Microbial Analysis and Proximate Composition of Boiled and Fried Local Cheese (WARA)

Ajayi, O. O[1]., Balogun, O. B[1]., Oriowo-Olaleye, M[1]. and Faturoti M. O[1].

Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji Arakeji, Osun State, Nigeria.

DOI: 10.29322/IJSRP.8.12.2018.p8491
http://dx.doi.org/10.29322/IJSRP.8.12.2018.p8491

Abstract- Fried cheese locally called Wara gets spoiled when attacked by pathogenic bacteria, as a result of poor hygiene practice by the producer and handlers. Wara samples were bought in Ilesha at Atakumosa market square Osun-state. This research was designed to isolate and enumerate microorganisms using standard microbial methods, proximate composition and the nutritional composition of boiled local and fried cheese (wara). Proximate analysis was carried out to determine nutritional composition. *Klebsiella* species and *Esherichia coli Staphylococcus epidermidis, Bacillus* species, *Escherichia coli, Salmonella* species, *Streptococcus* species, *Clostridium* species and *Lactobacillus* species were isolated using standard technique. The highest bacteria count for the fried cheese was 2.1×10^5cfu/ml the lowest bacterial count was 1.5×10^5cfu/ml unit highest bacterial count of the boiled local cheese was 4.4×10^5(cfu/ml), lowest bacterial count was 1.8×10^5(cfu/ml) while the range of the values was 2.6×10^5. Fungi isolated were *Penicillium* and *Gymnoase.* Fat content for the boiled cheese has the highest mean value of 44.78 ±0.028cfu/ml and Ash had lowest mean value of 2.46±0.014cfu/ml. The moisture content of fried cheese had the highest mean value 55.32±0.014 while the carbohydrate had the lowest mean value 0.63±0.02 for fried chesse. Fibre content was not detected in both samples. The microbial contamination might generally occur based on lack of standardization, fecal contamination and poor personal hygiene in the production, handling and storage. Therefore, all cheese producers and consumers should take care during processing and storage of the cheese to prevent contamination.

Index Terms- Pathogenic bacteria, Microorganisms, Proxinate composition and Microbial methods

I. INTRODUCTION

Cheese (wara) it is a Nigerian soft, white, unsalted and unripened cheese usually processed from cow milk by the Fulani tribes who are mainly cattle rearers in Nigeria (Raheem, 2006; Ogunbanwo *et al*., 2011). Wara making started with the Fulani's but as a result of their nomadic lifestyle, it spread to other parts of the country including Kwara, Oyo, Ogun, Ondo and the Benin Republic (Raheem, 2006).

It is obtained by coagulating the casein with rennette or similar enzymes in the presence of lactic acid produced by adding microorganisms (Adegoke *et al.,* 1992). However in many cases starter culture are not used as processing condition are not standardized (Adetunji, 2011). It could also be coagulated with the juice extract of leaf of sochom apple (*Colotropis procera*) or pawpaw). (Augusti, 1996; Adetunji and Alonge, 2009; FAO, 2009).

Cheese is usually stored in its whey and consumed fresh, but this can only last for 3-5 days after which spoilage occurs. It is sometimes fried and used as a meat-substitute in stews and soups, or smoke-dried to enhance its keeping qualities. However, all these increase its shelf life by only a few extra days or few weeks at best (Belewu *et al.,* 2005*).*

Cheese is made mostly from the milk of cows, buffaloes, sheeps or goats, and it is an important food component in the healthy diet of human's highly nutritious rich food and source of protein, peptides, aminoacids, vitamins, salt, and essential minerals including calcium (Augusti, 1996; Belewu and Aina, 2000). Cheese however, has been shown to have antimicrobial properties that prevent disease, it has been used as drugs for certain infection when common antimicrobial agents failed it had also been found that soft cheese has growth inhibitory activity against common bacteria that caused diarrhoea in South West Nigeria (Ibrahim and Falegan, 2013).

Microorganisms such as *Streptococci*, *Lactobacilli*, Coliform bacteria and some fungi are associated with milk and milk products (Baba *et al*., 2004; Raheem and Saris, 2009). The consumption of cheese is of utmost public health importance and high consumption rate in Nigeria calls for the periodical examination of cheese produced in different locality.. The study was designed to isolate and identify microorganisms found in both fried and boiled cheese; to determine the proximate composition and assess the best form of cheese for humans consumption.

