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MICROBIAL ASSESSMENT OF FRESH RAW MILK CHEESE SOLD IN RETAIL IN SOME SELECTED COMMUNITIES IN IBARAPA ZONE OF OYO STATE, NIGERIA

***Tairu, H.M, Adaramola[1], K.A, Kolawole[2], T.A and Olawale, B.R[3].**
Department of Science Laboratory Technology,
Oyo State College of Agriculture and Technology, P.M.B.10, Igboora,
Oyo State, Nigeria.

REVIEW ARTICLE

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Abstract: The safety of raw milk cheese is dependent upon a range of hurdles that influence the presence, growth, survival, and inactivation of pathogenic microorganisms. The objective of this study is to determine the microbial quality of fresh raw milk cheese sold in retail in some selected places in Ibarapa zone of Oyo State, Nigeria. For this reason, it is important to monitor the microbiological quality of dairy products and, in particular, the total viable count, total coliform count, and fungi count as they are indicators of the hygienic state of these products. Cheese samples were purchased at Igangan, Igboora, and Lanlate which are the three communities in Ibarapa namely; Ibarapa North, Ibarapa Central, and Ibarapa East Local government areas and labeled zone A, B, and C respectively. About eight (8) micro-organisms were isolated in all the samples, which are *Clostridium perfringens*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas spp*, *Listeria spp*, yeast and mould. The Proximate composition of the cheese samples exhibited high value in zone B on the crude protein which could curb protein deficiency, The pH values of the samples before fermentation were close to neutral (pH 7) but after fermentation, the pH values dropped indicating increasing acidity of the samples and the total titrable acidity of the cheese samples in which zone B and C had highest values compared to zone A. Microbiological analysis varied significantly ($P < 0.05$) among the three zones. TVC, TCC and TFC revealed were high in all the samples compared to WHO/USEPA standard for the count (/mL) and hence, the mandatory adoption of a food safety management system based on the Hazard Analysis Critical Control Point (HACCP) should improve the quality of dairy products.

Keywords: Cheese, pathogenic microorganism, Igangan, Igboora and Lanlate

INTRODUCTION.

Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms (Ruegg, 2003 and Rajagopal, et. al., 2005). The microbiological quality of milk and dairy products is influenced by the initial flora of raw milk, the processing conditions, and post-heat treatment contamination (Richter, et al., 1992).

Milk is the major source of raw materials used in the production of cheese with the influence of pathogenic microorganisms. The primary source of contamination in raw milk cheese is from the raw milk itself, as the milk does not receive a pathogen elimination process such as pasteurization. Other sources of contamination are the cheese-making environment including equipment, personnel, or cross-contamination between finished products and raw materials. These sources of contamination apply equally to both pasteurized and raw milk cheeses.

Cheese is the curd or hard substance formed by the coagulation of milk of certain mammals by rennet or similar enzymes in the presence of lactic acid produced by added or adventitious microorganisms in which part of the moisture has been removed by cutting, warming and or pressing, which has been shaped in a mould and then ripened by holding for sometime at suitable temperatures and humidity. The conventional method for the production of cheese has been discussed extensively by Frazier and Westhoff (1988). Starter cultures in cheese making is a medium of harmless, active microorganisms, which by growing in cheese milk and curd assist the development of mature cheese with desirable characteristics of flavour, aroma, pH, texture and body (Scott *et al*., 1998). Standard cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have been employed as starter cultures for cheese production (Frazier and Westhoff, 1988).

Cheese is generally made from cow milk, but in some countries and for making certain varieties of cheese, milk of other mammals is used (Helen and Elisabeth, 1990). For example, Ewe's milk is used for making Roquefort cheese and varieties such as Feta, Ricotta, Pecorino, etc.; goats' milk for making varieties of cheese in Italy and Greece and Buffalo's milk in India and Egypt.

They also observed that organisms present consist of psychrotrophs mostly *Pseudomonas*, *Aeromonas*, *Alcaligens*, a small number of lactic acid bacteria, spore-forming gram-positive rods, coryneform bacteria, *Micrococcus* and coliforms of these, only the psychrotrophs will multiply during transport and storage, particularly if the temperature in insulated tanks and milk silos is allowed to rise.

