British Journal of Dairy Sciences 3(2): 14-21, 2013
ISSN: 2044-2432; e-ISSN: 2044-2440

Submitted: February 05, 2013 Accepted: March 03, 2013 Published: June 25, 2013

Microbial Screening and Lipid Peroxidation Status of Fermented (Yoghurt) Milk Samples Sold in Maiduguri Metropolis and Commonly Consumed in University of Maiduguri, Borno State, Nigeria

[1]H.C.C. Maduka, [2]C.E. Ugwu, [3]A.A. Maduka, [1]N.H. Hashidu and [1]B.S. Gimba
[1]Department of Biochemistry, University Maidugury, Borno State Nigeria
[2]Department of Human Biochemistry, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria
[3]Department of Gender Studies, University of Hull, HU6 7RX, United Kingdom

Abstract: Fermented milk products are produced and consumed as supplements to normal diet especially in the Northern Nigeria. They could be contaminated by microorganisms due to poor quality control, thereby increasing the risk of food borne diseases in the community. Microbial screening and lipid peroxidation studies were carried out on some fermented milk products consumed within the University of Maiduguri campus to determine their safety levels. A total of seventy two samples comprising six differently prepared yoghurts were used in the study. Various microbial media were prepared and samples analyzed by the streak plate method after inoculation at 30ºC for 24 h. Lipid peroxidation was also determined in the samples using standard biochemical methods. The results show the presence of *Salmonella typhi, E. coli, Staphylococcus aureus* and *Staphylococcus epidemidis* only in the locally prepared kindrimu yoghurt while fungi and mould were isolated in all the samples. *Pseudomonas* and *Candida albicans* were not isolated in all the samples. The malonaldehyde was significantly higher in samples A and E compared to samples B and C. The results show that samples (A, C, D) with high malondialdehyde values had corresponding low ascorbic acid level also. The results suggest that the locally fermented yoghurt (Kindrimu) samples screened were contaminated with some food borne pathogens. The samples examined all showed some degrees of peroxidation. The public health importance of these results was highlighted.

Keywords: Lipid peroxidation, malonaldehyde, malondialdehyde, microbial, yoghurt

INTRODUCTION

Milk and its products (yoghurt, cheese, etc) form part of the human diet all over the world. Milk quality continues to be a topic of intense debate in the dairy industry and in medical and public health sectors (Oliver *et al*., 2009). Production of the best quantities of good quality milk and milk products is an important aspect of standard dairy practice. High-quality milk contains a low bacteria count and a low number of somatic cells and is free of human pathogens and antibiotic residues (Oliver *et al*., 2009). Different epidemiological studies have detected food borne pathogens in bulk tank milk (Umoh *et al*., 1990; Hassan *et al*., 2000; Jayaro and Henning, 2001; Waak *et al*., 2002; Murinda *et al*., 2002a, b, 2004b; Muraoka *et al*., 2003; Van Kessel *et al*., 2004; Oliver *et al*., 2005; Pangloli *et al*., 2008). Although the true incidence of milk borne disease in Maiduguri, Nigeria is not known, there are reports that link the consumption of contaminated raw milk, inadequate pasteurized milk or consumption of dairy products adulterated with contaminated raw milk to incidence of human food borne diseases in the in some countries (Fleming *et al*., 1985; Fahey *et al*., 1995; Evans *et al*., 1996).

The nutritive value of milk is widely documented (Huth *et al*., 2006). Increased consumption of low fat dairy products have been linked with lower incidence of chronic diseases, such as cancer, hypertension, osteoporosis (Huth *et al*., 2006) and atherosclerosis (Nettleton *et al*., 2008). Milk may also protect against oxidative stress (Hunter *et al*., 2012) as it contains antioxidant compounds, like whey and casein proteins, vitamin A, C and E (Zulueta *et al*., 2009). Studies have shown that there are increased demand on antioxidant nutrients, such as vitamins and polyphenols, which have an effective role in the optimization of human health (Mukhtar and Ahmad, 2000; Aoki *et al*., 2010). Therefore, the need to provide foods (milk) with antioxidant potentials and low lipid peroxide levels arises. An important problem in dairy industry is how to prevent lipid oxidation of milk fats, which gives rise to lipocatabolic odor (Lindermark-Mansson and Akesson, 2000). These odors result from unsaturated fatty acid

Corresponding Author: C.E. Ugwu, Department of Human Biochemistry, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria

oxidation by reactive oxygen during long-term preservation (Al-Mabruk *et al*., 2004). Research has shown that the supplementation of antioxidative nutrients like vitamin E and trehalose in the diets of dairy cows may result in milk with a low lipid peroxide value and greater antioxidant potentials (Al-Mabruk *et al*., 2004; Sympoura *et al*., 2009; Aoki *et al*., 2010).

