African Journal of Agriculture and Food Science
ISSN: 2689-5331
Volume 6, Issue 2, 2023 (pp. 93-101)
www.abjournals.org

MICROBIAL ANALYSIS OF STREET-VENDED FRESH AND FRIED LOCAL CHEESE (WARA) IN OWO AND OBA-ILE AKURE, ONDO STATE, NIGERIA

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Cite this article:

Owolabi O.T., Imoru A., Adeolu M.E., Akinrinmade B.P. (2023), Microbial Analysis of Street-Vended Fresh and Fried Local Cheese (Wara) in Owo and Oba-Ile Akure, Ondo State, Nigeria. African Journal of Agriculture and Food Science 6(2), 93-101. DOI: 10.52589/AJAFS-FSMGXSL1

Manuscript History

Received: 12 June 2023

Accepted: 3 Aug 2023

Published: 26 Sept 2023

ABSTRACT: *This work was based on microbial analysis of street-vended cheese, Wara (fresh and fried), in Owo and Oba-Ile Akure metropolis. The study was aimed at investigating the safety and quality of traditional ready-to-eat Wara vended in the streets. The results of the findings show that the Wara samples from Owo communities recorded higher counts of microorganisms than those from Akure. Also, Wara produced in Owo had the highest contamination from a microbiological point of view. It was therefore concluded that Wara could be exposed to contamination during the production process and sales when there is inadequate hygiene and knowledge of quality control during the production process and sales.*

KEYWORDS: Street-Vended Fresh, Fried Local Cheese, Wara Nigeria

Article DOI: 10.52589/AJAFS-FSMGXSL1
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INTRODUCTION

Wara is a Nigerian palatable, soft, white and unsalted concentrated milk product which is a locally made cheese usually processed from fresh cow milk by the Fulani tribes, mainly cattle rearers in Nigeria. Wara-making is thought to have started with the Fulanis, but as a result of their nomadic lifestyle, it has spread to other parts, including Kwara, Oyo, Ogun, Ondo and the Benin Republic (Raheem, 2006). Wara is made from unpasteurised, unfermented fresh whole cow milk by coagulation of the milk with the juice extract of *Calotropis procera* or *Carica papaya*. Wara is an excellent source of protein, fat and minerals such as calcium, iron and phosphorus, vitamins and essential amino acids and, therefore, an important food in the diet of both young and old people. It also provides an ideal vehicle for preserving the valuable nutrients in milk and making them available throughout the year (Adetunji and Chen 2011). Presently, the wara hawked by the vendors on the streets in Owo, Ondo State, Nigeria, is produced at dairy farms on a small scale using raw milk from cows and traditional techniques passed down from generation to generation (Raheem, 2006). All foods possess a finite risk of microbiological contamination. The highest risk factors include foods of animal origin and foods consumed without prior cooking (Roberts, 1990). Wara cheese is one of those kinds of products. Cheese microbial contamination may originate from various sources during cheese production and sales. At the same time, pathogenic organisms may be transferred to food by food handlers directly or by cross-contamination (Temelli *et al.*, 2006; Brito *et al.*, 2008). There have been outbreaks of infection associated with the consumption of cheese, and the predominant organisms responsible have included *Salmonella*, *Listeria monocytogenes*, verocytotoxin-producing *Escherichia coli* (VTEC), and *Staphylococcus aureus* (De Buyser *et al*., 2001; Callon *et al.*, 2008). Several outbreaks of foodborne illnesses associated with milk and dairy products indisputably revealed that cheeses, particularly those made from unpasteurised or improperly pasteurised milk, are the main source of Salmonellosis, Staphylococcal food poisoning and possibly E. coli infections (Doyle, 1991). Detailed investigations have demonstrated that the sources of contamination in cheese were raw milk, inadequately pasteurised milk, or post-pasteurisation contamination with organisms originally derived from raw milk or from manufacturing environments. The microbiological quality of Wara cheese hawked on the streets is of interest from a public health point of view since it is produced from raw milk, usually in small farms where basic facilities are absent, poor hygienic practices, marketed by vendors who lack basic education on food safety and consumed directly without further processing. In addition, several articles have implicated street-vended ready-to-eat food as positive vectors of foodborne illnesses (Edema and Omemu, 2004; Omemu *et al.*, 2005; Omemu and Aderoju, 2008).

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MATERIALS AND METHODS

Source of materials

The cheese samples (wara), both fresh and fried, used for this study were purchased from 10 different locations in Owo and Akure, in Ondo State, Nigeria. The samples were packed in sterile polythene bags and immediately transported to the laboratory for analysis.

