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Full Length Research Paper

Microbiological and physicochemical characterization of shea butter sold on Benin markets

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Shea butter, a fat from the nuts of shea tree, is of great nutritional and commercial value for local communities of Africa. The sanitary and physicochemical qualities of shea butter sold in Benin markets are unknown. This study assesses the quality characteristics of 54 samples of shea butter collected from eight markets in Benin, West Africa. Total germs, yeasts and mould varied with markets. Moisture content ranged between 2.5 to 6.2%. Iodine index was around 49 mgI_2/100 g, but acid index (4.1 to 6.0 mgKOH/g), peroxide value (9.4 to 11.8 meq O_2/kg) and saponification values (186.4 to 193.7 mgKOH/g) showed high variability both within and between samples of different markets. Quality characteristics were poorer for butter collected in the main urban markets (Cotonou, Bohicon and Malanville), due mainly to poor storage conditions. Shea butter could be stored in a clean package before sale to preserve its beneficial qualities.

Key words: Shea butter, microbial status, acid index, peroxide index, iodine index, saponification value, Benin market.

INTRODUCTION

Shea butter, a vegetable fat extracted from the kernels of the fruit of *Vitellaria paradoxa* Gaertner, Sapotaceae, is an ancient African commodity that still plays an important role in village life (Hall et al., 1996; Kengue and Ndo, 2003; Elias and Carney, 2004; Honfo et al., 2011). Shea tree is the main indigenous oil-producing wild plant spontaneously growing in Africa, and native of dry savannah zones from Senegal to Uganda. In *V. paradoxa* producing countries, such as Benin, shea butter is generally extracted by traditional processing that involves roasting, churning and boiling in the fruit-producing areas and then marketed in village or urban markets (Agbahungba and Depommier, 1989). However, the traditional extraction process in African countries implies unequal water qualities, at worst leading to increased oxidized material observable by high peroxide values of the resulting fat (Di Vincenzo et al., 2005). Locally, shea butter is widely used as cooking oil, for producing soap, and used in pharmacological and cosmetic products. Shea butter has an increasing international demand by cosmetic and pharmaceutical industries, and it is also used as a cocoa butter additive in chocolate manufacture (Elias and Carney, 2004; CNUCED, 2006).

Shea butter is composed of triglycerides and fatty acids including oleic acid (60 to 70%); stearic acid (15 to 25%); linolenic acid (5 to 15%); palmitic acid (2 to 6%); linoleic acid (<1%) and an unsaponifiable content (3 to 15%) (CENUCED, 2006). Due to their high content of unsaturated fatty acids (49 to 63%), shea nuts are susceptible to deterioration (Maranz et al., 2004; Di Vincenzo et al., 2005).

Shea butter is the main edible oil for the communities of northern Benin; it provides food oil for more than 80% of the population in this zone, it is therefore the most important source of fatty acids and glycerol in the diet

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Figure 1. Map of Benin showing the shea butter samples provenances.

(Agbahungba and Depommier, 1989). Its daily consumption in this zone is estimated at 26.3 g per person (Honfo et al., 2010), and its consumption is increasing due to the high cost of imported oils. Because of the dietary importance of shea butter, it is important to analyze the quality of the butter, and assess improvements opportunities if it is necessary. The objective for this study was to assess the major microbial and physicochemical characteristics of the shea butter sold in the different urban markets in Benin.

MATERIALS AND METHODS

Sample collection

Samples of shea butter were collected in the main urban markets of eight regions of Benin (Bohicon, Cotonou, Djougou, Kandi, Malanville, Natitingou, Parakou, and Tanguieta) (Figure 1). Most of the sample collection locations are the production zones except Bohicon and Cotonou. A total of 54 samples were collected, packed in aluminum foil, wrapped in polyethylene bags and numbered according to their origins (market, town and area). They were stored in an icebox containing ice cubes and transported to the laboratory, where they were kept at 4°C until analysis. For each parameter, sample determinations were made in triplicate.

