Nigerian Veterinary Journal 27(3) : 15 - 24

HAZARDS AND CRITICAL CONTROL POINTS OF YOGHURT PRODUCED IN A RESEARCH INSTITUTE FARM IN ZARIA, NIGERIA

OKOLOCHA*, E. C ., EGWU, P. T. A. ; UMOH, J. U. and LUGA, I. L. I
Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, Nigeria.

***Correspondence:E-mail: eokolocha@yahoo.com; Tel +234 082 373 8875**

SUMMARY

Hazard analyses of yoghurt, produced by the Dairy Unit of a Research Institute farm in Zaria were conducted to determine hazards associated with the processing of milk supplied to the unit into the milk product, and also to determine critical control points that are necessary for effective control of the hazards. The analyses consisted of watching all the steps leading to the processing of milk into yoghurt, recording temperatures and pH measurements throughout all these steps and collecting swab and milk samples and testing them for common food borne pathogens and indicator organisms. The temperature of the milk at the time it was freshly supplied to the Milk Processing Unit where the yoghurt was normally produced was 32^{0}C. At this temperature the microbial load was already more than 10log$_{10}$cfu/ml. The total aerobic plate count obtained for both the milk and the swab samples collected during the operations ranged from 8 Log$_{10}$ to 11log$_{10}$ cfu/ml or cm^2 as the case may be. The high aerobic plate counts recorded after pasteurization, and the isolation of Staphylococcus aureus from most of the samples indicate that the production of the milk product took place in a highly contaminated environment. Education of the producers on the hazards and the critical control points, and the importance of hygienic environment cannot be overemphasized. Some control measures bordering mainly on personal and general hygiene are suggested.

KEY WORDS: Hazards, Critical Control Points, Yoghurt, Zaria

INTRODUCTION

A great proportion of the milk produced in tropical countries is converted into indigenous products like ghee or some kind of fermented or concentrated product that can keep without artificial cooling. Traditional milk drinking societies in the tropics have long recognized that milk held at warm tropical temperatures quickly turns sour as bacteria convert the lactose to lactic acid, and that this action helps to keep milk longer. They have therefore developed simple fermented milk products (Chamberlain, 1990). Fermentation of milk is probably the earliest form of preservation (Scott, 1981).

It is difficult to classify fermented products but all the different names used refer to more or less the same product. In Spanish-speaking countries for example, the term *leche agria* is used while in South Eastern Europe, it is *yoghurt*, *kefir*, *matzoon* and *kumiss*. Many other local names are also used for fermented milk (Chamberlain, 1990). In Nigeria, it is generally referred to as *yoghurt*. There may however be other local Nigerian names.

Yoghurt is a semi-solid fermented milk product, which originated centuries ago in Bulgaria (Goff, 1995). Its popularity has grown and is now consumed in most parts of the world. Although the consistency, flavour and aroma may vary from one region to another the basic ingredients and manufacturing are essentially consistent. In Nigeria, it is mostly prepared by processing raw cow milk (the preparation steps are illustrated in figure 1) and is generally and freely consumed as a refreshing drink irrespective of tribe, religion, sex or culture.

There are many concerns about the sanitation of this milk product, which is often produced and packaged by individuals and organizations without regard to basic rules of personal and general hygiene. For example, Adesiyun (1984) found that many milk and milk products in Nigeria were highly contaminated with *Staphylococcus aureus* with counts ranging from 6.2×10^7 to 1.8×10^9 cfu/ml. Again, fermented milk and cheese depend upon a desired fermentation or succession of fermentations for their manufacture. Therefore any abnormality in these fermentations will affect the quality of the product and may even spoil it. The finished product too may be subject to spoilage by microorganisms. Coliform bacteria and lactose fermenting yeasts should not be present but may enter from equipment and other sources to produce bad flavours and gas (Frazier and Westhoff, 1991). In addition, most of the equipment and other facilities used in the processing of milk into the products are often improvised and provide room for sanitary deficiencies.

To develop a better understanding of the microbiological problems associated with *yoghurt* as commercially produced by the Research Institute farm in Zaria, and distributed to consumers within the immediate and neighbourng communities, the hazard analysis critical control point (HACCP) approach was adopted. The HACCP concept identifies hazards associated with the different stages of preparation and handling, assesses the relative risks and identifies points where control measures would be effective (Bryan, 1988, Ehiri *et al*, 2001).

The approach was adopted to identify and assess hazards and risks associated with *yoghurt* produced in the Research Institute farm in Zaria, Nigeria. Critical control points were determined and preventive measures are suggested.