II. MATERIALS AND METHOD

2.1 Study Area

The cheese sample (Wara) used for this study was purchased from Ilesa West Local Government at Atakumosa market square in Osun state, Nigeria. The market was sited close to the place of the king of the town (Ereja square) on the outskirt of the city of Ilesha. The total population according to 2006 census was 103,555 having the total area as 63km^2 (24 sq mi) and the coordinate of 7°39N 4°43'E.

2.2 Techniques for preparation of media

2.2.1 Sterilization Technique

The glassware used was thoroughly washed with detergent rinsed with distilled water and air dried. The glassware were wrapped with aluminum foil paper and sterilized in an autoclave at 121°C for 15 minutes.

2.2.2 Collection of Samples

Wara sample processed traditionally by Fulani woman were bought in highly populated location in Ilesha at Atakumosa Market Ilesa Osun State, Nigeria. The Fried cheese was bought into a sterile plastic plate and transported to Microbiology laboratory for analysis within 2 hours of purchase.

III. PREPARATION OF MEDIA

3.1 Nutrient agar: This was prepared according to manufacturer's description. Seven (7g) of nutrient agar powder was weighed and dissolved into 250 ml of distilled water. It was heated to get it homogenized and allowed to change to golden yellow before it was autoclave at 121° C for 15 minutes.

3.2 MacConkeyAgar: This was prepared according to manufacturer's description. 14g of MacConkey agar powder was weighed and dissolved in 250ml of distilled water and homogenized using heat to boil. This was then sterilized by autoclaving at 121°C for 15minutes.

3.3 Agar Slant Agar slant were prepared by dispensing 15ml of nutrient agar into different MacCartney bottles. The bottles were placed in a slanting position and allowed medium to gel.

3.4 Serial dilution of Samples Five grams (5g) of the cheese was meshed using the crucible and pestle, serial dilution was done by measuring 9ml of sterile water into first test tube till the tenth test tube. 1ml of the sample was then added into each labeled and properly covered test tube using a sterile syringe. 1ml was then taken from 10^5 or the fifth test tube into a petridish and gently swirled and allow to set. The petridishes were then incubated at 37°C for 24 hours. After the incubation, the colonies that developed on nutrient agar plate were counted and used to determine the total bacteria count of the sample (cfu/ml). The representative colonies on the plates were sub-cultured on a fresh nutrient agar to obtain a pure culture of isolates. The pure culture was then transformed into nutrient agar slant for biochemical test.

3.5 Isolation and identification of the bacteria

Distinct and well isolated colonies were sub-cultured and examined for various sizes, shapes, colours and texture, a series of tests such as Catalase, Oxidase, Indole, Nitrate reduction, and sugar fermentation were carried out to identify the bacteria according to (Cheesbrough, 2006).

3.6 Isolation of Fungi

Nine point eight gram (9.8g) of Potato Dextrose Agar (PDA) powder added with antibiotics (to inhibit the growth of bacteria) was dissolved into 250ml of sterile water and autoclaved at 121°C for 15 minutes and allowed to cool before pouring aseptically into the petridishes. The prepared potato dextrose agar (PDA) was aseptically dispensed into the Petri dish containing the sample and swirled gently before cooling. The plate was then incubated in an incubator at 26°C for five (5) days before observation (Onyeagba, 2004).

3.8 Proximate Analysis of Nutritional Composition of Fried Cheese

This refers to the determination of the major constituents of food sample and it is used to assess if a sample is within it's normal compositional parameters or somehow been adulterated. This method partitioned nutrient in food sample into six components: ash, crude protein, fat, crude fiber, moisture content, and carbohydrate (FAO, 2008).

IV. RESULT

Table 1: It shows the total bacterial count in fried cheese calculated in colony forming units per ml, the sample four had no bacterial population .

Table 2: Total bacterial load of boiled local cheese (wara) samples calculated in colony forming units per ml, all the sample had bacterial population which varies from sample one till sample five

Table 3: It shows the morphological characteristics and biochemical test of bacteria isolate from boiled local cheese (wara) based on size, texture, colour, opacity, surface, elevation and margin.

