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Research Article

Effect of Extraction and Preservation Methods on the Microbiological and Physicochemical Quality of *Pentadesma butyracea* Butter Produced in a Traditional Area in Benin

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P.butyracea butter, produced by different traditional methods, is often stored for further use in different types of packaging which may affect its quality. The present work aims to evaluate the effect of the production method and the type of packaging used on the physicochemical and microbiological quality of butter during storage. The extraction of Pentadesma butter was first carried out through production monitoring in three repetitions by three different butter producers according to the two most used traditional production methods. Then, butter from production was stored for three months in four types of packaging (aluminium bowls, calabashes, baskets, and black polyethylene bags) in the production environment. The microbiological and physicochemical quality of the stored butter was assessed at 0, 30, 60, and 90 days using normative reference methods. The production method and the type of packaging used had a significant effect on the variation of free fatty acid content ($1.54 \pm 0.07\%$–$2.6 \pm 0.2\%$), peroxide value (0.96 ± 0.09°meq·O_2/Kg–3.9 ± 0.7°meq·O_2/Kg), and colour of the butter during storage. In contrast, only the type of packaging material influenced the microbiological characteristics of the butter during storage. After three months of storage, the yeast and mould load was out of the standard range in all packages, i.e., 2.53 ± 0.4 log°CFU/g, 2.9 ± 0.2 log°CFU/g, 4.67 ± 0.2 log°CFU/g, and 1.4 ± 0.2 log°CFU/g for aluminium bowls, calabashes, baskets, and black polyethylene bags, respectively. The aerobic mesophilic germ load was within the standard in black polyethylene bags (3.22 ± 0.08 log°CFU/g), in contrast to the other packages (4.23 ± 0.08 log°CFU/g–6.45 ± 0.13 log°CFU/g). This shows that black polyethylene bags are the best packaging to guarantee the quality of butter. It is important to continue this investigation by storing butter for a longer period of time with more appropriate packaging.

1. Introduction

Pentadesma butyracea (butter tree) is a tropical species whose fruits contain kernels rich in edible butter, which is similar to shea butter [1]. Apart from its food uses (sauce preparation, frying, etc.), this butter is used in the production of traditional soap and ointment and for therapeutic purposes [2–4]. The physicochemical and organoleptic

characteristics of *P. butyracea* butter have shown its potential uses in the food, cosmetic, and pharmaceutical industries [5–8]. However, its use in these industries is conditioned by compliance with international standards established for butter quality, particularly that of unrefined shea butter [9]. *P. butyracea* kernels are available between April and June each year. The need to consume butter during lean periods leads women, producers, and consumers to store Pentadesma kernels and butter for a relatively long time. Storage of butter in unsuitable conditions could deteriorate the microbiological and physicochemical quality of butter. Indeed, it has been reported that production methods, packaging materials, and storage conditions significantly influence the quality of shea butter compared to international standards [10–12]. For example, shea butter produced from an optimised method and butter obtained from a traditional method present different physicochemical and microbiological characteristics, with the quality of traditional butter often not conforming to normative requirements [10]. Furthermore, the type of packaging used for the storage of shea butter has a significant effect on the microbiological quality of the product obtained under optimal conditions [11]. In addition, the duration of storage and the type of packaging would significantly influence the microbiological and physicochemical quality of shea butter produced by a traditional method in Benin [12]. In a relatively recent study, 10 production methods for *P. butyracea* butter have been identified in Benin, two of which are used by nearly 60% of female producers [13]. The evaluation of these two methods showed that several unit operations affect the physicochemical quality of the butter produced [7]. This leads to the question of to what degree the production method and the type of packaging of *P. butyracea* butter can affect the physicochemical and microbiological quality of the product during storage. The present work aims to evaluate the effect of production methods, type of packaging, and storage time of butter on the microbiological and physicochemical quality of *P. butyracea* butter.

