



# **Physicochemical, Sensory, and Microbial Characteristics of *Kindirmou* and *Pendidam*, Two Traditional Fermented Milks of Adamawa - Cameroon, as Affected by the Fermentation Vessels**

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## **Authors' contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

## **Article Information**

DOI: 10.9734/AJB2T/2021/v7i430107

### Editor(s):

(1) Dr. Fernando José Cebola Lidon, Universidade Nova de Lisboa, Portugal.

### Reviewers:

(1) Inês Filipa Mourão Ferreira, University of Porto, Portugal.

(2) Shehu Sarkiyayi, Modibbo Adama University, Nigeria.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/74207>

**Original Research Article**

**Received 05 July 2021  
Accepted 15 September 2021  
Published 22 September 2021**

## **ABSTRACT**

Recent data show that the containers traditionally used for fermentation, due to their nature, exchange with the fermented product, the consequence being a modification of the physico-chemical, microbiological and organoleptic properties of these products. The objective of this work is to study the influence of the fermentation vessel on the sensory, physicochemical, and microbial properties of *kindirmou* and *pendidam*, two traditional fermented milks from Adamawa - Cameroon.

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To this end, a descriptive test was used to generate the sensory properties of fermented milks in different containers, followed by biochemical (pH, titratable acidity, protein, sugar content, and total phenolic compounds) and microbiological analyses (total aerobic mesophilic flora, coliforms, lactic acid bacteria, yeasts and molds). Regarding sensory evaluation, samples of *kindirmou* and *pendidam* fermented in calabashes have a higher general acceptability than those fermented in plastic buckets and enamel plates. The total mesophilic flora and total coliforms were higher than the norm ( $\geq 6\log_{10}$ ) for *kindirmou* and *pendidam* regardless of the fermentation vessel. The absence of yeasts and molds is noted in the samples of *kindirmou*, while they are found at very high levels in the samples of *pendidam* fermented in the calabash and in the enamel plates ( $\geq 6\log_{10}$ ). Fecal coliforms are absent in samples of *kindirmou* and *pendidam* fermented in the calabash, while they are found in samples fermented in plastic buckets and enamel plates. On the physicochemical level, the results obtained showed that the *kindirmou* fermented in the calabash had the highest soluble protein content ( $1.47 \pm 0.04$  g / 100 mL of milk) than those fermented in plastic bucket and enamel plates. The phenolic compounds are present only in the samples of milk fermented in the calabash and the average contents are  $93.41 \pm 3.04$  mg / 100 mL of milk for *kindirmou* and  $111.20 \pm 2.01$  mg / 100 mL for *pendidam*.

To conclude, *kindirmou* and *pendidam* fermented in calabashes exhibit the best sensory and physicochemical characteristics, and are rich in bioactive compounds than those fermented in plastic buckets and enamel plates.

**Keywords:** Fermentation vessel; *kindirmou*; *pendidam*; physicochemical properties; microbiological quality; sensory properties.

## 1. INTRODUCTION

The transformation of fresh cow's milk into traditional fermented milk is experiencing an undeniable boom in the countries of Central Africa. These traditional fermented milks are found in urban centers where they occupy a prominent place among street foods. Among these traditional fermented milks, *kindirmou* and *pendidam* occupy a preponderant place in the northern part of Cameroon [1]. *kindirmou* is a traditional fermented milk obtained after inoculation of whole pasteurized cow's milk with 'old' *kindirmou* or 'old' *pendidam* playing the role of ferment. *kindirmou* has a titratable acidity comparable to that of yogurt. The fermentation occurs between 23°C and 24°C for 15h to 20h, brewing is then carried out, then conditioning and cooling follow [2]. *Pendidam* is obtained by spontaneous fermentation of fresh milk, resulting in curdled whitish, viscous and acidic milk containing large pieces of coagula and gas bubbles. Its acidity increases over time [2,3].

In the fermentation process of these milks, several containers are used: plastic buckets, stainless steel, aluminum or enamel plates, and calabashes (fruits of *Lagenaria* sp.). Studies on the comparison of traditional food fermentation vessels have revealed that calabash, like wood, is a lignocellulosic material that lends itself better to the traditional fermentation process of

products aimed at their physicochemical, organoleptic and microbiological valorization [4,5]. What is highlighted is the porous structure of their internal walls, which would promote exchange with food. These exchanges result from the phenomena of migration, sorption, and permeation [6,4]. In Kenya, the calabash is mainly used in the manufacture of *naoto*, *kule*, *murik* [7] and *suusac* [8] which are traditional fermented milks. These products are in great demand for their taste, their aromas, and their cultural and therapeutic values [7]. In addition, the calabash, thanks to the formation of a biofilm with microbial biodiversity, would limit the development of pathogenic bacteria and would constitute an inoculum reserve for subsequent fermentation [9]. In Cameroon, claims associate the best organoleptic characteristics with *pendidam* and *kindirmou* fermented in calabashes as well as the beneficial effects of their consumption on health. However, to the limit of our knowledge, there is no work on the study of the influence of the fermentation vessel on the physicochemical and microbiological characteristics of *kindirmou* and *pendidam*.

Thus, the objective of the present study was to investigate the influence of the fermentation vessel on the sensory, physicochemical and microbiological properties of *kindirmou* and *pendidam*.

## 2. MATERIALS AND METHODS

### 2.1 Process for Obtaining Fermented Milks

A preliminary survey in Mbéré show us the traditional dairy products in the area and the process to transform milk to traditional fermented milk. For this study, fresh milk known as *biradam* purchased from the same producer was processed into *kindirmou* and *pendidam* as described in Figure 1. Once the milk was collected, it underwent fine sieving to remove the impurities, and then this sieved milk was brought

to boil for 15 min followed by cooling to room temperature for 45 min; there followed the inoculation step which was done with the old *kindirmou* or the old *pendidam*. The milk, once inoculated, was allowed to ferment for 24 hours at room temperature in containers such as the calabash, the enamel plate, and the plastic seal; *kindirmou* or fermented whole milk was obtained from this first fermentation. This *kindirmou* was subsequently skimmed to give *ketoungole* or cream, on the one hand, and fermented skimmed milk or *bouadam* on the other. This then underwent a second fermentation at room temperature for 24 hours to give the *pendidam*.

