PHENOLIC COMPOUNDS EXTRACTION FROM SPENT COFFEE GROUNDS BY HYDROPHOBIC DEEP EUTECTIC SOLVENTS

Talita R. Prado[1], Cristiane N. Silva[2] & Bernardo D. Ribeiro[1]*

[1] *Chemistry School, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil.*
[2] *Chemistry Institute, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil.*

* *bernardo@eq.ufrj.br*

ABSTRACT

Spent coffee grounds are the main by-product of the coffee industry and are a rich source of phenolic compounds, mainly the chlorogenic acids, with potential antioxidant activity. Furthermore, deep eutectic solvents are a new, green and sustainable alternative to conventional extraction methods for extracting bioactive compounds from plant matrices. Hence, the objective of the present study was to evaluate the use of hydrophobic deep eutectic solvents (HDES) in the extraction of total phenolic compounds from spent coffee grounds (SCG). 12 different HDES were prepared based on terpenoids, fatty acids and fatty alcohols. Extractions were carried out for 2 h, 1000 rpm and 60ºC. The content of total phenolic compounds was determined by the Folin-Ciocalteu method. The results showed that the combination of borneol:dodecanoic acid (HDES 10) and camphor:dodecanoic acid (HDES 3) showed the best efficiency in the extraction of phenolic compounds from SCG, with yields of 289.68 ± 0.55 mg GAE/L and 264.46 ± 2.51 mg GAE/L, respectively, while the lowest yields were obtained by camphor: octanoic acid (HDES 1) and borneol:1-decanol (HDES 12), with values of 4.85 ± 0.13 mg GAE/L and 3.97 ± 0.04 mg GAE/L. Therefore, dodecanoic acid based HDES are promising solvents for extracting phenolic compounds from SCG.

Keywords: By-product. Green Solvent. Bioactive Compounds. Terpenoids. Fatty Acids.

1 INTRODUCTION

Spent coffee grounds (SCG) are one of the main by-products obtained from the coffee industry. It is generated by the instant coffee industry by preparing the drink in commercial establishments and domestic environments. It is estimated that approximately 10.39 million tons of coffee was consumed in 2023[1], with a probability of increasing over the coming years. Most SCG is disposed of inappropriately, resulting in negative environmental impacts. SCG is a rich source of phenolic compounds, caffeine, oil, proteins, and polysaccharides. Phenolic compounds stand out as the main bioactive compounds in SCG. Chlorogenic acids are the most important and abundant phenolics compounds in the by-product. They are highly recognized for their biological properties, including antioxidant, antimutagenic, anti-allergic, anti-inflammatory, neuroprotective and cardioprotective activities[2]. Phenolic compounds can be extracted using deep eutectic solvents (DESs), biocompatible, renewable, biodegradable, non-volatile solvents with lower toxicity and flammability than conventional ones[3]. DES is formed from the combination of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), with a lower melting point than their components. Hydrophobic deep eutectic solvents (HDES) are new green solvents formed from less polar compounds than hydrophilic deep eutectic solvents[3]. The first HDES were formed based on quaternary ammonium salts combined with long alkyl chain compounds, such as terpenoids, fatty acids and fatty alcohols, which weaken the hydrophilic areas in the chemical structure. HDES are characterized by immiscibility in water, due to the length of the carbon chain, and low water content. DES has been studied as a possible alternative to conventional solvents on extraction methods, such as methanol, hexane and chloroform, known for their environmental disadvantages, such as toxicity, flammability and volatility. Therefore, the objective of the present study was to evaluate the application of hydrophobic deep eutectic solvents in the extraction of total phenolic compounds (TPC) from spent coffee grounds.

2 MATERIAL & METHODS

2.1 Obtaining and preparing raw materials

The spent coffee grounds (SCG) were supplied by commercial establishments in Rio de Janeiro - RJ, Brazil. Subsequently, the byproduct was dried in an oven (Tedesco FTT 150 G) at 50 °C for 48 h, until the residual moisture content reached 5-6%. Next, the granulometry was standardized to <0.465 mm. The SCG was placed in polyethylene packaging and stored under refrigeration at <10°C, sheltered from light.

2.2 Preparation of deep eutectic solvents (DESs)

The HDES were prepared as described by Ribeiro et al.[3], in the molar proportions presented in Table 1. The mixture of components was heated to a temperature of 80 °C (IKA C-MAG HS 4), under constant stirring for 30 min until reaching complete homogenization and formation of a clear, colorless liquid. Afterwards, the solvents were stored at room temperature (25 °C).

