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Microwave-assisted solid-liquid extraction using propylene glycol as solvent in the recovery of bioactive compounds from umbu seeds

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Abstract: Umbu (*Spondias tuberosa*) is a Brazilian Caatinga native fruit that has a socioeconomic importance. Pulp is the main product of its agro-industrialization. However, depulping gives rise to about 25% of residue. It is known that fruit peels and seeds are composed of macro and micronutrients as well as bioactive compounds, becoming the use of umbu residue an opportunity to add value to the fruit agro-chain. As the recovery of bioactive compounds from umbu seeds has not yet been optimized, this work aimed to evaluate their recovery through a more sustainable approach, using propylene glycol as solvent in microwave-assisted solid-liquid extraction. For that, an experimental design varying the propylene glycol percentage (15-85%) and temperature (59-201 °C) was adopted. The total phenolic compounds (TPC) and antioxidant capacity by DPPH• were evaluated as responses. The highest values were found at 201 °C and 50% propylene glycol (3242 mg GAE/100 g and 162 µmol Trolox/g for TPC and DPPH•, respectively). The values observed were, at least, 8 times higher than those obtained in the worst experimental condition (25% propylene glycol and 80 °C), showing that the independent variables had effect on the evaluated responses (p<0.05). All models were significant, displaying *p* value <0.05 and no lack of fit was observed. High adjusted R^2 values (>0.95) confirm a good fit of the data to the models. Thus, microwave-assisted extraction employing propylene glycol as solvent can be a more sustainable way for the use of umbu seeds.

Keywords: Spondias tuberosa; antioxidant capacity; Brazilian Caatinga

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1. Introduction

The umbu tree (*Spondias tuberosa*) is a fruit tree native to the Brazilian Caatinga and belongs to the Anacardiaceae family, also known as the "sacred tree of the Sertão" named by Euclides da Cunha. Its name in Tupi-Guarani is "ymbu" which means "drinking tree" due to its ability to store water in its roots [1]. Relevant bioactive compounds, such as quercetin, rutin and vitamin C, are part of its phytochemical composition, as already reported by Ribeiro et al. [2]. Therefore, it has interesting biological properties for different areas of the industry. In addition, the fruit presents a relevant socioeconomic importance since it is present in the diet of the population and its cultivation can increase the income of families from semiarid region. The main product of the agro-industrialization of this fruit is the frozen pulp. However, the depulping process can generate a significant amount of residue. A total of 25% in mass can be obtained after processing. This residue is composed of the seeds, peels, and refining cake, which contains compounds with high added value, making the use of this material very advantageous [2].

The extraction process of bioactive compounds conventionally uses solvents known for their toxicity and inflammability, which makes the procedure dangerous and requires purification steps before their application. Thus, in order to avoid these problems and make the process more sustainable, safer and more environmentally friendly, green solvents have been evaluated [3].

Propylene glycol is a clear, slightly viscous, water-miscible liquid. It is used as solvent for aromas, essences and fragrances [4]. These characteristics make it a potential substitute for the most inflammable and toxic conventional solvents. Due to its non-toxic nature, a propylene glycol bioactive extract offers the advantage of being able to be used directly in a formulation [5].

In order to increase extraction efficiency and become more sustainable process, the solid-liquid extraction can be microwave-assisted. When compared with conventional heating methods, it exhibits a shorter processing time as it is possible to reach high temperatures in a short time as the heat is dissipated evenly in the plant material, reducing the degradation of the target compounds [6].

Therefore, this work aimed at evaluating recovery of bioactive compounds of umbu seeds through a more sustainable approach, using propylene glycol as solvent in microwave-assisted solid-liquid extraction.

2. Materials and Methods

2.1. Samples

The umbu seeds used in this study were obtained from the depulping process carried out in the pilot plant of Embrapa Agroindústria de Alimentos (Guaratiba, Rio de Janeiro). The residue was dried at 50 °C in an oven with forced air ventilation and disintegrated to obtain a pulverized material. By means of the granulometric analysis of the sample, it was possible to identify that approximately 54% of the material is composed of particles with a diameter between 1.00 and 1.68 mm.

2.2. Microwave-assisted solid-liquid extraction

In order to optimize the extraction process, an experimental design was used (Table 1). Different propylene glycol concentrations (15-85%) and temperatures (59-201 °C) were evaluated as independent variables. The extraction time and solid-liquid ratio were fixed at 10 min and 1:30 (m/v), respectively. The extraction was performed in microwave Ethos 1, Milestone. At the end of each extraction, the extracts were vacuum filtered and stored under freezing until analysis for total phenolic compounds (TPC) and antioxidant capacity by DPPH• assay. Experimental data were analyzed by response surface methodology using a second-order polynomial equation. Analysis of variance (ANOVA), test to determine the lack of fit of the models, and coefficient of determination (R^2) were used to verify the significance of the model considering a significance level of 5%.

