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Supercritical Fluid Extraction of Fat and Caffeine with Theobromine Retention in the Cocoa Shell

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Abstract: The cocoa shell is a residue of low commercial value, which represents an alternative for obtaining substances of added value for the food and pharmaceutical industry. Substances of interest in the shell include fat and methylxanthines (theobromine and caffeine). In order to obtain the extraction behavior with supercritical CO_2, a 2^3 factorial design was proposed with six central points, taking dynamic extraction into consideration. The following factors were involved: pressure (2,000–6,000 psi), temperature (313–333 K), and time (30–90 min). The obtained yield was between 3.66% and 15.30%. Fat was the substance that was extracted most effectively (94.73%). Caffeine demonstrated variability in the residue, with at least six treatments that exceeded a removal rate of more than 90%, while it was practically impossible to extract theobromine. The difference with regard to the extraction of theobromine may be attributed to its low solubility. Characterization using FT–IR showed the modifications before and after the process, providing clear evidence of the changes corresponding to the fat at 2,924, 2,854 and 1,745 cm^{-1}. The results presented establish the basis for the extraction of substances such as fats and methylxanthines from a cocoa shell with the use of CO_2.

Keywords: extraction of fat; caffeine; theobromine; cocoa shell; supercritical extraction

1. Introduction

Fruit from cocoa trees (*Theobroma cacao* L.) contain around 30–40 seeds or beans, and are covered by a layer or kind of pulp known as mucilage, which is rich in carbohydrates. These seeds consist of two cotyledons and a radicle surrounded by a testa [1,2]. The latter is made up of approximately 10–12% of the total dry bean used in the industry [2] and is used for the production of compost, food for ruminants [3], low-quality candy products and infusions [4]. In 2014, the global production of cocoa was more than four million tons [5]. Taking into consideration this production, the shell, as a residue, represents approximately 680–700 thousand tons [6].

Cocoa beans are conditioned for the industry using a post-harvest process, which consists of fruit selection, to stock before extracting the beans, fermentation, drying, storage, and transport. This is followed by a roasting process during which chemical changes occur [7,8]. During this stage, the cotyledon is separated from the shell and the germ. The shell is considered to be a byproduct of cocoa production and is considered to be a residue with its main use being fuel for heaters and, to a lesser degree, animal food and the preparation of fertilizers [6].

The bitter flavor of cocoa seeds is mainly due to theobromine and caffeine [9]. These substances belong to the xanthines or methylxanthines, which are not easy to eliminate or extract from the cocoa

bean. They are molecules with low solubility and are mainly contained in coffee, cocoa, mate and guarana, etc. [10]. Approximately 0.9% and 0.3% of theobromine and caffeine, respectively, can be found in the cocoa bean shell. Furthermore, the shell also contains a significant amount of fat, with a profile very similar to that of cocoa butter [6]. It has been demonstrated in humans that the consumption of cocoa provides cardiovascular benefits due to the vasodilator effect of theobromine and caffeine [11], which together with some fatty acids from the cocoa and flavonoids, increase high-density cholesterol and reduce low-density cholesterol, improving the health of people who consume it [12–16].

In order to obtain molecules or substances of high added value, the exploration of agricultural residue, as in the case of the shell, has received much attention. Various groups of researchers propose it has applications in food ingredients, supplements, and cosmetics due to its nutritional characteristics and the economically attractive potential of its recovery [6]. Nevertheless, emerging technologies are required for extraction, such as ultrasound, microwaves or supercritical fluids, and other green technologies [17–19]. The supercritical extraction process is characterized by the use of chemically inert solvents such as carbon dioxide, which is the most widely accepted and used due to its soluble, economic and ecological characteristics, in addition to the fact that it is accessible and highly effective [20–22].

Supercritical fluids are able to penetrate any solid matrix due to changes in their physicochemical characteristics under these conditions. Therefore, during the supercritical stage, the fluids present the peculiarities of a gas but also of a liquid [23–25]. This permits increased solubility and improves the extraction of compounds such as polyphenols, fatty acids, and alkaloids, among others that have previously been studied [21,23,25,26]. The supercritical extraction process involves a large number of operating variables that have an effect on the extraction yield of the compound of interest in a solid matrix. The most studied variables for the supercritical extraction system are the choice of the type of solvent, use of co-solvent, temperature, pressure, flow rate and extraction time. In addition, the amount of material to be used must be considered, in addition to the particle size, to reduce the problems of extracting compounds of interest from the solid matrix [25,27,28].

