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# Microbial Safety Evaluation of *Nunu* (Fermented Raw Milk) Produced in Southern Nigeria Using Titratable Acidity and Alkaline Phosphatase Activity Test

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## Abstract

Raw cow milk is nutritious and prone to microbial contamination resulting from unhygienic processing. This study evaluate the microbial safety of *Nunu* (fermented raw milk) using titratable acidity and Alkaline Phosphatase activity. *Nunu* was obtained from five locations, namely, Owerri, Obinze, Elele, Asaba and Enugu were subject to microbiological and chemical test using standard methods. The mean total bacterial count from five locations, $9.65\log_{10}$cfu/ml, $9.69\log_{10}$cfu/ml, $9.72\log_{10}$cfu/ml, $9.69\log_{10}$cfu/ml, and $9.6\log_{10}$Ccfu/ml showed no significant difference ($p>0.05$) in the different locations. The mean values were significantly higher ($p<0.05$) than the recommended level of $6.03\log_{10}$cfu/ml by East Africa Community Standards. The isolated bacteria belonging to the genus *Staphylococcus*, *Salmonella*, *Bacillus*, *Klebsiella*, *Campylobacter*, *Enterobacter*, *Enterococcus*, *Escherichia coli*, *Micrococcus* and *Shigella* are major contaminants of soil and water. The biochemical test used to determine the milk quality revealed high microbial contamination of the *Nunu*. The Titratable acidity and Alkaline phosphatase activity recorded was high and significant ($p<0.05$) than the Thai Agricultural Standard. This study concluded that the quality of *Nunu* produced and marketed in the five locations across the southern Nigeria falls short of acceptable limits and could pose health risk to numerous consumers of the product. It is therefore recommended that Good Manufacturing Practices (GMP) should be introduced by the Government to checkmate adulteration and sharp practices indulged in by produces.

**Keywords:** Titratable acidity, Alkaline Phosphatase activity, Bacterial load, *Nunu*

## Introduction

Milk is an important source of nutrients for humans. *Nunu* is one of the indigenous milk products in West Africa countries including Nigeria. It is a product of fermented cow milk. Milk has a complex biochemical composition and its high water activity and nutritional value serves as an excellent medium for growth and multiplication of many kinds of microorganisms when suitable conditions are maintained (Parekh and Subhash, 2008).

Milk is virtually sterile when secreted in the udder of a healthy cow; the milk may be contaminated with low level of bacteria (Karimuribo *et al*., 2005; Makerere University, 2011). Milk from a cow with mastitis (infection of the mammary gland) may harbour large numbers of the infectious bacteria (Kivaria *et al*., 2006). However, milk can be contaminated from the exterior of the cow (dirty cows), the environment and/or poorly cleaned equipment (Kivaria *et al*., 2006).

These microorganisms are indicators of the manner of handling milk from milking till consumption and the quality of the milk (Tassew and Seifu, 2010). Raw milk is an important vehicle for the transmission of milk-borne pathogens to humans, as it can be easily contaminated during milking and handling (Addo *et al*., 2011). *Nunu* is being highly consumed among the Fulani community in Nigeria. Because of the inadequate handling and processing, there is need to assess the safety of *Nunu* produced and sold by Fulani women in southern part of Nigeria. For this purpose, this study focuses on the microbial quality assessment of *Nunu* produced in southern Nigeria.

## Materials and Methods

### Description of Study Area

This research was carried out at Obinze and Ama-Hausa in Owerri, Imo State; Army Barracks mammy market Elele, Rivers state; mammy market and cattle market at Oko, Asaba, Delta State and trailer motor park along Enugu-Umuahia express way, Enugu State all in Nigeria. The study areas are dominated by small scale traders, livestock dealers and significant number of Fulani settlers.

### Sample Size Determination

A formula by Kothari (2004) for finite population was used to calculate the sample size for this study;

i.e. $n = \frac{Z^2.p.q.N}{e(N-1)+Z^2.p.q}$

Where Z= 1.96 (desired confidence level at 95% and value obtained from table)

P= 0.5 (Sample proportion according to Kothari, 2004, in which case 'N' will be the maximum and the sample will yield the desired precision).

q= 1-p

e= 0.07 (precision rate or acceptable error)

N= 30

$$n = \frac{1.96^2 x 0.5 x 0.5 x 30}{0.07(30-1) + 1.96^2 x 0.5 x 0.5}$$

= 9.635; approximately 10samples.

Based on the above formula, 10 samples were collected from each district.