The dairy industry in Maiduguri is still underdeveloped and crude with dietary supplementation with antioxidative nutrients not practiced. Maiduguri is the state capital of Borno State, North East Nigeria, with majority of the indigenes involved in farming and cattle rearing. It has an average daily temperature of between 35-40ºC during the peak heat period. In Nigeria and especially in the Northern part, fermented milk (yoghurt) of various types are produced and consumed as supplement to normal meals in homes and even for sale. Many farm families consume raw milk simply because it is a traditional practice and less expensive to buying pasteurized retailed milk (Hegarty *et al*., 2002).

Kindrimu refers to the local yoghurt in Hausa language that is produced without the use of the conventional method of producing commercial yoghurt. Kindrimu is produced by milking the cow into a container. This is followed by the addition of small quantity of already fermented yoghurt or milk and is covered for 24 h to ferment. This product is vulnerable to contamination by microorganisms due to poor quality control. Because of its relatively lower price compared to other commercially produced yoghurts, the consumption of this brand of fermented milk has gained popularity around Maiduguri metropolis despite the health implications. We carried out the safety examination of the local brand (kindrimu) and other commercially available yoghurt samples sold in shops in Maiduguri.

MATERIALS AND METHODS

Collection of samples: A total of seventy two samples comprising six differently prepared yoghurts were purchased from shops around the University of Maiduguri. Sample A comprised of twelve samples of kindrimu while samples B, C, D, E and F comprised twelve different samples of commercially prepared yoghurts. They were stored in a refrigerator and used within 24 h of collection. The commercially produced yoghurts were randomly labeled B-F respectively.

Media preparation: The following media were used in the study which includes sabouround dextrose agar, macconkey agar, nutrient agar, manitol salt agar, salinite feacal broth, salmonella shigella agar and blood agar. Different grams of each growth medium were added to 1 L of distilled water in a clean conical flask. They were each boiled to dissolve completely and then sterilized by autoclaving at 121ºC for 15 min. Each was allowed to cool to 5°-55°C before being poured aseptically into sterile petri dishes and allowed to set.

Plating technique: The poor plate technique of Van Soestbergen and Ching (1969) was used. Samples were taken after thorough mixing of the parent stock to enhance homogeneity. Plating of the serially diluted samples was done on sterilized petri dishes.

Microbial analyses: The samples were analyzed by the streak plate method. One ml of each sample was inoculated unto each medium that was sufficiently dried. Each plate was labeled and inoculated at 30 °C for 24 h. The colonies were smeared on a slide and stained. The smear was spread evenly covering an area of about 15-20 mm diameter on a slide. The sample was spread thinly using a sterile wire loop. The flame sterilized loop was allowed to cool before it was used for the other ones. Colonies were emulsified in distilled water before being spread thinly. After drying, the smears were stained with crystal violet stain for 30-60 sec and then rapidly washed with distilled water. The water was then tipped off and the smears were treated with lugolsloding for 30-60 sec and were then washed off with distilled water. They were decolorized with acetone alcohol solution and washed immediately with distilled water. The smears were also treated with neutral red stain for 1 min, washed off and air dried. The smears were examined under the microscope for the presence of microorganisms (Zall, 1981).