The temperature employed could also determine the type of starter to be employed. For example, a temperature of 38-40^{o}C will attract the use of a thermophilic starter. The study of the adaptation mechanism to temperature is useful in the

* Corresponding author:(Tairu, H.M, Adaramola1)
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optimization process, the choice of strain and fermentation protocols during cheese production.

The production of cheese involves the activity of microorganisms referred to as lactic acid bacteria (LAB). These bacteria are widely used as starter strains for fermentation. LAB has the ability to match various types of the temperature shift. They have two metabolic pathways which lead to either homolactic or heterolactic fermentation. It is possible to use a single-strain starter as in the case of *Streptococcus lactis* or a combination of both (Billie *et al*., 1985). It is also possible to employ multi-strain starters (Timson *et al*., 1982) or mixed strain starters involving the mixture of strains of *Streptococcus cremoris*, *Streptococcus lactis*, *Streptococcus diacetylactis* and *Leuconostoc* (Timson *et al*., 1982).

The microbiological quality of dairy products is influenced by the initial flora of raw milk, the processing conditions, and post-heat treatments. Spoilage bacteria and various bacteria of public health concern can be found in these products and their concentrations should be kept as low as possible (Varga, 2007). In contrast, lactic acid bacteria (**LAB**), occurring in the indigenous microflora of raw milk and being the major components of starter cultures used in fermentation, contribute to the quality of fermented cheese products by improving the taste and texture and inhibiting food spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid (Jana and Mandal, 2011). Thus, to be confident of fermented cheese quality, LAB concentration should be monitored during cheese production.

Undesirable microbes that can cause spoilage of dairy products include Gram-negative psychrotrophs, coliforms, lactic acid bacteria, yeasts, and molds. In addition, various bacteria of public health concern such as *Salmonella* spp, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, pathogenic strains of *Escherichia coli* and enterotoxigenic strains of *Staphylococcus aureus* may also be found in milk and dairy products (Tatini and Kauppi, 2003). For this reason, increased emphasis should be placed on the microbiological examination of milk and dairy foods.

Microbiological analyses are critical for the assessment of quality and safety, conformation with standards and specifications, and regulatory compliance. In the international literature, there is a relative scarcity of data pertaining to the levels of spoilage organisms and pathogens in commercially available milk products. Therefore, the aim of this study was to assess the microbial quality of fresh raw milk cheese sold in retail in some selected places in the Ibarapa zone of Oyo State, Nigeria.

LITERATURE REVIEW

European Union (EU) Directives define raw milk as “milk produced by secretion of the mammary glands of one or more cows, sheep, goats, or buffaloes from a single holding that has not been heated beyond 40°C or undergone any treatment having a similar effect”

Cheese is an ancient food whose origins may predate recorded history. Probably first discovered in Central Asia or the Middle East, cheese making then spread to Europe. The exact origins of cheese making are debated or unknown, and estimates range from around 8000 BC (when sheep were domesticated) to around 3000 BC.

When the milk is kept warm, it rapidly became sour and separated into curds and whey. In the absence of liquid milk, the curd is supplied as supplement as much of the milk value is retained. Cheese making likely began as a way of preserving soured and curdled milk through pressing and salting, with rennet introduced later, perhaps when it was noticed that cheese made in an animal stomach produced more solid and better-textured curds.

Classification of cheeses

According to Helen and Elisabeth (1990) cheese is classified into three categories-soft, blue-veined and hard-pressed cheese. This varies in moisture content and therefore in keeping quality and method of ripening. Soft cheese retains a high proportion of moisture (whey) 55- 80% and these varieties are eaten fresh, whilst others are ripened, usually by the growth of surface moulds. Semi-soft cheeses are made from slightly firm curds 45-55% moisture and are ripened by the surface growth of microorganisms particularly by *Brevibacterium linens*. These are the smear-ripened cheese (Helen and Elisabeth, 1990). Blue-veined cheeses such as Stilton, Roquefort, and Gorgonzola are made from semi-soft or semi-hard curd 42-52% moisture and are ripened by species of *Penicillium*, which grow with the cheese. Semi-hard cheeses such as Edam and Gouda are made from firmer curd with moisture content within ranges of 45-50%. These are ripened by bacteria and consumed within 2-3 months.

