





# ASSESSMENT OF LOCAL METHODS OF PROCESSING FOR THE PRESERVATION OF THE PHYSICO-CHEMICAL PROPERTIES AND MICROBIOLOGICAL QUALITY OF TWO LOCAL CHEESES IN ILORIN, NIGERIA

Bolanle Kudirat Saliu\*<sup>1</sup>, Toyin Olayemi Agbabiaka<sup>2</sup>, Ismaila Olawale Sule<sup>3</sup> and Rabiat Olaronke Gambari-Ambali<sup>4</sup>

Address(es): Dr. (Mrs.) Bolanle Kudirat SALIU

- <sup>1</sup>University of Ilorin, Faculty of Science, Department of Microbiology, P.M.B. 1515, Ilorin, Kwara State, Nigeria. 08023093294
- <sup>2</sup>University of Ilorin, Faculty of Science, Department of Microbiology, P.M.B. 1515, Ilorin, Kwara State, Nigeria.
- <sup>3</sup>University of Ilorin, Faculty of Science, Department of Microbiology, P.M.B. 1515, Ilorin, Kwara State, Nigeria.
- <sup>4</sup>University of Ilorin, Faculty of Science, Department of Microbiology, P.M.B. 1515, Ilorin, Kwara State, Nigeria.

\*Corresponding author: kudisaliu1964@yahoo.com, saliu.bk@unilorin.edu.ng

### ARTICLE INFO

Received 11. 5. 2013 Revised 18. 11. 2013 Accepted 21. 11. 2013 Published 1. 2. 2014

Regular article



# ABSTRACT

Nigerian locally produced cheese from milk, commonly known as wara is highly nutritious and highly prone to attack by spoilage and pathogenic microorganisms. Moist heat treatment and salting alone are the common methods used for processing. This study assessed the efficacy of these local processing methods in preserving the physicochemical properties and microbiological quality of wara. Samples were purchased from open markets in Ilorin; processed by boiling in water and with addition of salt; and stored at room temperature (28±2°C) to mimic the local method. The samples were observed at 24 hrs interval for a period of 96 hrs, for changes in pH, color, odor and texture. Microbiological analysis was done following standard methods. The pH of most of the samples increased while the color, odor and texture deteriorated within 48 hrs of storage. The cheese samples were preserved most by boiling with salt. Many spoilage and pathogenic microorganisms were isolated. The bacterial isolates were *Lactobaccillus acidophilus*, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Proteus vulgaris*, *Enterococcus feacalis*, *Aerobacter aerogenes*, *Klebsiella*, *Aerococcus*, *Micrococcus* and *Streptococcus* spp.; while the fungi were *Aspergillus flavus*, *A. fumigatus*, *A. flavus*, *Candida tropicalis*, *Rhizopus arrhizus*, *Penicillium* and *Mucor* spp. Some of the microorganisms were eliminated during treatments, others survived while some contaminated the samples during storage. Though boiling of wara with salt was shown to improve its keeping quality compared to other methods studied, further treatments such as frying, drying, and roasting; as well as storage at low temperature may significantly increase the shelf life.

Keywords: Wara, boiling, salting, bacteria, fungi

# INTRODUCTION

In Nigeria, cattle rearing are mainly for meat production. Milk is however collected by wives of pastorals and processed locally into products such as wara which are sold to the populace. Wara is a soft non-ripened cheese produced by coagulating the casein in whole milk from cattle with extract of the leaves and stem of Sodom apple plant Calotropis procera (Adeyemi and Umar, 1994; Aworh, 2008; Ovewole and Isah, 2012). Fresh milk is usually collected in the morning into calabash containers. The milk is heated briskly in earthenware pot, and extract of the leaves of C. procera is added to curdle it. The curd is separated from whey by sieving through small conical sieves which also divide the cheese into small portions and give it the characteristic conical shapes. This is white and soft cheese called warankashi which may be consumed directly. Warankashi may also be processed to semi-soft cheese known as wara kaiama by pressing the soft ones through bigger rounded sieves to further drain the moisture. The cheese obtained is often boiled with the stem of guinea corn and potash for coloring and to make it tough. The soft and semi soft cheeses may be boiled with salt, fried in vegetable oil, and consumed as snacks or added to soup as supplement for meat. Being a dairy product, wara is very rich in nutrients (Bamidele, 2006) and may serve as suitable media for the growth of spoilage and pathogenic microorganisms. It has a relatively short shelf life of 2-3 days (Adegoke et al., 1992) and spoilage may be caused by some gram negative psychrotrophic bacteria species such as Pseudomonas spp., Proteus spp. and Aeromonas spp. (Kosikowski and Brown, 1973; Brocklehurst and Lund, 1985, 1988). Spoilage by fungi of the genera Geotrichum, Penicillium, Mucor and Alternaria has also been reported (Chen and Hotchkiss, 1993). Food borne pathogens including Staphylococcus aureus, Bacillus cereus, Clostridium perfrigens, Clostridium botulinum, Brucella abortus, Escherichia coli, Listeria monocytogenes and Salmonella spp. have also been isolated from wara samples (Adetunji et al., 2003; Adeyemi et al., 2003).