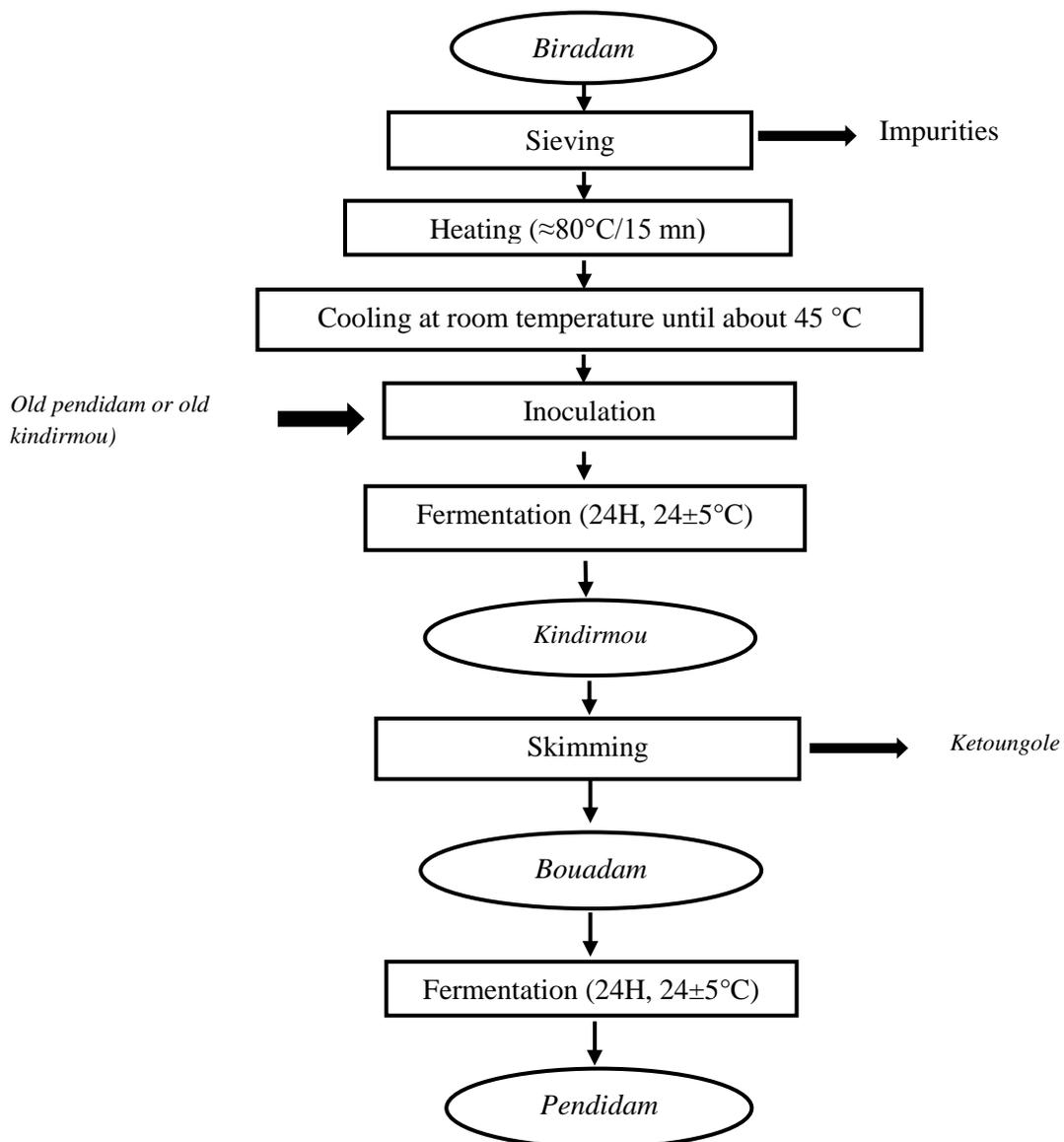


Fig. 1. Diagram of the stages of transformation of fresh cow milk into *kindirmou* and *pendidam*

## 2.2 Sensory Analysis

This is a descriptive test based on the ability of subjects to reliably describe the sensory perception of a product [10]. The particularity of this method lies in the description of the sensory qualities of a product tested by the panel, which develops and uses its own language. To do this, we first seek the maximum number of descriptors that can give maximum information on the sensory properties of the product to be analyzed, then to quantify the intensity of the sensory properties perceived for each descriptor chosen and finally to build the sensory profile of the product with different descriptors to quantify.

### 2.2.1 Pre-selection of descriptors

A group of 50 panelists (30 women and 20 men) chosen from among the students of the University of Ngaoundere was selected for the descriptive analysis based on their training, their knowledge and consumption of dairy products and their ability to distinguish fermented milk premises from different vessels or fermentation vessels through their sense organs.

### 2.2.2 Development of sensory descriptors

It consisted in looking for a large number of possible descriptors characterizing fermented milks; for this, the panelists were in a classroom equipped at the University of Ngaoundere and the different fermented milks were presented one by one to each subject. The panelists expressed any words that came to their mind that could describe their perceptions of the color, taste, texture, smoothness, smell, and flavor of the product. Subsequently, we proceeded by choosing the most relevant descriptive terms by successive sorting, which led to the elimination of irrelevant terms and the retention of the cited more than once. The panel did a second sorting by evaluating once more the samples from the previously selected descriptors, then; we defined the reference materials for the sensory characteristics listed. The references given were on the extreme limits of the intensity scale of each descriptor. Once the descriptors and references were determined, a pre-test of the analysis of the different descriptors was done using an unstructured 9 cm scale for each sensory descriptor developed.

### 2.2.3 Panel selection and training

To select the final panel, the preselected panel was trained to use three pre-tests as

recommended by Stone et al. [10]. To do this, the descriptors selected were well defined as well as the evaluation protocol and references likely to facilitate understanding and memorization of perceptions. The panel training resulted in repeatable and discriminating responses. The selection of the final panel was made on the basis of their ability to distinguish between different samples, the repeatability of their results, and the harmony of the results of the group described by Bannwart et al. [11].

