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Physico-chemical and microbiological quality of cocoa butter and cocoa cake from forastero beans in Cote D'ivoire

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Abstract

This study assessed the physico-chemical and microbiological quality of cocoa butter and cocoa cake derived from *Theobroma cacao* L. (Forastero variety) produced in Côte d'Ivoire. Twelve samples (six butter and six cocoa cake samples) were collected from a cocoa processing industry. Physicochemical parameters including moisture, pH, fat content, acidity, color, crystallization index, iron content, and sugars were analyzed using standard analytical methods. Microbiological quality was evaluated through the enumeration of total aerobic mesophilic flora, coliforms, *Escherichia coli*, yeasts, molds, and *Salmonella* spp. according to ISO standards. Results showed that most physicochemical parameters complied with international standards, except for cocoa butter moisture content (0.43%), which slightly exceeded the recommended limit (0.2%). Microbiological analysis revealed the absence of pathogenic microorganisms and low microbial loads (<100 CFU/g), indicating good hygienic quality. Principal Component Analysis (PCA) highlighted moisture, pH, fat content, and fineness as key quality determinants. Overall, the cocoa derivatives exhibited satisfactory quality, although improvements in moisture control are recommended.

Keywords: Cocoa; Cocoa Butter; Cocoa Cake; Physicochemical Quality; Microbiological Quality; Côte d'Ivoire.

1. Introduction

The cocoa tree (*Theobroma cacao* L.) is a tropical crop widely cultivated in agroforestry systems for its seeds, which are the main raw material for chocolate production [1]. In Côte d'Ivoire, the world's leading cocoa producer, cocoa cultivation has expanded significantly due to the availability of fertile forest land and labor [2]. As a key pillar of the Ivorian economy, cocoa accounts for approximately 30% of export revenues and contributes more than 15% to the gross domestic product (GDP) [3].

Industrial chocolate manufacturing involves post-harvest processing, in which fermentation plays a crucial role in developing the characteristic flavor of commercial cocoa beans. At the end of this process, properly processed cocoa beans are obtained. These beans are mostly exported in raw form or processed into cocoa liquor, from which cocoa butter and cocoa cake are derived [4, 5, 6].

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In Côte d'Ivoire, exports of semi-finished cocoa products have steadily increased, reaching 219 million tons for cocoa liquor [5]. Given this growing reliance on semi-finished products, it is essential to assess their commercial quality. Indeed, the quality of cocoa derivatives largely depends on their physicochemical and microbiological characteristics, which affect their stability, nutritional value, and safety.

To ensure consumer protection and compliance with international requirements, standards such as [7, 8] establish specific thresholds for various quality parameters. Therefore, this study aims to evaluate the quality of cocoa butter and cocoa cake produced from Forastero cocoa beans and to identify the main factors influencing their quality.

2. Material and methods

The biological material consisted of cocoa butter and cocoa cake obtained from samples of Forastero cocoa beans produced in Côte d'Ivoire (without added value) (Figure 1).

A : Cocoa cake ; B : Cocoa butter

Figure 1 Various cocoa products

2.1. Sample collection

Twelve samples of cocoa derivatives (six cocoa butter and six cocoa cake samples) were collected from a cocoa processing industry in Côte d'Ivoire. Samples were transported under controlled conditions for laboratory analysis.

2.2. Physicochemical analysis

2.2.1. Determination of moisture content and pH

The moisture content was determined using an Infralab analyser in accordance with the manufacturer's instructions. After homogenising 10 g of sample in 100 mL of distilled water, the pH was determined using the potentiometric method described by [9].

