An Approach to Value Cocoa Bean By-Product Based on Subcritical Water Extraction and Spray Drying Using Different Carriers

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Abstract: The aim of this study was to establish an efficient, sustainable technological procedure for valorization of food by-product, that is, cocoa bean shells (CBSs). The properties and stability of CBS extracts obtained by spray drying process with maltodextrin (MD) and whey protein (WP) as carrier agents were evaluated. For this purpose, phytochemicals of CBSs were extracted by subcritical water extraction. Physico-chemical properties, total phenolic (TP) and total flavonoid (TF) contents of the encapsulated extracts were determined in order to verify the efficiency of spray drying. Additional analyses for phytochemical characterization of the obtained powders were also performed. The efficiency of microencapsulation process was characterized by product recoveries higher than 58%. Both coating materials significantly influenced the encapsulation of phytochemicals in terms of rehydration, water solubility index and water absorption index, with WP being at an advantage. The best results for TP and TF contents were achieved when CBSs were encapsulated using WP (37.68 mg GAE/g and 7.66 mg CE/g, respectively). Microencapsulation using WP yielded higher content of gallic acid, caffeine, and theobromine than those with MD. According to the results, the formulation using 50% WP provided a better preservation of polyphenols compared to 50% MD. Therefore, spray drying with WP can be used as a method of choice for obtaining high quality CBS powders.

Keywords: cocoa bean shell; subcritical water extraction; spray drying technique; phytochemicals; maltodextrin; whey protein

1. Introduction

Cocoa (Theobroma cacao L.) bean shell is considered an industrial by-product of cocoa processing usually remaining underutilized. To reduce the impact of by-products’ disposal on the environment and the economy, and improve the value of by-products, suitable technologies have been investigated. Cocoa shells represent 12%–20% of the cocoa bean [1]. This valuable biowaste is mainly used as a fuel, animal feed additive or for preparation of fertilizers [2,3]. Recently, the value of cocoa bean shell (CBS) has received increasing attention due to high nutritional value and high content of phytochemicals, such as phenol compounds, dietary fibers, lipids, sugars, and proteins whose composition depends mostly on the variety, growing region, the fermentation, and processing operations [4]. Several studies revealed the presence of caffeine, theobromine, catechin, epicatechin, protocatechuic acid, quercetin-3-glucoside, quercetin-3-arabinoside, procyanidin B-type, and procyanidin A-type...
pentoside [5–7]. The recovery of phytochemicals from CBS is commonly performed by different extraction methods. Previous studies have reported the bioactivity of its extracts obtained by combined application of supercritical fluid and pressurized liquid extraction [8], pressurized liquid extraction with absolute ethanol [5], ultrasound and hydrodynamic cavitation [6], and extraction with ethanol [1]. Many researchers propose the use of this by-product as a food ingredient or other added-value applications [9–11]. In the patent published by Romanczyk and McClelland [9], cocoa shell fat was obtained enriched with phytosteros using solvent extraction, and as such, it can be included as an additive in dietary supplements, pharmaceuticals, and cosmetics. Rojo-Poveda et al. [11] demonstrated that CBS can be used as an ingredient for home-made beverages because of its potential health benefits.

Despite the resource-rich matrix of CBS, the efficient extraction of phytochemical compounds can be influenced by the applied extraction technique. Safe, green, cost-effective and energy-effective strategies are particularly attractive for obtaining bioactive compounds from natural sources. Due to its excellent features, subcritical water represents a competitive approach in the production of pharmacologically-active extracts and development of new pharmaceutical, medical, nutraceuticals or functional food products [12,13]. However, the high content of unstable active compounds susceptible to degradation and to the loss of activity represents a great challenge for technological processes. Microencapsulation represents a highly effective technology for the protection of unstable active compounds within a protective matrix resulting in powders with improved stability, low water activity, reduced bulk size, and prolonged shelf-life. The powder form of extracts ensures easy manipulation and shipping, lower storage and transportation costs, and easier standardization [14]. A lot of studies were conducted on the microencapsulation of different phytochemicals [15–17], but there is no published data on the stabilization of phytochemical compounds extracted from CBS by subcritical water.

Therefore, the present work aims to valorize CBS by applying subcritical water at optimal conditions of process parameters in order to uphold the efficient extraction of compounds with high-added value. In order to provide quality and stable bioactive products of CBS, the efficiency of spray drying technique with two carriers, maltodextrin (MD) and whey protein (WP), were estimated. The potential exploitation of the waste material has been evaluated by physico-chemical characterization, compound characterization and quantification, together with the determination of total phenol (TP) and flavonoid (TF) contents.