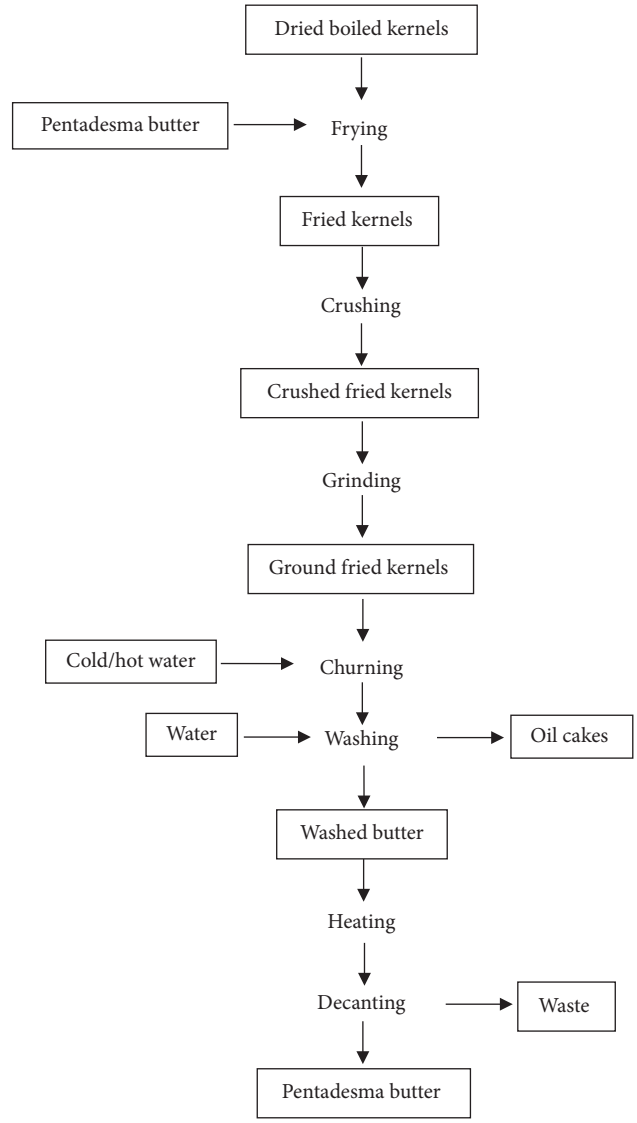

Figure 1: Traditional method 1 for extracting butter from *P. butyracea*.

2. Materials and Methods

First, the methodology adopted in this study consisted of producing butter from the two most commonly used traditional butter production methods. Second, the types of butter produced were packaged in the four main traditional butter-packaging materials for 90 days. During storage, the quality of butter was assessed microbiologically and physiochemically. The evaluation of butter quality was carried out after 0, 30, 60, and 90 days of storage.

2.1. Butter Production. Boiled and dried *Pentadesma butyracea* kernels were purchased from the market of Bassila in northern Benin. The change was made as follows: Six female butter producers were randomly selected based on their experience in butter production. This experience should be at least equal to 10 years. selected by randomisation, with at least 10 years of experience in butter production. These producers were organised in two groups of three producers each. Within each group, one of the two most common production methods was implemented. Three productions were carried out per production method. Method 1 consists of frying boiled and dried kernels in a pot with butter from a previous production method and then crushing them in a mortar. The crushed kernels were then finely ground in a mill, followed by churning until unpurified butter was obtained. The resulting butter was washed and heated in a pot to collect the supernatant, which was recovered and cooled to yield butter (Figure 1). For method 2, boiled and dried kernels were crushed in a mortar and then roasted in a pot. The rest of the process is identical to method 1 (Figure 2) The temperature applied during the heat treatments (frying and roasting) was measured at the end of each operation with a mercury thermometer graduated from 0 to 500°C.

2.2. Packaging and Storage of the Butter Produced. Immediately after production, 500 g of butter samples from different productions was then packaged in four different