### Sample Collection

A total of 50 samples comprising of 10 samples from each location were collected and analysed. The samples were collected between 9.00-11.00am and transported in an iced flask to the laboratory for immediate analysis.

### Microbial Analysis

Tenfolds serial dilution of the fermented cow milk was prepared aseptically by dispensing in sterile distilled water and homogenized by shaking followed by further decimal dilutions to up to $10^{-6}$ concentrations. Aliquot portion was inoculated onto freshly prepared and surface dried media, and incubated appropriately.Microbial count was determined using direct plate count method as described by (Cheesbrough, 2003; Sharma, 2005).

### Identification of isolates

The representative bacteria colonies that developed on the culture plates were characterized and identified (Benson, 2002; Sharma, 2005; Buchanan and Gibbon, 2004; Sneath *et al.*, 1986).

### Acidity test

Nine millilitres of the sample was mixed with 1ml of phenolphthalein in a test tube and then slow addition of 0.1N sodium hydroxide with a burette(NCE-MSTL, 2015).

### Determination of Alkaline Phosphatase activity

This was done using the procedures stated by University of Vermont (www.uvm.edu/-bio1and2/lab/%20manuals%20Fall%20201...).

Nine millilitres of the sample was mixed with 1ml of phenolphthalein in a dish/flask/test tube and then slow addition of 0.1N sodium hydroxide with a burette. The end point was pink colour appearance. (NCE-MSTL, 2015).

### Determination of Alkaline Phosphatase activity

This was done using the procedures stated by University of Vermont (www.uvm.edu/-bio1and2/lab/%20manuals%20Fall%20201...).

## Results

### Microbiological Result of *Nunu* samples

Fig.1 shows the mean total bacterial count ($log_{10}$ CFU/ml) of 10 samples each of five different locations respectively. The maximum value recorded was $log_{10}$ 9.689 from Elele and the minimum value recorded was $Log_{10}$9.614 from Enugu Express way.

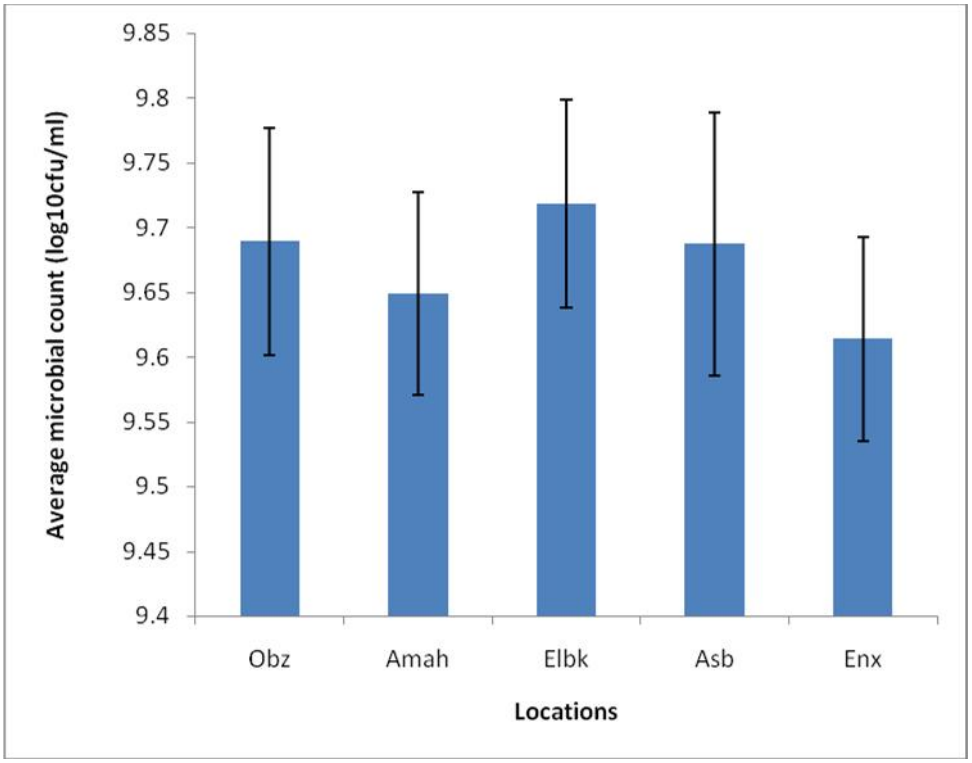


**Fig 1: Average of total Bacterial count of 10 samples each of five different locations**

Tables 1 shows colonial and microscopic characteristics of bacteria isolated from *Nunu*. Results of the biochemical and carbohydrate fermentation test of bacteria isolated from *Nunu* is shown in Table 2. The bacteria identified are *Micrococcus, Staphylococcus, Enterococcus, Escherichia coli, Bacillus, Campylobacter, Shigella, Salmonella, Klebsiella* and *Enterobacter* species. The distribution of bacterial isolates is shown in Table 3. All sample locations showed representative bacterial isolates.