Two supercritical extraction techniques have been reported, the first is known as static, where the pressure and temperature are controlled for a specific period of time in order to subsequently carry out the extraction by controlling the output flow of the extract (during this stage, there is no flow of supercritical fluid). The second technique is known as dynamic, and is carried out using a constant flow of supercritical fluid. This technique, normally, has a static stage of no more than 10 min in order for the supercritical fluid to penetrate the extraction matrix and obtain improved performances of the extracts [29]. Given the diversity of the variables, it is difficult to establish a simple model that predicts the behavior of the type of variable on the extraction performance or any compound to be extracted. However, it is possible to study the variables involved through a statistical experimental design by applying the response surface methodology, which has been used in different disciplines with positive results [28,30–33]. This information has served as a basis for modeling and optimizing the supercritical extraction with CO_2 of oils, fats, bioactive compounds, phenolic compounds, alkaloids, volatile oils, and purines, among others [28,30,34,35]. In all reported cases, there is a correlation between the efficiency of the process performance and in the reduction of time and costs for the estimation of operating conditions. A particular characteristic of the experimental design in surface response methodology to optimizing, is the obtainment a polynomial expression of second order; however, the methodology initially consists of proposing a screening of the factors and increasing from a first order to a second [36]. Because the solid matrices or waste of by-products are complex in terms of their composition and heterogeneity, coexisting in more than one phase, it is often difficult to generalize the studies with a particular type of model and, therefore, they must be analyzed case by case. The study of this type of matrix and of the extract requires analytical instrumental separation techniques to determine the compounds that are extracted or retained in the solid matrix. However, medium infrared spectroscopy has been reported as a useful tool for the qualitative and quantitative analysis of complex samples, without the need for previous separations stages, providing a mapping

of their chemical and structural composition. In addition, it is an almost ideal detector to be coupled to a system with flow [37,38]. Due to the importance of taking advantage of molecules of interest contained in agroindustry waste, such as the toasted cocoa shell, the purpose of this investigation was to evaluate the content of theobromine, caffeine, and fat contained in the cocoa shell powder by means of supercritical extraction with CO_2. For this purpose, a 2^3 factorial design was applied with six central points, taking into consideration dynamic extraction, with the following factors: operating pressure (2000–6000 psi), temperature (313–333 K), and extraction time (30–90 min), evaluated as a response in terms of the concentration in the residue of substances of interest in the study, in addition to the removal and residual ratios.

2. Materials and Methods

2.1. Raw Material

Cocoa shells were collected from a batch of approximately 80 kg of toasted Mexican Trinitario cocoa. The cocoa was obtained from the trading company Intercambio Mexicano de Comercio (IMCO), S.A. de C.V. located in Cárdenas, Tabasco, Mexico. Shelling of the toasted cocoa samples was carried out manually. The shell was washed with deionized water and dried in the sun. The obtained sample was taken to be milled (KRUPS, GX410011V, Mexico City, Mexico) and the particle size was standardized at 0.841 mm., using a No. 20 mesh screen (Montinox, Mexico City Mexico).

2.2. Extraction with Supercritical CO_2

Extractions were carried out using a supercritical extraction equipment model SFT-150 SFE from the company Supercritical Fluid Technologies, Inc., which has an operating temperature range of 298.15 to 473 K. It operates at a maximum pressure of 10,000 psi and its volumetric flow varies from 1 to 330 mL min^{-1} (250 g min^{-1}) of liquid CO_2. Furthermore, it has extraction chambers for samples of between 100 and 2,000 mL. The dry air is regulated at 75 psi by a KAESER 7.5 C compressor and gas was supplied using a CO_2 gas cylinder.

In order to extract theobromine, caffeine, and fat from the shell, 30 g of sample was used, to which 20 mL of absolute ethanol was added and the mix was then placed in the extraction chamber. The equipment was previously stabilized (30 min) before introducing the sample to ensure that the CO_2 entering the equipment is in a liquid state. Once stabilized, the sample was placed and the connections were closed and from this point, the extract collection bottle was weighed and placed in the collection site of the equipment extract. The extractions were carried out under a constant flow of 0.176 g CO_2 min^{-1}, with a static phase of 10 min (the static phase, also known as the soaking time, allows the critical CO_2 to penetrate the shell and improve extraction). After this time, the dynamic extraction begins for a time of 20 min, after this time the supply valves of both CO_2 and dry air are closed and the extract outlet valves are left open to perform the depressurization of the system and obtain complete recovery of the extract.