Efforts at improving the shelf life of wara has focused on the use of chemicals such as propionic acid, sodium benzoate and sorbic acid because of their reputation in inhibiting mesophilic and psychrotrophic bacteria, including coliforms (Aworh and Egounlety, 1985; Belewu, 2005). In addition, natural products such as nisin, a bacteriocin, produced by lactic acid bacteria (Uzeh et al., 2006) as well as extracts of the leaf of Carica papaya and Terminalia catappa, in combination with vacuum packaging (Adetunji, 2008; Adetunji, 2011) effectively controlled spoilage by aerobic and anaerobic bacteria. Local processing to increase the shelf life of wara uses a combination of heat and salt. These also improve the taste of the product. Moschetti et al. (2011) investigated the effect of salting on the chemico-physical and microbiological characteristics of Pecorino Siciliano cheeses. No report was found on the efficacy of the local processing methods in preserving the physicochemical properties and microbial quality of the Nigerian locally produced cheese, wara.

The objective of our study was to investigate the effects of boiling with or without addition of salt, on the physicochemical and microbial quality of wara.

# MATERIALS AND METHODS

# Sample collection

Two types of locally prepared cheeses generally referred to as wara were used. These were "wara kaiama", the semi soft pinkish red type (PRW) and "warankashi" the soft white type (SWW). Four pieces of "wara kaiama" (PRW) and twenty pieces of "warankashi" (SWW) samples were purchased from retailers at "Oja Oba" market in Ilorin, Kwara State, Nigeria and transported to the microbiology laboratory of the University of Ilorin, Nigeria within 1 hr for analysis.

#### **Treatments**

Each of the four pieces of "wara kaiama" (PRW) representing one part was sliced into five pieces with the aid of flame sterilized table knife while the twenty pieces of "warankashi" (SWW) were divided into four parts with each part having five pieces. Each part of PRW and SWW were treated differently as follow. One part was boiled in water for 15minutes at  $100^{\circ}$ C. A second part was sprinkled with salt at  $10^{\circ}$  w/w. Another portion was boiled in water containing  $10^{\circ}$  w/v cooking salt; and the last portion which served as the control was left untreated. Each of the five pieces in a part was placed in clean, sterile container and stored at  $28\pm 2^{\circ}$ C (room temperature), in the microbiology laboratory. Samples were withdrawn at 24 hour interval for physico-chemical and microbial analysis.

#### Determination of pH

Each sample was crushed and properly mixed with distilled water at 20% w/v. The mixture was poured into a 50 ml beaker and pH was taken using the CLIDA 25C precision table top pH meter.

#### Sensory parameters

The samples were rated for texture, color and odor. A panel made up of ten sensory assessors from the Department of food Science, University of Ilorin, was used. The rating was presented on a five point scale in a questionnaire. Color and color change was determined by visual examination using standard color guide. The options were 5 - pinkish red, 4 - faded pinkish red, 3 - light brown, 2 - brown and 1 - dark brown for "wara kaiama"; and 5 - white, 4 - off white, 3 - cream, 2 - light brown and 1 - brown for "warankashi".

The changes in odor were determined by holding the samples close to the nostrils. Odor changes were assigned 5, 4, 3, 2 and 1 representing pleasant odor, slight change, moderately bad, bad and pungent odor respectively.

The texture was determined by holding and pressing the samples between the fingers. The options were assigned numbers 5, 4, 3, 2, and 1 designating very firm, firm, soft, very soft and slippery respectively.

# Microbiological analysis

The plate count method was used to obtain the total bacterial, fungal and microbial counts. Nutrient agar and malt extract agar were used for isolating the bacteria and fungi respectively. One gram of sample was crushed with the aid of a flame sterilized forceps, and mixed properly with 9 ml sterile distilled water. A serial dilution up to  $10^{-5}$  was prepared and 1 ml each from the last two dilutions was used to inoculate the media by pour plate method. Nutrient agar plates were examined for growth every 24 hours. Colonies were counted after 48 hours for bacteria and 72 hours for fungi. A sum of the total bacterial and total fungal counts represents the total microbial count. Organisms were isolated in pure culture and maintained on agar slants at  $4^{\circ}$ C.

