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Extraction of Cocoa Butter from By-product Cocoa Bean Shells by Using SC-CO_2 Extraction and Investigation of Components and Antioxidant Capacities

Ertan Sahin[1,2], Esra Capanoglu[1], Asli Can Karaca[1] and Asli Barla Demirkoz[*,3,4]

[1]*Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, Istanbul Technical University, 34469, Maslak, Istanbul, Turkiye*

[2]*Department of Research and Development, Onem Food Industry and Trade Inc. Co., 34010, Zeytinburnu, Istanbul, Turkiye*

[3]*Department of Nutrition and Dietetics, Faculty of Health Sciences, Haliç University, 34060 Eyüp, İstanbul, Turkiye*

[4]*Department of Research and Development Center, Aromsa Flavours and Food Additives Industry and Trade Inc. Co., 41480, Gebze, Kocaeli, Turkiye*

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Abstract: Recently, sustainability in terms of making the life of humanity permanent is being by far the most important subject. In this study, the production of cocoa bean shell fat, a by-product obtained from supercritical carbon dioxide (SC-CO_2) extraction, and cocoa butter were compared in terms of antioxidant capacity, fatty acid composition, and flavor profile. In addition, Soxhlet method was used for the comparison of extraction yield. Extract of cocoa bean shell was obtained by supercritical extraction method with 70% yield. Fatty acid composition and flavor profile were determined using Gas Chromatography/Mass Spectrometry (GC-MS) and Dynamic Headspace-GC/MS, respectively. The yield of the cocoa bean shell extract was 4.15% at Soxhlet and 15.09% and 10.31% at SC-CO_2 extraction. The fatty acid constituents extracted using SC-CO_2 ranged from 23.62% to 23.72% palmitic acid, 30.83% to 31.28% stearic acid, 32.19% to 32.41% oleic acid, and 9.35% to 9.61% linoleic acid. Physicochemical properties and fatty acid composition of SC-CO_2 extracted cocoa bean shell fats content were found to be comparable to that of commercial cocoa butter. Antioxidant capacity of the obtained extract in terms of β-carotene linoleic acid method and total phenolic content were found to be lower than that of cocoa butter.

Keywords: Cocoa bean shell; cocoa butter; antioxidant capacity; supercritical extraction; sustainability; green chemistry.

[*] Corresponding author e-mail : aslibarla@gmail.com

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1. Introduction

Since the adoption of the 17 Sustainable Development Goals for Sustainable Development Solutions adopted by the United Nations on September 25, 2015, studies in the field of food and especially waste and by-product valorisation have gained momentum [1,2]. In the studies conducted in the area of food processing, it is observed that the issues related to waste management and efficient use of by-products have become prominent [3-5]. The issue of production of cocoa sustainably has also become very important recently [6]. Therefore, the pressure to increase cocoa production to meet the increasing chocolate demand worldwide has negative effects on the environment, economy and society of the producer countries [7]. Besides, each year, more than 4000 tons of cocoa beans are expended/roasted around the world, according to the International Cocoa Organization (ICCO) [8].

Cocoa name comes from the fruit of the cocoa tree (*Theobroma cocoa* L.) and cocoa seeds are generally known as cocoa beans [9]. The latter is used as the most valuable ingredients of confectionery, especially chocolate and of food, cosmetic and pharmaceutical industries [10]. Besides, cocoa bean is the main raw material for chocolate production, one of the most important products in the confectionery industry today. Cocoa mass, cocoa butter, and cocoa powder are produced from cocoa beans and they are also the main ingredients for chocolate production [11]. During the cocoa beans transformations, cocoa bean shells are produced as a by-product and sold as animal feed or used as fuel material. However, cocoa bean shells include dietary fiber, protein, and phenolic compounds, such as theobromine, caffeine, flavonoids, etc. Due to their rich nutrient compositions, they are recently used in various food formulations [12].

Cocoa butter has a valuable property that allows chocolate and bars to stay molded and contraction on solidification throughout their shelf life. The ratio of cocoa butter in the liquor used in chocolate making may not be sufficient in the formation of the desired rheological and sensory parameters in the final product chocolate. This problem is encountered especially in milky chocolate. Additional cocoa butter is added to ensure quality parameters in consumers and producers such as rheological and sensory [13].