MATERIALS AND METHODS

Description of the Milk Processing and *Yoghurt* Production Section of the Research Institute Farm.

The Milk Processing and *Yoghurt* Production Section is part of the Dairy Unit of the Research Institute farm. The Section starts with the Milk mixing and Pasteurization room. This room has a big gas burner improvised for the pasteurization of bulk milk supplied from the nearby Milk Production Section of the Dairy Unit and sometimes also from cows belonging to the Fulani nomads from the nearby villages. It also has two 200-litre water tanks also improvised for the cooling of the pasteurized milk. In this room also, the component parts of milk processing machine and other equipment meant for milk processing, which were yet to be assembled and / or installed were kept.

A door in the Milk Mixing and Pasteurization

room leads into the *Yoghurt* Production room. It is a smaller room. It contains an incubator and a deep freezer where the pasteurized milk from the Milk Mixing and Pasteurization room, and the *yoghurt* produced are usually stored. Part of the furnishing is a long working desk and a number of stools where the workers involved in the packaging of the finished product usually sit to do their work. The floor is roughly cemented. Adjoining the *Yoghurt* Production Section is the Laboratory room, which also serves as a Quality Control room. In this room instruments and equipment like the lactometer and the pH meter are kept.

Yoghurt is the only milk product produced by the Dairy Unit of the Research Institute farm. The finished product is normally dispensed with metal cups into PVC packs, sealed and sold to the staff of the Research Institute in Shika, the residents of nearby Samaru village and the University community with a population of about 60,000 people. Because of the limited quantity usually produced there are usually no leftovers.

Hazard Analyses

The hazard analyses were performed in June and consisted (a) of observing the steps in milk processing into the milk product *yoghurt*, to identify sources and modes of contamination (b) measuring temperatures and pH periodically during processing to evaluate survival, destruction and growth and (c) collecting samples at sequential stages of processing and testing them microbiologically (Bryan *et al*, 1991).

Temperatures were measured by inserting thermocouples (K type) with needle type sensors at various lengths into the milk at the different stages of processing so that the sensing point was near the geometric centre and the shaft nearly covered by the processed milk. Before use for measurement, the thermocouple was washed in soapy water, rinsed, wiped dry with a paper tissue, dipped in 95% alcohol and flamed after immersion. The thermocouple leads were plugged into a battery-powered hand-held digital potentiometer (Comark Ltd, Stevenage, Hertfordshire, UK). pH measurements were made using pH meter (Model 765 Calimatic, Knick). A lactometer was used to measure the specific gravity of milk supplied from the various sources.

Sampling

Sampling was done over a two-day period starting with the bulk milk supplied to the Milk Processing Unit and ending with samples of the final product. Samples of approximately 50ml of the liquid samples (milk, water, and *yoghurt*) and about 100g of the solid samples (sugar, preservative) were collected aseptically in sterile bijou bottles. Swab samples from equipment and utensils used at the various stages of production of the *yoghurt* were taken by rubbing surfaces with sterile swabs previously soaked in physiological saline solution.

All samples were appropriately labelled and were taken to the laboratory on the day of collection and either examined that day or kept refrigerated overnight and tested the following day. The sources of milk supply to the unit were the Milk Production Unit of the Farm, and additionally, sometimes, from some lactating cows belonging to Fulani nomads living in the nearby villages. The specific gravity of the milk supplied to the farm was usually taken to ensure that the milk supplies were not adulterated. The starter culture used is a mixture of *Streptococcus thermophilus* and *Lactobacilus bulgaricus*

Data were evaluated by diagramming the steps of production. Potential sources of contamination (raw milk, equipment and utensils and persons preparing the product) are noted on the diagram. Critical control points of the operations that require monitoring are indicated. The persons who were involved in production of the milk product did so in their usual manner and were not given any special instructions. Operations were observed, to identify sources and routes of contamination by the persons preparing the product, utensils, and equipment surfaces or by any other circumstances, which could have led to contamination.