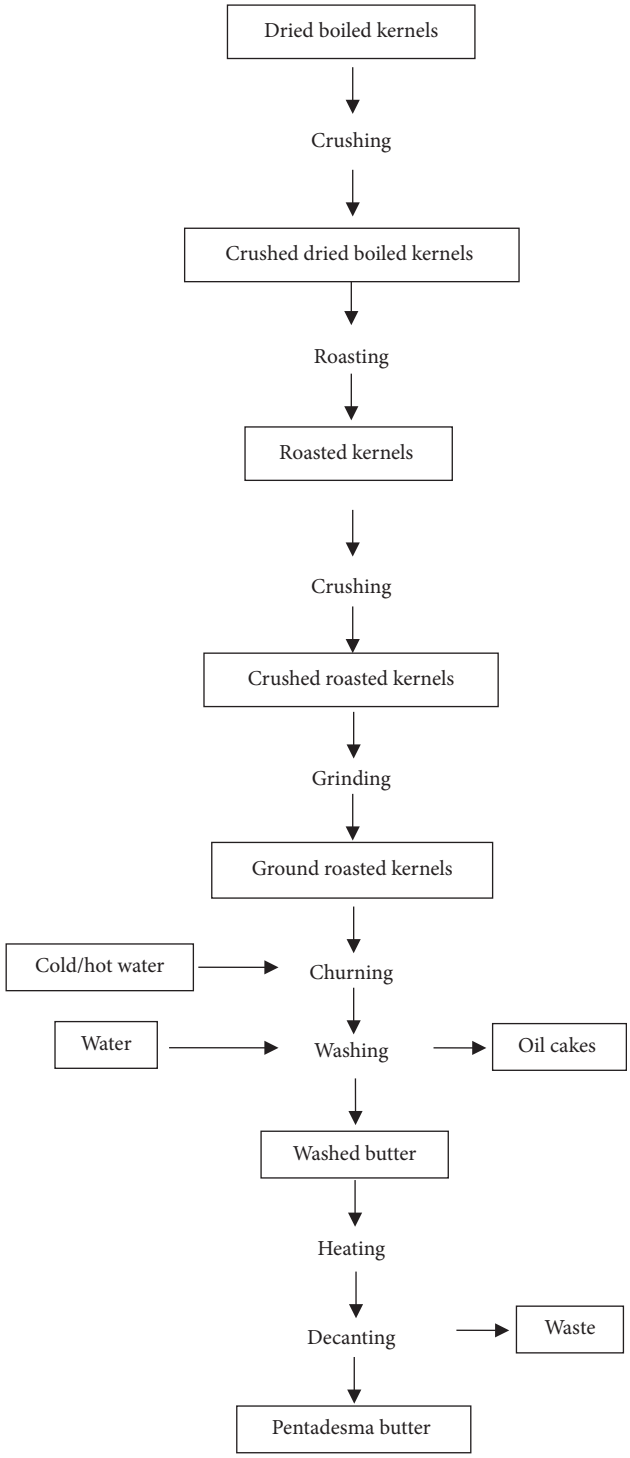

Figure 2: Traditional method 2 for extracting butter from *P. butyracea*.

types of packaging: aluminium bowls with lids, calabashes with lids, baskets lined with Tectona grandis leaves, and black polyethylene bags. The polyethylene bags used were low-density polyethylene (LDPE). These different types of packaging materials were the main packages used by producers and consumers to store butter and were identified in a previous survey [13]. The packaged butter was stored for three months by the producers (30 ± 1°C; relative humidity: 81 ± 3%). The temperature and relative humidity throughout the storage period were monitored by using a thermohygrometer recorder. Samples were taken after 0, 30, 60, and 90 days of storage for microbiological and physicochemical characterisation. 24 samples were analysed in each sampling moment.

2.3. Determination of the Microbiological Quality of P. butyracea Butter during Storage. The microbiological quality of butter was evaluated through the enumeration of aerobic mesophilic germs, indicators of the degree of microbial contamination of foodstuff [14, 15], coliforms (total and thermotolerant), indicator germs of faecal contamination, the presence of which in high numbers indicates a lack of hygiene [16, 17], and yeast and mould, which are the specific microorganisms that often contaminate butter [18].

Aerobic mesophilic germs were counted on PCA (Plate Count Agar, Oxoid, CM 463, Basingstoke, Hampshire, England) at 30°C for 72 h [19], total coliforms on VRBL (Oxoid, CM 485, Basingstoke, Hampshire, England) at 37°C for 24 h [20], faecal coliforms or thermotolerants on VRBL at 44°C for 48 h [21], and yeast and mould on MEA (Malt Extract Agar, Oxoid, CM 59, Basingstoke, Hampshire, England) incorporated with 10% lactic acid (Oxoid, SR 21, Basingstoke, Hampshire, England) and incubated at 25°C for 5 days [22]. To prepare the stock dilution (10^{-1}), 10 g of *P. butyracea* butter melted in a water bath (45–50°C) was added to 90 ml of salted peptone water. Successive decimal dilutions (10^{-2}, 10^{-3}, 10^{-4},10^{-5}, 10^{-6}, and 10^{-7}) were made from the mother dilution. Following dilutions, plating, incubation, and reading were carried out in accordance with the standards mentioned above.

2.4. Determination of the Physicochemical Quality of P. butyracea Butter during Storage. The physicochemical quality of Pentadesma butter was evaluated during storage by determining the main parameters that indicate butter quality, namely, free fatty acid content, peroxide value, colour, and water content.

2.4.1. Free Fatty Acid Content. The free fatty acid content was determined according to NF 60–204 [23]. 10 g of the Pentadesma butter sample was weighed in a conical flask and first dissolved in 150 ml of 1/1 (v/v) of 95% (v/v) ethanol and diethyl ether mixture. The mixture was then titrated with shaking against the ethanolic potassium hydroxide solution. The percentage of free fatty acids (FFAs), expressed as % of oleic acid, is as follows:

$$(\text{FFA}) = \frac{N \times V \times 282}{m \times 10}, \quad (1)$$

where V is the volume (ml) of the standard potassium hydroxide solution used, N is the exact normality of standard potassium hydroxide, and m is the sample weight.