Cocoa bean shell is obtained as a by-product. One valuable output is cocoa butter obtained from the cocoa bean shell, which is found between a 1.5-8.49 % ratio [14]. Because of the fact that economic gain will be achieved if recovery is achieved. It is important that the extraction method used for the reuse of the fat in the cocoa bean shell in food products does not leave any residue in the final product.

The quality of fat component extracts is strongly belonged to the extraction procedure and type of solvent used, which must be carefully chosen to provide an adequate balance to enhance yield and selectivity [15]. Hexane, toluene, chloroform and petroleum ether are traditional extraction solvents for fat components, especially using traditional method Soxhlet, but all of them are known hazardous to human health and pollutant [16]. Therefore, nontraditional procedures such as supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE), with green chemistry and environmental-friendly seem has been becoming more prefer for extraction from different natural matrices for recently [17,18]. The techniques using especially CO_2 as green solvents due to its low polarity, mild critical conditions, non-flammability, low cost and easiness to be removed from the extract are good alternative due to reduction in energy consumption and because they ensure hazardous solvent free extracts [10, 19, 20].

According to studies, cocoa bean in its different forms including hull, shell, bean and seed are extracted using different methods in terms of their functional properties and bio compounds. In previous studies, Cocoa shells are investigated for their compounds and applications in the food industry. These studies have revealed that cocoa shells have a variety of bio compounds, such as phenolic compounds, dietary fibers, and lipid profiles similar to that of cocoa butter. In previous studies, researchers especially have used traditional solvent extraction methods [14, 21, 22], however, some of them have preferred untraditional methods such as SC-CO_2, Deep Eutectic Solvents Extraction (DES), and PLE using a green solvent [10, 20, 23, 24]. When studying the scientific studies conducted to up to date, it was found that the volatile components of cocoa bean shell fat have not been studied in detail. There is one volatiles of cocoa seed study in the literature, which is a study performed in different cocoa cultures extracts using solvent extraction method and does not contain the necessary details [25].

In this study, we aimed to develop the supercritical fluid CO_2 extraction method necessary for the recovery of fat in the cocoa bean shell and the obtained extracts were compared in terms of antioxidant capacity, fatty acid composition, and flavor profile against reference cocoa butter.

2. Materials and Methods

2.1. Materials

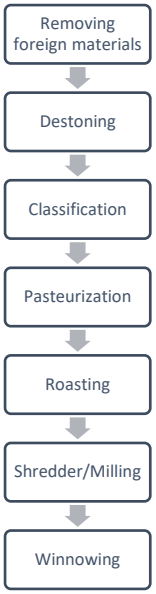
Cocoa bean shell (Ivory Coast) was obtained from Onem Food Industry Trade Inc. Co. (Istanbul, Turkey). It was stored at room temperature until being used. Ethanol, β-carotene, Trolox and α-tocopherol used were analytical grade. Methanol and chloroform used were analytical grade purchased from Merck (Darmstadt, Germany). Other chemicals also used were analytical grade purchased from Sigma-Aldrich (Dorset, UK). The water used was processed with the Mili-Q water purification system. CO_2, used in the Supercritical extraction device, was supplied from the Habas (Kocaeli, Turkey).

2.2. Sample Preparation

2.1.1. Production of Cocoa Bean Shell

By processing fermented and dried cocoa beans, cocoa liqueur, the raw material of chocolate, is produced. Cacao bean are cleaned physically and microbiologically first. Before pasteurization of cacao beans for physical cleaning of cocoa beans including removing foreign materials, de-stoning and classification process were applied.

Elimination of microbiological risk is done by pasteurization. Temperature-duration-pressure parameters, the total microbiological load is either eliminated. The indicator microorganism is *Salmonella* spp.