Laboratory Procedures

Samples for analyses were homogenized according to International Standards Organization (ISO) technique (ISO, 1980). 10ml or 10g were transferred into 90mls of 0.1% peptone water in sterile plastic bags, fastened and homogenized in a Stomacher machine (Stomacher Laboratory Blender 400) and by means of horizontal and vertical manual agitation for a few seconds. Appropriate serial dilutions of

all the samples were carried out and 0.1ml of the serially diluted homogenates were spread on nutrient agar plates using sterile glass spreaders. This technique was used for the enumeration of total aerobic viable count. *Escherichia coli* was isolated by plating loopfuls of the homogenates on eosin methylene blue (EMB) agar (Oxoid). *Staphylococcus aureus* was isolated by plating loopfuls of the homogenates on Staph 110 agar (Oxoid). All cultures were incubated at 37° C for 24 hours. Media used were prepared according to the manufacturer's instructions. *Escherichia coli* was confirmed by biochemical tests namely: Triple Sugar Iron (TSI), Simmon citrate, Indole, Motility, Hydrogen Sulphide, Urease and Methyl-red Vocks-Prockeur (MR-VP) tests. *S. aureus* was confirmed by the Gram Staining technique and on the basis of the results of coagulase production.

RESULTS

The steps involved in the processing of the bulk milk and production of *yoghurt* with indication of hazards and critical control points are illustrated in Figure I. Laboratory results for samples collected during the various stages of processing and of the finished products are listed in Table I. The figures obtained from the specific gravity measurements (1.028 1.032) were within the normal range.

Time temperature exposures and the pH measurements taken during production are shown in Table II. The total aerobic plate count for swab samples ranged from $7\log_{10}$ cfu/cm^2 to $10\log_{10}$ cfu/ cm^2 while the microbial counts obtained for milk before pasteurization and up to the final product ranged from $8\log_{10}$ cfu/ml to $11\log_{10}$ cfu/ ml. The microbial counts before pasteurization were very high, reaching $11\log_{10}$ cfu/ ml. High counts were also observed for the additives, namely, sugar and the preservative (benzyl benzoate) used. All the samples had counts exceeding 6log10 cfu/ml or cm^2 as the case may be (Table I). *E. coli* was not isolated from any of the samples tested (Table I) while *S. aureus* was isolated from 4 (14.8%) of the 27 samples examined.

The pH of the *yoghurt* was 5.9 after incubation, at a temperature of 50°C. This reduced to 4.2 at a temperature of 8.3°C, after being kept in the deep freezer until the following day and slightly increased to 4.6, about 30 minutes later (same day) at a temperature of 7.1°C (Table II)

Most of the facilities needed for the various stages of production were improvised as the automated milk processing machine whose parts had been lying packed in a corner of the building had neither been assembled nor installed. In effect the personnel involved in the production process had physical contact with every stage of the production process either through moving one improvised instrument or equipment, or through handling the utensils needed for transfer of milk or the milk product.

TABLE I: Total aerobic plate count and isolation of *Escherichia coli* and *Staphylococcus aureus* from the various stages of milk processing into *yoghurt*