**Table 1: colonial and Microscopic characteristics of Bacteria isolated from *Nunu* samples**

| Colonial morphology | Gram morphology | Motility | Sporulation | Capsule formation | Identity of isolates |
|---|---|---|---|---|---|
| Small circular moist and shiny low convex bright yellow colonies on Nutrient agar | Gram positive cocci predominantly in tetrads, few in clusters | Non motile | - | - | *Micrococcus* sp |
| Circular moist and shiny golden yellow colonies on Nutrient agar and Mannitol Salt agar | Gram positive cocci predominantly in clusters, few in pairs and tetrads | Non motile | - | - | *Staphylococcus* sp |
| Small smooth moist and shiny low convex cream colonies on Nutrient agar | Gram positive cocci in chains | Non motile | - | - | *Enterococcus* sp |
| Small circular moist and shiny low convex orange colonies on nutrient agar | Gram positive cocci predominantly in tetrads, few in clusters | Non motile | - | - | *Micrococcus* sp |
| Dull and dry serrated flat cream colonies on Nutrient agar | Gram positive central spores in short chains | Motile | + | - | *Bacillus* sp |
| Small smooth golden yellow colonies on Campylobacter Blood Free agar | Gram negative slender rods in short chains | Non motile | - | - | *Campylobacter* sp |
| Moist and shiny mucoid creamy white colonies on Campylobacter Blood Free agar | Gram negative slender rods in short chains | Non motile | - | - | *Campylobacter* sp |
| Small smooth light pink colonies on Salmonella Shigella agar | Short gram negative rods in chains | Non motile | - | - | *Shigella* sp |
| Shiny black fish eye colonies on Salmonella Shigella agar | Short slender gram negative rods predominantly in singles | Motile | - | - | *Salmonella* sp |
| Mucoid and shiny pink colonies on Eosin Methylene Blue agar | Gram negative rods in short chains and singles | Non motile | - | - | *Enterobacter*sp |
| Shiny greenish metallic sheen on Eosin methylene blue agar | Gram negative rods predominantly in singles | Motile | - | - | *Escherichia coli* |
| Mucoid and slimy pink colonies on Eosin methylene blues agar | Large Gram negative rods in chains | Motile | - | + | *Klebsiella*sp |
| Large raised slimy cream colonies on nutrient agar | Large gram positive rods in short chains | Motile | + | - | *Bacillus* sp |

**Table2: Biochemical and Carbohydrate Fermentation Test of Bacteria isolated from *Nunu* samples**

| CAT | OXI | COAG | IN | MR | VP | Cit | URS | $NO_3$ | GLU | SUC | LAC | MAL | XYL | Identity of isolates |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + | - | - | - | + | - | + | + | + | + | - | - | + | + | *Salmonella* sp |
| + | - | - | - | - | + | + | + | + | + | + | + | + | + | *Klebsiella* sp |
| + | - | - | - | + | - | + | - | - | + | - | + | - | - | *Enterobacter* sp |
| + | - | - | - | - | + | - | + | + | + | + | + | + | - | *Staphylococcus aureus* |
| + | - | - | - | - | + | + | - | + | + | - | - | - | - | *Bacillus cereus* |
| - | - | - | - | + | - | + | - | - | + | + | + | - | - | *Enterococcus faecalis* |
| + | - | - | + | - | + | - | - | + | + | + | + | + | + | *Escherichia coli* |
| + | - | - | - | - | + | + | - | + | + | - | - | + | - | *Bacillus subtilis* |
| + | - | - | - | - | + | - | - | + | + | - | - | - | + | *Campylobacter* sp |
| + | - | - | - | - | + | + | - | + | - | - | - | - | - | *Micrococcus luteus* |
| + | - | - | - | - | + | + | - | + | + | + | - | - | - | *Micrococcus roseus* |
| - | - | - | - | + | - | + | - | + | + | + | - | + | - | *Shigella* sp |

CAT, catalase; OXI, oxidase; COAG, coagulase; IN, indole; MR, methyl red; VP, VogesProskaeur, CIT, citrate, URS, urease production; $NO_3^-$; nitrate reduction; GLU, glucose; SUC, sucrose; LAC, lactose; MAL, maltose; XYL, xylose

