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# Valorization of cacao pod husk through supercritical fluid extraction of phenolic compounds

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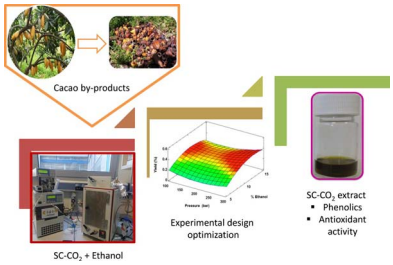
## GRAPHICAL ABSTRACT




## ARTICLE INFO

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## ABSTRACT

Cacao pod husk (CPH) is the main co-product of the chocolate industry. In the present work; a Box-Behnken design was employed to optimize the SC-$CO_2$ for the development of suitable and green process focused on obtaining an extract enriched in phenolic compounds from this by-product, considering temperature, pressure and co-solvent (ethanol) as factors. The response variables selected were yield, total phenolics and total antioxidant capacity (ABTS assay). Extraction pressure and ethanol percentage were the main factors influencing the yield of the target compounds. The extract obtained at the optimum conditions (60 °C, 299 bar and 13.7% of ethanol) presented 0.52% of yield, 12.97 mg GAE/g extract and, 0.213 mmol TE/g extract which were well adjusted to the ones predicted. The findings of this study showed that supercritical fluid extraction could be used as a technique to obtain an extract enriched in phenolic compounds from CPH.

## 1. Introduction

*Theobroma cacao* belonging to *Sterculiaceae* family; is one of the most important tropical crops worldwide. During chocolate manufacture it is necessary removing the beans from the pods generating huge quantities of by-products [1,2]. The pod husk is the major of these by-products accounting for, about 75% the weight of the whole fruit [3,4]. Unfortunately these by-products are discarded, unexploited and left on the cocoa plantation propagating diseases such as black pod rot and producing foul odors [5,6]. Nonetheless, cacao by-products can be employed as an alternative human/animal feeds or industrial applications. CPH has been used as a source of potash for soap manufacture, as

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green solid base catalysts for trans-esterification of oils, as a protein source and because of its high content of fibrous material as a pectin source, a dietary supplement for fish and pigs [1,4–7]. Due to the presence of phenolic compounds, CPH displays antioxidant properties.

Epidemiological studies indicate that phenolic compounds have the potential effect to prevent chronic diseases and also have anti-carcinogenic, anti-inflammatory, anti-microbial, vasodilator and analgesic activities [8–10]. Soxhlet, reflux, cold pressing, or maceration by organic solvents has been conducted as extraction processes to obtain phenolic compounds from different sources [11]. Though, these conventional methods have limitations concerning the long extraction times, low yield and low quality of the extracts, losses of volatile compounds, degradation of the bio-compounds due to the heat and, high solvent consumption [11,12]. Therefore, it is necessary the use of an extraction technique able to overcome the mentioned limitations. Supercritical fluid extraction (SFE) is a suitable technique with several advantages such as selectivity, low organic solvent consumption, higher speed, better reproducibility and environmental safety, compared to conventional extraction techniques [12,13] and also it has found applications recovering labile or easily oxidizable bioactive compounds [14].

Response surface methodology (RSM) is a statistical tool that can be used to define the effect between responses and independent variables as well as its interactions that allow finding the levels of input variables that optimize a particular response of a process [15,16]. Few studies have been conducted on the application of supercritical extraction using $CO_2$ as solvent not only for the extraction of xanthines (caffeine and theobromine), aromatic compounds and cocoa butter from cacao nibs [17–19], but also for the extraction of xanthines and some lipids from cacao hulls [20]. Besides, to our knowledge there is not a single application of SFE for the valorization of CPH. Thus, the objective of this study was to develop and optimize a SFE process to obtain a "green" phenolic extract and to evaluate the total antioxidant capacity of CPH extracts. A Box Behnken design was used to study and analyze the effects of three independent variables (pressure, temperature and co-solvent flow rate) on the extraction yield of total phenolic and antioxidant activity of CPH.

