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Research Article

Analyzing the Chemical Composition and Quality Attributes of Cocoa Butter from Different Producers: A Comparative Study

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Abstract

Cocoa butter is a highly prized and versatile product with a wide range of applications. It is a crucial component of chocolate due to its unique melting properties and ability to dissolve other ingredients. Therefore, it is essential to evaluate the compositional characteristics of cocoa butter from different sources to ensure its quality and authenticity. This study aimed to analyze the triacylglycerols, fatty acids, sterols, styrene compounds, extinction coefficient, and Solid Fat Content (SFC) of cocoa butter from nine diverse samples. The findings revealed that the main triacylglycerols were POS (39.12-40.25), SOS (25.73-28.91) and POP (16.07-17.74) while the primary fatty acids were stearic acid (32.46-35.64), oleic acid (31.51-32.47) and palmitic acid (26.25-28.96) The dominant sterols were (55.04-56.98 %), stigmasterol (25.4-26.2 %), and campesterol (9.22-10.53 %), with the ratio of stigmasterol to campesterol (2.5 to 2.7) serving as an indicator of authenticity. The study also found minimal levels of stigmastadiene (0.0001–0.0039 ppm), indicating low content dehydrated sterols, and measured the SFC content at different temperatures. Overall, this comprehensive analysis provides valuable insights into the composition of cocoa butter from various sources, offering important information for quality control and detection of adulteration.

Keywords: Triacylglycerol, Phytosterols, Fatty acid profile, SFC, Extinction coefficient

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1. Introduction

Chocolate is a widely used confectionary item worldwide, and supplies are growing. Chocolate's primary components are cocoa mass, cocoa butter (CB), sugar, and, in the case of milk chocolate, milk fat. 30 to 40% of the weight of chocolate can be made up of CB [1].

CB is the fat obtained from the *Theobroma cacao* tree, which is mainly composed of triacylglycerols (up to 98%) along with other minor components such as sterols, tocopherols, carotenoids, and other compounds [2].

The CB has unique fatty acids (FAs), i.e., stearic acid, oleic acid, palmitic acid, linoleic acid (36.5%, 33.5%, 25.8%, 2.4%, respectively), and triacylglycerols (TAG), i.e., (POS, SOS, POP: 40.2%, 25.7%, 17.6% respectively), making it appropriate for use as a foundation for confectionery products. At room temperatures (25 °C), the CB is mainly solid; however, it melts fully at temperatures of 37 °C or body temperature [3].

The cocoa tree's seeds contain high levels of phytosterols in their free and esterified forms (200–300 mg/100 g fat). βsitosterol and stigmasterol, the two most prevalent of them, represent 59 and 22%, respectively, in cocoa beans. Concentrations of campesterol, cycloartenol, 24-methylenecycloartanol, D5-avenasterol, and sitostanol are much lower. A significant class of substances in ways that are healthful for people is phytosterols [4].

Although the CB is ideal for chocolate formulation, there are a few weaknesses, including low harvest quality and technological defects. Hence, companies in the chocolate and confectionery sectors are looking for a cheap substitute compatible with CB's unique physicochemical characteristics [3]. In the food industry, verifying authenticity and discovering adulterations are becoming extremely important challenges, and methods for detecting variations between product labels and their actual composition are being investigated [5].

Sometimes it is problematic to distinguish CB from substitutes to monitor and control the quality of products on the market, and other fats may be sold as adulteration instead of CB. For this reason, it is necessary to determine the range of physicochemical properties of real CB to distinguish it from counterfeits. This study aims to determine some physicochemical properties, such as the profile of fatty acids, the construction of TAG, and the composition of phytosterols and styrene compounds, along with the extinction coefficient and SFC, in nine samples of original CB to determine suitable indicators for evaluation.

2. Materials and methods

2.1. Sample Preparation and Supplements

Methanol, sodium hydrogen sulfate, iso-octane, acetone, methylcyclohexane, pentanoate, butanoic acid, *n*-hexane, ethanol, anhydrous sodium sulfate, potassium hydroxide, silica gel, ethyl acetate, dichlorofluorescein, phenolphthalein, alpha cholestanol, 3, 5 cholestadiene, normal nonacosane, Stigmasta-3,5-diene, ethyl ether, aluminum oxide, diethyl ether, and botulin were purchased from Merck-Germany.