**Table 3: Distribution of Bacteria in the samples**

| Samples | Bacterial isolates |
|---|---|
| Obz 1 | *Staphylococcus* sp, *Enterococcus* sp, *Bacillus* sp, *Campylobacter* sp, *Enterobacter* sp |
| Obz 2 | *Staphylococcus* sp, *Enterococcus* sp, *Bacillus* sp, *Campylobacter* sp, *Escherichia coli* |
| Obz 3 | *Campylobacter* sp, *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp, *Salmonella* sp, *Escherichia coli* |
| Obz 4 | *Campylobacter* sp, *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp, *Salmonella* sp, *Escherichia coli*, *Enterococcus* sp |
| Obz 5 | *Staphylococcus* sp, *Enterococcus* sp, *Bacillus* sp, *Enterobacter* sp, *Escherichia coli*, *Campylobacter* sp, *Micrococcus* sp |
| Obz 6 | *Staphylococcus* sp, *Bacillus* sp; *Escherichia coli*, *Shigella* sp |
| Obz 7 | *Staphylococcus* sp , *Bacillus* sp, *Campylobacter* sp, *Micrococcus* sp |
| Obz 8 | *Staphylococcus* sp, *Enterococcus* sp, *Bacillus* sp, *Campylobacter* sp |
| Obz9 | *Bacillus* sp, *Staphylococcus* sp; *Micrococcus* sp, *Salmonella* sp, *Campylobacter* sp, *Escherichia coli*, *Klebsiella* sp |
| Obz 10 | *Klebsiella*sp, *Staphylococcus* sp, *Enterococcus* sp, *Bacillus* sp, *Salmonella* sp, *Shigella* sp |
| Amah 1 | *Staphylococcus* sp, *Salmonella* sp, *Bacillus* sp |
| Amah 2 | *Staphylococcus* sp, *Escherichia coli*, *Enterobacter*sp, *Bacillus* sp |
| Amah 3 | *Staphylococcus* sp, *Campylobacter* sp, *Bacillus* sp, *Micrococcus* sp, *Enterococcus* sp |
| Amah 4 | *Staphylococcus* sp,, *Enterobacter*sp, *Escherichia coli*, *Enterococcus* sp, *Micrococcus* sp, *Bacillus* sp |
| Amah 5 | *Staphylococcus* sp, *Bacillus* sp, *Micrococcus* sp, *Enterococcus* sp |

| | |
|---|---|
| Amah 6 | *Staphylococcus* sp, *Bacillus* sp, *Campylobacter* sp, *Bacillus* sp, *Micrococcus* sp, *Salmonella* sp |
| Amah 7 | *Enterococcus* sp, *Bacillus* sp, *Staphylococcus* sp |
| Amah 8 | *Enterococcus* sp, *Campylobacter* sp, *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp |
| Amah 9 | *Escherichia coli*, *Staphylococcus* sp, *Micrococcus* sp, *Bacillus* sp, *Enterococcus* sp |
| Amah 10 | *Campylobacter* sp, *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp, *Escherichia coli* |
| Elbk 1 | *Campylobacter* sp, *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp, *Escherichia coli* |
| Elbk2 | *Campylobacter* sp, *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp,*Salmonella* sp |
| Elbk 3 | *Campylobacter* sp, *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp, Salmonella sp |
| Elbk 4 | *Campylobacter* sp, *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp, *Salmonella* sp, *Escherichia coli* |
| Elbk 5 | *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp, *Escherichia coli* |
| Elbk 6 | *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp, *Enterococcus* sp |
| Elbk 7 | *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp |
| Elbk 8 | *Campylobacter* sp, *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp |
| Elbk9 | *Campylobacter* sp, *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp, *Salmonella* sp |

## Titratable Acidity

Table 4shows the titratable acidity of the milk samples (*Nunu*) from different locations. Titratable acidity is a test to determine the spoilage of milk samples. It quantifies the amount of lactic acid produced by lactic acid bacteria in the samples. The sample from Enugu had the highest mean acidity with 0.786±0.09 and samples from Asaba had the lowest mean acidity with 0.672±0.06.

