ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY. MAY 2014 ISBN 1595-689X VOL15 No.2
AJCEM/1412 **http://www.ajol.info/journals/ajcem**
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AFR. J. CLN. EXPER. MICROBIOL. 15(2):76-83

MICROBIAL CONTAMINATION OF LOCALLY PRODUCED CHEESE AND DETERMINATION OF THEIR ANTIMICROBIAL POTENTIAL IN NIGERIA

Ogbolu, D. O., Terry Alli, A. O., Oluremi , A. S., Olanrewaju, A. A.

Department of Biomedical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Correspondence: D. Olusoga Ogbolu, Department of Biomedical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. E-Mail: olusogadave@yahoo.com. Mobile: +2347055776547

ABSTRACT
The high consumption rate of soft cheese and manner of cheese production in Nigeria prompted the need to determine the microbial quality and antimicrobial properties of locally produced cheese in Nigeria. A total of 20 cheese samples were obtained from different points in 4 cities in southern Nigeria, 5 cheeses per city. They were investigated for some physico-chemical properties, isolation and microbial counts and determination of antimicrobial potential. There was no significant variation in the composition of physic-chemical properties of cheese samples from various cities except for the acidity of cheese sample obtained from Ilorin. All the 20 samples (100%) yielded low level of lactic acid bacteria (LAB) with counts ~ 103. *Escherichia coli* or *Klebsiella* species were constantly isolated in all the cheese samples. Similarly, yeast and *Aspergillus* species were isolated either alone or in a mixed culture. The result showed increase in total bacteria count from the point of production to the hawkers. Antimicrobial potential was not found in cheese against the microorganisms used in the study. The study identified local cheese ('wara') as a high risk food in Nigeria due to the high rate of contamination since they are ready-to-eat food item and no antimicrobial property detected in the soft cheese.

RUNNING TITLE: MICROBIAL CONTAMINATION OF CHEESE

Key Words: Cheese; Bacteria; Fungi; Nigeria, Susceptibility

LA CONTAMINATION MICROBIENNE DES FROMAGE PRODUITS LOCALEMENT ET DETERMINATION DE LEUR POTENTIEL ANTIMICROBIEN AU NIGERIA

D. Olusoga Ogbolu *, AO Terry Alli, AA Olanrewaju, AS Oluremi

Département des Sciences Biomédicales , Collège des Sciences de la Santé , Université de Technologie de Ladoke Akintola, Ogbomoso , Nigeria

Correspondance: D. Olusoga Ogbolu , Département des Sciences Biomédicales , Collège des Sciences de la Santé , Université de Technologie de Ladoke Akintola, Ogbomoso , Nigeria . E -Mail: olusogadave@yahoo.com. Téléphone Mobile: +2347055776547

RÉSUMÉ
Le taux de fromage à pâte molle et les modalités de production de fromage au Nigeria à forte consommation a incité la nécessité de déterminer la qualité microbienne et propriétés antimicrobiennes de fromage produit localement au Nigeria. Un total de 20 échantillons de fromage ont été obtenues à partir de différents points dans 4 villes au sud du Nigeria, 5 fromages par ville. Ils ont été étudiés pour certaines propriétés physico-chimiques, l'isolement et les numérations microbiennes et détermination du potentiel antimicrobien. Il n'y avait aucune variation significative dans la composition des propriétés physico- chimiques des échantillons de fromage à partir de différentes villes à l'exception de l'acidité de l'échantillon obtenu à partir de fromage de la ville d'Ilorin. Tous les 20 échantillons (100%) ont donné un faible niveau de bactéries lactiques (LAB) avec environs 103 espèces. *Escherichia coli* ou *Klebsiella* ont été constamment isolés dans tous les échantillons de fromage. De même, des espèces de levures et d'Aspergillus ont été isolés soit seuls, soit dans une culture mixte. Le résultat a montré l'augmentation des bactéries totales compté du point de production aux colporteurs. Potentiel antimicrobien n'a pas été trouvé dans le fromage contre les micro-organismes utilisés dans l'étude. L'étude a identifié fromage local (' wara ') comme un aliment à haut risque au Nigeria en raison du taux élevé de contamination, car ils sont prêts à consommer l'aliment et aucune propriété antimicrobienne détecté dans le fromage à pâte molle.
TITRE COURANT : LA CONTAMINATION MICROBIENNE DES FROMAGES