Lipid peroxidation measurement: The concentration of lipid hydroxides carbonyls present in the fermented milk (yoghurts) as malondialdehyde was determined by the method of Hunter *et al*. (1963) as modified by Kirkova *et al*. (1995). 0.175 ml of KCL Tris buffer (0.02 M) pH 7.4 was used as the medium for incubation after which 0.12 ml of 5N HCL was added. After mixing, 0.35 ml of 2% sodium barbituric acid solution was promptly added (TCA, HCL and thiobarbituric solution alone eliminate difficulties that arise due to absorption of colour due to protein precipitates),(Hunter *et al*., 1963). The tubes were then stopped with cotton wool and placed in boiling water for 10 min and the colour absorbance read at 532 nm. The concentration of the malondialdehyde formed was calculated using the molar extinction coefficient, 1.56×105 per m.cm using the formula:

$$\frac{\text{Absorbance} \times 46}{\text{Sample wt/Volume.}}$$

where,

46 = Constant or factor of lipid peroxide absorptivity.

The results were presented as means of triplicate determinations±standard deviations as described earlier (Maduka, 2008).

Malonaldehyde measurement: The lipid hydrogen peroxide aldehyde determined by malonaldehyde was carried out by the method of Wills (1987) by the thiobarbituric acid reactivity. The levels of malonaldehyde formed were calculated using molar extinction coefficient 1.56×10^4 cm^3mole/s. Results were expressed as mean±standard deviation and could be reproduced within±5%. The experiment was repeated three times.

Determinatiion of ascorbic acid concentration: The method used in the estimation of the ascorbic acid in the fruit juice was that of Roe and Kuetler (1943) and Natelson (1961) as modified by Tietz (1970). It was based on the conversion of ascorbic acid in strong acid to diketoglutonic acid in a strong acid and the reaction of diketogluconic acid with 2, 4-DNPH to form diphenyl hydrazine. The hydrazine dissolves in strong H_2SO_4 to produce a red colour which can be determined spectrophotometrically at 520 nm.

Statistical analysis: Data were subjected to Analysis Of Variance (ANOVA). In order to test whether or not significant differences exist between groups, we analyzed the mean values with the paired T-test. The acceptable level of significance was $p<0.05$. The analysis was carried out on SPSS windows version 13.0.

RESULTS

The results in Table 1 show the microbial screening of the Kindrimu yoghurts (sample A) and commercially prepared yoghurts respectively. The results show the presence of yeast in the six different samples but the absence of the other microorganisms in the Commercial yoghurts. *Salmonella typhi, E. coli* and *Staphylococcus aureus* were isolated in 25% of the Kindrimu yoghurts while *Staphylococcus epidermidis* was isolated in 75% of Kindrimu yoghurts. There were the absences *Pseudomonas, Candida albican* and fungi and mould in the commercially prepared yoghurts. Fungi and mould were isolated in all the twelve samples of Kindrimu yoghurts. Samples B-F was free from the microorganisms tested apart from yeast that was detected in various all the samples.

The results of the lipid peroxidation states of the yoghurt samples are shown in Table 1. The results show that samples A, C and E recorded high malondialdehyde values while samples B and F have low values. The malondialdehyde level in samples E and A were significantly higher ($p<0.05$) to those in samples B, C and F respectively. From the malonaldehyde assay, samples A and D had the higher concentrations (mg/dL) which were not significant ($p>0.05$) to samples B, C, E and F respectively. The results of the ascorbic acid content show that sample F recorded the highest ascorbic acid concentration which was significantly higher to sample D ($p<0.05$). Samples A, C and D which had the higher malondialdehyde values recorded lower ascorbic acid concentrations (Table 2).

DISCUSSION

The risk of food borne disease has increased remarkably in the last two decades, with nearly a quarter of the population at higher risk of illness. Therefore, preventing disease and death associated with food borne pathogens remains a major public health challenge. Furthermore, an increase in import and export of food products has made food safety a global issue and may lead to the introduction and establishment of new diseases in geographical areas that have not yet witnessed food borne pathogens (Oliver *et al*., 2005). Because of the inadequate regulation in the production, sale and consumption of fermented milk and milk products in Nigeria, the study screened for the presence of some pathogenic microorganisms in yoghurt samples consumed in the University of Maiduguri Metropolis.

The microbial screening of the samples of the commercially produced yoghurts (Table 1) showed the absence of pathogenic organisms except the growth of yeast. The presence of yeast could be a contaminant because yeast is not used as a starter culture since it will ferment the milk carbohydrate into alcohol which is an undesirable product (Kurmann and Rasic, 1988; Lee and Wong, 1993), however, they are also important because they produce desirable flavours as in cheese ripening (Fleet, 1990; Rohm *et al*., 1992; Jakobsen and Narvhus, 1996). There was also the presence of fungi and mould in the kindrimu yoghurt samples. Okpalugo *et al*. (2008) isolated 33 bacterial and 12 fungal isolates that belongs to 9 and 3 genera respectively in yoghurt samples sold in Abuja, Nigeria.