### Characterization of isolates

The isolates were characterized by colonial and microscopic examination. The bacterial isolates were further characterized by biochemical tests. All tests were performed following standard methods (Fawole and Oso, 2004).

## Analysis of data

All numerical data were subjected to analysis of variance and the sample means tested for significant differences using the Duncan Multiple Range Test. This was carried out with the statistical package, SPSS 15.0. Graphs were plotted with Microsoft Excel.

# RESULTS AND DISCUSSION

### Physico-chemical characters

The physico-chemical parameters of the various samples changed during storage. The changes obtained in the pH of the cheese samples are presented in figures 1

and 2. There was no significant increase in the pH of the treated samples in the first 24 hours of incubation. An exponential increase was however obtained afterwards from samples of "wara kaiama" treated with salt only; the pH of the samples that were boiled without salt dropped slightly before increasing, while that of samples boiled with salt increased slightly. The pH of the untreated samples increased exponentially within the first 48 hours of storage, after which no significant change occurred (Figure 1). The change in the pH of all the samples of "warankashi" followed the same pattern with increase in the first 48 hours and no significant change thereafter. Similar to the result obtained in "wara kaiama", the untreated samples of "warankashi" produced an exponential increase in pH within 48 hours of storage (Figure 2).

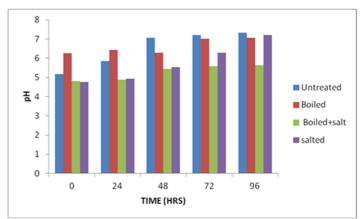


Figure 1 The effect of boiling and salting on the pH of "wara kaiama" during storage

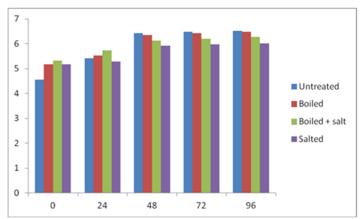


Figure 2 The effect of boiling and salting on the pH of "warankashi" during storage

The effect of preservative treatments on the color change of the cheese samples during storage is presented on table 1. All the treated samples retained the original color only for 24 hours and deteriorated afterwards. The worst color defect was obtained in the untreated "warankashi" while the sample that was boiled with salt had the best color rating. All the treated cheese samples retained the pleasant odor up to 48 hours of storage while that of the untreated samples started deteriorating within 24 hours. The best odor retention was obtained from "wara kaiama" boiled with salt (Table 2). A similar pattern was obtained for the texture of the cheese samples. Except for the untreated, all the samples maintained a firm texture up to 24 hours. The samples that were boiled with salt were fairly firm after 48 hours of storage (Table 3). The physico-chemical properties of the cheese samples were preserved by the boiling and salting treatments since the untreated samples deteriorated faster than the treated.

**Table 1** Change in color of the wara samples during storage

Storage Period (hrs)	Sample	Untreated	Boiled	Boiled with salt	Salted
0	PRW	$5.00 \pm 0.00^{a}$	$5.00\pm0.00^{a}$	$5.00\pm0.00^{a}$	$5.00 \pm 0.00^{a}$
0	SWW	$4.70 \pm 0.48^{a}$	$4.80 \pm 0.42^{a}$	$4.80 \pm 0.42^{a}$	$4.70 \pm 0.48^{a}$
24	PRW	$4.00\pm0.71^{b}$	$4.36 \pm 0.66^{b}$	$4.78 \pm 0.44^{a}$	$4.33 \pm 0.65^{b}$
24	SWW	$3.80 \pm 0.52^{b}$	$4.50 \pm 0.53^{b}$	$4.60 \pm 0.52^{a}$	$4.60 \pm 0.42^{ab}$
48	PRW	$3.24 \pm 0.59^{\circ}$	$2.77 \pm 0.73^{cd}$	$3.72 \pm 0.61^{b}$	$3.50 \pm 0.85^{\circ}$
48	SWW	$3.30 \pm 0.48^{c}$	$3.20 \pm 0.63^{\circ}$	$3.20 \pm 0.63^{b}$	$3.40 \pm 0.52^{c}$
72	PRW	$1.62 \pm 0.50^{e}$	$1.63 \pm 0.52^{e}$	$2.33 \pm 0.65^{c}$	$1.91 \pm 0.70^{d}$
72	SWW	$3.30 \pm 0.48^{\circ}$	$2.40 \pm 0.52^{d}$	$2.30 \pm 0.67^{c}$	$2.30 \pm 0.48^{d}$
06	PRW	$1.17 \pm 0.41^{e}$	$1.00\pm0.00^{\rm e}$	$1.31 \pm 0.48^{d}$	$1.30 \pm 0.48^{e}$
96	SWW	$2.80 \pm 0.42^{d}$	$2.00\pm0.47^{d}$	$1.30 \pm 0.48^{d}$	$1.50 \pm 0.53^{e}$

