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Physicochemical evaluation and microbiological properties of artisanal Yoghurt sold in Maroua-Cameroon

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Abstract

Malnutrition remains a real health problem in the Far North Region of Cameroon despite the presence of many food commodities. The objective of this work was to evaluate physicochemical and microbiological properties of ten artisanal yoghurt (kossam) sold in Maroua-Cameroun. Proximate composition, viscosity and titratable acidity were determined by standard Association of Official Analytical Chemists (AOAC) methods. Mineral contents: calcium, magnesium, sodium, potassium, iron, zinc and manganese were determined by atomic absorption spectrophotometry and phosphorus by Murphy Riley reagent. The microbiological analyses were carried out following the standard protocols. Analysis of variance revealed a significant difference in the levels of the parameters analysed in the different yoghurt samples ($p<0.05$). The results of this study revealed for all the samples an acid pH (3.20 - 4.37); titratable acidity (2.01-2.74%); viscosity (1.32-1.51). Other contents per 100g fresh matter are as fellow: Moisture (87.29- 92.45%); Protein (2.02-3.72g); Carbohydrate (4% for all the samples); ashes (0.28-0.49g); calcium (74-113mg); magnesium (4-20mg); zinc (0,117-0,333mg); potassium (60.4-165mg); sodium (10-17,25mg); iron (0.034-0.088mg) and phosphorus (51-80mg). The microbiological analyses revealed that fungal flora were only detected in few samples (1.2-4.47 log10cfu/ml); Total aerobic mesophilic flora (4.36 -9 log10cfu/ml); Staphylococcus (1.30-5.31 log10cfu/ml) and total coliforms (1- 3 log10cfu/ml). These yoghurts could therefore contribute nutrients for human nutrition and for the management of some nutritional diseases.

Keywords: Malnutrition; Kossam; Artisanal Yoghurts; Physicochemical Properties; Microbiological Analysis; Nutritional Disease

Introduction

In many regions of the world, milk and dairy products like yoghurt are essential component of the daily diet of human beings, especially for vulnerable groups such as preschoolers and elderly [1]. They are among the most diverse foods in terms of composition. They contain more than twenty different trace elements including copper, zinc, manganese and iron [2]. These minerals are cofactors of many enzymes and play an important role in many physiological functions in man. A deficiency in one of these minerals can cause serious troubles and pathologies [3].

Yoghurt is a traditional dairy product well known in all regions of the world. Where it exits in different forms and it has different names. Originally, it come from Bulgaria where it is known as '' yourt''. In Lebanon and some arab countries, yogurt is called ''Lebon''. In India and Pakistan, it is known as ''Dahi'' [4]. In Cameroon, it is ''Kossam'' Also called ''curdled milk'' Kossam is a traditional yoghurt obtained by fermenting cow's milk and it is widely consumed in the North region of Cameroon. When it is well made, it is uniform, dense and white. It comes from the North of Cameroon (Adamawa Region and Far North). For the majority of pastoral and agro-pastoral population kossam is a symbol, it represents an essential component in the daily food ration, It is an important source of income and a drink of great social value consumed by all ages [5].

Like all the other yoghurt, ''Kossam'' contains probiotics known for their therapeutic properties. They stimulate digestion; they boost the immune system and have anti-carcinogenic and cholesterol-lowering properties [6]. It contains proteins, lipids, carbohydrates, vitamins and minerals such as calcium, iron, phosphorus, magnesium and zinc [7]. It can significantly contribute to the recommended daily nutritional ratio of calcium and magnesium required to maintain the functioning of the physiological process in the body at a good level. Calcium plays an important role in the mineralization of bones and teeth as well as in the prevention of cardiovascular diseases osteoporosis and hypertension which constitute public health problems in Cameroon [8]. In addition to calcium, yoghurt is also an excellent dietary source of zinc and phosphorus which can contribute to more than 40% of nutritional phosphorus needs [9]. However, it is important to notice that its nutritional qualities depend on a certain number of factors such as: the production process, the chemical composition of the milk, the nature and the quality of the ingredient used the nature of the micro-organism, and incubation temperature and time [10]. As far as the physical and chemical properties are concerned, they are greatly influenced by water and protein content of the milk used [11].

