Food Science and Quality Management
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol.29, 2014


# Physico-Chemical and Microbiological Analysis of Fermented Cow Milk (Nono) Consumed Within Kaduna Town, North-Western Nigeria

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**Abstract**
10 Samples of two different types of fermented cow milk tsala (locally prepared diluted milk) and kindrimo (locally prepared concentrated milk) were collected from different locations around Kaduna metropolis, and analyzed for their physicochemical properties and microbiological quality. The average levels of major chemical components were for: tsala: acidity (0.106%), protein (2.732%), fat (6.54%), total solid (7.68%), ash content (0.638%), and carbohydrate (82.80%); and for kindirmo: acidity (0.122%), protein (3.59%), fat (8.2%), total solid (10.06%), ash content (0.436%) and carbohydrate (77.70%). The average pH values obtained were (4.09) and (4.42) respectively. The bacteria isolated includes; staphylococcus spp, lactobacillus spp, streptococcus spp, shigella spp, enterobacter, salmonella spp, protein Spp and mirococcus Spp. The fungi isolated included Aspergillus, yest, trichoderma, mucor and cardida. The result of the microbial count revealed that the total aerobic count on tsala ranged between 3 x $10^3$ – 25 x $10^3$ cfu/ml, while for Kindirmo the value ranged between 3 x $10^3$ – 24 x $10^3$ cfu/ml with sample 2E recording too numerous aerobic bacteria count. The coliform count on tsala ranged between 4 x $10^3$ – 10 x $10^3$ while kindirmo recorded a ranged of 1 x $10^3$ – 25 x $10^3$ cfu/ml. The fungi count in tsala ranged between 22 x $10^3$ – 28 x $10^3$ cfu/ml while that of kindirmo ranged between 10 x $10^3$ – 22 x $10^3$ cfu/ml. The microbiological quality of the two fermented cow milk shows a high level of bacteriological contamination which may pose public health threat and indicates poor hygienic practices and therefore the need for improved hygienic standards.
**Keywords:** microbiological quality, Physico-chemical composition, tsala, kindirmo, Kaduna.

## 1.0 INTRODUCTION

Milk is a food of outstanding interest and has been taken by humans since the earliest pre-historic times and still forms the basis of most nation economics [1]. A number of animal are used to produce milk for consumption by humans, although, cow is by far the most important in commercial terms [2] with White Fulani (Bunaji) recognized as the principal producer [3]. Milk is designed by nature to be a complete food for young animals and of high nutritional values. The principal constituents of milk include fat, protein, total solid, lactose, ash. In addition to this, milk contains several hundred minor constituents many of which include milk fat, vitamins, metal ion and flavor compounds, which have a major impact on the nutritional, technological and sensory properties of milk and dairy products [4]. Milk is an excellent culture medium for many kinds of micro-organisms and has been reported by several researchers [5, 6, 7, 8, 9, 10, 11, and 12]. Fresh milk drawn from a healthy cow normally contains a low micro activity particularly with bacterial load of less than $10^3$ cfu/ml [6,12], but the load may increase up to 100 fold or more once it is stored for sometime at ambient temperature[12]. Some of the factors that increase the bacterial activity in raw milk and its products include health of the animal, cleanliness of the housing area, the nature of feed, the water used at farm, the milk vessels / utensils for storage and essentially the hygiene of the milker / handler [6, 10, and 14]. The presence of this pathogenic bacteria in raw milk and its products have been reported to be a major threat to human health especially those who still drink raw milk[9, 8] and also reduces the keeping quality of milk [14].

Nono is local uncontrolled fermented cow milk which forms a major part of the staple food in Northern Nigeria. They are produced mainly by the nomadic Fulani. The fresh milk is directly obtained from a cow into a properly washed semi-dried calabash and kept wide open in the sun for approximately two hours to facilitate isolation of the far layer. Some quantity of overnight fermented milk is added therefore, to serve as a starter culture and the inoculated fresh milk is left overnight at room temperature for fermentation to get sour milk known as “Kindirmo” and the addition of large volume of water to the curdle sour milk which is then stirred with a T-shaped stick to a liquid of fine consistency gives rise to “Nono”. The most commonly product often mixed with nono is called “Fura” (a dumping made of millet or maize) to made a preparation called fura da nono.