**Table 4: Titratable Acidity of *Nunu* samples from five Different Samples**

| SAMPLE CODE | %Lactic Acid 1 | %Lactic Acid 2 | AV. % Lactic Acid |
|---|---|---|---|
| CONTROL | 0.70 | 0.71 | 0.71±0.005 |
| Obz1 | 1.04 | 1.03 | 1.04±0.005 |
| Obz2 | 0.98 | 0.98 | 0.98±0 |
| Obz3 | 1.00 | 1.00 | 1.00±0 |
| Obz4 | 0.55 | 0.56 | 0.56±0.0.005 |
| Obz 5 | 0.51 | 0.51 | 0.51±0 |
| Obz6 | 0.51 | 0.52 | 0.52±0.005 |
| Obz7 | 0.73 | 0.71 | 0.72±0.01 |
| Obz8 | 0.99 | 0.97 | 0.98±0.01 |
| Obz9 | 0.70 | 0.64 | 0.67±0.03 |
| Obz10 | 0.53 | 0.53 | 0.53±0 |
| Amah1 | 0.99 | 0.97 | 0.98±0.01 |
| Amah2 | 0.93 | 0.91 | 0.92±0.01 |
| Amah3 | 0.85 | 0.83 | 0.84±0.01 |
| Amah4 | 0.57 | 0.55 | 0.56±0.01 |
| Amah5 | 0.45 | 0.43 | 0.44±0.01 |
| Amah6 | 0.67 | 0.67 | 0.67±0 |
| Amah7 | 0.90 | 0.88 | 0.89±0.01 |
| Amah8 | 0.91 | 0.93 | 0.92±0.01 |
| Amah9 | 0.68 | 0.66 | 0.67±0.01 |
| Amah10 | 0.59 | 0.55 | 0.57±0.02 |
| Elbk1 | 0.68 | 0.66 | 0.67±0.01 |

| | | | |
|---|---|---|---|
| Elbk2 | 0.69 | 0.67 | 0.68±0.01 |
| Elbk3 | 0.79 | 0.77 | 0.78±0.01 |
| Elbk4 | 1.10 | 1.12 | 1.11±0.01 |
| Elbk5 | 0.93 | 0.91 | 0.92±0.01 |
| Elbk6 | 0.98 | 0.98 | 0.98±0 |
| Elbk7 | 0.90 | 0.88 | 0.89±0.01 |
| Elbk8 | 0.82 | 0.78 | 0.80±0.02 |
| Elbk9 | 0.68 | 0.66 | 0.67±0.01 |
| Elbk10 | 0.49 | 0.47 | 0.48±0.01 |
| Asb1 | 0.67 | 0.69 | 0.68±0.01 |
| Asb2 | 0.58 | 0.62 | 0.60±0.02 |
| Asb3 | 1.20 | 1.18 | 1.19±0.01 |
| Asb4 | 0.66 | 0.66 | 0.66±0 |
| Asb5 | 0.59 | 0.55 | 0.57±0.02 |
| Asb6 | 0.66 | 0.70 | 0.68±0.02 |
| Asb7 | 0.69 | 0.51 | 0.60±0.09 |
| Asb8 | 0.78 | 0.76 | 0.77±0.01 |
| Asb9 | 0.44 | 0.44 | 0.44±0 |
| Asb10 | 0.55 | 0.51 | 0.53±0.02 |
| Enx1 | 0.33 | 0.31 | 0.32±0.01 |
| Enx2 | 0.66 | 0.60 | 0.63±0.03 |
| Enx3 | 0.49 | 0.47 | 0.48±0.01 |
| Enx4 | 0.64 | 0.56 | 0.60±0.04 |
| Enx5 | 0.84 | 0.94 | 0.89±0.05 |
| Enx6 | 0.95 | 0.93 | 0.94±0.01 |
| Enx7 | 1.20 | 1.24 | 1.22±0.02 |
| Enx8 | 0.88 | 0.86 | 0.87±0.01 |
| Enx9 | 0.78 | 0.80 | 0.79±0.01 |
| Enx10 | 1.10 | 1.14 | 1.12±0.02 |

**Key: Elbk**=Elele Army Barracks mammy market; **Amah**= Amah Hausa market; **Obz**=Obinze Army Barracks mammy market; **Enx**, Enugu Express way mammy market; **Asb**, Asaba mammy market;

**Alkaline phosphatase activity**

Table 5 shows the alkaline phosphate activity in milk (*Nunu*) sample from different locations. Detection of enzyme indicates microbial contamination for unpasteurized milk and inadequate pasteurisation for pasteurised milk, the Fig.2 shows the standard calibration curve of p-nitrophenol plotted with absorbance at 410nm against concentration of the p-nitrophenol. The $R^2$ value indicates the closeness of the points to the regression line. The $R^2$ is between 0 to 1, and is regarded as perfect graph. The equation of the graph was y=1.123x where 'y' is the absorbance and x is the concentration of p-nitrophenol of the samples.