Sterilisation procedures.

The glass wares, such as conical flasks, beakers, test tubes and others, were thoroughly washed with detergent using a brush, rinsed under running water and finally with distilled water to remove the salt content of the tap water. After which, the wares were air dried and then sterilised in a hot air oven for 2 hours at 160^{o}c. Inoculating wire loops used were sterilised by flaming with a Bunsen burner until red hot and then allowed to cool before use. The workbench surfaces were sterilised with cotton wool soaked in 75% alcohol before and after each working period.

Culture media preparation

The media used for this research work include nutrient agar (NA) for total heterotrophic bacteria count, Eosin Methylene blue (EMB) agar selective for E. coli, MacConkey agar for enumeration of Enterobacteriaceae and Salmonella Shigella agar (SSA) selective for Salmonella and Shigella (Oyetayo *et al* ., 2020). All were prepared strictly according to the manufacturer's specification, autoclaved at 121°C for 15 minutes, allowed to cool to about 45°C before dispensing into Petri-dishes and MacCartney bottles and allowed to set.

Microbiological Assay

Isolation of bacterial species.

9mls of distilled water was pipetted into six clean test tubes each; they were covered with cotton wool and aluminium foil and then autoclaved at 121^{o}C for 20 minutes. The samples were macerated in a beaker, 5g of the sample was weighed into a sterile beaker and 45 ml of distilled water was added to make a 1:9 sample water ratio, i.e. 10-1. The mixture was shaken well; then a sterile pipette was used to measure 1ml from the supernatant into another test tube containing 9ml sterile distilled water. The mixture was shaken to homogenise this making 10-2, 0.1ml of suitable dilutions were transferred aseptically into Petri dishes, and molten sterile agar (at 45°C) was aseptically poured, and the mixture was swirled gently to ensure even distribution of the inocula. The medium was allowed to be set, after which plates were inverted and incubated at appropriate temperatures. Discrete colonies were counted at the end of the incubation period and expressed as colony-forming units.

Characterisation and identification of isolates

Various biochemical tests were carried out to identify the isolates from pure culture. This was done according to the scheme of (Oliver *et al*., 2005).

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Morphological and Biochemical tests on the isolates

Morphological test

Test isolates were observed for the discrete colonies' colour, shape, edge, elevation, and surface appearance.

Biochemical tests

The following biochemical tests were carried out on the isolates.

Gram staining: A smear of each isolate was made on a clean, grease-free glass slide with a sterile inoculating loop. It was air-dried and then heat-fixed by passing over a Bunsen burner flame three times. The slide was allowed to cool and then flooded with crystal violet solution for 30 seconds. It was washed off in running tap water and again was flooded with Gram's iodine for 30 seconds. It was again washed off in running tap water and then washed with ethanol for a few seconds to decolourise the smear; it was then washed off under running water. The smear was then counter-stained with safranin solution for 30 seconds. This was also washed off gently under the tap, allowing the smear to air dry. The stained smear was then examined under the microscope using X 100 oil immersion lens. Purplish blue indicated gram-positive, while pink to red indicated gram-negative.

Sugar fermentation

This test shows the ability of bacteria to ferment sugar with acid and/or gas production effectively. Peptone water containing 1% of the sugars (Mannitol, Arabinose, Fructose, Sucrose, Galactose, Lactose, Mannose, Ribose, Sorbitol, Inosotol) was dispensed in separate test tubes. 0.5ml of an indicator (3% phenol red) was incorporated into the medium, and a Durham tube was introduced into the set-up. The media was then sterilised by autoclaving at 121^0C for 15mins after which the indicator sugar broth was inoculated with the isolate and incubated at 35^0C for three days. The same organism was introduced into different test tubes containing different sugars, while some tubes were left uninoculated to serve as the control. The yellow colour indicated acid production, while gas production was indicated by bubbles in the Durham tube.

Catalase test

A smear of the isolate was made on a clean slide, and then two drops of hydrogen peroxide were added to the smear. Production of bubbles within 5 seconds signified positive catalase.

Coagulase test

This test differentiates the pathogenic S. aureus from non-pathogenic staphylococci. A discrete colony was emulsified in a drop of sterile normal saline, which had been placed on a clean oil-free slide. The mixture was homogenised, and then a drop of human plasma was added to it. Clumping within 10 seconds indicated a positive result.

Citrate test

A light suspension of the isolate is made in saline and is stab inoculated on Simmon's citrate agar. A growth of blue colour indicates a positive result.

