# Extraction of Antioxidant Phenolic Compounds by Autohydrolysis of Spent Coffee Grounds

P-SE24

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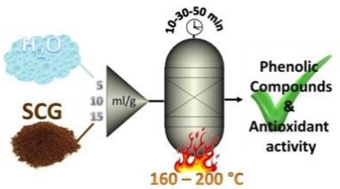


The extraction of antioxidant phenolic compounds from spent coffee grounds (SCG) was studied. Experimental assays were carried out by the autohydrolysis technique and the effect of the process variables temperature, water/SCG ratio and extraction time on the amount of phenolic compounds and antioxidant activity of the produced extracts was evaluated. The variables were combined according to a $2^3$ central composite design. The statistical model revealed that the optimum condition to produce extracts with high content of phenolic compounds (35.07 mg GAE/g SCG) and antioxidant activity (FRAP = 0.25 mmol Fe(II)/g SCG, DPPH = 121.7 µmol TE/g SCG, ABTS = 130.0 µmol TE/g SCG, TAA = 64.17 mg α-TOC/g SCG) was achieved when maximizing the process variables (temperature = 200 °C, ratio = 15 ml/g and time = 50 min).

## Introduction

Agro-industrial residues often contain high added-value substances that can be extracted by designing a proper bioprocess to exploit them in the food, chemical and pharmaceutical industries. Particularly, phenolic compounds are of great interest due to their enormous benefits for human health. Previous researches have shown that the potential of phenolic compounds is related to their antioxidant activity, protecting against chronic-degenerative diseases as cancer and diabetes [1]. Nonetheless, their properties are not limited the antioxidant activity, but they can also present anti-inflammatory, antiallergenic and/or antimicrobial effects [2].

Recently, spent coffee grounds (SCG) have been studied as a natural source of phenolic compounds [3, 4]. These findings showed the ability of a conventional solid-liquid extraction method to recover phenolic compounds from SCG using organic solvents such as ethanol [3] and methanol [4]. However, there is a necessity of evaluating and identifying more eco-friendly methodologies, which may enhance the extracts compatibility for the food industry and enable their use as added-value constituent for several applications such as natural preservatives against food degradation, raw material in the development of functional food and as especial components into edible food packaging, among others.

The aim of the present study was to extract antioxidant phenolic compounds from SCG using an eco-friendly technique, namely autohydrolysis. Additionally, the conditions able to produce a phenolic rich extract with high antioxidant activity were optimized.

## Materials and methods

SCG were supplied by the Portuguese coffee industry NovaDelta-Comércio e Indústria de Cafés S.A. The material was dried in an oven at 60 ºC until 6.8% moisture content and stored for further extractions.

Autohydrolysis assays were performed under different conditions of temperature (160 to 200 °C), liquid/solid ratio (5 to 15 ml water/g SCG) and extraction time (10 to 50 min), which were combined according to a $2^3$ central composite design. Total phenolic compounds concentration in the extracts was quantified by the Folin-Ciocalteu method and the antioxidant potential of the extracts was determined by FRAP [5], DPPH [6, 7], ABTS [8, 9] and total antioxidant activity (TAA) [10] procedures. 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. Finally, quadratic models describing the responses variation as a function of the process variables were determined.

## Results and discussion

The experimental conditions used in each assay and the respective results of total phenolic compounds concentration (TP), FRAP, DPPH, ABTS and TAA are presented in Table 1. The statistical analysis revealed a significant effect of the three variables on the total phenolic compounds extraction from SCG, being the water/SCG ratio the most significant variable on the process, as shown in Figure 1. As a result, similar trends on the antioxidant responses were expected, later evidenced by the model in which the ratio was found to exhibit greater effect on all the responses (FRAP, DPPH, ABTS and TAA).

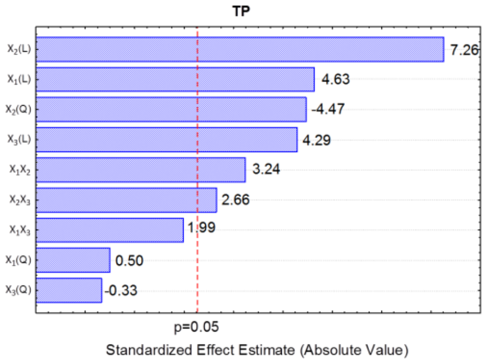


**Figure 1.** Pareto chart for the effects of temperature ($X_1$), water/SCG ratio ($X_2$) and extraction time ($X_3$), during the autohydrolysis of spent coffee grounds for the extraction of total phenolic compounds (TP).

All the responses were fitted to second-order polynomial equations, where the terms with non-statistical significance ($p<0.05$) were disregarded in order to improve the fitting and prediction of the model. The equation for each variable is presented in Table 2.

