GREEN EXTRACTION OF BIOACTIVE COMPOUNDS FROM COCOA HULLS

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ABSTRACT

The objective of this study was to extract total phenolic compounds (TPC) from cocoa hulls (CH), an agro-industrial residue, using two sustainable processes: heat-assisted extraction (HAE) and ultrasound-assisted extraction (UAE). The effects of pretreatment on the composition of phenolic compounds and the antioxidant activity of this residue were also evaluated. The raw cocoa hull was subjected to heat-assisted pretreatment.The modified Folin-Ciocalteu method was used to determine the phenolic compounds, and the ABTS (2,2-azino-bis (ethylbenzo-thiazoline6-sulfonic acid) diammonium salt)) method was used to determine antioxidant activity. The UAE method extracted 3.0 g/100g of TPC from the pretreated hulll and 5.7 g/100g from the raw hull, while the HAE method extracted higher values for both samples, 6.6 g/100g for and 16.7 for pretreated and raw hulls, respectively. In terms of antioxidant activity, raw CH showed the greatest antioxidant action, 15.6 g/100g. The HAE method proved to be more efficient for obtaining TPC and extracts with greater antioxidant activity from CH. Cocoa hull can be considered a promising residue to be used in food and pharmaceutical sectors because of it antioxidant activity.

Keywords: Agroindustrial residues. Phenolic compounds. Antioxidant activity. *Theobroma cacao*.

1 INTRODUCTION

The agroindustry is currently responsible for meeting the high demand for agricultural products through large-scale food production. However, the agroindustrial sector is equally responsible for generating solid residueand effluents, which can be toxic to humans or the global ecosystem.[1]

Among the various cultivated products, we can mention *Theobroma cacao*, popularly known as cocoa, a fruit widely used in the production of chocolate.[2] Countries on the African continent, such as Côte d'Ivoire and Ghana, are the world's primary cocoa producers, generating around 2,150,000 and 850,000 tons, respectively.[3] Latin American countries are also prominent in the cocoa production market, accounting for 15.3% of world production. For example, in the last estimate made in 2021, Brazil ranked seventh in the world cocoa production ranking.[4]

In cocoa processing, only 20-30% of the fruit is used, corresponding only to the cocoa bean and pulp. The main residue generated is the cocoa hull, representing around 70-80% of the total weight of the fruit, residue that is usually discarded incorrectly.[5] The cocoa hull has been used for producing biochar, animal feed, fuel, and cosmetics,[6,7,8,] and just like the fruit, it has different properties and components that can be widely used, especially the phenolics compounds. The inappropriate use or disposal of this residue can generate economic losses and environmental contamination.[9]

In recent years, there has been a growing interest in the development of less polluting processes for the extraction of phenolic compounds from lignocellulosic residues, mainly aiming to replace the use of organic solvents such as methanol, ethyl acetate, and hexane[10] by green solvents, such as water. Using solvents with lower toxicity for the extraction allows the recovery of bioactive compounds using a sustainable approach and providing a way to use them in food or pharmaceutical products. Additionally, combining solvents and using physical and physical-chemical processes, such as temperature and ultrasonication, can provide some advantages, such as shorter extraction times, reduced or eliminated wastewater generation, and improved quality of extracts.[9,11]

This study sought to valorize cocoa hulls (CH) by extracting phenolic compounds using two sustainable solvent extraction processes: heat-assisted extraction (HAE) and ultrasound-assisted extraction (UAE). The effects of pretreatment on the composition of phenolic compounds and the antioxidant activity of this residue were also evaluated.

2 MATERIAL & METHODS

The cocoa hull was supplied by ECOM Agroindustrial Corp. Limited (Mexico). The residue was dried at 50 °C for 24 h in an air-circulating oven (MA 415, Marconi, Piracicaba, Brazil) and ground to ensure homogeneity in particle size using a knife mill MA-680 (Marconi, Piracicaba, Brazil). All the reagents used in this study were of analytical grade PA (pure for analysis).