Microbiological characteristics

Aerobic mesophilics bacteria (on plate count agar at 30°C for 72 h), total coliforms (on violet red bile agar at 30°C for 24 h), faecal or thermotolerant coliforms (on Violet Red Bile Agar at 44°C for 24 h), yeasts and moulds (on malt extract agar at 25°C for 72 h) were determined in each shea butter sample according to the methods described by Megnanou et al. (2007) and AOAC (2002).

Physicochemical characteristics

Moisture content was determined according to AOAC (2002). Colour measurements were performed using the chromameter (Minolta (CR210b). Results were expressed as L* (brightness), b* (yellowness), and ΔE (difference of color from the white ceramic standard). Acid, peroxide, iodine and saponification values were determined according to the methods of the Beninese shea butter characterization standards using NB ISO 660 (2006), NB ISO 3960 (2006), NB ISO 3961 (2006), and NB ISO 3657 (2006) respectively.

Statistical analysis

The tests of conformity by Student's t-test were performed to compare the microbiological counts of butter with the international standards. The analysis of variance (Proc GLM) was used to compare the different parameters measured on the shea butter among the provenances and SNK (Student–Newman–Keuls) was used to classify these parameters using SAS 9.1 software. Correlations were also established between variables.

RESULTS

Microbial characteristics

Samples of shea butter had various microbial load with

Table 1. Microbiological characteristics of marketed shea butter from Benin.

Provenance	Germs identified (CFU/g)		
	Aerobic mesophilic bacteria	Yeast and mould	Total coliforms
Bohicon(n = 5)	$3.810^{5}\pm410^{3a}$	$1.810^{3}\pm1.910^{2a}$	79 ± 42^{a}
Cotonou (n = 4)	$4.710^{5}\pm4.210^{3a}$	$1.510^{3}\pm1.710^{2ab}$	56 ± 32^{abc}
Djougou (n = 8)	$10^{5}\pm210^{2a}$	$3.610^{2}\pm29^{bc}$	23 ± 13^{bc}
Kandi (n = 5)	$1.610^{4}\pm10^{2a}$	$3.710^{2}\pm35^{bc}$	24 ± 13^{bc}
Malanville (n = 7)	$9.110^{5}\pm1.810^{3a}$	$10^{3}\pm10^{2abc}$	64 ± 34^{ab}
Natitingou (n = 6)	$2.810^{5}\pm6.510^{3a}$	$2.510^{2}\pm10^{2c}$	15 ± 11^{c}
Parakou (n = 10)	$2.510^{5}\pm2.310^{2a}$	$8.910^{2}\pm85^{abc}$	13 ± 10^{c}
Tanguieta (n = 9)	$5.510^{4}\pm9.810^{2a}$	$3.410^{2}\pm85^{bc}$	11 ± 5^{c}
Norms*	10^{4a}	10^{c}	$25b^{c}$

*: Codex Alimentarius, 1992; NBF 01-005, 2006; For each parameter (in column), mean ± standard deviation with the same letter are not significantly different.

Table 2. Color and moisture content of marketed shea butter from Benin.

Provenance	Moisture content	Color characteristics		
		Luminance (L*)	Yellow saturation index (b*)	Color difference (ΔE)
Bohicon (n = 5)	6.06 ± 0.7^{a}	62.43 ± 2.6^{e}	19.77 ± 6.1^{bc}	44.15 ± 1.5^{a}
Cotonou (n = 4)	6.24 ± 1.7^{a}	68.53 ± 1.7^{bc}	19.81 ± 1.6^{bc}	38.74 ± 1.9^{b}
Djougou (n = 8)	6.17 ± 1.9^{a}	70.29 ± 2.5^{a}	22.12 ± 3.6^{ab}	42.13 ± 2.3^{a}
Kandi (n = 5)	5.67 ± 1.6^{a}	67.36 ± 1.1^{cd}	25.55 ± 5.9^{a}	43.45 ± 3.9^{a}
Malanville (n = 7)	6.13 ± 1.7^{a}	65.69 ± 2.6^{d}	22.09 ± 5.7^{ab}	42.69 ± 3.7^{a}
Natitingou (n = 6)	2.48 ± 1.7^{b}	69.69 ± 1.1^{abc}	21.88 ± 3.3^{ab}	39.26 ± 2.8^{b}
Parakou (n = 10)	3.64 ± 1.4^{b}	69.53 ± 2.6^{abc}	21.75 ± 3.1^{ab}	39.44 ± 2.4^{b}
Tanguieta (n = 9)	3.81 ± 1.1^{b}	71.24 ± 3.1^{a}	15.93 ± 2.8^{c}	34.69 ± 3.7^{c}