2.3. Statistical Analysis

A 2^3 factorial design was proposed with five central points for a total of 13 treatments (Table 1). The extraction conditions were: pressure (2,000–6,000 psi), temperature (313–333 K), and extraction time (30–90 min). The dependent variables were yield, concentration in the residue (theobromine, caffeine, fat), residual ratio (theobromine), and removal ratio (caffeine, fat). The effect of the independent variables on the responses (Y) was modeled using a polynomial response surface:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon_i \tag{1}$$

where Y is the answer, X_i, X_{ij} are the independent variables and their interaction, β_0 is the constant coefficient and β_i, and β_{ij} are the coefficients for linear regression and interaction, respectively, which

assess the effects. The polynomial model was used to evaluate the dependent variables (Y) analyzed by multiple linear regression, lack of fitness and analysis of variance (ANOVA), using Matlab R2012a. The response surfaces are generated using the polynomial.

Table 1. Concentration of theobromine, caffeine and fat in the residue, residual ratio of theobromine, removal ratio of caffeine and fat, concentration of theobromine, caffeine and fat in the extract obtained by supercritical extraction.

	Natural Variables				Concentration in the Residue			Residual Ratio	Removal Ratio	
Treat	P (psi)	T (K)	Time (min)	R_g	Th (mg·g^{-1})	Caf (mg·g^{-1})	Fat (g·g^{-1})	Th (%)	Caf (%)	Fat (%)
1	2000	333	90	3.66	17.593	0.563	0.011	106.89	32.67	86.99
2	2000	313	30	11.65	14.810	0.000	0.025	82.53	100.00	72.50
3	2000	333	30	8.61	12.113	0.000	0.009	69.81	100.00	89.60
4	2000	313	90	5.20	11.158	0.438	0.047	66.71	48.48	43.95
5	6000	333	90	8.11	13.189	0.280	0.010	76.43	68.10	88.97
6	6000	333	30	7.45	13.922	0.000	0.072	81.26	100.00	16.24
7	6000	313	90	6.31	17.415	0.071	0.005	102.90	91.80	94.73
8	6000	313	30	7.89	13.813	0.107	0.057	80.24	87.82	34.14
9	4000	323	60	15.30	15.778	0.113	0.043	84.28	88.17	54.26
10	4000	323	60	11.45	15.309	0.047	0.011	85.50	94.82	88.05
11	4000	323	60	6.68	16.557	0.000	0.006	97.45	100.00	92.53
12	4000	323	60	7.20	15.793	0.122	0.009	92.43	86.00	90.02
13	4000	323	60	10.99	16.885	0.170	0.009	94.79	81.26	90.32

R_g = yield, Th = Theobromine, Caf = Caffeine.

2.4. Determination of Fat Using the Soxhlet Method

Fat extraction was carried out using the methodology proposed by the Mexican standard [39]. For this purpose, 2 g of cocoa shell powder was used and fat was extracted using petroleum ether for 18 h. The percentage of fat was calculated using the following equation:

$$\% = \frac{(P - p)}{M} \times 100 \tag{2}$$

where P = mass in grams of the flat bottom flask with fat, p = mass in grams of the flat bottom flask without fat, and M = mass in grams of the sample.

2.5. Calculation of the Residual Ratio and the Removal Ratio for Each Variable

The removal and residual ratio for each variable after extraction with CO_2 was calculated using the following equations [40]:

$$Y(\%) = \frac{\left(Am_o - Bm_f\right)}{Am_o} \times 100 \tag{3}$$

$$X(\%) = 100 - Y(\%) \tag{4}$$

Y, *X*, signify the removal and residual ratio, respectively. *A* and *B* express the content of the response variables before and after extraction with supercritical CO_2, respectively. The quantities m_o and m_f represent the weight of the initial shell powder in the extraction unit and the weight of the residual shell dust in the extraction unit, respectively. The proportion of caffeine and fat were established as the removal ratio and theobromine as the residual ratio.

2.6. Global Yield

The extraction yield (Rg) was estimated by the relationship between the total extracted mass (m_e) after the extraction process for each treatment of the experimental design in relation to the initial mass (m_o) in the container of the supercritical extraction unit expressed as a percentage:

$$R_g = \frac{m_e}{m_o} \times 100 \tag{5}$$

2.7. Determination of Methylxanthines Using Visible Ultraviolet Spectroscopy

In order to extract theobromine and caffeine from the cocoa shell, a modification of the Peralta-Jiménez and Cañizares-Macías [41] technique was used where 1 g of fat-reduced cocoa shell powder was diluted in 100 mL of deionized water. Once aqueous and organic phases were obtained where theobromine and caffeine were solubilized, respectively, it was read in a spectrophotometer (Thermo Fisher Scientific, G10S Uv-Vis, Shanghai, China). In the case of theobromine, an aliquot of the aqueous phase was diluted in deionized water at a ratio of 1:25 and a reading was taken at 273 nm. In the case of caffeine, an aliquot of the organic phase was diluted in chloroform at a ratio of 1:10 and a reading was taken at 276 nm. In the case of theobromine and caffeine (Sigma Aldrich Corporation, St. Louis, MO, USA) standard curves were prepared between 0–50 μg in deionized water and chloroform, respectively.

