International Journal of Agricultural and Applied Sciences, December 2024, 5(2): 16-21
https://www.agetds.com/ijaas
ISSN: 2582-8053
https://doi.org/10.52804/ijaas2024.523

Research Article

OPEN ACCESS

Assessment of Microbial Quality of Milk Products (Wara and Nunu) Sold in Ilorin

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(Received: 30/07/2024; Revised: 10/10/2024; Accepted: 30/10/2024; Published: 30/11/2024)

ABSTRACT
Microbial contamination is a major problem due to unhygienic handling and processing. This study evaluated the microbial safety of milk products sold in Ilorin. The samples were obtained commercially from nine locations in the 3 local government areas of Ilorin respectively with a controlled sample. The samples collected were subjected to physicochemical and microbial parameters using standard methods. Physicochemical analyses of Wara samples showed that the titratable acidity and pH ranged from 0.07-1.19g/l and 5.74-7.19, respectively, while that of the Nunu sample had a value for titratable acidity and pH varying from 0.04-2.35 g/l and 4.23-4.61. Genomic identification of some of the isolates reported the presence of enteric bacteria such as *Serratia surfactantfaciens*, *Enterobacter soli*, *Salmonella bongori*, *Pseudomonas inefficax* and *Klebsiella oxytoca* from the commercial samples capable of causing gastrointestinal illnesses if consumed in sufficient amounts. This study showed that although the physicochemical and proximate properties of the commercial samples were good, the presence of pathogenic bacteria as a result of the poor hygienic handling of the producers of these milk products makes them relatively unsafe for human consumption, especially if large quantities of these bacteria are present in the product and consumed. Proper storage and good hygiene during production are thus important to ensure safety of the products.
Keywords: Hygiene, Safety, Genomic, Handling, Pathogen.

INTRODUCTION

Food safety has been of concern as poisoning caused by food consumption has increased, raising the need for processing and producing foods with high quality and safety (Sobukola *et al.*, 2010). Most small-scale food processing firms pay little or no attention to hygiene during food processing leading to the production of foods with low quality assurance (Adetunji and Chen, 2011). Unpasteurized milk contains microbes that are potential foodborne pathogens; however, the low pH obtained during fermentation of the milk to other products does not eradicate these microbes and could be transferred to the consumers. Viruses, rickettsia, bacteria and fungi may be found in raw milk as the udder of cattle often harbours several microorganisms (Okonkwo, 2011). Contamination of dairy products by microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* spp., *Bacillus* spp., *Micrococcus* spp. and *Klebsiella* spp. has been reported due to the poor hygienic conditions in which they are processed and the water source (Ifeanyi *et al.*, 2013; Afolabi *et al.*, 2017). Subsequently, this leads to cases of infections and in some cases intoxication as a result of toxins produced by these organisms (Stewart and Humphrey, 2002). Nunu means cow's milk in the Fulani language and is often hawked by women (Abdulkadir and Mugadi, 2012). It has a milky or opaque white colour and is produced traditionally by carrying out lactic acid fermentation of fresh cow milk (Omola *et al.*, 2019). In Nigeria, local cheese production is widely done and is known as Wara in the Northern region of Benin Republic, Wogachi is used to refer to locally made cheese. Wara production is believed to have originated in the Northern part of Nigeria as most of them are mainly nomadic farmers, however, this has spread to other parts of the country including Ondo, Oyo and Kwara States (Bamidele, 2006). Lack of knowledge and non-compliance to hazard analysis and critical control points associated with the production of milk and its production has led to the contamination of some locally made dairy products. Milk thus requires adequate attention during processing to ensure contaminants are not introduced in the final product. There is a dearth of information on the perception and the safety of these milk and milk products sold in Kwara state, Ilorin. The present study was aimed at filling this gap. This is necessary to ascertain the microbial quality and safety of some of the milk products sold in selected locations in Ilorin. Therefore, this study was aimed at carrying out the safety evaluation of some selected milk products (Wara and Nunu) in Ilorin.

MATERIALS AND METHODS

Study Location

This study was carried out in Ilorin metropolis in Kwara State, Nigeria. It is the capital of the State and is made up of indigenes who are farmers, public servants and students. Its houses some food, and pharmaceutical industries as well as a federal government-owned university with an estimated population of 77,667 (2006 Population Census). Three local governments, namely: Ilorin West, Ilorin South and Ilorin East were selected for this study.

Sample Collection and Preparation (Wara and Nunu)

Fresh cow milk was aseptically collected from cows in Osin farm, Ilorin, Kwara State for the production of Nunu and Wara between 6:30 am-8:00 am (during milking), which cooled and kept under refrigerated condition before processing. Collected samples were transported to the laboratory immediately where production was carried out.

