

MICROBIOLOGICAL ASSESSMENT OF SOME LOCALLY RETAILED CHEESES WITHIN ILORIN METROPOLIS, NIGERIA

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Abstract: Microbiological quality of locally made cheeses ("wara") from ten different locations within Ilorin metropolis was assessed. The pH of the cheeses ranged from 6.3 – 6.8, while the titratable acidity of its whey ranged from 3.1 – 3.4 ml of 0.1N sodium hydroxide per 25ml of the whey. Similarly, the bacterial counts of the cheeses ranged from 4.6×10^6 to 4.0×10^8 cfu/g while the fungal loads ranged from 2.0×10^2 to 2.5×10^3 cfu/g. Nine bacterial species were isolated from the cheese: *Lactobacillus casei*, *Streptococcus* sp., *Bacillus cereus*, *Micrococcus* sp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes* and *Aerococcus* sp. In addition, four fungal species were isolated. These were *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Mucor racemosus* and *Candida* sp. These microorganisms could be from the food handlers, production utensils, and the environment. The pathogenic microorganisms among them such as *S. aureus*, *P. aeruginosa*, and *E. coli* are of public health significance. Improvement in the sanitary practices during the preparation and retail of cheese is recommended.

Key words: Cheese, Quality, Bacteria, Fungi, Contaminants.

INTRODUCTION

Milk is considered the most satisfactory single food preparation elaborated by nature. It contains proteins, fats, carbohydrates, inorganic salts and vitamins (Uzeh *et al.*, 2006). It's therefore the best nutrient medium for the growth of different types of organisms. Milk cannot be preserved in its fresh condition for a long time and soon it is fermented as a result of the life activities of different types of microorganisms that gain entrance into the milk from various sources (Mahanta, 1995). The easy spoilage of milk led to the processing of milk to different dairy products to prolong its shelf life and to get foods with different tastes and flavours. One of these products is cheese. Cheeses are made in many countries of the world including Italy, France, United States, Belgium, Britain, Switzerland, Nigeria etc. The process of cheese making can be

Copyright © 2015 Nigerian Society for Microbiology divided into a number of relatively simple unit operations which are: sieving of milk to remove undesirable dirt, heating to a temperature which favour the growth of lactic acid bacteria, coagulation by rennin, curd separation from whey, cutting or moulding of curd, salting and maturing which generally produces ripened cheese (Pelczar *et al.*, 2005). Slight variation in these processes of production and the milk used combined to give the wide range of cheese available in the world today which is said to include 78 different types of blue cheese and 36 camemberts (Adam and Moss, 1999).

"Wara" the Yoruba name for the Nigerian cheese is the equivalent of the soft unripened cheese produced in some other part of the world. It is common and sometimes served as meat substitute in soups and stews in Oyun and Ilorin areas of Kwara State and some parts of Oyo and Osun States especially when fried. It sells like hotcake in most garages and motor parks in Kwara State. Most cheeses are

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made of cow's milk, while some types are made from the milk of other animals (Genicorgis *et al.*, 1979). If milk is improperly pasteurized or not pasteurized, it could become a vehicle for pathogenic organisms when processed into cheese (Saliu *et al.*, 2014). To make "Wara", milk collected fresh from the udder of a healthy cow is first sieved to remove hair and other adhering debris. The sieved milk is then heated slightly and allowed to cool. The extract from the leaves and stem of the plant *Calotropis procera* (Sodom apple) is then squeezed into the milk. The preparation is then left for at least 30 minutes to curdle. It is then sieved to remove the whey from the curd. The sieving is done in small basket-like sieves which gives the "wara" its shape. "Wara" is best preserved by boiling and frying with the addition of salt to taste. "Wara" has moisture content of 56%, pH of 6.4, fat content of 18.55%, protein 23%, ash 1.5%, and carbohydrate about 1% (Uzeh *et al.*, 2006). All these and more add to its quality as a good substance for the growth of many bacteria, yeast, and moulds (Atinmo and Akinyele, 1983).

