APPLICATION OF DEEP EUTECTIC SOLVENTS IN THE EXTRACTION OF PHENOLIC COMPOUNDS FROM ORANGE BY-PRODUCTS AND SPENT COFFEE GROUNDS

Cristiane N. da Silva[1], Ailton C. Lemes[2] & Bernardo D. Ribeiro[1,2]*

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

* *bernardo@eq.ufrj.br*

Orange by-products and spent coffee grounds are good sources of phenolic compounds, with potential applications in the food, cosmetic and pharmaceutical industries due to their antioxidant properties. Deep eutectic solvents have been explored as a sustainable alternative to organic solvents, mainly in extracting and purifying natural bioactive compounds. This work evaluated 11 DES combinations in extracting phenolic compounds from orange by-products and spent coffee grounds and compared them to the conventional 80% methanol method. The by-products were subjected to extraction for 2 h, 1000 rpm and 60 ºC. The total phenolic compound content of the extracts was evaluated using the Folin-Ciocalteau method. The results showed that DES 6, a combination of lactic acid and cinnamyl alcohol, was the most effective solvent in the extraction of total phenolic compounds from orange by-products and spent coffee grounds, with values of 859.14 ± 1.51 mg GAE/ L and 1306.96 ± 1.51 mg GAE/L, these values being higher than the conventional extraction method using 80% MeOH of 427.79 ± 6.43 mg GAE/L and 579.20 ± 5.75 mg GAE/ L. However, despite the good results, more studies are needed to consider DES as a potential alternative to conventional extraction methods.

Keywords: By-product. Extraction. Green Solvent. Extraction. Bioactive Compounds. Antioxidants.

1 INTRODUCTION

Food industries generate large quantities of by-products from the processing of fruits and vegetables, which are generally discarded inappropriately, resulting in adverse environmental impacts, such as soil contamination and water pollution due to the presence of phytotoxic organic matter, in addition to the growth of disease-carrying microorganisms. Brazil is the world's largest producer of oranges and coffee, and its processing generates large volumes of by-products, with an estimated production of 0.8 to 1 million tons of orange by-products and 1 million tons of SCG in Brazil [1,2]. Orange by-products, represented by the peel, pomace and seeds, are a rich source of phenolic compounds, mainly flavonoids [3]. On the other hand, SCG is the by-product of the industrial production of soluble coffee and the preparation of coffee for consumption in domestic and commercial establishments. SCG stands out for containing phenolic compounds in its composition, particularly chlorogenic acids. Phenolic compounds are biologically active constituents with antioxidant properties capable of delaying lipid oxidation in food products, prolonging their shelf life, and with cardioprotective, neuroprotective, antiallergic, anticancer and anti-inflammatory effects [4].

Phenolic compounds are generally recovered by conventional extraction methods such as soxhlet, maceration and percolation using solvents such as methanol, ethanol, hexane, and acetone. However, these methods require long process times and a high amount of toxic, flammable and volatile solvents, which are harmful to human health and pollutants to the environment [4]. In this context, deep eutectic solvents (DES) have been extensively studied as possible alternatives to conventional extraction methods. DES are a group of green solvents formed by combining two components, the hydrogen bond acceptor (HBA) and the hydrogen bond donor (HBD) [5]. Mixing these components in specific proportions forms a eutectic mixture with a lower melting point than its constituents. DES are biodegradable, versatile, and biocompatible solvents that allow the extraction of extracts rich in bioactive compounds without presenting toxic effects to the consumer. Furthermore, they can be recycled and reused in new extraction processes. [5]. Therefore, this study aims to explore the application of deep eutectic solvents in extracting total phenolic compounds from orange by-products and SCG, offering a more sustainable approach for recovering valuable compounds with high added value.

2 MATERIAL & METHODS

2.1 Obtaining and preparing raw materials

Orange by-products and SCG were used to carry out the experiments. The company Cutrale®, located in São Paulo – SP, Brazil, donated the orange by-products. The by-product was received dry containing a moisture content of 7%. The spent coffee grounds (SCG) were supplied by commercial establishments in Rio de Janeiro - RJ, Brazil. Subsequently, the by-product was dried in an oven (Tedesco FTT 150 G) at 50 °C for 48 h, until the residual moisture content reached 5%-6%. Afterwards, the orange by-products and SCG were milled and standardized for granulometry (<0.465 mm). The by-products were packaged in polyethene, stored under refrigeration at <10 °C and protected from light.