2.4.2. Peroxide Value. The peroxide value was determined according to NF T 60–220 [23]. 10 ml of chloroform was

Table 1: Technological parameters for the production of *Pentadesma* butter in a traditional environment ($n = 3$).

Technological parameters	Method 1 (frying)	Method 2 (roasting)
Roasting/frying temperature	$133 \pm 7.5^{\circ}C^{a}$	$119 \pm 2.88^{\circ}C^{b}$
Roasting/frying time	30 ± 8.5 min^{a}	27 ± 5.19 min^{a}
Churning time	62 ± 1.52 min^{a}	57 ± 3.21 min^{a}
Cooking temperature	$111 \pm 1.15^{\circ}C^{a}$	$105 \pm 2.3^{\circ}C^{a}$
Cooking time	60 ± 8.32 min^{a}	30 ± 4.5 min^{a}
Yield	$31.66 \pm 1.48\%^{a}$	$29.16 \pm 2.48\%^{a}$

Mean ± standard deviation; n = number of replication. In the same row, parameters with different numbers are significantly different at the 5% level.

added to about 2 g(m) of butter and homogenized to rapidly dissolve the sample. Fifteen millilitres of acetic acid and 1 ml of potassium iodide were added in succession. The flask was corked and agitated for about a minute and kept in the dark for about 5 min. At this stage, about 75 ml of distilled water was added. The solution was titrated against sodium thiosulphate solution using a starch indicator. The volume (V) was then noted. A blank test (without the sample) was carried out through the same procedure [24, 25]. The volume ($V0$) was then noted. The peroxide value (PV) in milliequivalents per kilogram is given as follows:

$$\mathrm{PV} = \frac{1000 \times N \times (V - V0)}{m}. \quad (2)$$

2.4.3. Colour. The colour of butter was measured in the L*, a*, b* space (CIELAB) using a chroma meter (Minolta CR 200 b) previously calibrated with a white ceramic reference. The colour parameter analysed is yellow saturation (b*).

2.4.4. Water and Volatile Matter Content. The water and volatile matter content was determined in accordance with NF 60–201 [23] 5°g of the butter sample was weighed in a crucible that was previously dried and tared. The crucible containing the test sample was kept in the oven set at $103 \pm 2^{\circ}C$ for 5 hours. The capsule was allowed to cool in the desiccator to room temperature and then weighed. The water and volatile matter content (WVMC) is calculated according to the following formula:

$$(\mathrm{WVMC}) = \frac{(m1 - m2) \times 100}{m1 - mo}, \quad (3)$$

where mo is mass in grams of the crucible, $m1$ is mass in grams of the crucible and the sample before heating, and $m2$ is mass in grams of the crucible and the sample after heating.

2.5. Statistical Analysis. A three-factor analysis of variance (ANOVA) (production method, packaging material, and storage time) was used to identify differences between treatments and interactions between the three factors at the 5% significance level. Compliance tests were performed to compare the microbiological and physicochemical characteristics of *P. butyracea* butter to international standards using Student's t-test. These statistical analyses were carried out using the STATISTICA software.

3. Results

3.1. Technological parameters of Butter Production. The technological parameters of butter production from the two production methods are presented in Table 1.

Churning time, cooking time, and temperature did not differ ($p > 0.05$) between the two methods. The same is true for frying or roasting time and yield. On the other hand, there was a significant difference ($p < 0.001$) between frying temperature (method 1) and roasting temperature (method 2).

3.2. Effect of Production Methods and Packaging Materials on the Microbiological Quality of Pentadesma butter during Storage. Table 2 shows the microbial load of butter obtained from the two production methods and stored in the four packages tested over different periods.

In contrast to the production method, which did not have a significant effect ($p > 0.05$) on the microbiological quality of the butter during storage, packaging, storage time, and the interaction between these two factors had significant effects ($p < 0.001$).

The number of aerobic mesophilic germs (AMGs) varied from 1.77 log°CFU/g on the day of production to 6.45 log°CFU/g after three (03) months of storage (Table 2). Regardless of the packaging used, an increase in the AMG load was observed with the increasing storage time. After three months of storage, only the butter contained in the plastic bag met the normative requirements for AMG (4 log°CFU/g). On the other hand, the yeast and mould load was out of the norm in all packaging used after three months of storage. The butter in the *Tectona grandis* leaf-lined basket had the highest yeast and mould and AMG loads (6.45 log°CFU/g and 4.67 log°CFU/g, respectively) after three months of storage, whereas the butter in the plastic bag had the lowest yeast and mould (1.4 log°CFU/g) and AMG (3.22 log°CFU/g) loads over the same period. As for coliforms (total and thermotolerant), they were absent in differently preserved butter. Overall, the plastic bag is the best packaging as it guarantees a better quality of butter regarding the microbial load compared to other packaging, in this case, basket packaging.