For this study, CB samples were chosen from various producers. The sources of the samples are shown in Table 1. The samples were carefully selected to represent well-known brands used by consumers from various economic classes. Before analysis, the samples were stored at 4°C in a refrigerator.

Table 1. Information on the origins of the examined cocoa butter samples

Sample name	Brand name	Origin
S1	Cargil	Netherlands
S2	Altin marka	Türkiye
S3	Afrighana	Ghana
S4	Panice	Germany
S5	Euromal	Germany
S6	Hakan	Germany
S7	DeZan beurre	Netherlands
S8	DeZaan olam	Netherlands
S9	K.L.Kepong	Malaysia

2.2. Triacylglycerols (TAGs) analysis

TAGs were determined using the approved AOCS Ce 5-86 procedure. A YL6500 GC gas chromatograph was used to conduct the GC analyses, which were done at 370°C with a flame ionization detector (FID) and a cold-on-column injector. The column was formed of a 30 m x 0.53 mm (0.1 m film thickness) REStEK Rtx-65 column connected in series with a 1 m x 0.53 mm precolumn/retention gap. 40 µL of fat was diluted in 2.0 mL of iso-octane to produce the samples, and 10 mL of the sample solutions were then further diluted in 1.5 mL of iso-octane. At a temperature of 100°C in the oven, samples (1 µL) were injected. The GC was heated after injection at 30°C/min gradient to 325°C, held for 1 min, and then heated again at 1°C/min to 345°C and finally at 5°C/min to 370°C [6].

2.3. Fatty acid profile analysis

The AOCS Ce 2–66 technique was employed for analyzing the fatty acid profile [7]. CB fatty acids were determined using Gas chromatography (SHIMADZO GC-2030) with a FID. The GC column (Dikmacap-2330) Capillary column 60 m × 0.25 mm, id 0.25 m, used Helium gas as the carrier phase. The injection volume and temperature were 1 µl and 250°C, respectively.

2.4. Phytosterols analysis

For soaping, which is carried out following standard 12228-1 (ISO, 2014) using alcoholic potassium hydroxide, 15 g of each sample was weighed. Diethyl ether is used to remove the non-saponifiable substance. After that, thin-layer chromatography (TLC) was used to separate the sterol content from the unaponifiable material. Sterols were then transformed into derivatives of silyl. Gas chromatography (SHIMADZO GC-2030) was used with a FID and hydrogen gas as the carrier phase. A 30 m 0.25 mm GC capillary column called the Equity-5 (SUPELCO) was used. Each sterol was identified by comparing its retention time to that of the reference sample [8].

2.5. Determination of styrene compounds (Stigmastadiene)

Around 20.0 g of CB was mixed with 1 mL of a standard cholesta-3,5-diene solution (0.3 mg mL-1). n-Hexane was used to remove the unsaponifiable material from previously saponified CB samples. The unsaponifiable component of the samples' substance was separated into its steroidal hydrocarbon component and extracted in a silica gel column. The target hydrocarbon fraction, which should have been in the center of the passing solution, was collected, and the drying procedure was completed by evaporating it. A GC-FID was used to analyze the residue after it dissolved in n-hexane. The following were the settings for the gas chromatograph: carrier gas is helium, pressure is 120 kPa, split ratio is 1:5, the temperature of the injector is 300 °C, and the temperature rises from 235 °C (6 minutes) to 285 °C by 2 °C each minute [9].

2.6. Extinction coefficient measurement

A spectrofluorometer method was used to determine the extinction coefficient. In 10 ml of isooctane, 0.05 g of CB samples were dissolved. A UV/VIS, U-2800-A spectrophotometer (Hitachi, Chiyoda, Tokyo, Japan) was used to determine CD and CT absorbance values at 232 and 268 nm, respectively, with the solvent supplied as a blank. The sample extinction E (1%, 1 cm) was given as a result of recalculating the sample concentration to be a 1% (w/v) solution of CB in isooctane [10].

2.7. Solid fat content (SFC) index

The standard method of ISO-8298 [11] was used to determine the SFCs of the CBs using P NMR. CB samples were melted for 30 minutes at 80 °C to complete melting and homogeneity. 3 g of fat samples were tempered at 0 °C for 90 minutes before analysis. The following situations were used to temper the fat samples: At 10 °C to 40 °C (after 30 minutes of equilibration at each 5 °C interval), the SFC values of the fat samples were measured at a 90° radio frequency pulse.