Table 4: It shows the morphological characteristics and biochemical test of bacteria isolate from fried local cheese (wara) is based on size, texture, colour, opacity, surface, elevation and margin.

Table 5: It shows the count for fungi isolated in the fried cheese sample. The fungi isolate were: *penicillium* spp and *Gymnoaseus* sp.

Table 6: Proximate analysis showing the nutrition evaluation of cheese, which is based on Moisture content, Fat, Fibre, Crude protein and Carbohydrate.

Table 7: Statistical analysis of proximate composition shows mean Standard deviation, Variance and Coefficient of variation.

Figure 1: Bacterial count in fried cheese which is calculated in colony forming units per ml. .

Figure 2: Bacterial load of local cheese (wara) samples.

Figure 3: The boxplot of the statistical analysis of proximate composition

TABLE 1: Total bacterial load of Fried Cheese (wara) sample

Replicate	Sample 1(Cfu/ml)	Sample 2(Cfu/ml)	Sample 3(Cfu/ml)	Sample 4 (Cfu/ml)
R I	1×10^5	2×10^5	2×10^5	0
R 2	2×10^5	1×10^5	2×10^5	2×10^5
Mean	1.5×10^5	1.5×10^5	2×10^5	1×10^5
SD	0.70710	0.70710	0	1.41421
CV	47.40	47.140	0	141.421
VAR	0.5	0.5	0	2

Keys:
SD: Standard deviation
VAR: Variance
CV: Coefficient of variance

Table 2: Total bacterial load of boiled local cheese (wara) samples.

Replicate	Sample 1 (Cfu/ml)	Sample 2 (Cfu/ml)	Sample 3 (Cfu/ml)	Sample 4 (Cfu/ml)	Sample 5 (Cfu/ml)	Sample 6 (Cfu/ml)
R1	4.5×10^5	3.3×10^5	2.7×10^5	1.8×10^5	2.1×10^5	1.7×10^5
R2	4.3×10^5	3.7×10^5	3.0×10^5	2.3×10^5	1.5×10^5	2.6×10^5
Mean	4.4×10^5	3.5×10^5	2.85×10^5	2.05×10^5	1.8×10^5	2.15×10^5
SD	1.4142136	2.8284271	2.1213203	3.5355339	4.2426407	6.363961
CV	3.2%	8%	7.4%	17%	24%%	29%
VAR	2	8	4.5	12.5	18	40.5

KEYS:

SD: Standard deviation VAR: Variance R2: Replicate 2
CV: Coefficient of variation R1:Replicate 1

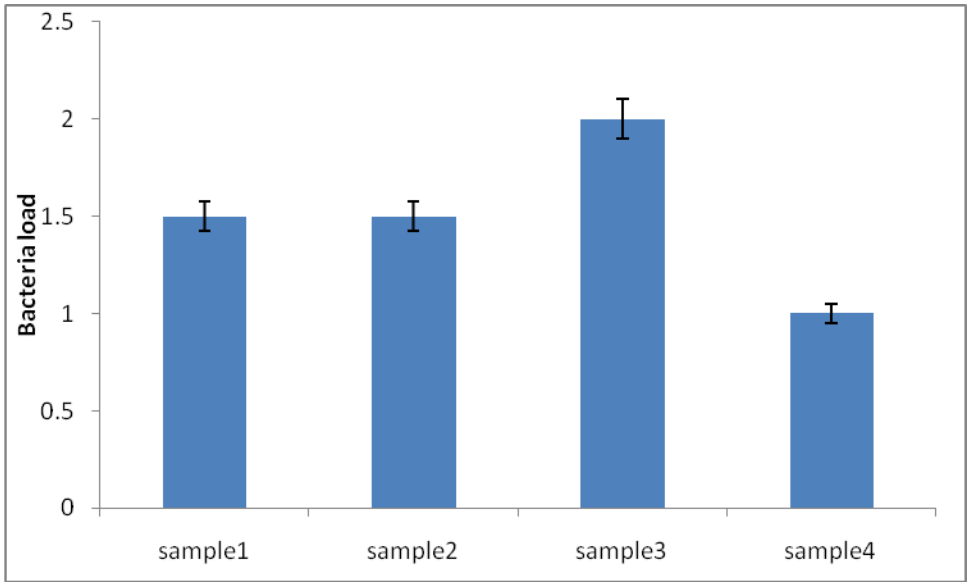
TABLE 3: Morphological characteristics and biochemical test of Bacterial isolates from fried local cheese (wara)