It is a common experience that in Northern part of Nigeria that direct consumption of locally processed raw milk in both cities and rural areas is much frequent and more popular than consumption of pasteurized milk because it is believed, especially in rural areas, that locally processed raw milk and its by-products have nutritional advantages over the pasteurized one. However, consumption of raw milk and its by-products is considered

 

potentially hazardous and has been associated with several types of infections including brucellosis, tuberculosis, salmonellosis, yersiniosis, Escherichia coli O157 and Staphylococcal entero toxin poisoning [15].
With the concern for quality of traditionally fermented dairy product like nono in northern Nigeria, it is imperative to ascertain a routine quality of this product consumed. Since it is locally processed and perhaps there is no microbial limit that has been established in this country for consumption of such kind of locally processed dairy product. The purpose of this study was to analyse the proximate nutritional value of kindirmo and tsala, asses the biochemical and microbial qualities of the collected fermented milk samples sold in Kaduna metropolis, so as to ascertain its general acceptability for consumption among the populace.

## 2.0 MATERIALS AND METHODS

### 2.1 Study Area

The study was conducted in Kaduna town, where the cow milk products are usually hawk by the Hausa/Fulani women in plastics and calabashes. Kaduna metropolis is the capital of Kaduna State. To the South-West, Kaduna shares a border with the Federal Capital Territory, Abuja. The global location of Kaduna is between longitude of 30'' east of the Greenwich meridian and also between latitude 0900 and 11 30'' North of the equator.

### 2.2 Sample collection

The fresh samples of kindirmo and tsala used for this study were obtained randomly around Kaduna State metropolis which includes: Unguwan rimi, Kano road, Ibrahim taiwo road, Sabon tasha, Tudun wada, Kawo, Hayin banki, kabalan doki, Marafa and Barnawa. Where most of the Hausa/Fulani women hawk the product in plastics and calabashes. The 10 samples were collected randomly at these different points and from different sellers into a sterile bottle and then taken immediately for analysis. The 10 samples comprises of 5 different samples of Kindirmo and 5 samples of tsala. The tsala was labeled as 1 (1A, 1B, 1C, 1D, 1E) while the Kindirmo was labeled as 2 (2A, 2B, 2C, 2D and 2E).

### 2.3 Physico-chemical analysis

The fermented milk samples of Kindirmo and Tsala were analyzed for pH, Acidity, protein, Extractable fats, and percentage dry weight, Total solid, ash and carbohydrate contents. Standard procedure was used according to [16].

### 2.4 Microbiological Analyses

Tenfold serial dilution of the fermented cow milk was prepared aseptically by dispensing 1ml of each of the milk samples into a test tube containing 9ml sterile distilled water and homogenized by shaking followed by further decimal dilutions to up to $10^{-6}$ concentrations. 1ml of dilutions was dispensed into appropriately labeled sterile Petri dishes with the appropriate media poured into each plate and gently swirled for proper mixture of the medium and sample. The plates were allowed to solidify before incubated at 37 $^{o}$C for 24 hours. The procedure was repeated at the $12^{th}$, $24^{th}$, $36^{th}$, and $48^{th}$ hours and microbial count was determined using direct plate count method as described by [17, 18, and 19]. Media employed for the isolation and enumeration of the organisms include: Nutrient Agar medium for Total Aerobic Plate Count, MacConkey Agar medium for
Coliform count and Saboraud Dextrose Agar for enumeration of moulds and yeasts. All calculations of colony forming units ($ML^{-1}$) were done using the formula:

$$\text{CFU/ML} = \frac{\text{Number of colony counted} \times \text{reciprocal of dilution}}{\text{Volume inoculated}}$$

### 2.5 Biochemical identification of the isolates

The representative bacteria colonies that developed on the culture plates were obtained. The various isolates were subjected to gram staining procedure, standard biochemical and physiological test were carried out. References were also made to stock cultures and different microbiology monographs in addition to colonial morphology in order to make proper identifications of the microbial isolate. The biochemical tests for the identification of the isolates were carried out using [20] procedures. Various tests include: Coagulate, catalase, indole, motility ureas, citrate utilization, methyl red, Voges Proskauer, Kligler Iron, Triple Sugar Iron tests.

## 3.0 RESULT AND DISCUSSION

### 3.1 Results

**Table 1: Results of the physicochemical analysis**

| Samples | 1A% | 1B% | 1C% | 1D% | 1E% | 2A% | 2B% | 2C% | 2D% | 2E% |
|---|---|---|---|---|---|---|---|---|---|---|
| Acidity | 0.13 | 0.09 | 0.08 | 0.10 | 0.13 | 0.15 | 0.09 | 0.14 | 0.12 | 0.11 |
| Protein | 2.93 | 1.95 | 1.56 | 1.95 | 3.32 | 3.12 | 1.95 | 2.54 | 7.02 | 3.32 |
| Fat | 13.50 | 2.70 | 4.60 | 6.60 | 5.30 | 6.50 | 3.00 | 11.20 | 11.90 | 8.40 |
| Total solid | 6.30 | 9.50 | 7.40 | 7.30 | 7.90 | 8.40 | 6.10 | 14.50 | 12.30 | 9.00 |
| Ash content | 0.19 | 0.60 | 0.20 | 1.80 | 0.40 | 0.20 | 0.19 | 1.20 | 0.19 | 0.40 |
| Carbohydrate | 77.08 | 85.25 | 86.24 | 82.35 | 83.08 | 81.78 | 88.76 | 70.56 | 68.51 | 78.88 |