3.3. Effect of the Extraction Method and Packaging Material on the Physicochemical Characteristics of Butter Storage. Figure 3 shows the evolution of free fatty acid content of butter during storage as a function of the method (A) and packaging (B) used. Very highly significant effects ($p < 0.0001$) of the production method, the storage time, and the type of packaging were observed on butter acidity. In general, an increase in free fatty acid content during storage was observed regardless of the method used, from 1.7% to 2.2% for the first method and from 1.4% oleic acid to 2% oleic acid for the second method. The butter produced by the first method had a higher acidity than that produced by

Table 2: Microbial flora of *P. butyracea* butter during storage in different packaging materials ($n = 6$).

Log germs (CFU/g) enumerated	Storage time (days)	Packaging material				Standards
		Aluminium container	Calabash	Basket	Plastic bag	
AMG	0	1.77 ± 0.06^{a}	1.77 ± 0.06^{a}	1.77 ± 0.06^{a}	1.77 ± 0.06^{a}	4^{g}
	30	2.59 ± 0.15^{c}	3.05 ± 0.04^{de}	3.18 ± 0.08^{ef}	2.24 ± 0.08^{b}	4^{g}
	60	3.06 ± 0.04^{de}	3.4 ± 0.09^{fg}	3.52 ± 0.05^{g}	2.85 ± 0.04^{d}	4^{g}
	90	4.23 ± 0.08^{h}	4.91 ± 0.2^{i}	6.45 ± 0.13^{j}	3.22 ± 0.08^{ef}	4^{g}
Y and M	0	Abs	Abs	Abs	Abs	1^{a}
	30	Abs	Abs	Abs	Abs	1^{a}
	60	Abs	1.55 ± 0.2^{b}	1.61 ± 0.4^{b}	Abs	1^{a}
	90	2.53 ± 0.4^{c}	2.9 ± 0.2^{c}	4.67 ± 0.2^{d}	1.4 ± 0.2^{b}	1^{a}
TC and ThC	0	Abs	Abs	Abs	Abs	1.40^{a}
	30	Abs	Abs	Abs	Abs	1.40^{a}
	60	Abs	Abs	Abs	Abs	1.40^{a}
	90	Abs	Abs	Abs	Abs	1.40^{a}

Mean ± standard deviation; n = number of replication. AMG: aerobic mesophilic germs; Y and M: yeast and mould; TC: total coliform; ThC: thermotolerant coliforms; Abs: absent. Numbers with the same letters are not significantly different at the 5% level.