Pretreatment of *raw CH*

Raw CH was subjected to heat-assisted pretreatment to eliminate possible interfering compounds in future analyses, such as pectic substances. The method described by Dimawarnita was used with modification.[12] The raw CH was placed in Erlenmeyer flasks containing distilled water, maintaining a ratio of 1:25 (CIN:water), and then the sample was autoclaved for 40 min, 121 °C

and 1 atm (Prismatec, SP, Brazil). After the process, the sample was filtered while still hot using polyester fabric. The resulting solid residue was dried for 24 h at 45 °C in an air-circulating oven (Marconi MA 035, Piracicaba, SP, Brazil). After 24 h of drying, the pretreated cocoa hulls (PTCH) were stored in plastic containers at room temperature for later use in the analysis of bioactive compounds and antioxidant activity.

Heat-Assisted Bioactive Extraction (HAE)

The heat-assisted extraction of total phenolic compounds (TPC) was carried out according to the Rebollo-Hernanz methodology with modifications.[13] Each sample was mixed with distilled water at a concentration of 0.02 $g.mL^{-1}$ in Erlenmeyer flasks, which were submitted to a Dubnoff bath (TECNAL, TE-053, SP Brazil) at 100 °C for 50 min, with continuous stirring. Once the extraction was complete, the samples were stored at -6 °C (ELUX Refrigerator, RDE37, Brazil) until further analysis.

Ultrasound-assisted Bioactive Extraction (UAE)

The extraction of bioactive compounds using ultrasound-assisted methodology was carried out according to Jafari with modifications.[14] Around 10 g of each sample were added to distilled water (170 mL). The material was subjected to ultrasonication (QSonics, Q700, Newtown, CT, United States) at 40°C for 30 min, at 20 KHz, a power of 700 W and amplitude of 60%, using a Qsonica probe (3/4"-19.1 mm, United States). The samples were then filtered through the polyester fabric, and the filtrate was frozen at -6 C° (ELUX Refrigerator, RDE37, Brazil) for further analysis.

Phenolic Compound Quantification

The modified Folin-Ciocalteu method was used to determine the phenolic compounds.[15] 100 µL of the extract from each sample, 8.6 mL of distilled water, 300 µL of Folin-Ciocalteu reagent (0.9 N), and 1.0 mL of 20% sodium carbonate solution (Na_2CO_3) were added to a test tube. After homogenization, the samples were kept for 1 h in a dark ambient for later analysis in triplicate by spectrophotometry at 765 nm (Libra S22 UV/Vis, Biochrom, United States).

Antioxidant activity determination

The ABTS (2,2-azino-bis (ethylbenzo-thiazoline6-sulfonic acid) diammonium salt) method was used to determine antioxidant activity. An aliquot of 10 µL of the extract of each sample was added to 4.0 mL of ABTS solution (5 mg/mL), and after 6 min, a reading was taken at 730 nm (Libra S22 UV/Vis, Biochrom, United States).[16]

Statistical Analysis

The quantification of phenolic compounds and the antioxidant activity of the samples were evaluated by one-way ANOVA analysis of variance, and the individual differences between the means were evaluated using the Tukey test. The statistical analyses were carried out using R software (version 4.3.1). The significance level was $p<0.05$.

3 RESULTS & DISCUSSION

Figure 1 shows that obtaining phenolic compounds by HAE was more efficient, doubling and tripling the TPC values compared to the samples obtained from AUE for pretreate and raw samples, respectively. The increase in temperature possible resulted degradation of plant structures and molecules, including lignin, favoring the release of phenolic compounds previously associated with other substances, resulting in a higher concentration of TPC accessible to extraction.[17] Exposure of the residue to different temperatures also interferes with the types of phenolic compounds extracted, as some molecules may be more resistant than others, requiring more energy to disassociate or degrade when exposed to small changes in temperature.[17,18,19]