2.2.2. Determination of titratable acidity

Acidity was determined by titration using NaOH (0.1 N) according to [10] methods. The results obtained are the average of three trials. The acidity level was determined using the following formula:

$$\text{Titratable acidity (\%)} = 0{,}045 \times V_{éq} \times 100$$

Véq : equivalent volume of sodium hydroxide solution used in millilitres

2.2.3. Determination of Total Sugars

Total sugars were quantified according to the method described by [11]. Briefly, 0.5 g of sample was mixed with 0.5 mL of 12 N sulfuric acid (H_2SO_4). The mixture was left at room temperature for 1 h, then heated in a boiling water bath for 2 h. After cooling, 5.5 mL of distilled water, 10 mL of 70% ethanol, 0.5 mL of zinc sulfate (2%), and 0.5 mL of potassium ferrocyanide (10.6%) were successively added.

The solution was filtered and adjusted to 50 mL. For the assay, 0.2 mL of the extract was mixed with 1 mL of 5% phenol and 5 mL of concentrated H_2SO_4. After standing for 10 min at room temperature, the mixture was homogenized and

allowed to cool for 20 min. Absorbance was measured at 485 nm using a spectrophotometer against a blank. Total sugar content was determined using a glucose standard curve (10 mg/100 mL).

2.2.4. Determination of Reducing Sugars

Reducing sugars were quantified according to the method of [12]. Five (5) grams of sample were homogenized in 20 mL of boiling water. The mixture was filtered and adjusted to 50 mL. One (1) mL of the extract was transferred into a test tube, followed by the addition of 0.5 mL of distilled water and 0.5 mL of 3,5-dinitrosalicylic acid (DNS) reagent.

The mixture was heated in a boiling water bath for 5 min. After cooling, 2 mL of distilled water were added, and absorbance was measured at 490 nm against a blank. Reducing sugar content was determined using a standard curve prepared under the same conditions from a glucose stock solution (1 mg/mL).

2.2.5. Determination of Iron Content

Iron content was determined by energy-dispersive X-ray fluorescence (EDXRF) using a LAB-X5000 analyzer (Hitachi High-Tech Analytical Science, formerly Oxford Instruments, UK). Disposable sample cups sealed with polypropylene film were filled with homogenized cocoa butter or cocoa cake and directly analyzed without chemical digestion. The iron concentration was measured according to the manufacturer's application method for cocoa products, and each analysis required approximately 1 min.

2.2.6. Determination of Color of Cocoa Mass and Cocoa Cake

Color was determined according to the method described by [13]. Five (5) grams of sample were weighed and mixed with 15 mL of distilled water preheated to 60 °C. The mixture was homogenized and allowed to stand for 1 min, then transferred into an 8 cm Petri dish for observation.

2.2.7. Determination of Cocoa Butter Clarity

Cocoa butter samples were prepared according to [14] ISO 661 :2003 (Preparation of test sample). The samples were heated to 63 °C until completely melted and homogeneous. The molten butter was poured into a standard Lovibond glass cell, ensuring that the sample was clear, limpid, and free from suspended particles or turbidity. Clarity (Lovibond colour value) was determined using a Lovibond Tintometer according to the manufacturer's operating instructions.

2.2.8. Determination of Fat Content

Refractive indices (n) were measured using an Abbe refractometer thermostated to within ±0.1 °C. Two (2) grams of sample were mixed with 4 g of 1-bromonaphthalene (n = 1.6535 at 25 °C). The resulting solution provided an average refractive index from three determinations. Oil content was then determined using a calibration curve relating refractive index to oil concentration.

2.2.9. Crystallization Index of Cocoa Butter

The crystallization index was determined using a tempermeter according to the manufacturer’s protocol. Cocoa butter samples were heated in an oven at 70 °C, after which 13.20 g were weighed and placed into the tempermeter vessel.

2.3. Microbiological Analysis

Microbiological analyses were performed according to standardized methods of the AFNOR (French Standards Association). A total of 10 g of each sample (cocoa butter and cocoa cake) was aseptically weighed and homogenized in 90 mL of sterile buffered peptone water to obtain a 10^{-1} dilution. Serial decimal dilutions were prepared under aseptic conditions.