Mots clés: Fromage; bactéries; champignons; Nigeria, sensibilité

INTRODUCTION

Soft cheese ('wara'), in one of the ethnic language from Nigeria, (Yoruba) is a very nutritious food obtained from cow milk. Milk is an aqueous colloidal suspension of proteins, fats, and carbohydrates that contains numerous vitamins and minerals. Cheese is one of the numerous products obtained from the processing of milk (1). The production of cheese in African countries has increased and about one third of the total volume of milk is used for this purpose (2). This soft cheese produced in some parts of southern Nigeria and predominantly in the northern parts makes use of local ingredients. Cheese making began about 8000 years ago and now there are in excess of 1000 cheese varieties worldwide (3) each unique with respect to its flavour and form. Manufacture of most cheese varieties involves combining 4 ingredients; milk, rennet, microorganisms and salt. Variations in ingredient blends and subsequent processing have led to the evolution of all these cheese varieties. The manufacturing process of soft cheese in Nigeria is undeveloped and at its infancy left with the natives to manufacture locally, hence there is difficulty in extending shelf life and conserving the nutritious components of milk.

Microorganisms found in cheese can be classified based on their biochemical types, temperature, response and ability to cause infection and disease (4). Organisms associated with milk and milk products include streptococci, lactobacilli, coliform bacteria and some fungi. All of these are from various sources and act on different substrates in cheese thereby producing various end products (5). Soft cheese has also been found to have outstanding antimicrobial properties. It contains a variety of factors and compounds which have been reported to have health promoting effects and prevent disease (6-8). Several studies have found lactic acid bacteria (LAB) to occur naturally as an indigenous microflora in cheese (9, 10). The lactic acid fermentation that these bacteria carry out has long been known and applied by human for making different food stuffs (11). Lactic acid bacteria had the highest percentage occurrence (76%), followed by enterobacteria (17%), and *Staphylococci* (7%). Four genera of lactic acid bacilli have been isolated, they were: *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Pediococcus* (11). Survey of soft cheese have found *E. coli* in 40-50% of the products (12) demonstrating that a mode of contamination with *E. coli* exist during the production or processing.

Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antimicrobial activities of cheese. Some of them have been seen to have antimicrobial effect which automatically has been conferred to the soft cheese (13,14). Lactic acid bacteria improve the quality of cheese and also play an important role in preventing the growth of undesirable bacteria like coliform (13). The lactic acid bacteria create an acidic environment conducive for proliferation of yeast while yeast provides growth factors such as vitamins and amino acid for lactic acid bacteria. Cheese however has been shown to have antimicrobial properties (6) and also prevent disease. It has been used as drug for certain infection when common antimicrobial agents failed. It has also been found that soft cheese has growth inhibitory activity against common bacteria that causes diarrhoea in South West Nigeria (15, 16).

The trend in manufacture of cheese all over the world is the production of flavoured, highly nutritive and good microbiological quality. Due to the high consumption rate of soft cheese and in view of manner of cheese production in Nigeria, there was need to determine the microbial quality which will be of utmost public health importance. Moreover, in order to continue in the search to developing natural novel antimicrobial agents that will be effective against a spectrum of commonly isolated microbial pathogens as done previously by the authors using various plant extracts with antimicrobial potentials, garlic (17); coconut oil (18). It was also important to determine the antimicrobial properties of cheese in view of multiple drug resistance nature of Gram negative bacteria pathogen in Nigeria (19) and presence of MRSA (20) and possible emergence of VRSA. This indicates a limited drug for use in such organisms. The study however investigates the extent of microbial contamination and antimicrobial properties of locally produced cheese in Nigeria.

MATERIALS AND METHODS

Cheese Samples

Twenty samples of soft cheese with the whey (water portion) were bought from hawkers and point of production at different locations of 4 cities in southern Nigeria; Osogbo, Ede, Ogbomosho and Ilorin. The soft cheese and the whey were put inside a sterile plastic universal container. The samples were transported immediately to the laboratory for processing or kept inside a refrigerator for few minutes if delay was envisaged.

Physico-chemical analysis

The moisture contents of cheese were determined by the oven drying method at 105ºC (21). About 5 g of the cheese sample was weighed and difference between the wet and dried weight of cheese represents the moisture content. The pH of cheese suspension at 2% strength was determined at room

temperature (29.7°C) using electrodes of a pH meter (Hanna instrument) placed directly into each suspension. The pH meter with accuracy of 0.1 was first standardised using buffer solution of pH 4 and 7. This was done in triplicate and the mean pH of each sample was determined.

