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Optimization of 5-CQA Extraction from Coffee Silverskin using Ultrasound-Assisted Extraction and Response Surface Methodology

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Abstract

Coffee production generates substantial amounts of by-products, particularly coffee silverskin (CSS), which has attracted attention due to its abundance, unique composition, and health-promoting properties. CSS valorization through green extraction technologies supports a circular and contribute to a more sustainable coffee industry. This study aimed to maximize the recovery of 5-caffeoylquinic acid (5-CQA) from this by-product through ultrasound-assisted extraction (UAE). Optimization was performed using Response Surface Methodology (RSM) based on a Central Composite Design (CCD). UAE was conducted with an ultrasonic probe under varying solid-to-liquid ratios (0.31–3.68 g/100 mL), extraction times (1–30 min), and ethanol concentrations (0–100%). Extracts were analyzed by HPLC-DAD. Statistical significance (p<0.05) and model fit were evaluated through ANOVA, regression analysis, and lack-of-fit tests. CSS extracts presented high levels of 5-CQA, with the solid-to-liquid ratio exerting the greatest influence on extraction yield, followed by extraction time. These findings confirm UAE as an efficient and sustainable extraction method for CSS valorization.

***Keywords*:** Coffee by-products; Ultrasounds; Green extraction; Chlorogenic acids; Sustainability.

1. Introduction

Coffee is one of the most widely consumed beverages worldwide and represents a major global commodity, generating substantial amounts of by-products throughout its processing chain, such as pulp or husks, mucilage, parchment, defective beans, silverskin, and spent coffee grounds [1]. Among these residues, CSS (a thin tegument released during beans roasting) has attracted increasing attention due to its abundance and reported health-promoting properties [2, 3]. Although it accounts for only about 1.2% of the coffee bean mass, its large-scale industrial production results in significant global disposal [4]. Often treated as waste, CSS is now recognized as a promising biomass resource for sustainable solutions in the agri-food sector [5]. From a compositional perspective, CSS is primarily constituted by structural polysaccharides, particularly cellulose and hemicellulose, and is rich in dietary fiber [6-8]. In addition, it also retains considerable amounts of bioactive compounds, including caffeine, trigonelline, chlorogenic acids (CGAs), and Maillard reaction products such as melanoidins [9-11]. Among phenolics, chlorogenic acids, especially 5-CQA, are the most abundant being strongly associated with the health benefits attributed to this by-product [8, 12,

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13]. The efficient recovery of these bioactives is highly dependent on the extraction method employed. In this context, UAE has emerged as a green and efficient alternative to conventional solvent-based techniques [14, 15]. Through acoustic cavitation, UAE promotes cell wall disruption, enhances solvent penetration, and improves mass transfer, thereby increasing extraction yields while reducing processing time as well as solvent and energy consumption [16-18]. When applied to coffee by-products, UAE has demonstrated high efficiency in extracting phenolic compounds, caffeine, and chlorogenic acids [19, 20]. Several studies have explored the extraction of bioactive compounds from this by-product, consistently highlighting ethanol and water as the most effective solvents. Ethanol is valued for its ability to dissolve a broad range of phenolics and for being safe and renewable, making it suitable for food and nutraceutical uses [15, 21]. Water is also effective, particularly for polar phenolics at higher temperatures [22]. Combining the use of ethanol and water can further improve phenolic recovery, reinforcing their role as green solvents in sustainable extraction processes [21]. Nevertheless, the performance of UAE is strongly influenced by multiple operational parameters. Parameters such as solvent composition, extraction time, temperature, and solid-to-liquid ratio play a critical role in determining extraction efficiency and compound selectivity. For this reason, the optimization of these parameters is essential not only to maximize the recovery of target bioactives but also to ensure process sustainability and environmental responsibility. In this work, UAE conditions were optimized using Response Surface Methodology based on a Central Composite Design to maximize the recovery of 5-caffeoylquinic acid from coffee silverskin, contributing to the valorization of this coffee industry by-product.