Physically and microbiologically cleaned cocoa beans go through the roasting process to form the precursors and components of the necessary flavour [26]. One of the advantages of roasting is that it facilitates the decomposition of the cocoa bean shell. In the roasting process, the core bark becomes easily decomposed because it loses water faster than nib. The kernels that are broken in the mill prior to shell separation are sent to the process of sequential sieves. The vacuum in each sire sieve ensures that the shell is separated from the nib. In the first sieve, larger parts are separated, while the last sieve separates the smaller parts. After processing roughly 10-12% cocoa bean shell is obtained from the cocoa beans. This process is called as winnowing. Process flow diagram is shared at Figure 1.

Figure 1. Cacao bean shell process flow diagram.

2.3. Fat Extraction from Cocoa Bean Shell

2.3.1. Supercritical CO_2 Extraction

SC-CO_2 extraction was operated using a Waters SFE 1000 supercritical fluid extraction system (Milford,MA, USA) equipped with a 1000 mL extractor under 450 bar pressure and 125 gCO_2/min CO_2 mass flow rate. Selected parameters (temperature, time, pressure, CO_2 flow rate) were optimized using a method that one parameter was changed in each experiment when the others were kept constant.

In this study, the particle size was fixed from the parameters affecting extraction efficiency; changes have been made to temperature, duration, pressure, CO_2 flow rate parameters and co-solvent was not used for the reuse of extracted fat in foods. In addition, standards require that cocoa butter should not include solvents (except hexane 1 mg/kg, propane-2-ol and methanol 10 mg/kg) for use in the food industry. Therefore, supercritical CO_2 extraction method complied with the standards when co-solvent is not used during extraction. Each study was replicated 3 times.

2.3.2. Soxhlet Extract

The fat content of the cocoa bean shells was calculated by weight loss after 12 h extraction with hexane under reflux in a Soxhlet apparatus Traditional extraction method of Soxhlet extraction of cocoa bean shell was performed according to Voigt *et al.* The obtained cocoa bean shell fat was stored in the darkness until further analysis [27].

2.4. Determination of the Volatile Compounds

The volatile compounds and flavour profile of the extract were analysed by dynamic headspace analysis -GC/MS (DHA/GC-MS). The total ion chromatogram was performed using a Gerstel DHS System (Germany) connected to an Agilent 7890A GC and 5975C MS (Inert MSD with Triple Axis Detector, USA. A total of 1 g of cocoa bean shell extract was placed in a 20 mL standard headspace vial. It was placed in a tray of a Gerstel Multi-Purpose Sampler. Desorbed compounds were injected automatically into a CP-WAX 52 GC column (60 m × 25 µm film thickness × 0.25 mm innerdia) (INNOWAX, Germany). The flow rate of the Helium carrier gas was adjusted to 1.2 mL/min. Each sample was injected in the spitless mode. The GC oven temperature was programmed from 40°C to 240°C at 5°C/min.

2.5. Analysis of the Fatty Acid Composition

The fatty acid composition analysis of deodorized cocoa butter, cocoa butter, and extracted cocoa butter obtained from cocoa bean shells was performed using GC/MS.

Cocoa bean shell fat was obtained from SC-CO_2-extraction method. Samples of the extracted fat were analysed by gas chromatography using fatty acid methyl esters (FAMEs) preparation [28]. FAMEs were analysed by a GC-MS, equipped with a flame ionization detector (FID) and a Hp-innowax 19091N-136GC column (60 m × 0.25 µm film thickness × 0.25 mm innerdia). The oven temperature was programmed from 40°C to 260°C at a 5°C/min heating rate. The injector and detector temperatures were held at 260°C. A reference standard composed of a mixture of FAMEs (Supelco Inc., Bellefonte PA, USA) was analysed under the same operating conditions to determine the peak identity. FAMEs value from the results of duplicate analyses were reported.

2.6. Antioxidant Capacity Analysis Methods

2.6.1. Determination of Total Amount of Phenolic Compounds

The total phenolic content of the samples was determined according to the method of Kabouche (2007) [29]. The extract was dissolved in ethanol (1000 ppm) and sampled from 500 µL and 1000 µL extracts. Approximately 0.1 mL Folin-Ciocalteu reagent was added to each sample. After 3 minutes, 0.3 mL of anhydrous sodium carbonate was added to the samples and diluted to 5 mL with distilled water. The sample was stored in the dark for 2 hours and the absorbance was recorded at 760 nm by using a Shimadzu UV-1280 spectrophotometer (Shimadzu, Kyoto, Japan). This study was conducted in triplicate.