2.2 Preparation of deep eutectic solvents (DESs)

The DESs were prepared as described by Ribeiro et al.[6], 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 forming a clear, colourless liquid. Afterwards, the solvents were stored at room temperature (25 °C).

Table 1 Components of deep eutectic solvents (DESs) and the molar ratio used in the study

DESs	Molar Ratio	Component 1	Component 2
DES 1	2.1: 1	Acetic Acid	Cinnamyl Alcohol
DES 2	1.7: 1	Acetic Acid	Glycerol
DES 3	2.5: 1	Acetic Acid	Proline
DES 4	1.8: 1	Hexanoic Acid	Cinnamyl Alcohol
DES 5	1.3: 1	Hexanoic Acid	Glycerol
DES 6	2.2: 1	Lactic Acid	Cinnamyl Alcohol
DES 7	1.7: 1	Lactic Acid	Glycerol
DES 8	1.9: 1	Lactic Acid	Proline
DES 9	4.5: 1	Lactic Acid	Sorbitol
DES 10	2.7: 1	Propionic Acid	Cinnamyl Alcohol
DES 11	1.8: 1	Propionic Acid	Glycerol

2.3 Extraction of total phenolic compounds by DESs

100 mg of SCG was weighed and 1000 mg of DES was added, in a solid-liquid ratio of 1:10 (*w/w*). The extraction of total phenolic compounds was carried out in a Thermomixer 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 (-18 ºC) for subsequent analyses. For comparison, orange and SCG by-products were subjected to the same process conditions using methanol and water (MeOH 80%:20%).

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.[7]. 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 presents the content of total phenolic compounds extracted from orange by-products. Most of the DES used provided a better yield in the extraction of TPCs for both by-products than extraction using 80% MeOH. However, the best results were obtained with DES 6, a combination of lactic acid and cinnamyl alcohol for orange by-products (859.14 ± 1.51 mg GAE/L) and SCG (1306.96 ± 1.51 mg GAE/L). These values were higher than those obtained by the conventional method using 80% MeOH, with 427.79 ± 6.43 mg GAE/L for orange by-products and 579.20 ± 5.75 mg GAE/L for SCG. Although methanol is a widely used organic solvent for the extraction of TPCs, due to its similarity in polarity and high solubilization capacity for these components, its toxicity encourages the use of alternative solvents in the extraction process, such as DESs [8]. The evaluation of DESs is important because their composition can affect their chemical properties and characteristics and, consequently, their performance in extracting the molecules of interest. The best yields of DES 6 may be related to the fact that the combination of lactic acid and cinnamyl alcohol provides a more significant intermolecular interaction with the target compound, promoting more excellent solubility of this compound and improving the mass transfer process and, consequently, the extraction efficiency [9]. It is worth noting that DES interacts with the phenolic molecule based on the availability of OH hydrogen bonds, facilitating the removal of the target compound. Therefore, compounds with greater capacity for interaction and formation of hydrogen bonds enable extraction with better performance [9]. In contrast, the lowest yields in TPC extraction were obtained using combinations of hexanoic acid with cinnamyl alcohol or glycerol for both by-products studied. The values were 216.84 ± 3.01 mg GAE/L (hexanoic acid: cinnamyl alcohol) and 126.51 ± 2.53 mg GAE/L (hexanoic acid: glycerol) for orange by-products, respectively, while for SCG they were 171.55 ± 2.61 (hexanoic acid: glycerol) and 70.75 ± 1.51 mg GAE/L (hexanoic acid: cinnamyl alcohol), respectively, values lower than extraction by 80% MeOH. These results show that the combination of hexanoic acid with cinnamyl alcohol or glycerol results in less intermolecular interaction with phenolic compounds, reflecting the low extraction yield. DES are good alternatives to conventional methods of extracting TPCs from agro-industrial by-products. Using lactic acid, cinnamyl alcohol efficiently extracted phenolic compounds from orange and SCG by-products.

