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Effect of Fermentation and Extraction Techniques on the Physicochemical Composition of Copoazú Butter (*Theobroma grandiflorum*) as an Ingredient for the Cosmetic Industry

Luisa L. Orduz-Díaz, Kimberly Lozano-Garzón, Willian Quintero-Mendoza, Raquel Díaz, Juliana E. C. Cardona-Jaramillo *, Marcela P. Carrillo, Diana Carolina Guerrero and Maria Soledad Hernández

Instituto Amazónico de Investigaciones Científicas Sinchi, Calle 20 # 5-44, Bogotá 110311, Colombia; lorduz@sinchi.org.co (L.L.O.-D.); wiquintero@sinchi.org.co (W.Q.-M.); rdiaz@sinchi.org.co (R.D.); mcarrillo@sinchi.org.co (M.P.C.); dguerrero@sinchi.org.co (D.C.G.); shernandez@sinchi.org.co (M.S.H.)
* Correspondence: jcardona@sinchi.org.co

Abstract: The Copoazú is a *Theobroma* species of Amazonian origin, and its derived products have a high content of lipids valuable for both the cosmetic and food industries. The composition of the butter extracted from its seeds can vary depending on the postharvest process and the diverse extraction techniques employed. In this study, the composition of this butter processed with and without seed fermentation was analyzed using two extraction techniques: expeller pressing and hydraulic pressing. Parameters such as lipid profile, quality indexes, melting point, and the content of phytosterols and glyceric compounds were compared with a highly sought-after commercial raw material assessed through standardized volumetric and spectroscopic methodologies. The results showed that non-fermentation and cold-pressing conditions preserved the properties of the butter. This analysis is the first step in a standardized process for developing high-quality cosmetic ingredients derived from Copoazú butter.

Keywords: copoazú; copoazú butter; fermentation; cosmetics; pressing

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1. Introduction

The utilization of natural ingredients (NIs) derived from diverse plant sources is emerging as a strategic approach for creating value during the shift towards a sustainable biobased economy, especially in biodiversity-rich countries. In these transitional models of the bioeconomy, Colombia has made efforts in various regions of the country through the implementation of agroforestry crops that provide both commodity and non-commodity benefits. Additionally, these models offer environmental advantages contributing to deforestation prevention, soil restoration, and socio-economic improvements for the local population [1,2].

Copoazú (*Theobroma grandiflorum*), also known as white cocoa or Amazonian cocoa, is a tropical tree from the Malvaceae family distributed across Brazil, Colombia, Peru, and Venezuela [3]. This widely distributed genus throughout the Amazon basin and the Orinoco region is one of the oldest with significant potential for local, regional, and global use. Cocoa (*Theobroma cacao*), Copoazú (*Theobroma grandiflorum*), and maraco (*Theobroma bicolor*) are the economically most-utilized species within the Theobroma genus. Unlike cocoa, all parts of the Copoazú fruit are utilized, either for direct consumption or for the extraction of natural ingredients and derivatives [4].

Cultivating Copoazú is integral to agroforestry systems in the Colombian Amazon, guided by a comprehensive management plan as a non-timber forest product (NTFP). This strategic approach not only supports the substitution of illicit crops but also contributes to the mitigation of deforestation and addresses the adverse effects of climate change. Additionally, Copoazú plays a role in the country's economic transition by strengthening rural

development and paving the way for the implementation of value chains for biodiversity products with high added value [1,5].

Copoazú seeds contain approximately 50% lipids, polar compounds with antioxidant properties, and emollients that are found attractive in the cosmetic and food industry, mainly due to their similarity with cocoa butter. This is why copoazú butter has been used as an alternative NI by several companies in the development of new products, primarily in the cosmetic field [6]. Regarding the cosmetic properties of the fat, this butter serves primarily as a natural emollient, featuring softening and moisturizing attributes. Its composition makes it suitable for use in emulsions, such as hydrating creams and lotions, as well as in soaps and other bioproducts with a high level of complexity [7]. For fat extraction, many mechanical and chemical techniques have been developed to preserve sample integrity and enhance yields. Since solvent-assisted and mechanical techniques are widely adopted for their simplicity and cost-effectiveness, newer approaches such as Supercritical Fluid Extraction (SFE), Ultrasound-Assisted Extraction (UAE), Enzyme-Assisted Extraction (EAE), and Microwave-Assisted Extraction (MAE) have shown considerable potential, albeit requiring more advanced technological infrastructure [8].