2. Materials and Methods

2.1. Material and Chemicals

The investigated material, cocoa shells, were obtained from the Kandit chocolate factory (Osijek, Croatia) in 2018. Prior to the extraction, the material was ground using a standard laboratory mill. Methanol (J.T. Chemicals Baker, Netherlands) and formic acid (Scharlau Chemie, Spain) were HPLC grade. Standards of methylxanthines (theobromine and caffeine) and phenolics (gallic acid, caffeic acid, p-coumaric acid, (+)-catechin, (-)-epicatechin and (+)-epicatechin gallate) were purchased from Sigma-Aldrich (St. Louis, USA) and all were suitable for HPLC analysis. The purity of used standards was as follows: theobromine, caffeine and (+)-catechin ≥ 99%, (-)-epicatechin gallate 98.6%, caffeic acid and p-coumaric acid ≥ 98%, gallic acid 95.5% and (-)-epicatechin ≥ 90%.

2.2. Subcritical Water Extraction

Subcritical water extraction (SWE) was carried out in a handmade subcritical water extraction system previously described by Jokić et al. [18]. The extraction cell was filled with 5 g of dried ground CBS and 50 mL of double-distilled water. Nitrogen was injected in the extractor to prevent possible oxidation at high temperatures in the presence of oxygen from air. SWE extraction was performed at temperature of 150 °C with extraction pressure of 30 bar and extraction time of 15 min. Obtained extracts were filtrated through filter paper under vacuum, collected into glass vials and stored in a dark place at 4 °C prior analysis.
2.3. Spray Drying Process and Process Efficiency

The carrier materials, maltodextrin (DE16) and whey protein (both in 50 mass (dry) percentages, calculated on extract dry weight), were used in the liquid feed. Carrier materials were dissolved in distilled water. These carrier solutions and liquid extracts were homogenized using a magnetic stirrer at a 30 °C temperature. During the spray drying process, the liquid feed was constantly mixed and pumped into the spray drying system Anhydro spray dryer (Anhydro AS, Denmark) via peristaltic pump. The process inlet temperature was 120 °C, while outlet air temperature ranged from 75 to 80 °C. During the production of the dry powder extract, atomizer speed ranged from 20,000 to 21,000 rpm. The obtained powder was separated from the heating medium in a cyclone and collected in glass bottles, sealed and kept protected from air and humidity. Efficiency of powder production (expressed as the weight percentage) is determined gravimetrically as ratio of mass of the powder obtained after spray drying and mass of total solids measured in the liquid feed.

2.4. Analysis of CBS Powder

2.4.1. Moisture Content

Moisture content in the obtained powder was determined according to standard procedure described in the official Pharmacopeia (Ph. Jug. IV). All experiments were performed in three replicates.

2.4.2. Hygroscopicity

Hygroscopicity of CBS powder was determined according to the method described in a study by Vladić et al. [16]. The hygroscopicity was monitored after 2, 5, 7, 10, and 14 days and expressed as a gram of absorbed water per 100 g of dry extract powder. All experiments were performed in three replicates.

2.4.3. Bulk Density

Bulk density was determined by measuring the volume of the dry extract mass. CBS powder (1 g) was placed in a 20 mL graduated glass cylinder which was exposed to vibration for 2 min. Next, bulk density was calculated from the difference of the empty glass cylinder and the mass of the glass cylinder with powder. Bulk density was expressed as mg of powder per mL. All experiments were performed in three replicates.

2.4.4. Rehydration

Time needed for powder to completely rehydrate (expressed in seconds) [19] was determined by adding 2 g of dry extract into 50 mL distilled water at room temperature. Mixture of powder and water was mixed via magnetic stirrer in glass flask. Rehydration was determined in dry extracts of CBSs immediately after their production and after 50 days of storage in desiccator. All experiments were performed in three replicates.

2.4.5. Water Solubility Index and Water Absorption Index

Water solubility index (WSI) and water absorption index (WAI) were determined according to a previously described method [20]. WSI, reconstitution property, is used as an indicator of degradation of powder constituents. WAI was calculated as the mass of solid pellets remaining after centrifugation divided by the mass of the dry sample. WAI is a measure of the product's ability to absorb water. The low WAI indicates better stability during the storage. All experiments were performed in three replicates.
2.4.6. Content of Total Phenols and Total Flavonoids

The contents of total phenolic compounds (TP) in CBS powders were determined by Kähkönen et al. [21]. Gallic acid was used as standard compound for preparation of calibration curve, and absorbance of the samples was measured at 750 nm (6300 Spectrophotometer, Jenway, Dunmow, UK). The content of phenolic compounds in dry extracts was expressed as mg of gallic acid equivalent per g of powder (mg GAE/g). All experiments were performed in three replicates and the results are expressed as mean values.