For each parameter (in column), mean ± standard deviation with the same letter are not significantly different.

high count of aerobic mesophilic germs (4.2 to 5.70 $\log_{10}$ CFU/g), yeasts and moulds (2.4 to 3.3 $\log_{10}$ CFU/g) and total coliforms (1.0 to 1.9 $\log_{10}$ CFU/g) (Table 1). Great variation were observed between and within the geographical locations for the number of aerobic mesophilic bacteria (CV = 142 to 225%), but the differences were not statistically significant. The number of aerobic mesophilic bacteria in all collected samples was close to the international standard of 4 $\log_{10}$ CFU/g (Codex Alimentarius 1992; NBF 01-005 2006). However, the highest microbial count was found in sample collected in Cotonou and Bohicon markets, which are the biggest markets in the Southern and Central regions respectively (Table 1). Differences between locations for the number of yeasts and moulds ($p = 0.0014$) and total coliforms ($p = 0.0056$) were observed, with samples collected at the Bohicon (Central Benin) market giving significantly higher values of yeasts and moulds compared with the samples from other markets. In addition, samples from some locations gave higher numbers of yeasts and moulds than the international standards value of 1.0 $\log_{10}$ CFU/g for yeasts and moulds. Total coliforms count for all sample were closed to the standard of 1.4 $\log_{10}$ CFU/g, but the higher value were observed in the sample collected at Bohicon. No faecal coliforms were detected.

Physicochemical characteristics

The moisture content ranged between 2.5 to 6.2% (Table 2), and significant differences between locations were found ($p = 0.0001$) with two distinctive groups: one with a moisture content ranging between 2 and 4% (from Natitingou, Parakou and Tanguieta) and the other with a higher moisture content (from Cotonou, Djougou, Malanville, Kandi and Bohicon).

Mean values for the color parameters of the shea butter samples are presented in Table 2. The highest L* value was observed for the samples collected in Tanguieta while the lowest L* value was from a sample from Bohicon. Reversely, low b* values and ΔE were observed in Tanguieta and the highest in Bohicon. Significant differences were observed between the shea butter provenance for L* value ($p = 0.0001$), b* value ($p = 0.0001$) and ΔE ($p = 0.0001$) in spite of great variability within locations.

Table 3. Biochemical characteristics of marketed shea butter from Benin.

Provenance	Acid index (mgKOH/g)	Peroxyde index ($meqO_2$/kg)	Iodine index (mgI_2/100 g)	Saponification index (mgKOH/g)
Bohicon (n = 5)	5.47 ± 1.4^{ab}	11.54 ± 2.4^{a}	49.37 ± 1.1^{a}	193.74 ± 6.0^{a}
Cotonou (n = 4)	5.70 ± 1.2^{a}	11.16 ± 1.2^{ab}	49.80 ± 1.2^{a}	187.19 ± 1.5^{b}
Djougou (n = 8)	4.88 ± 1.4^{ab}	9.78 ± 1.1^{ab}	50.26 ± 1.2^{a}	188.43 ± 2.4^{b}
Kandi (n = 5)	5.06 ± 1.3^{ab}	10.70 ± 2.5^{ab}	48.30 ± 1.3^{a}	190.74 ± 5.1^{ab}
Malanville (n = 7)	6.01 ± 1.2^{a}	11.76 ± 2.3^{a}	48.93 ± 1.5^{a}	190.39 ± 4.3^{ab}
Natitingou (n = 6)	4.51 ± 1.2^{ab}	9.41 ± 0.8^{b}	49.46 ± 1.5^{a}	188.59 ± 3.4^{b}
Parakou (n = 10)	4.90 ± 1.2^{ab}	10.91 ± 1.6^{ab}	49.07 ± 1.4^{a}	186.43 ± 2.6^{b}
Tanguieta (n = 9)	4.10 ± 1.6^{b}	9.45 ± 1.9^{b}	49.64 ± 1.7^{a}	186.69 ± 3.5^{b}

For each parameter (in column). mean ± standard deviation with the same letter are not significantly different.