2.8. Characterization Using Fourier Transform Infrared Spectroscopy (FT–IR)

An analysis was carried out on the initial sample and a central point was selected for characterization using Fourier Transform Infrared Spectroscopy (FT–IR). Each sample was mixed with IR-grade KBr (Sigma Aldrich Corporation, St. Louis, MO, USA) for the preparation of a tablet. They were subsequently analyzed in a FT–IR Shimadzu Mod. IRAffinity-1 spectrophotometer (Shimadzu Scientific Instrument, Columbia, MD, USA) in a wave number range of 4,000 to 400 cm^{-1}, at a resolution of 2 cm^{-1} and 40 scans. The data was processed using IRsolution TM Software and the graphs were analyzed in Origin Pro 8 (OriginLab Corporation, Northampton, MA, USA).

3. Results and Discussion

3.1. Fourier-Transform Infrared Spectroscopy (FT–IR)

The FT–IR technique was used to examine the modifications in the functional groups of cocoa shell powder before and after the supercritical extraction process (Figure 1). Treatment of the experimental design (central point) was used to carry out the analysis of the residue after the supercritical extraction.

Three signs of strong intensity in the X–H extension vibration region were identified. An absorption band of 3,431 cm^{-1} was simultaneously assigned to OH stretching [42] and vibrations corresponding to N–H groups of primary and secondary amides. The peaks at 2,924 and 2,853 cm^{-1} were assigned to asymmetric and symmetric CH stretching of the methyl and methylene groups of the lipids. Similarly, the CH group was presented in the FT–IR of the powder sample after supercritical extraction at 2,933 cm^{-1} (Figure 1), but with low intensity in relation to that in the initial shell and the peak at 2,854 showed no signal. As a result, significant changes in these groups took place as a result of the extraction process, which are associated with the extraction of lipids.

A peak at 1,745 cm^{-1} was assigned to C=O stretching of the carbonyl group of carboxylic acids in the shell powder and the intensity of this signal is reduced after the extraction process on the residue as a result of the extraction of fat. Furthermore, the signal associated with methylene groups of fat acids at 2,854 cm^{-1} is not evident in the spectrum. The peaks at 1,668, 1,637 and 1,455 cm^{-1} correspond to ring modes and are the result of the contracting and stretching of the carbon–carbon link of the aromatic ring [43]. The signals at 1,668 and 1,637 cm^{-1} are associated with C=O stretching and C–C

asymmetric stretching, respectively. Furthermore, some bands may be hidden by the C–H flexion vibrations of alkyl groups [42].
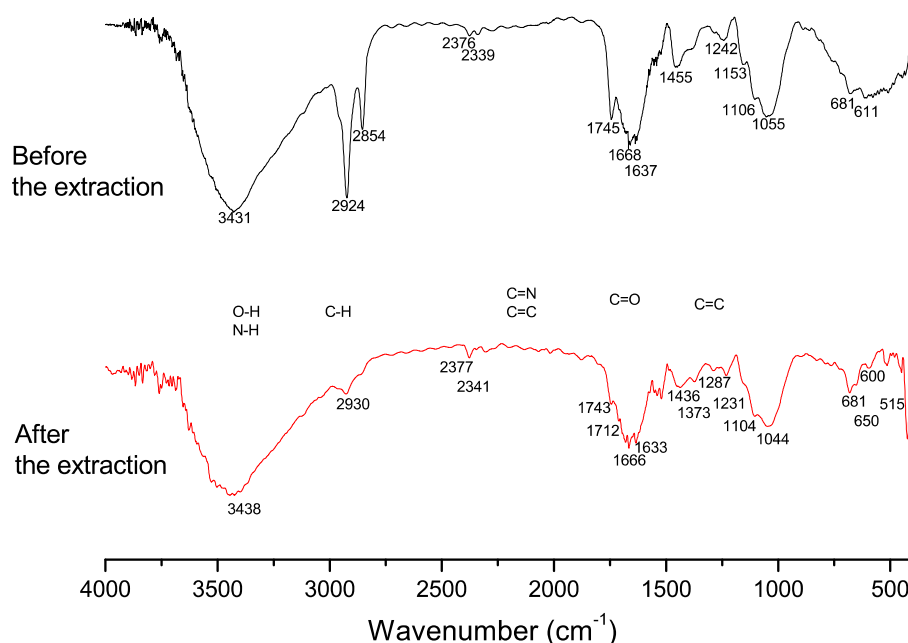

Figure 1. Changes in the shell after supercritical extraction.