The presence of *Salmonella* (25%), *Escherichia coli* (25%), *Staphylococcus aureus* (75%) and

Table 1: Microbial screening of the fermented milk (yoghurts) samples

Isolated organism	A	B	C	D	E	F
Salmonella typhi	+ (25%)	ND	ND	ND	ND	ND
E. coli	+ (25%)	ND	ND	ND	ND	ND
pseudomonas	ND	ND	ND	ND	ND	ND
Candida albican	ND	ND	ND	ND	ND	ND
Staphylococcus aureus	+ (25%)	ND	ND	ND	ND	ND
Staphylococcus epidermidis	+ (75%)	ND	ND	ND	ND	ND
Fungi and mould	+ (100%)	ND	ND	ND	ND	ND
Yeast	+ (100%)	+ (100%)	+ (25%)	+ (100%)	+ (75%)	+ (100%)

N = 12.0 + = Positive, ( ) = number of positive samples, ND = not detected

Table 2: Lipid peroxidation and ascorbic acid concentrations in the six sampled fermented milk (yoghurt) products

Sample	Malondialdehyde (mg/mL)	Malonaldehyde (mg/mL)	Ascorbic acid (mg/mL)
A	11.30±1.90 [a]	2.80±0.10[a]	1.50±0.30
B	1.93±0.05[b]	1.20±0.15[a]	3.90±0.10
C	11.70±0.50[a]	1.40±0.70[a]	1.00±0.20
D	2.50±1.60[b]	3.50±0.80[a]	0.90±0.10[b]
E	16.80±0.10[a]	0.60±0.10[a]	1.40±0.10
F	0.81±0.01[b]	0.55±0.10[a]	4.50±0.70[a]

Results are mean±SD of triplicate determinations; values with different superscripts in a column are statistically significant ($p<0.05$).

epidemidis (75%) in the kindrimu yoghurt (sample A) sold within the University community is quite high to attract public health attention. The results obtained for percentage *E. coli* content was higher than that obtained from other studies (Okonkwo, 2011; Soomro *et al*., 2002; Ekici *et al*., 2004; Mohamed and El-Zubeir, 2007). The results are also in agreement with Schlegelova *et al*. (2002), Guta *et al*. (2002), Okpalugo *et al*. (2008), Adeleke *et al*. (2000) and Mahami *et al*. (2011) who found bulk cow milk in Czech Repulic, cow foremilk in Botswana, pasteurized milk in Nigeria and soy milk in Nigeria and cow milk in Ghana respectively to be contaminated with bacteria pathogens. *E. coli* has been associated with the contamination of milk and milk products (Kulshrestha, 1990; Asmahan and Warda, 2011; Okpalugo *et al*., 2008; Dadie *et al*., 2010). Reports have linked the presence of *E. coli* as an index organism indicative of the presence of other pathogenic organisms like *Klebsiella* and *Staphylococcus aureus* (Okpalugo *et al*., 2008; Adesiyun *et al*., 1995; Smooth and Pierson, 1997; Okonkwo, 2011). Recovery and counting of *E. coli* is used as reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute public health hazard (Asmahan and Warda, 2011). Most *E. coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man (Kaper *et al*., 2004; Asmahan and Warda, 2011).

