MICROBIAL LOAD OF PUNCTURED EVAPORATED MILK SOAKED IN Cold WATER

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ABSTRACT

A total of four samples of evaporated milk (Peak, Nunu, Luna and 3 Crown) were analyzed for their microbial load when punctured and left open for 48h without proper storage. The percentage occurrence of *Staphylococcus aureus* and *Escherichia coli* obtained from Peak and Luna milk was 20% each while that of *Aspergillus species* was 50%. The percentage occurrence of *Pseudomonas fragi, Micrococus leteus, Shigella* species, *Klebsiella pneumoniae, Serratia marscens* and *Proteus mirabilis* were 10% each while *Rhodotorula* species *Penicillum chrysogenum* and *Rhizopus species* had a frequency occurrence of 20%, 20% and 10% respectively. The study shows that it is a health risk to consume evaporated milk exposed to air for or long period after opening.

INTRODUCTION

Milk is an opaque white liquid produced by the mammary gland of female animals. Milk has a high nutrient composition. It contains an abundance of water, minerals, protein, butterfats, sugar and vitamins. Because milk is a nearly perfect culture medium, it has been known to support microbial growth (Kathleen and Arthier, 2002).

There are different varieties of milk such as evaporated milk, condensed milk and dry milk. The microbial load of punctured liquid tin milk is simply the number of organisms that will be found when the punctured can is exposed to air. Evaporated milk is a shelf stable canned milk product from which about 60% of water has been removed.

Milk is canned and heat processed under steam pressure in an attempt to destroy all the micro-organisms present. Spoilage can take place only when the heat process is inadequate or if defects in the can permit the entrance of microorganisms. (Frazier and Westhoff, 1995).

Milk and dairy products are generally very rich in nutrients which provide an ideal growth environment for many microorganisms. The microorganisms associated with milk spoilage include: Psychrotrophic bacteria, which are bacteria that are capable of growing at 7^0C or less. They are of primary concern to dairy industry since they can grow and cause spoilage in raw and processed dairy products commonly held under refrigeration. Most commonly found in milk are the gram-negative rods belonging to the genus *Pseudomonas.*

These bacteria produce some enzymes that are neither inactivated by pasteurization nor by other local treatment. They continue to degrade milk products even when the bacterium is destroyed (Lee and Lin, 2002).

Serious taste and odour defects can appear duet to an accumulation of products resulting either from cell metabolism or from the effect of complex enzyme systems on the milk constituents. Many undesirable changes in organoleptic quality are possible when environmental conditions are conducive to microbial proliferation and enzyme activity. The following list of adverse changes is not restrictive. Most frequently one speaks of milk that is sour, bitter, fruity, rancid, malty, with an off -flavour taste, and also of dirty milk, etc. these forms of spoilage are associated with the growth of yeasts, moulds and bacteria. In view of its ecological characteristics, bacterials contamination is the most frequent and the greatest, and its potential development should be feared most of all. This contamination is responsible for two main types of defects: souring and lipolysis. The defects due to acidification are the most frequently encountered since lactic flora is one of the main natural contaminants of milk, being predominantly mesophilic in character.

Thermodieric bacteria are responsible for the spoilage of milk due to their ability to survive pasteurization and other local treatment while coliform bactier when detected in pasteurized milk generally indicate recontamination after pasteurization. These coliforms include *Escherichia coli, Klebsiella species, Enterobacter species*, and *citrobacter species* (Kathleen and Arthur, 2002).

The lactic acid bacteria are a group of bacteria capable of fermenting lactose to lactic acid which may be responsible for the sour taste and odour associated with spoilt milk. These bacteria are normally present in the milk and are used as starter culture in products such as yoghurt. (Pulusani, 1979). Lactic bacteria generally are not heat resistant and most of them are destroyed by low temperature pasteurization. However the survivors or recontaminating bacteria can be responsible for further souring if temperature conditions are favourable to their development. The predominant lactic flora in milk is mesophilic and the natural environmental conditions in warm and hot regions are often favourable to its proliferation. The use of cold treatment at different stages of production, processing and marketing makes it possible in practice to decrease considerably the dangers of spoilage due to uncontrolled proliferation of these lactic bacteria. However, it should be noted that in these conditions even with cold techniques the multiplication of microorganisms and enzyme changes are not wholly blocked and that irreversible changes in the organoleptic quality of the product can still occur.