Mean acid values ranged from 4.1 to 6.0 mgKOH/g, and mean peroxide values from 9.4 to 11.8 meq O_2/kg (Table 3). Samples collected in the markets of Tanguieta and Natitingou (Northern Benin) seemed to have low acid and peroxide values.

The mean values of the iodine index were around 49 mgI_2/100 g for the samples from all locations. No significant differences for this index were observed between the different sample provenances. However, relatively higher values were found in the samples collected in Djougou (Table 3). The mean of the saponification values varied from 186.4 to 193.7 mgKOH/g, with significant differences between locations for this index ($p = 0.0001$) (Table 3).

DISCUSSION

Traditional shea butter sold on the urban markets of Benin presented a great variability in microbial and physicochemical characteristics. Total coliform counts of most of the shea butter samples were close to the microbiological international standard for edible fat (Table 1) (Codex Alimentarius, 1992). However, difference was observed between samples provenances; the highest values of the total coliforms yeasts and moulds observed in samples collected in the three biggest markets (Bohicon, Cotonou and Malanville) could be explained basically by the suboptimal transportation conditions and storage materials used by traders or by the exposure to the atmospheric air during sale, engine exhaust and dust, since these elements could be vectors of microbial germs (Roquebert, 1997; Pfohl-Leszkowicz, 2000). Indeed, most of the butter is stored for 6 months before sale in major urban markets (Honfo et al., 2011). Two locations (Bohicon and Cotonou) are outside the shea butter production zone and during transportation traders watered the butter to maintain its humidity. This practice could lead to favourable environmental conditions for the development of microorganisms, such as yeasts and moulds, as showed by the high value in these two markets. In addition, these locations also have a climate with a high relative humidity (65 to 85%), which conditions could also influence the sanitary quality of the butter.

The moisture content, acid, and peroxide values of the butters sold in Benin were lower than those of shea butter from Côte d'Ivoire (Megnanou et al., 2007), while the iodine values of shea butter in Benin were higher. This variability could be explained by the diversity of butters sold which come from many locations, but also by the different traditional processes used for their extraction (Dieffenbacher et al., 2000, Kapseu et al., 2005; Honfo et al., 2011). The traditional process is generally without control of the unit operations (Kapseu et al., 2005) and could result in the presence of low quality shea butter in the market.

Irrespective of locations, the moisture content in all samples was higher than the international standard (0.05 to 2%) for non refined shea butters (NBF 01-005, 2006), but lower than values found in shea butter (10.2%) processed by the Bangoua method in Cameroon (Kapseu et al., 2005). The high moisture content of shea butter can activate lipase, which can potentially catalyze the hydrolysis of triglycerides leading to rapid deterioration of shea butter (Mittal and Paul, 1997). The hydrolysis of the triglycerides leads to high levels of acidity in the butter; this is corroborated by the positive and significant correlation between moisture content and acid index ($r = 0.437$, $p = 0.0001$). A high moisture content can also promote the growth of microorganisms and in this study high levels of total germs were positively correlated with moisture content ($r = 0.306$; $p = 0.0032$).

The high level of the acid index in samples collected in Malanville, Cotonou, and Bohicon, might be related to the hydrolysis of triglycerides that occurred during the storage of butter. In addition, the high number of yeasts and moulds found on the samples in these locations could increase the enzymatic hydrolysis since some of these microorganisms would have the capacity to secret