Our results on the locally produced yoghurt samples (Kindrimu) showed significant growth of *Salmonella typhi*, *E. coli*, *S. epidermidis*, fungi and yeast. The prevalence of food borne pathogens in fermented milk products is influenced by many factors including farm size, number of animals on the farm, hygiene, farm management practices, variation in sampling and types of samples evaluated, differences in detection methodologies used, geographic location and season (Oliver *et al*., 2005). These factors demonstrate that milk can be a major source of food borne pathogens of human health significance. Introduction of raw milk contaminated with food borne pathogens into processing plants and their persistence in biofilms represents an important risk of post-pasteurization contamination that could lead to exposure of the consumer to pathogenic bacteria (Arizcun *et al*., 1998; Roberts and Weidmann, 2003; Wong, 1998). The outbreak of human Salmonellesis have been associated with *Salmonella* (De Buyer *et al*., 2001). Results shows that *Salmonella spp.* is one of the most etiologic agents responsible for several outbreaks associated with the consumption of raw milk (De Buyer *et al*., 2001). Our results showed 25% contamination of the locally produced yoghurt (Kindrimu). Human infection of Salmonella come from different sources including fecal contamination of food products and water; consumption of unpasteurized milk and dairy products, particularly by farm families etc (Wells *et al*., 2001, Pangloli *et al*., 2008, Headrick *et al*., 1998; Lejeune and Rajala-Schultz, 2009). Since cull dairy cows and cattle are primary reservoirs and source of Salmonella, control of the pathogen on farm through management strategies may prevent or reduce contamination of foods in the food chain.

The presence of *Staphylococcus aureus* and *Staphylococcus epidermidis* is in line with the results of Okpalugo *et al*. (2008), Okonkwo (2011) and Tormo *et al*. (2011). *Staphylococcus aureu* has been linked to gastroenteritis by producing enterotoxins, boils and skin infections. Because *Staphylococcus aureus* is highly vulnerable to destruction by heat treatment and nearly all sterilizing agents, its presence in pasteurized yoghurt is an indication of poor sanitation or post-pasteurization contamination (Okpalugo *et al*., 2008). Results show that *Staphylococci spp* (Kaplan, 2005) and *Salmonella typhi* (Fontaine *et al*., 1980) are agents for the cause of mastitis in dairy animals and may have contaminated milk from udder of infected animals.

Many studies have been published that support the health benefits of increased antioxidant content in foods, such as prevention of disease and aging and improved health. These reported effects are mediated by the reduced damage caused by reactive oxygen species (Aoki *et al*., 2010; Herrera *et al*., 2009). Milk is a complex biological matrix which contains many factors that can exert antioxidant and/or prooxidant effects. The rate of and extent of lipid oxidation in milk and milk products is influenced by a range of variables including oxygen, light, tocopherols, carotenoids, thiols, proteins and enzymes and storage temperature. It is well known that lipid peroxidation occurring in food products causes deteriorations in food quality, like rancid flavor, unacceptable taste and shortening of shelf life. Furthermore, it has been accepted that oxidative stress plays a significant role in a number of age specific disease (Philanto, 2006; Halliwell, 2001; Collins, 2005).

To prevent foods from undergoing deterioration and to provide protection against serious disease, it is important to inhibit the peroxidation of lipids and formation of free radicals occurring in the living body and food stuffs. This study also, examined the lipid peroxidation states of locally and commercially produced fermented milk (yoghurt) samples in order to

assess their safety levels. The results from this study indicate that the fermented milk samples had varying concentrations of malondialdehyde with samples A, C and E having appreciably higher concentrations than samples B, D and F. We also observed from the study that the malonadeyde levels were not correspondingly high in samples A, C and E. From the ascorbic acid determination the samples with lesser levels of malondialdehyde values had higher ascorbic acid concentrations respectively. Ascorbic acid is a known effective scavenger of alkoxy radicals (RO^*).

Historically, the seasonal variation in milk production made it important for milk to be preserved (Haug *et al*., 2007). The relationship between food and health is well established (Halliwell, 2001; Liu *et al*., 2003; Abuja and Albertini, 2001) and some results have indicated that modifiable risk factors seem to be of greater significance for health than previously anticipated (Yusuf *et al*., 2004). Prevention of disease may in future be just as important as treatment of diseases. Indeed, many consumers are highly conscious of the health-properties of food and they take antioxidant supplements to improve the antioxidant capabilities of the body (Prior *et al*., 2000; Leonard *et al*., 2002). Ascorbic acid is only one aspect of the Reactive Oxygen Species (ROS) scavenging capacity of milk (Lindermark-Mansson and Akesson, 2000). Therefore, regular vitamin C consumption in the form of food (fermented milk) or supplements may reduce risk of disease pathology by reducing oxidative stress in vivo (Johnston *et al*., 2003; Joshipura *et al*., 2001; Lennie, 2001).

