysis

A two factorial 2^2 central composite design (CCD) was employed to evaluate the effects of time (X_1) and temperature (X_2) on TPC, RSC, caffeine and TotCQAs (as the sum of 3-CQA, 4-CQA and 5-CQA) values of the extracts obtained using UAE, MAE and CSE methods. Extraction experiments were performed in triplicate for each experimental condition of the Central Composite Design (Table 1).

As reported in Table 1, variables were codified such that their values ranged between ± 1.414 and the central point was repeated five times.

The values of the independent variables were coded using following equation:

$$x_i = (X_i - X_m) / \Delta X$$

where x_i is the coded value of an independent variable, X_i is the real value of an independent variable, X_m is the mean of the real values of an independent variable at the central point, and ΔX is the step change value.

According to PAVLIĆ *et al.* (2016), response surface regressions were used to analyse TPC, RSC, TotCQAs and caffeine of obtained extracts and were fitted to the following second-order polynomial model:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$$

where Y are the predicted responses (TPC, RSC, TotCQAs and caffeine), X_1 and X_2 correspond to independent variables time and temperature, b_0 is the constant coefficient, b_1 and b_2 represent the linear coefficients, b_{11} and b_{22} the quadratic effects and b_{12} the cross-product coefficient.

The 24 second-degree polynomial equations, describing the four predicted responses obtained for each of the extraction method applied on the two CS particle sizes, were calculated using STATISTICA software (version 7.0, StatSoft Inc., Tulsa, OK, USA). Also the respective surface plots were developed using STATISTICA software. The values of TPC, RSC, TotCQAs and caffeine registered in the 13 experiments of CCD and observed at optimal conditions were compared among the extraction methods by analysis of variance (ANOVA) in order to evaluate the extraction efficiency of UAE and MAE compared to CSE; the means of the triplicates were separated at a 95% confidence interval using Duncan's test.

3. RESULTS AND DISCUSSIONS

3.1. Model fitting

Second order polynomial model is the empirical model most commonly used for optimization methodology. Least-squares regression analysis of variables was used to determine the corresponding coefficients within the quadratic models and their ability to predict the responses (Table 2).

The quality of the generated models was evaluated by analysis of variance (ANOVA) and R square of the models; as shown in Table 2, ANOVA results showed that all the models had very low p values (≤ 0.0001). In addition, high R square values suggest that the proposed models were generally adequate to explain most of the variability (Table 2).

3.2. Effects of the process variables on total phenolic content (TPC)

Experimental data of TPC obtained using UAE, CSE and MAE on CS at 80- and 250 μm particle size (p.s.) are reported in Table 3.

TPC value ranged from 5.23 GAE kg^{-1} dw (MAE, 30 min, 80°C, 250 μm p.s.) to 10.58 GAE kg^{-1} dw (CSE, 30 min, 80°C, 80 μm p.s.). As shown in the ANOVA test reported in Table 2, TPC value was affected by the linear and quadratic terms of time for UAE applied on CS at 80- and 250 μm and for MAE applied on CS at 80 μm ; the linear and quadratic terms of temperature affected TPC value for all the extraction methods applied on CS at 80 μm and for CSE and MAE applied on CS at 250 μm . According to BALLESTEROS *et al.* (2014), 30 min may be considered an enough time to be used in the three extraction processes to obtain a high phenolic content. Temperature exerted a higher effect on TPC than extraction time, especially for CSE and MAE; an increase in temperature favors the extraction of phenolics by enhancing the diffusion coefficient of solvent, solubility of solutes, diffusion rate of analytes, and reducing solvent viscosity and surface tension (JU and HOWARD, 2003).

Response surface plots representing the effect of time and temperature on total phenolic content (TPC) of the coffee silverskin at 80 μm particle size extracts obtained using conventional solvent extraction (CSE), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) have been reported in Figs. 1, 2 and 3 respectively. Since no significant differences were observed in the extraction efficiency using the two CS particle sizes, data related to 250 μm particle size have been reported in the text without graphical representations.

Considering MAE, above 51.5°C temperature exerted a negative effect on TPC value (Fig. 3).

Other studies have shown that chlorogenic acids, phenolic compounds to whom the selected caffeoylquinic acids belong, decreased at temperatures higher than 50°C using MAE (UPADHYAY *et al.*, 2012). Phenolic compounds with several hydroxyl groups in their aromatic rings such as caffeoylquinic acids are unstable in a solvent and can easily degrade under microwave radiation combined with high temperatures (INCE *et al.*, 2014). Finally the increase of TPC value at high temperatures using CSE and UAE could confirm this hypothesis, since temperature without microwaves did not produce a degradation of the phenolic compounds (Figs. 1 and 2).

Table 2. Regression coefficients of the predicted quadratic polynomial models of TPC, RSC, TotCQAs and caffeine obtained using CSE, UAE, and MAE extraction methods on coffee silverskin at 80- and 250 μm particle sizes.

