

PROCESS OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION OF POLYPHENOLS FROM COCOA BEAN SHELLS AND THEIR ANTIOXIDANT POTENTIAL

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ABSTRACT

Food waste is a valuable source of bioactive compounds, and cocoa bean shells (*Theobroma cacao* L.) are rich in phenolic compounds with antioxidant potential. This study optimized ultrasound-assisted extraction of polyphenols by varying solvent type, solid-to-solvent ratio, and time, and evaluated the extracts' antioxidant activity. The results showed that extraction conditions significantly affected both total polyphenol content (TPC) and IC₅₀ values. The highest TPC was obtained with 50% ethanol, while optimal conditions were a 1:30 ratio and 30 min extraction. Antioxidant activity improved with higher TPC, but only a weak correlation was observed ($r = -0.37$), indicating that activity depends not only on total phenolics but also on the extract's chemical profile. Ultrasound-assisted extraction was confirmed as an efficient method for obtaining antioxidant-rich cocoa bean shell extracts.

INTRODUCTION

Food waste represents a significant environmental problem, but it can also serve as a source of bioactive compounds, dietary fibers, and natural antioxidants for the development of functional food products. Valorization of food waste materials contributes to the principles of the circular economy, the reduction of environmental pollution, and the more efficient use of agro-industrial by-products [1,2]. Cocoa bean shells from *Theobroma cacao* L. are a valuable by-product rich in flavonoids, phenolic acids, methylxanthines (theobromine and caffeine), dietary fibers, phytosterols, amino acids, and microelements, thus showing significant antioxidant and functional potential [1,3]. Ultrasound-assisted extraction allows for shorter extraction times, lower solvent consumption, and higher efficiency of bioactive components separation due to

the cavitation effect and improved mass transfer [4]. Therefore, this study aimed to optimize the ultrasound extraction process from cocoa shell *via* evaluation of polyphenol yield and antioxidant potential of developed extracts.

EXPERIMENTAL

The optimization of the extraction from cocoa bean shells was performed by varying the solid-to-solvent ratio (1:10, 1:20, 1:30, and 1:40), the extraction medium (30%, 50%, and 70% ethanol, and water), and time (15, 30, 45, and 50 min), using an ultrasound bath. Total polyphenol content (TPC) was measured using the previously described Folin-Ciocalteu method [4]. Antioxidant potential of the obtained extracts was evaluated using the DPPH• assay by measuring absorbance changes at 517 nm [5].

RESULTS AND DISCUSSION

The effects of ultrasound extraction conditions on the TPC and antioxidant activity of cocoa bean shell extracts are presented in Table 1.

Table 1. Total polyphenol content (TPC) and antioxidant potential of cocoa bean shell extracts obtained by ultrasound extraction.

Extraction conditions	TPC (mg GAE*/g plant material)	IC ₅₀ DPPH• (mg/mL)
1:10, 30% EtOH, 30 min	12.09±1.18 ^c	60.71±2.00 ^d
1:20, 30% EtOH, 30 min	14.32±1.04 ^{bc}	29.65±1.61 ^b
1:30, 30% EtOH, 30 min	15.77±1.23 ^b	21.99±0.55 ^a
1:40, 30% EtOH, 30 min	14.22±1.11 ^{bc}	23.44±1.02 ^a
1:20, H ₂ O, 30 min	9.46±0.74 ^d	39.46±1.42 ^c
1:20, 50% EtOH, 30 min	18.18±1.07 ^a	29.27±0.75 ^b
1:20, 70% EtOH, 30 min	9.00±0.44 ^d	29.26±1.00 ^b
1:20, 30% EtOH, 15 min	13.22±0.91 ^c	29.00±1.24 ^b
1:20, 30% EtOH, 45 min	16.24±1.25 ^b	29.97±0.87 ^b
1:20, 30% EtOH, 60 min	12.42±0.75 ^c	30.50±1.34 ^b

*GAE, gallic acid equivalent; IC₅₀, concentration for 50% inhibition of DPPH radicals; EtOH, ethanol; different letters in superscript indicate statistically significant differences between samples (n = 3, p < 0.05, one-way ANOVA, Duncan *post hoc* test).

The results presented in Table 1 show that the ultrasound extraction conditions significantly affect the TPC in cocoa bean shell extracts. The highest TPC was recorded with 50% ethanol (~18.18 mg gallic acid equivalent/g of plant material), indicating that the mixture of water and ethanol provides more efficient extraction of phenolic compounds compared to pure water or higher ethanol concentrations. This result is in accordance with literature data stating that mixtures of water and ethanol often show higher efficiency in polyphenol extraction compared to pure solvents, due to better solubility of different groups of phenolic compounds [4,6]. Increasing the solid-to-solvent ratio to 1:30 led to an increase in the TPC value, while further increase to 1:40 had no positive effect. Also, extending the extraction time to 45 min increased the TPC, while after 60 min, there was a decrease, probably due to the degradation of sensitive phenolic compounds and possible oxidation of the extracted components [4,7]. The results obtained indicate that all tested variables are key factors for the efficient extraction of polyphenols from cocoa bean shells. The results of the IC₅₀ DPPH analysis indicate that the extraction conditions also significantly affect the antioxidant activity of cocoa bean shell extracts (Table 1). Namely, lower IC₅₀ values indicate a higher ability to neutralize free radicals. The most pronounced antioxidant activity was achieved at the solid-to-solvent ratio of 1:30 and 1:40 with 30% ethanol for 30 min, where the lowest IC₅₀ values were recorded (~21.99 and ~23.44 mg/mL, respectively). Water extract showed significantly weaker antioxidant activity (~39.46 mg/mL), while the ratio of 1:10 yielded the highest IC₅₀ value (~60.71 mg/mL), indicating the lowest extraction efficiency of antioxidants. Changes in ethanol concentration and extraction time did not significantly affect the IC₅₀ values. The results confirm that selecting an appropriate solvent type and solid-to-solvent ratio increases the antioxidant potential of cocoa bean shell extracts. The Pearson correlation analysis showed a weak-to-moderate negative correlation between TPC and IC₅₀ values ($r = -0.37$). The negative sign of the correlation indicates that an increase in polyphenol content is accompanied by a decrease in IC₅₀ values, *i.e.*, an increase in the antioxidant activity. However, the obtained correlation is not statistically significant ($p > 0.05$), which suggests that the antioxidant capacity of the extracts does not depend solely on the TPC, but also on the composition and structure of individual phenolic compounds present in the extracts [8].

CONCLUSION

Based on the results obtained, it can be concluded that the ultrasound extraction conditions significantly affect the TPC and the antioxidant activity of cocoa bean shell extracts. The most effective solvent was 50% ethanol, while the optimal ratio and extraction time were 1:30 and 30 min, respectively. Although the increase in TPC was followed by an improvement in antioxidant activity, correlation analysis showed only a weak relationship between these parameters.

The data confirm that ultrasonic extraction is an effective procedure for obtaining antioxidant-rich extracts from cocoa bean shells and indicate their potential for further valorization as a functional ingredient.

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