	Edge	Colour	Surface	Size	Optical	Spore staining	Gram staining	Methyl red	VP	Indole	Motility	Hydrogen sulphide	Gluose	Sucrose	Probabale organism
l	Round	Cream	Smooth	Small	Transluscent	-	-	+	+	-	+ +		G	G	*Klebsiellia* spp
l	Entire	Cream	Smooth	Small	Opaque	-	+	+	+	+	+	-	G	G	*Lactobacillus* spp
l	Entire	Cream	Dry smooth	Big	Opaque	-	-	+	-	+	-	-	G	G	*Escherichia coli*
l	Round	cream	Dry smooth	Small	Translucent	-	-	+	-	+	-	-	G	G	*Salmonella* spp

Table 4: Morphological characteristics and biochemical test of bacterial isolates from boiled local cheese (wara)

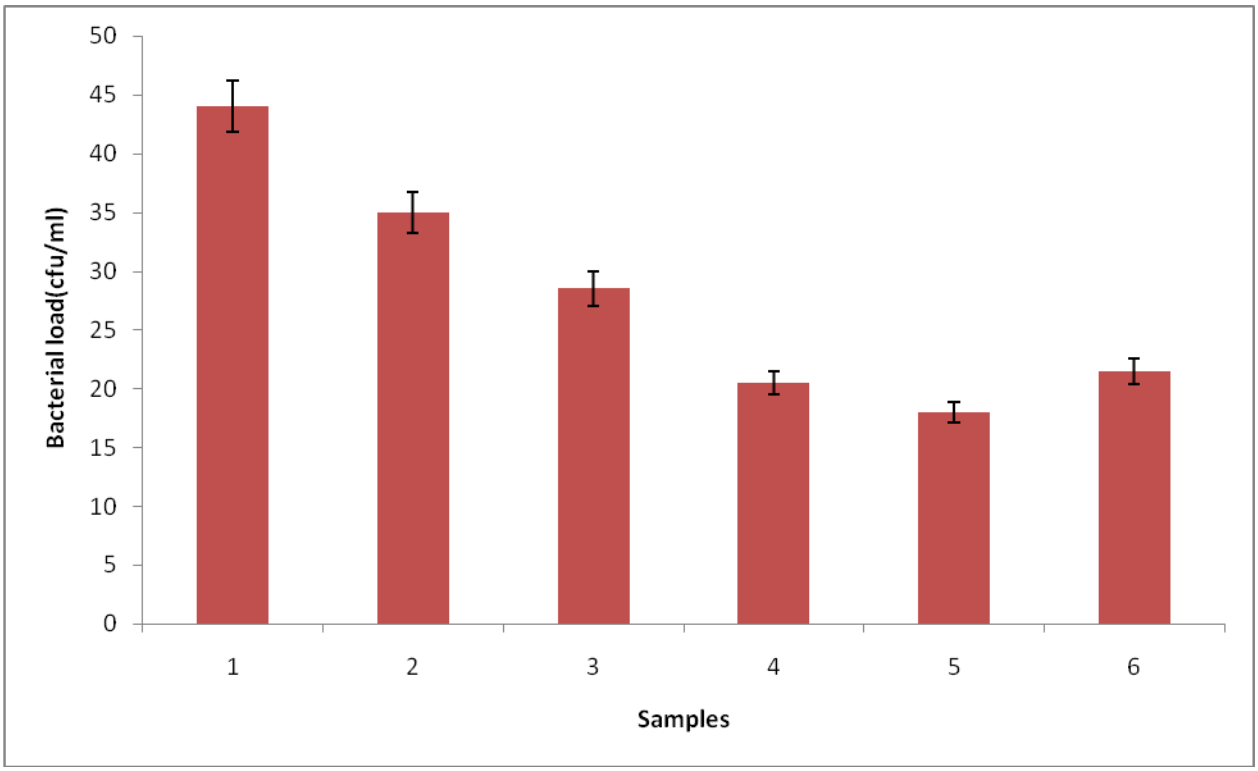
Probable Organisms	**Shape**	**Size**	**Texture**	**Color**	**Opacity**	**Edge**	**Gram stain**	**Spore stain**	**Methyl red**	**catalase**	**Indole**	**Motility**	**VP**	**H_2S**	**Lactose**	**Glucose**	**Sucrose**
Bacillus spp	Rod	Small	rough	Cream	opaque	entire	+	+	+	+	+	+	+	-	A	A	A
Streptococcus spp	Cocci	Small	rough	Cream	opaque	entire	+	-	+	+	+	-	-	-	-	A	A
Clostridium spp	Rod	Small	smooth	Yellow	opaque	center	+	+	+	+	+	-	+	+	-	AG	AG
Lactobacillus spp	Cocci	Small	smooth	Cream	opaque	entire	+	-	-	+	-	-	-	+	G	AG	AG