| CSE ^a | Coffee silverskin (80- μm particle size) | | | | Coffee silverskin (250- μm particle size) | | | | |
|---------------------|---|------------------------|-------------------------|-------------------------|--|------------------------|------------------------|-------------------------|-------------------------|
| | TPC | RSC | TotCQAs | Caffeine | CSE ^a | TPC | RSC | TotCQAs | Caffeine |
| b_0 | 7.9106 ^{***} | 33.9350 ^{***} | 180.0969 ^{***} | 35.97 ^{ns} | b_0 | 5.6448 ^{***} | 22.0388 ^{***} | 156.2306 ^{***} | 192.9056 ^{**} |
| b_1 , time | 0.0239 ^{ns} | 0.0358 [*] | 2.0294 ^{***} | 28.13 ^{***} | b_1 , time | 0.0441 [*] | 0.1896 ^{***} | 3.3476 ^{***} | 21.4610 ^{***} |
| b_2 , temperature | -0.0064 ^{ns} | 0.1387 ^{***} | 0.5358 ^{ns} | 21.63 ^{***} | b_2 , temperature | 0.0597 ^{***} | 0.4139 ^{***} | 1.2105 ^{***} | 19.2199 ^{***} |
| b_{11} | -0.0003 ^{ns} | -0.0001 ^{ns} | -0.0039 ^{ns} | -0.24 ^{***} | b_{11} | -0.0006 ^{ns} | -0.0015 ^{**} | -0.0427 ^{***} | -0.1459 ^{***} |
| b_{22} | 0.0003 ^{***} | -0.0009 ^{**} | 0.0069 ^{**} | -0.11 ^{***} | b_{22} | -0.0003 ^{**} | -0.0027 ^{***} | -0.0080 ^{***} | -0.1030 ^{***} |
| b_{12} | 0.0002 ^{ns} | -0.0004 ^{***} | -0.0230 ^{***} | -0.19 ^{***} | b_{12} | 0.0002 ^{ns} | -0.0014 ^{***} | -0.0036 ^{ns} | -0.1533 ^{***} |
| R^2 | 0.96 | 0.96 | 0.91 | 0.92 | R^2 | 0.92 | 0.97 | 0.90 | 0.94 |
| p value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | p value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| UAE ^b | TPC | RSC | TotCQAs | Caffeine | UAE ^b | TPC | RSC | TotCQAs | Caffeine |
| b_0 | 4.1174 ^{***} | -5.0522 [*] | -30.0050 ^{ns} | 573.9909 ^{***} | b_0 | 4.6483 ^{**} | 16.8912 ^{***} | -62.2672 [*] | 691.4779 ^{***} |
| b_1 , time | 0.1676 ^{***} | 0.7384 ^{***} | 4.9254 ^{***} | -2.6802 ^{ns} | b_1 , time | 0.2595 ^{***} | 0.8034 ^{***} | 7.8861 ^{***} | 7.7372 ^{**} |
| b_2 , temperature | 0.0714 ^{***} | 1.0476 ^{***} | 6.0426 ^{***} | 27.6387 ^{***} | b_2 , temperature | -0.0321 ^{ns} | 0.2369 ^{***} | 5.9860 ^{***} | 16.4356 ^{***} |
| b_{11} | -0.0018 ^{***} | -0.0022 ^{ns} | -0.0201 ^{ns} | 0.0956 [*] | b_{11} | -0.0029 ^{***} | -0.0075 ^{***} | -0.0648 ^{***} | -0.0045 ^{ns} |
| b_{22} | -0.0002 [*] | -0.0057 ^{***} | -0.0277 ^{***} | -0.2021 ^{***} | b_{22} | 0.0009 ^{**} | -0.0005 ^{ns} | -0.0279 ^{***} | -0.0866 ^{***} |
| b_{12} | -0.0011 ^{***} | -0.0098 ^{***} | -0.0703 ^{***} | -0.0704 [*] | b_{12} | -0.0011 [*] | -0.0042 ^{***} | -0.0679 ^{***} | -0.1212 ^{***} |
| R^2 | 0.92 | 0.94 | 0.86 | 0.94 | R^2 | 0.74 | 0.90 | 0.86 | 0.89 |
| p value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | p value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| MAE ^c | TPC | RSC | TotCQAs | Caffeine | MAE ^c | TPC | RSC | TotCQAs | Caffeine |
| b_0 | 1.9917 ^{***} | 26.2296 ^{***} | 125.0379 ^{***} | 108.9807 ^{ns} | b_0 | 3.6921 ^{***} | 25.2296 ^{***} | 110.1193 ^{***} | 119.3925 ^{ns} |
| b_1 , time | 0.1011 ^{***} | -0.0880 ^{**} | -1.0855 ^{ns} | 33.1830 ^{***} | b_1 , time | 0.0543 ^{ns} | -0.0880 ^{**} | -0.1901 ^{ns} | 39.3521 ^{***} |
| b_2 , temperature | 0.1680 ^{***} | 0.2242 ^{***} | 4.5193 ^{***} | 18.0169 ^{***} | b_2 , temperature | 0.1100 ^{***} | 0.2242 ^{***} | 4.6963 ^{***} | 14.0377 ^{***} |
| b_{11} | -0.0015 ^{***} | 0.0011 ^{**} | -0.0009 ^{ns} | -0.3733 ^{***} | b_{11} | -0.0015 ^{**} | 0.0011 ^{**} | -0.0136 ^{ns} | -0.3500 ^{***} |
| b_{22} | -0.0016 ^{***} | -0.0024 ^{***} | -0.0627 ^{***} | -0.0791 ^{***} | b_{22} | -0.0015 ^{***} | -0.0024 ^{***} | -0.0630 ^{***} | -0.0254 ^{ns} |
| b_{12} | -0.0001 ^{ns} | 0.0005 ^{ns} | 0.0281 [*] | -0.1912 ^{***} | b_{12} | 0.0008 [*] | 0.0005 ^{ns} | 0.0241 [*] | -0.2891 ^{***} |
| R^2 | 0.92 | 0.94 | 0.92 | 0.84 | R^2 | 0.86 | 0.94 | 0.93 | 0.89 |
| p value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | p value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

^aConventional solvent extraction; ^bUltrasound-assisted extraction, ^cMicrowave-assisted extraction.

P value, probability of F value for the model.

* P < 0.05, ** P < 0.01, *** P < 0.001, ns= Not significant.

Table 3. Central composite design with the observed TPC, RSC, TotCQAs and caffeine values of coffee silverskin (80- and 250 μm particle sizes) extracts obtained using CSE, UAE, and MAE.