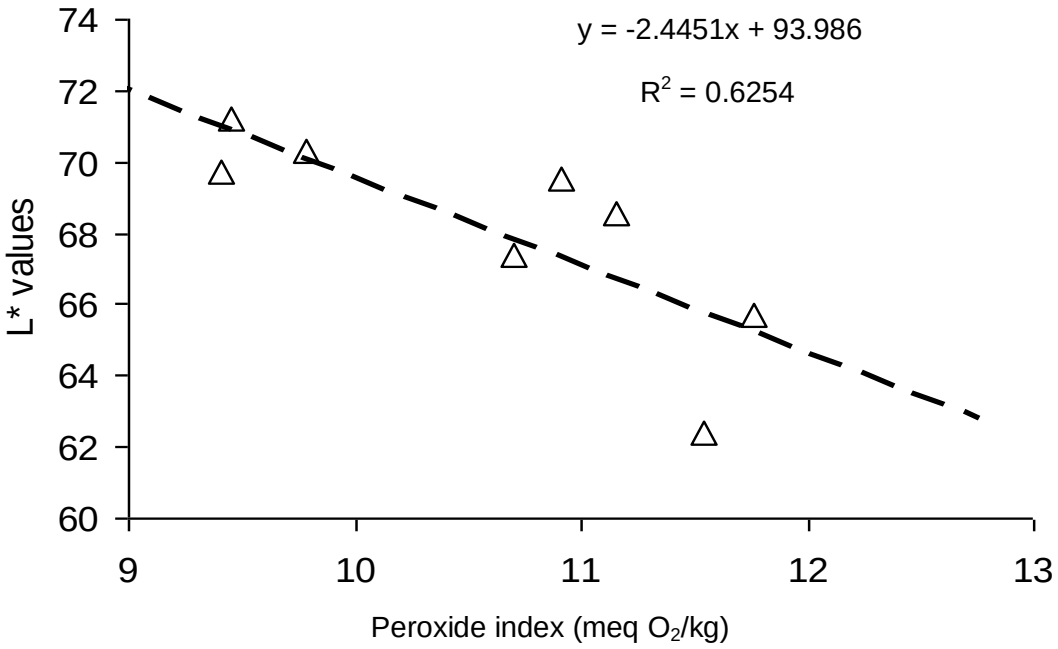

Figure 2. Relationship between luminance (l*) and peroxide index of shea butter.

lipase, responsible for enzymatic hydrolysis in lipids (Hultin, 1994). This result is in agreement with the positive correlation between the acid value and the number of yeast and moulds (*r = 0.306; p = 0.0032*).

All samples of shea butter collected had peroxide values close to the maximal value of 10 meq02/kg tolerated by the cosmetic and pharmaceutical industry (Codex Alimentarius, 1992; NBF 01-005, 2006). Furthermore, the negative correlation between L* value and peroxide index (*r = -0.79; p = 0.0013*) (Figure 2) is consistent with the previous work of Akissoe et al. (2003), who observed the increase of brown index (100-L*) with the increase in peroxidase activity in yam cultivars (*D. rotundata* spp). Then, any deterioration factor (enzymic, hydrolysis) which increases peroxide values, can result in the low luminance of the shea butter. In addition, a positive correlation was observed between the peroxide index and the moisture content (*r = 0.509; p = 0.0001*). The relatively high acid and peroxide values in marketed shea butter of Benin could be associated with the lack of quality control in the traditional process; in particular, these high values could be explained by the long drying period for nuts and prolonged roasting of kernels (Kapseu et al., 2005; Womeni et al., 2006). Exposure of shea butter to sun and air assumedly causes hydrolysis of glycerides and the oxidation of the unsaturated fatty acids (Schreckenberg, 2004), potentially resulting in high acid and peroxide indices as observed in the presented data.

The value of the iodine index, which is a measure for the level of unsaturation of oils, was lower than the norms (58-72 mgI_2/100g) (NBF 01-005, 2006). The oxidation of unsaturated fatty acids could be responsible for the relatively low iodine values of the marketed shea butter (Dieffenbacher et al., 2000).

Conclusions

The quality of shea butters sold in urban markets of Benin varied widely in terms of microbiological quality (yeasts and moulds, total coliforms) and physicochemical characteristics (color parameters, acid, peroxide and saponification values). This variation is probably associated with the transportation and storage conditions since the butter is often stored in makeshift packages for up to six months before being sold. It is recommended to transport and store shea butter in a clean package, safe from heat (solar), air and dust to preserve its beneficial qualities.

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