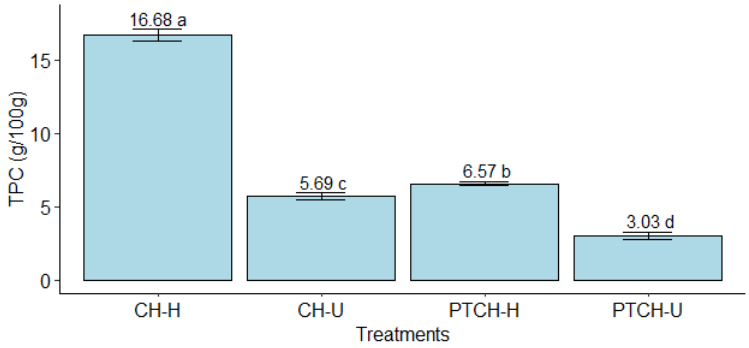

Figure 1 Concentration of the content of total phenolic compounds (TPC) in cocoa hulls from two extraction methods: heat-assisted extraction (HAE) and ultrasound-assisted extraction (UAE). CH-H: raw CH submitted to HAE; CH-U: raw CH submitted to UAE; PTCH-H: pretreated CH submitted to HAE; PTCH-U: pretreated CH submitted to UAE.

With regard antioxidant action, the samples that obtained the highest antioxidant activity were those obtained by HAE and the PTCH-H sample (Figure 2). Phenolic compounds have antioxidant properties and can inhibit free radicals in solution; however, the effectiveness of the antioxidant action depends on the type of phenolic compound, its concentration and which free radicals.[20] The high antioxidant activity may be associated with the concentration of total phenolic compounds.

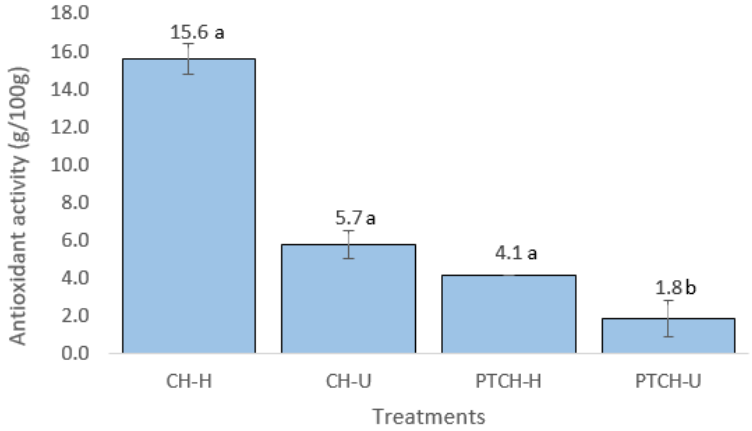

Figure 2 Antioxidant activity of cocoa hulls from two extraction methods: heat-assisted extraction (HAE) and ultrasound-assisted extraction (UAE).CH-H: raw CH submitted to HAE; CH-U: raw CH submitted to UAE; PTCH-H: pretreated CH submitted to HAE; PTCH-U: pretreated CH submitted to UAE.

The antioxidant capacity of cocoa hulls allows them to be widely applied in the food and pharmaceutical industries, among others. In the literature, there are reports of the benefits obtained from consuming CH, which showed stable antioxidants in gastric juice, causing beneficial effects on the endothelial cells of the blood vessels of rats, and could be applied for cardiovascular treatment.[21] Other studies have reported the potential application of cocoa hulls for enriching foods, where their application brought higher values of phenolic compounds and antioxidant activity.[22,23] Thus, the study and development of alternative phenolic compound extraction methods allows cocoa hulls to be applied in different areas of industry.

4 CONCLUSION

Heat-assisted extraction (HAE) proved to be more effective than ultrasound-assisted extraction (UAE) to obtain extracts with higher total phenolic compounds and higher antioxidant action. In addition, this study showed that the pretreatment of CH did not favour the extraction of phenolic compounds. Cocoa hull can be considered a promising residue to be used in food and pharmaceutical sectors because of it antioxidant activity.

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