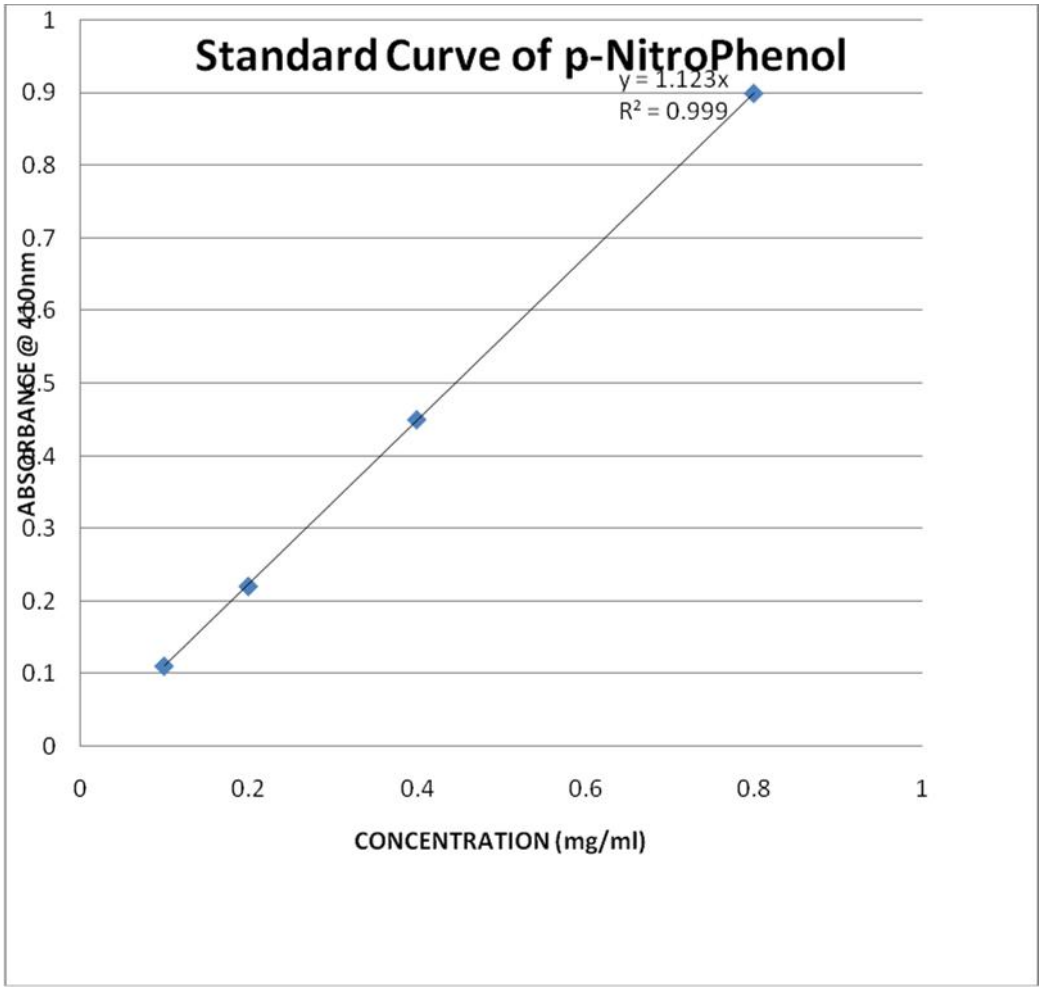


**Fig.2: Standard Curve of p-NitroPhenol**

**Table 5: Alkaline Phosphatase activity of *Nunu* samples from five different locations.**

| SAMPLE CODE | ABSORBANCE @ 410nm | | | CONCENTRATION (mg/ml) |
|---|---|---|---|---|
| | 1 | 2 | AVERAGE | |
| CONTROL | 1.257 | 1.259 | 1.258 | 1.120±0.001 |
| Obz1 | 0.208 | 0.188 | 0.198 | 0.176±0.025 |
| Obz2 | 0.569 | 0.536 | 0.552 | 0.276±0.025 |
| Obz3 | 0.464 | 0.414 | 0.439 | 0.391±0.025 |
| Obz4 | 0.396 | 0.335 | 0.366 | 0.326±0.025 |
| Obz5 | 0.322 | 0.288 | 0.305 | 0.272±0.025 |
| Obz6 | 0.491 | 0.506 | 0.496 | 0.441±0.025 |
| Obz7 | 0.384 | 0.360 | 0.372 | 0.331±0.025 |
| Obz8 | 0.407 | 0.401 | 0.404 | 0.360±0.025 |
| Obz9 | 0.475 | 0.469 | 0.472 | 0.420±0.025 |
| Obz10 | 0.335 | 0.315 | 0.325 | 0.289±0.025 |
| Amah1 | 0.306 | 0.298 | 0.302 | 0.269±0.021 |
| Amah 2 | 0.384 | 0.378 | 0.381 | 0.340±0.021 |
| Amah3 | 0.471 | 0.453 | 0.462 | 0.411±0.021 |
| Amah4 | 0.399 | 0.379 | 0.389 | 0.346±0.021 |