2. Material and methods

2.1. Sample preparation

Coffee silverskin was provided by JMV - José Maria Vieira, S.A. (Portugal). After delivery, the sample was milled to a fine powder (< 500 µm) using a Thermomix® TM5 (VORWERK, Portugal), vacuum-sealed, and stored at room temperature, protected from light, until further analysis.

2.2. Ultrasound-assisted extraction

The extraction of 5-CQA from coffee silverskin was performed using an ultrasonic homogenizer probe (LUHS-A16, Labtron, Frimley, United Kingdom). Experimental conditions included solid-to-liquid ratios ranging from 0.31 to 3.68 g/100 mL, extraction times between 1 and 30 min, and ethanol concentrations from 0 to 100%, as detailed in Table 1. After the extraction, samples were centrifuged at 4,500 rpm for 10 minutes (Heraeus Megafuge 16, Thermo Fisher Scientific, United States) to remove insoluble material. The resulting supernatants were subsequently centrifuged at 13,000 rpm for an additional 10 minutes (Biofuge Pico, Heraeus, Germany) prior to chromatographic analysis.

Table 1. Independent variables, their coded levels, and corresponding symbols used in the RSM design for optimizing the UAE extraction conditions.

	Solid-to-liquid ratio (X_1)	Time (X_2)	Ethanol (X_3)
Units	g.100 mL^{-1}	min	%
(-) α	0.31	1.00	0.00
-1	0.99	6.88	20.27
0	2.00	15.50	50.00
1	3.00	24.12	79.72
α	3.68	30.00	100.00

2.2.1. Design of the experiments (DoE)

This study evaluated the effect of UAE process variables on the extraction of 5-CQA from CSS, as described in Section 2.2. Optimization was performed using Response Surface Methodology (RSM) based on a Central Composite Design (CCD). The CCD for the 2^3 factorial design consisted of 20 experimental runs: 8 at cube points (factors at +1, 0, and −1), 6 at axial points (factors at ±α, where α = 1.682 and the central level = 0), and 6 replicates at the center point. A detailed overview of the experimental design matrix is presented in Table 2. The selection of factor levels was based on experiments and relevant literature data. A multiple linear regression based on a full quadratic model was applied to analyze the responses across all CCD runs, according to the following equation:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i . X_i + \sum_{i=1}^{k} \beta_{ii} . X_i^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k-1} \beta_{ij} . X_i X_j \quad (1)$$

Where: y represents the response, β_0 the model constant, β_i the coefficients of the

linear terms (Xi), β_{ii} the coefficients of the quadratic terms (X_i^2), and β_{ij} the coefficients of the first-order interaction terms (X_iX_j).

2.3. Chromatographic analyses

2.3.1. Preparation of Extracts

For HPLC-DAD (High-performance liquid chromatography with diode-array detection) analysis, 100 μL of CSS extracts were diluted in 900 μL of ultrapure water. The solutions were homogenized using a vortex mixer and then centrifuged for 10 minutes at 10000 g. The resulting supernatants were collected and analysed.

2.3.2. HPLC-DAD

Chromatographic analyses were performed using an integrated HPLC system (Jasco, Tokyo, Japan). The setup included an LC-Net II/ADC hardware interface, an autosampler (AS-2057 Plus), a pump (PU-2089 Plus), a multi-wavelength diode-array detector (MD-2018 Plus), and a column oven (CO-2060 Plus), all from Jasco (Tokyo, Japan). Separation was achieved using a Zorbax SB-C18 column (5 μm, 250 mm × 4.6 mm; Agilent Technologies, Santa Clara, CA, USA) at 28 °C. The gradient elution was carried out under the following conditions: 0 min, 5% B; 40 min, 25% B; 55 min, 45% B; 60 min, 60% B; 65 min, 5% B (solvent A: 0.5% acetic acid; solvent B: 100% methanol). The flow rate was maintained at 1.1 mL/min, and 5-CQA was monitored at 320 nm. The compound was identified by comparing its retention time and UV spectrum with those of an analytical standard. The 5-CQA content was expressed as mg/100 mL of extract. All measurements were performed in triplicate.