MATERIALS AND METHODS

Four different brands of evaporated milk were purchased from four different stores in Abraka for this study. They include Peak and 3 Crown milk (Fresh land Foods Nig. Plc), Luna milk (Ganivas Nig. Ltd) and Nunu milk (Nutricima Nig. Plc). The four samples were left open to the air for two days.

MICROBIOLOGICAL ANALYSIS

Bacterial Counts

The four milk types were punctured and left open to the air for two days. 1ml from each sample was diluted ten-fold serially under strict aseptic conditions in the laboratory. The diluted samples were pour plated aseptically into triplicate plates of MacConkey agar and nutrient agar to obtain coliform counts and total aerobic viable counts respectively as previously described by Harrigan and MacCance (1982). The plates were left undisturbed for about 20 minutes to set after which they were incubated at 37^0C for 24h in an inverted position.

Bacteria growth was counted after 24h and suspected colonies were subcultured into freshly prepared nutrient and MacConkey agar plates to obtain pure colonies. The isolated pure colonies were transferred into slants and stored in the refrigerator until ready for identification.

Preparation of Milk Samples

Sixteen test tubes were sterilized for the preparation of the milk samples. The test tubes were shared into four tubes for each milk type and labelled accordingly.

Nine mililiter distilled water was dispensed into each of the test tube using a sterilized disposable pipette. Making use of fresh pipettes, 1ml of the different milk types was pipetted into the first tube in each group and mixed thoroughly. With the help of new pipettes, 1ml of the content of tube 1 from each group was transferred into tube 2 and mixed thoroughly. This process was continued in all the milk samples to tube 4. Finally, from tube 4 in each sample, 1ml was discarded. The content of tube 3 (10^3) in each milk sample was used for analysis.

Bacterial Culture

The pour plate method was used to determine the bacterial standard plate count.

Eight sterile Petri dishes, two for each different milk sample, were prepared for the culture of the bacteria. One milliliter from 10^3 dilution from each of the milk sample was transferred asceptically into teach of the two Petri dishes prepared for that sample using a sterile disposable pipette. Twenty milliliter of nutrient and Macconkey agar respectively was poured into each plates for each milk sample and mixed gently in a circular motion for 15 seconds to ensure uniform distribution of innoculum. The plates were left undisturbed for about 2o minutes after which they were put in

the incubator at 37^0C for 24h in an inverted position.

Bacteria growth after 24h of incubation was counted and suspected colonies were subcultured into freshly prepared nutrient and MacConkey agar by streaking method to obtain pure colonies. The isolated pure colonies were transferred into slants and stored in the refrigerator until ready for identification.

Fungi Culture

The diluted spoilt milk samples were also pour plated into sterile sabourand agar plates under strict aseptic condition as was previous described by Haringan and McCancel (1982). The agar plates were allowed to set and incubated at 28^0C for 7 days. Fungi growth observed were subcultured into freshly prepared sabourand agar plates using a sterile inoculating needle and incubated as above in order to obtain pure cultures of the various fungi isolates. These subcultures were carried out about five times in order to obtain pure fungi culture.

Identification of Fungi

The fungi were identified by picking a little quality of the fungi isolates with a piece of sterile wire loop and placing it on a clean glass slide. This was strained with lactophenol cotton blue stain and viewed with X40 objective. Their morphological characteristics, colour, size, shapes of colonies and spores as described by Harrigan and McCance (1982) were noted.

Identification of Bacteria

Bacteria identification was carried out using the string test (Archi, *et al* 2003) and Biochemical methods based on the Scheme of Buchanan and Gibbons (1974). The tests include catalase test, oxidase test, urease test, indole test. Glucose, lactase and fructose fermentation and production of hydrogen sulphide.

String Test

A loopful from the pure colony of the various bacterial isolates were emulsified respectively on the surface of a clean glass slide in a suspension of 3% KOH. The suspension was stirred continuously for about 60 seconds. The test is positive if stringing occurred within the first 30 seconds of mixing the bacteria in KOH solution. Those that are string positive are gram negative while those that are string negative are gram positive.