| | | | | |
|---|---|---|---|---|
| Amah5 | 0.528 | 0.520 | 0.524 | 0.467±0.021 |
| Amah6 | 0.377 | 0.365 | 0.371 | 0.330±0.021 |
| Amah7 | 0.300 | 0.296 | 0.298 | 0.267±0.021 |
| Amah8 | 0.382 | 0.372 | 0.377 | 0.336±0.021 |
| Amah9 | 0.338 | 0.314 | 0.326 | 0.290±0.021 |
| Amah10 | 0.288 | 0.284 | 0.286 | 0.255±0.021 |
| Elbk1 | 0.522 | 0.526 | 0.524 | 0.467±0.020 |
| Elbk2 | 0.414 | 0.410 | 0.412 | 0.367±0.020 |
| Elbk3 | 0.412 | 0.386 | 0.399 | 0.355±0.020 |
| Elbk4 | 0.373 | 0.379 | 0.376 | 0.335±0.020 |
| Elbk5 | 0.538 | 0.510 | 0.524 | 0.467±0.020 |
| Elbk6 | 0.558 | 0.514 | 0.536 | 0.477±0.020 |
| Elbk7 | 0.434 | 0.440 | 0.437 | 0.389±0.020 |
| Elbk8 | 0.339 | 0.311 | 0.485 | 0.289±0.020 |
| Elbk9 | 0.549 | 0.421 | 0.485 | 0.432±0.020 |
| Elbk10 | 0.476 | 0.444 | 0.460 | 0.410±0.020 |
| Abs1 | 0.342 | 0.344 | 0.343 | 0.305±0.037 |
| Abs2 | 0.263 | 0.261 | 0.262 | 0.233±0.037 |
| Abs3 | 0.474 | 0.470 | 0.472 | 0.420±0.037 |
| Abs4 | 0.483 | 0.483 | 0.483 | 0.430±0.037 |
| Abs5 | 0.244 | 0.250 | 0.247 | 0.220±0.037 |
| Abs6 | 0.160 | 0.168 | 0.164 | 0.146±0.037 |
| Abs7 | 0.376 | 0.374 | 0.375 | 0.334±0.037 |
| Abs8 | 0.492 | 0.496 | 0.494 | 0.440±0.037 |
| Abs9 | 0.256 | 0.260 | 0.258 | 0.230±0.037 |
| Abs10 | 0.134 | 0.142 | 0.138 | 0.123±0.037 |
| Enx 1 | 0.321 | 0.329 | 0.325 | 0.289±0.025 |
| Enx2 | 0.246 | 0.248 | 0.247 | 0.220±0.025 |
| Enx3 | 0.378 | 0.372 | 0.375 | 0.334±0.025 |
| Enx4 | 0.496 | 0.492 | 0.494 | 0.440±0.025 |
| Enx5 | 0.454 | 0.466 | 0.460 | 0.410±0.025 |
| Enx6 | 0.384 | 0.390 | 0.387 | 0.345±0.025 |
| Enx7 | 0.259 | 0.257 | 0.258 | 0.230±0.025 |
| Enx8 | 0.370 | 0.378 | 0.374 | 0.333±0.025 |
| Enx9 | 0.474 | 0.470 | 0.472 | 0.420±0.025 |
| Enx10 | 0.462 | 4.580 | 0.460 | 0.410±0.025 |

**Key:Elbk**=Elele Army Barracks mammy market; **Amah**= Amah Hausa market; **Obz**=Obinze Army Barracks mammy market; **Enx**, Enugu Express way mammy market; **Asb**, Asaba mammy market;

## Discussion

### Microbiological quality of *Nunu* samples

Total bacterial count was used as important indicator of the microbial quality of the milk sample (*Nunu*). A total of 50 samples from five locations (10 from each location) were cultured for total bacterial counts on different bacteriological media. The mean total bacterial count from the five locations is recorded as follows; $\log_{10}$ 9.7 CFU/ml for Obinze Army Barracks mammy market,$\log_{10}$ 9.94 CFU/ml for OwerriAmah-Hausa market,$\log_{10}$ 9.64 CFU/ml for Elele Army Barracks mammy market,$\log_{10}$ 9.04 CFU/ml for Asaba mammy market; and $\log_{10}$ 9.46 CFU/ml for Enugu Express way mammy market. The values obtained from the different locations does not show any significant difference ($p>0.05$).