2.8. Statistical analysis

The influence of CB origin and brands on the physicochemical parameters of CB samples was investigated using a one-factor ANOVA. With the help of Duncan's multiple range tests, the significance of mean differences was determined ($P<0.05$). With the SPSS software (version 25.0 software, SPSS Inc., Chicago, IL, USA), all analyses were conducted.

3. Result and discussion

3.1. Triacylglycerols (TAGs)

TAGs are significant from a technical and dietary perspective. They are one of the key components in vegetable oils and fats that can be employed to detect adulteration as well as determine authenticity [12,13].

The TAG contents of CB samples were evaluated, revealing variances between samples (Table 2 and Figure 1). Identi-

fied TAGs for the CB samples were PPP, MOP, PPS, POP, PLP, PSS, POS, POO, PLS, PLO, SSS, SOS, SOO, OOO, SLO, SOA, and AOO. CB samples exhibit complete differentiation for POS, SOS, and POP. These data agree with the previously published data (Dionisi, et al. [14]. According to their findings, three POP, PLS, and POS were shown to be most useful in differentiating between CB and Cocoa Butter Equivalents (CBE). Specifically, for this usage, the POP would be most beneficial. Moreover, two TAG ratios (POS/PLP and POP/PLS) can differentiate between CB and CBE [14].

3.2. Fatty acid profile analysis

The fatty acid composition of edible oils is used to assess their oxidative stability and nutritional value. However, the distribution of fatty acids on the TAGs is also determined by the physical and chemical characteristics of the oils and fats [15]. For all CB samples, the most significant fatty acids were C16:0 (>26%), C18:0 (>32%), and C18:1 (>32%). These findings corroborated previously reported data [16].

According to the obtained results, the following fatty acids C14:0, C16:0, C17:0, C18:0, C16:1 and C17:1 and C18:1 was significantly affected by the origin and CB production conditions (Table 3). These findings support previous observations that the geographic origin of CB affected its fatty acid content. Therefore, variations should be considered in the analysis and determination of the fatty acid profile of the CB and evaluation of its authenticity and purity.

3.3. Phytosterols analysis

For each sample, the quantity of both individual and total phytosterols was determined (Table 4). The total sterols in all samples were 2159 to 2912 mg/kg, which were significantly different among all samples. As with other vegetable oils and fats, β -sitosterol was the most important and main phytosterol in all CB samples (55-58%), followed by stigmasterol (25%), and campesterol (9%). These results are in agreement with the previously published. The major phytosterol in pure CB was discovered to be sitosterol, followed by campesterol, and stigmasterol, which were 123.3, 18.7, 60.1, and 8.6 mg/100 g, respectively [17]. Another study revealed that CB is one of the sources of phytosterols and can be affected by geographic factors [18].

In a previously published research, two different varieties of cocoa beans were examined to determine how the roasting procedures affected the phytosterol concentration. In all raw samples, They discovered that of all the phytosterols, b-sitosterol (56–61%) made the most considerable contribution, followed by stigmasterol (30–39%), campesterol (6-7%), and D5-avenasterol (0.5–0.7%) [4]. Other studies reported that, roasted cocoa beans, like unroasted samples, were primarily composed of b-sitosterol, followed by stigmasterol, campesterol, and D5-avenasterol. Overall, the content of phytosterols decreased after heat treatment, and the specific reduction depended on the roasting conditions and variety of cocoa beans analyzed [4].

3.4. Styrene compounds (Stigmastadiene)

Analytical methods such as counting the numbers of steroidal hydrocarbons and the specific extinction at 270 nm (E 270) can determine whether refined oils are present in virgin oils (Steradienes). During the refining procedure, molecules with conjugated double bonds may be formed due to the dehydration of desmethylsterols from the unsaponifiable fraction and the conjugation of unsaturated fatty acids to glycerol. First, the process of bleaching the earth results in the oxidation of polyunsaturated fatty acids (such as linoleic acid), which promotes the creation of conjugated double bonds. Vegetable oils containing these compounds can be identified by their enhanced UV absorbance at 270 nm [9].

Furthermore, during the refining processes, a minor portion of 4-demethylsterols undergoes dehydration reactions, resulting in unsaturated hydrocarbons. The most prevalent steroidal molecules, stigmata-3,5-diene, is produced when beta-sitosterol is dehydrated. Consequently, stigmasterol, campesterol, and beta-sitosterol all result in stigmasta-3,5-diene, stigmastan-3,5,22-triene, and campesterol also result in campesta-3,5-diene. These residues of compounds, which are formed from sterols during the bleaching process, indicate that virgin oils contain refined oils. A well-known method for figuring out how much edible oils have been refined (especially bleached) is the measurement of stigmasta-3,5-diene and other steroidal hydrocarbons, also known as steradians. Evidence of a refining process, such as stigmastadiene, is now of utmost relevance because of the rising concern about food safety [9].