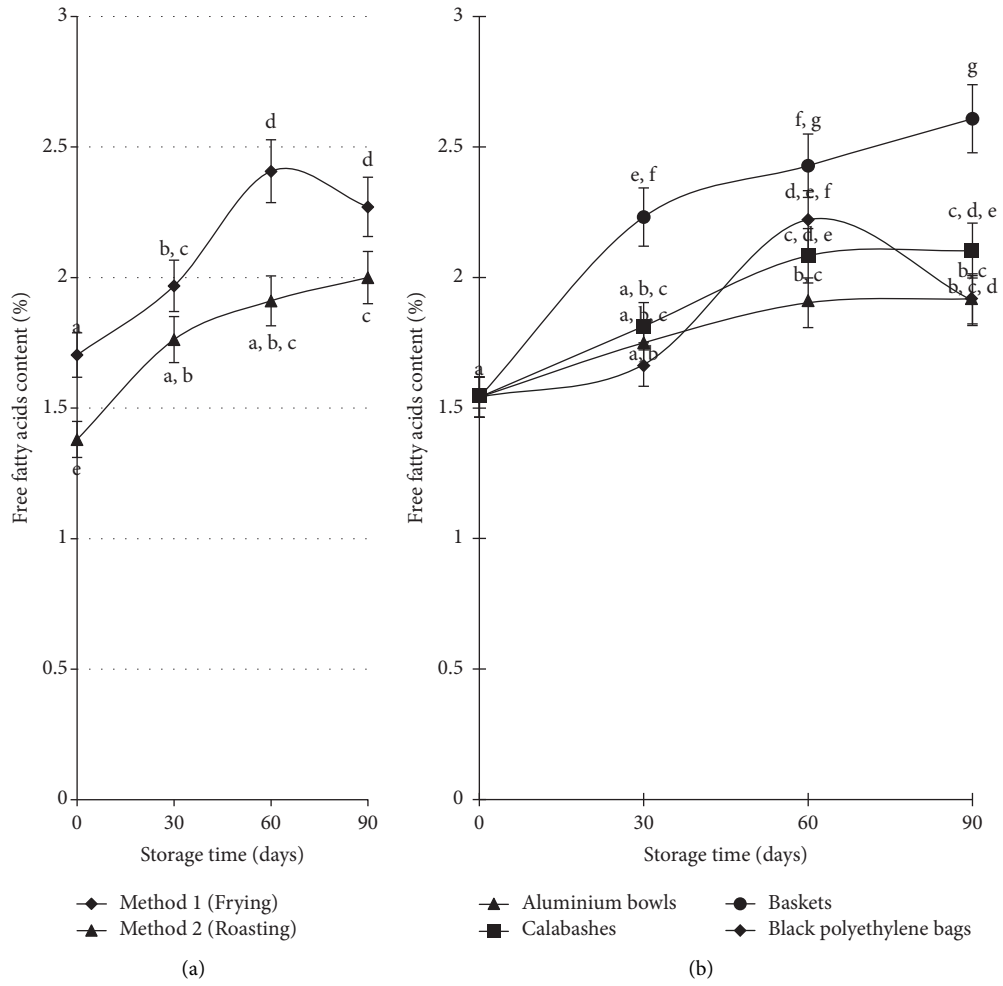

Figure 3: Effect of the production method (a) ($n = 12$) and packaging material (b) ($n = 6$) on free fatty acid of Pentadesma butter during storage.

method 2 (Figure 3(a)). Regardless of the production method, the acidity of butter varies according to the packaging used during storage (Figure 3(b)). Thus, the butter in the basket had the highest acidity regardless of the storage time. In three months of storage, the butter packed in black polyethylene bags had the lowest acidity (1.91%).