Table 1 Hydrophobic Deep Eutectic Solvents (HDESs) used for TPCs extractions in the SCGs

HDES	Component 1 (x1)	Component 2 (x2)	Molar Ratio (x1:x2)
HDES 1	Camphor	Octanoic Acid	1.59:1
HDES 2	Camphor	Decanoic Acid	1.75:1
HDES 3	Camphor	Dodecanoic Acid	1.99:1
HDES 4	Camphor	Oleic Acid	1.94:1
HDES 5	Camphor	1-Octanol	1:1.62
HDES 6	Camphor	1-Decanol	1:1.16
HDES 7	Camphor	1-Dodecanol	1.10:1
HDES 8	Camphor	Oleyl Alcohol	1.08:1
HDES 9	Borneol	Decanoic Acid	1:2.15
HDES 10	Borneol	Dodecanoic Acid	0.56:1
HDES 11	Borneol	1-Octanol	1:6.61
HDES 12	Borneol	1-Decanol	1:4.62

2.3 Extraction of total phenolic compounds by HDESs

100 mg of SCG was weighed and 1000 mg of HDES was added, in a solid-liquid ratio of 1:10 (*w/w*). The extraction of total phenolic compounds was carried out in a Thermomixer (ThermoMixer C) for 2 h, 1000 rpm and 60 °C. After the samples were centrifuged for 15 min at 10,000 rpm (Centrifuge 5804 R), the supernatant was collected and stored (-18ºC) for subsequent analyses.

2.4 Determination of total phenolic compounds (TPC)

Total phenolic compounds were determined using the Folin-Ciocalteu methodology, with some modifications made by Almeida et al.[4]. The analysis was carried out in 96-well microplates, mixing 10 µL of the sample and 200 µL of Folin-Ciocalteu solution (1:10 *v/v*). After 3 minutes, the reaction between the sample and the Folin-Ciocalteu solution is stopped by adding 100 µL of sodium carbonate solution (20% *w/v*). Absorbance was measured at 765 nm on a spectrophotometer (Spectramax M2). The standard curve was made with gallic acid equivalent, and the results were expressed in mg of gallic acid per litre of samples (mg GAE/L).

2.5 Statistical analyses

All analyses were performed in triplicate and results were presented as mean values and standard deviation. Mean values were evaluated by analysis of variance (ANOVA) and Turkey mean test at a 5% significance level ($p<0.05$) using Statistica 8.0 software (version 8.0, StatSoft).

3 RESULTS & DISCUSSION

Table 2 shows the results of total phenolic compound content extracted from SCG by hydrophobic deep eutectic solvents.The effect of 12 different HDESs, formed from the combination of terpenoids with fatty acids and alcohols, on the extraction of total phenolic compounds (TPC) from SCG, was evaluated. It is worth mentioning that HDES are solvents normally applied to extract hydrophobic bioactive molecules, due to their low solubility in water. The highest TPC yields were obtained using HDES 10 (borneol + dodecanoic acid) followed by HDES 3 (camphor + dodecanoic acid) with values of 289.68 ± 0.55 mg GAE/L and 264.46 ± 2.51 mg GAE/L, respectively. The highest TPC contents were obtained using HDES based on dodecanoic acid as a component of the eutectic mixture, showing that the combination of this acid with borneol or camphor improved mass transfer and dissolution of the target compounds in the solvent resulting, consequently, in a better efficiency in the extraction of TPCs from SCG. It is important to highlight that for combinations of fatty acids containing 12 or fewer carbon atoms with camphor and borneol, the extraction yield increased as the alkyl chain of the fatty acid increased, showing that the length of the alkyl chain of the HDES can significantly influence ($p \leq 0.05$) the performance of TPC extraction from SCG. However, for combinations using oleic acid and oleyl alcohol, the same behavior was not observed. These results may be attributed to the fact that changes in the length and type of alkyl chain of the HBD (hydrogen bond donor) enable a change in the polarity of the solvent and, therefore, influences the yield of TPC extraction by different HDES[5]. Furthermore, the phenolic compound present in the plant matrice may have different polarities, thus, certain phenolic compounds may be insoluble in a particular solvent[6]. On the other hand, the lowest yields were obtained using HDES1 (4.85 ± 0.13 mg GAE/L) and HDES 12 (3.97 ± 0.40 mg GAE/L), which do not present significant differences ($p \leq 0.05$). The lower yields can be attributed to the alkyl chain size of the HBD, as generally, the longer the carbon chain, the higher the hydrophobicity. Consequently, the lower polarity of the compound and the extraction yield[7]. In general, the lowest efficiency in TPC extraction was obtained using HDESs based on borneol as the HBA (hydrogen bond acceptor). Considering that both camphor and borneol have 10 carbon atoms in their composition, the results can be explained by the fact that not only the hydrophobicity of the solvent influences the extraction yield, but also the viscosity of the HDES, as a higher viscosity results in a slower mass transfer during the extraction[8]. In fact, borneol-based solvents showed a higher viscosity and, therefore, lower TPC extraction yields.