In the Colombian Amazon region, diverse communities and organizations of copoazú producers and processors possess the production capacity to potentially fulfill the increasing demand for copoazú butter [9]. Accordingly, the comprehensive utilization of Copoazú has become a tool for generating resources that enable the improvement of the quality of life of the inhabitants of the Amazon, and it is crucial to comprehend the implementation process required to meet market demands effectively. The value chain of Copoazú currently involves two production hubs (one well-established and expanding and one in development), four local grassroots organizations that bring together over 500 families, 240 hectares of sustainable production systems (agroforestry arrangements), and 4000 hectares of conserved forest designated as such via agreements [10].

To potentially contribute to a real sustainable use of nature and promote Natural Ingredient Value Chains (NIVCs), efforts need to be directed towards research and strengthening the knowledge base of NIs in accordance with a value chain (VC) approach [1,11]. There is a gap in terms of strengthening the technical and technological capacities of these NIs, as well as scientific research based on the processes of obtaining them, to ensure they meet the required quality standards and are truly competitive in the market [1,5].

This study aims to evaluate various copoazú butter extraction treatments (including both fermented and non-fermented processes, using an expeller or hydraulic pressing) conducted by an association of producers and processors in the Caquetá region of the Colombian Amazon. This assessment focuses on their impact on physicochemical composition, comparing the results with a sample of commercially sought-after copoazú butter available on the international market. The objective is to provide information supported by scientific evidence to inform decision-making in processes that enhance the value chain of this country's copoazú butter. To achieve this, this evaluation involves the quality parameters of the butter (quality indices), its fatty acid profile, infrared spectroscopy (IR) findings, phytosterol analysis, triglyceride analysis, and the color of each extraction treatment.

2. Materials and Methods

2.1. Copoazú Butter Extraction

Copoazú seeds obtained from pulping, containing approximately 30% natural pulp, were placed in a fermentation box and covered with clean cloth or canvas to prevent insect infestation. Manual turning was performed every two days, stirring the fermenting mass from the bottom of the container. Fermentation was evidenced by the seeds heating up (45 to 50 °C) in the initial days and subsequently exhibiting acidity. Cutting tests were also conducted to monitor the fermentation process, which lasted a total of 10 days.

After fermentation, the seeds were dried in a solar drying system with temperatures ranging between 37 and 60 °C for 10 days until reaching a moisture content of 6 to 10%. Non-fermented seeds were also dried under the same conditions.

Once dried, the seeds were subjected to mechanical extraction processes using two types of presses: an expeller press and a hydraulic press. The extraction technologies evaluated were selected not only because they are clean technologies but also because of their ease of operation and the availability of machinery in the processing plants of the community organizations with which this study was conducted. The operating parameters for both pressing methods are not described as they are part of proprietary development. Temperature ranges between 40 and 60 °C were utilized.

For our research purposes, four treatments using Copoazú butter from the Caquetá department of Colombia were conducted, namely, extracting non-fermented butter via expeller pressing (WF_EXP), extracting fermented butter using expeller pressing (FER_EXP), extracting non-fermented butter via hydraulic pressing (WF_HP), and extracting fermented butter using hydraulic pressing (FER_HP), while a trading pattern was used as control (COM). Butter extraction was conducted using the same batch of copoazú from the same producer to ensure the reliability of this study.

2.2. List of Applied Reagents

Glacial acetic acid, potassium iodine, sodium thiosulfate, sodium hydroxide, soluble starch, analytical-grade chlorophorm, pyridine, isoctone, THF, and methanol were purchased from Merck (Darmstadt, Germany). Methanolic boron trifluoride (BF_3) (10%), Fatty Acid Methyl Esters (Standard FAME 37), N, and O-bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSFTA + TMCS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The external phytosterols standards (cholesterol, stigmasterol, campesterol, brassicasterol, and β-sitosterol) were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

2.3. Instrumental Color Determination

The instrumental color was determined using a HunterLab MiniScan™ XE Plus colorimeter (HunterLab, Reston, VA, USA) via the HunterLAB scale and by performing mathematical conversion via the CieLAB scale.

2.4. Evaluation of Butter Quality Indices and Physicochemical Properties

The acidity value, saponification value, and iodine value were determined, following the same methodology described by Lozano-Garzón et al. [12], wherein titration, according to the AOCS methods, was carried out. All the analyses were performed in triplicate.