Aerobic mesophilic bacteria were enumerated on Plate Count Agar (PCA) according to [15]. *Coliforms* and *Enterobacteriaceae* were counted on Violet Red Bile Lactose (VRBL) agar following [15]. Detection and enumeration of *Escherichia coli* were performed using Tryptone Bile X-glucuronide (TBX) agar after incubation at 44°C for 24 h according to [16]. The detection of *Salmonella* was carried out using a pre-enrichment step in buffered peptone water followed by selective enrichment and plating on selective media according to [17]. Results were expressed as presence or absence in 25 g of sample. Yeasts and molds were enumerated on Sabouraud Chloramphenicol Agar after incubation at 25°C for 3-5 days following [18]. Microbial counts were calculated according to the formula:

$$N(CFU/g) = \frac{\sum C}{V(n_1 + 0{,}1n_2)d}$$

V : volume of inoculum (0.1 mL or 1 mL) ; n_1: number of plates retained at the first dilution n_2: number of plates retained at the second dilution ; d: dilution factor of the first selected dilution ; N: number of colony-forming units (CFU/mL) ; C: total number of colonies counted on selected plates from two successive dilutions.

Table I summarizes the reference microbiological criteria applicable to cocoa-derived products.

Table 1 Microbiological reference criteria for cocoa derivatives (cocoa butter and cocoa cake)

Microorganisms	**Microbiological critera**	**Acceptable limits**	**Reference methods (AFNOR/ISO)**
Total aerobic mesophilic flora	Hygiene indicator	≤10^5 CFU /g	NF EN ISO-4833-1
Yeasts and molds	Fungal contamination	≤10^3 CFU /g	NF ISO 21527-1
Enterobateriaceae	Hygiene indicator	≤10^2 CFU /g	NF ISO 21528-2
Escherichia coli	Fecal contamination	≤10 CFU /g or absence	NF ISO 16649-2
Staphylococcus aureus	Toxigenic risk	≤10^2 CFU /g	NF EN ISO 6888-1
Salmonella spp	Pathogenic bacteria	Absence in 25 g	NF EN ISO 6579-1

Microbiological limits were established based on international recommendations (Codex Alimentarius and European Commission Regulation EC N° 2073/2005) adapted to low water activity foods such as cocoa products.

3. Results

3.1. Physicochemical parameters

The various parameters analyzed complied with the standards established by ISO 22000 (Table II). However, for the cocoa cake, the fineness value obtained (98.42 ± 0.67) was close to the recommended reference value (98%). Regarding the butter, the mean moisture content (0.43 ± 0.57) exceeded the maximum limit set by ISO 22000 (0.2%), indicating non-compliance. Nevertheless, the other physicochemical parameters, including free fatty acids (FFA), lipid compounds (CL), the BCI index, and FFAFEVES, were within the acceptable limits defined by this standard, namely ≤ 2% for FFA, ≤ 5% for CL, and a tolerance of +0.10% FFA for FFAFEVES (Table III).

Table 2 Physicochemical parameters measured in the oilcake

	Parameters							
	Hu (%)	**pH**	**Fi (%)**	**MG (%)**	**TF (ppm)**	**Col (%)**	**ST (g/L)**	**SR (mg/mL)**
	2,37 ± 0,19	5,69 ± 0,12	98,42 ± 0,67	10,48 ± 0,25	631,92 ± 0,1	45,01 ± 0,46	0,05 ± 0,01	0,51 ± 0,28
ISO 22000 standards	4 % maxi	6 maxi	98 % mini	10 à 12% maxi	700 ppm maxi	45 à 55	-	-

Hu : humidity **; ST :** total sugars **; SR :** reducing sugar **; Fi :** fineness **; MG :** fat content **; TF :** iron content **; Col :** color

Table 3 Physicochemical parameters measured in butter

	Parameters					
	FFA (%)	BCI (%)	CL (%)	Hu (%)	ST (g/L)	SR (mg/mL)
	1,91±0,45	4,87±0,45	3,06±0,5	0,43±0,57	0,52±0,01	0,78±0,02
ISO 22000 standards	2 maxi	4 mini	5 maxi	0,2 maxi	-	-