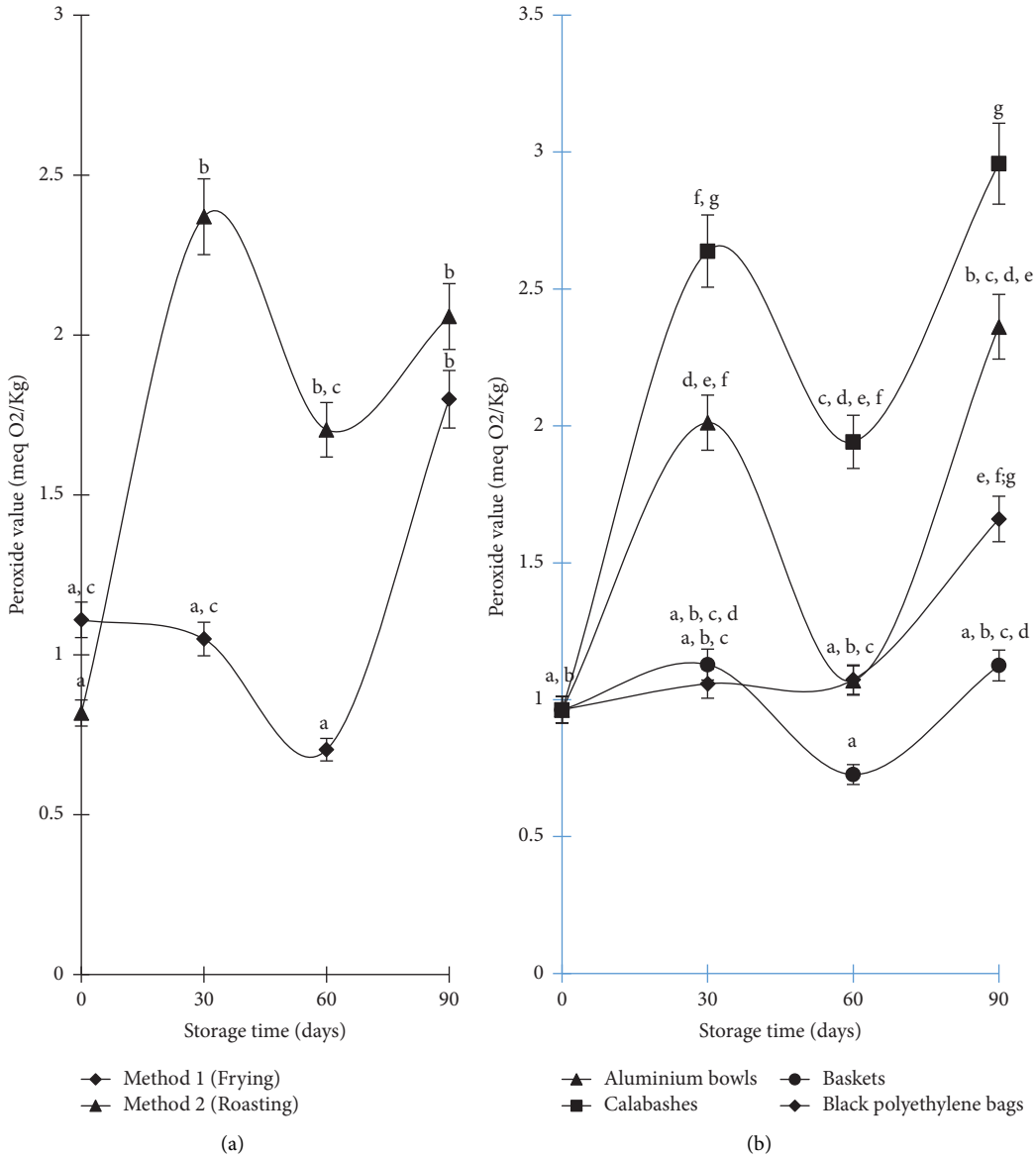

Figure 4: Effect of the production method (a) ($n = 12$) and packaging material (b) ($n = 6$) on the peroxide value of Pentadesma butter during storage.

Figure 4 shows the evolution of the peroxide value of butter during storage according to the production method (A) and packaging (B). The production method, storage time, and packaging material used had very highly significant effects ($p < 0.0001$) on the peroxide value of butter (Figure 4(a)). The second method resulted in butter with a higher peroxide value than the butter obtained by the first method. The peroxide value increases during the first month of storage, followed by a decrease in the second month of storage and an increase in the third month with differences in each type of packaging (Figure 4(b)).

The degree of yellow saturation of butter was significantly influenced by the packaging material (p‹0.0001) and the storage time ($p < 0.01$). The production method did not influence ($p > 0.05$) the colour of butter. The average of the data obtained from the two methods as a function of the production time and the packaging material was used to design the curve in Figure 3. A decrease in the degree of yellow saturation of butter was observed as a function of the storage time regardless of the storage material used (Figure 5). Moreover, the plastic bag retains the yellow saturation level of butter better regardless of the storage time compared to other packages, with the basket lined with *Tectona grandis* leaves having the lowest yellow saturation level.

The water and volatile matter content of the butter was not influenced by any of the factors studied and averaged 0.32% ± 0.24.

4. Discussion

Mesophilic aerobic germs are indicators of the degree of microbial contamination of foodstuff [14]. Their presence in

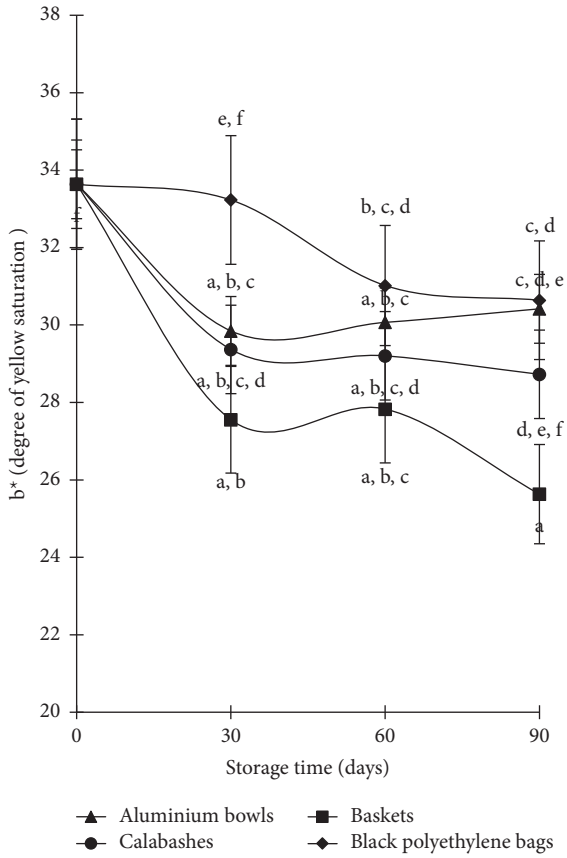

Figure 5: Effect of packaging on the yellow saturation index of butter during storage ($n = 6$).