One litre of sterilized fresh cow milk was put in a metallic pot to which 10 ml of starter culture was added. The starter culture is a culture of lactic acid bacteria which is added to the milk to acidify it, inhibit unwanted bacteria as well as enhance the quality of the drying process. The heating was done at 68°C for 20 min for the milk to clot. The resultant mixture was allowed to cool at a renneting temperature (the appropriate temperature for coagulation of milk) of 31°C. The curd (coagulated portion) was separated from the whey (non-coagulated portion) using a sterilized sieve; 150 ml of sterile water with a temperature of 50°C was added to the curd while stirring continuously for 45 min and this washing process was maintained at 34°C to remove the leftover whey in the curd. The washing process also aids in reducing the lactose concentration thus reducing the acidity of the curd. Stirring was stopped and the curd collected in porcelain cloth and left for 15 min to drain out the water. The curd was further pressed into mould to obtain the final Wara product.

A modified method by Owuzu-Kwateng *et al*. (2010) and Akabanda *et al*. (2010) was used for the production of Nunu. Collected cow milk in a sterile container was screened and allowed to ferment for 48 hours at room temperature. Using a wooden ladle, the fermented milk was churned; fat was removed while the excess whey drained off to obtain the product (Nunu) with a thick consistency.

Physicochemical Analyses

Titratable acidity

This was determined using the method by AOAC (2000)

Determination of pH

The pH of all samples was determined at room temperature (29 °C) using a digital pH meter (JENWAY 3505) according to AOAC, 2005 (Nielsen, 2002).

Microbiological Analysis

Sterilization of materials

The glassware including test tubes, conical flasks, measuring cylinder and McCartney bottles used in this study were properly washed with detergents, rinsed with water, wrapped with aluminum foil and sterilized in the hot air oven at a temperature of 170 C for 1 hour. Inoculating needles and loops were flamed to redness on the Bunsen burner. All media were sterilized in the autoclave at 121 C and 15psi for 15 minutes; upon cooling (43±2 C) of the sterile medium, the discrete conical flask's brim was flamed before dispensing the respective medium. Sterile Petri dishes and micropipette tips were used during analyses. The workbench was properly disinfected using 70% ethanol-soaked cotton wool.

Sample collection

The experimental analyses of the discrete samples were undertaken at the Microbiology laboratory of Nigerian Stored Products Research Institute (Headquarters), Ilorin.

Preparation of Culture Media

All media were prepared according to the specifications of the manufacturer(s).

Characterization and identification of isolates

Characterization and subsequent identification of bacterial isolates (Bergey's Manual) were based on colonial and microscopic observations, biochemical tests and molecular studies. The colonial morphology observed includes the shape, colour of colony, elevation, edge, optical characteristics and surface texture of the colonies. The cellular morphology of the bacteria isolates was determined through Gram staining with photomicrography. Catalase, Coagulase, Citrate, Indole, Sugar fermentation, Starch hydrolysis, Oxygen relationship, Methyl red and Vogues Proskauer were undertaken biochemical tests. Molecular studies were done at the International Institute for Tropical Agriculture (IITA), Ibadan.

RESULTS AND DISCUSSIONS

Physicochemical property

The titratable acidity and ph were as presented in Table 1. TTA of wara samples collected within the Ilorin metropolis varied significantly ($p>0.05$) and ranged from 0.07 - 1.19g/l. The highest TTA value was recorded for MDW, while the lowest value was recorded for GKW. These values are in line with the findings of Zalan *et al*. (2005), however a slightly higher than the values reported by Veljovic *et al.* (2007). The variation in TTA values might be attributed to different levels of fermentation that take place during the production and sales of Wara. The case was quite different for pH values obtained for these samples as samples collected from Ilorin East and West recorded pH values of above 7. The lowest pH value was recorded for TKW. This is in line with the reports of Laba *et al*. (2015) who reported different pH values for Wara samples collected at different locations in Ilorin. The variation in pH values of Wara obtained from the Ilorin metropolis might be attributed to non-standardized processing and handling techniques for Wara production in these regions.