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Motility test

Motility was shown by the diffused movement of the wet mount of the isolate when viewed under the microscope.

RESULTS AND DISCUSSION

Table 1: Total bacterial count for fresh *wara* sample (x 10^3 cfu/g)

SAMPLE	NA (cfu/ml)	EMB (cfu/ml)	MCA (cfu/ml)	SSA (cfu/ml)
IKARE JUNCTION, OWO	181	193	170	370
ISUADA, OWO	62	109	116	121
TISCO, OWO	22	110	101	152
IJEBU, OWO	11	07	15	21
HOUSING A	8	11	30	-
HOUSING B	13	15	21	-
HOUSING C	41	14	27	7
AKURE 1	109	71	121	-
AKURE 2	100	52	94	-
AKURE 3	37	-	77	-

Key: NA – Nutrient Agar, EMB – Eosine Methylene Blue, MCA – MacConkey Agar, SSA - Salmonella Shigella Agar.

Table 2: Total bacterial count for fried *wara* samples (x 10^3 cfu/g)

SAMPLE	NA (cfu/ml)	EMB (cfu/ml)	MCA (cfu/ml)	SSA (cfu/ml)
IKARE JUNCTION	142	-	61	-
ISUADA, OWO	101	-	31	-
TISCO, OWO	29	-	38	-
EMURE, OWO	37	-	17	-
AKURE A	59	-	05	-
AKURE B	71	-	26	-

Key: NA – Nutrient Agar, EMB – Eosine Methylene Blue, MCA – MacConkey Agar, SSA - Salmonella Shigella Agar.

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Table 3: Morphological and biochemical characterization of isolates

GRAM	SHAPE	MOT	CAT	COAG	CIT	GLU	SUC	LAC	ORGANISM
-	Rod	+	+	N.D	-	+	+	+	*E. coli*
-	Rod	+	+	N.D	+	+	-	+	*Enterobacter*spp
+	Cocci	-	+	+	-	+	+	+	*Staphylococcus aureus*
+	Cocci	-	+	-	-	+	-	+	*Staphylococcus epidemidis*
+	Rod	+	+	N.D	-	+	+	+	*Bacillus subtilis*
-	Rod	-	+	N.D	+	+	+	+	*K. preumomiac*
-	Rod	-	+	N.D	-	+	-	-	*Pseudomonas aeruginosa*
+	Cocci	-	-	-	+	+	+	-	*Enterococcus faecalis*
-	Rod	-	+	N.D	-	+	+	-	*Preteus mirabilis*
-	Rod	-	+	N.D	-	+	+	+	*Salmonella* spp
-	Rod	-	+	N.D	-	+	+	+	*Shigella*spp

Key:+ = positive, - = negative, N.D = not done

Table 4: Distribution of the isolates on fresh *wara* samples

Sample	E.C	E.S	S.A	S.E	B.S	K.P	P.A	E.F	P.M	SaS	ShS
IK	+	-	+	+	+	+	+	+	-	+	+
IS	+	-	+	-	+	+	-	+	-	+	-
TIS	+	+	+	+	-	-	-	+	+	+	+
IJ	+	-	+	-	-	+	-	+	-	+	-
HS.A	+	-	+	-	-	+	-	+	-	+	-
HS.B	+	+	+	+	-	+	+	-	-	-	-
HS.C	+	+	+	+	-	+	+	-	+	-	-
AK.1	+	+	+	+	-	-	+	-	+	-	-
AK.2	+	+	+	+	-	-	+	+	+	-	-
AK.3	+	+	+	+	-	+	-	+	-	-	-

Key: IK – Ikare, IS – Isuada, TIS – Tisco, IJ – Ijebu, HS.A – Housing A, HS.B –

Housing B, HS.C – Housing C, AK.1 – Akure 1, AK.2 – Akure 2, AK.3 – Akure 3, E.C – *Enterbacter*spp, S.A - *Staphylococcus aureus*, S.E – *Staphylococcus epidermitis*, B.S - *Bacillus subtilis*, K.P - *K. preumomiac*, P.S - *Pseudomonas aeruginosa*, E.F - *Enterococcus faecalis*, P.M - *Proteus mirabilis*, Sa.S – *Salmonella*spp and Sh.S – *Shigella* spp.