Contamination of cheese by bacteria occurs during or after processing. Other sources of contamination include water, food utensils, air, soil, dust, food handlers, packaging materials, milking machine, tanks, animals, and manure (Frazier and Westhoff, 2004). Contamination of cheese is a serious problem since many consumers believed that it is a ready to eat food and as such they might not cook it before eating. Some organisms that have been found to be pathogenic contaminants from infected cows to milk and then to man include *Mycobacterium* spp. causing tuberculosis; *Staphylococcus aureus* causing mastitis; *Brucella* spp. causing brucellosis; and *Coxiella burnetii* causing Q-fever (Nester *et al.*, 2005).

This research was conducted so as to ascertain the quality of locally retailed

cheeses. Therefore, the objectives of this research were to determine: the bacterial and fungal loads, and their species present in locally retailed cheeses; some of the physicochemical characteristics of cheeses; and proffer ways of preventing contamination of cheeses.

MATERIALS AND METHODS

Collection of cheese samples

Fresh samples of cheese ("Wara") were obtained from ten different locations within Ilorin metropolis. These were near Unilorin Senate Car Park, Unilorin Bus Stop, Tanke Oke-Odo, Tanke Tipper Garage, Oja-Oba Ile film, Oja-Oba Idiape, Sawmill Garage, Sawmill Motor Park, Saboline old Bridge, and Saboline Opomalu Road. Samples from each location were collected into clean, sterile polythene bags to prevent contamination and the samples were taken to the laboratory immediately for analysis. The cheese samples were collected in the morning between the hours of 8.00 – 9.00 am.

Physicochemical analyses

pH determination

The pH of the sample was taken using pH meter (Denver instrument model 20) which was first standardized with buffer solutions of pH 4, 7 and 9. About 5 gram of the macerated sample was weighed and 20ml of sterile distilled water was added and the mixture shaken on rotatory orbital for 30 minutes. The pH of the suspension was then determined by inserting the electrode of the pH meter into the solution and the pH value was read when the reading was stable (Daniyan *et al.*, 2011).

Titrateable acidity

The whey collected from the cheese sample was used for the titrateable acidity since it is the liquid left after curding of the milk to form cheese, and it is usually used to bath the cheese by the sellers so as to

prevent it from drying. Twenty five millilitre of the whey was added into a conical flask and 2 drops of phenolphthalein indicator was introduced. Then, 0.1N sodium hydroxide was used to titrate the solution until it turned from colourless to faint pink. The titratable acidity was expressed as the volume in millilitre of 0.1N NaOH used per 25ml of the filtrate (AOAC, 1990).

Microbiological analyses

Total bacterial counts

In order to isolate and enumerate bacteria, 1g of the cheese sample was macerated and serially diluted up to 10^{-5} . Nutrient agar was used for the isolation using pour plate method (Fawole and Oso, 2004).

Total and faecal coliform counts

Plating of 0.5ml of the aliquot was done at 10^{-1} and 10^{-2} dilutions using MacConkey agar and Eosin methylene blue agar medium for the isolation of total and faecal coliforms respectively. Spread plate technique was used for the isolations. Typical coliforms gave pink or red colonies on MacConkey agar, while faecal coliform (*Escherichia coli*) produced colonies with greenish metallic sheen on Eosin methylene blue agar medium (Willey *et al.*, 2011).

Fungal counts

Spread plate method was used to isolate and enumerate fungal population. The medium of choice was potato dextrose agar. Plating of the sample was done at 10^{-2} dilution (Fawole and Oso, 2004).

Preparation of pure culture of isolates

Subculturing was done in order to obtain pure culture of each isolate from the mixed cultures. The pure culture obtained was then preserved in a refrigerator until required for identification (Fawole and Oso, 2004).

Characterization and identification of isolates

The bacterial isolates were characterized on the basis of their colonial, cellular, and biochemical characteristics (Buchanan and Gibbon, 1974), while the fungal isolates were identified based on their macroscopic and microscopic characteristics (Onions *et al.*, 1981).

Statistical analyses

The statistical analysis package SPSS 15.0 was used to determine the mean, range, and standard deviation of the data, while the means obtained were then separated using Duncan's Multiple Range Test (SPSS, 2010).