2.2.2. Statistical analysis

Statistical significance was established at $p<0.05$. Data were analyzed using STATISTICA version 12 (StatSoft, Inc., Germany). Data normality was assessed with the Shapiro-Wilk test.

3. Results and discussion

3.1. UAE

A CCD was employed to optimize UAE conditions, comprising 20 randomized extractions to minimize potential bias. The input values for each independent variable and the corresponding responses are presented in Table 2. Data normality was verified using the Shapiro–Wilk test. Stepwise regression ($\alpha < 0.05$) was applied to identify significant predictors and refine the model. ANOVA was performed to assess the effects of the studied variables on the selected responses and to evaluate the reliability of the fitted models (Table 3).

Table 2. RSM experimental design matrix and response values of silverskin extracts, expressed on mg/100 mL of extract

Standard order	Run order	S/L ratio X_1	Time X_2	Ethanol X_3	5-CQA (mg/100 mL)
1	3	-1	-1	-1	0.03382
2	4	+1	-1	-1	0.28914
3	5	-1	+1	-1	0.18625
4	6	+1	+1	-1	0.81495
5	10	-1	-1	+1	0.07083
6	11	+1	-1	+1	0.57727
7	12	-1	+1	+1	0.08773
8	13	+1	+1	+1	0.74079
9	14	-1.682	0	0	0
10	8	+1.682	0	0	1.02083
11	9	0	-1.682	0	0.35031
12	7	0	+1.682	0	0.54108
13	1	0	0	-1.682	0
14	2	0	0	+1.682	0
15	15	0	0	0	0.58006
16	16	0	0	0	0.59597
17	17	0	0	0	0.59178
18	18	0	0	0	0.45157
19	19	0	0	0	0.54834
20	20	0	0	0	0.59748

Table 3. ANOVA results for response surface quadratic models of 5-CQA

Source	Sum of Squares	Df	Mean Square	F-value	*p*-value
Linear					
X_1	583.531	1	583.531	72.6696	0.0003
X_2	231.576	1	231.576	28.8392	0.0030
X_3	0.057	1	0.057	0.0070	0.9364
Square					
X_1* X_1	256.332	1	256.332	31.9221	0.0024
X_2 * X_2	21.791	1	21.791	2.7138	0.1604
X_3 * X_3	1196.443	1	1196.443	148.9983	<0.0001
2-Way Interaction					
X_1*X_2	4.452	1	4.452	0.5544	0.4900
X_1*X_3	22.583	1	22.583	2.8124	0.1544
X_2*X_3	83.941	1	83.941	10.4536	0.0231
Error	129.635	10			
Lack of Fit	89.485	5	17.897	2.2288	0.1998
Pure Error	40.150	5	8.030		
Total	2411.856	19			
R^2	0.9463		**Adj. R^2**	0.8979	

The model exhibited a high coefficient of determination (R^2 = 0.9463), with an adjusted R^2 value close to the corresponding R^2, indicating good model fit. Furthermore, the lack-of-fit test yielded a high *p*-value (0.1998), confirming that the lack of fit was not significant ($p > 0.05$).

To develop the mathematical model, all dependent variables were expressed as functions of the independent variables using a second-order polynomial equation (Equation 1), incorporating the significant factors ($p<0.05$). The resulting polynomial equations for the responses are given in Equation (2), where X_1, X_2, and X_3 represent the S/L ratio, extraction time, and ethanol concentration, respectively.

$$5-CQA = 27.9132 + 6.5363X_1 + 4.1177X_2 - 4.2167X_1^2 - 9.1099X_3^2 - 3.2392X_2X_3 \quad (2)$$