## 2. Materials and methods

### 2.1. Chemicals

Carbon dioxide (99% purity), purchased from Carburos Metálicos (Barcelona, Spain), and ethanol (99.5%), provided by VWR Chemicals (Fontenay-sous-Bois, France), were used for supercritical fluid extraction (SFE). Ultrapure water was obtained from a Millipore system (Billerica, MA, USA). Gallic acid, 6- hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, ≥97%) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS, ≥99%) were purchased from Sigma-Aldrich (Madrid, Spain). Folin– Ciocalteu phenol reagent was provided by Merck (Darmstadt, Germany).

### 2.2. Raw material

Cacao (*Theobroma cacao* L.) pods were harvested from Tapachula (14°54′00″N92°16′00″O) in Chiapas, Mexico. Whole cacao pods were rinsed with water, the cacao seeds were manually removed from the pods, and the husk was triturated into a paste using a semi-industrial blender (Crypto Peerless K55, Birmingham, England). Then, CPH paste was dried in a drying chamber at 60 °C until a dry matter having a water content of < 8% was obtained. The dried CPH was milled using a laboratory mill with grinding tank and sieved to have a particle size ≤ 0.5 mm.

### 2.3. Extraction process

#### 2.3.1. Conventional extraction

The CPH powder was extracted with ethanol (1:30) and continuously stirrer at 400 rpm for 2.5 h at room temperature. The experiments were performed in triplicate. After extraction was completed,

**Table 1**
Experimental design matrix including coded and uncoded extraction conditions and results for each response variables for the Box-Behnken design at 150 min of extraction.

| Variation levels | | Variables | | |
|---|---|---|---|---|
| | | Extraction Temperature (°C) | Extraction Pressure (bar) | % Co-solvent |
| Low level | (−1) | 40 | 100 | 5 |
| High level | (1) | 60 | 300 | 15 |
| Medium level | (0) | 50 | 200 | 10 |

| Run | Operation variables | | | | | | Responses | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Temperature (°C) | | Pressure (bar) | | Co-solvent (%) | | | | | |
| | Coded | Un coded | Coded | Un coded | Coded | Un coded | TPC (mg GAE $g^{-1}$ extract) | TAA (mmol TE $g^{-1}$ extract) | Yield (%) | PR (mg/100 g RM) |
| 1 | (−1) | 40 | (−1) | 100 | (0) | 10 | 4.12 | 0.12 | 0.36 | 1.48 |
| 2 | (1) | 60 | (−1) | 100 | (0) | 10 | 5.19 | 0.16 | 0.35 | 1.8 |
| 3 | (−1) | 40 | (1) | 300 | (0) | 10 | 9.94 | 0.18 | 0.44 | 4.3 |
| 4 | (1) | 60 | (1) | 300 | (0) | 10 | 12.42 | 0.24 | 0.47 | 5.8 |
| 5 | (−1) | 40 | (0) | 200 | (−1) | 5 | 6.83 | 0.21 | 0.15 | 1.0 |
| 6 | (1) | 60 | (0) | 200 | (−1) | 5 | 3.97 | 0.17 | 0.15 | 0.6 |
| 7 | (−1) | 40 | (0) | 200 | (1) | 15 | 5.52 | 0.13 | 0.33 | 1.8 |
| 8 | (1) | 60 | (0) | 200 | (1) | 15 | 5.59 | 0.14 | 0.29 | 1.6 |
| 9 | (0) | 50 | (−1) | 100 | (−1) | 5 | 8.68 | 0.18 | 0.20 | 1.7 |
| 10 | (0) | 50 | (1) | 300 | (−1) | 5 | 3.03 | 0.17 | 0.15 | 0.4 |
| 11 | (0) | 50 | (−1) | 100 | (1) | 15 | 7.74 | 0.14 | 0.30 | 2.3 |
| 12 | (0) | 50 | (1) | 300 | (1) | 15 | 13.63 | 0.18 | 0.37 | 5.0 |
| 13 | (0) | 50 | (0) | 200 | (0) | 10 | 6.94 | 0.20 | 0.34 | 2.3 |
| 14 | (0) | 50 | (0) | 200 | (0) | 10 | 8.23 | 0.21 | 0.27 | 2.2 |
| 15 | (0) | 50 | (0) | 200 | (0) | 10 | 10.58 | 0.15 | 0.26 | 2.7 |

TPC: Total phenolic content, GAE: Gallic acid equivalent, TAA: Total antioxidant activity, TE: Trolox equivalent, PR: Phenolic recovery, RM: Raw material.