Like all other fermented or unfermented dairy products and despite of its natural quality, Kossam constitutes a culture medium of choice for a number of pathogenic or non-pathogenic microorganisms [12]. Mould, fungies and a wide range of bacteries such as: *Listeria monocyogenes, Salmonella species, Staphylococcus aureus, Campylocaterjejuni,* and *Yersinia enterocolitica.*

The presence of microorganisms in Kossam has several origins. Because of their safety, innocuousness, organoleptic and probiotic properties, they can be added intentionally in the preparation of their fermentable properties. They can be accidentally added during the preparation, the packing or the marketing if rules of hygiene guaranteed by good manufacturing practices are not observed [13]. Beyond the considerable economic losses attributable to this contamination, many infectious bones illnesses can be caused by the consumption of infected Kossam.

The objective of this study was to determine physicochemical characteristics and the microbiological quality of the different varieties of Kossam sold in Maroua, Far North region of Cameroon. In the perspective of evaluating nutritional intake by children with malnutrition and controling hygienic quality.

Materials and Methods

Samples Collection

The samples of local yoghurts (Kossam) where purchased in detail in Maroua town between August and September 2017. They were then transported in frigirated box to the laboratory where the analyses were carried out. Ten samples of kossam were collected in 10 points of sale which were used to constitute the samples on which the microbiological and physicochemical analyses were carried out. Samples of each variety were collected after 7-10 days preceding the production. Sample were labeled: A1 (Myl lait), A2 (N'djaren yaourt), A3 (Yaourt Socropole), A4 Yaourt des femmes de Pete), A5 (Kossam WaldeApif- Cameroun), A6 (Diamare Laban Kossam), A7 (Kossam de boutique), A8 (Sahel yaourt , A9 (Had yaourt) and A10 (Yaourt sodelait).

Physicochemical Analysis

The pH and viscosity of the different samples were determined using a pH meter (Geneq) with an ice electrode and a viscosimeter (DV-E model). It is also provided with glass electrode and a standard ball, equipped with stopwatch at respectively 20°C. Viscosity was expressed in centipiosis (cP). The titratable acidity was determined according to the AOAC [14] method and expressed in percentage of lactic

acid. Moisture content; Total ash, lipids and proteins were analysed according to the methodology developed by AOAC [15]. Total carbohydrates were calculated following this formula: Total carbohydrates = Total solid particles – (Fats + proteins + Total ash) [16].

Mineral Analysis

The mineral composition (sodium, potassium, calcium, magnesium, zinc, and iron) of the different samples was determined by atomic absorption spectrophotometric equipped with a flame ionization detector following the methodology described by Benton and Vernon [17]. For the determination of phosphorus content, Murphy Riley reagent was used [18].

Microbiological Analysis

The microbiological analysis was carried out in triplicate following the standard protocols. The enumeration of the mesophilic aerobic flora was carried out in an agar medium after incubation at 30°C for 24-48hrs. The results were expressed in colony forming units per ml (cfu/ml). The yeast and mould population were counted on the Sabouraud CAF agar culture medium (supplemented with chloramphenicol) after 3-5 days of incubation. The agar culture medium Man, Rogosa and Sharp (MRS) served to enumerate lactic bacteria after an incubation of 24-48h at 37°C aerobically. The total coliforms were listed on the lactose agar and after an incubation period of 24h at 37°C. The researches and the numbering of staphylococcoccus and salmonella were carried out on Baird-Parker agar media (supplemented with egg yolk incubated at 37° C for 24-48 hours) and Salmonella shigella (incubated at 37° C for 24 hours) respectively.

Statistical Analysis

All the analyses were done in triplicate and the data obtained were statistically analyzed with IBM SPSS software 20.0 for Windows. One-way Analysis of Variance (ANOVA) test and the Least Significant Difference (LSD) at the critical point 5% was done to compared the different value.

Result and Discussion

Physicochemical Proprieties

Table 1 shows the physicochemical proprieties (pH, viscosity, titratable activities) of the different samples of kossam.