Staphylococcus epidermidis	Cocci	Small	smooth	Cream	opaque	center	+	-	-	+	-	-	+	+	-	AG	AG
Escherichia coli	Rod	Small	rough	Pink	opaque	center	-	-	+	+	+	+	-	-	AG	AG	AG



Error bar ±1 SE

Figure 1: Bacteria count in fried cheese.



Error bar ±1 SE

Figure 2: Bacterial load of local boiled cheese (wara) samples.

Table 5: Total fungi count in fried cheese (Wara)

Sample	**10**	**Cfu/ml**	**Fungi organism**
	10^3	6×10^3	*Penicillium* spp
	10^5	4×10^5	*Gymnoase* spp

Table 6: Proximate analysis result showing nutritional evaluation in fried cheese

S/N	%Ash	% MC	%CP	%Fat	%Fibre	%CHO
R1	2.45	10.33	21.11	44.8	0	21.31
R2	2.47	10.31	21.1	44.76	0	21.36
Mean	2.46	10.32	21.105	44.78	0	21.335
SD	0.014142	0.014142	0.007071	0.028284	0	0.035355
VAR	0.002	0.002	5E-05	0.008	0	0.0125
CV	0.05749	0.0137	0.335	0.0632	0	0.01657

Sample	2.46±0.014	10.31±0.014	21.1±0.007	44.76±0.02	0	21.36±0.03

Keys:
MC**:** Moisture Content
SD: standard deviation CP: Crude protein
VAR: variance CHO: Carbohydrate
CV: coefficient of variance

Table 7: Statistical analysis of proximate composition in boiled cheese

Replicate	**ASH**	**MC**	**CP**	**FAT**	**FIBRE**	**CHO**
R1	1.16	55.31	18.11	24.78	0	0.64
R2	1.18	55.33	18.12	24.76	0	0.61
Mean	1.17	55.32	18.115	24.77	0	0.625
SD	0.014142	0.014142	0.007071	0.014142	0	0.021213
CV	1.41%	0.03%	0.04%	0.1%	0	3.4%
VAR	0.0002	0.0002	5E-05	0.0002	0	0.00045

Sample	1.17±0.014[a]	55.32±0.014[b]	18.2±0.007[c]	24.8±0.014[d]	0[e]	0.63±0.021[f]

Data are presented as Mean±SD (n=2) from duplicate determination. Different superscripts in the same column are significantly different (P<0.05).

Keys:

MC: Moisture content
CP: Crude protein
CHO: Carbohydrate
ND: Not detected

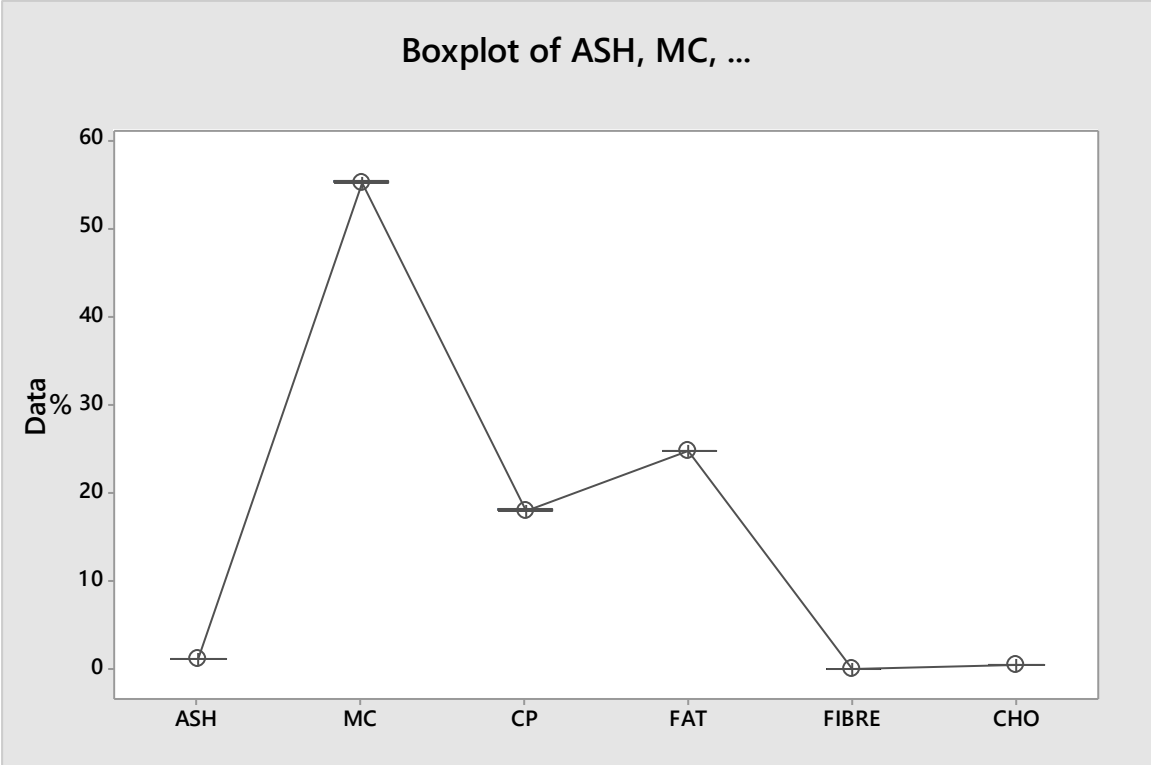


Figure 3: The boxplot of the statistical analysis of proximate composition

V. Discussion and Conclusion

The results obtained from the microbial analysis of fried cheese (wara) show that the products were contaminated with microorganisms of public health concern. The bacterial count in the sample may be a consequence of low level of hygiene maintained during the processing and sale of the products. This includes the handlers, quality of water used and the utensils. During the sale of fried cheese (wara), dirty hands and spoons are dipped into the bowl for product selection by both hawkers and consumers.

The exposure of wara and while they are displayed for sale in bowls can serve as source of contamination. The detection *Klebsiella* spp and *E.coli* in wara may indicates possible faecal contamination because the fulani's do not disinfect the teats and udders prior to milking despite the fact that the cow lies in a muddy barnyard and dirty environment which inevitably contaminate the milk and could increase the microbial load. The presence of *Staphyloccus epidermidis*, as supported by the study of Ajayi *et al*. (2016) may lead to contamination of food and eventually affects the health of the consumers. The presence of *Bacillus* species which produces several toxins, being isolated in this study probably is an indication of poor hygienic habit of the milker milking the cow. *Clostridium* species isolated which are important cause of diarrhea inhabits the soil and intestinal tract of animals including humans and can cause food intoxication which is congruent to the findings of Ajayi *et al*. (2016). Being enteric bacteria, their presence indicates poor hygienic practices among handlers of wara. Due to the significance of the faecal-oral route transmission for many bacterial food-borne diseases, basic hygienic measures assume a decisive importance in food safety management (Alalade and Adeneye, 2006).

The detection of *Lactobacillus* species isolated from sample show that they are organism used for the production of cheese. *Lactobacillus* species allows the production of gas in lactose, sucrose and glucose Aworh and Egounlety (1985). They are motile, gram positive bacteria which tests positive for

catalase test. The fungal isolates: *Gymnoase* and *Penicillium*, species which were isolated are known as spore formers, which therefore means that they can easily contaminate the dairy products which are usually exposed during processing, storage, and hawking. They are major spoilage organisms of carbohydrate foods (Rhodes and Fletcher, 1966). However, their growth can result in the production and accumulation of mycotoxins which are of public health and economic importance (Burnett and Beuchat, 2001). The nutritional analysis of fried cheese shows that they are of appreciable nutritional status especially in the protein and fat content. The dairy products particularly cheese are good sources of protein. Higher fat content was observed in fried cheese wara. This could be due to vegetable oil used in frying of the cheese.