As shown in Table 3, all independent variables significantly influenced the responses evaluated. Among them, ethanol concentration (X_3) had the greatest impact on 5-CQA extraction yields. According to the model, the solid-to-liquid ratio (X_1) exhibited a positive linear effect, indicating that increasing the sample mass at a constant solvent volume enhanced 5-CQA extraction. However, its quadratic term was negative, suggesting the existence of an optimal intermediate S/L ratio. Similarly, the quadratic term of ethanol concentration negatively affected 5-CQA levels, indicating that higher ethanol percentages reduce extraction efficiency. In contrast, extraction time (X_2) showed a positive linear effect, with longer extraction times increasing 5-CQA concentration. The only significant interaction observed in the model was between extraction time and ethanol concentration (X_2*X_3), which was negative, suggesting that the simultaneous increase of both variables negatively affects 5-CQA extraction. The order of significance of the factors for 5-CQA concentration was $X_3>X_1>X_2$ (Table 3). Complementing the results presented in Table 3, the interactions between extraction variables can be visualized through 3D response surface plots (Figure 1) and contour plots (Figure 2). As shown in Figure 2b and 2c, extraction time had a strong positive effect on 5-CQA levels, with higher concentrations observed above 25 min and reaching a maximum at times exceeding 28 min. Regarding ethanol concentration, increased 5-CQA levels were observed at intermediate ethanol percentages, particularly between 30 and 50% (Figure 2a and 2b). Similarly, the solid-to-liquid (S/L) ratio showed higher responses at intermediate values, around 2.0–3.5 g/100 mL (Figure 2a and 2c).

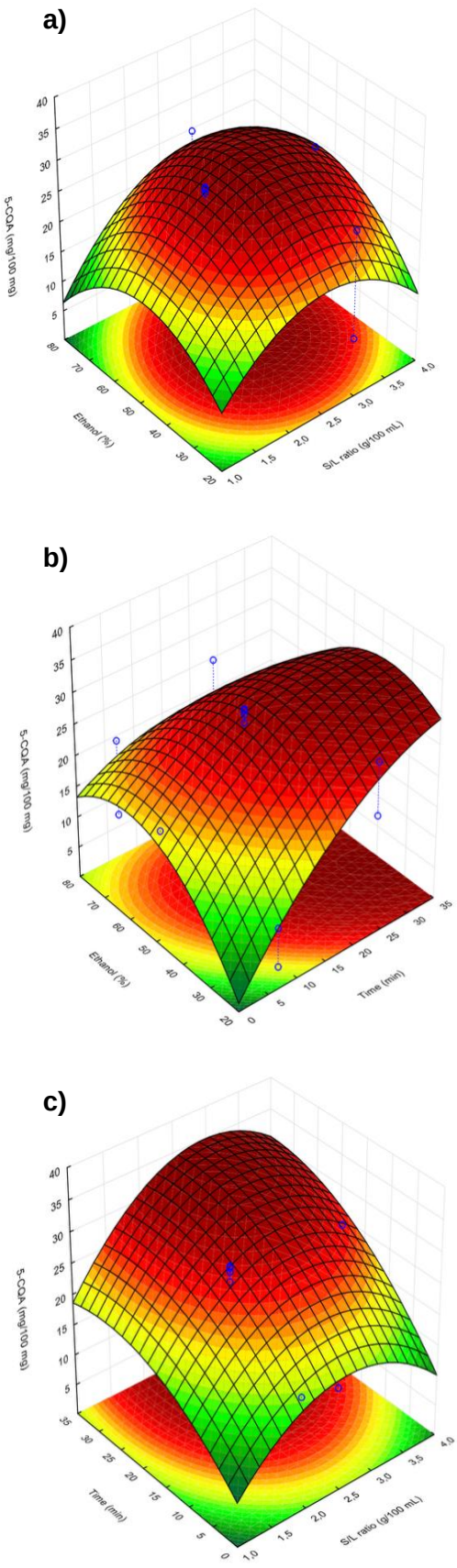

Figure 1. UAE 3D response surface plots.