RESULTS

The result presented in tables 2 and 3 show a total of eight bacteria species namely S*taphylolococcus aureus, Pseudomnas fragi, Micrococcus Leteus*, *Eschericai coli, Shigella species, Klebsiella pneduromiae, Serratia marcescens and Proteus mirabilis* and four fungi species namely *Rhodotorula species, Aspergillus species, Penicillium Chrysogenum* and *Rhizopus species* were isolated from the milk samples used in this study.

Table 1, shows the total bacteria and fungi count from the milk samples. The result shows that the highest bacteria count of 7.2 x 10^4cfu/ml was recorded in Luna milk while the lowest, 4.0 x 10^4 cfu/ml was recorded in 3 Crown milk. Peak milk recorded the highest fungal population, 6.4 x 10^4 cfu/ml while the 3 Crown milk had the lowest fungal population, 3.6 x 10^4cfu/ml.

The percentage occurrences of both bacteria and fungi are shown in Table 2 and 3. *Staphylococcus aeureus and Rhodotorula species,* both have the same percentage of occurrence 20% for the Peak milk. The highest percentage of occurrence of 50% was recorded in Nunu milk by the *Aspergillus species,* while the lowest of 10% was recorded in 3 Crown, Luna and Peak milk by *Pseudonomas fragi*, *Serratia marcescens* and Proteus mirabilis respectively.

The bacteria isolated and identified are shown in table 4. They include *Staphylococcus aureus, Pseudonomas fragi, Micrococcus luteum, Escherichia coli, Klebsiella Pneumoniae, Shigella species, Sarretia marscens and Proteus species.*

Table 1: Total Bacterial and Fungal Count from Milk Samples

MILK	TOTAL BACTERIA COUNT (CFU/mL)	TOTAL FUNGAL COUNT (CFU/mL)
Peak Milk	4.8×10^4	6.4×10^4
Nunu Milk	6.4×10^4	5.2×10^4
3 Crown Milk	4.0×10^4	3.6×10^4
Luna Milk	7.2×10^4	4.4×10^4

Table 2: Percentage Occurrence of Bacterial Isolates from Spoilt Milk

Bacterial Isolates	Percentage Occurrence			
	Peak	Nunu	3 Crowns	Luna
Staphylococcus aureus	2(20)			
Pseudonomas fragi			1(10)	
Escherichia coli				2(20)
Shigella species				
Klebsiella Pneumoniae			2(20)	
Sarretia marscesns				1(10)
Proteus mirabilis	1(10)			
Micrococcus luteum			1(10)	

Table 3: Percentage Occurrence of Fungal Isolates from Spoilt Milk

Bacterial Isolates	Percentage Occurrence			
	Peak	Nunu	3 Crowns	Luna
Rhodotorula species	2(20)			
Aspergilus species		5(5)		
Penicillum chrysogenum			2(20)	
Shigella species				
Rhizopus species	1(10)			

Table 4: Biochemical Test for various Isolates on Milk Samples

Isolates	Cultural characteristics	String test	Grain reaction	Catalase	Oxidase	Coagulase	Urease	Indole	Methyl red	Citrate	Glucose	Latose	Sucrose	H_2S	Gas	Milk
1) taphylococcus aureus	Round smooth, raised and glistering, grey to deep golden yellow in colour.	-	+	+	-	+										Pea
2) taphylococcus aureus	Round smooth, raised and glistering, grey to deep golden yellow in colour.	-	+	+	-	+										Pea
3) Pseudonomas fragi	Green in colour, red in shape	+	-	+	+	+										Nu
4) Micrococcus luteum	Yellow in colour, cocci in shape	-	+	+	-	-										
5) Escherichia coli	Pink colour, raised, smooth colonies with distinct edges	+	-				-	+	+	-	+	+	+	-	+	Lu
6) Escherichia coli	Pink colour, raised, smooth colonies with distinct edges	+	-				-	+	+	-	+	+	+	-	+	Lu
7)Shigella species	Cream-white colour, circular, convex with entire margin	+	-				-	+	+	-	+	+	-	-	-	Nu
8) Klebsiella Pneumoniae	Pink colour with large mucoid colonies	+	-				+	-	+	+	+	+	+	-	+	3 C
9) Sarretia marscesns	Brilliant red pigment with serrated edges	+	-				+	-	+	+	+	+	+	-	+	Lu
10) Proteus mirabilis	Cream colour, wide spread over the entire surface of the plate with undulated edges	+	-				+	-	+	+	+	-	+	+	+	pea