2.3. Methods

2.3.1. DPPH• assay

The DPPH• method was performed according to the methodology proposed by Hidalgo, Sanchez-Moreno and Pascual-Teresa [7]. For the reactions, 100 μL of each extract was added to 2900 μL of DPPH• solution (6×10^{-5} M in methanol and diluted to obtain an absorbance of 0.700 at 517 nm) and allowed to react for 30 minutes at room temperature. Then, absorbance was read at 517 nm using methanol as a blank. The results were obtained from the elaboration of a standard curve of Trolox with different concentrations in a range of 80-700 μmol. Results were expressed as μmol Trolox/g.

2.3.2. Total phenolic compounds (TPC)

This analysis was performed with the Folin-Ciocalteu reagent according to the method described by Georgé et al. [8] For that, 250 μL of each extract were mixed with 1250 μL of 10% Folin-Ciocalteu (v/v) and 1000 μL of 7.5% Na_2CO_3 (w/v). Subsequently, the mixtures were heated to 50 °C for 15 minutes, being cooled in an ice bath to read the absorbance at 760 nm. The results were obtained with the aid of a calibration curve prepared from gallic acid solutions with concentrations ranging from 10 to 100 mg/L. The content of TPC in the extracts was expressed as mg gallic acid equivalents per 100 g of sample (mg GAE/100 g).

2.4. Statistical analysis of data

The experimental data obtained was evaluated on Statistica software version 13 (Dell Inc.), through analysis of variance (ANOVA) and Pareto chart. The test to determine the model's lack of fit and determination of R^2 were employed to verify the model significance, considering a confidence interval of 95%.

3. Results and Discussions

By means Table 1 it is possible to verify that both solvent composition and the processing temperature influenced the evaluated responses, since the antioxidant capacity and the content of TPC varied considerably among the obtained extracts. The analysis of the antioxidant capacity of the extracts by the DPPH• method shows that this potential ranged from 16 to 162 μmol Trolox/g reached a value ten times higher in the trial 8, 201 °C and 50% propylene glycol.

The content TPC in the extracts ranged from 406 to 3242 mg GAE/100 g, resulting in a value eight times higher compared to the worst experimental condition (trial 1: 25% propylene glycol, 80 °C), corroborating the results for antioxidant capacity. It is worth noting that the best response is twice as high as the result reported by Petchsomrit et al. [5] (1503 mg GAE/100 g), which evaluated the extraction of bioactive compounds from *Buchanania siamensis*, in a microwave-assisted extraction process at a temperature of 60 ◦C, using a solvent of 50% propylene glycol in water for 20 min.

Extract obtained in the best condition of the experimental design (trial 8) also showed a higher concentration of TPC (3242 mg GAE/100 g) when compared to extracts obtained using microwaves as in the case of red onion (1239 GAE/100 g) [9], different pepper species (82-2939 mg GAE/100 g) [10], cherry processing residue (1414 GAE/100 g) [11], and umbu seeds by conventional extraction using acetone as solvent (947 g GAE/100 g) [12].

The Pareto diagram (Figures 1 and 2) presents the effects of the independent variables on responses. It is possible to observe that the temperature and propylene glycol had an effect on TPC and DPPH•. The linear effect of the temperature showed a positive value ($p<0.05$), demonstrating that the increase in the temperature favors the recovery of bioactive compounds. Furthermore, the quadratic effect was also significant, proving that there is a limit to this behavior. Higher temperatures favor the extraction of biocompounds, by reducing the solvent´s viscosity, making easier the solvent-sample interaction, increasing the solubility of the target compounds and the diffusion coefficient [13]. However, very high temperatures can result in the degradation of certain substances, reducing the efficiency of the extraction process, which requires studies of process optimization [14,5]. The positive linear effect of propylene glycol concentration in the solvent was also significant, showing that increase of propylene glycol concentration in the extractive solution improves the efficiency of the process. This behavior is explained by the chemical affinity of propylene glycol with the sample's compounds, that is, a solvent with an intermediate polarity interacts well with a wide range of bioactive compounds, such as phenolic compounds. Furthermore, the combination of the propylene glycol with water is required in order to reduce the viscosity of the solvent system [15]. Regarding the statistical analysis, all models were significant for predicting the behavior of the responses, since the calculated F values (36 and 295 for TPC and DPPH$^\bullet$, respectively) were higher than the listed F value ($F_{5,5}$=5.05) at α=0.05. Adjusted R^2 values were higher than 0.95, which explains at least 95% of the data variability.