| Coffee silverskin at 80- μm particle size | | | | | | | | |
|---|---------------------------------|-------------------------|------------------------|----------|-------------------------------------|-------------------------|-------------------------|----------|
| Run | TPC (g GAE kg ⁻¹ dw) | | | | RSC ($\mu\text{mol TE g}^{-1}$ dw) | | | |
| | CSE ^a | UAE ^b | MAE ^c | <i>p</i> | CSE ^a | UAE ^b | MAE ^c | <i>p</i> |
| 1 | 8.73±0.02 ^A | 8.35±0.10 ^B | 7.51±0.02 ^C | *** | 38.28±0.21 ^A | 32.05±0.72 ^B | 30.47±0.02 ^C | *** |
| 2 | 8.94±0.06 ^A | 8.58±0.01 ^B | 7.55±0.02 ^C | *** | 38.63±0.04 ^A | 38.41±0.12 ^B | 30.01±0.04 ^C | *** |
| 3 | 10.15±0.06 ^A | 8.76±0.02 ^B | 7.14±0.04 ^C | *** | 39.40±0.04 ^A | 38.47±0.16 ^B | 29.45±0.04 ^C | *** |
| 4 | 9.77±0.04 ^A | 9.35±0.05 ^B | 7.17±0.03 ^C | *** | 39.38±0.02 ^B | 39.47±0.06 ^A | 29.55±0.04 ^C | *** |
| 5 | 8.98±0.08 ^A | 8.60±0.02 ^B | 7.55±0.10 ^C | *** | 39.06±0.08 ^A | 38.67±0.27 ^B | 30.57±0.09 ^C | *** |
| 6 | 9.57±0.14 ^A | 8.67±0.02 ^B | 7.89±0.01 ^C | *** | 39.16±0.08 ^A | 39.02±0.33 ^A | 31.09±0.10 ^B | *** |
| 7 | 8.53±0.06 ^A | 8.46±0.04 ^A | 7.15±0.04 ^B | *** | 37.80±0.11 ^A | 32.27±0.28 ^B | 29.62±0.06 ^C | *** |
| 8 | 10.58±0.10 ^A | 9.41±0.06 ^B | 7.01±0.07 ^C | *** | 39.37±0.04 ^A | 39.22±0.38 ^A | 28.62±0.08 ^B | *** |
| 9 | 9.31±0.07 ^A | 8.92±0.05 ^B | 7.90±0.08 ^C | *** | 39.30±0.05 ^A | 39.16±0.22 ^A | 30.75±0.03 ^B | *** |
| 10 | 9.33±0.13 ^A | 9.19±0.08 ^A | 8.01±0.06 ^B | *** | 39.10±0.02 ^B | 39.29±0.12 ^A | 30.63±0.07 ^C | *** |
| 11 | 9.43±0.02 ^A | 9.03±0.11 ^B | 7.90±0.04 ^C | *** | 39.17±0.10 ^B | 39.34±0.05 ^A | 30.44±0.06 ^C | *** |
| 12 | 9.26±0.09 ^A | 8.93±0.03 ^B | 8.16±0.11 ^C | *** | 39.16±0.07 ^A | 38.97±0.35 ^A | 30.44±0.02 ^B | *** |
| 13 | 9.38±0.06 ^A | 9.13±0.03 ^B | 8.17±0.06 ^C | *** | 39.12±0.09 ^B | 39.41±0.10 ^A | 30.44±0.16 ^C | *** |
| Run | TotCQAs (g kg ⁻¹ dw) | | | | Caffeine (g kg ⁻¹ dw) | | | |
| | CSE ^a | UAE ^b | MAE ^c | <i>p</i> | CSE ^a | UAE ^b | MAE ^c | <i>p</i> |
| 1 | 2.34±0.00 ^A | 2.04±0.01 ^B | 2.00±0.07 ^B | *** | 9.81.00±0.03 ^C | 12.86±0.05 ^A | 9.93±0.04 ^B | *** |
| 2 | 2.51±0.01 ^A | 2.20±0.09 ^B | 2.00±0.03 ^C | *** | 11.31±0.18 ^B | 12.89±0.12 ^A | 11.01±0.10 ^C | *** |
| 3 | 2.63±0.03 ^A | 2.18±0.06 ^B | 1.71±0.01 ^C | *** | 12.03±0.02 ^B | 13.49±0.06 ^A | 11.93±0.06 ^B | *** |
| 4 | 2.63±0.02 ^A | 2.54±0.02 ^B | 1.50±0.03 ^C | *** | 11.91±0.04 ^C | 13.98±0.03 ^A | 12.29±0.07 ^B | *** |
| 5 | 2.38±0.01 ^A | 2.21±0.02 ^B | 1.89±0.06 ^C | *** | 11.10±0.09 ^C | 13.91±0.17 ^A | 11.47±0.06 ^B | *** |
| 6 | 2.58±0.03 ^A | 2.26±0.01 ^B | 1.99±0.01 ^C | *** | 11.99±0.09 ^B | 13.75±0.15 ^A | 11.12±0.08 ^C | *** |
| 7 | 2.37±0.02 ^A | 1.84±0.01 ^C | 2.04±0.02 ^B | *** | 10.63±0.07 ^C | 11.31±0.05 ^A | 11.01±0.04 ^B | *** |
| 8 | 2.68±0.02 ^A | 2.38±0.07 ^B | 1.06±0.03 ^C | *** | 12.12±0.15 ^B | 13.39±0.13 ^A | 12.27±0.08 ^B | *** |
| 9 | 2.53±0.01 ^A | 2.31±0.02 ^B | 1.98±0.07 ^C | *** | 11.81±0.17 ^B | 13.70±0.10 ^A | 11.70±0.35 ^B | *** |
| 10 | 2.49±0.01 ^A | 2.31±0.04 ^B | 1.96±0.02 ^C | *** | 11.86±0.21 ^B | 13.74±0.05 ^A | 11.94±0.33 ^B | *** |
| 11 | 2.53±0.04 ^A | 2.37±0.02 ^B | 1.87±0.03 ^C | *** | 12.05±0.19 ^B | 13.75±0.09 ^A | 12.08±0.08 ^B | *** |
| 12 | 2.49±0.05 ^A | 2.32±0.06 ^B | 2.02±0.07 ^C | *** | 12.08±0.38 ^B | 13.73±0.13 ^A | 12.28±0.11 ^B | *** |
| 13 | 2.46±0.03 ^A | 2.28±0.07 ^B | 2.03±0.04 ^C | *** | 12.17±0.28 ^B | 13.69±0.07 ^A | 12.23±0.22 ^B | *** |
| Coffee silverskin at 250- μm particle size | | | | | | | | |
| Run | TPC (g GAE kg ⁻¹ dw) | | | | RSC ($\mu\text{mol TE g}^{-1}$ dw) | | | |
| | CSE ^a | UAE ^b | MAE ^c | <i>p</i> | CSE ^a | UAE ^b | MAE ^c | <i>p</i> |
| 1 | 8.31±0.05 ^A | 8.14 ±0.02 ^A | 6.67±0.25 ^B | *** | 36.13±0.09 ^A | 34.42±0.31 ^B | 29.47±0.02 ^C | *** |
| 2 | 8.75±0.02 ^A | 8.59 ±0.09 ^B | 6.61±0.05 ^C | *** | 37.13±0.17 ^B | 38.41±0.25 ^A | 29.01±0.04 ^C | *** |
| 3 | 9.87±0.04 ^A | 8.87 ±0.01 ^B | 5.88±0.29 ^C | *** | 39.00±0.09 ^B | 39.38±0.07 ^A | 28.45±0.04 ^C | *** |
| 4 | 9.25±0.18 ^A | 9.28 ±0.01 ^A | 5.35±0.16 ^B | *** | 39.05±0.14 ^A | 38.56±0.67 ^A | 28.55±0.04 ^B | *** |
| 5 | 8.59±0.02 ^A | 7.56 ±0.13 ^B | 6.52±0.35 ^C | *** | 38.07±0.31 ^A | 35.18±0.85 ^B | 29.57±0.09 ^C | *** |
| 6 | 9.24±0.12 ^A | 9.00 ±0.08 ^A | 6.63±0.18 ^B | *** | 38.62±0.07 ^B | 39.20±0.07 ^A | 30.09±0.10 ^C | *** |
| 7 | 8.05±0.21 ^A | 8.49 ±0.34 ^A | 6.73±0.14 ^B | *** | 34.74±0.19 ^B | 37.68±0.39 ^A | 28.62±0.06 ^C | *** |
| 8 | 9.63±0.04 ^B | 10.46±0.07 ^A | 5.23±0.13 ^C | *** | 39.31±0.06 ^A | 39.40±0.50 ^A | 27.62±0.08 ^B | *** |
| 9 | 9.29±0.05 ^A | 8.88±0.51 ^A | 6.95±0.16 ^B | *** | 38.84±0.13 ^A | 38.53±0.19 ^B | 29.75±0.03 ^C | *** |
| 10 | 9.25±0.09 ^A | 9.35±0.05 ^A | 6.92±0.21 ^B | *** | 38.83±0.19 ^B | 39.13±0.04 ^A | 29.63±0.07 ^C | *** |
| 11 | 8.98±0.16 ^A | 8.53±0.08 ^A | 6.80±0.37 ^B | *** | 38.65±0.21 ^A | 38.81±0.31 ^A | 29.44±0.06 ^B | *** |
| 12 | 9.06±0.20 ^A | 8.73±0.30 ^A | 6.53±0.25 ^B | *** | 38.66±0.20 ^A | 38.61±0.52 ^A | 29.44±0.02 ^B | *** |
| 13 | 9.08±0.09 ^A | 8.74±0.27 ^A | 6.97±0.10 ^B | *** | 38.81±0.19 ^A | 38.80±0.83 ^A | 29.44±0.16 ^B | *** |
| Run | TotCQAs (g kg ⁻¹ dw) | | | | Caffeine (g kg ⁻¹ dw) | | | |
| | CSE ^a | UAE ^b | MAE ^c | <i>p</i> | CSE ^a | UAE ^b | MAE ^c | <i>p</i> |
| 1 | 2.38±0.01 ^A | 2.09±0.01 ^A | 2.03±0.05 ^B | *** | 9.88±0.07 ^B | 12.64±0.04 ^A | 9.93±0.03 ^B | *** |
| 2 | 2.52±0.02 ^A | 2.38±0.02 ^B | 1.99±0.05 ^C | *** | 11.69±0.09 ^C | 13.41±0.07 ^A | 11.85±0.05 ^B | *** |
| 3 | 2.58±0.02 ^A | 2.43±0.01 ^A | 1.60±0.15 ^B | *** | 12.40±0.02 ^B | 13.80±0.08 ^A | 12.02±0.02 ^C | *** |
| 4 | 2.47±0.03 ^B | 2.64±0.02 ^A | 1.47±0.08 ^C | *** | 11.73±0.05 ^C | 13.93±0.03 ^A | 12.27±0.08 ^B | *** |
| 5 | 2.35±0.01 ^A | 2.23±0.03 ^B | 1.91±0.07 ^C | *** | 11.31±0.18 ^B | 13.48±0.10 ^A | 11.14±0.03 ^B | *** |
| 6 | 2.52±0.004 ^A | 2.34±0.03 ^B | 2.04±0.01 ^C | *** | 12.12±0.04 ^B | 13.51±0.02 ^A | 11.43±0.13 ^C | *** |
| 7 | 2.42±0.02 ^A | 2.03±0.03 ^B | 2.03±0.03 ^B | *** | 10.66±0.06 ^C | 11.92±0.06 ^A | 11.52±0.03 ^B | *** |

| | | | | | | | | |
|----|------------------------|------------------------|------------------------|-----|-------------------------|-------------------------|-------------------------|-----|
| 8 | 2.54±0.02 ^A | 2.48±0.04 ^A | 1.19±0.05 ^B | *** | 12.14±0.07 ^C | 14.01±0.03 ^A | 12.31±0.08 ^B | *** |
| 9 | 2.57±0.02 ^A | 2.55±0.03 ^A | 2.02±0.04 ^B | *** | 11.97±0.09 ^B | 13.67±0.15 ^A | 11.99±0.07 ^B | *** |
| 10 | 2.55±0.03 ^A | 2.53±0.03 ^A | 2.02±0.01 ^B | *** | 11.94±0.03 ^C | 13.54±0.12 ^A | 12.10±0.05 ^B | *** |
| 11 | 2.54±0.04 ^A | 2.53±0.05 ^A | 1.94±0.07 ^B | *** | 11.99±0.03 ^B | 13.54±0.15 ^A | 11.94±0.17 ^B | *** |
| 12 | 2.55±0.02 ^A | 2.43±0.06 ^A | 1.93±0.09 ^B | *** | 12.06±0.13 ^B | 13.61±0.09 ^A | 12.02±0.24 ^B | *** |
| 13 | 2.52±0.02 ^A | 2.39±0.05 ^B | 2.06±0.04 ^C | *** | 11.93±0.12 ^B | 13.70±0.06 ^A | 12.11±0.09 ^B | *** |

Data are expressed as the means±standard deviation (n = 3).

Values in each row having different capitals letters indicate significant differences at P < 0.05 (Duncan's test).

* P < 0.05, ** P < 0.01, *** P < 0.001, ns= Not significant

dw: dry weight; TotCQAs: total caffeoylquinic acids.

^aConventional solvent extraction; ^bUltrasound-assisted extraction, ^cMicrowave-assisted extraction.

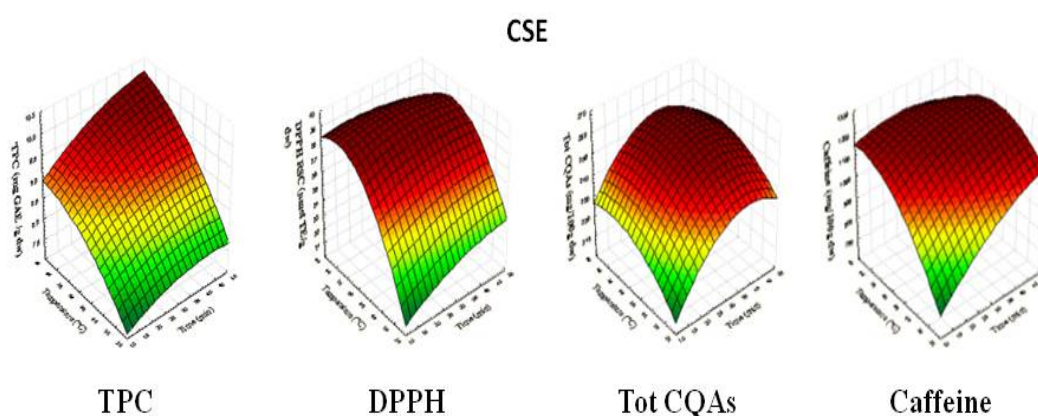


Figure 1. Response surface plots representing the effect of time and temperature on total phenolic content (TPC), radical scavenging capacity (RSC), total caffeoylquinic acids (TotCQAs) and caffeine content of the coffee silverskin (80 μ m particle size) extracts obtained using conventional solvent extraction (CSE).

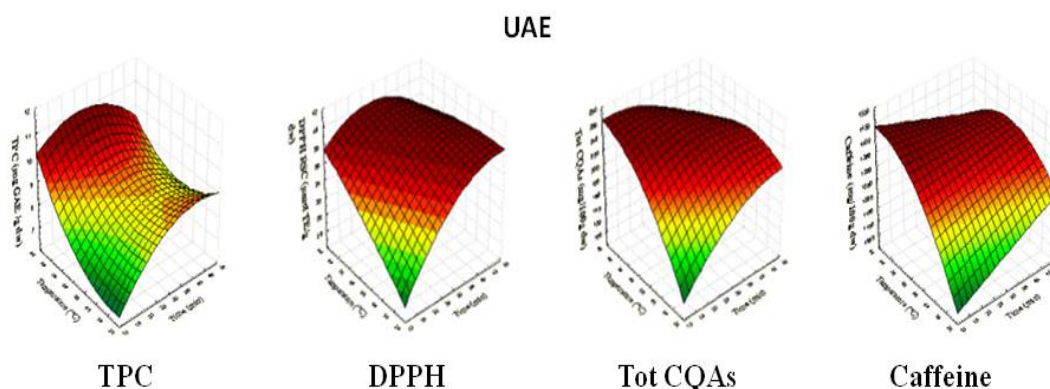


Figure 2. Response surface plots representing the effect of time and temperature on total phenolic content (TPC), radical scavenging capacity (RSC), total caffeoylquinic acids (TotCQAs) and caffeine content of the coffee silverskin (80 μ m particle size) extracts obtained using ultrasound-assisted extraction (UAE).

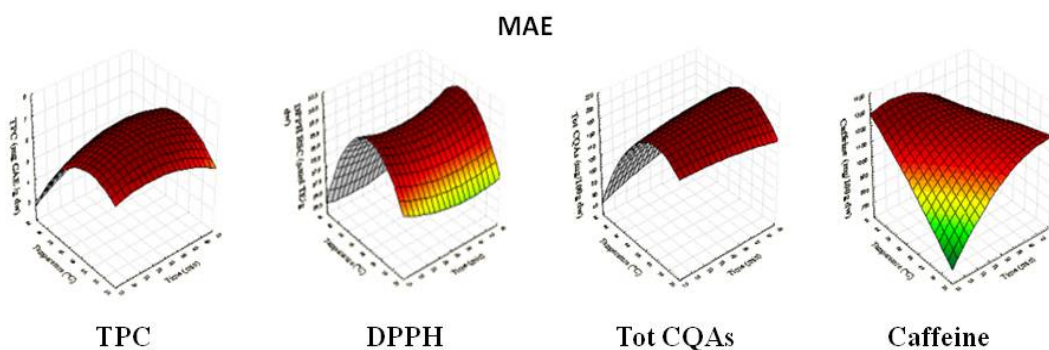


Figure 3. Response surface plots representing the effect of time and temperature on total phenolic content (TPC), radical scavenging capacity (RSC), total caffeoylquinic acids (TotCQAs) and caffeine content of the coffee silverskin (80 μm particle size) extracts obtained using microwave-assisted extraction (MAE).

3.3. Effects of the process variables on the DPPH radical scavenging capacity (RSC)

As shown in Table 3, RSC values ranged from 27.62 $\mu\text{mol TE/g dw}$ (MAE, 30 min, 80°C, 250 μm p.s.) to 39.47 $\mu\text{mol TE/g dw}$ (UAE, 19 min, 73°C, 80 μm p.s.). Table 2 shows how RSC value was affected by linear and quadratic terms of temperature for all the extraction methods applied on CS at 80 μm and for CSE and MAE applied on CS at 250 μm particle size. Moreover RSC value was affected by both linear and quadratic terms of extraction time for MAE applied on CS at 80 μm . As seen for TPC, also RSC was generally more affected by temperature than time, especially for CSE and MAE methods.

Considering the surface plots reported in Figs. 1, 2 and 3, a positive effect of extraction time was observed for UAE while a temperature increase caused a rapid increase of RSC value for all the extraction methods, except MAE applied at temperatures higher than 50 °C, as seen for TPC.

According to NARITA and INOUYE (2012), a correlation between TPC and RSC trends has been observed meaning the high contribute of phenolic compounds in antioxidant activity of CS extracts.

3.4. Effects of the process variables on total caffeoylquinic acids (TotCQAs)

Table 3 shows how TotCQAs ranged from 1.06 g kg^{-1} dw (MAE, 30 min, 80°C, 80 μm p.s.) to 2.68 g kg^{-1} (CSE, 30 min, 80°C, 80 μm p.s.). Considering the ANOVA test on CS extracts at 80- and 250 μm particle sizes (Table 2), TotCQAs value was affected by linear terms of extraction time and temperature for UAE and by linear and quadratic terms of temperature for MAE. As seen for TPC and RSC, TotCQAs was more affected by temperature than time for all the extraction methods. Also surface plots of TotCQAs were generally comparable, for each extraction method, to that observed for TPC and RSC (Figs. 1, 2 and 3); these similar trends could confirm that 3-CQA, 4-CQA and 5-CQA are the main compounds in phenolic content of CS and the main responsible of CS antioxidant activity, as reported by BRESCIANI *et al.* (2014).

According to UPADHYAY *et al.* (2012), caffeoylquinic acids content decreased at temperatures above 50°C using MAE, confirming a possible degradation of the selected compounds caused by the combination of microwaves and high temperatures (Fig. 3).

3.5. Effects of the process variables on caffeine content

As reported in Table 3, caffeine content ranged from 9.81 g kg⁻¹ dw (CSE, 19 min, 37°C, 80 μm p.s.) to 14.01 g kg⁻¹ dw (UAE, 30 min, 80°C, 250 μm p.s.).

Table 2 shows how the caffeine content was affected by linear and quadratic terms of time and temperature for CSE and MAE applied on CS at the two particle sizes while UAE was especially affected by extraction temperature. As seen for TPC, RSC and TotCQAs, also in this case temperature was the variable that produced the most relevant effects on the extraction processes.

In contrast with TotCQAs values, caffeine content obtained by MAE was rapidly increased also at high temperatures (Fig. 3); caffeine stability in these conditions has been reported by LIU *et al.* (2012) that optimised microwave-assisted extraction of caffeine from coffee at 120°C.