RESULTS

Physicochemical analyses

The pH of the cheese ranged from 6.3 – 6.8, while the titratable acidity of its whey ranged from 3.1 – 4.8ml of 0.1N NaOH per 25ml of the whey. The cheese from Saboline (old bridge) had the highest titratable acidity of 4.8ml of 0.1N NaOH, while the cheese from Tanke (Oke Odo), Oja-Oba (Ile film), and Unilorin (Senate car park) had the highest pH (Table 1).

Microbiological analyses

The bacterial count ranged from 4.6×10^6 – 4.0×10^8 cfu/g, while the fungal count ranged from 2.0×10^2 – 2.5×10^3 cfu/g. Similarly, the total and faecal coliform count ranged from 0 – 30 cfu/g, and 0 – 6 cfu/g respectively (Table 2).

Characterization and identification of isolates

Based on the colonial, cellular, and biochemical characteristics; nine bacterial species were identified as follows: *Lactobacillus casei*, *Streptococcus* sp., *Bacillus cereus*, *Micrococcus* sp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*,

Enterobacter aerogenes, and *Aerococcus* sp. (Table 3). The four fungal species isolated

were: *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Mucor racemosus*, and *Candida* sp.

Occurrence of isolates

The occurrences of the bacterial and fungal isolates in the different cheeses collected from different locations were as presented in Table 4.

Table 1: Physicochemical properties of some cheeses with their whey being retailed within Ilorin metropolis

Sampling locations	Physicochemical parameters	
	pH	Titrateable acidity (per 25ml of the whey)
Unilorin Senate Car Park	6.8 ^d ± 0.2	3.1 ^a ± 0.1
Unilorin Bus Stop	6.4 ^{ab} ± 0.1	4.0 ^{ef} ± 0.2
Sawmill Garage	6.4 ^{ab} ± 0.1	3.8 ^{de} ± 0.1
Sawmill Motor Park	6.3 ^a ± 0.15	4.1 ^{fg} ± 0.2
Oja-Oba Ile film	6.8 ^d ± 0.2	3.3 ^{ab} ± 0.1
Oja-Oba Idiape	6.7 ^{cd} ± 0.15	3.4 ^{bc} ± 0.1
Tanke Oke-Odo	6.8 ^d ± 0.2	3.3 ^{ab} ± 0.1
Tanke Tipper Garage	6.5 ^{abc} ± 0.1	3.6 ^{cd} ± 0.2
Saboline old Bridge	6.3 ^a ± 0.1	4.3 ^g ± 0.2
Saboline Opomalu Road	6.6 ^{bcd} ± 0.15	3.6 ^{cd} ± 0.1

All values are mean of 3 replicates.

Values followed by the same superscript within the same column are not significantly different at $\alpha = 0.05$ based on Duncan's multiple range test.

Table 2: Microbiological counts of some cheeses retailed within Ilorin metropolis

Sampling locations	Bacterial count (x 10 ⁶ cfu/g)	Fungal count (x 10 ² cfu/g)	Total coliform count (cfu/g)	Faecal coliform count (cfu/g)
Unilorin Senate Car Park	5.0 ^a ± 1	20 ^d ± 3	3 ^b ± 1	0 ^a ± 0
Unilorin Bus Stop	250 ^e ± 10	21 ^d ± 2	0 ^a ± 0	0 ^a ± 0
Sawmill Garage	400 ^f ± 20	12 ^c ± 2	30 ^g ± 2	6 ^d ± 1
Sawmill Motor Park	190 ^d ± 15	6.6 ^b ± 0.2	25 ^f ± 2	5 ^{cd} ± 1
Oja-Oba Ile film	37 ^b ± 1	11 ^c ± 2	0 ^a ± 0	0 ^a ± 0
Oja-Oba Idiape	50 ^b ± 5	12 ^c ± 2	15 ^d ± 1	4 ^c ± 1
Tanke Oke-Odo	4.6 ^a ± 0.2	6.0 ^b ± 1	2 ^{ab} ± 1	0 ^a ± 0
Tanke Tipper Garage	40 ^b ± 3	2.0 ^a ± 1	10 ^c ± 2	0 ^a ± 0
Saboline old Bridge	73 ^c ± 4	4.2 ^b ± 0.2	20 ^e ± 3	2 ^b ± 1
Saboline Opomalu Road	12 ^a ± 2	25 ^e ± 2	0 ^a ± 0	0 ^a ± 0

All values are mean of 3 replicates

Values followed by the same superscript within the same column are not significantly different at $\alpha = 0.05$ based on Duncan's multiple range test.