In the fingerprint region (1,500–600 cm^{-1}), the methylxanthines (theobromine and caffeine) show slightly different bands in relation to the standards. The theobromine spectrum presented peaks at 614, 681, 730, 757, 860, 938, 1,068, 1,135, 1,174, 1,225, 1,290, 1,363, 1,417, 1,454, 1,548, 1,590, 1,689 cm^{-1} assigned to flexion vibrations of C=O–C, O=C–N, C=C–N and C=C–C corresponding to the pyrimidine ring, N=C–H and N–C–H deformation vibrations, C–N stretching vibrations of pyrimidine rings, C–N stretching vibrations of imidazole; C=C, C–C, C–N stretching vibrations of aromatic rings, respectively.

The caffeine spectrum demonstrated peaks at 609, 741, 862, 970, 1,022, 1,067, 1,121, 1,184, 1,236, 1,283, 1,356, 1,430, 1,450, 1,480, 1,547, 1,659 cm^{-1}. Theobromine and caffeine structurally contain two carbonyl vibration groups in the meta position and the bands around 1,658 and 567 cm^{-1} are, therefore, assigned to this type of group and correspond to asymmetric and symmetric stretching vibrations. In the study samples, the bands assigned to the methylxanthines are better appreciated in the spectrum after extraction with the definition of an increased number of peaks in the fingerprint region and the associated signals were 681, 1,044, 1,231, 1,287, 1,373, 1,436, 1,520, 1,666 cm^{-1}. It is evident that, after extraction, during which increased fat extraction occurred, hidden peaks were defined, as in the case of the signal at 1,520 cm^{-1}.

A band at 1,244 cm^{-1}, and an intense band at 1,043 cm^{-1}, were identified as C–O stretching of phenolic compounds corresponding to lignin and alcohols [44]. The vibrations of these groups were reduced in both cases and the bands moved slightly, to 1,230 and 1,046 cm^{-1}, after the supercritical extraction process.

The O–C–C band of secondary alcohol esters occurs around 1,100 cm^{-1}, while the aromatic esters of primary alcohols demonstrate absorption at approximately 1,111 cm^{-1}; therefore, the peak found at 1,106 cm^{-1}, corresponds to this type of functional group.

3.2. Supercritical Fluid Extraction of Cocoa Shell Powder

Cocoa shell powder is a complex study matrix with regards to composition, multi-phases and heterogeneity. The results of the initial evaluation for the substances of interest in this study found initial concentrations for fat, theobromine and caffeine of 80.00 mg·g^{-1} (±0.014), 15.85 mg·g^{-1} (±0.002),

and 0.80 (±0.009) mg g^{-1}, respectively. During the different treatments of the proposed design, the fat was the substance that was most effectively recovered in the extract (Table 1), with the maximum extraction conditions being 6,000 psi, 313 K and 90 min (Treatment 7). The caffeine showed significant changes in relation to concentration in the residue, with at least six treatments that exceeded more than 90% of the removal ratio (Table 1, Treatments 2, 3, 6, 7, 10, and 11). Treatment 7 was found to be the best treatment that simultaneously extracted fat and caffeine.

Theobromine was the most difficult substance to extract, even without obvious changes under the different operating conditions imposed on the different treatments, for example, the residual ratio showed results of over 100% (Treatments 1 and 7), which indicated that the extraction conditions were not capable of removing theobromine and, with the elimination of the fat, it was concentrated in the residue. Li and Hartland [45] have reported the difficulty of extracting fat and methylxanthines from cocoa beans based on the low solubility of pure CO_2, indicating the importance of using polar solvents, such as ethanol, together with supercritical CO_2, which improves the effectiveness of the extraction of fat. Nevertheless, the same effect does not occur with the methylxanthines, for example, the solubility reported for theobromine is approximately two orders of magnitude lower than caffeine in conditions of supercritical CO_2, which is attributed to the differences in the fusion point and fusion enthalpy. Unlike the results reported by Li and Hartland [45], which indicated a low level of solubility of fat or fat in the cocoa beans, the removal of more than 90% was demonstrated in various treatments on the shell, which suggested two explanations: (a) different conditions between the vegetable matrixes are based on the components of each one and (b) a wider exploration of the study factors where the extraction time is significant.

In the case of caffeine, Kopcak and Mohamed [46] explained that the addition of a co-solvent and an increase in pressure increases the density of the supercritical fluid and contributes to increased solubility. The results for caffeine in the residue indicate that the extraction of caffeine was practically complete (not detected using the UV-VIS method) in some treatments (Table 1). The conditions of these treatments in relation to pressure are similar (6,000 psi), which was the maximum pressure used in this study and confirms that which was established by Kopcak and Mohamed [46], that the increase in pressure permits increased solubility of the caffeine. Otherwise, the minimum pressure used (Table 1, Treatment 1) presented maximum retention of caffeine in the residue.