Samples	pH	Viscosity (cP)	Titratable acidity (% of lactic acidity)
A1	3.92 ± 0.14^{ab}	1.32 ± 0.11^{ab}	2.81 ± 0.41^{a}
A2	4.14 ± 0.02^{ab}	1.43 ± 0.08^{ab}	2.02 ± 0.41^{ab}
A3	3.20 ± 0.13^{ab}	1.37 ± 0.09^{ab}	2.83 ± 0.06^{a}
A4	3.48 ± 0.09^{ab}	1.46 ± 0.10^{a}	2.28 ± 0.81^{ab}
A5	4.37 ± 0.24^{a}	1.51 ± 0.11^{a}	2.03 ± 0.45^{ab}
A6	3.36 ± 0.11^{ab}	1.43 ± 0.05^{ab}	2.24 ± 0.81^{ab}
A7	3.22 ± 0.02^{ab}	1.43 ± 0.11^{ab}	2.94 ± 0.81^{a}
A8	4.42 ± 0.013^{a}	1.37 ± 0.11^{ab}	2.01 ± 0.61^{ab}
A9	3.69 ± 0.11^{ab}	1.46 ± 0.07^{a}	2.32 ± 0.4^{ab}
A10	4.33 ± 0.20^{a}	$1.34 \pm 0.08a^{b}$	2.05 ± 0.71^{ab}

Table 1: Physicochemical proprieties (pH, viscosity, titratable activities) of the different samples of kossam
Mean values in the same column with different superscript letters are significantly different ($P< 0.05$).

The pH values obtained are between 3.20 in sample A3 and 4.42 in sample A8 which express the acid character of these foods. These values are similar for some samples (A1, A3, A4, A6, A7, and A9). They are similar to the results obtained by Djoulde et al.[19] in Kossam samples collected in the same region. Generally the reduced pH in artisanal yoghurts ''Kossam'' is due to the activity of microorganisms and lactic acid bacteria in particular which sometimes exist in large numbers in these products which are obtained under uncontrolled conditions. These conditions concern: the starter impurity, which is made up of part of the previous preparation for incubation and uncontrolled conditions, storage and conservation conditions, and the hygienic quality of the containers. All these factors can have a positive impact on the activity of organic acids by microorganisms present in Kossam.

Titratable acidity designates the level of lactic acid produced during fermentation. There is a slight difference in titratable acidity in these samples. The values of this parameter are between 2.01% of lactic acid in sample A8 and 2.94 of lactic acid in A7. These values comply with the standard applicable in East Africa which is at least 0.6 % of titratable acidity in yoghurts. There is a negative correlation between the pH values and those of the titratable acidity.

Acidity is a determining factor in the reduction of the bacterial load, especially on the spontaneously fermented product. This acidity is due to the accumulation of the lactic acid produced by lactic acid bacteria and other microorganisms present during fermentation [20]. As for viscosity, no significant difference is observed between the different samples analyzed. The smallest value was recorded in the sample A1 (1.32) and the highest values were recorded with the samples A5 (1.51), A4 and A9 (1.46). This also indicates that they are the most viscous samples. There is also a negative correlation between water content and viscosity.

Proximate Composition Of Kossam Samples Collecte

Table 2 presents the proximate composition of the different varieties of kossam collected in Maroua.

Samples	Moisture	Ash	Protein	Lipids	Carbohydrates
A1	89.6 ± 0.056^{ab}	0.49 ± 0.12^{a}	3.72 ± 0.014^{a}	3.1 ± 1.41^{ab}	4.90 ±1.01^{ab}
A2	88.96 ± 0.310^{ab}	0.35 ± 0.09^{bc}	2.02 ± 0.056^{d}	2.2 ± 1.61^{c}	4.70 ± 1.21^{ab}
A3	89.84 ± 0.260^{ab}	0.40 ± 0.16^{b}	3.11 ± 0.014^{bc}	3.3± 1.9^{a}	4.80 ± 1.16^{ab}
A4	89.71 ± 0.155^{ab}	0.43 ± 0.19^{ab}	3.16 ± 0.127^{b}	2.2 ± 0.91^{c}	4.60 ± 1.51^{b}
A5	89.41 ± 0.125^{ab}	0.39 ± 0.08^{b}	2.77 ± 0.09^{c}	2.5± 0.91^{b}	4.75 ± 1.07^{ab}
A6	88.57 ± 0.565^{ab}	0.28 ± 0.11^{c}	2.12 ± 0.042^{cd}	2.3 ± 1.22^{c}	4.90 ± 1.21^{ab}
A7	92.45 ± 0.318^{a}	0.31 ± 0.15^{c}	2.39 ± 0.063^{cd}	2.2 ± 1.49^{c}	4.60 ± 1.61^{b}
A8	87,38 ± 0,806^{b}	0.40 ± 0.19^{b}	2.85 ± 0.106^{bc}	2.4 ± 1.41^{bc}	4.80 ± 1.41^{ab}
A9	87.29 ± 0.2199^{b}	0.44± 0.09^{ab}	2.58 ± 0.028^{cd}	3.1 ± 1.48^{ab}	4.20 ± 1.81^{a}
A10	86.57 ± 0.268^{b}	0.30 ± 0.012^{c}	2.96 ± 0.091^{bc}	2.8 ± 1.42^{ab}	4.90 ± 1.21^{ab}