2.6.2. β-Carotene-Linoleic Acid System Model

Antioxidant capacity of extracts was measured in β-carotene-linoleic acid system model [30]. Approximately 2.5 mg of β-carotene was dissolved in 5 mL chloroform and then 125 µL linoleic acid and 1 g Tween 40 were added. Chloroform was evaporated in a vacuum of 450 bar at a temperature of 40 °C, and the residue was diluted to 500 mL with oxygenated water. Extract (2000 ppm) and synthetic antioxidant (2000 ppm) standards dissolved in ethanol. Samples (700 µl) were removed from solution and kept for 120 minutes in a 50 °C water bath (Nuve BM402 Model, Turkey). Zero-time absorption (T0) was measured at 470 nm after waiting time. After waiting for another 2 hours, the reabsorbing value (T120) was measured at 470 nm. B-carotene-free control sample (T0) was prepared and measured. This study was repeated for each extract and synthetic antioxidant reagent (α-tocopherol).

Table 1. Crude fat content of cocoa bean shells

Day	Shift	Crude fat content (g/100 g)
1	1	4.0
1	2	4.0
1	3	3.9
2	1	4.0
2	2	3.5
2	3	4.7
3	1	3.9
3	2	3.3
3	3	3.8
4	1	5.2
4	2	4.5
4	3	5.0
5	1	3.9
5	2	3.6
5	3	3.7
6	1	5.6
6	2	5.0
6	3	3.7
7	1	3.9
7	2	4.6
7	3	3.6

3. Results and Discussion

3.1. Soxhlet Extraction

3.1.1. Total Fat Analysis

Approximately 10-12% cocoa bean shell is obtained from the cocoa bean process as a waste. Soxhlet analysis was performed based on fat in cocoa bean shells. Samples were taken in 7 days and 3 shifts to observe the fluctuation in cocoa beans and processing. Table 1 presents the crude fat content determined by the Soxhlet method of total cocoa bean shell. The average value was 4.15 ±0.6%.

After total fat analysis of cocoa bean shell obtained from production line, sieve analysis was the next step aim to fix the particle size. Cocoa bean shells passed through five different sieves aim to a good separation of shells from nib. Samples were taken from each sieve and fat content was analysed with Soxhlet method (Table 2).

Tablo 2. The sieve-based analysis results

	1.Sieve %	**2.Sieve %**	**3.Sieve %**	**4.Sieve %**	**5. Sieve %**
Run 1	1.6	1.0	1.8	9.2	12.9
Run 2	1.5	1.0	1.9	9.2	13.0
Run 3	1.6	1.0	1.9	9.2	13.0
Average Value	1.57	1.00	1.87	9.20	12.97

Analysis was carried out in samples encoded from each sieve. According to the sieve-based analysis results, 12.98% fat was found on average in the cocoa bean obtained from the smallest size, called 5th sieve. That is why, the study was progressed through the shells of cocoa bean extracted from sieve no. 5.

3.2. Process Yield of SC-CO_2 Extraction: Parameter Optimization

The yield of the extract in SC-CO_2 extraction is affected by several factors including pressure, temperature, time, CO_2 flow rate, and particle size. As a result of the Soxhlet analysis, cocoa bean shell, obtained from the top of the 1 mm x 1 mm sieve (5^{th} sieve) with the highest fat content, was used (Table 2).

Table 3. Process parameters

Run Order	**Pressure (bar)**	**T (°C)**	**Flow rate (gCO_2/min)**	**Time (h)**	**Shell (g)**	**Extract (g)**	**Yield (%)**
1	250	60	40	6	130	9.83	7.56
2	350	60	40	6	130	11.95	9.19
3	250	70	40	6	130	9.10	7.00
4	350	70	40	6	130	12.63	9.72
5	250	60	60	6	130	9.84	7.57
6	350	60	60	6	130	19.62	15.09
7	250	70	60	6	130	11.90	9.15
8	350	70	60	6	130	13.40	10.31

The selected process parameters (time, CO_2 flow rate, pressure, temperature) were optimized using a method that one parameter was changed in each experiment when the others were kept constant [31]. Process parameters are presented in Table 3.