The hard-pressed cheeses are made from relatively dry curd 35-45% moisture, ripened by bacteria, and mature slowly within 3-12 months. The very hard, grating cheeses such as Parmesan, Romano, and Asiago are made from curd below moisture 26-34%, made partly from skimmed milk and are ripened by bacteria slowly over a period of one to two years (Specialist Cheese Makers Association, 2002).

Microbial contamination of cheeses

Microbial contamination, causing approximately one-fourth of the world’s food supply loss, has become an enormous economic and ethical problem worldwide (Huis in ‘t Veld, 1998). Dairy products are an excellent growth medium for a wide range of microorganisms and, thus, display a reduced shelf life (Ruegg, 2003). The microorganisms that may be present in the contamination of cheese are psychrotrophs; mostly *Pseudomonas*, *Aeromonas*, *Alcaligens*, a small number of lactic acid bacteria, spore-forming gram-positive rods, coryneform bacteria, *Micrococcus*, and coliforms. Of these, only the psychrotrophs will multiply during transport and storage, particularly if the temperature in insulated tanks and milk silos is allowed to rise

METHODOLOGY

Description of the study environment

The study environments were three selected communities in the local government areas of Ibarapa zone in which the cheese samples were purchased at Igangan, Igboora and Lanlate which

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are the three communities in Ibarapa namely; Ibarapa North, Ibarapa Central and Ibarapa East respectively.

Sample collection

The cheese samples were purchased at three different locations of Ibarapa communities in Oyo State Nigeria. The samples were kept in an ice flask and taken to the Department of Science Laboratory Technology of Oyo State College of Agriculture and Technology, Igboora where the experiment was carried out.

Microbiological analysis of the cheese samples

Total viable count (TVC), Total Coliform Count (TCC), and Fungi count (TFC) were evaluated according to the methods described by Olusegun and Jacob, 2013.

Nutrient agar

This was used for the determination of total viable bacteria in the sample. The plates were incubated at 37^{oC} for 24-48 hours.

MacConkey agar

This was used for the enumeration of total coliform organisms in the sample. The plates were incubated at 35^{oC} for 24- 48 hours.

Sabouraud dextrose agar

This was used for the enumeration of mould and yeast in the sample. The plates were incubated at 30^{oC} for 24 h for yeasts and 3-5 days for mould.

PH determination

About 10 ml of the cheese sample was dispensed into a conical flask and its pH was determined. The pH meter was standardized using a standard buffer of pH 4.0 and 7.0.

Determination of the total titrable acidity

This was done by dispensing 10 ml of the fermented cheese sample into conical flasks and adding 3 drops of phenolphthalein indicator. Thereafter, 0.1N NaOH was used for titration to a noticeable pink colour for endpoint determination.

Proximate Composition of the samples

Crude protein, Crude fibre, protein content, fat contents, Moisture contents, ash contents, Dry matter and carbohydrates were estimated as per AOAC (2002).

Statistical Analysis:

The data generated from these investigations were analysed using Statistical Package for Social Sciences (SPSS) version 20.0. They were subjected to one-way analysis of variance (ANOVA) and test for significance were carried out using Duncan multiple range tests (DMRT)

RESULTS

Table 1

The average pH values of the fresh milk samples in each zone during cheese production

Zone	pH	
	Before production	**After production**
A	6.83	4.02
B	6.45	3.84
C	6.76	3.92

Key: A= IBARAPA NORTH
B= IBARAPA EAST
C= IBARAPA CENTRAL

Table 2

Total titrable acidity of the sample in each zone during cheese production

Zone	Titre values			Lactic acid (%)
	1st titre	2nd titre	3rd titre	Average titre
A	1.41	3.02	4.12	2.85
		0.10		
B	1.98	3.45	4.75	3.39
		0.12		
C	1.67	3.11	4.31	3.03
		0.11		

Note: The acidity was calculated as lactic acid using the relationship:

$$\textbf{Lactic acid (\%)} = \frac{\text{Titre value x Normality of Alkali x 9}}{\text{Volume of sample}}$$

Normality of alkali = 0.1, **Volume of sample** = 25 ml

Table 3

Determination of the average values of proximate composition of fresh milk cheese samples