**Table 2.** Quadratic models describing the responses variation (total phenolic compounds (TP) and antioxidant activity by the FRAP, DPPH, ABTS and TAA methods) as function of the process variables ($X_1$, temperature; $X_2$, water/SCG ratio; $X_3$, extraction time) and their correspondent $R^2$ coefficients.

| MODEL EQUATION | $R^2$ |
|---|---|
| **TP** = $24.18 + 3.84X_1 + 6.61X_2 + 3.56X_3 + 3.00X_1X_2 + 2.46X_2X_3 – 8.59X_2^2$ | 0.93 |
| **FRAP** = $0.14 + 0.026X_1 + 0.056X_2 + 0.029X_3 + 0.012X_1X_2 + 0.013X_2X_3 – 0.03X_2^2$ | 0.96 |
| **DPPH** = $77.20 + 8.46X_1 + 30.21X_2 + 13.75X_3 + 7.02X_2 X_3 – 14.89X_1^2$ | 0.94 |
| **ABTS** = $76.19 + 11.81X_1 + 29.37X_2 + 13.43X_3 + 6.50X_1 X_2 + 6.65X_2 X_3 – 13.93X_2^2$ | 0.95 |
| **TAA** = $31.39 + 6.27X_1 + 14.02X_2 + 6.96X_3 + 3.19X_1X_2 + 2.06X_1X_3 + 3.29X_2X_3 – 3.00X_3^2$ | 0.98 |

A graphical optimization was carried out in order to determine the extraction condition to obtain an extract with high content of total phenolic compounds as well as high antioxidant activity. The optimization process was conducted by overlapping the responses curves obtained in the model, based on the mathematical description of each variable (*cf.* in Table 2) and imposing the following criteria: TP > 35, FRAP > 0.23, TAA > 60, ABTS > 110, and DPPH > 100. Figure 2 shows the result of the described methodology where the ratio and the time variables are presented in the range studied, while the temperature was limited to 200 °C. The graph reveals an area in which these criteria are satisfied (shadow area). The extraction results are then maximized when the processes variables are also maximized (200 °C, 15 ml/g and 50 min).

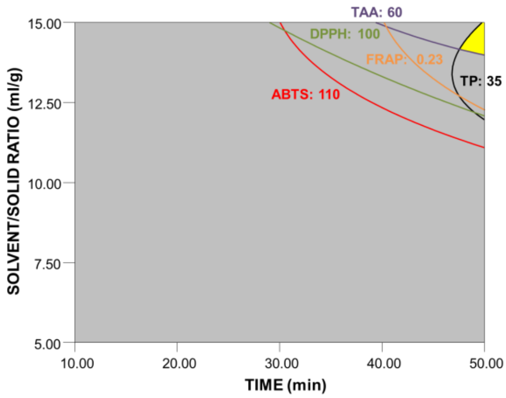


**Figure 2.** Optimum region by overlaying the curves of the responses total phenolic compounds (TP), FRAP, DPPH, ABTS and antioxidant activity (TAA) as a function of the extraction time and water/SCG ratio used for spent coffee grounds extraction.

The predicted values are in agreement with the values obtained experimentally (Table 1, conditions +1, +1, +1). The optimal point was later reproduced to validate the results, obtaining values within 5 % of relative standard deviation, which demonstrates a good degree of prediction. Furthermore, this study also revealed two-times higher phenolic compound extraction by autohydrolysis when compared to solid/liquid extractions using organic solvents such ethanol [3] or methanol [4].

## Conclusion

Spent coffee grounds are a raw material of great interest for use on biotechnological processes due to their low cost and availability, and mainly due to their antioxidant capacity and presence of phenolic compounds. The eco-friendly extraction method used in the present study revealed to be of value to extract antioxidant phenolic compounds from SCG. The optimal condition predicted an extract with 35.07 mg GAE/g SCG of total phenolic compounds and antioxidant activity of

FRAP = 0.25 mmol Fe(II)/g SCG, DPPH = 121.75 µmol TE/g SCG, ABTS= 130.01 µmol TE/g SCG and TAA= 64.17 mg α-TOC/g SCG.