3.2.1. Process Time

The extraction yield was meant to improve as the procedure time increased [29]. Studies shows that 6 h is sufficient to obtain the highest extraction efficiency and over 6 h of extraction time it remains unchanged due to reaching equilibrium state level [32]. Process times were studied as 2 and 6 h while pressure, extraction temperature, CO_2 flow rate were kept constant. The extraction yield results are presented in Table 4. Based on the yield values obtained, 6 h of process time was selected to be a constant parameter for further analyses.

SC-CO_2 extraction method introduces several advantages over traditional methods such as Soxhlet. While the Soxhlet method is employed at 12 hours, SC-CO_2 extraction method can be completed in a few hours for yielding pure extracts with higher quality compared to traditional methods, which is the biggest advantage of SC-CO_2 extraction method for large-scale production in food industry.

Table 4. Comparison of extraction yields of 2 and 6 hours of process time

Run Order	Pressure (bar)	T (°C)	Flow rate (gCO_2/dk)	Time (hour)	Shell (gr)	Extract (gr)	Yield (%)
1	250	60	40	2	130	3.16	2.43
2	250	60	40	6	130	9.83	7.56
3	350	60	40	2	130	6.53	5.02
4	350	60	40	6	130	11.95	9.19
5	250	70	40	2	130	4.52	3.48
6	250	70	40	6	130	9.10	7.00

3.2.2. CO_2 Flow Rate

The yield of an extraction process increases with increasing flow rate. When the flow rate is comparably high, the mass transfer resistance around the feed particles decreases and the yield enhances [33]. For this purpose of optimizing of the flow rate; pressure, extraction temperature, process time, and feed amount were kept constant while 40 and 60 gCO_2/min flow rates were applied on to cocoa bean shells. Optimum CO_2 flow rate was specified as "60 g CO_2/min" because the maximum yield (15.09%) were obtained with 60 g CO_2/min of flow rate (Table 3).

3.2.3. Process Pressure

Pressure affects the yield and the selectivity of the extraction process. The solubility is increased, and the volume of the fluid is decreased by increasing pressure at a constant extraction temperature [29]. Temperature, CO_2 flow rate, process time and feed amount were kept constant while 250 and 350 Bar pressures were applied during the extraction process (Table 3).

3.2.4. Temperature

When temperature increased at a constant pressure, density of a supercritical fluid decreased, and its solubility is affected [34]. However, the process temperature is directly related to the raw material and the

fat part which is expected to be extracted. While optimizing the temperature, heat sensitive raw materials should be considered. SC-CO_2 extraction of cocoa bean shells were performed at two temperatures (60 and 70°C) while keeping the other process conditions constant. 60 °C was confirmed to be the most suitable temperature since maximum yield was obtained (Table 3).

The process yield of the cocoa bean shell extraction is related with the extraction efficiency. In SC-CO_2 method, detected optimum process parameters were used and extraction yield was calculated as 15.09% while in Soxhlet, it was 12.98%. Thus, SC-CO_2 technique can be appreciated as a preferable method to Soxhlet for cocoa fat extraction from cocoa bean shell.

3.3. Volatile Compounds of CBS Fat Extracts and Deodorized Cocoa Butter (DHA/GC-MS)

Cocoa Bean Shell fat extracts obtained by SC-CO_2 extraction and deodorized cocoa butter as a reference were performed using DHA/GC-MS. Cocoa volatiles generally occur during fermentation and bean drying. During the roasting stage, due to the Maillard reactions and Strecker degradation of flavour precursors and intermediates provide the characteristic chocolate flavour [35]. In a previous study, volatile compounds such as alcohols, carboxylic acids, aldehydes, ketones, esters, pyrazines, etc. of cocoa products and cocoa were identified as odour-active components [36].