FFA : Acidity content ; **BCI** : Crystallisation index ; **CL** : clarity; **Hu** : humidity; **ST** : total sugars ; **SR** : reducing sugar

3.2. Principal Component Analysis (PCA)

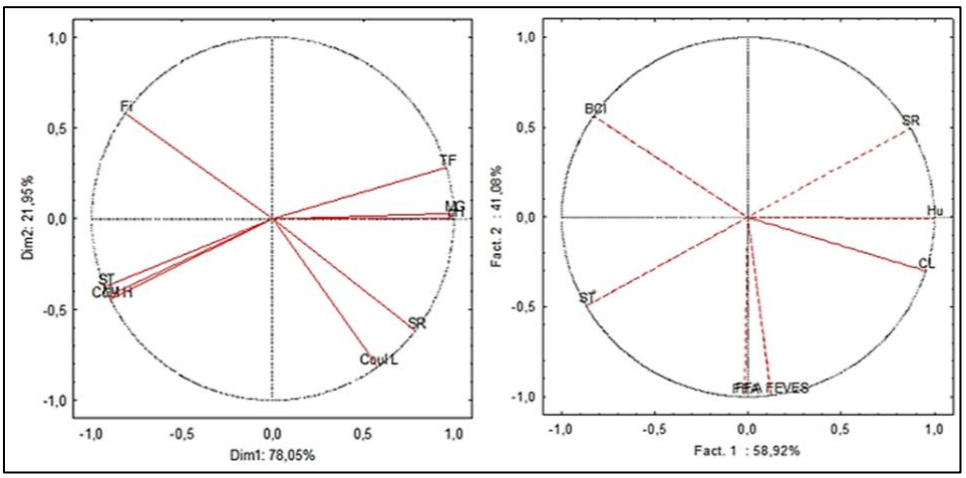
The principal axes, denoted Dim 1 and Dim 2, accounted for 100% of the total variability. For the oilcake, the individual contributions were 78.05% for Dim 1 and 21.95% for Dim 2, whereas for the butter, they were 58.92% for Dim 1 and 41.08% for Dim 2 (Figure 2a).

The variables fineness, pH, fat content (MG), and iron content (TF) measured in the oilcake showed higher contributions to Dim 1. These findings suggest that the quality of the oilcake is mainly influenced by these four parameters, with contributions of approximately 18% for fat content and pH each, around 15% for fineness, and about 17% for iron content.

In butter, the variables lipid compounds (CL), moisture (Hu), FFAFEVES, and SR exhibited higher contributions to Dim 1. On Dim 2, the BCI variable showed the highest contribution (Figure 2b).

3.3. Microbiological Analysis

The results presented in Table IV indicate an overall satisfactory microbiological quality of cocoa by-products (cake and butter). The total aerobic mesophilic flora (TAMF) load was below 100 CFU/g for both sample types, which is well below the acceptable limits established by Codex Alimentarius ($\leq 10^5$ CFU/g). This low microbial load reflects good hygienic conditions during processing and handling.



a) cake ; **b)** butter **ST :** total sugars **; SR :** reducing sugar **; Fi :** finess **; MG :** fat content **; TF :** iron content **; Coul :** color **; BCI** : Crystallisation index ; **CL** : clarity ; **Hu** : humidity ; **FFAFEVES** : acidity level of the beans ;

Figure 2 Principal Component Analysis (PCA)

Furthermore, yeast and mold counts were also very low (< 10 CFU/g), remaining far below the recommended maximum threshold ($\leq 10^3$ CFU /g). These results suggest good microbiological stability and a low risk of spoilage during storage.

Regarding *Enterobacteriaceae*, their complete absence in all samples is a strong indicator of sanitary quality, suggesting no contamination of fecal or environmental origin. Similarly, *Escherichia coli*, commonly used as an indicator of fecal contamination, was not detected, in compliance with the standard requirements ($\leq 10^2$ CFU /g).