**Legend:** Each value represents mean of ten independent readings  $\pm$  standard deviation. Means displayed with homogenous superscript within the same column are insignificantly different P = 0.05. PRW denotes "wara kaiama"; SWW denotes "warankashi"

Table 2 Odor change of stored wara samples during storage

Storage Period (hrs)	Sample	Untreated	Boiled	Boiled with salt	Salted
0	PRW	$4.80 \pm 0.42^{a}$	$4.90 \pm 0.32^{a}$	$4.80 \pm 0.42^{a}$	$4.80\pm0.42^{a}$
U	SWW	$4.80 \pm 0.42^{a}$	$4.90 \pm 0.32^{a}$	$4.80 \pm 0.42^{a}$	$4.70\pm0.48^{a}$
24	PRW	$4.00\pm0.67^{b}$	$4.20 \pm 0.42^{b}$	$4.40 \pm 0.63^{b}$	4.30±0.57 <sup>a</sup>
	SWW	$3.60 \pm 0.52^{b}$	$4.60 \pm 0.52^{a}$	$4.70 \pm 0.48^{a}$	$4.40\pm0.52^{b}$
48	PRW	$3.00\pm0.67^{c}$	$3.20 \pm 0.42^{c}$	$3.60 \pm 0.52^{\circ}$	3.00±0.67°
40	SWW	$3.10 \pm 0.52^{\circ}$	$3.80 \pm 0.79^{\circ}$	$3.90 \pm 0.57^{\circ}$	$4.00\pm0.47^{c}$
72	PRW	$1.80 \pm 0.63^{d}$	$2.00 \pm 0.47^{d}$	$3.20 \pm 0.42^{\circ}$	1.40±0.52 <sup>e</sup>
	SWW	$2.20 \pm 0.63^{d}$	$3.50 \pm 0.53^{\circ}$	$2.50 \pm 0.53^{b}$	$2.50\pm0.71^{cd}$
96	PRW	$1.20 \pm 0.42^{e}$	$1.40 \pm 0.52^{e}$	$2.20 \pm 0.63^{d}$	1.20±0.42 <sup>e</sup>
70	SWW	$1.20 \pm 0.42^{d}$	$3.60 \pm 0.52^{c}$	$2.90 \pm 0.74^{d}$	1.60±0.52 <sup>d</sup>

**Legend:** Each value represents mean of ten independent readings  $\pm$  standard deviation. Means displayed with homogenous superscript within the same column are insignificantly different P = 0.05. PRW denotes "wara kaiama"; SWW denotes "warankashi"

Table 3 Change in texture of stored wara samples during storage

Storage	Sample	Untreated	Boiled	Boiled	Salted
Period (hrs)	•			with salt	
0	PRW	$4.80 \pm 0.42^{a}$	$4.80 \pm 0.42^{a}$	$4.70 \pm 0.48^{a}$	$4.60\pm0.52^{a}$
	SWW	$4.60 \pm 0.52^{a}$	$4.60 \pm 0.52^{a}$	$4.50 \pm 0.53^{a}$	4.60±0.53 <sup>a</sup>
24	PRW	$3.80 \pm 0.63^{b}$	$4.20 \pm 0.57^{b}$	$4.50 \pm 0.53^{b}$	4.10±0.56 <sup>b</sup>
	SWW	$3.90 \pm 0.57^{b}$	$4.10 \pm 0.57^{b}$	$4.30\pm0.48^{ab}$	$3.30\pm0.82^{ab}$
48	PRW	$2.40 \pm 0.52^{c}$	$2.80 \pm 0.63^{\circ}$	$3.90 \pm 0.57^{c}$	2.90±0.57°
	SWW	$2.50 \pm 0.53^{\circ}$	$2.90 \pm 0.57^{c}$	$2.50 \pm 0.53^{b}$	2.20±0.42 <sup>b</sup>
72	PRW	$1.80 \pm 0.42^{d}$	$1.90 \pm 0.57^{c}$	$2.50 \pm 0.53^{d}$	$2.10\pm0.57^{d}$
	SWW	$1.70 \pm 0.48^{d}$	$2.10 \pm 0.57^{d}$	$2.10 \pm 0.57^{c}$	1.80±0.63°
96	PRW	$1.40 \pm 0.51^{e}$	$1.30 \pm 0.48^{d}$	$1.80 \pm 0.63^{d}$	1.50±0.53 <sup>e</sup>
	SWW	$1.30 \pm 0.48^{e}$	$1.50 \pm 0.53^{e}$	$1.70 \pm 0.48^{cd}$	$1.30\pm0.48^{d}$