Table 3: Characterization and identification of bacterial isolates

Bacterial isolates	Colonial colour	Colonial shape	Optical characteristics	Colonial elevation	Gram's reaction	Cell's shape	Cells' arrangement	Spore staining	Lactose fermentation	Oxidase	Starch hydrolysis	Citrate	Coagulase	Catalase	Methyl- red	Voges –Proskauer	Indole	Oxidation-Fermentation	Probable identity
I	W	Ci	Op	F	+	R	P	-	+	-	-	-	-	-	N	-	N	Fe	<i>Lactobacillus casei</i>
II	Cr	Ci	Op	Ra	+	C	Ch	-	+	-	-	-	-	-	-	-	-	Fe	<i>Streptococcus</i> sp.
III	Cr	Ir	Op	F	+	R	P	+	+	-	+	+	-	+	+	+	-	Fe	<i>Bacillus cereus</i>
IV	Cr	Rh	Op	Um	+	C	S	-	+	-	-	+	-	+	+	-	-	Ox	<i>Micrococcus</i> sp.
V	W	Ci	Op	Ra	+	C	Cl	-	+	-	+	-	+	+	+	+	-	Fe	<i>Staphylococcus aureus</i>
VI	Yg	Ci	Tl	F	-	R	S	-	-	+	-	+	-	+	-	-	-	Ox	<i>Pseudomonas aeruginosa</i>
VII	Cr	Ci	Tl	Ra	-	R	Ch	-	+	-	+	-	-	+	+	-	+	Fe	<i>Escherichia coli</i>
VIII	Cr	Ci	Op	Ra	-	R	P	-	+	-	-	+	-	+	-	+	-	Fe	<i>Enterobacter aerogenes</i>
IX	W	Ci	Op	Ra	+	C	Cl	-	+	-	-	-	-	-	-	-	+	Fe	<i>Aerococcus</i> sp.

Key: Cr = cream; W = white; Yg = yellow green; Ci = circular; Rh = rhizoid ;Ir = irregular; R = rod; C = cocci; Tl = translucent; Op = opaque; F, = flat; Ra = raised; Um = umbonate; S = Single; Ch = chain; P = pair; Cl = cluster Fe = Fermentative; Ox = oxidative; N = not determined - = negative reaction; + = positive reaction

Table 4: Occurrence of bacterial and fungal species in some cheeses retailed within Ilorin metropolis

S/N	Microbial isolates	Sampling locations									
		A	B	C	D	E	F	G	H	I	J
I	<i>Lactobacillus casei</i> *	+	+	+	+	+	+	+	+	+	+
	<i>Aspergillus fumigates</i> **	-	-	+	-	-	+	-	-	-	-
II	<i>Streptococcus</i> sp. *	+	-	+	-	-	+	-	+	-	+
	<i>Saccharomyces cerevisiae</i> **	+	+	-	+	+	+	+	-	+	-
III	<i>Bacillus cereus</i> *	+	+	+	-	-	+	+	+	-	-
	<i>Mucor racemosus</i> **	+	-	-	+	-	+	-	+	+	-
IV	<i>Micrococcus</i> sp. *	-	+	+	+	-	-	+	-	-	-
	<i>Candida</i> sp. **	+	-	+	+	+	+	-	+	+	-
V	<i>Staphylococcus aureus</i> *	+	+	+	+	+	+	+	+	+	+
VI	<i>Pseudomonas aeruginosa</i> *	-	+	-	-	+	+	-	-	-	-
VII	<i>Escherichia coli</i> *	-	-	+	+	-	+	-	-	+	-
VIII	<i>Enterobacter aerogenes</i> *	+	-	+	+	-	+	+	+	+	-
IX	<i>Aerococcus</i> sp. *	-	-	-	+	-	-	+	-	-	-