The total flavonoids content (TF) was determined in CBS powders using aluminum chloride colorimetric assay [22]. Catechin was used as a standard for preparation of calibration curve and absorbance of the samples was measured at 510 nm. The content of flavonoids in dry extracts was expressed as mg of catechin equivalents per g of powder (mg CE/g). All experiments were performed in triplicate and the results are expressed as mean values.

2.4.7. HPLC Analysis

Identification and quantification of methylxanthines (theobromine and caffeine) and phenolic components (gallic acid, caffeic acid, p-coumaric acid, (+)-catechin, (-)-epicatechin and (-)-epicatechin gallate) in cocoa shell powders was performed using HPLC method with photo-diode array (PDA) detector as described by Barišić et al. [23]. Bioactive compounds were extracted with 5 mL of 70% methanol, the solution was ultrasonicated for 30 minutes and centrifugated for 10 minutes at 3000 rpm. Supernatant was collected and procedure repeated. Obtained supernatants were combined in a flask and the final volume (10 mL) was adjusted with 70% methanol. Before injection, the extracts were filtered through 0.45 μm Chromafil Xtra nylon membrane filter (Machery-Nagel, Germany). The HPLC analysis was performed on Shimadzu liquid chromatograph consisting of Shimadzu LC-20AD solvent delivery module, Shimadzu CTO-20AC column oven, Shimadzu autosampler SIL-10AF, and Shimadzu SPD-M20A photodiode array detector. The instrument was supported with LabSolution Lite software (Release 5.52). Separation of bioactive components was achieved on Inertsil ODS-3V (GL Sciences, 250 mm × 4.6 mm, 5 μm particle size) using mixture of HPLC grade methanol (solvent A) and 1% formic acid (solvent B) under gradient elution at the flow rate of 0.8 mL/min. The gradient elution conditions were as follows: solvent A percentage in mobile phase at the beginning was 10%, then it linearly increased to 32% A at 15 min, 40% A at 20 min to 25 min and 60% A at 30 min. The column and detector temperatures were set at 30 °C and the injection volume was 20 μL. The chromatograms were recorded at 278 nm. Identification of separated components was achieved based on the comparison of the retention times and UV spectra with reference materials, and the quantification was performed using external calibration method.

2.5. Statistical Analysis

All analyses were run in triplicate and the results were expressed as means ± standard deviation (SD). Mean values were considered significantly different at \( p < 0.05 \) confidence level, after the performance of the one-way ANOVA statistical analysis followed by Tukey’s test.

3. Results and Discussion

3.1. Process Efficiency

The main purpose of the present study was to estimate the efficiency of spray drying technology to microencapsulate phytochemical compounds from CBS subcritical water extract. The effect of two carrier materials on quality of dried powders was examined regarding their physico-chemical properties, TP and TF contents.

Efficient recovery of different constituents and bioactive fractions requires selection of an opportune extraction technique. In the last decade, subcritical water extraction (SWE) is being more and more implemented due to its excellent features. This technique, relying on heated and pressurized water,
improves extraction efficiency due to lower solvent tension and viscosity, and improvement in solubility and mass transfer effects [24]. Moreover, SWE process does not consume organic solvents, making it more advantageous in terms of safety and compatibility with consecutive applications, environmental concerns and solvent recovery operations. However, there are numerous obstacles to the application of extracts as functional ingredients due to their vulnerability to an oxidizing environment (light, pH, temperature, oxygen, moisture, enzymes). In this context, encapsulation by spray drying is an alternative process to overcome the disadvantages of bioactive molecule instability. The physico-chemical properties of encapsulated powders are influenced by characteristics of the feed (carrier type and its concentration) and drying condition (air inlet/outlet temperature, pressure and feed flow rate) [25]. Hence, it is essential to monitor and optimize spray drying parameters in order to obtain superior quality powder with optimum yield and absence of wall deposition or stickiness phenomena. According to Jayasundera et al. [26], some process-based (low outlet temperature and low humidity air) and material-based (high molecular weight and high glass transition temperature carriers) approaches have been developed in order to prevent or reduce the stickiness during spray drying. High molecular weight carrier agents such as MD and gum Arabic contributed significantly to powder stability mainly due to their low viscosity change in the feed material [26]. WP has been used in encapsulation studies due to their surface-active properties [27]. In addition, recovery of powders higher than 50% and obtained from a pilot scale spray dryer has been used to define efficient spray drying process [28]. The results revealed that the carrier used for encapsulation had an important role in the retention of phytochemicals within the matrix. Better result was achieved when using MD as carrier material with an approximately 74% efficacy of the process. Using WP as carrier material, the content of phytochemicals retained in the encapsulated sample corresponded to 58.61%. Ezhilarasi et al. [29] observed similar results when Garcinia cowa fruit extracts were spray-dried using MD, WP, and their combination. MD encapsulates’ yield was higher in comparison to other powder yields.