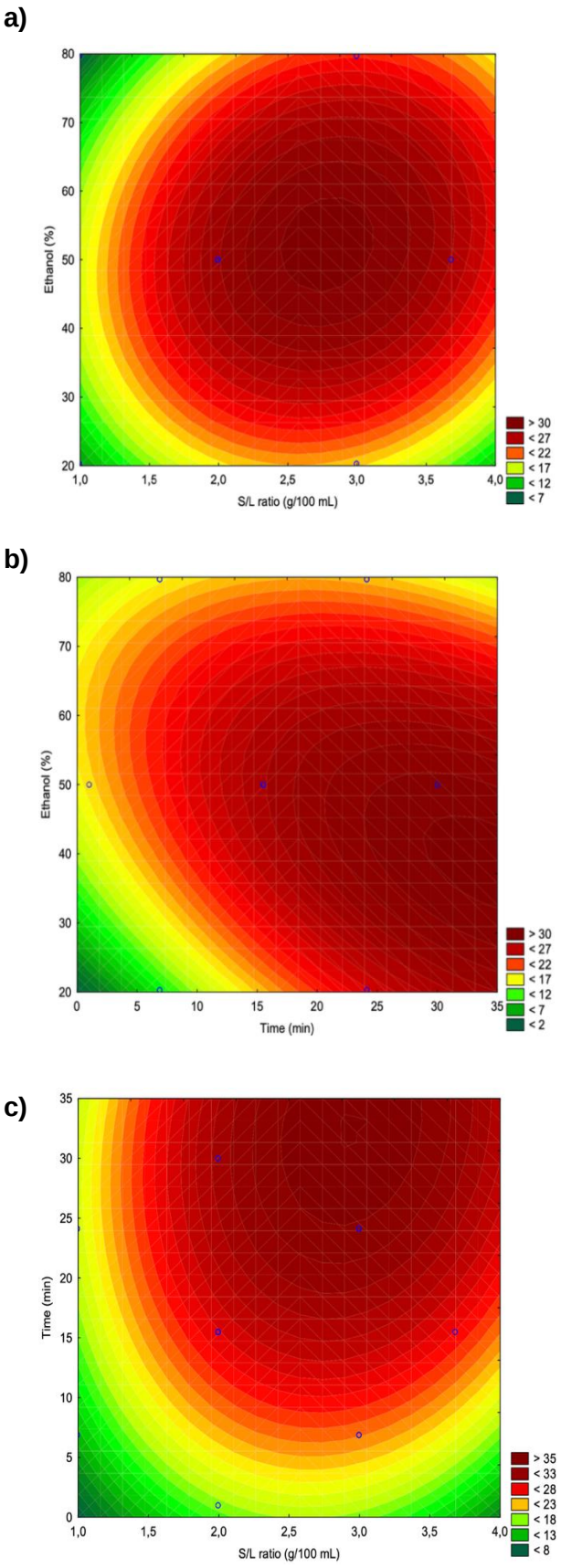

Figure 2. UAE response surface contour plots.

The interaction between extraction time and ethanol concentration (X_2*X_3), as shown in Figure 2b, resulted in the highest 5-CQA levels at extraction times above 28 min combined with ethanol percentages between 35 and 45%. Overall, the model predicted that the optimal conditions for maximizing 5-CQA recovery were an S/L ratio of 2.7949 g/100 mL, an extraction time of 27.70 min, and an ethanol concentration of 41.41%, corresponding to a predicted 5-CQA concentration of 35.8992 mg/100 g.

4. Conclusion

This study confirmed that coffee silverskin is a valuable source of 5-CQA. UAE optimization through RSM, proved to be an efficient and green approach for maximizing 5-CQA recovery. Ethanol concentration was the most influential parameter, followed by the solid-to-liquid ratio and extraction time, emphasizing the need to carefully tailor operational conditions to the specific target compound to maximize extraction efficiency and selectivity. Overall, the results support UAE as an efficient and sustainable technique for the recovery of high-value bioactive compounds from coffee by-products, supporting their circular valorization.

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Ethics approval: No ethic situation.

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