Finally, the absence of *Salmonella*, a major foodborne pathogen, confirms the microbiological safety of the analyzed products.

Overall, these findings demonstrate that cocoa cake and butter exhibit excellent microbiological quality and comply with the requirements of Codex Alimentarius, supporting their potential suitability for food or industrial applications under controlled conditions.

Table 4 Microbiological composition of cocoa derivated

	Microorganisms					
	TAMF	Yeast	Molds	*Enterobacteria*	*E.coli*	*Salmonella*
Cake	<100	< 10	< 10	Absent	Absent	Absent
Butter	<100	< 10	< 10	Absent	Absent	Absent
Acceptable limits (Codex Alimentarius)	$\leq 10^5$ CFU /g	$\leq 10^3$ CFU /g		$\leq 10^2$ CFU /g	≤10 CFU /g or absence	Absence in 25 g

TAMF : Total Aerobic Mesophilic Flora ; ***E. coli :*** *Escherichia coli* ; CFU : Colony-Forming Unit

4. Discussion

The physicochemical analyses demonstrated that most quality parameters of cocoa butter and cocoa cake complied with the limits recommended by ISO standards, indicating that the products were obtained under appropriate processing conditions. The moisture content of cocoa cake (2.37%) remained below the maximum acceptable limit (4%), suggesting effective drying after pressing. Low moisture is essential because it reduces microbial proliferation and delays lipid oxidation during storage. Similar observations have been reported by [1], who emphasized that adequate drying is one of the main determinants of cocoa product stability. Likewise, [19] reported that moisture contents below 3–4% are generally associated with prolonged shelf life and preservation of cocoa quality.

The fat content of cocoa cake (10.48%) was within the expected range (10–12%), confirming efficient extraction of cocoa butter during pressing. Residual fat levels are known to influence both nutritional value and industrial applications of cocoa cake, particularly in bakery and confectionery products [4]. Likewise, the fineness value (98.42%) was consistent with industrial specifications, indicating satisfactory particle size reduction, an important factor affecting dispersibility and sensory quality.

The pH value (5.69) was characteristic of well-fermented cocoa products. During fermentation, organic acids produced by yeasts and acetic acid bacteria diffuse into the beans, contributing to flavor precursor formation while maintaining the slightly acidic pH generally observed in commercial cocoa derivatives [6]. Similar pH values have been reported for cocoa powders produced from well-fermented beans in West Africa.

The iron concentration (631.92 ppm) remained below the maximum recommended limit (700 ppm), suggesting limited contamination during processing. Elevated iron concentrations may result from contact between cocoa products and metallic equipment during grinding or pressing. Maintaining iron levels below the recommended threshold is important because transition metals catalyze lipid oxidation and may reduce butter stability [20].

For cocoa butter, all physicochemical parameters complied with quality standards except moisture content, which reached 0.43%. Although this value exceeded the maximum recommended limit (0.20%), it remained relatively low and is unlikely to compromise product stability if appropriate storage conditions are maintained. Residual moisture in cocoa butter may originate from incomplete separation of the aqueous phase during hydraulic pressing or inadequate drying of cocoa liquor before extraction. Similar observations have been reported in industrial cocoa processing, where slight increases in moisture were attributed to processing conditions rather than deterioration of product quality [21].

The free fatty acid (FFA) content (1.91%) remained below the maximum permissible value (2%), indicating limited hydrolytic degradation of triglycerides. Low FFA values generally reflect good post-harvest handling practices and rapid drying of cocoa beans, which limit lipase activity. According to [22, 1], low acidity contributes to improved flavor development and longer shelf life.

Similarly, the lipid compound (CL) value (3.06%) and crystallization index (BCI = 4.87%) complied with quality standards, suggesting good crystallization behavior and desirable physical properties for chocolate manufacture. Proper crystallization is essential for obtaining cocoa butter with optimal hardness, gloss, and resistance to fat bloom [23].