**Legend:** Each value represents mean of ten independent readings  $\pm$  standard deviation. Means displayed with homogenous superscript within the same column are insignificantly different P = 0.05. PRW denotes "wara kaiama"; SWW denotes "warankashi"

# Microbial isolates

The bacterial population of the untreated samples of the two cheese types increased steadily during storage. In the treated samples, the bacterial count was low during the first 24 hours of storage but increased afterwards. In general, the bacterial count of the samples that were boiled with salt was comparatively low (Table 4). Excluding the untreated, the initial samples of cheese had very low fungal count and growth during the time of storage was relatively low compared to that of bacteria (Table 5). Overall, the microbial load of samples of "warankashi" was higher than that of "wara kaiama". A total of 12 bacterial and 8 fungal species belonging to different genera were isolated from the cheese samples. The bacteria were identified by the conventional morphological and biochemical characteristics (Table 6). The bacteria, *Lactobaccillus acidophilus*, *Escherichia coli*, *Bacillus cereus*, and fungus *Saccharomyces cerevisae* were

present in all the samples. Klebsiella sp., Staphylococcus aureus and Proteus vulgaris were not found in "warankashi" while Enterococcus feacalis and Aerococcus sp. were not isolated from "wara kaiama". Among the bacterial species isolated, P. vulgaris and E. feacalis were the least predominant. Aerobacter aerogenes, Micrococcus and Streptococcus spp. were also isolated from many samples of the two types of cheese. S. cerevisae was the only fungi isolated from all the samples while Aspergillus flavus was the least predominant. Higher diversity of bacteria were isolated from "wara kaiama" while "warankashi" had a higher diversity of fungal species. The other fungi isolated from the samples were A. fumigatus, A. flavus, Penicillium sp., Candida tropicalis, Rhizopus arrhizus and Mucor sp. Some of the organisms that were not present in the fresh samples of cheese were isolated from the treated and untreated samples during storage (Tables 7 and 8).

Table 4 Total Bacteria count obtained from the wara samples during storage

Storage	Sample	Bacterial Count (log cfu/g)	Bacterial Count (log cfu/g)							
Period (hrs)	Sample	Untreated	Boiled	Boiled with salt	Salted					
0	PRW	4.1139	4.0972	4.0792	4.0414					
U	SWW	5.0828	4.2553	4.0792	4.1139					
24	PRW	4.4624	4.0972	4.1761	4.1461					
24	SWW	5.1173	4.6990	4.2041	4.7404					
48	PRW	4.5563	4.4914	4.2788	4.2305					
40	SWW	5.2201	4.7076	4.2553	5.0969					
72	PRW	4.7404	5.1335	5.0531	5.0645					
12	SWW	5.3010	5.2355	5.0531	5.0899					
96	PRW	4.4314	5.2068	4.1761	5.0607					
<i>7</i> 0	SWW	5.3802	5.2788	5.1072	5.2788					

Legend: Each value represents mean of two different dilutions. PRW denotes "wara kaiama"; SWW denotes "warankashi"

Table 5 Total fungal counts obtained from wara samples during storage

Storage	Sampla	Fungal Count (log cf	Fungal Count (log cfu/g)						
Period (hrs)	Sample	Untreated	Boiled	Boiled with salt	Salted				
0	PRW	5.1844	3.6021	3.9542	4.1761				
U	SWW	5.2553	4.1761	5.0719	5.0414				
24	PRW	5.3704	4.0414	4.4624	4.7076				
	SWW	5.5911	5.1289	5.0000	5.1139				
40	PRW	5.2833	4.3010	4.4624	4.8062				
48	SWW	5.6857	5.1761	5.0607	5.1139				
72	PRW	5.2529	4.5682	4.6233	4.8325				
	SWW	5.4669	5.2405	5.0792	5.2175				
96	PRW	5.3054	4.8195	4.9638	4.8808				
	SWW	5.3655	5.2810	5.0792	5.2833				

Legend: Each value represents mean of two different dilutions. PRW denotes "wara kaiama"; SWW denotes "warankashi"