### 2.2.4 Descriptive sensory analysis itself

To do this, a classroom subdivided into well-lit boxes was used. These boxes did not communicate with each other and were equipped with a tablet and a chair. For the experiment, each panelist was placed in a box at his disposal dry bread and mineral water to rinse his mouth after analyzing the sample. The analysis of the samples was carried out monardically. Thus, approximately 100 mL of the sample contained in a disposable glass previously coded and an analysis sheets was presented to each panelist. Scoring was performed using an unstructured intensity scale formed by a 9 cm segment. The intensity of perception of a product corresponds to the length between the left end of the scale and the mark made by the panelist on the segment.

## 2.3 Sampling

Thirteen samples of fermented milk (*kindirmou* and *pendidam*) having undergone sensory analysis were taken aseptically and were divided into 3 batches. The first batch consisted of milk fermented in the calabash, the second batch, meanwhile, consisted of samples of fermented milk in the plastic bucket, and the third batch consisted of the fermented milk in the enamel plate. These 30 samples of fermented milk were collected in sterile jars, placed in a cooler containing freezer blocks and transported to the Microbiology and Food Biotechnology Laboratory at the University of Ngaoundere for immediate analysis.

## 2.4 Physicochemical Analyses

### 2.4.1 Measurement of pH and acidity

The pH was measured using a pH meter (pH meter AS218 SMART SENSOR) according to the method of Soro et al. [12]. The device was calibrated with a buffer solution (pH 7.0 and pH

4.0), the pH is obtained by immersing the electrodes in 10 ml of fermented milk introduced into a beaker containing 10 ml of each sample.

The acidity was determined by titrimetry according to the AOAC [13] method which consists of determining the total acid content of the product. For this, to 10 mL of milk contained in the beaker, 4 drops phenolphthalein (0.05%) were added. The titration was carried out at room temperature by adding dropwise 1 / 9 N sodium hydroxide solution until the change to pale pink, the final volume of sodium hydroxide was noted, and the acidity expressed in degrees Dornic (1 °Dornic corresponds to 0.1 g lactic acid).

#### 2.4.2 Viscosity measurement

The viscosity of the various fermented milks was measured using a viscometer (model 800, viscostart plus fungi lap Francophonie). The samples were placed in a stainless steel measuring the cylinder, the measuring modules were inserted into it, and the dynamic viscosity readings were taken with the viscometer set at 600 rpm for each sample of fermented milk.

#### 2.4.3 Determination of the total polyphenol content of fermented milks

To 0.02 mL of samples, 1.58 mL of distilled water and 0.1 mL of Folin's reagent were added. The resulting mixture was stirred and allowed to stand for 3 min at room temperature. Then 0.4 mL of sodium carbonate was added and the mixture was incubated in the dark for 20 min and the optical density read with a spectrophotometer at 760 nm. The concentration of total crude phenolic compounds for each test was calculated using the gallic acid standard line equation in mg gallic acid equivalent per 100 mL of fermented milk.

#### 2.4.4 Determination of flavonoids content of *kindirmou* and *pendidam*

The determination of the flavonoids was carried out according to the method of Abd Ghafar et al. [14]. For this, to 2 mL of milk were added aluminum chloride (2%) followed by an incubation of 10 min in the dark and measurement of the absorbance at 430 nm. The flavonoid content is expressed in mg quercetin equivalents per 100 g of milk (mg EQ / 100g), with reference to the calibration curve.

#### 2.4.5 Determination of the soluble protein content of *kindirmou* and *pendidam*

The soluble protein content was determined in the samples according to the method of Lowry et al. [15]. The soluble protein content was calculated using a standard made from bovine serum albumin (BSA). The results were expressed in mg BSA / 100 mL equivalent of fermented milks with reference to the calibration curve.

All these physicochemical analyses were carried out in triplicate.

#### 2.5 Microbiological Analyses of *Kindirmou* and *Pendidam*

The samples of *kindirmou* and *pendidam* were homogenized and 25 mL of each sample introduced aseptically with 225 mL of saline solution (8.5‰). After mixing, decimal dilutions were made ( $10^{-1}$  to  $10^{-9}$ ) and each dilution was inoculated in triplicate on agar culture media. The dishes were then incubated at precise temperatures depending on the microorganism to be counted. For the isolation of the total flora, 0.1 mL of the sample was spread on PCA agar (Plate Count Agar), and then the inoculated dishes were incubated at 30°C for 24 hours and the colonies obtained were counted [16]. The lactic acid bacteria were counted after inoculation of 0.1 mL of each dilution on MRS medium; after 48 hours of incubation at 37°C, the colonies obtained were counted. For coliform testing, 0.1 mL of each dilution was spread onto Endo agar and the plates were incubated at 37°C for total coliforms and at 45°C for fecal coliforms. After 24 to 48 hours of incubation, the red-violet colonies were counted. Yeast and molds were enumerated after inoculation of 0.1 mL of dilution on the Yeast Extract Peptone Dextrose agar and Malt Extract Agar (MEA) respectively, and incubation at 25°C for 3 to 5 days [17]. All enumerations were transformed to  $\log_{10}$  cfu/mL.

#### 2.6 Statistical Analysis

A one-way analysis of variance (ANOVA) was performed on the data obtained for each type of traditional fermented milk to determine whether there are significant differences among the fermentation vessels. DUNCAN's multiple range tests for significant differences were performed using Statgraphics 16.1.18 software (Manugistics, Rockville, Maryland, USA, 1997) for sensory, physicochemical and microbiological

parameters. The principal component analysis allowed grouping the data according to the similarities and similarities between the parameters evaluated. Statistical significance was defined for  $P= 0.05$ .