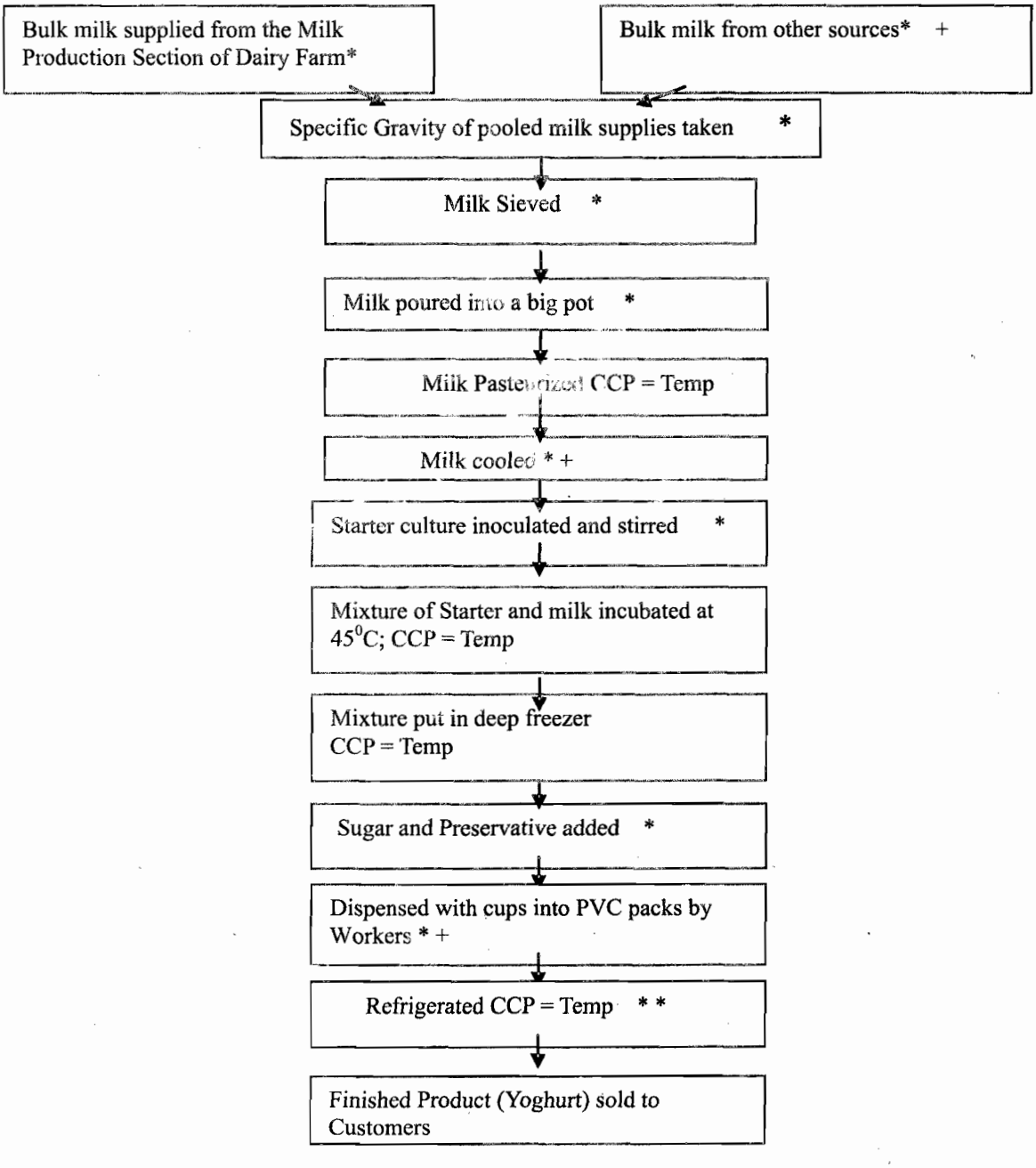
Sample	TAPC ([a, b, c])	*E. coli*	*S. aureus*
Swab of Churner 1	8[b]	-	+
Swab of Churner 2	7b	-	+
Swab of Pot used for Pasteurization of the bulk milk	10b	-	-
Swab of Sieve	8b	-	-
Swab of Lactometer	8b	-	-
Milk before sieving and Pasteurization	11a	-	+
Milk before mixing with starter culture, ready for freezing	8a	-	+
Sugar before adding to milk / starter	10c	-	-
Preservative before adding to milk / starter	10c	-	-
Mixture of milk, starter culture, sugar and preservative	10a	-	-
Swab of big spoon used in stirring mixture	10b	-	-
Swab of cup used in transferring milk into big bowl	8b	-	-
Swab of Bowl	8b	-	-
Swab of Steel bucket for receiving milk supply	8b	-	-
Swab of hands of Worker 1	9b	-	-
Swab of hands of Worker2	7b	-	-
Swab of hands of Worker 3	8b	-	-
Swab of PVC pack 1 before yoghurt was dispensed into it	7b	-	-
Swab of PVC pack 2 before yoghurt was dispensed into it	7b	-	-
Swab of PVC pack 3 before yoghurt was dispensed into it	8b	-	-
Swab of PVC pack 4 before yoghurt was dispensed into it	7b	-	-
Swab of PVC pack 5 before yoghurt was dispensed into it	7b	-	-
Yoghurt 1 in PVC pack	10a	-	-
Yoghurt 2 in PVC pack	9a	-	-
Yoghurt 3 in PVC pack	10a	-	-
Yoghurt 4 in PVC pack	9a	-	-
Yoghurt 5 in PVC pack	9a	-	-

Table II Temperature and pH measurements recorded at various stages in the production of *yoghurt*

Status/Process	Time (Hours)	Temp (^0C)	pH
Bulk Milk before pasteurization	09.00	32	ND
Milk just before end of pasteurization	10.56	75	ND
Milk before mixing with starter culture, ready for freezing	11.16	42.2	ND
Milk and starter culture mixture immediately after incubation	13.17	50	5.9
Mixture after freezing **(Following day)**	09.30	8.3	4.2
Yoghurt after freezing **(Same day)**	09.50	7.1	4.6

LEGEND: ND = Not Determined

Flow chart of milk processing and *yoghurt* production method used in the research farm indicating hazards and critical control points



LEGEND

*** Hazards of Contamination likely; ** Hazards of survival likely; + Hazards of microbial growth likely;**

CCP = Critical Control Point

NB: It is important to point out that improvisations were made at stages where appropriate facilities were lacking during the production process.