3.6. Optimization and verification of the models

Optimization of CSE, UAE and MAE methods was carried out to obtain extracts with the highest predicted TPC, RSC, TotCQAs and caffeine values (Table 4).

Following the model proposed by ZIELINSKI *et al.* (2015), the errors in relation to the predicted models of the optimization variables were verified. An external validation was performed using the optimal conditions of time and temperature obtained by the predict models; all the observed values were within the predicted interval to a level of 95 % (Table 4), meaning that experimental data matched well the predicted values and interval ranges generated by RSM. Comparing the observed values at optimal conditions with the ones reported in literature, TPC values of 10.01 g GAE kg⁻¹ dw (CSE, 45 min, 80°C, 80 μm p.s.) and 9.91 g GAE kg⁻¹ dw (UAE, 29.5 min, 80°C, 250 μm p.s.) were higher than the one obtained during CS extraction with water at 80°C during 60 min (7.00 g GAE kg⁻¹ dw) performed by NARITA and INOUYE (2012); moreover UAE allowed to split in half the extraction time. Observed TotCQAs values of 2.61 g kg⁻¹ dw (CSE, 24.5 min, 80°C, 80 μm p.s.) and 2.57 g kg⁻¹ dw (UAE, 15 min, 80°C, 80 μm p.s.) can be compared with the sum of 3-CQA, 4-CQA and 5-CQA (4.31 g kg⁻¹ dw) obtained by BRESCIANI *et al.* (2014) using two times a sonic bath for 30 min and then a Dubnoff bath for 1 h at 70°C; CSE and UAE optimized in our study allowed to obtain a slightly lower TotCQAs value balanced by a significant time reduction (86.4 % for CSE and 91.7 % for UAE). Finally the highest caffeine content of 14.24 g kg⁻¹ dw (UAE, 15 min, 66°C, 80 μm p.s.) obtained in our study was higher than the one reported by NARITA and INOUYE (2012) using subcritical water at 210°C (4.1 g kg⁻¹ dw). Considering UAE, highest predicted and observed values were generally obtained at high temperatures according to other studies that reported a maximum UAE extraction of several bioactive compounds at 80°C (TOMŠIK *et al.*, 2016; ZHANG *et al.*, 2011; ZHU *et al.*, 2017).

3.7. Comparison among the combinations of extraction method and CS particle size

The observed values of TPC, RSC, TotCQAs and caffeine obtained at optimal time and temperature conditions by each combination of extraction method and CS particle size were compared in order to evaluate the most efficient combination for each response (Fig. 4).

Table 4. Predicted and observed values of TPC, RSC, TotCQAs and caffeine at optimal time and temperature conditions obtained using CSE, UAE, and MAE extraction methods on CS at 80- and 250- μm particle sizes.

| CS 80 μm Response variables | Optimal conditions | | CSE | | | | | |
|---|--------------------|--------------------------|----------------|-------|------|-----------------|------------|------------|
| | T (min) | T ($^{\circ}\text{C}$) | Observed value | | | Predicted value | -95 % Pred | +95 % Pred |
| TPC (g GAE kg^{-1} dw) | 45 | 80 | 10.01 | \pm | 0.21 | 10.51 | 9.97 | 10.75 |
| RSC ($\mu\text{mol TE g}^{-1}$ dw) | 45 | 67 | 39.01 | \pm | 0.46 | 39.41 | 38.96 | 39.86 |
| TotCQAs (g kg^{-1} dw) | 24.5 | 80 | 2.61 | \pm | 0.05 | 2.69 | 2.61 | 2.78 |
| Caffeine (g kg^{-1} dw) | 31.5 | 69 | 12.11 | \pm | 0.12 | 12.26 | 11.80 | 12.72 |
| CS 250 μm | | | CSE | | | | | |
| Response variables | Optimal conditions | | Observed value | | | Predicted value | -95 % Pred | +95 % Pred |
| | T (min) | T ($^{\circ}\text{C}$) | | | | | | |
| TPC (g GAE kg^{-1} dw) | 45 | 80 | 9.83 | \pm | 0.15 | 9.99 | 9.59 | 10.39 |
| RSC ($\mu\text{mol TE g}^{-1}$ dw) | 31 | 68.5 | 39.01 | \pm | 0.50 | 39.19 | 38.66 | 39.72 |
| TotCQAs (g kg^{-1} dw) | 36.5 | 67.5 | 2.54 | \pm | 0.05 | 2.58 | 2.53 | 2.63 |
| Caffeine (g kg^{-1} dw) | 40 | 63.5 | 12.13 | \pm | 0.11 | 12.34 | 11.94 | 12.73 |
| CS 80 μm | | | UAE | | | | | |
| Response variables | Optimal conditions | | Observed value | | | Predicted value | -95 % Pred | +95 % Pred |
| | T (min) | T ($^{\circ}\text{C}$) | | | | | | |
| TPC (g GAE kg^{-1} dw) | 22 | 80 | 9.25 | \pm | 0.16 | 9.43 | 9.25 | 9.61 |
| RSC ($\mu\text{mol TE g}^{-1}$ dw) | 15 | 79 | 40.81 | \pm | 0.76 | 41.1 | 39.39 | 42.81 |
| TotCQAs (g kg^{-1} dw) | 15 | 80 | 2.57 | \pm | 0.05 | 2.61 | 2.42 | 2.80 |
| Caffeine (g kg^{-1} dw) | 15 | 66 | 14.24 | \pm | 0.15 | 14.29 | 13.86 | 14.72 |
| CS 250 μm | | | UAE | | | | | |
| Response variables | Optimal conditions | | Observed value | | | Predicted value | -95 % Pred | +95 % Pred |
| | T (min) | T ($^{\circ}\text{C}$) | | | | | | |
| TPC (g GAE kg^{-1} dw) | 29.5 | 80 | 9.91 | \pm | 0.24 | 10.38 | 9.54 | 11.22 |
| RSC ($\mu\text{mol TE g}^{-1}$ dw) | 31 | 80 | 39.21 | \pm | 1.80 | 39.93 | 38.73 | 41.13 |
| TotCQAs (g kg^{-1} dw) | 19 | 80 | 2.54 | \pm | 0.05 | 2.61 | 2.44 | 2.79 |
| Caffeine (g kg^{-1} dw) | 15 | 80 | 14.02 | \pm | 0.21 | 14.22 | 13.69 | 14.74 |
| CS 80 μm | | | MAE | | | | | |
| Response variables | Optimal conditions | | Observed value | | | Predicted value | -95 % Pred | +95 % Pred |
| | T (min) | T ($^{\circ}\text{C}$) | | | | | | |
| TPC (g GAE kg^{-1} dw) | 32 | 51.5 | 7.34 | \pm | 0.23 | 7.78 | 7.02 | 8.55 |
| RSC ($\mu\text{mol TE g}^{-1}$ dw) | 45 | 51.5 | 26.44 | \pm | 0.72 | 26.61 | 26.20 | 27.01 |
| TotCQAs (g kg^{-1} dw) | 45 | 46 | 1.98 | \pm | 0.06 | 2.02 | 1.82 | 2.22 |
| Caffeine (g kg^{-1} dw) | 24 | 80 | 11.58 | \pm | 0.21 | 11.96 | 11.28 | 12.64 |
| CS 250 μm | | | MAE | | | | | |
| Response variables | Optimal conditions | | Observed value | | | Predicted value | -95 % Pred | +95 % Pred |
| | T (min) | T ($^{\circ}\text{C}$) | | | | | | |
| TPC (g GAE kg^{-1} dw) | 30 | 44.5 | 6.76 | \pm | 0.20 | 6.85 | 6.35 | 7.35 |
| RSC ($\mu\text{mol TE g}^{-1}$ dw) | 45 | 51.5 | 28.44 | \pm | 0.26 | 28.68 | 28.28 | 29.09 |
| TotCQAs (g kg^{-1} dw) | 31.5 | 43.5 | 2.08 | \pm | 0.02 | 2.08 | 1.93 | 2.24 |
| Caffeine (g kg^{-1} dw) | 23 | 80 | 7.18 | \pm | 0.02 | 7.86 | 7.16 | 8.57 |