To summarize, regarding powder recovery higher than 50%, absence of stickiness and wall deposition phenomenon, process of CBS powder production can be considered as efficient and suitable in both cases.

3.2. Moisture Content and Hygroscopicity

Moisture content represents an essential property in determining the storage stability of dry extracts and their handling and flowability due to its effect on glass transition and crystallization behavior [30]. The powders obtained with MD and WP had moisture content of 5.54 ± 0.03 and 5.83 ± 0.09%, respectively, which secures the extended stability of the extract if it is stored in adequate conditions. Powder obtained with WP showed a slightly higher moisture content probably caused by greater ability of proteins to maintain moisture trapped in the particles [27]. This study can be supported by Souza et al. [31] for spray drying of lycopene-rich tomato concentrate with WP isolate. The same property change was observed for other protein-based carrier materials such as gelatin [32] and soy protein isolate [33]. On the contrary, lower moisture content of carbohydrate-based carrier materials (MD, gum Arabic) was ascribed to the difficulty of the diffusion of water among their large molecules [34].

Another critical quality parameter is hygroscopicity, i.e., the capacity of the material to absorb environmental moisture. Composition of the material, feed flow rate, size of microcapsules, and concentration of carrier agent influence hygroscopicity significantly [35]. High hygroscopicity leads to quality deterioration and modification of the physical properties (stickiness, flowability, agglomeration) of powder [36]. According to GEA Niro [37], powder is considered hygroscopic if hygroscopicity is in range of 15%–20%, slightly hygroscopic for values in range of 10%–15%, while in non-hygroscopic powder this value should be <10%. The hygroscopicity of CBS powders was monitored after 2, 5, 7, 10, and 14 days. Figure 1 showed that hygroscopicity of CBS powders increased from 12.67 ± 0.25 to 16.93 ± 0.1% during the 14-day storage. CBS powder with MD showed slightly lower but statistically insignificant hygroscopicity for storage time up to 48 h as compared to WP. For both carriers, there was
no significant difference in hygroscopicity of CBS powders over the 2-day storage time. In general, variations in hygroscopicity may occur due to the type of carrier agents and their chemical structure. The higher hygroscopicity of powder produced with WP might be attributed to more hydrophilic nature of proteins in comparison to polysaccharides [38]. Hygroscopicity value can also be correlated with moisture content of CBS powder following the same trend of increase as previously observed by Ferrari et al. [39] and Mohd Nawi et al. [40].

![Figure 1. Effect of storage time on the hygroscopicity (%) of CBS powders. Different letters indicate significant differences (p < 0.05). Different letters indicate significant differences between samples (p < 0.05).](image-url)

### 3.3. Water Solubility Index, Water Absorption Index and Rehydration

The instant properties of a powder involve the ability of wetting, submerging, dispersing and dissolving. Water solubility and water absorption are inversely related and demonstrate the abilities of powders to form a solution in water, i.e., to absorb water. The most important factors that affect solubility are the carrier agents, compressed air flow rates, and low feed rates [27,41]. According to Shittu and Lawal [42], soluble compounds of the original cocoa powder, as well as those generated during the processing stages, could also contribute to the solubility of powder. In this study, the effect of different carrier agents on WSI and WAI was determined. Encapsulated CBS extract with MD exhibited a lower water solubility index (62.4%) in comparison to WP (72.8%). The observed lower solubility of MD powder may be attributed to the high concentration of carrier agent. According to Lee et al. [43], this can be because of the low moisture content of the product or the high amount of air in the particles, as MD is a skin-forming material. Oliveira et al. [44] and Vladić et al. [16] reported similar WSI in the passion fruit juice powder (57.59%) and Achillea millefolium “herbal dust” powder (72.12%) using MD. Conversely, using WP reduced the water absorption capacity of the CBS powder (Table 1). According to Ahmed et al. [45], an increase in WAI might be associated with different degrees of engagement of hydroxyl groups to form hydrogen and covalent bonds between MD chains. An increase in WAI might be also due to the loss of the crystalline configuration of the powders [46].