The results obtained showed that all of the milk samples have higher TBC (total bacterial count) than the maximum recommended level of $\log_{10}$ 6.30 CFU/ml (2.0 x $10^6$ CFU/ml) as the standard set by East Africa Community Standards (EAS 67: 2007), and $\log_{10}$ 5.70CFU/ml given by Thai Agricultural Standard (TAS 6003—2010). The mean TBC obtained was higher than that reported by Briade *et al.* (2015) ($\log_{10}$ 9.64 CFU/ml) and Worku *et al.* (2012) ($\log_{10}$7.22 CFU/ml). These high counts suggest that the product (*Nunu*) is not safe for human consumption. The implication of these results is that *Nunu* from these locations is of poor microbial quality. Presence of high total bacterial load in raw milk indicate contamination possibly from lactating cows, milking equipment, storage containers, unsatisfactory hygiene/sanitation practiced at farm level, unsuitable storage condition, unclean udder/ or teats, poor quality of water used for cleaning and poor personal hygiene of the milkers (Bukuku, 2013; Kenyaka, 2014).

The morphological, cultural and biological characteristics of microbial isolates revealed the following bacteria genera; *Micrococcus, Enterobacter, Staphylococcus, Enterococcus, Escherichia coli, Bacillus, Campylobacter, Shigella, Salmonella* and *Klebsiella*. The detection of these bacteria strongly suggest gross contamination from the environment, milking machine, diseased animal and poor hygiene practice among others (Donkor *et al*., 2007). The detection of these organisms in milk sample was also reported by Egwaikhide *et al*. (2014) in Nigeria. These organisms have been reported to be responsible for the spoilage of milk by different researchers (Donkor *et al.*, 2007; Tamime, 2009 and Bukuku, 2013).

However, the higher microbial counts recorded in this study, especially, TBC at the different locations compared to EACS (2010) and TAS (2007) standards could be attributed to the cumulative results of milk contamination at different levels as reported by Karimuribo *et al*. (2005) and Makerere University, (2011). They incriminated insufficient pre-milking udder preparation, insufficient cleaning of milkers' hands, and milking utensils, use of poor quality and inadequate sterile water for cleaning of milk equipment and storage containers as the predisposing factors for gross contamination.

## Biochemical Quality of Milk (*Nunu*) Samples

### Titratable Acidity

The high percentage of acid present in milk samples at any time indicates the age of the milk and possible contamination of the milk (Wanjala *et al*., 2017). The mean acidity was recorded as 0.751±0.07, 0.746±0.06, 0.798±0.06, 0.672±0.06 and 0.786±0.09 from Obinze, Owerri, Elele, Asaba and Enugu respectively. There was no significant difference ($P>0.05$) between the mean acidity of the samples from different locations. However, there was significant difference ($p<0.05$) between the mean acidity of samples from different locations and the declared standard value of ≤0.16% by Thai Agricultural Standard (TAS 6003-2010). Wanjala *et al*. (2017) suggested that high acidity may indicate high microbial and enzyme activity in the samples, which may results from lack of adherence to the cold chain in the distribution channels and the long duration taken from the source to market. This however, confirms the high total bacterial count obtained which reflects the substandard hygienic conditions during production and handling of milk in the areas studied.

### Alkaline Phosphatase activity

The mean p-nitrophenol concentrations were recorded as 0.411±0.08, 0.335±0.02, 0.401±0.03, 0.288±0.04 and 0.343±0.02 from Obinze, Owerri, Elele, Asaba, and Enugu respectively. There was no significant difference ($p>0.05$) between the mean p-nitrophenol of the samples from different locations. However, there was significant difference ($p<0.05$) between the standard value of 0.01mg/ml published by Collins and Lyne (2004) and the mean of samples from different locations. Although this test is best used for pasteurised milk sample (Collins and Lyne, 2004), using it for this unpasteurised *Nunu* samples also give an information on possible microbial contamination because of high value of p-nitrophenol concentration indicating possible microbial contamination and inadequate pasteurisation of milk samples.

Routine assessment of *Nunu* microbial quality produced by small-scale producers and consumed by public has to be mandatory in order to safeguard the public from milk-borne zoonotic infections which may radiate through consumption of unsafe milk and milk products. The production of *Nunu* for public consumption by individuals without appropriate permission by the appropriate authority should be discouraged.

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