The data are expressed as the means \pm standard deviation ($n = 3$).

^aConventional solvent extraction; ^bUltrasound-assisted extraction, ^cMicrowave-assisted extraction.

Generally lower values were obtained using MAE than CSE or UAE, regardless of CS particle size. Other studies showed a better capacity of UAE compared to MAE to extract chlorogenic acids (ROUTRAY and ORSAT, 2014); as shown in Fig. 4, UAE and CSE exhibited comparable maximum observed values of TPC and TotCQAs, while RSC was higher using UAE than CSE, regardless of the particle size; considering TPC, RSC and TotCQAs values, UAE allowed to obtain higher or similar values to CSE with a significant reduction of extraction time, especially at 80 μm particle size (Table 4). According to CHOUNG *et al.* (2014), caffeine content was significantly ($p \leq 0.05$) higher using UAE than CSE or MAE (Fig. 4). Generally maximum observed values were significantly affected by extraction method while no significant differences were observed between the two particle sizes of CS, with the only exception of caffeine content for MAE (Fig. 4). Nevertheless a lower CS particle size allowed to reduce extraction time up to 32.9% for TotCQAs obtained with CSE and up to 51.6% for RSC obtained with UAE (Table 4).

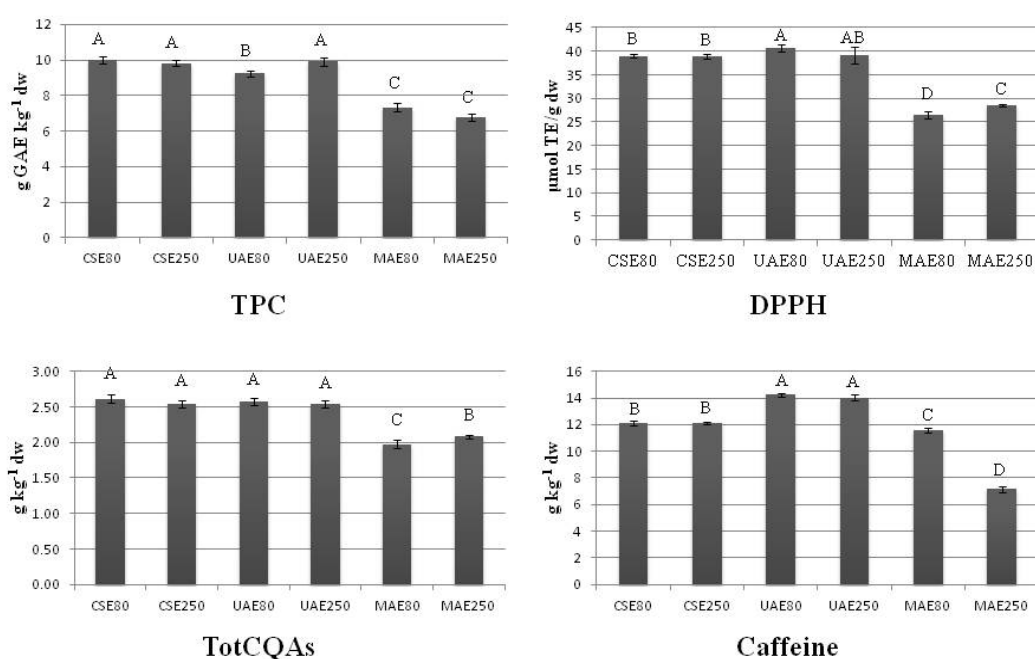


Figure 4. Comparison of the observed values (mean \pm standard deviation) of total phenolic content (TPC), radical scavenging capacity (RSC), total caffeoylquinic acids (TotCQAs) and caffeine content obtained at optimal time and temperature conditions by each combination of extraction method and CS particle size. The standard deviation bars with different letters are significant different ($p \leq 0.05$).

4. CONCLUSIONS

CSE, UAE and MAE of phenolic compounds and caffeine from coffee silverskin at 80- and 250 μm particle sizes were optimised using RSM approach. All the quadratic polynomial models were able to predict and optimise CSE, UAE and MAE processes.

ANOVA showed that temperature was the process variable that most affected the extraction processes. In particular a positive correlation was observed between an increase of temperature and TPC, RSC, TotCQAs and caffeine values for CSE and UAE; using MAE above 50°C, a negative effect on TPC, RSC and TotCQAs was observed meaning a possible degradation of the selected caffeoylquinic acids due to a combination of high temperatures

and microwaves. Comparing extraction methods, lowest values of TPC, RSC, TotCQAs and caffeine were obtained using MAE; UAE allowed to obtain extracts with values of TPC, RSC and TotCQAs higher or similar to CSE with a significant reduction of extraction time, especially at 80 μm particle size. Moreover, UAE produced a significant higher content of caffeine compared to CSE in the half of the extraction time. Generally maximum observed values were not affected by the particle size of CS. Nevertheless, using the lower particle size, a reduction of extraction time was observed. In conclusion, UAE applied at high temperatures represented a fast and efficient substitute for conventional solvent extraction of bioactive compounds from coffee silverskin.