The global yield of the shell extraction process using critical CO_2 is approximate due to the fact that the extraction of the fat is not a pure component and the weight of the extract contains other components such as methylxanthines, among others. The obtained performance was between 3.66% and 15.30%, where some treatments demonstrate values higher than those obtained using the Soxhlet method, indicating that the latter does not completely remove fat from the sample. The shell is a raw material of interest due to its bioactive properties as a result of the type of compounds it possesses [6]. Supercritical extraction is an alternative to conventional processes that use solvents, as in the case of Soxhlet or others, such as distillation and mechanical pressing for the extraction of oils or fats, as reported by Cornelio-Santiago et al. [47].

The results obtained in this study indicate that there are conditions for the proper extraction of the fat contained in the shell; nevertheless, there are other compounds still present that possess a significant added value. The solubility of the fat competes in parallel with the other compounds in orders of magnitude and the preliminary elimination of this substance must, therefore, allow the extraction of other molecules. Thus, a test was carried out in order to understand the influence of the fat by submitting a residue where the majority of the fat was eliminated (Treatments 9–13), once again submitted to extraction with supercritical CO_2 using the central point conditions (4,000 psi, 323 K, and 60 min). The results demonstrated a yield of 2.58% with a 6.18 mg g^{-1} concentration of theobromine in the residue and a residual ratio of 39.17%; therefore, the presence of fat in the shell leads to interference in the extraction of other molecules of interest inside the vegetable matrix. The foregoing shows that any trace of fatty material needs to be eliminated before recovering other substances of interest, as is the case of theobromine and caffeine.

3.3. Statistical Analysis

The variance analysis of the variables (fat, theobromine, and caffeine) in the residue, the residual ratio (theobromine), and removal ratio (fat and caffeine) are shown in Table 2. The dependent and independent variables were adjusted to a first-order polynomial with interaction effects. The replicas in the central point are necessary for an adjustment shortage test on the regression model [48]. The variability detected in some central points, including in the quantified response variables, was attributed to the depressurization time of the equipment, despite having controlled the output flow of CO_2. The depressurization of the equipment was highly variable and it is, therefore, indicated as a critical parameter in this study.

Table 2. Analysis of variance in the residue, residual ratio, and removal ratio.

Sum of Squares	Source	Regression	Linear	Non-Linear	Residual	Lack of Fit	Pure Error	Total	R^2
Residue	**Th**	36.40	9.96 *	26.43	12.40	10.76 *	1.64	48.81	0.75
	Caf	0.185	0.119	0.065	0.178	0.170 *	0.008	0.364	0.51
	Fat	0.004	0.0039 *	0.000	0.002	0.001	0.001	0.006	0.71
Residual ratio	**Th**	1457.10 *	540.92	916.14 *	310.52	177.23	133.29	1767.6	0.82
Removal ratio	**Caf**	5994.70	5355.00 *	639.66	2325.30	1280.00	1045.30	8320.0	0.72
	Fat	2635.80	1697.50	938.27	2746.50	2527.6 *	218.92	5382.3	0.49

Th = Theobromine, Caf = Caffeine. * Significant $p < 0.05$.

The results showed that the proposed model was suitable based on the fact that the determination coefficient (R^2) presented a value higher than 0.7 [48], with the exception of caffeine in the residue and the removal ratio. Although the values of R^2 are acceptable for theobromine and residual fat, in addition to the residual ratio of theobromine, the prediction resulting from the model must be taken with caution because the values of R^2 are between 0.7 and 0.82. Furthermore, the validity of the model was assessed based on an adjustment shortage analysis. The use of a model with adjustment shortage is attributed to the fact that there are other factors not taken into consideration, mainly, the use of co-solvent (our equipment does not have this service). Another factor to be investigated is porosity, which can be explored with a smaller number of experiments because, to this end, these factors of the system have been evaluated.

Table 2 shows the study variables as a function of linear and non-linear terms. Pressure, temperature and extraction time were only significant factors ($p < 0.05$) for the residual ratio of theobromine. The combined effect (interaction) of pressure and extraction time had a positive effect (Table 3) on the residual ratio of theobromine, which indicates that high conditions of these variables in the combined form will increase the percentage in the aforementioned ratio. Although the regression model was not significant ($p > 0.05$) for theobromine and caffeine, a linear effect for at least one study factor occurred and there was a lack of adjustment ($p < 0.05$), which implies the consideration of a more complex model, taking into account the extension of the intervals of the design conditions reported in Table 1. Nevertheless, we also need to consider prior removal of the fat before establishing an experimental design for any substance inside the cocoa shell. The fat in the residue and in the removal ratio yielded an R^2 of 0.71 and 0.72, respectively, behaving similarly to the theobromine and caffeine in the residue; nevertheless, there was no adjustment shortage ($p > 0.05$).