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Table 5: Distribution of the isolates on fried *wara* samples

Sample	E.C	E.S	S.A	S.E	B.S	K.P	P.A	E.F	P.M	SaS	ShS
IK	-	+	+	-	+	-	+	+	+	-	-
IS	-	-	+	+	-	-	+	-	+	-	-
TIS	-	+	+	-	-	-	+	-	+	-	-
EM	-	+	+	-	+	+	+	-	+	-	-
AK.A	-	+	+	+	-	-	+	+	-	-	-
AK.B	-	-	+	+	-	-	+	-	-	-	-

Key: IK – Ikare, IS – Isuada, TIS – Tisco, E.M – Emure, AK.A – AkureA, AK.B – AkureB, E.C – *E. coli*, E.S - *Enterbacter*spp, S.A - *Staphylococcus aureus*, S.E – *Staphylococcus epidermitis*, B.S - *Bacillus subtilis*, K.P - *K. preumomiac*, P.S - *Pseudomonas aeruginosa*, E.F - *Enterococcus faecalis*, P.M - *Proteus mirabilis*, Sa.S – *Salmonella*spp and Sh.S – *Shigella* spp.

DISCUSSION

The total plate count for the samples ranges from 8 x 103 to 181 x 103cfc/ g for fresh wara

samples and 29 – 142 x 103 cfu/ g) for fried wara samples from all the markets. The total coliform count for the samples ranges from 7 x 103 to 193 x 103cfu/g for fresh wara samples, while there was no coliform in the fried samples. The total Enterobacteriaceae count range from 15 x 103 to 170 x 103 cfu/g) for fresh wara samples and 7 – 370 x 103 cfu/g) for fried wara samples from all the markets. The Salmonella-Shigella count ranges (15 x 103 – 170 x 103 cfu/g) for fresh wara samples except for Housing A, Housing B, Akure 1, Akure 2 and Akure 3, where no growths existed. Also, there was no salmonella or shigella on all the fried samples. Summarily, wara samples from Owo communities recorded higher counts than those from the Akure metropolis. This suggests that wara produced in Owo had higher contamination from a microbiological point of view. The organisms isolated were: *E. coli, Enterbacter spp, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, K. preumomiae, Pseudomonas aeruginosa, Enterococcus faecalis, Proteus mirabilis, Salmonella spp* and *Shigella spp* respectively.

Tables 4 and 5 indicate the distribution of the organisms across the two samples in all the locations. All the organisms isolated and identified were well distributed in all the samples and their locations except *E. coli, K. preumomiac, Salmonella spp* and *Shigella spp,* which were not detected in fried samples. *Staphylococcus aureus* was found in all wara samples of both fresh and fried across all the locations. All the bacterial species encountered in the wara samples can affect the keeping quality or the health of the consumers (Oliver *et al*., 2005). *Staphylococcus aureus* is associated specifically with the hands and nasal cavity. The presence of these microorganisms in wara could be an indication of poor sanitary practices by handlers. The growth of microorganisms in food can lead to decomposition and spoilage. Pathogenic microbes may result in the transmission of diseases. Also, the appearance, flavour, colour, and other properties of food may change as a result of the degradative action of microorganisms (Pelczar *et al.,* 2005).

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This present study agrees with the findings of Abdulmalik and Abdulaziz (2011) that isolated pathogenic flora Salmonella and Listeria from local Yemeni cheese. *Bacillus spp* could come from soil or dust since a large number of *Bacillus spp.* occurs in the soil (Atlass, 2007). *Bacillus spp* can cause spoilage of dairy products due to its phospholipase activity (Pelczar *et al.*, 2005). *Staphylococcus aureus* could be introduced into the milk during the hand-milking process and dirty utensils Adam and Moss (2009). *Staphylococcus saprophyticus* is an agent of food spoilage as it causes ropiness or sliminess of food (Mahanta, 2005).

The wara samples from both Owo and Akure were both contaminated but those of Owo were more contaminated owing to the fact that there were more microbial counts than those of Akure. The contamination noticed could be due to unhygienic conditions under which the cheeses were produced or, probably, the unsterilised nature of the Utensils used (Adetunji *et al.*, 2008). The contamination could have equally emanated from the raw milk (Ibrahim and Falegan, 2013).

CONCLUSION

This present study revealed the degree of contamination of wara sold in Owo and Akure metropolis by street vendors. The consumption of contaminated wara as a ready-to-eat food is of public health concern. Wara can be exposed to contamination during production or at sales points due to inadequate knowledge of quality control in food processing and unhygienic practices. All the bacterial species encountered in the wara samples can affect the keeping quality and the health of the consumers.

RECOMMENDATIONS

Adequate public awareness about hygiene practices in food manufacturing should be given to local food processors and handlers. Also, the regulatory body saddled with the responsibility of overseeing the quality of food in the country (NAFDAC) should seriously look into the quality of local wara being hawked around for public consumption.

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