Principal Component Analysis (PCA) highlighted the variables contributing most strongly to cocoa product quality. For cocoa cake, fineness, pH, fat content, and iron concentration explained most of the total variability, indicating that these parameters are the principal indicators of technological quality. Their strong contribution suggests that manufacturing conditions such as roasting intensity, grinding efficiency, and butter extraction largely determine the physicochemical characteristics of cocoa cake.

For cocoa butter, moisture, free fatty acids, lipid compounds, and reducing sugars were the principal discriminating variables. Moisture and acidity are widely recognized as key indicators of cocoa butter stability because they directly influence lipid oxidation and shelf life [21]. The high contribution of the crystallization index on the second principal component further emphasizes the importance of fat crystallization in determining butter quality, particularly for chocolate manufacturing where polymorphic stability is essential [23].

The microbiological analyses revealed excellent hygienic quality of both cocoa butter and cocoa cake. Total aerobic mesophilic flora remained below 100 CFU/g, which is substantially lower than the maximum limits established by the [8]**.** Such low microbial loads indicate effective implementation of hygienic practices throughout cocoa processing and storage.

Yeasts and molds were also detected at very low levels (<10 CFU/g), demonstrating good microbiological stability. These microorganisms are frequently associated with inadequate drying or excessive storage humidity. Their near absence in the present study confirms that drying and storage conditions effectively prevented fungal development. Comparable findings have been reported for cocoa products processed under good manufacturing practices [24].

No *Enterobacteriaceae* or *Escherichia coli* were detected, indicating the absence of fecal or environmental contamination during processing. These microorganisms are widely recognized as indicators of sanitary quality and process hygiene. Their absence reflects compliance with Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Point (HACCP) principles during cocoa processing.

Likewise, *Salmonella* was absent from all analyzed samples. This finding is particularly important because *Salmonella* represents one of the most significant microbiological hazards associated with low-moisture foods, including cocoa products. Although cocoa generally has low water activity, contamination may occur after roasting through contact with contaminated equipment or the processing environment. The absence of *Salmonella* therefore demonstrates effective hygienic control throughout production. Similar microbiological profiles have recently been reported for commercially processed cocoa products, where strict implementation of food safety management systems resulted in pathogen-free products [25, 8].

Overall, the physicochemical conformity together with the excellent microbiological quality confirms that both cocoa butter and cocoa cake possess characteristics suitable for food and industrial applications. These findings further demonstrate the effectiveness of current processing practices in preserving the nutritional, technological, and sanitary quality of cocoa derivatives produced from Forastero beans in Côte d'Ivoire

5. Conclusion

The objective of this study was to contribute to the characterization of cocoa-derived products. The products investigated were cocoa oilcake and cocoa butter. The physicochemical parameters analyzed, including moisture content, dry matter, total and reducing sugars, pH, fineness, fat content, iron content, color, and acidity, did not negatively affect the hygienic quality of the studied oilcake and butter.

Microbiological analysis revealed the absence of *Enterobacteriaceae*, *E. coli*, yeasts, and *Salmonella*, while total aerobic mesophilic flora (TAMF) was present at levels below the regulatory standards. The low microbial load observed in both oilcake and cocoa butter indicates satisfactory microbiological quality.

However, the results obtained in this study remain preliminary. Further in-depth investigations are recommended, focusing on the parameters already studied as well as exploring additional quality and safety indicators.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

This research did not involve human participants, animals, or any procedures requiring ethical approval. Therefore, formal ethical approval was not required for this study.

Statement of informed consent

Formal informed consent was not required for this study because it did not involve human participants, clinical trials, or direct human interventions.

Declaration of AI Use

This manuscript was prepared through the joint contributions of all authors, particularly with respect to the study design, data collection and analysis, content development, results, interpretation, and scientific work. The authors acknowledge the use of ChatGPT to assist with grammatical proofreading, language refinement, and reference formatting. These AI-assisted tools were not used as authors and did not replace the authors' intellectual contributions or scientific judgment. All AI-generated outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the authors. The authors take full responsibility for the accuracy, integrity, and final content of the manuscript.

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