The peroxide value was determined by modifying the protocol proposed in USP (2013) [13]. For this purpose, butter samples were dissolved in a mixture of chloroform and glacial acetic acid in a 2:3 ratio. Immediately, 0.5 mL of saturated potassium iodine solution was added and shaken vigorously for a minute; then, 30 mL of water was added. The mixture was titrated with 0.01 N sodium thiosulfate until the yellow color almost disappeared. Finally, 5 mL of saturated starch solution was added, and titration was continued until the blue color vanished. The peroxide value is expressed in mEq of active O_2 per kg of sample.

The melting point was determined using a Thiele tube containing mineral oil and a mercury thermometer; all samples were measured in triplicate.

2.5. Determination of Fatty Acid Profiles

To obtain fatty acid profiles, derivatization with methanolic boron trifluoride (BF_3) was carried out using the methodology reported by Lozano-Garzón and Cardona Jaramillo [12,14]. The analysis was conducted utilizing an Agilent 7890B Gas Chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a Flame Ionization Detector (FID). Chromatographic separation was achieved using an Agilent DB23 column (60 m × 0.25 µm × 250 µm) (Agilent Technologies, Santa Clara, CA, USA), with Helium employed as the carrier gas at a constant flow rate of 1.1 mL/min. The injector temperature was set to 270 °C in split mode with a split ratio of 30:1. The initial oven temperature was maintained at 60 °C for 1 min, followed by a programmed increase of 6 °C per minute until reaching

210 °C, and this temperature was maintained 24 min. The detector temperature was set to 310 °C. The air flow rate was maintained at 400 mL/min, hydrogen flow was maintained at 40 mL/min, and makeup gas (helium) was maintained at 30 mL/min, compensated for with column flow. Additionally, a solvent delay of 9 min was implemented.

2.6. Determination of Phytosterol Profiles

An analysis of phytosterols was carried out according to the methodology outlined by Lozano-Garzón [12]. This involved obtaining the unsaponifiable fraction, followed by derivatization using N, O-bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSFTA + TMCS) and pyridine. The analysis was conducted using an Agilent 7890B Gas Chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled with a Flame Ionization Detector (FID). Separation of compounds was achieved using an Agilent HP5-MS column (30 m × 0.25 µm × 250 µm) (Agilent Technologies, Santa Clara, CA, USA), with helium serving as the carrier gas at a constant flow rate of 1 mL/min. The injector temperature was set to 270 °C in split mode with a split ratio of 15:1. Initially, the oven temperature was maintained at 250 °C for 1 min, followed by a programmed increase of 2 °C per minute until reaching 300 °C, holding this temperature for 7 min. The detector temperature was maintained at 300 °C. The air flow rate was set to 400 mL/min, with hydrogen flow set to 40 mL/min and makeup gas (Helium) set to 30 mL/min. Additionally, a solvent delay of 9 min was incorporated into the method [15].

2.7. Evaluation of Triglyceride Profiles

Triglyceride analysis was performed via GC-FID, employing samples prepared in THF at a concentration of 5 mg/mL. The analysis was performed using an Agilent 7890B Gas Chromatograph coupled with an FID detector under the following conditions using an Agilent DB17 HT column (15 m × 0.25 µm × 150 µm) and helium as a carrier gas at 1.02 mL/min: injector temperature, 360 °C (20:1); initial oven temperature, 250 °C; 5 °C/min increase up to 350 °C (20 min); detector temperature, 310 °C; air flow, 400 mL/min; hydrogen flow, 40 mL/min; makeup (helium), 30 mL/min (compensated for with column flow); solvent delay, 1 min [16].

2.8. Attenuated Total Reflectance Fourier Transform Infrared (FTIR-ATR) Analysis

FTIR-ATR spectra of all samples were acquired with a Jasco 4600 FT/IR spectrometer (Jasco, Tokyo, Japan) with 4 cm^{-1} resolution in the 3500–400 cm^{-1} wavelength region using an attenuated total reflectance device (ATR) with single-diamond reflection and a zero-fill factor of 2. The spectra were obtained with 32 scans, and the average of each sample was calculated automatically using Jasco Spectra Manager software version 2.15.01.

2.9. Statistical Analysis

ANOVA was performed to analyze variables, namely, quality values, instrumental color, and melting point, followed by multiple comparisons with the Tukey test (maximum significant difference) using Statistix9 v8.1 software and a significance of 5%. Principal component analyses (PCAs) were performed using Simca 14.1 developed by Umetrics, using the lipid profile data and the percentage of the areas of each signal, along with the TAG profile (each retention time was an input variable), as input variables.