Table 6 Biochemical characteristics of bacterial isolates

Chamastana	Organisı	ns										
Characters	A	В	С	D	E	F	G	Н	I	J	K	L
Gram rxn	+	-	-	-	+	+	-	+	+	+	-	+
Shape of cell	R	R	R	R	С	R	R	С	С	С	R	C
Motility	-	+	+	-	-	+	+	-	-	-	+	-
Sporulation	-	-	-	-	-	+	-	-	-	-	-	-
Mac	Nd	Pk	Pl	Pk	Nd							
EMB	Nd	MS	Pk		Nd	Nd	Nd	Nd	Nd	Nd		Nd
Urease	+	-	-	+	+	+	-	+	+	+	+	+
Catalase	-	+	+	+	+	+	+	+	-	-	+	+
Starch	+	-	-	-	-	+	-	+	-	+	-	-
Oxidase	+	-	+	-	-	+	-	-	+	-	-	-
Citrate	-	-	+	+	+	-	-	-	+	-	-	-
Indole	Nd	+	-	-	Nd	Nd	-	Nd	Nd	Nd	+	-
MR	Nd	+	-	-	Nd	Nd	-	Nd	Nd	Nd	+	-
VP	Nd	-	+	+	Nd	+	Nd	Nd	Nd	Nd	-	Nd
Glu	+	+	+	+	-	-	+	+	+	+	+	+
Suc	-		+	+	-	+	+	+	+	+	+	-
Lac	+	+	+	-	-	+	+	+	+	+	+	-
Gas	+	+	+	-	-	+	-	-	+	+	+	-
H <sub>2</sub> S	+	-	-	-	+	-	-	-	-	-	+	-
O <sub>2</sub> Rq	MA	MA	FA	FA	MA	FA	AE	AΕ	MA	AE	FA	FA

Legend: Tests were performed following standard methods and organisms identified by making reference to Bergeys Manual of Determinative Bacteriology. + denotes positive result, - denotes negative result, R - rod, C - coccus, Nd - not determined, Pk - pink, Pl - pale, MA - microaerophilic, FA - facultative anaerobe, AE - aerobic, Mac - growth in MacConkey agar, EMB - growth in Eosin Methylene Blue agar, MR - Methyl Red test, VP - Voges Proskuaer test, Glu - glucose utilization, Suc - sucrose utilization, Lac - lactose utilization, O<sub>2</sub> Rq - oxygen requirement. Organisms: A - L denote L. acidophilus, E. coli, E. aerogenes, klebsiella sp., Micrococcus sp., Bacillus cereus, P. aeruginosa, Streptococcus sp., E. faecalis, Aerococcus sp., P. vulgaris and S. aureus.

 Table 7 Distribution of bacterial isolates obtained from the different samples of wara during storage

Organism	Sample	Fresh	Untreated	Boiled	Boiled with salt	Salted
La ataka sillus a sidambilus	PRW	+	+	+	+	+
Lactobacillus acidophilus	SWW	+	+	+	+	+
Escherichia coli	PRW	+	+	+	+	+
Escherichia cott	SWW	+	+	+	+	+
Entanahaatan aanaganas	PRW	+	+	+	+	-
Enterobacter aerogenes	SWW	-	-	+	+	-
Klebsiella sp.	PRW	-	+	+	+	+
Kieosieuu sp.	SWW	-	-	-	-	-
Migragagus sp	PRW	+	+	+	+	+
Micrococcus sp.	SWW	-	+	+	+	+
Staphyloccus aureus	PRW	-	+	+	+	+
	SWW	-	-	-	-	-
Bacillus cereus	PRW	+	+	+	+	+
bacillus cereus	SWW	+	+	+	+	+
Passidam anna anniain ann	PRW	-	+	+	+	+
Pseudomonas aeruginosa	SWW	+	+	-	-	-
D	PRW	-	+	-	-	-
Proteus vulgaris	SWW	-	-	-	-	-
C44	PRW	+	+	+	-	-
Streptococcus sp.	SWW	+	+	-	+	+
Entangagana fagaalia	PRW	-	-	-	-	-
Enterococcus faecalis	SWW	+	+	-	-	-
Aerococcus sp.	PRW	-	-	-	-	-
	SWW	+	+	+	+	+

Legend: + denotes present - denotes absent

Table 8 Distribution of fungal isolates obtained from the different samples of wara during storage

Organism	Sample	Fresh	Untreated	Boiled	Boiled with salt	Salted
4	PRW	-	+	+	-	-
Aspergillus niger	SWW	+	+	+	+	+
Asmanaillus fuminatus	PRW	+	+	+	+	+
Aspergillus fumigatus	SWW	-	-	+	+	-
Penicillium sp.	PRW	+	+	+	-	+
Peniculium sp.	SWW	+	+	+	+	+
C	PRW	+	+	+	+	+
Saccharomyces cerevisiae	SWW	+	+	+	+	+
Candida tropicalis	PRW	+	+	+	-	+
Canataa tropicatis	SWW	+	+	-	+	-
Aspergillus flavus	PRW	-	+	-	-	-
Asperguius juivus	SWW	-	-	-	-	-
Rhizopus arrhizus	PRW	+	+	+	+	+
Knizopus arrnizus	SWW	-	-	-	-	-
Mucor sp.	PRW	-	+	+	+	+
Mucor sp.	SWW	-	+	+	+	+