DISCUSSION

After microorganisms have entered milk, it is difficult to remove them effectively (Frazier and Westhoff, 1991). The data appreciably show the value of conducting hazard analysis critical control point to detect food borne disease hazards and to focus attention on situations where control action is needed. This procedure is particularly valuable in countries that have either no or rudimentary food borne diseases surveillance activities (Bryan *et al*, 1992).

The containers, equipment and utensils that were used in storing the milk, including the bulk milk that was supplied to the Milk Processing Unit had microbial counts exceeding 6log10 cfu/ml or cm^2. The highest microbial counts recorded before sieving and pasteurization was therefore not surprising (Table I). It is most likely that the utensils or the instruments and / or other facilities often handled during processing were always easily contaminated. In other words the level of environmental contamination was appreciable. The specific gravity values indicate the milk supplied to the farm was not adulterated.

Pasteurization as a critical control point led to the reduction in the level of contamination of the milk. In production of *yoghurt*, heating is necessary not only to remove microorganisms of potential public health significance but also to produce favourable conditions for the growth of the starter. From the viewpoint of intrinsic quality the behaviour of the starter culture is of great importance (Scott, 1981). Despite the fact that a temperature of 75°C was attained in the process, a count of 8log10 cfu/ml was still recorded, after inoculation of the starter, and cooling. This points to contamination from either the handlers or the environment or both, as the presence of coliforms after high temperature-short time pasteurization is indicative of contamination (Frazier and Westhoff, 1991).

Freezing is another critical control point. This may not be very effective because of the unsanitary practices observed within the environment leading to post freezing contamination. Nevertheless freezing is an important control measure to prevent growth of bacteria in ice cream mixes and milk products (Bryan *et al.*, 1992). Hence it should be done as soon as practicable after preparation of a batch of *yoghurt*. Otherwise the fresh product should be stored in a deep freezer (or a refrigerator, if available) after preparation until put into the freezing dispenser or at the point of manual dispensing (as was observed in the *Yoghurt* Production Section).

Yoghurt employs a mixed culture of *Streptococcocus thermophilus* and *Lactobacillus bulgaricus*. Though the acidity of the fermented milk is sufficient to prevent spoilage by proteolytic or other bnacteria that are not acid tolerant, chilling is necessary to stop acid formation by the starter bacteria at the desired stage and packaging and sealing to also avoid mold growth (Frazier and Westhoff, 1991). In addition, the primary objective in adding a preservative to *Yoghurt* is to prevent growth of molds and to extend its shelf life (Frazier and Westhoff, 1991). However, as stated earlier, in the introduction, any abnormality in the fermentation processes will affect the quality of the product and may even spoil it. This was not the case in the *yoghurt* produced in the farm at the time of this study. It could rather be inferred that unclean equipment, contaminated milk or poor hygiene of the production staff more than any other thing played a very important role in the contamination of the product.

It is interesting to note that despite the high aerobic plate counts obtained from the swab samples taken, *E. coli* was not isolated from the PVC packs and the *yoghurt* samples that were tested, an indication that there may have been no contamination of faecal origin. The handling of utensils, cups, and spoons and other vessels that were often dipped in the milk were probable sources of hazards. Touching cooked foods is a commonly identified factor that leads to outbreaks of staphylococcal food poisoning (Bryan, 1988). Facilities for hand washing though available were not readily used. Hand washing was seldom observed but when done it usually consisted of rinsing hands under tap of running water. Utensils and vessels were usually rinsed in water and rubbed by hand. Poorly cleaned and not disinfected, utensils are additional sources of contamination.

The high aerobic microbial counts recorded for the milk product could have come from the raw milk or may have reached the product later

during handling. The large numbers suggest either bacterial survival and subsequent growth, (despite the reasonably low pH of the yoghurt) or bacterial growth in the milk before fermentation. Fermentation, so as to sufficiently lower the pH of the yoghurt, would be considered a critical control point, but the potential for the presence of staphyloenterotoxin exists ((Bryan *et al,* 1988).

Varadaraj and Ranganathan, for example showed that *S. aureus* grew and produced thermostable deoxyribonuclease in curd and other fermented milks and in association with lactic cultures (Varadaraj and Ranganathan, 1988).