1 = Tsala 2 = Kindirmo

 

**Table 2: pH value**

| Samples | 1A | 1B | 1C | 1D | 1E | 2A | 2B | 2C | 2D | 2E |
|---|---|---|---|---|---|---|---|---|---|---|
| pH | 4.11 | 3.59 | 4.19 | 4.39 | 4.22 | 4.22 | 4.47 | 4.58 | 4.28 | 4.54 |

**Table 3: Microbiological Count of fermented milk (kindirmo and tsala)**

| **Samples** | **Total aerobic count (cfu/ml)** | **Coliform count (cfu/ml)** | **Fungi count (cfu/ml)** |
|---|---|---|---|
| 1A | 25 x $10^3$ | 12 x $10^3$ | 28 x$10^3$ |
| 1B | 16 x $10^3$ | 10 x $10^3$ | 22 x $10^3$ |
| 1C | 23 x $10^3$ | 5 x $10^3$ | 31 x $10^3$ |
| 1D | 5 x $10^3$ | Too numerous to count | Too numerous to count |
| 1E | 3 x $10^3$ | 4 x $10^3$ | 22 x $10^3$ |
| 2A | 24 x $10^4$ | 13 x $10^3$ | 13 x $10^3$ |
| 2B | 24 x $10^3$ | 25 x $10^3$ | 21 x $10^3$ |
| 2C | 21 x $10^3$ | 1 x $10^3$ | 10 x $10^3$ |
| 2D | 3 x $10^3$ | 8 x $10^3$ | 18 x $10^3$ |
| 2E | Too numerous to count | Too numerous to count | 10 x $10^3$ |

**Table 4: Fungi identification table**

| **S/No** | **Species Morphology** | **Probable genera as observed under microscope** |
|---|---|---|
| 1. | Whitish, flat round growth on 1A, 1B, 1C, 1E, 2A, 2B, 2C, 2D and 2E | Mucor |
| 2. | Greenish growth on sample 1A, 1C, 1D, 1E, and 2A, 2B, 2C, 2D | Trichoderma |
| 3. | Black growth on all sample except sample 1B, 1A, 1C, 1D, 1E, 2B and 2D | Aspergillus |
| 4. | Numerous pinkish growth on sample 1A, 1D, 2A, 2D, and 2E | Cardida or phoma |

## 4.0 DISCUSSION

The physicochemical results of fermented milk product samples are shown in Table 1 and 2. The pH average of the milk samples tsala and kindirmo were 4.092 and 4.418 respectively. In other studies, various rates of pH readings were reported for both raw milk and fermented milk as between 6.44-6.99 by [21, 22, 23, 12, 24]. Milk pH gives an indication of milk hygiene and a milk pH should not be <6.6 or >6.8 when the milk temperature is at 20$^o$C [25]. The acidity obtained (Table 1) ranged between 0.08-0.13; average of 0.106 and 0.09-0.15; average of 0.122 respectively for tsala and kindirmo. Result of present study is in line with that of different researchers from other countries who have reported 0.13, 0.15, and 0.15 by [26, 22, 27] respectively. The mean protein content for tsala is (2.73%) and for kindirmo is (3.59%), these are similar to the values reported by [28, 12]. The percentage solid (% dry weight) ranges from 6.3-9.5 in tsala and 6.1-14.5 in kindrimo. The high percentage solid in kindirmo may arise from the presence of lumps and less water. The ash content ranges from 0.20 – 1.80 in tsala with sample 1D having the highest at 1.80. In kindrimo, sample 2A has the lowest, while the highest is in sample 2C; the highest percentage of ash recorded in tsala may arises from high water content, high percentage of fermentable bacteria and higher mineral content, similar result was recorded by [12]. The carbohydrate content ranges from 77.08 – 86.24 in tsala and 68-51 – 88.76 in kindirmo. All samples have a very high percentage of carbohydrate with similar close values; and thus can be use as an energy serving food. Kindirmo has the highest percentage of carbohydrate; this high percentage in carbohydrate may arise due to the low level of ash content or storage at ambient temperature may be attributed to utilization of available carbohydrate and other organic matters by spoilage micro-organisms leading to a decrease in proportion of available carbohydrate to inorganic ash content [29].