| Sample     | WSI (%)  | WAI (%)  | Bulk Density (mg/mL) | Rehydration (s) |
|------------|----------|----------|----------------------|-----------------|
| SWE + MD   | 62.4 ± 0.85<sup>a</sup> | 29.6 ± 0.12<sup>a</sup> | 421.58 ± 4.1<sup>a</sup> | 5.3 ± 0.2<sup>a</sup> |
| SWE + WP   | 72.8 ± 0.5<sup>b</sup>  | 12.8 ± 0.2<sup>b</sup>  | 302.42 ± 2.4<sup>b</sup> | 4.3 ± 0.1<sup>b</sup> |

Different letters indicate significant difference between samples at p < 0.05.
Rehydration, i.e., ability of subsequent absorption of water, represents an essential attribute of dried products, because many of them are dissolved before use [47]. Improvement in the powder rehydration properties is highly important. CBS powders must be fully dispersed and dissolved to express their functional properties. Therefore, powder rehydration in water should be fast and complete. CBS powders spray-dried with MD presented a higher rehydration time (5.3 s) in comparison to powders dried with WP (4.3 s). According to Caliskan and Dirim [48], MD as a modified starch has dissolution time longer than most hydrophilic compounds present in plant extracts. Therefore, it is expected that MD powders have a prolonged rehydration time [49].

3.4. Bulk Density

Bulk density is a quality parameter of dried microcapsules relevant to the storage, processing, packaging and distribution conditions in industrial processes. It is well known that bulk density is mainly related to size, shape, and surface properties of powder particles and powder composition [50]. Powders with higher bulk density show a smooth, uniform surface and are spherical in shape. Moreover, higher bulk density implies filling the inter-particle voids with smaller particles, consequently decreasing the volume of trapped air within the powders and reducing the possibility for product oxidation [51,52]. Bulk density of CBS powder with MD showed denser particles (421.58 mg/mL) in comparison to WP (302.42 mg/mL) (Table 1) probably due to more compact physical structure and hydrophilic wall matrix of MD. According to Suhag and Nanda [53], low bulk density of powder with WP was related to the lower stickiness of particles, high viscosity changes in the feed material, and skin-forming character of WP. A similar observation was confirmed during spray drying of beetroot juice concentrate [54], Roselle’s calyx [55], and tamarind pulp [56]. Furthermore, there was a correlation between bulk density and moisture content of CBS powders. Particles with higher moisture content have a tendency to become sticky and to form larger agglomerates, resulting in a smaller surface area and hence, in a lower bulk density [57].

3.5. Phytochemical Profile

An increasing number of studies have been conducted using different phytochemical compounds to develop functional products [10,58,59]. Phenolic compounds of natural origin enjoy substantial interest due to their notable antioxidant and other beneficial properties [60–62]. However, due to easy thermal degradation/oxidation of polyphenolic compounds, they need to be encapsulated to enhance their stability.

Encapsulation efficiency was determined in dry powders in terms of TP and TF content. Subcritical water was used to extract phytochemicals from CBSs. The content of TP and TF in obtained CBS powders was determined using standard spectrophotometric procedures. Subcritical water exhibited high extracting capacity leading to TP content in MD powder of 16.14 ± 0.35 mg GAE/g, being more than two-fold higher than the values observed in other works dealing with different CBS extracts without using spray drying [8,63]. In the case of WP powder, a significantly higher (p < 0.05) TP content was obtained (37.68 ± 0.72 mg GAE/g), confirming previous data that indicated a higher TP content of WP tamarind pulp powders in comparison to MD [64]. WP also provided higher TP content in the encapsulated black rice anthocyanin microcapsules [65]. According to Norkaew et al. [65], WP may encapsulate or react with some phenolics better than the other carrier materials due to its nature. Everette et al. [66] found that Folin-Ciocalteu reagent could be reduced by several proteins, including bovine serum albumin probably present in WP. The change of the TF content followed the same trend as in the case of TP content. A two-fold increase of TF content was determined in WP dry powder (7.66 ± 0.21 mg CE/g).