KEY: *+ = Positive; - = Negative; d = Different strains give different results*

DISCUSSION

From the result obtained from this study it could be seen that the bacterial load for Peak milk and Luna milk were highest with *Staphylococcus aureus* and *Escherichia coli* showing 20% occurrence for each brand of milk (table 2). *Staphylococcus aureus, Escherichia coli, pseudomonas species and Bacillus species* are common flora of raw milk (Sherikar *et al* 2004). Therefore the occurrence of these bacteria in the different brand of milk could be as a result of improper pasteurization. The probable way these microorganisms could have contaminated the milk could be through the ubiquitous domestic insects, dust particles and aerosoil which might be the source of the microorganisms that contaminated the milk after opening.

Improper sterilization of equipment and containers used for milk can result in milk contamination. This is not at variance with the work of Birhanu, *et al* (2008) who isolated species of Pseudomonas and Bacillus from

milking vessel samples and, therefore concluded that they were the common environmental contaminants.

The Nunu and 3 Crown milk are less prone to bacterial contamination than the Peak and Luna milk. This could be as result of the inability of these microorganisms to fully metabolize the contents of the milk as materials for growth.

The fungi most involved in the spoilage of milk in this study was the *Aspergillus species* with a high percentage occurrence of 50% in the Peak milk while the Rhizopus species has a low occurrence of 10% in Luna milk. The high bacterial count of 7.2 x 10^4cfu/ml from Luna milk and fungi count of 6.4 x10^4cfu/ml from Peak milk indicate that it is a health hazard to consume evaporated milk exposed to the air for a prolonged period of time after opening.

CONCLUSION

This study has shown that the evaporated milk can serve as a suitable medium for the growth of bacteria and some fungi species as a total of 12 organisms were isolated from the four different samples. The presence of these microorganisms in these milk constitute great health hazard.

The result of this study suggested that if evaporated milk is not consumed immediately after opening it may serve as a source of infection if not properly kept in a clean container and stored at refrigerator temperature to prevent the growth of contaminating microorganisms, particularly the anaerobic sporeformers.

REFERENCES

Arthi, K., Appalara, B. and Paruathi, S. (2003) Vancomycin Sensitivity and KOH string test as an alternative to Gram-staining of bacteria. *Indian Journal of Microbiology* **21:**121-123.

Birhanu, H., Etsay, K. and Hailay, K. (2008). Assessment of bacteriological quality of raw camel's milk in Ab-Ala, North-Eastern Ethiopia. *Livestock Research for Rural Development* 20 (9)

Buchanan, R. E. and Gibbsons, N. E. (1974) *Manual of determinative bacteriology. 8th edn*. Williams and Wilkins. Company, Baltimore.

Frazier, W. C. and Westhotl, D. C. (1995) *Food Microbiology.* 4th edn. Tata McGraw-Hill Publishing Co. Ltd, New Delhi.

Harrington, W. F. and McCance, (1982) *Laboratory Methods in Food and Dairy Microbiology*. Academy Press Inc. London.

Kathleen, P. T. and Arthur, T. (2002) *Foundations in Microbiology.* 4th edn. McGraw-Hill, Inc. New York.

Lee, S. and Lin, C. W. (2002) The Relationship Between Psychrotrophic Bacteria in Raw Milk Quality. *Journal of Taiwan Livestock Research.* **35:** 127-134.

Pitt, J. I., and Hocking, A.D. (1997) *Fungi and Food Spoilages Book,* 3rd edn. Academic Press. New York.

Pulusani, I. (1979) Lactic Acid Bacteria, Antibacteria Substances, *Journal of Food Science.* **44:** 575-578.

Sherikar, A. T., Bachhil, V. N. and Thaphliyal, D. C. (2004) *Text Book of Element of Veterinary Public Health*. India Council of Agricultural Research. New Delhi. 75-120.