Table 2: Proximate composition of kossam samples collected (g/100g) fresh weight.
Mean values in the same column with different superscript letters are significantly different (P< 0.05).

For each of the nutritional parameters analyzed, there is a significant difference between the samples analyzed (P< 0.05). Lipids content of food products has a positive influence on the physical characteristics (viscosity and density) and sensory properties [21]. The analysis of fat shows that the sample A2, A4, A5, A6, A7, A8 and A10 have concentration values less than 3%. This is the maximum lipid concentration in yoghurt set by Codex alimentarius (*CODEX STAN A-11(a)*. They have a lipids content that match the Codex alimentarius standard. If we must stick to this standard, the samples that have a lipid content greater than 3% do not comply. It is the case of samples A1 (3.1 %), A3 (3.3 %) and A9 (3.1 %). Low lipid contents also have a beneficial effect on the health and the stability of yoghurts [22].

We can note that these products have moisture contents between 87.29 % in the sample A9 and 92.45 % in the sample A7. Muhammed et al. [23] proposed that the moisture standard in yoghurts should be between 83 and 86%. Almost all the yoghurts analyzed do not meet this standard. In general, the chemical composition of yoghurts and moisture content in particular significantly influences the firmness, accessibility and the syneresis degree of fermented milk products [24].

The protein content also varies between 2.02 g/100g in sample A2 and 3.72 g/100g in sample A1. These values are comparable to those obtained by Ponka et al. [25] in the sample of traditional yoghurt collected in Maroua.

The carbohydrate concentration is around 4%, just like that obtained by Ponka et al. (2013) in the traditional yoghurts of Maroua town. The low sugar contents observed are the results of fermentation. This fermentation takes place and transforms lactose into lactic acid. That process makes yoghurt a great food for people with lactose intolerance [26].

Ash contents observed in the analyzed samples is between 0.28 in sample A6 and 0. 49 in sample A1. Usually, there is a very large difference in the ash content in dairy

products. This can be explained by the difference in origin of the main ingredients such as milk. Milk is the major ingredient used in the manufacture of these products. Ash content analysis gives an idea on the nutritional importance of the inorganic mineral elements in the samples analyzed. It was reported that the ash content of a food sample gives an idea on the mineral elements present in the food sample which is needed for bone development, teeth formation and body functions [27].

Minerals Contents

Table 3 presents mineral contents in samples (mg/100g). Minerals such as phophorus, calcium, magnesium, potassuim and sodium are the most abundant with a respective average of 58.9; 85.9; 12.93; 92.64 and 14.02 mg in 100 g of kossam. In a study published by Ponka et al. [25] on the analysis of the mineral composition of yoghurt in Maroua (Cameroon), the authors noted the preponderance of phophorus, calcium, magnesium, potassium and sodium over the seven minerals analyzed. The average content in Ca, Na and P were lower than those obtained by Ponka et al.[25] which respectively were 99.1; 29.3 and 75.5 mg/100 g of the traditional yoghurt in Maroua town. However, our samples contained more Mg (12.93 mg/100 g) and Fe (0.060 mg/100 g) than the samples analysed by Ponka et al. [25] which respectively were 10.03 and 0.056 mg/100 g of yoghurt. A similar observation was made with the iron contained in the traditional yoghurt analyzed by Enb et al. [28]. All those differences observed in the mineral composition of traditional yoghurts made from cow's milk can be explained by the differences in the origin of the ingredients used and the preparation protocol.