Table 2 Total phenolic compounds (TPC) content extracted from SCG by HDESs

HDES	TPC (mg GAE/L)
HDES 1	4.85 ± 0.13^{k}
HDES 2	64.97 ± 3.23^{c}
HDES 3	264.46 ± 2.51^{b}
HDES 4	40.53 ± 1.42^{e}
HDES 5	8.30 ± 0.47^{j}
HDES 6	24.32 ± 0.31^{g}
HDES 7	53.41 ± 0.05^{d}
HDES 8	10.67 ± 0.52^{i}
HDES 9	22.58 ± 0.18^{h}
HDES 10	289.68 ± 0.55^{a}
HDES 11	27.78 ± 0.00^{f}
HDES 12	3.97± 0.40^{k}

Means with different lowercase letters in the same column are significantly different (p≤0.05).

4 CONCLUSION

This study evaluated the efficiency of 12 hydrophobic deep eutectic solvents in extracting total phenolic compounds from spent coffee grounds. Overall, the combinations of borneol + dodecanoic acid (HDES 10) and camphor + dodecanoic acid (HDES 3) provided the highest extraction yields of phenolic compounds from SCG (289.68 ± 0.55 mg GAE/L and 264.46 ± 2.51 mg GAE/L), indicating that hydrophobic deep eutectic solvents based on dodecanoic acid enabled the best mass transfer and efficiency in extracting phenolic compounds present in SCG. Additionally, the HDESs used in this study are less viscous solvents compared to hydrophilic deep eutectic solvents (DESs). Besides, HDES are from natural origin and non-toxic solvents, which allows the obtained extracts to be incorporated into the formulation of food products, pharmaceuticals, and cosmetics. Hence, HDESs are promising alternatives in extracting total phenolic compounds from SCG and further studies are needed to optimize process parameters (molar ratio, temperature, solid-liquid ratio, and time) to increase the yield of phenolic compounds and their application as a possible alternative to conventional extraction solvents such as hexane, methanol, and chloroform, used mainly for extraction of polar molecules..

REFERENCES

1 INTERNATIONAL COFFEE ORGANIZATION (ICO). Coffee Report And Outlook, 2023.

2 SOLOMAKOU, N., LOUKRI, A., TSAFRAKIDOU, P., MICHAELIDOU, A. M., MOURTZINOS, I., GOULA, A. M. 2022. Recovery from phenolic compounds from spent coffee grounds through optimized extraction processes. Sustain. Chem. Pharm. Volume 25. 100592. ISSN 2352-5541.

3 RIBEIRO, B. D., FLORINDO, C., IFF L. C., COELHO, M. A. Z., MARRUCHO, I. M. 2015. Menthol-based Eutectic Mixtures: Hydrophobic Low Viscosity Solvents. ACS. Sustain. Chem. Eng. Volume 3. Issue 10. 2359-2590. ISSN 2168-0485.

4 ALMEIDA, F. D. L., CAVALCANTE, R.S., CULLEN, P.J., FRIAS, J.M., BOURKE, P., FERNANDES, F.A., RODRIGUES, S. 2015. Effects of atmospheric cold plasma and ozone on prebiotic orange juice. Inn. Food Sci. Emerg. Techn. Volume. 127–135.

5 CAO, J., YANG, M., CAO, F., WANG, J., SU, E. 2017. Tailor-made hydrophobic deep eutectic solvents for cleaner extraction of polyprenyl acetates from Ginkgo biloba leaves. J. Clean. Prod. Volume 152. 399-405. ISSN 0959-6526.

6 TURKMEN, N., SARI, F., VELIOGLU, Y. S. 2006. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. Food Chem. Volume 99. Issue 4. 835-841. ISSN 0308-8146.

7 WEI, J., AN, M., ZHANG, X., WU, G., KANG, S., WU, L., YANG, D., ZHAO, L. 2023. Ultrasonic-assisted hydrophobic deep eutectic solvents for the extraction of seven compounds from Piperis longi fructus-Rhei radix et rhizoma drug pair and their vitro antioxidant evaluation. Sustain. Chem. Pharm. Volume 32. ISSN 2352-5541.

8 ALSAUD, N., SHABAZ, K., Farid, M. 2021. Antioxidant and antibacterial evaluation of Manuka leaves (Leptospermum scoparium) extracted by hydrophobic deep eutectic solvent. Food Chem. Chem. Eng. Res. Des. Volume 174. 96-106. ISSN 0263-8762.

ACKNOWLEDGEMENTS

This work was supported by Faperj and CAPES.