REFERENCES

- Astill C., Birch M.R., Dacombe C., Humphrey P.G. and Martin P.T. 2001. Factors affecting the caffeine and polyphenol contents of black and green tea infusions. *J. Agric. Food Chem.* 49:5340-5347.
- Bae I.K., Ham H.M., Jeong M.H., Kim D.H., Kim H.J. 2015. Simultaneous determination of 15 phenolic compounds and caffeine in teas and mate using RP-HPLC/UV detection: method development and optimization of extraction process. *Food Chem.* 172:469–475.
- Ballesteros L.F., Teixeira J.A. and Mussatto S.I. 2014. Selection of the solvent and extraction conditions for maximum recovery of antioxidant phenolic compounds from coffee silverskin. *Food Bioprocess Tech.* 7:1322-1332.
- Bresciani L., Calani L., Bruni R., Brighenti F. and Del Rio D. 2014. Phenolic composition, caffeine content and antioxidant capacity of coffee silverskin. *Food Res. Int.* 61:196-201.
- Chan C.-H., Yusoff R., Ngoh G.-C. and Kung, F.W.-L. 2011. Microwave-assisted extractions of active ingredients from plants. *J. Chromatogr. A* 1218:6213-6225.
- Chemat F., Zill-e-Huma and Khan M.K. 2011. Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrason. sonochem.* 18:813-835.
- Choung M.-G., Hwang Y.-S., Lee M.-S., Lee J., Kang S.-T. and Jun T.-H. 2014. Comparison of extraction and isolation efficiency of catechins and caffeine from green tea leaves using different solvent systems. *Int. J. Food Sci. Tech.* 49:1572-1578.
- Costa A.S.G., Alves R.C., Vinha A.F., Barreira S.V.P., Nunes M.A., Cunha L.M. and Oliveira M.B.P.P. 2014. Optimization of antioxidants extraction from coffee silverskin, a roasting by-product, having in view a sustainable process. *Ind. Crop Prod.* 53:350-357.
- D'Archivio A.A., Maggi M.A. and Ruggieri F. 2016. Investigation by response surface methodology of extraction of caffeine, gallic acid and selected catechins from tea using water-ethanol mixture. *Food Anal. Method.* 9:2773–2779.
- del Castillo M.D., Fernandez-Gomez B., Martinez-Saez N., Iriondo A. and Mesa M.D. Coffee by-products. Ch. In press. In: "Coffee: Chemistry, quality and health implications". A. Farah (Ed.), Cambridge: RSC Publishing Inc..
- Gadow A.V., Joubert E. and Hansmann C.F. 1997. Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. *Food Chem.* 60:73-77.
- Glade M. 2010. Caffeine – not just a stimulant. *Nutrition.* 26:932-938.
- Ince A.E., Sahin S. and Sumnu G. 2014. Comparison of microwave and ultrasound-assisted extraction techniques for leaching of phenolic compounds from nettle. *J. Food Sci. Tech.* 51:2776-2782.
- International Coffee Organization. Historical data on the global coffee trade. <http://www.ico.org/>.
- Ju Z.Y. and Howard L.R. 2003. Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin. *J. Agric. Food Chem.* 51:5207-5213.
- Liu H., Shao J., Li Q., Li Y., Yan H.M. and He L. 2012. Determination of trigonelline, nicotinic acid, and caffeine in yunnan arabica coffee by microwave-assisted extraction and HPLC with two columns in series. *J. AOAC Int.* 95:1138-1141.

- Machado E.M.S., Rodriguez-Jasso R.M., Teixeira J.A. and Mussatto S.I. 2012. Growth of fungal strains on coffee industry residues with removal of polyphenolic compounds. *Biochem. Eng. J.* 60:87-90.
- Murthy P.S. and Naidu M.M. 2010. Recovery of phenolic antioxidants and functional compounds from coffee industry by-products. *Food Bioprocess Tech.* 5:897-903.
- Narita Y. and Inouye K. 2012. High antioxidant activity of coffee silverskin extracts obtained by the treatment of coffee silverskin with subcritical water. *Food Chem.* 135:943-949.
- Pavlič B., Vidović S., Vladić J., Radosavljević R., Cindrić M. and Zeković Z. 2016. Subcritical water extraction of sage (*Salvia officinalis* L.) by-products - Process optimization by response surface methodology. *J. Supercrit. Fluid* 116:36-45.
- Pinelo M., Tress A.G., Pedersen M., Arnous A. and Meyer A.S. 2007. Effect of cellulases, solvent type and particle size distribution on the extraction of chlorogenic acid and other phenols from spent coffee grounds. *Am. J. Food Tech.* 2:641-651.
- Routray W. and Orsat V. 2014. MAE of phenolic compounds from blueberry leaves and comparison with other extraction methods. *Ind. Crop Prod.* 58:36-45.
- Severini C., Derossi A., Fiore A.G. 2016. Ultrasound-assisted extraction to improve the recovery of phenols and antioxidants from spent espresso coffee ground: a study by response surface methodology and desirability approach. *Eur Food Res. Technol.* DOI:10.1007/s00217-016-2797-7
- Sharma O. P. and Bhat T.K. 2009. DPPH antioxidant assay revisited. *Food Chem.* 113:1202-1205.
- Singleton V.L. and Rossi J.A.Jr. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.* 16:144-158.
- Tomšik A., Pavlič B., Vladić J., Ramić M., Brindza J. and Vidović S. 2016. Optimization of ultrasound-assisted extraction of bioactive compounds from wild garlic (*Allium ursinum* L.). *Ultrason. sonochem.* 29:502-511.
- Upadhyay R., Ramalakshmi K. and Jagan Mohan Rao L. 2012. Microwave-assisted extraction of chlorogenic acids from green coffee beans. *Food Chem.* 130:184-188.
- Zhang G., He L. and Hu M. 2011. Optimized ultrasonic-assisted extraction of flavonoids from *Prunella vulgaris* L. and evaluation of antioxidant activities in vitro. *Innov. Food Sci. Emerg. Technol.* 12:18-25.
- Zhou K. and Yu L. 2006. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. *LWT-Food Sci. Technol.* 39:1155-1162.
- Zhu Z., Guan Q., Koubaa M., Barba F.J., Roohinejad S., Cravotto G., Yang X., Li S. and He J. 2017. HPLC-DAD-ESI-MS, analytical profile of extracts obtained from purple sweet potato after green ultrasound-assisted extraction. *Food Chem.* 215:391-400.
- Zielinski A.A.F., Granato D., Alberti A., Nogueira A., Demiate I.M. and Haminiuk C.W.I. 2015. Modelling the extraction of phenolic compounds and in vitro antioxidant activity of mixtures of green, white and black teas (*Camellia sinensis* L. Kuntze). *J Food Sci. Tech.* 52:6966-6977.

Paper Received January 2, 2017 Accepted March 15, 2017