Zone	% Crude protein	% Crude fibre	% Protein content	% Fat content	% Moisture content	% Ash content	% Dry matter	% Carbohydrate
A	58.47	11.00	12.07	9.10	65.96	0.29	0.01	12.58
B	59.04	13.04	12.34	11.01	65.13	0.45	0.01	11.07
C	53.96	12.99	11.89	9.08	68.02	0.41	0.01	10..60

Note: Carbohydrate (%) = 100-(sum of moisture, protein, ash and fat)

Table 4

The average value of Total aerobic counts, Total coliform counts and fungi counts of fresh milk cheese samples in each zone compared to World Health Organization or United State Environmental Protection Agency

Zone	Total viable count (log cfu/ml)	Total coliform count (log cfu/ml)	Total Fungi count (log cfu/ml)	WHO/USEPA standard for the count (/mL)
A	1.04±0.11	1.02±0.13	1.03±0.12	0.00
B	0.02±0.12	0.09±0.16	0.06±0.18	0.00
C	1.02±0.32	1.03±0.14	1.01±0.17	0.00

Five different dilutions of each bacterial strain were tested in duplicate. The values are mean± SD of 5 experiments carried out in triplicate in each zone.

Table 5

Microorganisms isolated from the fresh milk cheese samples in each zone

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Zone	Bacteria (both gram-positive and negative)		Fungi
A	*Clostridium perfringens*	*Staphylococcus aureus*	Yeast and mould
	Escherichia coli	*Bacillus cereus*	
	Pseudomonas spp	*Listeria spp*	
B	*Clostridium perfringens*	*Staphylococcus aureus*	Yeast
	Escherichia coli	*Bacillus cereus*	
C	*Clostridium perfringens*	*Staphylococcus aureus*	Yeast and mould
	Escherichia coli	*Bacillus cereus*	
	Pseudomonas spp	*Listeria spp*	

DISCUSSION

Table 1 shows that the pH values of the samples before fermentation were close to neutral (pH 7) but after fermentation, the pH values dropped indicating increasing acidity of the samples. Zone B and zone C which had the lowest pH compared to zone A. These results are in line with findings of Salji *et al*., 1985, Varnam *et al*., 1994, Seo *et al*., 2009 and Uaboi-Egbenni et al.,2010 in their studies of yoghurt samples in which they obtained a pH value range from 3.00 - 3.82.

Table 2 shows the total titrable acidity of the cheese samples in which zone B had the highest values compared to zone A and C. The enhanced titrable acidity is due to the presence of lactic acid produced by lactic acid culture during fermentation. These results are in line with the findings of titrable acidity obtained in the study reported by Uaboi-Egbenni et al.,2010 in which they obtained a value of 0.049-0.137.

The average value of the proximate composition of fresh cheese samples is shown in Table 3 where zone B had higher crude protein content (59.04%) followed by zone A (58.47) and C (53.96). These results obtained were similar to those reported by Aworh and Akinniyi (1989); Fasakin and Unokiwedi (1992) and Uaboi-Egbenni et al.,2010. The entire zones had the same value of 0.01% in their dry matter and this was due to higher values in their moisture content. The higher the moisture content the lower the crude protein which shows the protein quality of the sample. The high protein content of this product shows that its consumption will help eliminate protein deficiencies that have become the bane of poor nations including Nigeria.

Table 4 shows that the microbial load varied significantly ($P < 0.05$) among the three zones. The results of an average value of total viable counts, total coliform counts and fungi counts of the cheese samples of each zone were contaminated with coliform and other bacteria. The results obtained in this present work indicate that cheese sold by retailers have a high microbial load indicating the low hygienic quality of the product. This may have originated from the initial microflora of raw milk and/or the other ingredients as well as the environment, insufficient heat treatment and poor personal hygiene (Jay, 1996). The TVC gives a quantitative idea of the presence of mesophilic aerobic microorganisms of animal origin. It serves as an important criterion to evaluate the microbial quality of various foods and also the degree of freshness of food (Nanu et al., 2007).

It was recorded that zone A had the highest value of TVC (1.04±0.11) followed by zone C (1.02±0.32) while zone B had the least and very close (0.02±0.15) compared to WHO/USEPA standard for the count (/mL). The higher TCC was recorded in zone C (1.03±0.14), followed by zone A (1.02±0.13) and the least value was recorded in zone B. Although the poor quality of water used to wash the serving scoop may also be a significant source of contamination for aerobic mesophilic bacteria, coliforms and *Escherichia coli* (Ergun and Civan, 1992; Kanbakan *et al*., 2004; Wilson *et al*., 1997).