Legend: + denotes present; - denotes absent

### Discussion

The increase in pH obtained from samples of cheese during storage indicates that organisms that release alkaline products and raise pH may be growing in the samples particularly the untreated sample. Low metabolic activity due to preservative treatments may however adduce for the initial balance obtained in the pH of the treated samples which increased slightly when activity resumed, to that which favors the growth of coliforms and other organisms (Ledenbach and Marshall, 2009). Among dairy products, cheese is highly valued for its reduced water content, and acidic pH which significantly inhibit bacterial growth to prolong the shelf life (Yim, 2003). Though the moisture content of "wara" is low, an acidic pH is not usually attained and it may be prone to spoilage compared to conventional cheeses that are produced by fermentation with lactic acid bacteria and ripened with bacteria or fungi.

In general, milk and dairy products are prone to spoilage due to their rich nutrient content, and must be properly stored. Storage in refrigerator will significantly increase the shelf life. However, the epileptic nature of electricity supply in most part of Nigeria has been an impediment to effective storage of dairy products including "wara". The organoleptic properties of the samples treated with a combination of salt and moist heat were retained up to 48 hours which makes these treatments suitable as a method of increasing the shelf life of cheese particularly in the rural settlements where electricity is lacking. The presence of proteolytic bacteria such as B. cereus and P. aeruginosa causes the cheese samples to lose color, odor and texture. B. cereus is one of the most common spore forming bacteria causing spoilage of dairy products (Meer et al., 1991) and has been implicated in the spoilage of wara (Adetunji et al., 2003; Adeyemi et al., 2003). It is an endospore forming bacterium which may not be destroyed by mild treatments. Treatment may actually promote germination of spores leading to proliferation of the bacterium in the samples (Table 7). P. aeruginosa and Proteus vulgaris were isolated from fresh samples of "warankashi" but destroyed by the treatments; while they were found as heavy contaminants of "wara kaiama" after treatment. The proteolytic activity of these bacteria is evident in the rapid spoilage of the cheese samples. Streptococcus sp. and L. acidophilus are homofermenters producing lactic acid and hence low pH from fermentation of lactose in milk and dairy products; and may be responsible for the softening texture of the cheese samples during storage; while the low pH favors the growth of the yeast S. cerevisae, a major cause of spoilage of dairy foods (Ledenbach and Marshall, 2009).

Enterococcus feacalis, E. coli, Klebsiella sp., and P. vulgaris are known enteric bacteria and their presence in a sample is indicative of fecal contamination. They are also pathogenic organisms and may cause diseases of the gastro-intestinal tracts. Their presence in the cheese samples therefore renders the cheese unsuitable for consumption. E. feacalis was however eliminated from the treated sample, an indication that it is susceptible to salt and moist heat treatments. Isolation of Enterobacter aerogenes, Staphylococcus aureus and Klebsiella sp. from the treated samples may be a result of post treatment contamination since the organisms were not found in the fresh samples. Staphylococcus aureus is an opportunistic pathogen and is undesirable in food as its growth may result into food poisoning. Klebsiella sp., E. coli and S. aureus were among the pathogenic bacteria isolated from wara and nono (Olasupo et al., 2002; Ogbonna, 2011); and from cheese during ripening (Brooks et al., 2012). Micrococcus sp. was also found as contaminant of 'warankashi'. Some of the microorganisms isolated in the fresh sample were not eliminated by the boiling and salting treatments. In addition, quite a number were not present in the fresh samples and may have contaminated the cheese after the treatments. These underscore the importance of further treatments such as frying, roasting and drying; as well the conditions for storage in prolonging the shelf life of Nigeria indigenous cheeses.

### CONCLUSION

In this study, the local processing method of boiling wara with salt and water was shown to preserve its physico-chemical properties and thereby increase the shelf life. Boiling without salt and salting alone produced some preservative effects which were not as significant as the treatments that combined the two. However, other treatments such as frying, drying and roasting; as well as storage at low temperatures are recommended in order to further increase the shelf life.

#### REFERENCES

ADEGOKE, G.O., NSE, E.N., AKANNI, A.O. 1992. Effects of heat, processing time, and ph on the microflora, aflatoxin content, and storability of wara, a soft white cheese. *Die Nahrung*, 36(3), 259–264.

ADETUNJI, V.O. 2008. Comparative assessment of the effect of crude extracts of *carica papaya* and *terminalia cattapa*, and a bacteriocin on vacuum-packed West African soft cheese ('wara'). *African Journal of Microbiology Research*, 2, 272-276

ADETUNJI, V.O. 2011. Effects of packaging, treatments, and storage conditions on the survivability of aerobes and anaerobes in vacuum packaged 'wara'; a soft white cheese. *Advance Journal of Food Science and Technology*, 3(4), 289-293.