The regression coefficients of the concentration in the residue, the residual ratio, and the removal ratio can be observed in Table 3, and were used to generate the response surfaces for theobromine and fat in the residue, the residual ratio of theobromine and the removal ratio of fat, which are shown in Figure 2. The foregoing explains how pressure and time are factors that simultaneously influence the extraction of methylxanthines and fat, according to Li and Hartland [46] and the use of polar co-solvents such as ethanol and water may encourage the extraction of both compounds. Other

factors also exist which influence the extraction of molecules in the shell, such as genetic origin, the fermentation process (diffusion of theobromine from the bean to the shell), and roasting process (rotary drum system, tray or fluidized bed), as well as commonly studied factors such as flow rate, co-solvent, and extraction time, which have been reported by Asep et al. [28,49]. Specific studies of these factors are necessary, in addition to the fact that the fat extracted from the shell and the fat from the cocoa bean present differences in their fatty acid profiles, for example, the quantity of linoleic acid is almost double in the shell than in the cocoa fat [6]. In addition to the study variables, a separation analysis by liquid or gas chromatography coupled with a mass detector is required to identify other components of great value, such as different phenolic compounds, proteins, sugars, and lipids among others. The use of successive supercritical extraction or in sequential steps may be considered necessary to extract the previously mentioned compounds.

Table 3. Regression coefficients of the response variables in the concentration of the residue, residual ratio and removal ratio.

	Coefficients	β_0	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}
Residue	**Theobromine**	14.949	−0.743	0.108	0.825	−0.599	−1.683 *	−0.333
	Caffeine	0.152	−0.112	−0.014	0.046	−0.055	0.023	0.067
	Fat	0.024	−0.001	0.008	−0.021 *	0.004	0.001	−0.007
Residual Ratio	**Theobromine**	13.753	5.838	−1.205	−5.666	3.175	10.049 *	1.860
Removal Ratio	**Caffeine**	72.486	0.937	−9.147	24.183 *	−4.761	−1.724	7.370
	Fat	83.008	13.417	1.515	−5.466	6.461	−2.509	−8.321

* Significant $p < 0.05$.

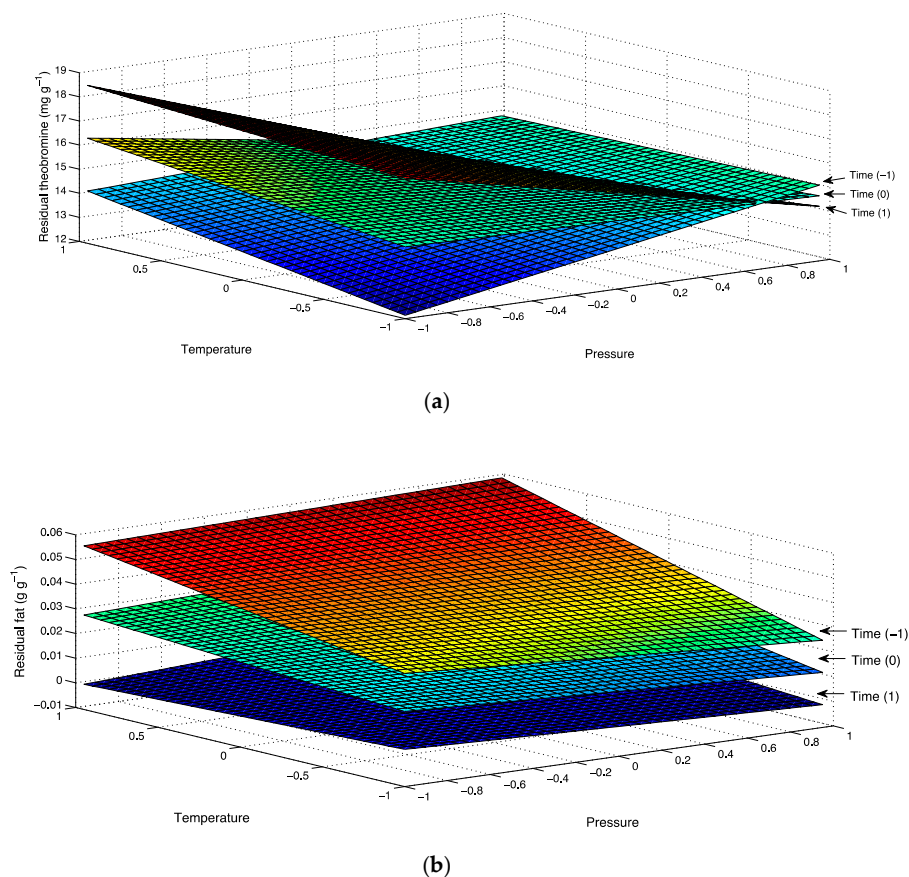(**a**)