### 3. RESULTS AND DISCUSSION

#### 3.1 Sensory Characteristics of *kindirmou* and *pendidam* Samples

The sensory descriptors generated by the panel according to the fermentation vessels are presented in Figs. 2 and 3. Fifteen descriptors grouped into five groups were identified in particular: 3 for appearance (color, homogeneity, and syneresis), 3 for odor (fresh milk, fermented milk and butter), 2 for texture (smoothness, viscosity), 4 for taste (sour, sweet, bland and rancid) and 3 for flavor (acid, sweet, oily). The general acceptability of the samples made it possible to evaluate the products by assigning them a score. These sensory attributes were defined with reference objects and therefore made it possible to evaluate the samples according to a reference scale ranging from 1 to 9. There is a positive and significant correlation between the general acceptability and homogeneity, the smell of butter, viscosity and smoothness as well for *kindirmou* as for

*pendidam*. Samples of *kindirmou* and *pendidam* fermented in calabashes have a higher general acceptability than those fermented in plastic buckets and enamel plates. These results corroborate the studies carried out on the fermentation of *rob* (Sudan) [18], *nunu* (Ghana) [19], *ititu* (South Africa) [20] in gourds from fermentation where the products obtained had better viscosity, good flavor, good taste, good texture and consistency similar to yogurt. Likewise, studies carried out on the traditional fermentation of *Kivuguto* in Rwanda showed that the calabash contributes to improving the organoleptic properties of these products [21].

#### 3.2 Physicochemical of *kindirmou* and *pendidam* Samples

##### 3.2.1 Acidity and viscosity

The results of acidity and viscosity of *kindirmou* and *pendidam* according to the fermentation vessel are presented in Table 1. The average pH of the samples was between  $3.20 \pm 0.02$  (fermented milk in the enamel plates) and  $4.68 \pm 0.01$  (fermented milk in calabashes). Specifically, traditional fermented milks produced in enamel plates have the lowest pH (3.20 - 4.11), followed by the milk fermented in plastic buckets (3.22 - 4.44) and finally, the milks obtained

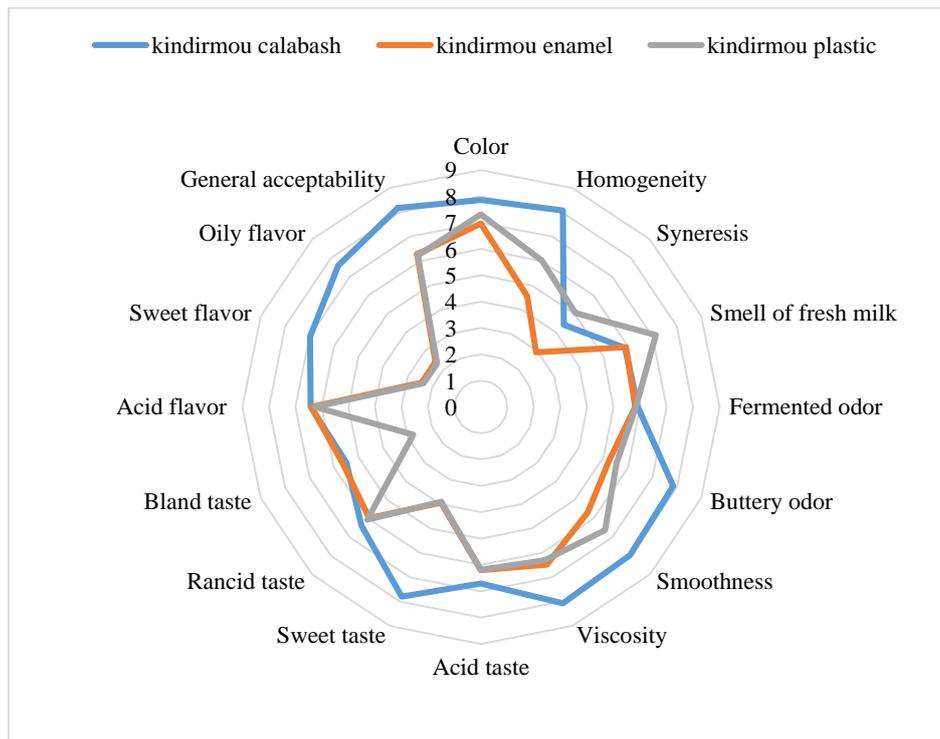
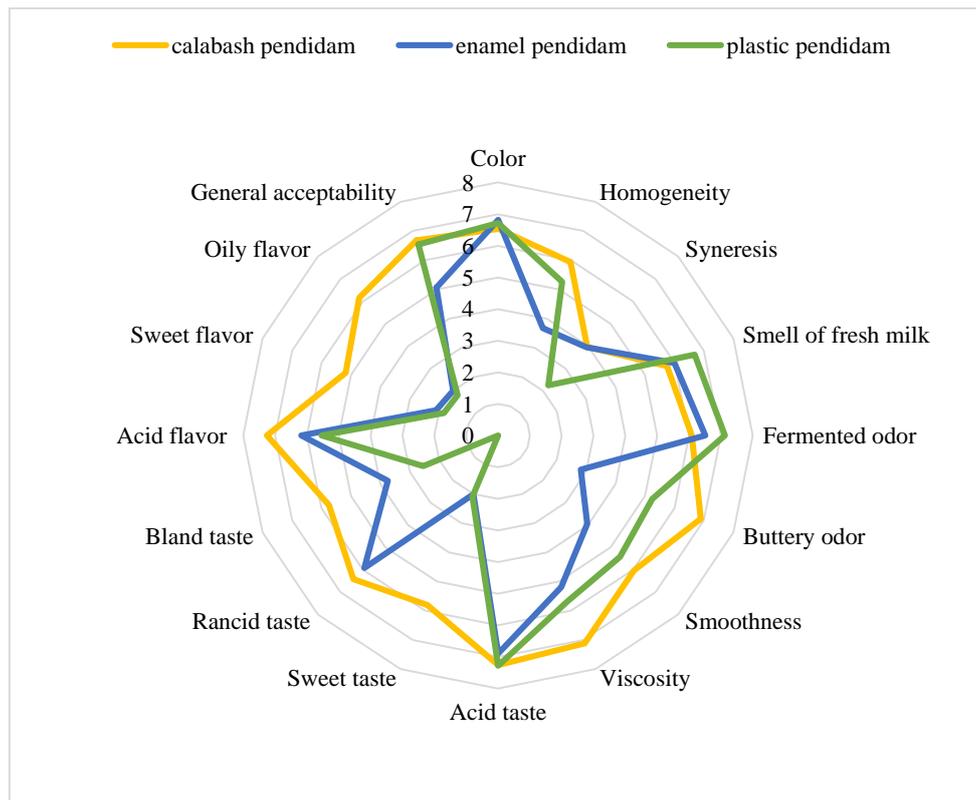