REFERENCES

[1] Alalade, O.A. and Adeneye, J.A. (2006). The effects of short term frozen storage on chemical composition and coliform and coliform microflora of Wara cheese "Wara cheese under frozen storage. *Advance Journal of Food Technology*. 2(1): 44-44

[2] Augusti, K.T. (1996). Therapeutic values of onion and garlic. *Indian J ExpBiol* 64: 334- 336.

[3] Aworh, O.C. and Egounlety, M. (1985). Preservation of West African soft cheese by chemical treatment. *Journal of Dairy production Research*. 52: 189-195.

[4] Baba, K.M., Orire, A.O. and Suleiman, H W. (2004). Profitability of cheese production and marketing among rural women in Moro local Government area of Kwara state. Proc. *American Journal of Food and Nutrition*., 3(1): 13-21

[5] Belewu M.A and Aina (2000). Microbial evaluation of indigenous milk products with special reference to the bacterial flora of public health importance in Nigeria. *African Journal of Experimental Microbial analysis*. 1 (1): 13-19.

[6] Belewu, M.A., Belewu, K.Y and Nkwunonwo C.C. (2005). Effect of biological and chemical preservatives on the shelflife of West African soft cheese. *African Journal of Biotechnology*. 4: 1076-1079.

[7] Belewu, M.A., Belewu, K.Y. and Ganiyu, R.O. (2011). Evaluation of lemon Grass *Cymbopogon citrates*) oil and pure natural honey on the shelf life of cheese. *Electronic Journal of Environmental, Agricultural and Food Chemistry (EJEAFChe)*, 10(4), 2129-2134.

[8] Burnett and Beuchat, (2001). Emerging infectious Diseases. Produce Handling and Processing Practices; 5:6

[9] Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. Part 2 Press Syndicate of the university of Cambridge, United Kingdom pp. 63-70.

[10] FAO: Food and Agricultural organization.(2008). Milk for health and wealth .FAO, Rome, Italy. *Journal of agricultural*, 19:34-12.

[11] Ibrahim, T.A. and Falegan C.R. (2013). Anti-bacterial activities of crude cell free supernatants of Lactic acid bacteria from wara (Nigeria soft cheese). Research and Reviews..*Journal of food and Dairy Technology*, 1 (2):1-4.

[12] Ogunbanwo, S. T., Sanni, A. I. and Onilude, A. A. (2011). Effect of bacteriocinogenic *Lactobacillus* spp. On the shelf life of fufu, a traditional fermented cassava product. *World Journal of Microbiology and Biotechnology*, 20:57-63.

[13] Oladipo I.C., Onyeruke I.C. and Adebiyi, A.O. (2009); Microbiological analysis of some Vended cheese in Osun, Nigeria. *Afrrican Journal of Food Science*. 3 (12) pp: 406-412.

[14] Onyeagba , A. (2004) Laboratory guide for microbiology. Crystal Publishers, Okigwe, Imo State. pp 95-97.

[15] Raheem, V.D. and Saris, D.E.J. (2009). Characterization and Application of Calotropisprocera, a coagulant in Nigeria wara cheese. *International Journal of Food Science and Technology*. 20:23-12.

[16] Sanni A.I., Onilude A.A. and Momoh M.O. (2011). Selection of starters and a Starter-mediated novel procedure for production of wara, a West African soft cheese. *International Journal of Food Science Technology*, 34: 325-333.

AUTHORS

First Author – Ajayi, O. O, Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji Arakeji, Osun State, Nigeria.

Second Author – Balogun, O. B, Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji Arakeji, Osun State, Nigeria.

Third Author – Oriowo-Olaleye, M, Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji Arakeji, Osun State, Nigeria., Corresponding Author e-mail: ooajayi@jabu.edu.ng.

Fourth Author – Faturoti M. O, Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji Arakeji, Osun State, Nigeria.