3. Results

Color is a significant attribute that not only serves as an initial indicator of product quality but also has implications for the formulation of food or cosmetic products. L*, a*, b* notation is used to represent different aspects of color. L* measures the difference between brightness and darkness, a* indicates the difference between red and green, and b* signifies the difference between yellow and blue. This notation was employed to evaluate the color of the butter. According to the results presented in Table 1 and Figure 1, a notable difference was observed between the various treatments evaluated and the control sample.

Table 1. Instrumental color on copoazú butter.

Sample	L*	a*	b*
COM	78.79 ± 5.80 A	2.13 ± 0.26 B	17.74 ± 0.91 A
FER EXP	69.22 ± 1.76 B	3.20 ± 0.19 B	7.78 ± 0.69 C
FER HP	75.43 ± 1.31 AB	5.78 ± 0.48 A	11.61 ± 0.09 B
WF EXP	70.10 ± 2.94 AB	4.90 ± 0.30 A	10.96 ± 1.38 B
WF HP	77.19 ± 2.28 AB	5.18 ± 0.63 A	10.15 ± 0.97 BC
p-Value	0.0154	0.0000	0.0000

Rows with the same letter (A, B, C, AB, BC) do not show a significant difference (n = 3, α= 0.05).

Figure 1. Color of the control sample (commercial) and the four evaluated treatments. WF EXP: extracted non-fermented butter via expeller pressing; FER EXP: extracted fermented butter using expeller pressing; WF HP: extracted non-fermented butter via hydraulic pressing; FER HP: extracted fermented butter using hydraulic pressing; COM: trading pattern used as control.

Regarding the quality indexes, in all four treatments, the most significant difference was the saponification value (Table 2). This value is a measure of the average molecular weight of the fatty acids in a fat or an oil. It is based on saponification, a chemical reaction that involves breaking the ester bonds present in a sample.

Table 2. Quality indexes and melting points of control and evaluated treatments.

Sample	Acidity Index (mg KOH/g Sample)	Iodine Value (g I_2/100 g Sample)	Saponification Index (mg KOH/g Sample)	Peroxide Index meq of Active O_2/kg Sample	Melting Point (°C)
COM	4.83 ± 0.12 C	48.83 ± 0.42 A	86.50 ± 4.69 B	2.67 ± 0.23 B	34.33 ± 1.21 AB
FER EXP	8.37 ± 0.31 A	43.13 ± 2.82 B	59.80 ± 5.79 C	3.27 ± 0.12 B	32.33 ± 1.86 B
FER HP	8.30 ± 0.00 A	40.30 ± 2.44 B	99.77 ± 6.81 AB	3.50 ± 0.10 AB	32.50 ± 1.05 B
WF EXP	6.17 ± 0.12 B	41.60 ± 0.62 B	103.93 ± 9.87 AB	4.33 ± 0.31 A	34.33 ± 1.21 AB
WF HP	4.87 ± 0.29 C	41.07 ± 1.10 B	117.93 ± 10.24 A	3.47 ± 0.61 AB	35.33 ± 1.51 A
p-Value	0.0000	0.0010	0.0000	0.0017	0.0035

Rows with the same letter (A, B, C, AB) do not show a significant difference (n = 3, α= 0.05).

The variations in iodine values were non-significant; nevertheless, COM exhibited a slightly higher value, implying a potentially greater amount of unsaturated fatty acids. For the acidity index, only WF_HP was comparable with the commercial sample; fermentation processes produce free organic acids, which could cause a decrease in pH.

On the other hand, fatty acids were identified via comparison with a fatty acid methyl ester (FAME) Sigma Supelco 37 standard, and the relative composition was determined by integrating the obtained peaks (Table 3).

Table 3. Fatty acid relative compositions.

ID	COM (%)	WF EXP (%)	FER HP (%)	FER EXP (%)	WF HP (%)
Palmitic (C16)	9.72	9.62	9.90	9.72	10.39
Palmitoleic (C16:1)	0.21	0.20	0.19	0.00	0.21
Stearic (C18)	34.74	32.77	32.67	34.50	32.33
Oleic (C18:1)	40.17	41.37	39.64	40.57	40.51
Linoleaidic (C18:2)	0.53	0.52	0.55	0.50	0.57
Linoleic (C18:2)	3.52	4.45	6.80	4.54	4.86
Arachidic (C20)	9.72	9.65	9.07	9.15	9.75
Gadoleic (C20:1)	0.27	0.31	0.21	0.17	0.27
Behenic (C22)	1.13	1.11	0.97	0.86	1.12

On the other hand, quantitative data were submitted to multivariate analysis, and a principal component analysis (PCA) (Figure 2a) was performed, finding that the main differences between the samples were due to the concentrations of linoleic and stearic acid (Figure 2b). This analysis also showed that there is a greater resemblance between COM and the non-fermented samples.