Samples	P	Fe	Ca	Mg	Zn	K	Na	Na/k	Ca/Mg
A1	57 ± 3.00^{a}	0.034 ± 0.0015^{a}	80 ± 3.00^{a}	11 ± 1.00^{bcd}	0.151 ± 0.003^{b}	165 ± 3,00^{d}	17.25 ± 0.03^{e}	0.10 ± 0.01^{a}	7.27 ± 0.02^{e}
A2	51 ± 3.00^{a}	0.054 ± 0.004^{abc}	112 ± 2.00^{d}	19 ± 2.00^{e}	0.241 ± 0.005^{e}	65.9 ± 0.10^{a}	13.6 ± 0.40^{b}	0.21 ± 0.03^{a}	5.89 ± 0.03^{d}
A3	54 ± 3.00^{a}	0.035 ±0.004^{a}	74 ± 2.00^{a}	20 ± 2.00^{e}	0.323 ±0.003^{g}	80.5 ± 0.3^{b}	15.8 ± 0.40^{cde}	0.20 ± 0.03^{a}	3.70 ± 0.03^{a}
A4	54 ± 3.00^{a}	0.08 ± 0.02^{c}	88 ± 2.00^{b}	12 ± 2.00^{cd}	0.189 ± 0.003^{c}	80.9 ± 0.3^{b}	9.35 ± 0.05^{a}	0.12 ± 0.03a	7.33 ± 0.03^{e}
A5	56 ± 3.00^{a}	0.067 ± 0.003^{abc}	76 ± 3.00^{a}	16 ± 3.00d^{e}	0.333 ± 0.003^{g}	84.2 ± 0.20^{b}	14.2 ± 0.20^{bc}	0.17 ± 0.06^{a}	4.75 ± 0.050^{c}
A6	55± 3.00^{a}	0.04± 0.002^{ab}	98 ± 2.00^{c}	4 ± 2.00^{a}	0.285 ± 0.003^{f}	60.4 ± 0.3^{a}	10 ± 2.00^{a}	0.17 ± 0.03^{a}	24.50 ± 0.030^{i}
A7	76±3.00^{b}	0.06± 0.03^{abc}	113 ± 3.00^{d}	5,3 ± 0.30^{ab}	0.117 ± 0.04^{a}	110 ± 5.00^{c}	11.1 ± 0.10^{a}	0.31 ± 0.33^{a}	21.32 ± 0.040^{h}
A8	56± 3.00^{a}	0.071± 0.002^{abc}	97 ± 3.00^{c}	12 ±3.00^{cd}	0.229 ± 0.003^{d}	84.3 ± 0 .30^{b}	17.15 ± 0.05^{de}	0.20 ± 0.03^{a}	8.08 ± 0.04^{f}
A9	50± 3.00^{a}	0.076 ± 0.003^{bc}	102 ±2.00^{c}	10 ±1.00^{bc}	0.147 ± 0.003^{b}	86.2 ± 0.20^{b}	16.23 ± 0.25^{de}	0.19 ± 0.03^{a}	10.20 ± 0.05^{g}
A10	80± 3.00^{b}	0.088 ± 0.002^{c}	89 ± 2.00^{b}	20 ±2.00^{e}	0.144 ± 0.004^{b}	109 ± 5.00c	15.3 ± 0.30^{bcd}	0.14 ± 0.04^{a}	4.45 ± 0.05^{b}

Table 3: Minerals contents (mg/100g) fresh weight.
Mean values in the same column with different superscript letters are significantly different (P< 0.05).

The presence of these minerals in an acid environment like in yoghurt is important as acidity facilitates their absorption, thus increasing bioavailability [29]. The Ca/K ratio, when low, is a good indicator of the effect of Na and K on blood pressure regulation. The values of this parameter are shown in table 3. It varies from 0.10 to 0.21. This indicates that these yoghurts could be used to regulate blood pressure [30]. Alinnor and Oze [30] recommend that the Ca/Mg ratio should be greater than 1 in food products. In all kossam samples analyzed, this ratio (Ca/Mg) was between 3 and 24. K^{+} and Ca^{2}+ are the most important elements for bone growth, development, metabolism, and health maintenance.

The differences observed between the samples of kossam manufactured in Maroua town demonstrate the lack of standardization in the manufacturing process and in the composition of yoghurt in Cameroon. A similar analysis was done by Djoulde et al. [19] who proposed a standardized

protocol for the production of kossam. The differences can also be explained by a number of factors such as: the chemical composition of the milk, the production process, the nature and quality of the ingredients used, the nature of the fermentable microorganisms used, the incubation time and temperature [10].