**Table 1.** Experimental conditions used for extraction of total phenolic compounds (TP) by autohydrolysis of spent coffee grounds, concentration of TP and antioxidant potential of the obtained extracts.

| **Process variables (original and (coded) values)** | | | **Responses [a]** | | | | |
|---|---|---|---|---|---|---|---|
| Temp. | Ratio | Time | TP | FRAP | DPPH | ABTS | TAA |
| (ºC) | (ml/g) | (min) | (mg GAE)* | (mmol Fe(II))* | (µmol TE)* | (µmol TE)* | (mg α-TOC)* |
| 160 (-1) | 5 (-1) | 10 (-1) | 6.09 ± 0.07 | 0.03 ± 0.001 | 18.28 ± 0.09 | 21.53 ± 1.83 | 8.14 ± 0.23 |
| 200 (+1) | 5 (-1) | 10 (-1) | 8.59 ± 0.09 | 0.05 ± 0.002 | 33.06 ± 0.06 | 32.08 ± 0.01 | 12.61 ± 0.27 |
| 160 (-1) | 5 (-1) | 50 (+1) | 8.59 ± 0.14 | 0.06 ± 0.004 | 35.33 ± 0.73 | 34.83 ± 0.15 | 14.15 ± 0.23 |
| 200 (+1) | 5 (-1) | 50 (+1) | 10.95 ± 0.24 | 0.08 ± 0.006 | 40.01 ± 0.19 | 41.39 ± 0.65 | 20.94 ± 0.08 |
| 160 (-1) | 15 (+1) | 10 (-1) | 12.63 ± 0.27 | 0.10 ± 0.004 | 55.74 ± 0.50 | 51.94 ± 0.61 | 26.06 ± 0.26 |
| 200 (+1) | 15 (+1) | 10 (-1) | 19.55 ± 0.77 | 0.15 ± 0.006 | 78.42 ± 0.04 | 85.75 ± 0.28 | 37.38 ± 1.38 |
| 160 (-1) | 15 (+1) | 50 (+1) | 17.39 ± 0.30 | 0.16 ± 0.009 | 96.16 ± 6.94 | 88.59 ± 0.47 | 39.30 ± 1.38 |
| 200 (+1) | 15 (+1) | 50 (+1) | 39.29 ± 0.83 | 0.25 ± 0.008 | 118.15 ± 0.27 | 124.39 ± 3.21 | 64.79 ± 0.98 |
| 160 (-1) | 10 (0) | 30 (0) | 23.57 ± 0.47 | 0.13 ± 0.005 | 63.73 ± 0.45 | 61.50 ± 0.78 | 25.63 ± 0.42 |
| 200 (+1) | 10 (0) | 30 (0) | 28.26 ± 0.23 | 0.19 ± 0.014 | 84.22 ± 0.37 | 57.01 ± 0.20 | 40.29 ± 0.55 |
| 180 (0) | 10 (0) | 10 (-1) | 21.42 ± 0.47 | 0.12 ± 0.007 | 60.70 ± 0.37 | 70.34 ± 0.58 | 22.95 ± 0.18 |
| 180 (0) | 10 (0) | 50 (+1) | 27.57 ± 0.32 | 0.18 ± 0.006 | 93.97 ± 0.88 | 92.89 ± 0.05 | 37.58 ± 1.85 |
| 180 (0) | 5 (-1) | 30 (0) | 10.62 ± 0.07 | 0.07 ± 0.002 | 40.94 ± 0.19 | 34.61 ± 0.34 | 17.08 ± 0.04 |
| 180 (0) | 15 (+1) | 30 (0) | 36.88 ± 0.51 | 0.20 ± 0.002 | 119.01 ± 1.60 | 107.98 ± 0.43 | 45.48 ± 0.36 |
| 180 (0) | 10 (0) | 30 (0) | 21.52 ± 0.50 | 0.14 ± 0.003 | 80.10 ± 6.65 | 70.34 ± 0.58 | 29.80 ± 0.20 |
| 180 (0) | 10 (0) | 30 (0) | 21.40 ± 0.52 | 0.13 ± 0.005 | 78.31 ± 0.31 | 69.80 ± 0.17 | 28.72 ± 0.96 |
| 180 (0) | 10 (0) | 30 (0) | 23.21 ± 0.29 | 0.14 ± 0.003 | 79.05 ± 1.14 | 73.45 ± 1.09 | 31.33 ± 0.88 |
| 180 (0) | 10 (0) | 30 (0) | 25.10 ± 0.65 | 0.14 ± 0.006 | 79.76 ± 0.38 | 72.88 ± 0.98 | 29.12 ± 0.48 |
| 180 (0) | 10 (0) | 30 (0) | 23.77 ± 0.13 | 0.13 ± 0.001 | 81.64 ± 1.35 | 84.65 ± 0.87 | 33.99 ± 1.14 |
| 180 (0) | 10 (0) | 30 (0) | 25.95 ± 0.18 | 0.14 ± 0.003 | 79.05 ± 0.85 | 85.94 ± 0.68 | 32.46 ± 0.84 |

*All the units are expressed per gram of spent coffee grounds.

[a] TP: Total phenolic compounds; FRAP: antioxidant activity by the ferric reducing antioxidant power assay; DPPH: antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl assay; ABTS: antioxidant activity by the 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid assay; TAA: Total antioxidant activity


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