Zone A recorded a high TFC value (1.03±0.12) and followed by zone C (1.01±0.17) while zone C had the least TFC value of 0.06±0.18 compared to WHO/USEPA standard for the count (/mL).

These high counts recorded from this work might have arisen due to the poor level of hygiene and sanitation observed in each zone. These results, therefore, points to the need for implementing regulatory measures like good manufacturing practices, hygienic distribution and retail storage practices for ensuring microbiological safety of cheese sold in open places. Likewise, personal hygiene should be emphasized as Kanbakan *et al*.(2004) stated that coliform contamination from the hands of personnel in the sales department was higher than from the hands of factory workers and handwashing with soap only may not be enough for the cleaning of hands.

The high counts of microorganisms above the recommended level and the presence of some groups of pathogenic bacteria may pose a risk to public health particularly of children and vulnerable elderly people. There is a necessity for ensuring a hygienic level of locally produced cheese in domestic or catering premises in Nigeria.

Table 5 shows the microorganisms isolated from the cheese samples in each zone where six (6) bacteria and two (2) fungi organisms were isolated from zone A and C while four (4) bacteria and a fungi organisms were isolated from B. The presence of *Clostridium perfringens*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas spp*, *Listeria spp*, yeast and mould in cheese samples may possibly be due to the unhygienic materials used in the cheese production as stated in previous works. The count for *E. coli*, *Pseudomonas*, *Staphylococcus*, *Clostridium* and fungi is considered as one of the parameters used for food hygiene quality. These organisms may have been from either insufficient pasteurization of milk, or human exposure. They find their way into the skin and wound either directly or indirectly. The most common skin sources are arms, hands, and face. In addition to skin and nasal cavities, *Staphylococcus aureus* may be found in the eyes, throat and intestinal tract. From these sources, the organism finds its way into air and dust, onto clothing, and in other places from which it may contaminate foods (Jay, 1996). It is obvious from the previous and the present data that cheese samples are frequently subjected to *Staphylococci* contamination which may indicate inadequate personal hygiene of workers or salespeople. *Escherichia coli* are commensal organisms that reside within the host gut, but some pathogenic strains are recognized as a cause of gastroenteritis (Callaway et

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al., 2003). Contamination from human and animal waste is traditionally indicated by the presence of commensal *E. coli*. Although these organisms are essentially non-pathogenic, their presence warns of the possible concurrent existence of pathogenic microbes (Sherfi et al., 2006).

The analyses concern the presence of pathogenic and contaminating bacteria but are still insufficient to guarantee the constant microbiological control needed for a particular product such as cheese. In this case in fact, it would be important to assess the microbiological quality before the product is sold and consumed to allow rapid intervention. Analysis should, therefore, be rapid, reliable, economical, and executable by the personnel without relying on external laboratories.

CONCLUSION

The presence of possible pathogenic organisms in the analyzed raw milk cheese samples in the three selected zones of Ibarapa should be monitored with concern by the producing company and the Government because food poisoning by *Bacillus, Staphylococcus, E.coli, Clostridium, Pseudomonas, listeria* and fungi species is possible through consumption of cheese. Some species of fungi are known to produce toxins which are very harmful to man, thus their occurrence in cheese is undesirable. A high level of hygiene is expected in the production of quality cheese especially at pre-and post-pasteurization stages and at retail. An increased level of staff training and transfer of knowledge, especially in relation to food safety, handling, maintenance and cleaning of machines and tools may improve the quality of production. The quality production of cheese is hereby recommended for both growing children and adults due to the retention of a high percentage of protein after fermentation and its expected ability to correct protein deficiencies. The mandatory adoption of a food safety management system based on the Hazard Analysis Critical Control Point (HACCP) should improve the quality of dairy products. The quality of the raw material prior to processing, manufacturing, and storage of the products under appropriate conditions should be given high priority.

In conclusion, the current investigation has indicated a poor overall level of hygiene in the services of openly sold raw milk cheese in some selected communities in Ibarapa zone of Oyo State.

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