Key: +, isolated; -, not isolated; A, Unilorin Senate Car Park; B, Unilorin Bus Stop; C, Tanke Oke-Odo; D, Tanke Tipper Garage; E, Oja-Oba Ile film; F, Oja-Oba Idiape; G, Sawmill Garage; H, Sawmill Motor Park; I, Saboline old Bridge; J, Saboline Opomalu Road; *, bacterial isolate; **, fungal isolate

All the bacterial species encountered in the cheese samples can affect the keeping quality or the health of the consumers (Willey *et al.*, 2011). *Staphylococcus aureus* and *Micrococcus* sp. are associated specifically with the hands and nasal cavity. The deposition of these microorganisms in cheeses can occur if good sanitary practices are not followed by the food handlers. Growth of microorganisms on food can lead to its 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 degradative action of microorganisms (Ojokoh and Tabowei, 2002). The colour of cheeses used for this study was white. No discolouration was observed since the cheeses were most likely being sold in the market on the day they were produced. Abdulmalik and Abdulaziz (2011) isolated pathogenic flora *Salmonella* and *Listeria* from local Yemeni cheese. *Bacillus cereus* could come from soil dust since large number of *Bacillus* spp. occur in the soil (Atlass, 1997). *Bacillus cereus* can cause spoilage of dairy product due to its phospholipinase activity (Pelczar *et al.*, 2005). *Streptococcus* sp. could be introduced into the milk during hand milking process, and dirty utensils, while *Pseudomonas aeruginosa* could arise due to wound contamination (Adam and Moss, 1999). *Enterobacter aerogenes* is an agent of food spoilage as it causes ropiness or slimeness of food. The presence of *E. coli* in the cheese samples suggested a very poor level of sanitation either during or after the production. *Lactobacillus* spp. are both homofermenters and heterofermenters that ferment glucose or other fermentable hexose sugars to form lactic acid, acetic acid, and succinic acid in dairy products. *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus casei* can tolerate temperature in the range of 40 – 45°C. They cause normal fermentation of milk and are not pathogenic (Mahanta, 1995).

The fungal species encountered in the cheese (*Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Mucor racemosus*, and *Candida* sp.) could also come from the environment, production process, and the food handlers.

Tudor *et al.* (2009) reported that certain species of *Aspergillus* produce toxic metabolite, while *Mucor* sp. could degrade the biochemical structure of proteins and lipids thereby altering its organoleptic property. Frazier and Westhoff (2004) have implicated species of *Aspergillus* and *Mucor* in food poisoning and in the spoilage of cheese. *Saccharomyces cerevisiae* occurred in the cheese samples than the other fungi and this may be due to the fact that they are one of the most dominant organisms found in curdled milk. *Candida* sp. could come from the female handler's genital tract if their hands are not properly washed after urination.

CONCLUSION

It can be concluded from this study that most of the cheese samples obtained from the different markets are not fit for consumption since they have been contaminated by both pathogenic and spoilage microorganisms. It is important to keep cheese fit for consumption by taking adequate measures to prevent contamination during and after production of cheese.

Hence, the following recommendations would help to maintain the keeping quality of cheese. There is need to improve on the hygienic conditions surrounding cheese production. Pre-production and post-production operations should be adequately checked. Care should be taken not to use contaminated water in the course of production; and contact with soil and dust should be avoided. Clean bowls with covers should be used to keep the cheese; and spoons should be used for the selection of cheese for the consumers. Healthy cows should be used for milking. Food handlers with open wound should not be involved either in the production or sales of cheese. Utensils used for preparation of cheese should be washed well after each use to prevent contamination. Cheese should not be exposed to contamination from air and flies while hawking. The hair, nose, and mouth should not be touched during cheese preparation. Ring and other jewelries especially those worn on the wrist and fingers should not be worn during cheese preparation as they can

harbour dirt and germs. Finally, cheese should be boiled or fried before eaten by the consumers.

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