Fig. 2. Sensory attributes of *kindirmou* as affected by fermentation vessel



**Fig. 3. Sensory attributes of *pendidam* as affected by fermentation vessel**

from the calabash have the highest pH (3.85-4.68). Whatever the fermentation vessel considered, the average pH values vary from 3.20 to 3.85 for *pendidam* and from 4.11 to 4.68 for *kindirmou*. Average pH values significantly vary from containers to containers. However, there is no significant difference between the pH values of fermented milks in the enamel plate and those of fermented milks in the plastic buckets. These results could be justified by the fermentation vessel used, the variability of the production technologies and the ferments used which were often uncontrolled since the ferment most used for the production of these different milks is old milk. Indeed, the microorganisms contained in these milks have the role of hydrolyzing lactose into lactic acid, aromatic compounds, and enzymes; this is accompanied by a drop in the pH of the medium [22].

These pH values obtained are similar to those of traditional fermented milks reported in the literature, in particular *Amasi* (pH = 4.5) [23], *Nunu* (pH = 3.4) [19], *Omashikwa* (pH = 3.3) [4]. The results observed corroborate those of Mbawala et al. [24] who found *pendidam* pH values between 3.38 to 4.47 in the city of Ngaoundere (Cameroon).

The average acidity value regardless of the fermentation vessel vary between  $122.40 \pm 10.37$  °D (fermented milks in the calabash) and  $218.40 \pm 22.64$  °D (fermented milks in the enamel plate). Additionally, whatever the fermentation vessel considered, the average acidity values vary from  $128.7 \pm 1.8$  °D to  $151.5 \pm 15.38$  °D for *kindirmou* and  $122.4 \pm 10.37$  °D to  $218.4 \pm 22.64$  °D for *pendidam*. The *pendidam* samples are more acidic than those of *kindirmou*, this would be linked to the much longer fermentation time of *pendidam*, the drop in pH which favors the increase in titratable acidity and consequently, a coagulation of the casein of the milk [25]. These results are different from those obtained by Mbawala et al. [24] ( $93.31-129.7$  °D) for *pendidam* and that of Libouga et al. [3] who obtained values of 60 - 90 °D for *kindirmou*. This difference could be related to the difference in the site of sampling and in the fermentation vessel used.

The viscosity varies significantly from one fermented milk to another and from one fermentation vessel to another. It is between  $107.84 \pm 0.67$  cp,  $223.29 \pm 1.09$  cp for *kindirmou*,  $43.27 \pm 3.53$  cp, and  $115.41 \pm 2.28$  cp for *pendidam*. However, there was no significant

difference between the average viscosity value of the *pendidam* of the calabashes ( $115.41 \pm 2.28$  Cp) and those of the *kindirmou* of the Enamel plates and of the plastic buckets, which are respectively  $107.84 \pm 0.67$  Cp and  $109.73 \pm 3.10$  Cp. It appeared that the value of the viscosity of *pendidam* decreases from one fermentation vessel to another, so the average value depending on the fermentation vessels are  $115.41 \pm 2.28$  Cp;  $99.03 \pm 0.62$  Cp and  $43.27 \pm 3.53$  Cp respectively for calabash milks, plastic buckets and Enamel plates. The high viscosity measured in the samples of fermented milk in the calabash could be explained by its chemical composition, its adiabatic character reported during the investigation. This is because the proteins in the reaction medium can interact with other proteins or other components such as carbohydrates, fats, and minerals, which help to improve the texture of the final product, on the one hand. On the other hand, bacteria and yeast present in the media produce aromatic compounds, enzymes, and other compounds that affect the texture of dairy products [25,26], during the fermentation of milks, there is a change in the rheological properties going from a liquid to a viscoelastic gel characteristic of fermented milks. This increase in viscosity helps to improve the acceptability of milk by reducing the size of the suspended particles, increasing the charge density or the viscosity of the continuous phase. This implies that the length of fermentation affects the texture and the amount of lactic acid produced.

### 3.2.2 Chemical parameters

The results of chemical parameters of different samples are presented in Table 2. The protein content in various traditional fermented milks depends on the type of fermented milk and the fermentation vessel. It varies from  $0.89 \pm 0.07$  g/100 mL to  $1.47 \pm 0.04$  g/100 mL for *kindirmou* and from  $0.89 \pm 0.04$  g/100 mL and  $1.01 \pm 0.07$  g/100 mL for *pendidam*. This high value of proteins in the *kindirmou* compared to *pendidam* would be linked to their composition, because *kindirmou* represents whole milk while *pendidam* represents skimmed milk, the microbial load contained in each and the fermentation time. Depending on the fermentation vessel, *kindirmou* and *pendidam* produced in the calabash were higher than that of those produced in the other two containers. This difference can be associated with a possible migration of proteins from the inner wall of the calabash to the milk,

contributing to the increase the content in the medium.

The sugar contents in the various traditional fermented milks are quite low ( $0.28 \pm 0.08$  g / 100 mL to  $0.64 \pm 0.03$  g / 100 mL for *kindirmou* and  $0.13 \pm 0.03$  g /100 mL to  $0.48 \pm 0.05$  g /100 mL for *pendidam*) and provide information on the level of use of sugars by lactic acid bacteria for metabolic purposes. Depending on the type of milk, the fermented milk in the calabashes has a higher sugar content than in the other two containers. The secretion of hydrolytic enzymes by the microorganisms present in the medium during fermentation can explain this relatively high sugar content in traditional milk fermented in the calabash. These hydrolytic enzymes would degrade the macromolecules (cellulose, hemicellulose, and lignin) of the calabash into simple sugars for their metabolic needs, thus promoting an increase in the sugar content in the medium. It may also reflect the diffusion of carbohydrate particles from the calabash to the milk during fermentation. Indeed, fermentation contributes to reducing the soluble sugar content of the medium, whatever the type of milk and the fermentation vessel.