In this study, 31 compounds were detected in run 6 and 28 compounds were detected in run 8, while 14 compounds were detected in deodorized cocoa butter which was used as a reference (Table 5). Table 5 summarizes the volatile compounds of CBS fat extracts and cocoa butter as a reference and also, includes several chemical classes such as alcohols, acids, esters, pyrazines, etc.. In Table 5, “a” indicates unpleasant flavours whereas “b” indicates pleasant flavours in cocoa butter and cocoa products.

According to flavour profiles of extracts and cocoa butter, due to the deodorization process of cocoa, cocoa butter contains a few unpleasant flavour compounds. Another important parameter is that a small amount of acetic acid is observed in cocoa butter, due to the improvement process of cocoa. Theobromine, caffeine, and pyrazines are characteristic compounds of cocoa and cocoa products. Theobromine was only observed in cocoa butter reference because of the production process. Cocoa butter is obtained from cocoa nib directly. Hence, these types of compounds are naturally observed in the cocoa direct process than the other extraction techniques.

In a recent study SC-CO_2 cocoa hull extract was performed using GC/MS in term of its volatile compounds. Caffeine, theobromine, tetramethyl pyrazine, ethyl oleate were not detected in cocoa hull extract [10]. These volatiles are characteristics for cocoa butter, our analysis results are similar with Mazzuti’s research.

In this study, it was observed that the characteristic flavouring compounds were higher in the extract obtained run 8. There are two alcohols, five aldehydes and ketones, six acids, ten esters, 1 lactone, two pyrrols, one pyrazine and one alkaloid. 2,3,5,6-Tetramehylpyrazine is detected only run 8. Benzyl alcohol, 1-phenylethanol, isovaleraldehyde, 2-phenyl-2-butanal, 5-methyl-2-phenyl-2-hexenal, phenylacetic acid, ethyl laurate, ethyl oleate, 2-acetylpyrrole, Pyrrole-2-carboxaldehyde, tetramethyl pyrazine and caffeine, which are considered the main contributors to the flavour profile and the key odour-active compounds of cocoa products, are observed in run 8 [36].

In order to completely understand the changes in flavor profiles, sensory analysis should be performed to quantify the variances in the final product's taste profile.

3.4. The Fatty Acid Composition of the Extracts and Cocoa Butter

In a previous study related to fatty acids profile from the cocoa nib, Okiyama *et al.* (2018) studied cocoa shell fat. The major components of cocoa shell fat were stearic (C18:0), oleic (C18:1) and palmitic (C16:0) acids, with concentrations of 12.1%, 28.2%, and 22.3% respectively [9].

In this study, the major components of cocoa bean shell extract of run 6 were stearic (C18:0), oleic (C18:1), and palmitic (C16:0) acids, with concentrations of 31.28%, 32.41%, and 23.62%, respectively. The major components of cocoa bean shell extract of run 8 were stearic (C18:0), oleic (C18:1) and palmitic (C16:0) acids, with concentrations of 30.83%, 32.1%, and 23.72%, respectively. According to these results,

run 6th and 8th were found to be similar for fatty acid compositions, while these findings are higher than the results reported in literature. In conversely, these results were found to be lower than that of cocoa butter in terms of palmitic acid, stearic acid, and oleic acid contents. The differences in fatty acid composition are indicated that fats from cocoa bean shells and cocoa nib are not formed by the passage of fat from the shell to the nib of cocoa.

Cocoa butter is obtained by using processes including pressing, centrifugations, and filtration. Therefore, there is a high amount of sediment observed in the fat. For this reason, cocoa bean shell fat obtained from SC-CO_2 extraction was expected to contain a higher number of fatty acids than that of deodorized cocoa butter. Nevertheless, both results of cocoa butter (deodorized and SC-CO_2 extract) were found to be similar.

Cocoa butter fatty acid composition included stearic (C18:0), oleic (C18:1) and palmitic (C16:0) acids, with concentrations of 36.5%, 33.04% and 25.95%, respectively. In contrary, deodorized cocoa butter fatty acid composition included stearic (C18:0), oleic (C18:1) and palmitic (C16:0) acids, with concentrations of 36.5%, 33.12% and 26.05%, respectively. These results are higher than the literature values.