Qualitative estimation of protein and glucose was done for all the samples using Combo 2 strips. The strip was dipped into the samples and allowed to stay for 45 sec; colour change was checked with the standard gradients of colour provided by the manufacturer. Sterile distilled water and standard protein or glucose solutions were used as controls.

Microbiological analysis

Viable microbial count analyses were performed on samples of cheese as follows; a 10-fold serial dilution of up to 10-10 for each sample was prepared in 0.1% peptone water. For viable bacterial count, each dilution was subsequently plated onto standard plate count agar (PCA). The PCA plates were incubated at 37°C for 48 hours. The colony forming units (CFU) were counted on plates having between 30 and 300 colonies using Quebec colony counter (22). The enumeration of viable bacteria count was carried out in duplicate on each sample and the isolated bacteria were identified using standard bacteriological procedures (23). Similarly, for fungi Saborauds dextrose agar (SDA) was used to plate the dilutions. The plates were incubated at room temperature in a moistened and dark environment for 3 to 5 days. The mean of colony forming unit per gram and log10 cfu were calculated and recorded. Middlebrook agar was also inoculated after previous treatment of the sample with NaOH to decontaminate the samples for possible isolation of *Mycobacterium* species. This was then incubated for 2 weeks.

Antimicrobial potential

In order to determine antimicrobial property of the cheese samples 10 g of each 20 samples of cheese was dissolved in 100 ml of whey. After extraction, filtration was done with the aid of sterile membrane filter of pore size 0.22 µm (Curringham, UK). The sterile extracts obtained were used to determine antimicrobial property using 45 microorganisms (*Escherichia coli*, 10; *Klebsiella pneumoniae*, 10; *Pseudomonas aeruginosa*, 10; *Staphylococcus aureus*, 10; *Candida albicans*, 5) from our collections. They were previously identified using API strips (bioMérieux, Marcy l'Etoile, France) accordingly and comprise of susceptible and multiresistant strains. These strains were from various clinical specimens (wound, urine, blood culture, sputum, catheter tip and ear swab). Inoculum obtained from overnight culture, suspended with sterile distilled water was adjusted to 0.5 MacFarland standards at 500 nm absorbance (24). Reference strains *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 and *Candida krusei* ATCC 6258 were included as control strains. The minimum inhibitory concentration of cheese for each isolate was determined using punch-hole agar diffusion method. With the aid of sterile swab sticks, the bacterial suspensions were inoculated separately on plates and wells of appropriate diameters were made on the seeded plates. Varying concentrations of each extracts 100, 80, 70, 50, 40, 30, 20 and 10% were introduced into the wells of different plates with the aid of sterile automatic pipettes. The plates were labelled and incubated at 37°C for 18 to 24 h. After incubation, clear zones of inhibition around the wells indicate sensitivity; diameters of the clear zones were measured as index of the degree of sensitivity. Tests were carried out in triplicates to ensure accuracy and reproducibility of results. Sensitivity pattern was compared with the control strains

Statistical analysis

Collation of data was carried out using Epi-info software from Centre for Disease control and prevention, USA. Data were analysed using statistical package within the Epi-info software. ANOVA was used to determine whether there were significant differences in moisture content and pH values. Similarly, for bacteria or fungi count between or within cheese samples collected from varying locations. The p value less than 0.05 was considered to be significant.

RESULTS

Composition of cheese

The moisture contents of the cheese expressed in terms of water availability aw were found to range between 3.3 and 4.6. The average aw for Ede, Ilorin, Osogbo and Ogbomoso were 3.9, 3.8, 4.0 and 3.9 respectively, there was no statistical significant difference between aw of these samples within the city or across the cities; $P > 0.05$. Similarly, pH ranged between 5.5 and 6.8. The average pH from each city was as follows; Ede, 6.5; Ilorin, 5.9, Osogbo, 6.4; and Ogbomoso, 6.6. Cheese samples from Ilorin are more acidic than rest of the cities, $P>0.05$. The glucose and protein were measured qualitatively and results ranged from trace to ++ as shown in Table 1.

Viable bacterial count

All the 20 samples (100%) yielded low level of lactic acid bacteria (LAB) with counts ~ 103 (data not shown).