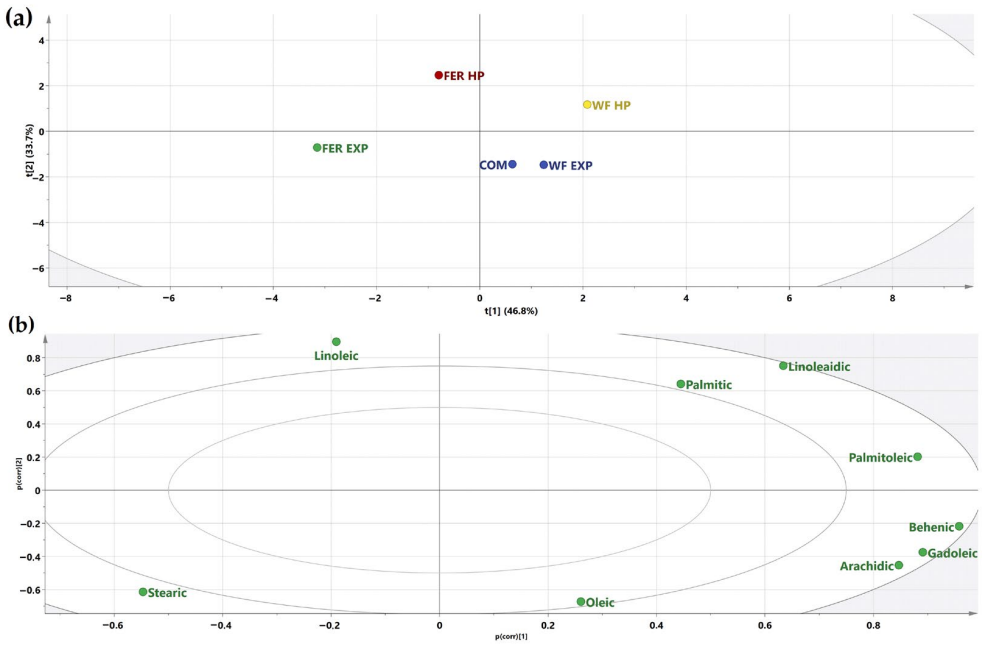

Figure 2. (**a**) Score plot based on the composition of fatty acids in the treated copoazú butter. The x-axis (T [1]) explains 46.8% of the total variance, while the y-axis (T [2]) explains 33.7%, for a total variance of 70%. (**b**) The axes represent Pearson correlation coefficients in the loading plot based on the composition of fatty acids in the treated copoazú butter.

Triglycerides showed a high complexity pattern but remained very similar (Figure 3) in all cases. All the profiles presented signals between 20 and 40 min, with WF EXP presenting the lowest intensities. This uniformity in the observed patterns suggests a notable consistency in triglyceride composition, while the subtle variations in signal intensities among cases provide valuable insights into the nuanced distinctions within these lipid profiles.