ADETUNJI, V.O., IKHELOA, J.O., ADEDEJI, A.M., ALONGE, D.O. 2003. An evaluation of the bacteria in milk products sold in South Western Nigeria. *Nigerian Veterinary Journal*, 24(3), 21-25.

ADEYEMI, I.A., UMAR, S. 1994. Effect of method of manufacture on quality characteristics of kunun-zaki, a millet-based beverage. *Nigerian Food Journal*, 12, 34-41.

ADEYEMI, I.G., IKHELOA, J.O., OGUNLEYE, A.O., ORIOKE, O.O. 2003. Food-borne pathogens found in local cheese ('wara'nkasi) sold as take-away food in Ibadan, Nigeria. *Nigerian Veterinary Journal*, 24(3), 86-89.

AWORH, O.C. 2008. The role of traditional food processing technologies in national development: The West African experience. *International Union of Food Science & Technology*, 1-18.

AWORH, O.C., EGOUNLETY, M. 1985. Preservation of West African soft cheese by chemical treatment. *Journal of Dairy Research*, 52, 189-195.

BAMIDELE, R.D. 2006. Development and microbiological applications in african foods: Emphasis on Nigerian wara cheese. Department of Applied Chemistry and Microbiology, Division of Microbiology, University of Helsinki Finland. 1(1), 7-20.

BELEWU, M.A., BELEWU, K.Y., NKWUNONWO, C.C. 2005. Effect of biological and chemical preservatives on the shelf life of West African soft cheese. *African Journal of Biotechnology*, 4, 1076-1079.

BROCKLEHURST, T.F., LUND, B.M. 1985. Microbiological changes in cottage cheese varieties during storage at 7°C. *Food Microbiology*, 2, 207.

BROCKLEHURST, T.F., LUND, B.M. 1988. The Effect of pH on the initiation of growth of cottage cheese spoilage bacteria. *International Journal of Food Microbiology*, 6, 43.

Microbiology, 6, 43.
BROOKS, J.C., MARTINEZ, B., STRATTON, J., BIANCHINI, A., KROKSTROM, R., HUTKINS, R. 2012. Survey of raw milk cheeses for microbiological quality and prevalence of foodborne pathogens. Food Microbiology, 31(2), 154-8.

CHEN, J. H., HOTCHKISS, J. H. 1993. Growth of *Listeria monocytogenes* and *Clostridium sporogenes* in cottage cheese in modified atmosphere packaging. *Journal of Dairy Science*, 76, 972-977.

FAWOLE, M.O., OSO, B.A. 2004. Characterization of bacteria. In Laboratory Manual of Microbiology. Spectrum Books Limited, Nigeria. pp 22-33.

KOSIKOWSKI, F.V., BROWN, D.P. 1973. Influence of carbon dioxide and nitrogen on microbial populations and shelf life of cottage cheese and sour cream. *Journal of Dairy Science*, 56, 12–18.

- LEDENBACH, L.H., MARSHALL, R.T. 2009. Microbiological spoilage of dairy products. In W.H. Sperber and M.P. Doyle (eds.), *Compendium of the Microbiological Spoilage of Foods and Beverages*, *Food Microbiology and Food Safety*, 41–67.
- MASSIMO, T., NICOLA, F., STEFANO, R., GIANCARLO, M., FABRIZIO, V., LUCA, S. 2011. Effect of different salting technologies on the chemical and microbiological characteristics of PDO pecorino siciliano cheese. *European Food Research and Technology*, 233(6), 931 940.
- MEER, R.R., BAKER, J., BODYFELT, F.W., GRIFFITHS, M.W. 1991. Psychrotrophic *Bacillus* spp. in fluid milk products. *Journal of Food Protection*, 54, 969–979.
- OGBONNA I.O. 2011. Microbiological analyses and safety evaluation of nono: A fermented milk product consumed in most parts of Northern Nigeria. *International Journal of Dairy Science*, 6, 181-189.
- OLASUPO, N.A., SMITH, S.I., AKINSINDE, K.A. 2002. Examination of the microbial status of selected indigenous fermented foods in Nigeria. *Journal of Food Safety*, 22(2), 85–93.
- OYEWOLE O.A., ISAH P. 2012. Locally fermented foods in Nigeria and their significance to national economy: a Review. *Journal of Recent Advances in Agriculture*, 1(4), 92-102.
- UZEH R.E., OHENHEN, R.E., ROJUGBOKAN, A.K. 2006. Microbiological and nutritional qualities of dairy products: Nono and Wara. *Nature and Science*, 4(3), 37 40.
- YIM, G., GLOVER, C. 2013. Food Microbiology: The Basics and the details of cheese production. *The Science Creative Quarterly*.