The encapsulated extracts obtained by subcritical water were characterized for their phytochemical profile by HPLC-PDA. Typical HPLC chromatogram of identified phytochemicals in CBS powder is presented in Figure 2. The contents of phytochemical compounds in CBS powders were estimated from calibration curves constructed with the mixture of the selected phytochemicals.
Sample Gallic phenolics, namely procyanidins, protocatechuic and phenolic profile from encapsulated CBSs extracted by sonication and magnetic stirring and reported...}

Figure 2. HPLC chromatogram of identified phytochemicals in cocoa bean shell (CBS) powder spray-dried with whey protein (WP) recorded at 278 nm. Peaks: GA (gallic acid), TEO (theobromine), CAT ((+)-catechin), CAF (caffeine), EPI ((-)-epicatechin), CA (caffeic acid), EPG ((-)-epicatechin gallate), p-KA (p-coumaric acid).

Table 2 shows the quantities of the main compounds identified in these samples. Six major phenolic compounds, gallic acid, caffeic acid, p-coumaric acid, (+)-catechin, (-)-epicatechin and (-)-epicatechin gallate were identified, with (+)-catechin being the most abundant (1.48 mg/g). The main phenolic acid recovered in the powders was gallic acid, with its concentration being highest in WP powder (0.48 mg/g). On the other hand, p-coumaric and caffeic acids were the phenolic compounds found in lower concentration in both samples. Recoveries of (+)-catechin, (-)-epicatechin and (-)-epicatechin gallate were slightly lower when MD was added as a carrier. Papillo et al. [67] investigated the phenolic profile from encapsulated CBSs extracted by sonication and magnetic stirring and reported that epicatechin and protocatechuic acid were the prominent phenolic compounds in all powders obtained using different combinations of MD and gum Arabic. These authors also quantified other phenolics, namely procyanidins, protocatechuic and p-hydroxybenzoic acids, which were not identified in the CBS-SWE powders. Regarding the catechin and gallic acid, their presence was detected in the aforementioned study, but SWE+WP enabled the recovery of higher amounts of these phenols comparing to conventional extraction+MD+gum Arabic, demonstrating the potential of SWE technique coupled with spray drying (WP) for the recovery and stability of phenolic compounds.

Table 2. Content of the identified phytochemicals detected by HPLC-PDA in subcritical water extract of CBSs after spray drying (mg per g of powder (mg/g)).

| Sample   | Gallic Acid | Caffeic Acid | p-Coumaric Acid | (+)-Catechin | (-)-Epicatechin | (-)-Epicatechin Gallate | Theobromine | Caffeine |
|----------|-------------|--------------|-----------------|--------------|-----------------|--------------------------|-------------|----------|
| SWE + MD | 0.37        | 0.04         | 0.02            | 1.48         | 0.15            | 0.07                     | 5.98        | 1.10     |
| SWE + WP | 0.48        | 0.03         | 0.03            | 1.47         | 0.11            | 0.06                     | 7.34        | 1.34     |

The results concerning methylxanthines, caffeine, and theobromine in CBS powders were in accordance with the range of data previously reported for the cocoa bean by-product [5,68,69]. Higher content of methylxanthines was obtained when WP was used as a carrier. The caffeine content in obtained powders was not far from the values observed in other works dealing with different cocoa shell extracts [5,68]. Okiyama et al. [5] determined a slightly higher concentration of theobromine (9.89 mg/g) in CBS extracts obtained by pressurized liquid extraction when compared with the present study. In general, high content of theobromine in CBSs might be a result of the migration of methylxanthines from the bean into the shell during processing in the fermentation stage [70].
4. Conclusions

In the present study, the effect of the carrier type on physical and chemical properties of CBS powders was investigated. Subcritical water extract encapsulated with MD and WP was analyzed in terms of physico-chemical properties, total phenolic and flavonoid contents, and phytochemical composition. It was found that both carriers improved the CBS powder recovery, with MD giving a higher value (73.52%). Bulk density of the powder was significantly affected by the carrier type (MD > WP). The best results for WSI and WAI and rehydration time were achieved when CBSs were encapsulated using WP as the carrier material (72.8%, 13.8% and 4.3 s, respectively). On the basis of the higher TP and TF contents, it can be considered that the formulation using 50% WP provided a better preservation of polyphenols compared to 50% MD. Microencapsulation using WP yielded a higher content of gallic acid, caffeine, and theobromine than those with MD. According to all mentioned facts, it can be concluded that microencapsulation spray drying process might be used for preparation of whey protein-based microcapsules of cocoa bean by-product, retaining its phytochemical value and improving its stability.

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