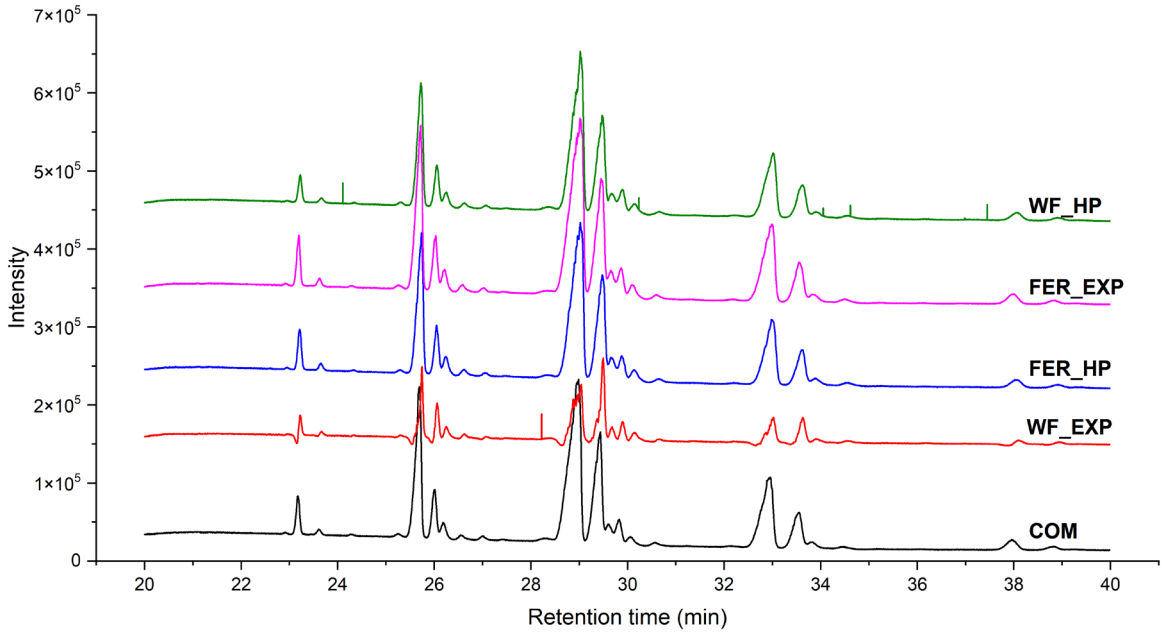

Figure 3. Chromatographic profile of triacylglycerols in copoazú butter samples obtained via GC-FID-HT analysis.

Despite the chromatograms exhibiting low quality, characterized by broad signals with low resolution (due to the non-volatile nature of triglyceride molecules), a principal component analysis (PCA) was conducted to assess the similarities among the samples. The results shown in Figure 4 corroborate the differences observed regarding WF EXP and the similarity between COM and FER HP.

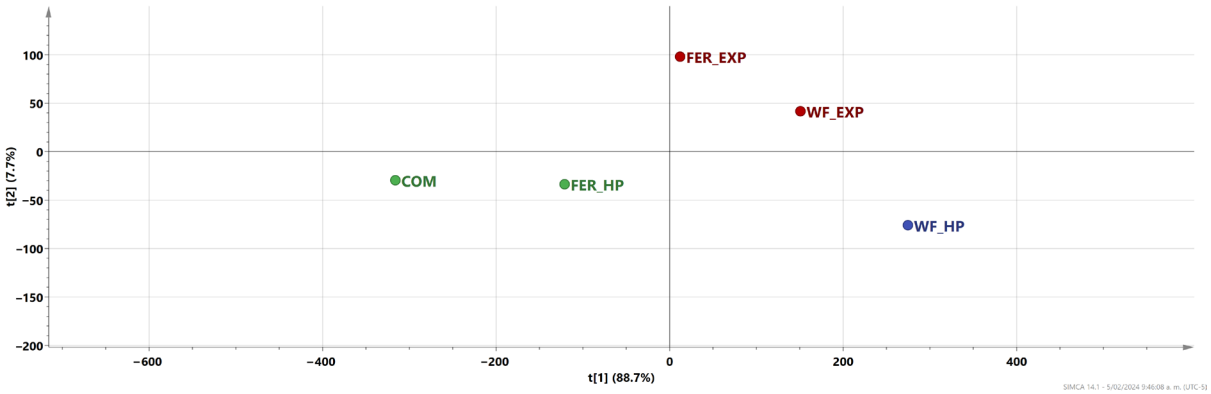

Figure 4. Multivariate analysis based on the chromatographic data of the triacylglycerol profiles. Here, 95% of the total variance of the data is presented in the first two components.

Phytosterols stand out as remarkable molecules within the cosmetics industry, primarily because of their well-documented photoprotective capabilities. These compounds function as an effective barrier shielding the skin from dehydration. Notably, when compared to cholesterol, phytosterols have a lower probability of inducing acne when included in topical products [17,18], which can be considered a comparative advantage in the formulation of cosmetics. Table 4 shows the results of the composition of phytosterols present in the unsaponifiable fraction of copoazú butter.

Table 4. Phytosterols profile in the unsaponifiable fraction of copoazú butter.

	Campesterol (µg/g)	Stigmaterol (µg/g)	β-Sitosterol (µg/g)
COM	-	680 ± 5	5244 ± 10
WF_EXP	-	632 ± 5	3532 ± 10
FER_HP	322 ± 2	812 ± 7	6006 ± 10
FER_EXP	300 ± 3	860 ± 5	6326 ± 11
WF_HP	-	880 ± 7	6180 ± 10

Complementarily, an IR analysis was carried out to establish differences between treatments and obtain a profile that allows quick a diagnosis of the samples. As can be observed in Figure 5, the spectral comparison revealed minimal differences between samples, suggesting that this technique may not be conducive to the intended quality control procedures.