The variation in fatty acid composition is attributed to the fact that cocoa nib is an agricultural product that is affected by climatic conditions, soil type, and fermentation process.

3.5. Total Phenolic Content

The total phenolic content (TPC) of the samples is presented as the equivalent of gallic acid. The solubility of phenolic components is relatively lower in CO_2, while solubility in ethanol is higher [10]. Mazzutti *et al.* showed the lowest TPC was observed in the extractions obtained only by applying SC-CO_2. The average results of the TPC analyses conducted in our study were found to be 51.17±1.27 mg GAE/100 g in run 6 and 53.38±1.27 mg GAE/100 g in run 8 sample. The result of the reference cocoa butter sample was observed to be 56.34±1.28 mg GAE/100 g. When the extraction samples obtained by applying SC-CO_2 were compared with reference cocoa butter, the difference was found to be significant ($p<0.05$). Total phenolic compounds of SC-CO_2 extraction samples were found to be lower than reference cocoa butter.
In a study of HPLC analysis in extracts with SC-CO_2, it was noted that there was no presence of phenolic compounds [23]. In another SC-CO_2 study with cocoa pod shell, ethanol was used as co-solvent. The total phenolic compounds ratios in the extract obtained in the method where the highest yield was obtained were determined as 12.87 mg GAE/g extract [37].

3.6. β-carotene-Linoleic Acid Analysis

Inhibition values, calculated from absorbance values measured at t=0 and t=120 in the study, were 21.5±1.28% in run 6th sample; run 8th measured as 21.7±1.37% in the sample. The inhibition percentage of the reference cocoa butter sample was calculated as 26.3±0.26%. As standard α-tocopherol measurement was also performed. α inhibition percentage of α-tocopherol was calculated as 36.7±2.43%.
Inhibition percentages of SC-CO_2 extraction samples were found to be significantly higher compared to the reference cocoa butter ($p<0.05$). Inhibition values (ID) of SC-CO_2 extraction samples were found to be lower than reference cocoa butter.

Inhibition rates obtained by β-carotene inhibition method in cocoa butter samples reported by Mazzutti *et al.* [10] were 15-26%. It was also reported in the same study that samples obtained with SC-CO_2 did not have a significant difference in terms of antioxidant performance. In another study, it was indicated that cocoa bean phenolic compounds poorly inhibited β-carotene oxidation [38].

Table 5. Volatile compounds of cocoa bean shell butter and deoderized cocoa butter

Run 6 CBS butter			**Run 8 CBS butter**			**Deodorized Cocoa Butter (Reference)**		
Compounds	**% Area**	**Odor quality**	**Compounds**	**% Area**	**Odor quality**	**Compounds**	**% Area**	**Odor quality**
Alcohols and phenols			**Alcohols and phenols**			**Aldehydes and Ketones**		
Benzyl alcohol[b]	0.11	Sweet, floral	Benzyl alcohol[b]	0.07	Sweet, floral	2-Heptadecanone	0.26	-
1-phenylethanol[b]	0.07	Honey-floral	1-phenylethanol[b]	0.04	Honey-floral	2-Nonadecanone	0.26	-
Aldehydes and Ketones			**Aldehydes and Ketones**			**Acids**		
Isovaleraldehyde[b]	0.07	Chocolate	Isovaleraldehyde[b]	0.05	Chocolate	Acetic acid[a]	0.18	Sour, vinegar
2-fenil-2-butenal[b]	0.06	Sweet	2-fenil-2-butenal[b]	0.08	Sweet	Myristic acid[a]	0.47	Waxy, fatty, soapy
2-Pyrollidinon	0.25	-	2-Pyrollidinon	0.18	-	**Esters**		
5-Methyl-2-phenyl-2-hexenal[b]	0.39	Cocoa	5-Methyl-2-phenyl-2-hexenal[b]	0.18	Cocoa	Ethyl myristate[a]	0.1	Soapy
Acids			2-Heptadecanone	0.17	-	Methyl palmitate[a]	0.71	Waxy
Acetic acid[a]	1.16	Sour, vinegar	**Acids**			Ethyl palmitate[a]	1.39	Waxy
Isovaleric acid[a]	0.05	Rancit, sour	Acetic acid[a]	0.75	Sour, vinegar	Methyl stearate[a]	0.68	Fatty
Hexanoic acid[a]	0.09	Sour, astringent	Isovaleric acid[a]	0.04	Rancit, sour	Methyl octa-8-decanoate	0.82	-
Octanoic acid[a]	0.16	Yağsı	Hexanoic acid[a]	0.08	Sour, astringent	Ethyl oleate[b]	2.2	Fatty, oily, milky
Phenylacetic acid[b]	0.67	Sweet, floral, chocolate	Phenylacetic acid[b]	0.65	Sweet, floral, chocolate	Methyl linoleate	0.33	Oily, fatty
Myristic acid[a]	1.42	Waxy, fatty, soapy	Myristic acid[a]	0.75	Waxy, fatty, soapy	Ethyl linoleate	1.63	-
Pentadecanoic acid	0.32	-	Pentadecanoic acid	0.14	-	Alkaloids		