The investigation of stigmastadiene in nine samples of CB ranged from 0.0001-0.0039 mg/kg (Table 5). Then it was clear that these samples are natural, and the reported range can be used as a finger test to determine the original CB from other fats.

The recent research revealed that the reduction in β-sitosterol levels might be caused by the sterols conversion to steradiene when water is removed during the high-temperature frying, bleaching, and deodorization of vegetable oils [4]. While refined butter may contain up to several hundred mg/kg, the stigmastadiene level in unrefined cocoa butter is much below 0.1 mg/kg [19].

Table 2. Triacylglycerols (TAGs) composition of nine different samples of CB (%)

TAGs	S1	S2	S3	S4	S5	S6	S7	S8	S9
PPP	0.23±0.02^{a}	0.16±0.01^{bc}	0.24±0.01^{a}	0.23±0.02^{a}	0.18±0.02^{b}	0.13±0.01^{e}	0.14±0.00^{de}	0.15±0.00^{cd}	0.15±0.02^{cd}
MOP	0.26±0.04^{b}	0.19±0.01^{cd}	0.28±0.02	0.31±0.03^{a}	0.22±0.02^{c}	0.16±0.01^{d}	0.18±0.02^{d}	0.15±0.03^{d}	0.17±0.02^{d}
PPS	0.53±0.09^{cd}	0.49±0.06^{d}	1.0.37±0.097^{a}	0.68±0.08^{b}	0.62±0.07^{bc}	0.47±0.06^{de}	0.45±0.06^{de}	0.44±0.06^{de}	0.37±0.04^{e}
POP	16.65±0.12^{b}	16.61±0.09^{b}	16.07±0.06^{d}	16.40±0.09^{c}	16.27±0.12^{b}	16.64±0.12^{b}	16.61±0.06	17.74±0.1^{5}a	16.68±0.09^{b}
PLP	1.60±0.03^{c}	1.63±0.05^{c}	1.69±0.05^{bc}	1.91±0.08^{a}	1.74±0.09^{bc}	1.79±0.17^{ab}	1.72±0.09^{bc}	1.65±0.07^{bc}	1.61±0.08
PSS	0.85±0.04^{a}	0.75±0.03^{b}	0.07±0.03^{b}	0.87±0.03^{a}	0.57±0.02^{d}	0.63±0.03^{c}	0.39±0.02^{e}	0.66±0.03^{c}	0.30±0.02^{f}
POS	39.87±0.16^{b}	39.62±0.29^{bc}	39.12±0.11^{e}	.39.21±0.11^{e}	39.40±0.12^{cde}	39.32±0.19^{de}	39.52±0.21^{cd}	40.25±0.18	39.91±0.09
POO	1.73±0.09^{c}	1.78±0.07^{bc}	1.71±0.10^{c}	1.72±0.14^{c}	1.94±0.09^{ab}	2.1±0.18^{a}	1.78±0.14^{bc}	1.84±0.11^{bc}	1.79±0.06^{bc}
PLS	3.18±0.07^{c}	3.34±0.12^{bc}	3.24±0.10^{c}	3.56±0.17^{a}	3.2±0.05^{c}	3.28±0.13^{bc}	3.26±0.08^{c}	3.45±0.13	2.83±0.04^{d}
PLO	0.25±0.03^{a}	0.23±0.02^{a}	0.26±0.03^{a}	0.26±0.02^{a}	0.24±0.025^{a}	0.26±0.02^{a}	0.25±0.04^{a}	0.29±0.02^{a}	0.26±0.03^{a}
SSS	0.44±0.02	0.39±0.06^{a}	0.39±0.03^{a}	0.35±0.03^{a}	0.39±0.05^{a}	0.33±0.00^{a}	0.34±0.02^{a}	0.40±0.06^{a}	0.32±0.02^{a}
SOS	27.27±0.05^{bc}	27.23±0.12^{c}	27.44±0.12^{b}	27.35±0.08^{bc}	26.51±0.06^{d}	26.68±0.18^{d}	27.18±0.09^{c}	25.73±0.1^{1}e	28.91±0.09^{a}
SOO	2.55±0.09^{c}	2.54±0.03^{c}	2.56±0.09^{c}	2.65±0.04^{c}	3.11±0.07^{a}	3.16±0.06^{a}	2.97±0.12^{b}	2.59±0.07^{c}	2.21±0.02^{d}
OOO	2.83±0.16^{d}	1.97±0.06^{c}	2.21±0.079^{a}	2.12±0.07^{ab}	2.03±0.07^{bc}	2.11±0.07^{ab}	2.11±0.07 ^{ab}	2.06±0.05^{bc}	2.19±0.02^{a}
SLO	0.23±0.02^{d}	0.28±0.04^{cd}	0.36±0.04^{ab}	0.38±0.05^{ab}	0.39±0.07^{a}	0.28±0.06^{cd}	0.33±0.04^{abc}	0.32±0.04^{abc}	0.30±0.03^{bcd}
SOA	1.32±0.07^{c}	1.51±0.06^{a}	1.43±0.06^{ab}	1.41±0.00^{b}	1.43±0.03^{ab}	1.32±0.05^{c}	1.40±0.03^{bc}	1.20±0.05^{d}	1.45±0.05^{ab}
AOO	0.05±0.03^{b}	0.07±0.02^{b}	0.06±0.03^{b}	ND	0.06±0.03^{b}	ND	0.24±0.03^{a}	0.05±0.03^{b}	0.06±0.01^{b}

[a] Mean and standard deviation of three replicates is reported. Superscript letters represent statistically significant differences between CB samples ($P<0.05$). P: palmitic acid, O: oleic acid, S: stearic acid, M: myristic acid, L: linoleic acid, A: arachidonic acid.

Sample names S1, S2, and... are mentioned in Table 1.

Table 3. Fatty acid profile of nine different samples of CB (%)

Fatty acids	S1	S2	S3	S4	S5	S6	S7	S8	S9
Lauric acid (C12:0)	0.01±0.00^{a}	0.01±0.01^{a}	0.02±0.01^{a}	0.01±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.04±0.01^{a}	0.02±0.01^{a}
Myristic acid (C14:0)	0.12±0.01^{b}	0.12±0.01	0.12±0.01^{b}	0.12±0.01^{b}	0.13±0.01^{b}	0.13±0.01^{b}	0.13±0.00^{ab}	0.14±0.01^{a}	0.12±0.00^{b}
Palmitic acid (C16:0)	27.343±0.051^{d}	27.047±0.031^{e}	26.82±0.08^{f}	28.4±0.02^{b}	27.82±0.07^{c}	26.89±0.05^{f}	26.25±0.05^{h}	28.96±0.16^{a}	26.62±0.06^{g}
Palmitoleic acid (C16:1)	0.40±0.00^{bcde}	0.40±0.00^{bcde}	0.38±0.02^{de}	0.39±0.01^{cde}	0.45±0.03^{a}	0.37±0.02^{e}	0.42±0.03^{abc}	0.43±0.03^{ab}	0.41±0.02^{bcd}
Heptadecanoic acid (C17:0)	0.27±0.01^{cde}	0.29±0.01^{bcd}	0.29±0.03^{bc}	0.26±0.02^{de}	0.37±0.02^{a}	0.25±0.01^{e}	0.32±0.02^{b}	0.28±0.03^{cde}	0.28±0.01^{cde}
Margaric acid (C17:1)	0.07±0.02^{bc}	0.06±0.01^{bc}	0.04±0.02^{de}	0.04±0.01^{de}	0.11±0.02^{a}	0.02±0.01^{e}	0.08±0.02^{b}	0.04±0.01^{cde}	0.05±0.02^{cd}
Stearic acid (C18:0)	34.36±0.09^{b}	33.97±015^{c}	35.64±0.07^{a}	33.68±0.09^{d}	32.46±0.06^{f}	33.02±0.11^{e}	33.71±0.09^{d}	32.59±0.09^{f}	35.51±0.09^{a}
Vaccenic acid (C18:1T)	ND	ND	ND	ND	0.37±0.05^{a}	0.31±0.05^{bc}	0.39±0.04^{a}	0.28±0.02^{c}	0.35±0.05^{ab}
Oleic acid (C18:1C)	32.17±0.11^{bc}	32.24±0.04^{b}	32.47±0.08^{a}	31.75±0.09^{de}	31.61±0.09^{ef}	32.02±0.12^{c}	32.23±0.07^{b}	31.51±0.09^{f}	31.81±0.09^{ef}
Linolelaidic acid (C18:2T)	0.01±0.00^{cd}	0.03±0.00^{ab}	0.03±0.01^{ab}	0.02±0.01^{ab}	0.03±0.01^{ab}	0.03±0.00^{ab}	0.02±0.01^{bc}	ND	0.03±0.01^{a}
Linoleic acid (C18:2C)	2.69±0.12^{d}	3.05±0.02^{c}	2.80±0.122^{d}	3.573±0.065^{a}	3.573±0.065^{a}	3.337±0.087^{b}	3.403±0.175^{b}	2.713±0.064^{d}	2.73^{d}
Arachidic acid (C20:0)	0.887±0.045^{e}	1.433±0.029^{b}	0.86±0.056^{e}	1.04±0.057^{d}	1.69±0.06^{a}	1.44±0.05^{c}	1.61±0.083^{a}	0.93±0.03^{e}	1.15±0.03^{c}
Linolenic acid (C18:3N3)	0.13±.015^{e}	0.28±0.00^{c}	0.14±0.01^{e}	0.36±0.03^{b}	0.42±0.03^{a}	0.33±0.03^{bc}	0.31±0.35^{bc}	0.28±0.03^{c}	0.23±0.02^{d}
Eicosenoic acid (C20:1)	0.12±0.03^{de}	0.17±0.01^{c}	0.05±0.01^{f}	0.27±0.01^{b}	0.37±0.01^{a}	0.25±0.02^{b}	0.10±0.01^{e}	0.14±0.03^{cd}	0.11±0.02^{de}
Behenic acid (C22:0)	0.21±0.03^{a}	0.18±0.01^{a}	0.15±0.01^{a}	0.23±0.03^{a}	0.15±0.04^{a}	0.18±0.02^{a}	0.18±0.02^{a}	0.18±0.02^{a}	0.16±0.02^{a}
Lignoceric acid (C24:0)	0.01±0.01^{d}	0.09±0.01^{ab}	0.08±0.02^{abc}	0.05±0.02^{c}	0.10±0.02^{ab}	0.08±0.03^{abc}	0.11±0.01^{a}	0.06±0.01^{c}	0.07±0.02^{bc}

[a] Mean and standard deviation of three replicates is reported. Superscript letters represent statistically significant differences between CB samples ($P<0.05$).

Sample names S1, S2, and... are mentioned in Table 1.

Table 4. Sterol's composition of nine different samples of CB (%)

Samples	S1	S2	S3	S4	S5	S6	S7	S8	S9
cholesterol	1.94±0.00[a]	1.99±0.01[a]	0.71±0.01[e]	1.78±0.01[c]	1.84±0.01[b]	1.58±0.01[d]	1.84±0.00[b]	1.76±0.01[c]	1.84±0.09[b]
Brassicasterol	0.33±0.01[ef]	0.36±0.01[d]	0.29±0.01[g]	0.38±0.01[c]	0.437±0.01[a]	0.03±0.00[e]	0.20±0.00[h]	0.41±0.01[b]	0.33±0.01[f]
Campesterol	9.73±0.01[c]	9.68±0.01[c]	9.22±0.00[f]	9.73±0.03[c]	9.92±0.02[b]	9.53±0.01[e]	9.74±0.01[c]	10.05±0.01[a]	9.61±0.12[d]
Stigmasterol (%)	26.51±2.89[a]	25.79±0.02[a]	25.91±0.01[a]	25.35±0.01[a]	25.72±0.01[a]	25.99±0.01[a]	25.19±0.03[a]	25.4±0.01[a]	25.43±0.43[a]
β -sitosterol (%)	56.34±0.00[c]	55.04±0.02[d]	58.26±0.01[a]	56.79±0.02[b]	55.14±0.01[d]	56.95±0.02[b]	56.86±0.05[b]	56.20±0.57[c]	56.18±0.14[c]
Sitosterol (%)	1.12±0.00[a]	0.96±0.01[c]	ND	0.92±0.01[d]	1.11±0.01[a]	ND	0.98±0.01[c]	1.06±0.01[b]	1.05±0.06[b]
Delta-5-avenas-terol (%)	2.66±0.00[a]	2.53±0.01[a]	2.71±0.00[a]	2.46±0.06[a]	2.62±0.01[a]	2.70±0.01[a]	2.68±0.01[a]	2.08±1.19[a]	2.63±0.03[a]
Delta-7-avenas-terol (%)	0.34±0.00[b]	0.40±0.00[a]	0.20±0.00[d]	0.17±0.01[g]	0.21±0.01[c]	0.19±0.01[e]	0.15±0.00[h]	0.18±0.01[f]	0.15±0.00[h]
Total Sterols (mg/kg)	2400.12±0.19[f]	2439.65±1.12[e]	2912.26±0.22[a]	2535.14±0.94[b]	2317.39±0.52[h]	2328.32±0.67[g]	2528.24±2.32[c]	2159.63±0.38[i]	2454.49±0.25[d]

[a] Mean and standard deviation of three replicates are reported. Superscript letters represent statistically significant differences between CB samples ($P<0.05$).

Sample names S1, S2, and... are mentioned in Table 1

3.5. Extinction coefficient measurement

During storage, oils and fats undergo chemical changes that result in oxidation stability, which is one of the most important quality characteristics. These interactions result in hydroperoxides, a class of moderately unstable chemicals that, over time, resemble secondary oxidation compounds, as well as the conversion of aldehydes. The extinction coefficient is an excellent metric for evaluating the oxidative stability of oils and fats. The value of the extinction coefficient in all of the CB samples was found to be 0.001 (Table 5), indicating the lack of oxidation.

3.6. Solid fat content (SFC) index

The SFC parameter measures the solid and liquid mass ratio of fat at different temperatures, which influences important sensory and physical properties, including consistency and stability. The SFC curve shows how well CB and its combinations with other fats work technologically. CB hardness is determined by SFC between 20 and 25 °C. While the quick melting between 32 and 35 °C gives the taste sensations of cooling and creaminess, the temperature range at which there is a conspicuous decrease in SFC reveals the heating resistance. The difference between the SFC at 25 and 35 °C is one of the more important and valuable metrics employed by the industry to assess the quality of CB. At temperatures above 35 °C, the presence of solid fat is identified as a waxy feeling and is easily recognized when eating. Therefore, the combinations must show appropriate melting qualities at oral temperature with no waxy residual (no SFC over 35 °C) to be employed in the manufacture of chocolate. They must also be brittle and being hard at room temperature (SFC higher than 50% at 25 °C) [20].

Figure 2 displays the SFC value found in CB samples. Sample S8 had the most excellent SFC value at 20°C, with a value of 74.97, and sample S7 had the lowest, 70.24. S8 had the most significant value at 25 °C, 70.10, while S7 had the lowest, 63.95. S8 and S6 had the highest and lowest values at 30°C, 46.38 and 40.30, respectively. The S8 sample had the highest value at 35 degrees Celsius (1.22), whereas the S7 sample had the lowest value (0.2).

The SFC curve for pure CB reported in another study differs from the one observed in this particular study [20].

4. Conclusion

In conclusion, this study evaluated the compositional characteristics of cocoa butter (CB) from different sources, focusing on triacylglycerol, fatty acid, and sterol composition, as well as styrene compounds, extinction coefficient, and solid fat content (SFC). The results revealed that POS, SOS, and POP were the main triacylglycerols, while stearic (C18:0) oleic (C18:1) and palmitic (C16:0) acids were the major fatty acids. β-sitosterol, stigmasterol, and campesterol were the predominant sterols. The stigmastadiene content was negligible, indicating the authenticity of the tested CB samples. The extinction coefficient measurement showed no oxidation products in the CB samples. The SFC values varied across different temperatures, with sample S8 exhibiting the highest values. These findings provide a comprehensive understanding of the composition of cocoa butter from various sources and can help identify and detect adulteration. The evaluated parameters serve as valuable indicators for assessing the authenticity and quality of cocoa butter. Further research can focus on exploring additional physicochemical properties and conducting sensory evaluations to enhance the characterization of cocoa butter samples.

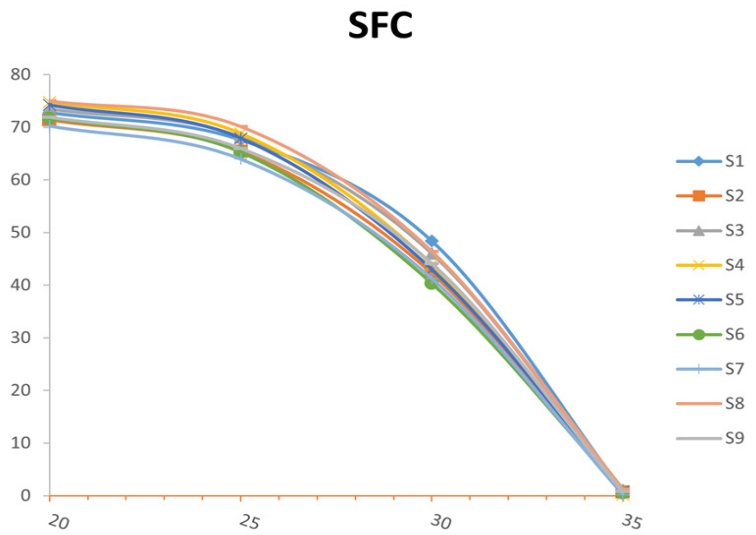

Fig 2. SFC of nine different samples of CB

Abbreviations

CB	Cocoa butter
POP, SOS, POS, PLS, SOO, OOO, POO, PLP, SOA, PPP, MOP, PPS, PSS, PLO, SSS, SLO, and AOO	P: palmitic acid, O: oleic acid, S: stearic acid, M: myristic acid, L: linoleic acid, A: arachidonic acid.
SFC	Solid Fat Content
FAs	Fatty Acids
TAG	Triacylglycerols
AOCS	American Oil Chemists' Society
GC	Gas Chromatography
FID	Flame Ionization Detector
TLC	Thin-Layer Chromatography
UV/VIS	Ultraviolet/ Visible
ANOVA	Analysis of Variance
CBE	Cocoa Butter Equivalents
EVOO	Extra Virgin Olive Oil

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مقاله پژوهشی

آنالیز ترکیب شیمیایی و ویژگی‌های کیفی کره کاکائو از تولیدکنندگان مختلف: بررسی مقایسه ای

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چکیده

کره کاکائو یک محصول بسیار ارزشمند و تطبیق پذیر با کاربردهای گسترده است. به دلیل خواص ذوب منحصر به فرد و توانایی حل کردن سایر مواد تشکیل دهنده، جزء حیاتی شکلات است. بنابراین، ارزیابی ویژگیهای ترکیبی کره کاکائو از منابع تامین مختلف جهت اطمینان از کیفیت و اصالت آن ضروری است. این مطالعه با هدف آنالیز تری آسیل گلیسرول‌ها، اسیدهای چرب، استرول‌ها، ترکیبات استایرن، ضریب خاموشی و محتوای چربی جامد (SFC) کره کاکائو از ۹ نمونه مختلف انجام شد. یافته ها نشان داد که تری آسیل گلیسرول های اصلی POS (۴۰.۲۵-۳۹.۱۲ ٪)، SOS (۲۸.۹۱-۲۵.۷۳ ٪) و POP (۱۷.۷۴-۱۶.۰۷ ٪) بودند، در حالی که اسیدهای چرب عمده استئاریک اسید (۳۲.۴۶-۳۵.۶۴٪)، اولئیک اسید (۳۱.۵۱-۳۲.۴۷٪) و پالمیتیک اسید (۲۶.۲۵-۲۸.۹۸٪) بودند. استرول های غالب شامل بتاسیتوسترول(۵۵.۰۴-۵۶.۹۸٪)، استیگمااسترول (۲۶.۲-۲۵.۴ ٪) و کامپسترول (۱۰.۵۳-۹.۲۲٪) بودند که نسبت استیگمااسترول به کامپسترول (۲.۵ تا ۲.۷) به عنوان شاخص اصالت در نظر گرفته شد. این مطالعه همچنین سطوح حداقلی از استیگمااستادی‌ان (ppm ۰.۰۰۳۹- ۰.۰۰۰۱) را نشان داد که بیانگر مقدار کم استرول های دهیدراته است. همچنین در این مطالعه محتوای SFC در دماهای مختلف اندازه‌گیری شد. به طور کلی، این بررسی جامع، دیدگاه ارزشمندی در خصوص ترکیب کره کاکائو از منابع مختلف ارائه میدهد و اطلاعات مهمی را جهت کنترل کیفیت و تشخیص تقلب ارائه می نماید.

واژگان کلیدی: **تری آسیل گلیسرول، فیتواسترول ها، پروفایل اسیدهای چرب، SFC، ضریب خاموشی**

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