The contents of total phenolic compounds are  $93.41 \pm 3.04$  and  $111.20 \pm 2.01$  mg / 100 mL of milk, respectively, for *kindirmou* and *pendidam* fermented in a calabash. These compounds were absent in samples of milk fermented in a plastic buckets and enamel plates. Furthermore, these results show that the flavonoids are present only in the milk fermented in the calabash. Their presence in milk is the result of the interaction between the calabash and the milk. These exchanges occur through the phenomena of diffusion, permeation, and migration; because during fermentation, these characteristic plant compounds diffuse from the inner wall of the calabash into milk. The significant difference observed between the milks is due to the fermentation time, which is relatively long for the *pendidam*. These observations are similar to those reported on the use of the cucurbit for the formulation of milk drinks and yogurt in Algeria, where an increase in total phenolic compounds was observed in the products after processing [27,28]. Regarding carotenoids, they were present in all samples, although the contents were lower in milks fermented in plastic buckets and enamel plates than those fermented in calabashes.

**Table 1. Physicochemical parameters of fermented milks as affected by fermentation vessel**

Fermented milks	Fermentation vessel	pH	Titration acidity (°D)	Viscosity (Cp)
<i>Kindirmou</i>	Calabash (n=5)	4.68 ± 0.01 <sup>a</sup>	148.50 ± 4.50 <sup>a</sup>	223.29 ± 1.09 <sup>a</sup>
	Plastic bucket (n=5)	4.44 ± 0.02 <sup>b</sup>	151.50 ± 15.38 <sup>a</sup>	109.73 ± 3.10 <sup>b</sup>
	Enamel plate (n=5)	4.11 ± 0.05 <sup>c</sup>	128.70 ± 1.80 <sup>b</sup>	107.84 ± 0.67 <sup>b</sup>
<i>Pendidam</i>	Calabash (n=5)	3.85 ± 0.01 <sup>b</sup>	122.40 ± 10.37 <sup>c</sup>	115.41 ± 2.28 <sup>a</sup>
	Plastic bucket (n=5)	3.22 ± 0.01 <sup>a</sup>	174.01 ± 22.64 <sup>b</sup>	99.03 ± 0.62 <sup>b</sup>
	Enamel plate (n=5)	3.20 ± 0.02 <sup>a</sup>	218.40 ± 22.64 <sup>a</sup>	43.27 ± 3.53 <sup>c</sup>

Identical letters with superscript in the same column, and for the same type of traditional fermented milk, indicate that the values are not significantly different at the probability threshold ( $P = 0.05$ ).

**Table 2. Biochemical composition of *kindirmou* and *pendidam* as affected by fermentation vessel**

Fermented milks	Fermentation vessel	Fermentation vessel	Proteins (g / 100 mL)	Sugar (g / 100 mL)	Total phenolic compounds (mg / 100 mL)	Flavonoids (mg / 100 mL)	Carotenoids (mg / 100 mL)
<i>Kindirmou</i>	Calabash (n=5)	Calabash	1.47 ± 0.04 <sup>a</sup>	0.64 ± 0.03 <sup>a</sup>	93.41 ± 3.04	14.51 ± 3.71	0.21 ± 0.007 <sup>e</sup>
	Plastic bucket (n=5)	Plastic bucket	0.89 ± 0.07 <sup>c</sup>	0.08 ± 0.02 <sup>c</sup>	ND	ND	0.204 ± 0.01 <sup>b</sup>
	Enamel plate (n=5)	Enamel plate	1.01 ± 0.07 <sup>b</sup>	0.28 ± 0.08 <sup>b</sup>	ND	ND	0.15 ± 0.04 <sup>c</sup>
<i>Pendidam</i>	Calabash (n=5)	Calabash	0.92 ± 0.10 <sup>a</sup>	0.48 ± 0.05 <sup>a</sup>	111.20 ± 2.01	17.63 ± 2,74	0.106 ± 0.01 <sup>a</sup>
	Plastic bucket (n=5)	Plastic bucket	0.80 ± 0.07 <sup>b</sup>	0.32 ± 0.04 <sup>b</sup>	ND	ND	0.08 ± 0.01 <sup>b</sup>
	Enamel plate (n=5)	Enamel plate	0.89 ± 0.04 <sup>b</sup>	0.13 ± 0.03 <sup>c</sup>	ND	ND	0.09 ± 0.02 <sup>a</sup>

Identical letters with superscript in the same column, and for the same type of traditional fermented milk, indicate that the values are not significantly different at the probability threshold ( $P = 0.05$ ).