All 10 samples of Kindirmo and tsala analyzed in the study yielded numerous growths. In table 3, the total aerobic plate count ranges from 3 x $10^3$ CFU/ML to too numerous to count on the cultured plates of Nutrient Agar. More growth where seen in sample 1C, 1A, 2A, 2B, 2C and 2E respectively. Next to too numerous to count is sample 1A with 25 x $10^3$ CFU/ML of total aerobic plate count. Coliform count was analyzed using MacConkey Agar (MA). Sample 1D and 2E recorded too numerous to count, sample 2B recorded about 25 x $10^3$ CFU/ML. None of the samples indicated less than one (<1 x $10^3$ CFU/ML). Since the coliform counted exceeded

 

10 -20 for sample 1A, 1B, 1D, 2A, 2B, 2D, and 2E. The presence of coliform bacteria in the samples indicate a gross contamination except for sample 1C, 1E, and 2C; despite their low coliform count, non is less than 1, which mean they are also contaminated. The result obtained reveals high bacteria and coliform count in milk samples thus indicating significant low level hygiene maintained during the processing and sales of the products. This includes the handlers, quality of water used and utensils. During the sales of nono, dirty hands and bowls dipped into the calabash or plastic by the hawkers and the exposure of it while been displayed for sale in their containers can serve as sources of contamination. Due to the presence of coliform growth, which is a major contaminant, it simply means staphylococcus and fungi are present as well. The result of coliform count support reports previously obtained by [30, 31] but disagree with lower values obtained by [32, 33, 34]. The fungi count was analyzed using Saboraud Dextrose Agar (SDA). With reference to table 3, the fungi count values ranges from 10 x $10^3$ - 28 x $10^3$ CFU/ML. Tsala has the highest fungi count in sample 1A while the lowest was recorded in Kindirmo to be 10 x $10^3$ CFU/ML and too numerous to count in sample 1D (tsala). This result indicate that there are possible contaminations and can cause serious problems when consumed. The fungi species viewed under the microscope, indicated isolates of trichoderma, aspergillums, mucurs and cardida Spp. Trichoderma and Aspergillus species which were isolated are known spore formers, which therefore means that they can easily contaminate the diary product which are usually exposed during processing, storage and hawking. They are the major spoilage organisms of carbohydrate food. However, their growth can result in the production and accumulation of mycotoxins which are of public health and economic importance (Rhodes and Fletcher 1996).
The morphological, cultural and biological characteristics of microbial isolates (Table 4) revealed the following probable bacteria genera: entro bacter, salmonella, streptococcus, lactobacillus, staphylococcus among others. The detection of these bacteria indicates possible faecal contamination and being enteric bacteria, their presences indicate poor hygiene practice among handlers (Roseline, 2007). The lactobacillus Spp are recorded elsewhere as catalese negative and gram positive, the ferment sugar to yield lactic acid as the main product. They ferment sugar chiefly to lactic acid if they are homo fermentative, with small amount of lactic acid, carbondioxide and trace products; if they are hetero fermentative, they produce appreciable amount of volatile products, including alcohol, in addition to lactic acid (Smith and Berry 1975). The identification of fungal isolates (Table 5) include Aspergillus, yest, trichoderma, mucor and cardida, most of this isolate were reported to be present also in the studies conducted.

## 5.0 CONCLUSION

It is quite evident from this study that the samples of both kindirmo and tsala analyzed do not meet the requirement for total aerobic, hemophilic count standard; these organisms which have played a role in the fermentation of the samples and where found to be too many in most of the sample culture, where the fungal and aerobic plate count exceed 1 x 10 cfu/ml that is set for beverages under regulations, which mean that the method of preparation and handling is unhygienic. Due to this unhygienic method of preparation of nono it is expected that the level of contamination would be very high. However, the nono samples containing coliform, staphylococcus and fungi can constitute a lot of health hazard to consumers, this is because, their route of transmission and method of preparation have not been given much attention by the hawkers, consumers and authorities. There are no microbiological standard put in place for locally fermented nunu in Nigeria and even in most African countries where they are consumed. This is applicable to many other locally processed foods in Nigeria. Due to the fact that nunu is already-to-eat food which are consumed without further processing, great attention should therefore be given to the microbiological safety of these products because their direct consumption may cause health hazard to the consumers. Kindirimo and tsala where discovered to have good nutritional value, with kindirimo with the highest nutritional values. But the microbial analysis carried out proves with no daunt that the consumption of such unhygienic nunu may lead to a very significant health hazard to the consumers.

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