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

Deep Eutectic Solvents (DES)	Total Phenolic Compounds (mg GAE/L)	
	Orange By-products	Spent Coffee Grounds
MeOH 80%	427.79 ± 6.43^{i}	579.20 ± 5.75^{g}
DES 1	451.35 ± 2.61^{h}	677.44 ± 3.98^{e}
DES 2	625.97 ± 1.51^{b}	595.54 ± 2.80^{f}
DES 3	420.69 ± 2.61^{j}	339.30 ± 3.98^{i}
DES 4	216.84 ± 3.01^{k}	70.75 ± 1.51^{l}
DES 5	126.51 ± 2.53^{l}	171.55 ± 2.61^{k}
DES 6	859.14 ± 1.51^{a}	1306.96 ± 1.51^{a}
DES 7	486.09 ± 3.01^{g}	827.83 ± 1.51^{c}
DES 8	537.71 ± 3.01^{c}	909.02 ± 2.61^{b}
DES 9	502.74 ± 2.61^{f}	749.69 ± 3.98^{d}
DES 10	527.36 ± 3.98^{e}	269.97 ± 3.00^{j}
DES 11	534.10 ± 1.51^{d}	393.78 ± 3.98^{h}

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

4 CONCLUSION

This work presents the application of DES in extracting total phenolic compounds from orange by-products and coffee grounds. DES based on lactic acid and cinnamyl alcohol provided higher yields in the extraction of total phenolic compounds from orange and SCG by-products, with values of 859.14 ± 1.51 mg GAE/L, and 1306.96 ± 1.51 mg GAE/ L, respectively, being higher than those obtained by the conventional method using 80% MeOH. These results show that the combination of lactic acid and cinnamyl alcohol is more efficient in extracting total phenolic compounds from the by-products under study when compared to 80% MeOH. The use of DES can increase the stability of phenolic compounds during the extraction process, providing greater yields in the extraction of these bioactive compounds. In addition to being a more sustainable alternative, due to the lower toxicity, flammability, and volatility of these solvents, it allows obtaining an extract rich in phenolic compounds and safe, which can be applied in the formulation of new products in the food, cosmetic and pharmaceutical industries. The high availability of HBAs and HBDs gives DES enormous versatility and, therefore, allows the extraction of different phenolic compounds from plant matrices. Thus, DESs are a promising alternative for extracting phenolic compounds with antioxidant activity from orange by-products and SCG. However, optimization studies must evaluate the best process conditions (solid-liquid ratio, temperature and time) to recover phenolic compounds from these agro-industrial by-products.

REFERENCES

1 CASTRO, L. A., LIZI, J. M., CHAGAS, E. G. L., CARVALHO, R. A., VANIN, F. M. 2020. From the by-product of orange juice in the food industry to a functional ingredient: application in the circular economy. Foods. Volume 9. Issue 5. 593.

2 FREITAS, C. P. M., MARANGON, B. B., PEREIRA, E. G., RENATO, N. S. 2023. Exploring spent coffee grounds energy potential in the Brazilian Scenario. Eng. Agric. Volume 43. ISSN 1809 4430.

3 SILVA, C. N., ALMEIDA, L. M. P., LEMES, A. C., RIBEIRO, B. D. 2024. Citrus by-products valorization using deep eutectic solvents – A review. Food Biosci. Volume 60. 104446. ISSN: 2212-4292.

4 ZHAO, N., LIU, Z., YU, T., YAN, F. S. 2024. Spent coffee grounds: Present and future of environmentally friendly applications on industries-A review. Tends Food Sci Tech. Volume 143. 104312.

5 POSTA, D. S., GALLO, V., GENTILI, A., FANALI, C. 2022. Strategies for the recovery of bioactive molecules from deep eutectic solvent extracts. TrAC Trend. Anal. Chem. Volume 157, 116798. ISSN 0165-9936.

6 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.

7 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. ISNN 1466 8564.

8 OZTURK, B., PARKINSON, C., GONZALEZ-MIQUEL, M. 2018. Extraction of polyphenolic antioxidants from orange peel waste using deep eutectic solvents. Sep. Purif. Technol. Volume. 206, 1-13. ISSN 1383 5866.

9 ALSAUD, N., SHAHBAZ, K., FARID, M. 2021. Application of deep eutectic solvents in the extraction of polyphenolic antioxidants from New Zealand Manuka leaves (*Leptospermum scoparium*): Optimization and antioxidant activity. J. Mol. Liq. Volume 337, 116385, 2021. ISSN 0167 7322.

ACKNOWLEDGEMENTS

This work was supported by Foundation Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil.