

Influence of Fermentation and Roasting Degree on Tocopherols and Sterols of Cocoa Butter Extracted from Taiwan Cocoa Bean

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Abstract This study used Taiwan cocoa beans as the material and processed these cocoa beans either by natural fermentation in wooden boxes or blanching without fermentation with three different roasting temperatures (60°C for light, 130°C for medium, and 155°C for heavy roasting) applied. The resulting six types of cocoa beans were extracted by using a low-temperature high-pressure expeller to produce six types of cocoa butter, which are ULRCB (Unfermented Light Roasting Cocoa Butter), UMRCB (Unfermented Medium Roasting Cocoa Butter), UHRCB (Unfermented Heavy Roasting Cocoa Butter), FLRCB (Fermented Light Roasting Cocoa Butter), FMRCB (Fermented Medium Roasting Cocoa Butter), and FHRCB (Fermented Heavy Roasting Cocoa Butter), respectively. The contents of four isomers of tocopherols in each type of cocoa butter were analyzed by HPLC, while GC was used to assess the sterol composition. The total tocopherols content in the six cocoa butter ranged from 279 to 355 mg/kg fat, with ULRCB having the highest level, and UMRCB and FMRCB the lowest. Among the six types of cocoa butter, γ -tocopherol has the highest content, accounted for 87-91% of the total tocopherols, while β -tocopherol was the least abundant. The total sterol content ranged from 2388 to 3784 mg/kg fat, with ULRCB containing the highest level and FHRCB the lowest. β -Sitosterol was the most abundant sterol, comprising about 60% of the total sterols, whereas stigmastanol was the least abundant. In conclusion, ULRCB had significantly higher sterols content than other samples ($p < 0.05$). Although ULRCB also exhibited higher tocopherols content compared to UMRCB, UHRCB, FLRCB, and FMRCB, it showed no significant difference compared to FHRCB ($p > 0.05$). Our results suggest that higher roasting temperatures may lead to the breakdown of tocopherols and sterols, and appropriate fermentation and roasting conditions might enhance the bioactive compounds in cocoa beans and the derivatives. ULRCB, with its high tocopherols and sterols contents, shows high potential for applications in food, pharmaceutical, and cosmetic industries.

Keywords: cocoa butter, fermentation, roasting, tocopherols, sterols

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1. Introduction

Chocolate is a delicious food, with flavors ranging from very sweet white chocolate to intensely bitter 100% dark chocolate, and it is enjoyed by consumers of all ages around the world. Cocoa beans are the main raw material for making chocolate, with cocoa butter accounting for approximately 45-55% of their content. Cocoa butter can be extracted from cocoa beans through various methods [1,2]. It plays a crucial role in chocolate processing, forming the continuous phase and ensuring the flowability of the chocolate paste so that it can be molded into desired shapes. It also gives the product a glossy surface, a snap sound when bitten, and a smooth, melt-in-the-mouth texture [3,4]. Moreover, cocoa butter is rich in bioactive

compounds such as tocopherols and sterols. These substances greatly enhance the nutritional value and health benefits of cocoa beans. According to literature data [5], the tocopherols content in cocoa butter ranges from 100 to 300 mg/kg fat, with γ -tocopherol being the primary isomer. It is well-known that tocopherol is a natural antioxidant that effectively protects biological systems from damage caused by free radicals and reactive oxygen species. Thus, tocopherols prevent the oxidation of fatty acids in cocoa butter, ensuring the long-term stability of cocoa butter during storage and its nutritional value after processing [6].

Many studies have reported that the sterols content in cocoa butter ranges from 2,000 to 3,000 mg/kg fat, with β -sitosterol being the most abundant. Sterols are known for their ability to lower LDL cholesterol by binding to receptors in the intestines and forming complexes with proteins and phospholipids, thus hindering the absorption

of animal cholesterol. This helps reduce the risks of cardiovascular disease, coronary atherosclerosis, hypertension, and prostate enlargement [7,8,9]. Research also suggests that sterols can interact synergistically with tocopherols, further preventing the oxidation of fats [10]. Cocoa beans are usually fermented for 5-7 days using the box method, which involves an initial anaerobic fermentation led by yeast and a subsequent aerobic fermentation driven by acetic acid bacteria. The latter stage transforms alcohol into acids and is an exothermic reaction that raises the temperature to around 48°C. The fermentation step is essential for developing the chocolate's flavor [11]. However, as consumer interest in health products grows, cocoa-based supplements such as polyphenol capsules are becoming more popular. Some studies have found that skipping fermentation and using blanching to inactivate polyphenol oxidase in raw cocoa beans can help retain more bioactive compounds, such as polyphenol and flavanol [12,13]. This study aims to extract cocoa butter from both fermented and unfermented cocoa beans.

Roasting is a necessary step in chocolate production, typically performed at 120-160°C to reduce undesirable compounds and remove potential contaminants. Roasting also induces the Maillard reaction, which contributes to the development of chocolate's flavor and aroma. However, high-temperature roasting may negatively impact the quality of cocoa beans by promoting lipid oxidation and degrading bioactive compounds such as tocopherols and sterols. Some studies have shown that roasting reduces tocopherols content in various plant seeds [14,15], while others have reported no change or even an increase in tocopherols levels after heat treatment [16]. These discrepancies may be due to differences in roasting conditions, seed types, or plant varieties. Oracz et al. [10] studied the effects of roasting temperature and humidity on cocoa butters extracted from the cocoa beans from seven countries (cultivar): Brazil (Forastero), Ecuador (Nacional), Papua New Guinea (Trinitario), Venezuela (Trinitario), Ghana (UAF, Upper Amazon Forastero hybrid clone), Cameroon (T x UAF, Trinitario x Upper Amazon Forastero hybrid clone), and Indonesia (T x UAF, Trinitario x Upper Amazon Forastero hybrid clone), and found that high-temperature roasting led to a reduction in tocopherols and sterols level. They also reported significant differences in the bioactive compound content across cocoa butter from different regions. Although many studies have disclosed how roasting temperature, time, and plant variety affect the tocopherols and sterols content in plant seeds, data on how these factors impact cocoa butter are still limited, especially for cocoa beans produced in Taiwan. In addition, most cocoa butter is traditionally obtained by mechanically pressing chocolate liquor made from fermented cocoa beans, but there is little research on the tocopherols and sterols content in cocoa butter from unfermented beans. Therefore, this study is the first to explore the combined effects of fermentation and roasting conditions on the bioactive compounds in cocoa butter extracted from Taiwanese cocoa beans.

The objective of this study is to prepare fermented and unfermented cocoa beans and subject them to three different roasting conditions. We aim to investigate how fermentation (fermented and unfermented) and roasting

levels (light, medium, and heavy) influence the tocopherols and sterols content of cocoa butter derived from Taiwanese cocoa beans. Our goal is to produce cocoa butter with high levels of these bioactive compounds, which could have potential applications in the food, pharmaceutical, and cosmetic industries.

2. Materials and Methods

2.1. Cocoa Bean Preparation

Cocoa pods were purchased from Pingtung, Taiwan. After one day of pod storage, the husk and damaged parts of the ripe pods were removed and cocoa seeds obtained were in boiling water for 10 min at 95°C and at once cooled in ice water. Then, the boiled cocoa beans were washed and oven-dried (60°C) until the moisture level dropped to 6-7%. These bean samples were known as unfermented and light roasting beans (ULRB). The ULRB (2000g) were randomly sample and roasted for 25 min at a temperature of 130°C. The resulting beans were known as unfermented and medium roasting beans (UMRB). Another batch of ULRB (2000g) were roasted for 25 min at a temperature of 155°C. The resulting beans were known as unfermented and heavy roasting beans (UHRB). On the other hand, the harvested cocoa seeds were fermented for 7 days in a wooden box using the box method and then oven-dried (60°C) until the moisture level was 6-7%. These beans were known as fermented and light roasting beans (FLRB). The FLRB (2000g) were randomly sample and roasted for 25 min at a temperature of 130°C. The resulting beans were known as fermented and medium roasting bean (FMRB). Another batch of FLRB (2000g) were roasted for 25 min at a temperature of 155°C. The resulting beans were known as fermented and heavy roasting beans (FHRB). The above six types of beans with different fermentation and roasting degree were cooled and kept in hermetically closed plastic containers and stored at 4°C until cocoa butter extraction.

2.2. Cocoa Butter Preparation

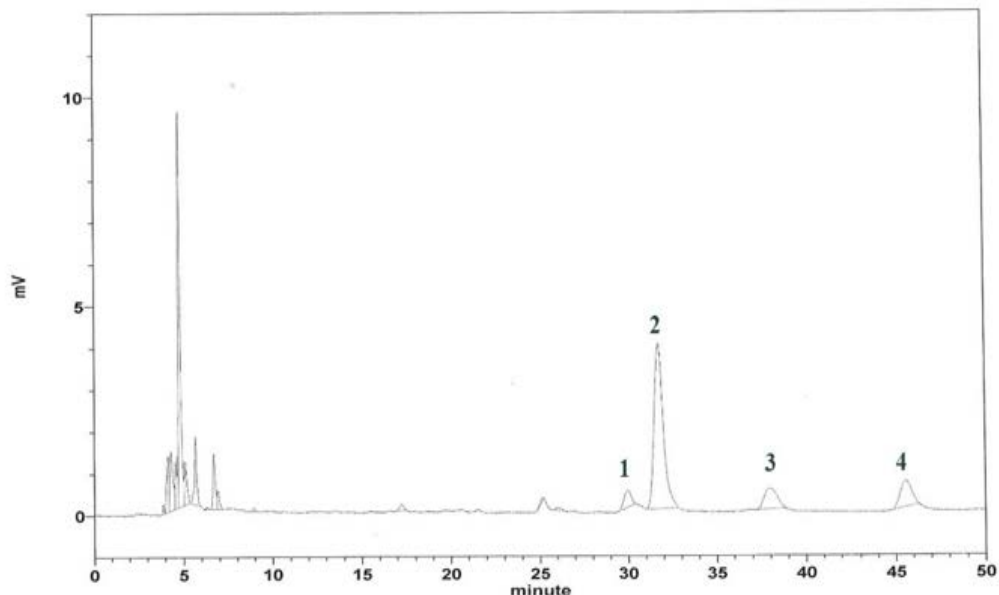
Six cocoa butters were obtained by mechanical press with operating conditions: 54MPa and 60°C from six whole cocoa beans under different fermentation and roasting degree: ULRB, UMRB, UHRB, FLRB, FMRB and FHRB. The resulting crude cocoa butters were known as ULRCB, UMRCB, UHRCB, FLRCB, FMRCB and FHRCB, respectively. They were placed in dark glass vials and stored at -20°C for subsequent analysis.

2.3. Tocopherols Measurement

The measurement of the tocopherols is based on Konings [17]. Samples after saponification and extraction process were analyzed through HPLC (Shimadzu Prominence LC-20AD Liquid Chromatograph, SpectraLab Scientific Inc., Canada). The procedures were as the followings: cocoa butter (2-5g) was placed in a 50 ml capped serum bottle with the addition of 0.1% BHT/methanol solution (10 ml) and underwent saponification process through 40%KOH/methanol

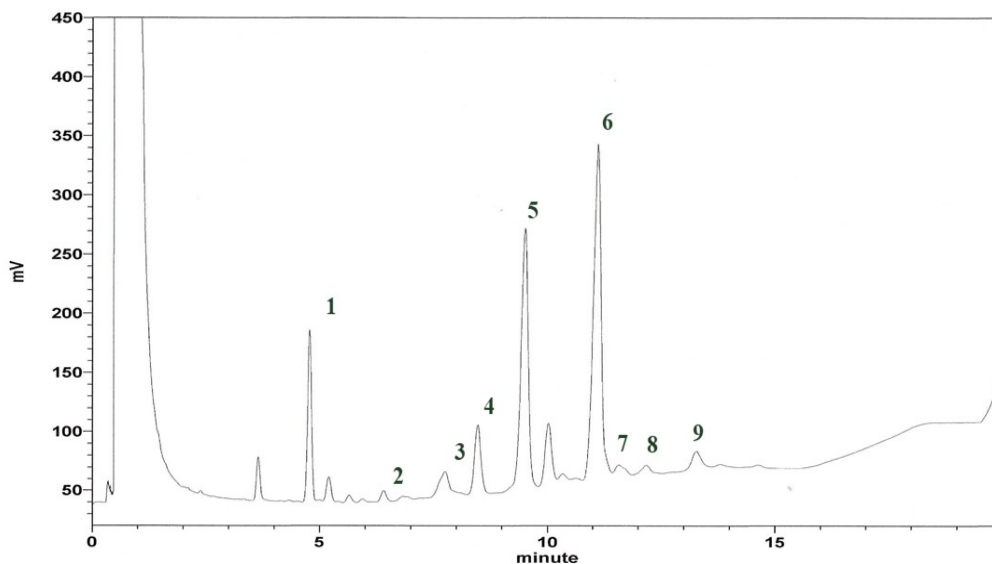
solution, then being water-washed to remove the lipid. Tocopherols (unsaponifiable matter) were extracted through ether and concentrated under reduced pressure, making the residue resolve into the methanol solution. The resulting solution was filtrated through a 0.22 μm filter for HPLC analysis. The analysis condition is as the following: column: C18 Column, 250 $\times$ 4.6 mm, 5 μm ; fluorescence

detector: Ex: 290 nm, Em: 340 nm; mobile phase: methanol: water = 98:2 (v/v); flow rate: 1 mL/min. Column temperature: 30°C; injection volume: 20 μL . The HPLC analysis for each sample was performed in quadruplicate. Exemplary sample chromatograms are presented in Figure 1.



Peaks 1 = β -tocopherol, 2 = γ -tocopherol, 3 = δ -tocopherol, 4 = α -tocopherol.

Figure 1. Chromatograms for evaluation of α -, β -, γ -, δ -tocopherol by HPLC



Peaks 1 = 5 α -cholestane (Internal standard, IS), 2 = cholesterol, 3 = brassicasterol, 4 = campesterol, 5 = stigmasterol, 6 = β -sitosterol, 7 = stigmasterol, 8 = Δ^5 -avenasterol, 9 = Δ^7 -stigmasterol.

Figure 2. Chromatograms for evaluation of cholesterol, brassicasterol, campesterol, stigmasterol, β -sitosterol, stigmasterol, Δ^5 -avenasterol and Δ^7 -stigmasterol by GC

2.4. Sterols Measurement

The measurement of the sterols is based on AOCS (revised 2011) Ch 6-91 [18]. The procedure is as the followings: cocoa fat samples (0.2-0.5g) were placed in a 100ml dull-polished flask with the addition of pure ethanol 2ml and 2N KOH/ methanol solution (10ml). The saponification process last for 30-50 min in a 90°C water bath, with a shake of the flask per 10 min during the saponification. After the saponification process, 10% of NaCl (2ml) and diethyl ether (20ml) were added in the flask. The mixture was extracted through a separatory funnel and vibrated for 1 min and still stratified. The diethyl ether layer was taken and the extraction was replicated for three time. All the diethyl layer solution was collected and the solvent was removed and dried through nitrogen. Pyridine (1ml) for dissolving, 0.1ml of TFA (Trifluoro acetic acid), 0.5 ml of HMDS (1, 1, 1, 3, 3, 3-hexamethyl disilazane), as well as 0.5 ml of internal standard solution (5 α -cholestane) were all mixed together for a 5 min vibration to conduct the GC analysis. The brand model of GC equipment was GL Science GC-4000. The analysis condition is as follows: column: 0.53mm $\times$ 30m, fused silica column; stationary phase: CP-5 (film thickness 1.0 μ m); flow rate: 3-5 mL/min (splitter ratio = 1:50); FID detector: H₂ flow rate 30 mL/min, Air flow rate 300 mL/min, Injection temperature = 320°C, Detector temperature = 335°C; the process of raising temperature in the oven is to maintain at 260°C for 5 min, and then raise 2°C per minute until reaching 290°C and maintain at 290°C for 3 min. Next, 10°C were raised per minute until reaching 320°C and maintain at 320°C for 2 min. The GC analysis for each sample was performed in quadruplicate. Exemplary sample chromatograms are presented in Figure 2.

2.5. Statistical Analysis

All the measurements were run in quadruplicate, using analytical grade reagents. Results were expressed as means $\pm$ SD. Analysis of Variances (ANOVA) and Tukey's Test were applied to analyze the significant difference of the means among the treatments. The significant level (α) was set at 0.05. The statistical computing software R 4.0 was used to perform all statistical analysis.

3. Results and Discussions

3.1. Tocopherols Contents

The analysis results of the six types of cocoa butter extracted from cocoa beans subjected to different fermentation and roasting conditions showed that all samples contained four types of tocopherols isomers: α -tocopherol, β -tocopherol, γ -tocopherol, and δ -tocopherol. According to Mbida et al. [19], cocoa butter extracted from 10 different cocoa varieties contained varying isomers of tocopherols. Among these, two cocoa butters had only β -tocopherol and γ -tocopherol; four contained β -

tocopherol, γ -tocopherol, and δ -tocopherol; and the remaining four contained all four isomers. Their total tocopherols content ranged from 96 to 307 mg/kg fat. Bruni et al. [20] also reported the presence of these four tocopherols isomers in cocoa butter, while Carpenter et al. [21] observed the presence of α -, γ -, and δ -tocopherol. Figure 3 illustrates the total tocopherols content across different samples, showing significant differences between them ($p < 0.05$). The total tocopherols content ranged from 279.1 to 355.5 mg/kg fat. ULRCB had the highest level (355.5 ± 33.48 mg/kg fat), followed by FHRCB (321.88 ± 14.92 mg/kg fat), UHRCB (302.5 ± 12.88 mg/kg fat), FLRCB (290.92 ± 14.66 mg/kg fat), FMRCB (279.12 ± 9.31 mg/kg fat), and UMRCB, which had the lowest (279.1 ± 10.15 mg/kg fat). Although the total tocopherols content in ULRCB was significantly higher than in UMRCB, UHRCB, FLRCB, and FMRCB, there was no significant difference between ULRCB and FHRCB ($p > 0.05$).

Table 1 presents the concentrations of the four tocopherols isomers in the six cocoa butter samples. The results indicated that γ -tocopherol was the most abundant isomer, accounting for 87-91% of the total tocopherols content across all samples. It was followed by α -tocopherol (4.5-8.1% of total tocopherols), with β -tocopherol being the least abundant (0.04-0.3% of total tocopherols). Significant differences ($p < 0.05$) were observed between the tocopherol concentrations across samples. ULRCB had the highest γ -tocopherol content (314.88 ± 28.18 mg/kg fat), while FMRCB had the lowest (241.92 ± 6.45 mg/kg fat). The detected levels of β -tocopherol were minimal, ranging from 0.13 to 1.21 mg/kg fat, with significant differences between samples ($p < 0.05$) as well. For unfermented cocoa beans, the total tocopherols content in cocoa butter extracted from lightly roasted beans was significantly higher than that from medium and heavy roasted beans. This may be due to increased oxidation of lipids and degradation of tocopherols during high-temperature roasting, leading to a reduction in tocopherols content. This result is consistent with the findings of Barrera-Arellano et al. [14]. For fermented cocoa beans, the total tocopherols content in cocoa butter extracted from heavy roasted beans was significantly higher than that from medium-roasted beans. This suggests that the combined effects of fermentation and high-temperature roasting may release more tocopherols from the cellular matrix of fermented cocoa beans, increasing its level. According to Kim et al. [16], the increase in tocopherols level may also result from the thermal degradation of cell structures during roasting, which enhances the extraction efficiency of tocopherols. When considering both fermentation and roasting conditions, we found that although ULRCB exhibited the highest tocopherols content, there was no significant difference compared to FHRCB ($p > 0.05$). Moreau et al. [22] suggested that tocopherols forms complexes with proteins, phosphates, and phospholipids, providing thermal stability. However, these bonds may break at higher temperatures, releasing more free tocopherols, which leads to an increase in the tocopherols level of the extracted oil.

Table 1. Tocopherol contents in six cocoa butters extracted from cocoa bean under different processing conditions (mg/kg)

Types of cocoa butter	α - tocopherol	β - tocopherol	γ - tocopherol	δ - tocopherol
ULRCB	27.36 $\pm$ 7.61 ^a	1.21 $\pm$ 0.06 ^a	314.88 $\pm$ 28.18 ^a	12.04 $\pm$ 2.21 ^c
UMRCB	12.44 $\pm$ 1.57 ^a	0.3 $\pm$ 0.12 ^b	252.66 $\pm$ 7.22 ^{bc}	13.7 $\pm$ 1.65 ^{bc}
UHRCB	13.9 $\pm$ 2.52 ^a	0.13 $\pm$ 0.05 ^b	272.62 $\pm$ 13.87 ^{bc}	15.86 $\pm$ 1.42 ^{ab}
FLRCB	17.96 $\pm$ 3.27 ^a	0.35 $\pm$ 0.09 ^b	255.21 $\pm$ 17.32 ^{bc}	17.4 $\pm$ 1.28 ^a
FMRCB	22.51 $\pm$ 13.17 ^a	1.02 $\pm$ 0.14 ^a	241.92 $\pm$ 6.45 ^c	13.67 $\pm$ 0.62 ^{bc}
FHRCB	21.64 $\pm$ 5.03 ^a	0.26 $\pm$ 0.13 ^b	283.71 $\pm$ 10.41 ^{ab}	16.27 $\pm$ 0.89 ^{ab}

Values are expressed as the means $\pm$ SD.

Different letters in the same column indicate significant differences ($p < 0.05$)

Table 2. Sterol contents of the six extracted cocoa butters obtained from cocoa bean under different processing conditions (mg/kg)

Sterol	ULRCB	UMRCB	UHRCB	FLRCB	FMRCB	FHRCB
Cholesterol	9.47 $\pm$ 1.14 ^a	8.18 $\pm$ 0.68 ^{ab}	7.57 $\pm$ 0.22 ^{bc}	7.33 $\pm$ 0.37 ^{bcd}	6.44 $\pm$ 0.7 ^{cd}	5.85 $\pm$ 0.43 ^d
Brassicasterol	30.13 $\pm$ 2.85 ^a	26.14 $\pm$ 2.96 ^{ab}	24.46 $\pm$ 4.55 ^{ab}	23.74 $\pm$ 4.87 ^{ab}	21.09 $\pm$ 6.11 ^{ab}	19.1 $\pm$ 4.9 ^b
Campesterol	385.83 $\pm$ 18.24 ^a	334.51 $\pm$ 22.35 ^{ab}	312.28 $\pm$ 44.85 ^{ab}	302.8 $\pm$ 48.54 ^{ab}	268.38 $\pm$ 66.53 ^b	243.3 $\pm$ 52.4 ^b
Stigmasterol	855.18 $\pm$ 28.22 ^a	741.41 $\pm$ 41.49 ^{ab}	691.16 $\pm$ 86.56 ^{ab}	669.96 $\pm$ 95.63 ^{ab}	593.43 $\pm$ 138.14 ^b	538.2 $\pm$ 107.49 ^b
β -Sitosterol	2256.58 $\pm$ 124.12 ^a	1957.38 $\pm$ 157.85 ^{ab}	1828.6 $\pm$ 286.78 ^{ab}	1773.37 $\pm$ 307.97 ^{ab}	1573.68 $\pm$ 412.56 ^b	1426.08 $\pm$ 327.36 ^b
Stigmastanol	2.12 $\pm$ 0.94 ^a	1.86 $\pm$ 0.88 ^a	1.78 $\pm$ 0.95 ^a	1.73 $\pm$ 0.94 ^a	1.57 $\pm$ 0.96 ^a	1.41 $\pm$ 0.84 ^a
Δ^5 -avenasterol	12.96 $\pm$ 2.94 ^a	11.28 $\pm$ 2.85 ^a	10.64 $\pm$ 3.42 ^a	10.34 $\pm$ 3.52 ^a	9.28 $\pm$ 3.91 ^a	8.37 $\pm$ 3.26 ^a
Δ^7 -stigmasterol	232.49 $\pm$ 10.11 ^a	201.62 $\pm$ 13.72 ^{ab}	187.86 $\pm$ 23.56 ^{ab}	182.03 $\pm$ 25.4 ^{ab}	161.26 $\pm$ 37.19 ^b	146.3 $\pm$ 29.1 ^b

Values are expressed as the means $\pm$ SD.

Different letters in the same row indicate significant differences ($p < 0.05$)

3.2. Sterols Contents

The analysis of cocoa butter extracted from cocoa beans with different fermentation and roasting levels revealed the presence of eight sterols: cholesterol, brassicasterol, campesterol, stigmasterol, β -sitosterol, stigmastanol, Δ^5 -avenasterol, and Δ^7 -stigmasterol. Oracz et al. [10] investigated the effects of roasting temperature and humidity on cocoa butter from seven countries, as mentioned above, and they reported that cocoa butter contained four major sterols: campesterol, stigmasterol, β -sitosterol, and Δ^5 -avenasterol. Figure 4 shows the total sterol content across different cocoa butter samples, which ranged from 2,388 to 3,784 mg/kg fat. ULRCB had the highest sterols content (3,784.79 $\pm$ 167.98 mg/kg fat), followed by UMRCB (3,282.39 $\pm$ 231.37 mg/kg fat), UHRCB (3,064.35 $\pm$ 447.34 mg/kg fat), FLRCB (2,971.31 $\pm$ 484 mg/kg fat), FMRCB (2,635.12 $\pm$ 664.53 mg/kg fat), and FHRCB, which had the lowest content (2,388.61 $\pm$ 524.04 mg/kg fat). There were significant differences in total sterols content between the samples ($p < 0.05$). The total sterols content of ULRCB was significantly higher than those in FMRCB and FHRCB ($p < 0.05$). However, there was no significant difference between ULRCB and UMRCB, UHRCB, or FLRCB ($p > 0.05$). Table 2 presents the concentrations of the eight individual sterols in the six cocoa butter samples. The results showed that β -sitosterol comprises about 60% of the total sterols across all samples, which aligns with findings from Verleyen et al. [23] and Menéndez-Carreño et al. [24] that β -sitosterol was the most abundant in the sterols. Stigmasterol accounted for about 23%, campesterol for 10%, and Δ^7 -stigmasterol for 6%, with the remaining four sterols each accounting for less than 1%. Significant differences ($p < 0.05$) were found between the samples. ULRCB had the highest β -sitosterol content (2,256.58 $\pm$ 124.12 mg/kg fat), while FHRCB had the lowest (1,426.08 $\pm$ 327.36 mg/kg fat). Stigmastanol was present only in trace amounts,

ranging from 1.41 to 2.12 mg/kg fat and accounting for 0.1% of sterols content, with no significant differences between samples ($p > 0.05$). Kochhar [25] reported that the sterols composition of cocoa butter generally consists of β -sitosterol (59-63%), stigmasterol (24-31%), campesterol (8-11%), Δ^5 -avenasterol (3%), cholesterol (1-2%), Δ^7 -stigmasterol (1%), and trace amounts of brassicasterol and Δ^7 -avenasterol. The percentages of most sterols in our samples were consistent with these findings, except for some variation in Δ^7 -stigmasterol and Δ^5 -avenasterol, which may be attributed to differences in cocoa variety, fermentation, roasting conditions, or extraction methods.

For unfermented cocoa beans, the total sterol content in cocoa butter ranged from 3,064 to 3,784 mg/kg fat. The sterol content decreased by 19% as roasting levels increased, which aligns with the findings of Amaral et al. [26]. They reported that roasting hazelnuts at 125-200°C for 5-30 minutes resulted in a 14% loss of sterols. This decrease may be due to the intact structure of unfermented cocoa beans, which reduces the impact of degradation of sterols caused by high temperatures. Although the sterols content decreased with higher roasting temperatures, the differences were not statistically significant ($p > 0.05$). For fermented cocoa beans, the total sterols content decreased gradually from 2,971.31 $\pm$ 484.34 mg/kg fat in lightly roasted beans to 2,635.12 $\pm$ 664.53 mg/kg fat in medium-roasted beans, and finally to 2,388.61 $\pm$ 524.04 mg/kg fat in heavily roasted beans. This trend was similar to that observed in unfermented cocoa beans, with no significant differences between the roasting levels ($p > 0.05$). When considering both fermentation and roasting effects, ULRCB had the highest sterols content, significantly higher than FMRCB and FHRCB ($p < 0.05$). This result indicates that the combination of fermentation and high-temperature roasting leads to greater disruption of the cellular matrix in fermented cocoa beans, causing sterol loss of 30-37%. Studies have shown that oxidation

at high temperatures can cause sterols degradation [27]. Singh [28] reported that β -sitosterol may dehydrate under high-temperature roasting to form steradiene, leading to a reduction in sterols content. Although sterols may reduce lipid oxidation, this benefit comes at the cost of sterols loss. Our findings align with previous studies, which emphasized the effects of roasting temperature, duration, and humidity on sterols content. This study is the first to examine the impact of fermentation degree on the sterols content of cocoa butter.

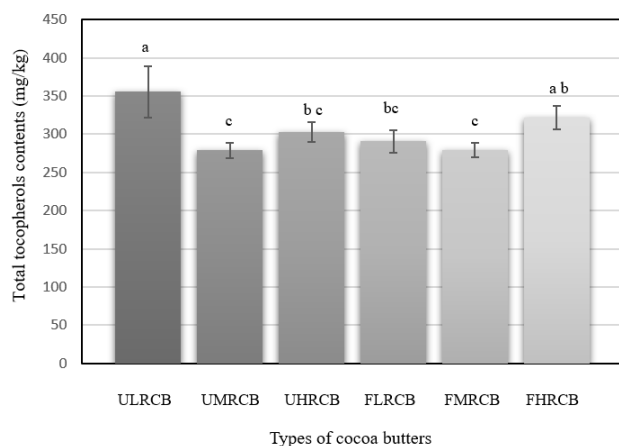


Figure 3. Total tocopherols contents in six cocoa butters obtained from cocoa beans under different processing conditions. Data were indicated as means $\pm$ SD. Different letters indicate significant differences ($p < 0.05$)

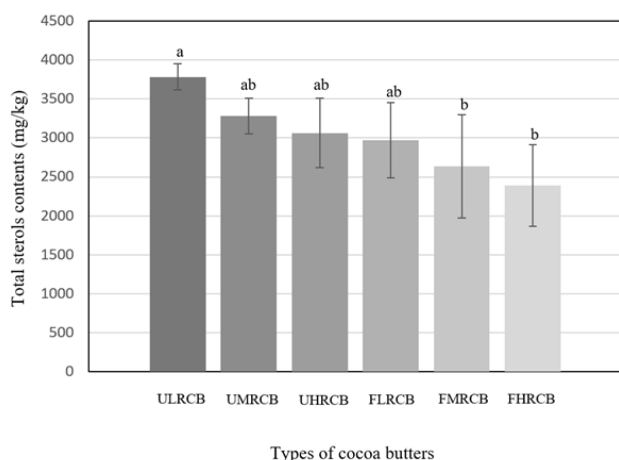


Figure 4. Total sterols contents in six cocoa butters extracted from cocoa beans under different processing conditions. Data were indicated as means $\pm$ SD. Different letters indicate significant differences ($p < 0.05$)

4. Conclusions

The results of this study reveal that different fermentation and roasting levels significantly affect the content of bioactive compounds: tocopherols and sterols in cocoa butter. The total tocopherols content across the tested cocoa butter samples ranged from 279 to 355 mg/kg fat, with ULRCB having the highest content. Among the four isomers, γ -tocopherol was the most abundant, accounting for 87-91% of the total tocopherols content. The total sterols content across the samples ranged from 2,388 to 3,784 mg/kg fat, with ULRCB showing the highest concentration and FHRCB the lowest. β -sitosterol

was the most prevalent sterol, comprising about 60% of the total sterols content. In general, medium and heavy roasting reduced the tocopherols and sterols content of cocoa butter compared to light roasting. However, in the case of fermented cocoa beans, roasting at the highest temperature (155°C) only slightly decreased the tocopherols content but significantly reduced the sterols content. This study demonstrates that selecting appropriate fermentation and roasting conditions can enhance the bioactive compounds level of cocoa beans and their derived products. Cocoa butter obtained from unfermented and lightly roasted beans (ULRCB), with its high levels of tocopherols and sterols, shows great potential for applications in the food, pharmaceutical, and cosmetic industries.

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Conflicts of Interest

The authors declared no conflicts of interest.

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