TABLE 1: SOME PHYSICOCHEMICAL PROPERTIES OF CHEESE

Sample	Location	aw	pH	Glucose	Protein
P	Ede	3.7	6.5	+	+
1	Ede	4.0	6.5	+	+
2	Ede	4.2	6.5	+	Trace
3	Ede	3.9	6.5	+	+
4	Ede	3.8	6.5	+	+
P	Ilorin	3.3	6.0	-	Trace
1	Ilorin	3.8	5.5	-	-
2	Ilorin	4.0	6.0	-	Trace
3	Ilorin	3.8	5.5	-	-
4	Ilorin	3.9	6.5	-	-
P	Osogbo	4.6	6.5	Trace	++
1	Osogbo	3.9	6.5	-	+
2	Osogbo	4.1	6.5	-	+
3	Osogbo	3.7	6.0	+	+
4	Osogbo	3.7	6.5	+	+
P	Ogbomoso	3.6	6.5	+	+
1	Ogbomoso	3.7	6.5	+	+
2	Ogbomoso	4.1	6.8	-	+
3	Ogbomoso	4.1	6.5	Trace	+
4	Ogbomoso	3.8	6.8	Trace	++

P - point of production; 1 - 1st Hawker; 2 - 2nd Hawker 3 - 3rd Hawker; 4 - 4th Hawker; aw - water availability. + - Low; ++ - Moderate

Also, *Escherichia coli* or *Klebsiella* species were constantly isolated in all the cheese samples. On Sabouraud agar, yeast and *Aspergillus* species were isolated either alone or in a mixed culture. Nine of the samples yielded *Aspergillus* species while 12 yielded yeast and none of the samples yielded *Mycobacterium species*. There was high viable bacterial count in all the samples of soft cheese. The result showed increase in total bacteria count from the point of production to the hawkers (Table 2). Statistically, there was no significant difference in the proportion of bacteria or fungi count detected from the samples between locations ($P = 0.29$; $P > 0.05$) and within each of the 4 locations ($P = 0.19$; $P > 0.05$, a representative value for one of the within location).

Antimicrobial property

There was however no antimicrobial properties detected in the soft cheese to the isolates. All the isolates tested were resistant to the aqueous or whey extracts of cheese, no zone of inhibition (0 mm diameter) (Table 3). *E. coli*, ATCC 25922; *P. aeruginosa*, ATCC 27853; *S. aureus*, ATCC 25923; *Candida krusei*, ATCC 6258 were used as control strains.

DISCUSSION

The presence of Gram negative bacteria (lactose fermenters), yeast and mould indicate that the soft cheeses hawks in Nigeria are contaminated. Gram negative bacteria had the highest number of occurrence followed by yeast and mould. There was no difference in the microbial load of these cheese samples obtained from different locations. This is in line with the works of Sangoyomi et al. (25) that isolated some members of *Enterobacteriaceae* including *E. coli*, and yeast from soft cheese, and Abou Dawood et al. (26) who obtained high counts for aerobic bacteria, mould and yeast. In this study, the yeast was present in more than 50% of the 20 samples bought at these locations. It is evident that yeast strains which have activities of amylase, protease and lipase will have an impact on the textural and taste profile of soft cheese. The trend in cheese manufacture is production of nature flavoured cheese made in short time with highly nutritive value and good microbiological quality for human consumption (27, 28).