Incubation of the milk starter mixture also served as a critical control point but as observed in some steps of the production process, post process contamination could have come through unhygienic handling, and

As hazards are identified by hazard analyses or epidemiological or other scientific studies, preventive measures which are practical under prevailing customs and circumstances must be chosen from known measures or they must be devised. The high microbial counts recorded in this study can be attributed to mainly lack of appropriate infrastructure and poor and unhygienic handling. It is obvious from the foregoing that the main quality control points to consider concern hygiene. Food safety activities must therefore concentrate on informing personnel involved in the preparation of foods about specific hazards of the foods they usually prepare and practical preventive measures that can be applied at critical control points. Hazard analysis critical control point evaluations indicate where attention for preventive measures needs to be focused. There is need to educate the producers on the hazards and critical control points of *yoghurt* preparation. Such control and monitoring procedures as washing hands at intervals with soap, washing pots, equipment and utensils thoroughly with soap before and after use, preparing the *yoghurt* in quantities that can be easily sold off especially where there are no means of refrigeration, are necessary for the preparation of a safe product (Oranusi *et al,* 2003).

ACKNOWLEDGEMENTS

The authors are grateful to Ahmadu Bello University, Zaria, Nigeria for providing part of the funds for this work and also to the laboratory staff in the Department of Veterinary Public Health & Preventive Medicine Ahmadu Bello University, Zaria, Nigeria especially Mr. M. B. Odoba, Mr. Martin Mgbegha, Mr. Kevin Iwuanyanwu and Mr. Joseph Damsa for their assistance and to all those who may have contributed to the success of this work.

REFERENCES

ADESIYUN, A.A. (1984): Enterotoxogenicity of Staphylococcus auerus strain isolated from Nigeria Ready to eat foods J. of Food Prot., 3: 438-440.

BRYAN F. L. (1988): Risks associated with practices, procedure on the processes that lead to outbreak of food-borne disease. J. Food Prot., 51: 663-673.

BRYAN F.L., TEUFEL, P., RIAZ, S., ROOHP, S., QUADAR, F. AND MALIK, Z. (1992): Hazards and Critical Point of Street Vending Operations in a Mountain Resort Town in Pakistan. J. of Food Prot., 55(9): 701-707.

BRYAN, F. L., MICHANIE, S., FERNANDEZ, N. M., VIZCARRA, M. M., TABOADA, D. P., NAVARROS, S. O., ALONSO, A. B. AND REQUEJO, E. G. (1988): Hazard analyses of foods prepared by migrants living in a new settlement at the outskirts of Lima, Peru.

BRYAN, F. L., BARTLESON, C. O., COOK, C. O., FISHER, P., GUZEWICH, J., HUMM, B., SWANSON, R. C., AND TODD, E. C. D. (1991): Procedures to implement the hazard analysis critical control point system. International Association of Milk, Food and Environmental Sanitarians, Ames, Iowa.

CHAMBERLAIN, A. (1990): Milk and milk products. In: An introduction to animal husbandry in the tropics 4th Ed. W. J. A. Payne, Ed. Longman Scientific & Technical, New York; 747 789.

EHIRI, J. E., AZUBUIKE, M. C. UBAONU, C. N., ANYANWU, E. C., IBE, K. M., OGBONNA, M. O. (2001): Critical control points of complementary food preparation and handling in Eastern Nigeria. Bull. World Health Org., 79: 423 433.

FRAZIER, W. C. AND WESTHOFF, D. C. (1991): Fermented milk products. In: Food Microbiology, 3rd Ed. Frazier, W. C. and Westhoff, D. C. (Eds) McGraw Hill Book Company, New York. 281 382.

GOFF, D (1995). Yoghurt. http: www.foodsc.uoguelph.ca.dairyedu/yogurt.html.starter, Dairy Science Education, University of Guelph, Guelph ,Canada

INTERNATIONAL STANDARDS ORGANISATION (1980): ISO 6687 Microbiology General guidance for the preparation of dilutions for microbiological examinations. International Standards Organisation, Geneva, Switzerland.

ORANUSI, S. U. UMOH, V. J. and KWAGA, J. K. P. (2003): Hazards and critical control points of kunun zaki, a non alcoholic beverage in Northern Nigeria. Food Microbiol. 20: 127 132.

SCOTT, R. (1981): Cheese making practice. In: A colour atlas of food quality control. Sutherland, J. P., Varnam, A. H. and Evans G. A Wolfe Science Book; 17 20.

VARADARAJ, M. C. and RANGNATHAN (1988): Growth and production of thermostable deoxyribonuclease by Staphylococcus aureus in Shirkland. J. Food Sci. Technol., 25: 23 27.