butter before conservation could be due to the questionable quality of water used and that of the materials used during the butter extraction process. Water quality is a fundamental aspect for the quality of butter produced and must be controlled before considering applying any type of packaging material. Packaging does not improve the initial quality of a food product. Indeed, the equipment used to produce butter is rudimentary [13], and butter is often produced under unhygienic conditions. Moreover, cooling of butter after production is performed in the open air, with an increased risk of contamination by environmental microorganisms. Once present in butter before storage, the increase in their relatively high load in the butter stored in the basket and calabashes during storage would be linked to an increased metabolism generated by the optimal conditions for their growth, favoured mainly by the contamination of the packaging materials used, which are made of biodegradable organic materials. Indeed, evaluation of the aerobic mesophilic flora of *Tectona grandis* leaves showed the presence of 8.00×10^4UFC/g of leaf [26]. In addition, the same authors detected the presence of yeast and mould (40 CFU/g) in these leaves. This observation would justify the relatively high load of yeast and mould observed in the butter stored in the teak leaf-lined basket compared to the other packages after three months of storage. For short storage periods of less than two months, people can use these vegetable packages. The AMG load and yeast and mould load of our butter after two months of storage, regardless of the type of packaging, are lower than those reported in samples of shea butter collected from markets in Côte d'Ivoire and Benin [10, 27]. In addition, the authors in [12] showed that the AMG load and yeast and mould load of shea butter stored for two months in different traditional packaging (calabashes, plastic container, and paper lined with jute sacking) were out of range. The water content of shea butter stored by these authors was on average 4.9%, whereas that of our butter was on average 0.32%. Therefore, the water content of butter determines the length of storage of butter.

The influence of the method of production of butter on the physicochemical characteristics, including free fatty acid content, peroxide value, and clarity of butter, is due to the parameters of execution of unit operations. The two methods differ from each other only in the heat treatment of pretreated kernels, where in the first production method, dried boiled kernels are fried in Pentadesma butter, while in the second production method, dried boiled kernels are roasted [7]. The quality of the Pentadesma butter used for frying kernels could be the reason for high free fatty acid content of the butter produced by method 1. Moreover, the frying temperature is on average 135°C compared to 115°C for roasting. This relatively high frying temperature could result in increased hydrolysis of butter triglycerides![24]. It has been reported that frying and roasting of *P. butyracea* kernels are unitary operations that significantly affect the quality of the butter produced [7]. The increase in free fatty acid content of butter during storage is due to the hydrolysis of triglycerides through the combined action of lipase, light, and heat. Indeed, the highest acidity is obtained in the butter stored in the basket lined with teak leaves where yeast and mould have been counted. These microorganisms have the capacity to produce lipases, responsible for the enzymatic hydrolysis of lipids [25]. The increase in the peroxide value during the first month of storage, regardless of the storage material, is probably due to the oxidation of butter by oxygen from air and light. On the other hand, the decrease observed during the second month of storage is due to conversion of primary compounds into secondary oxidation compounds, which are mostly volatile. The authors in [28] reported that the number of volatile compounds in shea butter increases with the storage time. However, the difference observed at the level of the type of packaging used is due to the barriers that each type of packaging offers to the precursors of oxidation reactions. Indeed, the low peroxide value observed in butter stored in the black polyethylene bag compared to other packaging is related to the limitation of contact of butter with light and oxygen, which are contributing factors for the formation of peroxide precursors [28]. As the peroxide index only measures primary oxidation compounds, the oxidation state of fat should be assessed in combination with the measurement of secondary oxidation compounds [29–31], which was not performed in this work. The decrease in the degree of yellow saturation of butter as a function of the storage time is due to the degradation of carotenoids, responsible for the yellow colour of butter through oxidation [28]. This hypothesis is reinforced by the fact that the yellow colour of butter stored in the black bag does not change

regardless of the storage time. As the yellow colour is a quality attribute for *P. butyracea* butter, the use of the polyethylene bag seems to offer more protection to the quality of butter.

5. Conclusion

The production method and packaging material had a significant influence on the physicochemical and microbiological characteristics of *P. butyracea* butter during storage. An increase in the microbial load and free fatty acid content of the butter was observed as a function of the storage time, regardless of the packaging material used. After one month of storage, the microbiological quality of the butter complied with the standards whatever the storage material used, whereas after two months of storage, the load of yeast and mould in the butter stored in baskets lined with *Tectona grandis* leaves and in calabashes was out of the norm. In view of the results obtained, the butter produced by the method with the heat treatment of roasting and preserved in black polyethylene packaging during the two months of storage presented the best physicochemical and microbiological characteristics. It is important to continue this investigation by using other types of packaging for the storage of butter for a longer period.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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