(**b**)
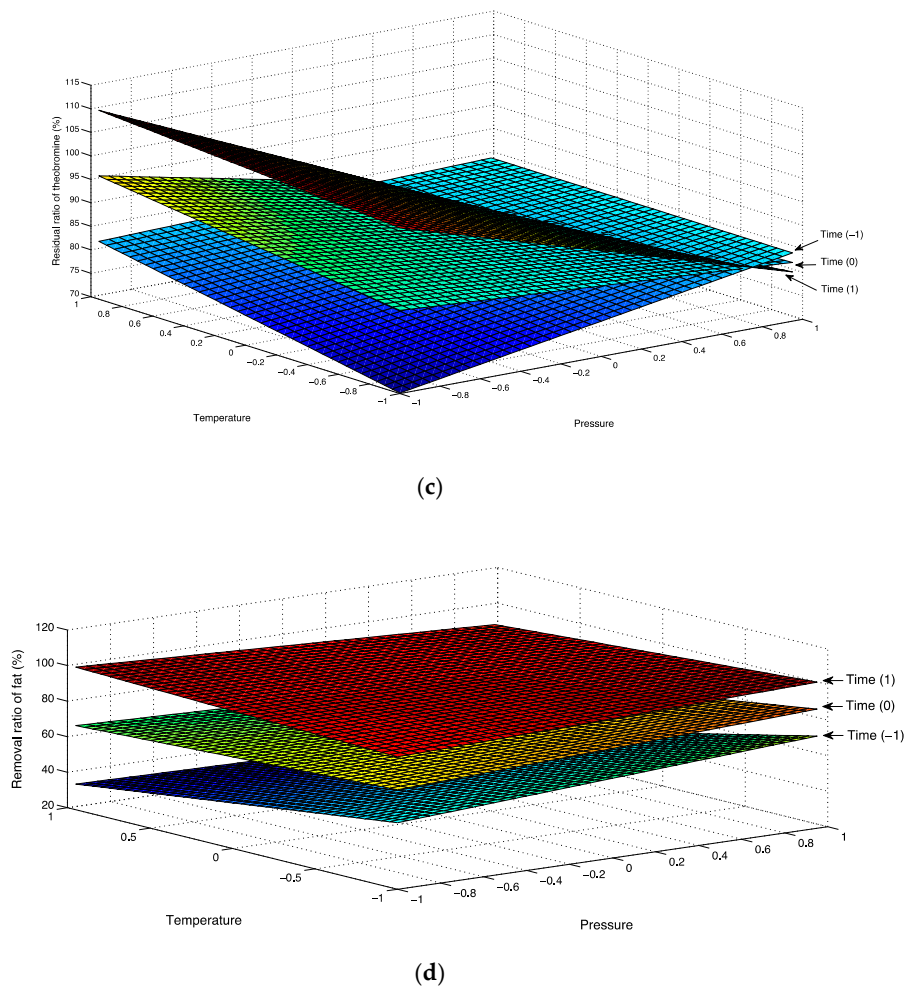
Figure 2. *Cont.*

(**c**)

(**d**)

Figure 2. The surface plots of residual theobromine (**a**); residual fat (**b**); residual ratio of theobromine (**c**); removal ratio of fat (**d**), as affected by temperature, pressure and extraction time with supercritical CO_2.

Caffeine has an anti-physiological effect; therefore, reduction or elimination preconditions the vegetable waste as a source of animal nutrition. Furthermore, the presence of phenolic compounds in the residue could modify the metabolism in a positive way. However, this will depend on the concentrations. The use of waste due to the amount of compounds present allows reflection on the level of extraction, which will be a function of production costs.

4. Conclusions

The results allow us to conclude that the supercritical extraction process in cocoa shell powder using CO_2 as a solvent was an efficient method to remove fat and caffeine; however, theobromine was retained in the residue. The changes in the vibrations of the functional groups evidenced the extraction of the fat in the vegetable matrix. The products of the extraction of cocoa shell powder and the residue can be considered as being of high value due to the fact that components such as fat and caffeine were recovered, in addition to a residue with functional character upon retaining the theobromine. The present work may be used with reference to the additional studies to elucidate the optimal extraction conditions of theobromine using one co-solvent and potentially recover other components by performing sequential steps.

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