pleasant flavour and [b] is pleasant flavour

Table 5. continued

Run 6 CBS butter			**Run 8 CBS butter**			**Deodorized Cocoa Butter (Reference)**		
Margaric acid (Heptadecanoic acid)	0.45	-	**Esters**			**Alkaloids**		
Esters			Ethyl decanoate	0.06	Fruity (pear, grape)	Teobromin[b]	0.89	Cocoa
Ethyl laurate[b]	0.23	Floral, fruity	Ethyl laurate[b]	0.15	Floral, fruity	Kafein[b]	8.05	Coffee
Methyl myristate[a]	0.05	Fatty	Ethyl myristate[a]	0.35	Soapy			
Ethyl myristate[a]	0.39	Soapy	Methyl palmitate[a]	0.45	Waxy			
Ethyl palmitate[a]	0.56	Waxy	Ethyl palmitate[a]	2.92	Waxy			
Ethyl palmitate[a]	4.12	Waxy	Methyl stearate[a]	0.24	Fatty			
Ethyl heptadecanoate	0.19	-	Ethyl oleate[b]	1.99	Fatty, oily, milky			
Methyl stearate[a]	0.4	Fatty	Metil linoleate[a]	0.53	Oily, fatty			
Pyrroles			**Alkaloids**					
2-Acetylpyrrole[b]	0.09	Chocolate, hazelnut	Cafein[b]	4.09	Coffee			
Tiazols								
Benzothiazole[b]	0.15	Brown, coffee like						
Alkaloids								
Cafein[b]	5.8	Coffee						

[a] is unpleasant flavour and [b] is pleasant flavour

4. Conclusion

In the GC analysis, the difference in fatty acid composition between the SC-CO_2 extraction samples and the reference cocoa butter was examined. For cocoa butter, oleic, palmitic, and stearic acids are known to be naturally present in cocoa butter. It was observed that the related fatty acid ratios in the SC-CO_2 extraction samples were lower than the reference. To better understand this difference, it is necessary to measure their effectiveness in processes such as shelf-life test and tempering in the final product.

It was observed that SC-CO_2 extraction samples, for which flavour profiles were investigated, contained more components compared to the reference cocoa butter. The fat obtained from the cocoa bean shell by SC-CO_2 extraction can be mixed with 5% of the amount of cocoa butter in the final product formula.

It is known that cocoa beans can show antioxidant effect which is attributed to theobromine and phenolic compounds. However, it was determined that the cocoa bean shell, which is a waste or evaluated as an animal feed, was less effective than the cocoa butter. At the same time, its effect appears to be lower than that of cocoa nib in its cocoa butter. It is lower than expected due to the excessive heat treatment of the reference during manufacture. Accordingly, in this study, it was observed that cocoa bean shell extracts obtained by SC-CO_2 extraction do not have a significant antioxidant capacity. The process parameters determined after optimization should also be tested and verified at industrial scale.

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ORCID
Ertan Sahin: 0000-0002-2961-4941
Esra Çapanoğlu: 0000-0003-0335-9433
Aslı Can Karaça: 0000-0002-4137-0644
Aslı Barla Demirkoz: 0000-0002-3261-0212

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