Microbiological Analysis on the Kossam Samples

The results of the microbiological analysis on the Kossam samples collected are presented in Table 4.

Samples	Ffo log_{10} (cfu/ml)	TMAF log_{10} (cfu/ml)	Sta total log_{10} (cfu/ml)	TC log_{10} (cfu/ml)
A1	4.47 ± 0.04a	9.00 ± 2.13a	3.16 ± 1.98ab	3.00 ± 1.32a
A2	1.30 ± 0.06 b	6.62 ± 1.38ab	3.17 ± 1.02 ab	1.70 ± 0.13b
A3	ND	ND	5.31 ± 1.42a	1.00 ±0.04b
A4	ND	8 ± 2.26 a	3.85 ± 0.92ab	ND
A5	ND	4.36 ±1.72b	1.30 ± 0.33c	ND
A6	ND	5 ±1.20ab	4.47 ± 1.43a	ND
A7	ND	6.13 ± 2.23^{ab}	4.74 ± 1.05^{a}	ND
A8	1.20 ± 0.10^{b}	ND	1.84 ± 0.09^{c}	ND
A9	ND	ND	ND	ND
A10	ND	ND	ND	ND

Table 4: Values of germs isolated (log 10 cfu/ml) and enumerated in kossam.
Mean values in the same column with different superscript letters are significantly different (P< 0.05).
TC: Total Coliforms, Ffo : Fongal flora, Sta : Staphylococci, TMAF: Total Mesophilic Aerobic Flora, ND: not detected.

Four indicators of hygiene and microbiological quality were used (identification and enumeration of Total Coliforms, Fungal Flora, Staphylococci, Total Aerobic Mesophilic Flora). Fungal flora were not detected in most of the samples tested (A3, A4, A5, A6, A7, A9 and A10). It was detected in samples A1, A2 and A8 and its load ranged from 1.2 to 4.47 log_{10} cfu/ml. These values are above the maximum acceptable limit of 10 cfu/ml in yoghurt according to the East African Standard [31].

Total Mesophilic Aerobic Flora (TMAF) varies between 4.36 and 9 Log $_{10}$ cfu/ml in samples where it has been detected. Staphylococci ranged from 1.30 to 5.31 Log_{10} cfu/ml in A5 and A3 samples respectively where their presence was detected.

Total coliforms were detected in A1, A2 and A3 samples; their concentration varies between 1 and 3 log_{10} cfu/ml in A3 and A1 samples respectively. The total coliform load in these samples is well above the maximum total coliform load recommended by Tamine et Robinson [32] which is 1 log_{10} cfu/ml. approximately 66% of the samples analysed were found to be free of total coliforms. These samples comply with the maximum limit allowed in similar dairy products according to the standard applicable in the East African Community [31].

The results of the microbiological analysis indicate that the majority of kossam samples collected are poor in microbiological quality. The quality of the traditional yoghurt thus described can be attributed to the poor hygiene conditions observed during the different phases of preparation, packaging and marketing of this consumer product. Other factors such as the quality of the starting milk and the unhealthy environment can also significantly contribute to the presence of these undesirable microorganisms in kossam [33]. Bonfoh et al. [34] reported that a number of factors such as the quality of the water used, good hygiene practices such as hand washing before and after each operation, cleaning and disinfection of containers are all key factors that can justify the contamination of dairy products such as locally produced kossam. Generally, CPC greater than 100 cfu/ml would indicate poor milking hygiene [35].

Conclusion

Dairy products in general and yoghurt in particular, are important sources of nutrients for all age groups of the population. Yoghurts produced and marketed in Maroua town have shown a great difference in nutritional composition and microbiological quality. Some of these yoghurts contain total coliforms and staphylococci, which make these products a

health hazard for consumers. On the other hand, the yoghurts analyzed are important sources of protein and minerals such as Ca and K. The Na/K ratio values obtained also indicate that these yoghurts can be used to regulate blood pressure. The differences observed between the different samples analyzed require the need to standardise the production of Kossam in Cameroon. There is also a great need to train the producers and sellers of these products in the good hygiene practices to be implemented during the transport, production and conservation phases of Kossam.

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