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Optimization of the extraction conditions for antioxidant phenolic compounds recovery from coffee silverskin

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Interest in the development of bioprocesses for the production or extraction of bioactive compounds from natural sources has increased in recent years due to the potential applications of these compounds in food, chemical, and pharmaceutical industries [1]. In this sense, increased attention has been given to the agro-industrial residues as abundantly available and cheap renewable feedstocks for the production of value-added compounds. Coffee silverskin (CS, Fig. 1), the outer casing of the seed separated during the beans roasting, is one of the main residues proceeding from the coffee industry [2].

Fig. 1. Coffee silverskin [2].

In a previous study, the effect of different organic solvents (methanol, ethanol and acetone) on the extraction of antioxidant phenolic compounds from CS was evaluated. Methanol and ethanol gave similar extraction results; however, since ethanol is less toxic than methanol, it was selected for use in the present work, which aimed to maximize the extraction of antioxidant phenolic compounds from CS. It is well known that the establishment of the most suitable process conditions is of relevance to achieve elevated yields. The use of experimental design statistical methodology is a useful tool to define such conditions performing a minimal number of experiments. Considering the above mentioned reasons, the present study consisted in using a statistical experimental design to optimize the process conditions to maximize the extraction of antioxidant phenolic compounds from CS.

Experimental assays were performed at 60 ºC, using different conditions of ethanol concentration (20 to 90%), solid/liquid ratio (1/10 to 1/40 g CS/ml solvent) and extraction time (30 to 90 min), which were combined according to a 2^3 central composite design. Total phenolic compounds concentration in the extracts was determined by the method of Folin-Ciocalteu, the value being converted to mg/g CS. The antioxidant potential of the extracts was determined by the methods of FRAP and DPPH. Statistical analysis of the results was performed to identify the influence of each variable on the responses, and the conditions able to maximize the extraction of antioxidant phenolic compounds were established. Statistica 5.0 was the software used for data analyses.

The experimental conditions used in each experimental assay and the respective results of phenolic compounds concentration (PC), FRAP and DPPH, are presented in Table 1. Note that the results obtained for these responses strongly varied according to the extraction conditions. PC extraction, for example, varied in a range between 5.26 and 13.53 mg/g CS. Similar variation (around 2.7-fold) was observed for the FRAP results. Statistical analysis of these data revealed that the solid/liquid ratio was the variable with greater influence in all the responses. The ethanol concentration did not present significant (p<0.05) influence on the extraction of phenolic compounds, but affected the antioxidant potential of the extracts. On the other hand, the extraction time did not influence any of the responses, and thus, 30 min may be considered an enough time to be used for extraction.
Quadratic models describing the responses variation as function of the process variables (ethanol concentration, x_1, and solid/liquid ratio, x_2) were established (Eq. 1-3, coded values). All the models presented high determination coefficient R^2, which demonstrate that they were well adjusted to the experimental data and explain more than 86% of the variability in the responses.

$$PC\ (mg/g\ CS) = 11.82 - 0.58x_1 - 0.91x_1^2 + 3.30x_2 - 1.99x_2^2 - 0.37x_1x_2 \quad (R^2 = 0.86)\ \text{Eq. 1}$$

$$FRAP\ (mM\ Fe(II)/g) = 0.074 - 0.001x_1 - 0.005x_1^2 + 0.023x_2 - 0.013x_2^2 - 0.002x_1x_2 \quad (R^2 = 0.98)\ \text{Eq. 2}$$

$$DPPH\ (\%) = 81.37 + 5.83x_1 + 0.40x_1^2 + 3.55x_2 - 3.06x_2^2 - 2.50x_1x_2 \quad (R^2 = 0.95)\ \text{Eq. 3}$$

Table 1. Experimental conditions (real and (coded) values) used for extraction of antioxidant phenolic compounds (PC) from CS, concentration of PC extracted from CS, and antioxidant potential of the obtained extracts by FRAP and DPPH methods.

Assay	Variables [a]						Responses		
	x_1		x_2		x_3		PC (mg/g)	FRAP (mM Fe(II)/g)	DPPH (%)
1	90	(+1)	1/40	(+1)	90	(+1)	12.15	0.076	86.27
2	20	(-1)	1/40	(+1)	90	(+1)	13.53	0.079	78.19
3	90	(+1)	1/40	(+1)	30	(-1)	12.19	0.077	85.89
4	20	(-1)	1/40	(+1)	30	(-1)	12.29	0.080	77.47
5	90	(+1)	1/10	(-1)	90	(+1)	5.54	0.038	84.80
6	20	(-1)	1/10	(-1)	90	(+1)	5.63	0.031	66.06
7	90	(+1)	1/10	(-1)	30	(-1)	6.82	0.035	84.12
8	20	(-1)	1/10	(-1)	30	(-1)	5.26	0.033	66.39
9	90	(+1)	1/25	(0)	60	(0)	7.00	0.064	84.65
10	20	(-1)	1/25	(0)	60	(0)	12.80	0.073	79.31
11	55	(0)	1/25	(0)	90	(+1)	12.78	0.075	81.35
12	55	(0)	1/25	(0)	30	(-1)	12.94	0.077	80.05
13	55	(0)	1/40	(+1)	60	(0)	11.88	0.088	83.04
14	55	(0)	1/10	(-1)	60	(0)	5.76	0.032	74.00
15	55	(0)	1/25	(0)	60	(0)	12.52	0.076	81.59
16	55	(0)	1/25	(0)	60	(0)	11.60	0.074	81.81
17	55	(0)	1/25	(0)	60	(0)	11.33	0.072	81.55
18	55	(0)	1/25	(0)	60	(0)	11.77	0.073	81.42

* x_1, ethanol concentration; x_2, solid/liquid ratio; x_3, extraction time

Based on the three models obtained, a graphical optimization was conducted. This method consists in overlaying the curves of all the models according to the criteria imposed. The optimal working conditions were defined to attain maximum values for all the responses. The criteria adopted were: PC ≥ 12, FRAP ≥ 0.082, and DPPH ≥ 82. The overlaying plot attained showed an area where all the imposed criteria were satisfied. A point was chosen in this area as optimum point and corresponded to the use of an ethanol concentration of 60% and solid/liquid ratio of 1/35 g/ml. Under these conditions, the model predicts a PC extraction of 12.63 mg/g CS, FRAP of 0.083 mM Fe(II)/g, and DPPH of 82%, in the confidence range of 95%.

This study allowed to establish the optimum conditions to extract phenolic compounds with antioxidant potential from CS. Extraction conditions consisted in using ethanol 60% as solvent, 1 g CS/ 35 ml ethanol, during 30 min, at 60 ºC. These findings are of great relevance for two main reasons: I) the increased use of antioxidant compounds in food, chemical and pharmaceutical areas, and II) the valorization of this unused agro-industrial waste.

References

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