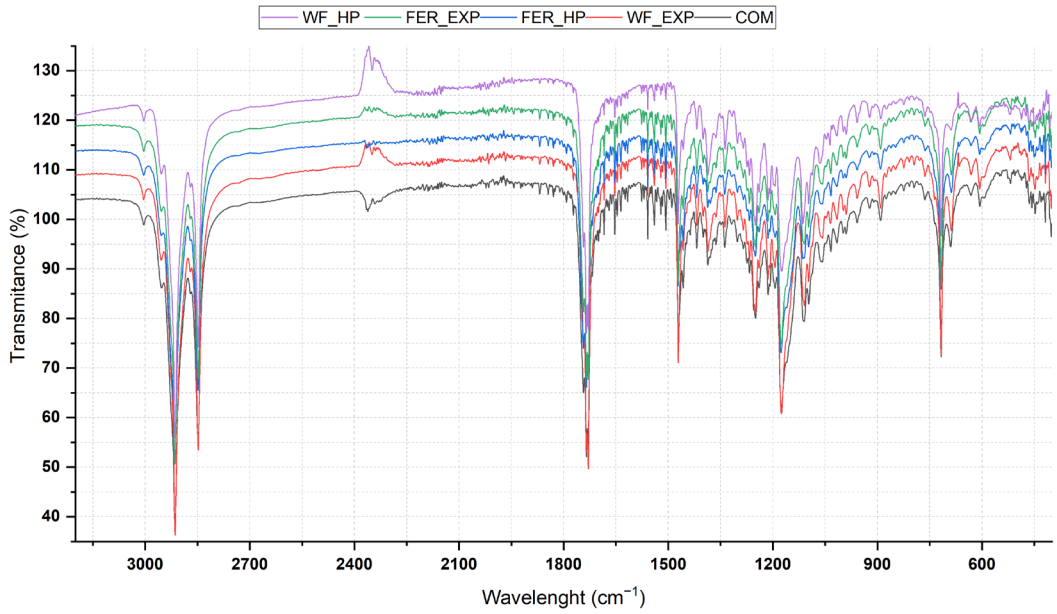

Figure 5. Average FTIR-ATR spectrum of copoazú butter samples.

4. Discussion

When evaluating color, a noticeable change in the b* parameter was observed, signifying a more pronounced intensity of the yellow color in COM. This resulted in stronger tones significantly differing from the other samples with a lighter coloration, particularly FER_EXP. This may be due to the pH and temperature changes occurring during pressing that could induce the degradation of the triglycerides present in the butter, as suggested by the decreased luminosity. In contrast, FER_HP and WF_HP showed an increase in a*, indicating a slightly redder tone, which might be attributed to preserved carotenoids in the cold-pressed samples.

The copoazú butter obtained through hydraulic pressing exhibited higher levels of brightness, possibly due to a process conducted at lower temperatures, resulting in a butter with a distinct crystalline structure and, consequently, varying opacity compared to that obtained through expeller pressing [19]. On the other hand, the darker coloration observed in the four treatments compared to the commercial sample may be linked to factors related to the soil conditions of copoazú growth. However, it is more likely associated with the fermentation process, as noted in a study on *Theobroma cacao*, where it was shown that fermentation significantly influences butter coloration [20,21]. This difference is also influenced by the unknown fermentation process of the commercial sample. As extensively researched, the fermentation of *Theobroma* grains leads to changes in both physical and

chemical properties. Various enzymatic reactions alter components such as polyphenols, proteins, and carbohydrates, among others [20,22].

Saponification value evaluation is crucial as it provides insight into the number of bonds per gram of a sample, offering a detailed reflection of the chemical composition and structure under examination. A lower saponification index usually hints at a reduced number of triglycerides in both fermented samples. The most significant contrast was noted in FER_EXP. The fermentation process induces acidification of the matrix, potentially causing partial triglyceride hydrolysis. During expeller pressing, temperatures of 80 to 100 °C can be reached, caused either by friction or the temperature ramp programed. Hydraulic pressing is performed with lower temperatures (approximately 40 °C), which are just enough for melting the butter. Higher temperatures during extractions may result in partial hydrolysis too. This divergence underscores the distinct impact of the fermentation process on the chemical dynamics and potential transformations within the matrix.

A high acidity index usually indicates poor quality since it is related to compounds such as lactic acid, citric acid, and other derivatives of fermentative processes that affect pH and some organoleptic properties [18,23]. Lipid peroxidation derived from fermentation can be ruled out since there were no significant differences in the values of the peroxide index. The results exhibit similarities with those reported for Copoazú butter by Carrillo and Moreno [7,24]. The acidity levels range from 1.0 to 15 mg of KOH/g sample; additionally, the values of the iodine and saponification indices align with the fatty acid profiles of the evaluated samples, showcasing a predominant presence of oleic and stearic acids. Furthermore, the low peroxide index values indicate that Copoazú butter has reduced susceptibility to oxidation.