Table 1. TTA and pH of *Wara* samples

Sample	TTA (g/l)	pH
MDW	1.19^{f}±0.00	7.1^{c}±0.01
ASW	1.03^{e}±0.04	6.77^{b}±0.02
IDW	0.93^{d}±0.05	7.04^{c}±0.00
GBW	0.02^{a}±0.00	7.19^{c}±0.00
PFW	0.18^{c}±0.01	7.18^{c}±0.00
SGW	0.18^{c}±0.01	5.90^{a}±0.00
TKW	0.11^{b}±0.00	5.74^{a}±0.02
OGW	0.09^{b}±0.00	5.82^{a}±0.00
GKW	0.07^{b}±0.00	5.90^{a}±0.00
CCW	1.00^{e}±0.00	6.12^{c}±0.00

Results represent Mean±SD of triplicate determinations. Means with different superscripts are significantly different (p=0.05) along the rows. TTA- Titratable acidity, MDW=Mandate Wara, ASW=Asa-dam Wara, IDW= Idiape Wara, GBW= Gambari Wara, PFW=Post office Wara, SGW=Songo Wara, TKW=Tanke Wara, OGW=Offa Garage Wara, GKW=Gaa Akanbi Wara

The Titratable acidity and pH of Nunu samples are shown in Table 2. The total titratable acidity (TTA) of the Nunu samples ranged from 0.03 to 2.35 g/l whereas that of the control prepared sample was 0.08 g/l. The peak value obtained in this study is quite higher than the peak values reported for Nunu sold in the Kano metropolis by Omola *et al*. (2019). The high acidity explains why Nunu has a sour taste and may be due to the variation in the duration of the fermentation period and method of production. There was no significant difference in the pH of Nunu samples. However, the peak value (4.70) was recorded for control Nunu while that of the Nunu sold in Ilorin ranged from 4.23 to 4.61. pH value obtained corroborates with the findings of Shibdawa *et al.* (2018), who reported a pH range of 4.6 – 4.8 for Nunu sold in the Bauchi metropolis. Beresford *et al.* (2001) reported that the optimum pH for the growth of most common bacteria is around neutral and growth is often poor at pH values < 5.0. This implies that Nunu sold in Ilorin are not prone to microbial growth and cannot therefore cause any form of microbial food poisoning.

Table 2. TTA and pH of *Nunu* sample

Sample	TTA (g/l)	pH
MDN	0.04^{b}±0.00	4.44^{a}±0.00
ASN	0.04^{b}±0.00	4.44^{a}±0.00
IDN	0.04^{b}±0.00	4.44^{a}±0.00
GBN	1.35^{f}±0.00	4.35^{a}±0.00
PFN	2.29^{g}±0.00	4.25^{a}±0.00
SGN	2.35^{g}±0.00	4.23^{a}±0.00
TKN	1.27^{d}±0.00	4.32^{a}±0.00
OGN	0.03^{a}±0.00	4.31^{a}±0.00
GKN	1.34^{e}±0.00	4.61^{a}±0.00
CCN	0.08^{c}±0.00	4.70^{a}±0.00

Results represent Mean±SD of triplicate determinations. Means with different superscripts are significantly different (p=0.05) along the rows. TTA- Titratable acidity, MDN=Mandate Nunu, ASN=Asa-dam Nunu, IDN= Idiape Nunu, GBN= Gambari Nunu, PFN=Post office Nunu, SGN=Songo Nunu, TKN=Tanke Nunu, OGN=Offa Garage Nunu, GKN=Gaa Akanbi Nunu

Identification and Characterization of Bacteria Isolates of Wara and Nunu Sold In Ilorin

The genomic characterization of microbial isolates is shown in Table 3 respectively. The percentage identity shows the relatedness of the isolates to the bacterium also found in the NCBI database (National Center for Biotechnology Information). The result shows the presence of *Serratia surfactantfaciens*, *Enterobacter soli*, *Salmonella bongori*, *Pseudomonas inefficax* and *Klebsiella oxytoca* which are all enteric pathogens. The genomic technique makes use of the nucleotide sequence of the isolates and, as such is a more reliable method for the identification of organism. The use of genomics in the identification of microorganisms has been effective in the study of pathogenic organisms as the method is more reliable compared with the use of biochemical tests and serology.

DNA fingerprints bacteria were generated by polymerase chain reaction using a primer based on the repetitive elements found in the genome the isolates. The molecular analyses of isolated bacteria were identified on 1.5% agorose gel electrophoresis that showed Size of the amplicon at 1500 bp (base pair).

Identification of some the isolates using genomic (molecular) technique showed the presence of enteric bacteria including *Klebsiella oxytoca*, *Serratia surfactantfaciens*, *Salmonella bongori*, *Pseudomonas inefficax* and *Enterobacter coli.* These are Gram negative bacteria capable of causing gastrointestinal illnesses such as Salmonellosis, Urinary Tract infection, septiceamia and outbreaks of nosocomial infections (Chavez Hernandez, 2014; Singh *et al.*, 2016).