CONCLUSION

Milk can harbor a variety of microorganisms and can be an important source of food borne diseases. The results obtained in this study suggest that the locally fermented milk (kindrimu) available to consumers in Maiduguri was contaminated with food borne pathogens unlike the commercially prepared yoghurts. Since these organisms like *Salmonella, Staphylococuss aureus and Escherichia coli* were not isolated from the laboratory prepared yoghurts, it is possible to have pathogen-free yoghurts through high and strict preventive measures. The study shows that some of the fermented milk samples marketed in Maiduguri has appreciable degree of peroxidation in them as indicated on their malondialdehyde levels and corresponding low levels of ascorbic acids. The public perception of food quality is critical in the marketing of any product. It is, therefore, very important that high aseptic conditions be maintained in the Nigerian milk products industry.

REFERENCES

Abuja, P. and R. Albertini, 2001. Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. Clin. Chim. Acta, 306: 1-17.

Adeleke, O.E., B.A. Adeniyi and A.A. Akinrinmisi, 2000. Microbiological quality of local soy milk: A public health appraisal. Afr. J. Biomed. Res., 3: 89-92.

Adesiyun, A.A., L. Webb and S. Rahaman, 1995. Microbiological quality of raw cow's milk at collection centers in Trinidad. J. Food Protect., 58: 139-146.

Al-Mabruk, R.M., N.F. Beck and R.J. Dewburst, 2004. Effect of silage species and supplemental vitamin E on the oxidative stability of milk. J . Dairy Sci., 87: 406-412.

Aoki, N., S. Furukawa, K. Sato, Y. Kurokawa, S. Kanda, Y. Takahashi, H. Mitsuzumi and H. Itabashi, 2010. Supplementation of the diet of dairy cows with trehalose results in milk with low lipid peroxidation and high antioxidant content. J. Dairy Sci., 93: 4189-4195.

Arizcun, C., C. Vasseur and J.C. Labadie, 1998. Effect of several decontamination procedures on *Listeria monocytogenes* growing in biofilms. J. Food Protect., 61: 731-734.

Asmahan, A.A. and S.A. Warda, 2011. Incidence of *Escherichia coli* in raw cow's milk in Khartoum State. British J. Dairy Sci., 2(1): 23-26.

Collins, A., 2005. Antioxidant intervention as a route to cancer prevention. Eur. J. Cancer., 41: 1923-1930.

Dadie, A., G. Tagro, L. Ochoanin, E. Dako, M. Dje and M. Dosso, 2010. Gastroenteritis *E. coli* carried by milk products sold in the street of Abidjan, Cote d'Ivoire. Eur. J. Sci. Res., 39(1): 143-152.

De Buyer, M.L., B. Dufour, M. Maire and V. Lafarge, 2001. Implication of milk and milk product in food-borne disease in France and different industrialized countries. Int. J. Food Microb., 67: 1-17.

Ekici, K., H. Bozkurt and O. Islyici, 2004. Isolation of some pathogens from raw milk of different milch animals. Pak. J. Nutr., 3: 161-162.

Evans, M.R., R.J. Roberts, C.D. Ribeiro, D. Gardner and D. Kembrey, 1996. A milk-borne Comylobacter outbreak following an educational farm visit. Epidemiol. Infect., 117: 457-462.

Fahey, T., D. Morgan, C. Gunneburg, G.K. Adak, F. Maji and E. Kaczmarski, 1995. An out break of *Campylobacter jejuni* enteritis associated with failed milk pasteurization. J. Infect., 31: 137-143.

Fleet, G.H., 1990. Yeast in dairy products. J. Appl. Bacteriol., 68: 199-211.

Fleming, D.W., S.L. Cochi, K.L. MacDonald, D.V.M. Brondum, M.S. Pegsy and S. Hayes, 1985. Pasteurized milk as a vehicle of infection in an outbreak of Listeriosis. New Engl. J. Med., 312: 404-407.

Fontaine, R.E., M.L. Cohen, W.T. Martin and T.M. Vernon, 1980. Epidemic Salmonellosis from Chedder cheese-surveillance and prevention. Am. J. Epidemiol., 111: 247-254.