The primary difference in fatty acid composition among the samples was attributed to the content of linoleic acid, with COM exhibiting a lower amount of this substance. Polyunsaturated fatty acids (PUFAs) applied to the skin help to restore the lipid barrier, integrating into the lipid layers and interfering in inflammatory processes related to prostaglandin synthesis, for which a higher proportion of PUFAs can improve the properties of the final product [25]. The technical data sheet of the commercial sample provides detailed ranges for each of the fatty acids: when compared with the expected values, FER HP and FER EXP were 0.14% lower for behenic acid (1–2.5%), and FER HP also showed a 0.36% lower amount of oleic acid, while the other samples presented a higher content (5–9%) of palmitic acid than expected.

The fatty acid profiles exhibit similarities with those reported in Copoazú butter studies [7,24,26], identifying oleic acid as the predominant component, followed by stearic acid. This composition has a direct effect on the melting point of butter due to the presence of unsaturated fatty acids [27]. Moreover, fatty acids have a determining role in cellular functions and influence proliferation processes. Essential unsaturated fatty acids (linoleic and arachidonic), which can be found in Copoazú butter, are highly recognized for their impact on inflammation and tissue-healing processes [28–30]. The presence of oleic acid further accelerates healing and scar tissue formation, making it a versatile matrix with applications in cosmeceuticals [26].

For *Theobroma grandiflorum*, the following triglyceride values were reported per kilogram of sample: PLiP, 6 (Palmitic Linoleic Palmitic triglyceride, 6); OOO 20, (Oleic Oleic Oleic triglyceride, 20); POO, 43, (Palmitic Oleic Oleic triglyceride, 43); PliS, 11 (Palmitic Linoleic Stearic triglyceride, 11); POP, 11 (Palmitic Oleic Palmitic triglyceride, 11); SOO, 161 (Stearic Oleic Oleic triglyceride, 161); SLiS, 18 (Stearic Linoleic Stearic triglyceride, 18); POS, 120 (Palmitic Oleic Stearic triglyceride, 120); OOA, 78 (Oleic Oleic Arachidic triglyceride, 78); SOS, 3.14 (Stearic Oleic Stearic triglyceride, 3.14); SOA, 181 (Stearic Oleic Arachidic triglyceride, 181) [31]. In this study, the signals were neither identified nor quantified as the analysis was conducted solely for comparative purposes. However, this TAG profile is correlated with the fatty acid profile presented in Table 3.

The fermented samples contained campesterol (Table 4), which could be a result of microbial development, such as the formation of cell membranes. Phytosterols support

microbial growth by acting as photoprotectors and maintaining cell integrity regardless of the pH and temperature changes associated with the fermentation processes [18]. During the extraction of the fermented material, the compounds resulting from the metabolism of microorganisms are also extracted.

The IR spectra (Figure 5) show C-H stretching bands in aliphatic chains for methyl and methylene close to 2900 and 2850 cm^{-1}, as well as an intense band of C=O stretching characteristic of carboxylic acids and asymmetric C-H-C methylene bending at 1471 cm^{-1}. The spectra obtained (Figure 5) for the samples mainly present characteristic fatty acid bands. When comparing the spectra of each sample, slight differences were observed, both in frequency and intensity, showing that this technique is not helpful for the desired quality control process [17].

5. Conclusions

To obtain a product with similar characteristics to the commercial sample, it is advisable to avoid fermentation and to conduct copoazú butter extraction using hydraulic pressing. The standardization of the copoazú butter extraction process can contribute to the development of local industry by strengthening the capabilities of local producers and enhancing the quality of the natural ingredient obtained, aiming to make it competitive in both national and international markets. This, in turn, encourages processes that have the potential to contribute to a genuinely sustainable use of nature and promote Natural Ingredient Value Chains (NIVCs).

On the other hand, according to the physicochemical results, the presence of campesterol could be used as a diagnostic parameter to determine whether fermentation was carried out prior to the extraction of copoazú butter from the seeds.

Although heating during the extraction step can help improve yields, it can also lead to the degradation of some compounds of interest, such as carotenoids and acylglycerols.

For future work, we recommend assessing the physicochemical composition concerning pressing yield, as this factor plays a crucial role when considering scaling up for industrial processes.

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