Klebsiella oxytoca is a non-motile, gram-negative rod-shaped bacterium belonging to the family *Enterobacteriaceae*. *K. oxytoca* is ubiquitous in the environment (Gokiewicz, 2009) and can be cultured from the skin, mucous membranes, oropharynx and intestines of healthy humans and animals, as well as a variety of tissues from clinically affected humans and animals has been reported to exhibit multiple antibiotics resistance which also makes it a microorganism of public health concern.

Their presence in the commercial samples may be attributed to the poor hygiene condition in which the products are made (Utermann, 1998). These include the source of bottles used in packaging Nunu, the source of water for processing and storage methods. If not stored properly, dairy products provide a good environment for microbial proliferation.

Serratia spp. are Gram-negative bacteria that are similar in structure to *Escherichia coli and Klebsiella* spp., yet the treatment and control of these organisms remain difficult. The most common mastitis-causing species is *Serratia marcescens*. However, the treatment and

control of these organisms is similar across all species of Serratia.

Pseudomonas has been identified as a predominant milk-associated psychrotrophic bacteria, making it one of the most important bacterial groups in the dairy industry (Wiedmann *et al.*, 2000; Marchand *et al.*, 2009a). The most commonly detected *Pseudomonas* species in milk and milk products are *P. fluorescens, P. gessardii, P. fragi,* and *P. lundensis* (Mallet *et al.*, 2012). Several bacteria genera have been described as psychrotolerant microorganisms and *Pseudomonas* is the genus of most technological relevance (Hantsis-Zacharov and Halpern 2007). Since psychrotolerant microorganisms are commonly found in natural environment, dairy products can be contaminated by contact with water, the inner surface of bulk tanks during storage of refrigerated raw milk, surface of cows' teats and equipment used throughout the milking process (Leriche *et al.*, 2004, Fagundes *et al.*, 2006, Teh *et al.*, 2011). Therefore, the adoption of hygienic measures during milking process is necessary to reduce contamination with psychrotolerant microorganisms (Elmoslemany *et al.*, 2010).

Table 3. Isolates Characterized Using Molecular Genomic Technique

Code	Identification	Strain	E-value	Percentage	Accession Number
TT1	*Serratia surfactant faciens*	YD25	0.0	96.45 %	NZ_CP016948.1
TT2	*Enterobacter soli*		0.0	95.71 %	NC_015968.1
TT3	*Klebsiella oxytoca*	10-5243	$7x10^{-120}$	82.79 %	NZ_JH603144.1
TT4	*Pseudomonas inefficax*	JV551A3	$5x10^{-169}$	89.20 %	NZ_OPYN01000207.1
TT5	*Salmonella bongori*	85-0051	$5x10^{-24}$	80.62 %	NZ_CP053416.1

Key: TT1 – Milk Products 1, TT2 – Milk Products 2, TT3 – Milk Products 3, TT4 – Milk Products 4, TT5 – Milk products 5

Enterobacter soli is an important source of food-borne pathogens. These pathogens in milk have been linked to the environment on the farm, mixing clean milk with mastitis milk and from livestock (Marco *et al*., 2008). E. *coli* frequently contaminates food organisms and it is a good indicator of faecal pollution (Benkerroun *et al*., 2004). The presence of E. *coli* in milk products indicates the presence of pathogenic microorganisms, which constitute a public health hazard (Yohannes, 2018). E. *coli* is among many pathogenic microorganisms which can access to milk and some of dairy products which is considered a reliable indicator of contamination by manure, soil and contaminated water (Somroo *et al*., 2002). This shows that microorganisms including *Proteus* sp., *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus* sp., *Streptococcus* sp., *Enterobacter* sp., *Aeromonas* sp., *Bacillus* sp., *Bacillus subtilis*, *Klebsiella pneumoniae* and *Serratia* sp. were isolated from the samples.

CONCLUSION

This study evaluated the nutrition and safety of milk products (Wara and Nunu) sold in Ilorin; all the milk product samples contained pathogenic bacteria in high quantities which may pose a great danger to the consumer of these ready-to-eat products. There is therefore need to develop standard for the producers and handler to prevent contamination.

CONFLICT OF INTEREST

The author here declares that there is no conflict of interest in the publication of this article.

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Citation: Adediji, A.Y., Joseph, J.K. and Kayode, R.M.O 2024. Assessment of Microbial Quality of Milk Products (Wara and Nunu) Sold in Ilorin. *International Journal of Agricultural and Applied Sciences*, 5(2): 16-21.

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