Thus, these results show the potential of the umbu seeds to obtain an antioxidant extract and that the more sustainable approach by combining microwaves and propylene glycol provided promising results.

Table 1. Experimental design for optimization of microwave-assisted extraction of antioxidant compounds from umbu seeds using propylene glycol as solvent and results for content of total phenolic compounds (TPC) and antioxidant capacity by DPPH$^\bullet$ assay. .

Trial	Propylene glycol (%)	Temperature (°C)	DPPH$^\bullet$ (µmol Trolox/g)	TPC (mg GAE/100 g)
1	25	80	16	406
2	25	180	99	2289
3	75	80	21	801
4	75	180	143	3187
5	15	130	22	970
6	85	130	56	1159
7	50	59	20	551
8	50	201	162	3242
9	50	130	71	1603
10	50	130	65	1498
11	50	130	64	1413

GAE: Gallic acid equivalent.

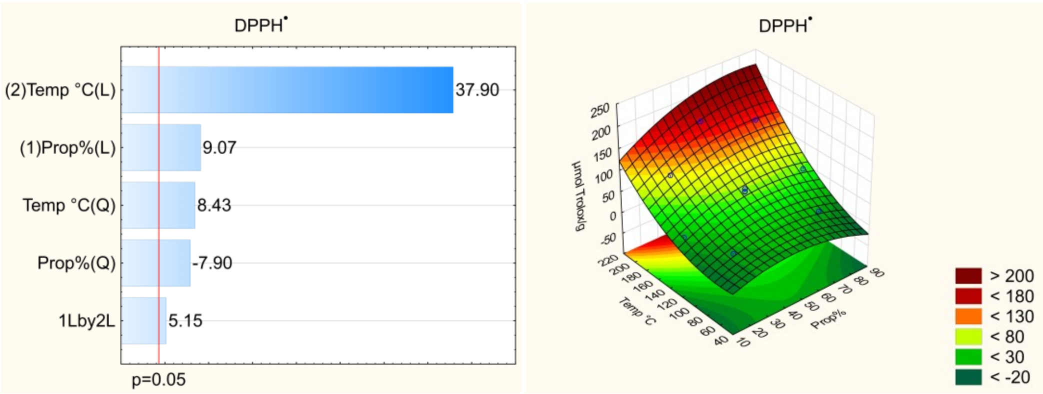

Figure 1. Effect of the independent variables on the antioxidant capacity by DPPH$^\bullet$ assay and response surface.

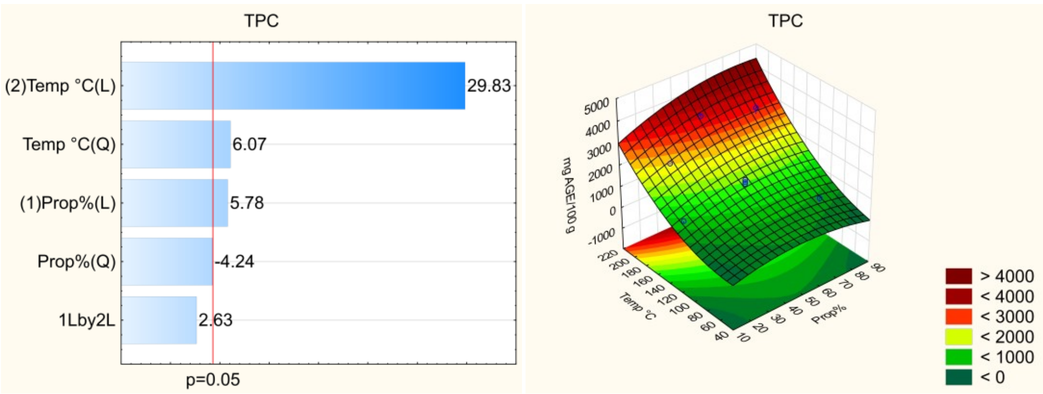

Figure 2. Effect of the independent variables on the total phenolic compounds (TPC) and response surface.

4. Conclusion

From the results, it is possible to conclude that using propylene glycol as a green solvent in microwave-assisted solid-liquid extraction favored the recovery of bioactive compounds from the umbu seeds. Also, the condition that produces extract with higher values of antioxidant capacity and content of total phenolic compounds was at 201 °C, using a 50% propylene glycol solution as solvent. Thus, microwave-assisted extraction employing propylene glycol as solvent can be a more sustainable way to add value to the umbu seeds, considered a residue in this agro-chain.

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