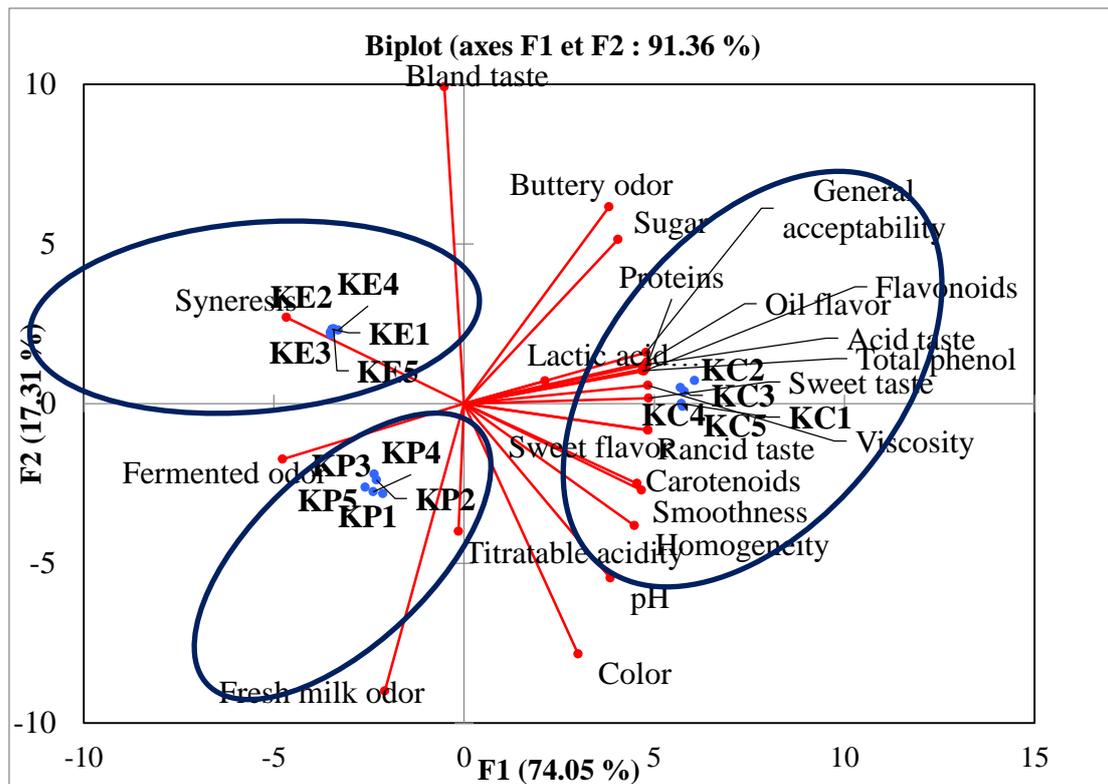


Fig. 4. Biplot describing the mapping of *kindirmou* and correlation between sensory attributes, chemical composition and fermentation vessel

### 3.3 Microbiological Characteristics of *kindirmou* and *pendidam* Samples

The results of microbiological analysis are presented in Table 3. The microbial load varies to the type of traditional fermented milk and with the type of fermentation vessel used. Generally, the total flora varies from  $8.17 \pm 0.15 \log_{10}$  to  $9.39 \pm 0.22 \log_{10}$  cfu / mL and  $6.95 \pm 0.26$  to  $8.11 \pm 0.32 \log_{10}$  cfu / mL respectively for *kindirmou* and *pendidam*. The high concentration of the total flora observed in the samples of fermented milk would be justified by the lack of good hygiene practices during the process, the fermentation vessels used, the very high concentration of ferment, contamination by wind, and flies which are deposited on the utensils during processing, thus constituting a source of contamination of the contamination samples. Millogo et al. [29] Report that containers used during milk processing can be a source of contamination of the finished product with the consequence of increasing the microbial load. All samples of *kindirmou* and *pendidam* analyzed have a considerable lactic flora although it is below the ANFOR standard, which recommends a lactic flora greater than or equal to  $8 \cdot \log_{10}$  cfu / mL. The initial microbial

load of the milk, the quality and concentration of the ferment used, the temperature, and the use of an inappropriate ferment could have explained this. Indeed, the pH of the milk is favorable to the development of lactic acid bacteria, but their number decreases as the fermented milk ages. The lactic flora produces metabolites such as lactic acid and bacteriocin with antibacterial activities. In addition, the use of bacteriocin in food prevents the development of pathogenic bacteria [30]. Yeast were mostly found in samples of *pendidam* fermented in the calabash and enamel plate ( $8.54 \pm 1.07 \log_{10}$  cfu / mL and  $6.02 \pm 0.60 \log_{10}$  cfu / mL respectively) while molds were mostly found in the *pendidam* fermented in the calabash ( $6.00 \pm 0.5 \log_{10}$  cfu / mL).

These results would be due to poor conditions of transport and storage of samples, incomplete pasteurization, and the type of container used. These results are not an isolated case because Coulibaly et al. [31] revealed their presence on yogurts sold in Côte d'Ivoire. According to Dieng [32] yeast and molds have an optimal pH between 4.5 and 6.5, which means that they can develop perfectly in fermented milks and cause

**Table 3. Microbial loads ( $\log_{10}$  cfu / mL) of *kindirmou* and *pendidam* as affected by fermentation vessel**

Fermented milks	Fermentation vessel	Total flora	Lactic bacteria	Yeast	Molds	Total coliforms	Fecal coliforms
<i>Kindirmou</i>	Calabash (n=5)	9.39 ± 0.22 <sup>a</sup>	9.26 ± 3.30 <sup>a</sup>	0	0	6.08 ± 0.10 <sup>b</sup>	0
	Plastic bucket (n=5)	8.17 ± 0.15 <sup>b</sup>	9.93 ± 1.23 <sup>a</sup>	0	0	7.69 ± 0.50 <sup>a</sup>	6.41 ± 0.12 <sup>a</sup>
	Enamel plate (n=5)	8.22 ± 0.23 <sup>b</sup>	8.80 ± 1.03 <sup>a</sup>	0	0	7.42 ± 0.51 <sup>a</sup>	6.41 ± 0.10 <sup>a</sup>
<i>Pendidam</i>	Calabash (n=5)	8.11 ± 0.32 <sup>a</sup>	8.97 ± 2.80 <sup>a</sup>	8.54 ± 1.07 <sup>a</sup>	6.00 ± 0.5	6.55 ± 0.50 <sup>b</sup>	0
	Plastic bucket (n=5)	8.02 ± 0.48 <sup>a</sup>	9.79 ± 1.75 <sup>a</sup>	0	0	6.79 ± 0.25 <sup>b</sup>	7.57 ± 0.80 <sup>a</sup>
	Enamel plate (n=5)	6.95 ± 0.26 <sup>b</sup>	8.22 ± 1.35 <sup>a</sup>	6.02 ± 0.60 <sup>b</sup>	0	7.39 ± 0.15 <sup>a</sup>	7.00 ± 0.10 <sup>b</sup>

Identical letters with superscript in the same column, and for the same type of traditional fermented milk, indicate that the values are not significantly different at the probability threshold ( $p < 0.05$ ).

various alterations in the product. The presence of yeast has been reported in several studies on the spontaneous fermentation of Kenyan milks [33,34]. Their main role may be flavor development due to their limited ability to assimilate carbohydrates [32]. The majority of yeast species found in most African fermented milks include *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* [35]. Total coliforms are present in the samples; however, their rate is more pronounced in milks fermented in plastic buckets and enamel plates, notwithstanding, this rate varies from  $6.08 \pm 0.10 \text{ Log}_{10} \text{ cfu / mL}$  to  $7.69 \pm 0.50 \text{ Log}_{10} \text{ cfu / mL}$ . These results may be associated with a hygienic failure: either during processing and handling of the product, these results corroborate those obtained by Ngassam [26] on curdled milk sold in Senegal where the majority of their samples possessed coliforms.