TABLE 2: VIABLE COUNTS OF ORGANISMS ISOLATED FROM CHEESE CONTAMINATION

Location	Sample	Bacteria	Count (cfu/ml)	Fungi	Count (cfu/ml)
Ede	P	*E. coli*	2.9 × 106	ND	-
	1	*E. coli/Klebsiella* spp	3.0 × 106/2.5 × 104	ND	-
	2	*E. coli*	3.0 × 106	Yeast	1.0 × 103
	3	*E. coli/Klebsiella* spp	3.0 × 108/3.0 × 106	Yeast/*Aspergillus* spp	1.0 × 102/1.0 × 103
	4	*E. coli*	3.0 × 108	Yeast/*Aspergillus* spp	1.5 × 103/1.5 × 102
Ilorin	P	*E. coli/Klebsiella* spp	8.0 × 103/5.0 × 102	Yeast	1.2 × 104
	1	*E. coli/Klebsiella* spp	9.0 × 103/5.0 × 103	Yeast	2.0 × 103
	2	*E. coli*	8.4 × 103	Yeast/*Aspergillus* spp	1.5 × 103/2.0 × 103
	3	*E. coli*	1.2 × 104	Yeast	1.0 × 105
	4	*E. coli*	1.4 × 104	Yeast/*Aspergillus* spp	1.5 × 103/1.0 × 103
Osogbo	P	*E. coli*	1.7 × 104	*Aspergillus* spp	1.2 × 103
	1	*E. coli*	1.9 × 106	ND	-
Location	Sample	Bacteria	Count (cfu/ml)	Fungi	Count (cfu/ml)
	3	*E. coli/Klebsiella* spp	3.0 × 106/1.0 × 103	ND	-
	4	*E. coli/Klebsiella* spp	2.0 × 108/2.0 × 104	Yeast/*Aspergillus* spp	1.0 × 104/1.8 × 102
Ogbomosho	P	*E. coli*	1.9 × 106	*Aspergillus* spp	1.0 × 103
	1	*E. coli*	2.0 × 106	*Aspergillus* spp	1.8 × 102
	2	*E. coli*	2.9 × 106	Yeast	2.0 × 105
	3	*E. coli*	2.7 × 106	Yeast/*Aspergillus* spp	1.0 × 104/1.5 × 103
	4	*E. coli/Klebsiella* spp	2.7 × 108/1.5 × 103	ND	-

ND- not detected

TABLE 3. NUMBER AND PERCENTAGES OF STRAINS INHIBITED WITH DIFFERENT CONCENTRATIONS OF CHEESE

Organisms	100% x (%)	80% x (%)	70% x (%)	60% x (%)	50% x (%)	40% x (%)	30% x (%)	20% x (%)	10% x (%)	5% x (%)
E. coli, n = 10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
K. pneumonia, n = 10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
P. aeruginosa, n = 10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
S. aureus, n = 10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
C. albicans, n = 5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

n = number of strains; x = number of strains that show inhibition

The samples consistently contained *E. coli* and *Klebsiella* species; this is similar to the study of Vigano et al. (29) where 98% of milk samples grew *E. coli*. Also, retail surveys by Aureli et al. (12) in Italy of soft and semi cheeses found *E. coli* in 40 - 50% of these cheeses demonstrating that a mode of contamination of *E. coli* exists during the chain of production or processing. *E. coli* is a consistent inhabitant of the human intestinal tracts and regular presence of the bacterium in the human intestine and faeces has led to tracking the bacterium in nature as an indicator of faecal pollution. Through this, it means wherever *E. coli* was found, there may be faecal contamination (30). The methods of transportation,

handling and sale of cheese or cheese products are not hygienic or sterile enough. One major observable problem is that the producers or vendors are really not educated and locals which consequentially affect the methods and handling of these products. Bhat et al. (31) had earlier opined that unclean hands of workers, poor quality of materials used and water supplied for washing utensils could be the source of accelerating the bacterial contamination of milk products. It has been reported that contamination of raw milk and cheese poses a significant risk to humans (32).

The high water availability and a neutral pH; these physical conditions favour the growth and survival of these bacteria, and these consequently caused the low level of bacteria count in LAB and vice versa. An increase in the moisture content of cheese could lead to increased susceptibility to spoilage. Similarly, the optimum pH for the growth of most common bacteria is around neutral, and growth is often poor at pH values <5.0. This explains in part the reasons why the samples were heavily contaminated.

It was observed from the present work that soft cheese did not have antimicrobial property against tested bacteria. This however is contrary to the work done by Olorunfemi et al. (33) who reported that soft cheese had antimicrobial properties against different organisms. There is a high level of contamination of soft cheese samples bought and this could account for the absence of antimicrobial properties in the sample. Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate antibacterial compounds in plants (14, 34) and food and food products (18).

In conclusion, local cheese ('wara') has been identified as a high risk food in Nigeria due to the high rate of contamination since they are ready-to-eat food item. They however do not have any antimicrobial activity. There is a need for standardising the production methods in order to set a benchmark for minimum standard of cheese quality.

Conflict of interest

We declare that we have no conflict of interest.

Authors Contributions

OAT contributed and advised DOO on concept and design of the experiment. DOO, AAO and ASO did preliminary work while the rest of the experiment was done by AAO and ASO under the supervision of OAT who also did the statistical analysis. The write-up was executed by DOO with contributions and proof-reading by other authors.

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