Fecal coliforms were absent in the samples of milk fermented in the calabash and were high in *pendidam* fermented in the plastic bucket. The presence of fecal coliforms in some samples could be explained by exogenous contamination of fecal origin. Their presence indicates poor hygiene during processing [36]. Poor hand hygiene, manufacturing environment, the addition of ferment after pasteurization, defective or contaminated equipment are likely sources of contamination [31].

### 3.4 Grouping of All Evaluated Parameters

The principal component analysis, taking into account the results of the sensory, physico-chemical, and microbiological analyzes of the various samples of *kindirmou* and *pendidam* according to the fermentation vessels,

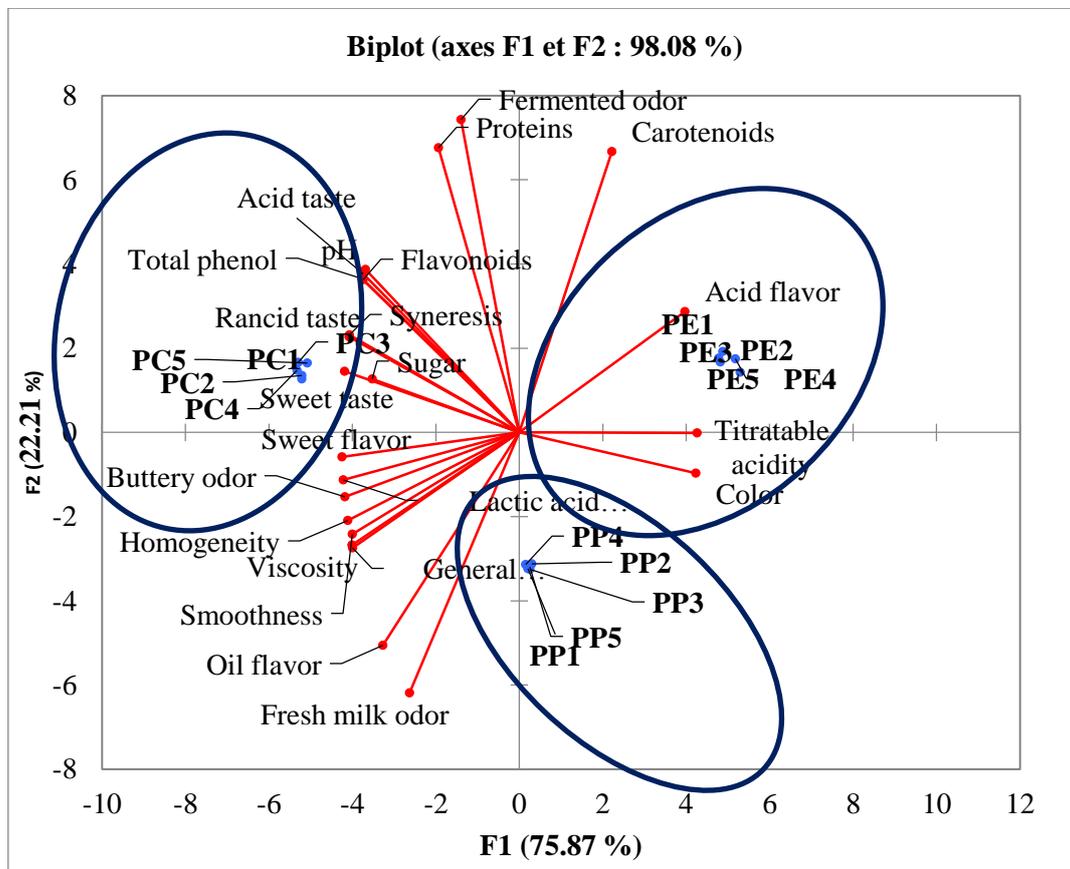


Fig. 5. Biplot describing the mapping of *pendidam* and correlation between sensory attributes, chemical composition and fermentation vessel

**Legend:**

- KC: *kindirmou* fermented in calabash
- KE: *kindirmou* fermented in enamel plate
- KP: *kindirmou* fermented in plastic bucket
- PC: *pendidam* fermented in calabash
- PE: *pendidam* fermented in enamel plate
- PP: *pendidam* fermented in plastic bucket

are presented in Figure 4 and 5. The main components F1x F2 explains 91.36% of the total variability of the parameters measured between the 15 samples of *kindirmou* fermented in the 3 vessels and 98.36% for the samples of *pendidam*. The main axes described these same data at 74.05% on the F1 axis and 17.31% on the F2 axis for the *kindirmou* samples, and 75.87% on the F1 axis and 22.21% on the F2 axis for *pendidam* samples. As well for the *kindirmou* as for the *pendidam*, positive correlations are noted between the F1 axis and the sensory parameters (color, smoothness, viscosity, homogeneity, sweet taste, buttery odor, oily flavor, sweet flavor, general acceptability), chemical parameters (total phenol content, carotenoids, flavonoids, soluble sugars content soluble protein content) and microbiological characteristics (lactic acid bacteria).

#### 4. CONCLUSION

At the end of this work, which the objective was to assess the influence of the fermentation vessel on the sensory, physicochemical, and microbiological qualities of *kindirmou* and *pendidam*, it emerges on the sensory level, the samples produced in the calabash have the best general acceptability. On the physicochemical level, milks fermented in calabashes have the highest content of total phenolic compounds, flavonoids, carotenoids, soluble sugars and soluble proteins. Finally, from a microbiological point of view, these milks are richer in yeast and lactic acid bacteria. Phenolic compounds are present only in milk fermented in the calabash compared to other containers. *kindirmou* and *pendidam* produced in the calabash exhibit better sensory and physicochemical characteristics and better phenolic compound content compared to those produced in plastic buckets and enamel plates.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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