Guta, C., T.K. Sebuanya and B.A. Gashe, 2002. Antimicrobial susceptibility of *Staphylococci spp* from cow foremilk originating from dairy farms around Gaborone, Botswana. East Afr. Med. J., 79(1): 1-4.

Halliwell, B., 2001. Role of free radicals in the neurodegenerative diseases: Therapeutic implications for antioxidant treatment. Drug Aging, 18: 479-484.

Hassan, L., H.O. Mohammed, P.L. McDonough and R.N. Gonzalez, 2000. A cross-sectional study on the prevalence of *Listeria monocytogenes* and *Salmonella* in New York dairy herds. J. Dairy Sci., 83: 2441-2447.

Haug, A., A.T. Hostmrk and O.M. Harstad, 2007. Bovine milk in human nutrition: A review. Lipids Health Dis., 6: 25, DOI: 1186/1476-511x-6-25.

Headrick, M.L., S. Korangy, N.H. Bean, F.J. Angulo, S.F. Altekruse, M.E. Potter and K.C. Klontz, 1998. The epidemiology of raw milk-associated foodborne disease outbreaks reported in the United States, 1973 through 1992. Am. J. Public Health, 88: 1219-1221.

Hegarty, H., M.B. O'Sullivan, J. Buckley and C. Foley-Nolan, 2002. Continued raw milk consumption of farms: Why? Commun. Dis. Public Health, 5: 151-156.

Herrera, E.R., R. Jimenez, O.I. Aruoma, S. Hercherg, T. Sanchez-Garcia and C. Fraga, 2009. Aspects of antioxidant foods and supplements in health and disease. Nutr. Rev., 67: S140-S144.

Hunter, D.C., R. Brown, T. Green, C. Thomson, M. Skeaff, S. Williams, J.M. Todd, C.E. Lister, T. McGhie, J. Zhang, H. Martin, P. Rippon, R. Stanley and M.A. Skinner, 2012. Changes in markers of inflammation, antioxidant capacity and oxidative stress in smokers following consumption of milk and milk supplemented with fruit and vegetable extracts and vitamin C. Int. J. Food Sci. Nutr., 63(1): 90-102.

Hunter, F.E. Jr., J.M. Gebicki, P.E. Hoffstein, J. Weinstein and A. Scott, 1963. Welling and lysis of rat liver mitochondria induced by ferrous ions. J. Biol. Chem., 238(2): 828-835.

Huth, P.J., D.B., DiRienzo and G.D. Miller, 2006. Major scientific advances with dairy foods in nutrition and health. J. Dairy Sc., 89: 1207-1221.

Jakobsen, M. and J. Narvhus, 1996. Yeast and their possible beneficial and negative effects on the quality of dairy products. Int. Dairy J., 60: 755-768.

Jayaro, B.M. and D.R. Henning, 2001. Prevalence of foodborne pathogens in bulk tank milk. J. Dairy Sci., 84: 2157-2162.

Johnston, C.S., C.L. Dancho and G.M. Strong, 2003. Orange juice ingestion and supplemental vitamin C are equally effective at reducing plasma lipid peroxidation in healthy adult women. J. Am. Coll. Nutr., 22(6): 519-523.

Joshipura, K.J., F.B. Hu, J.E. Manson, M.J. Stamfer, E.B. Rimm, F.E. Speizer, G. Colditz, A. Ascherio, B. Rosner, D. Spiegelman and W.C. Willette, 2001. The effect of fruit and vegetable intake on risk for coronary heart disease. Ann. Int. Med., 134(12): 1106-1114.

Kaper, J.B., J.P. Nataro and H.L.T. Mobley, 2004. Pathogenic *Escherichia coli.* Nat. Rev. Microb., 2: 123-140.

Kaplan, S.L., 2005. Implications of methicillin-resistant *Staphylococcus aureus* as a community-acquired pathogen in pediatric patients. Infect. Dis. Clin. North Am., 19: 747-757.

Kulshrestha, S.B., 1990. Prevalence of enteropathogenic serogroups of *E. coli* in milk products samples from Bareilly and their multiple drug resistance. Indian J. Dairy Sci., 43: 373-378.

Kurmann, J.A. and J.L.J. Rasic, 1988. Technology of Fermented Special Products. Fermented Milks: Science and Technology. IDF Bulletin No. 277, International Dairy Federation, Brussels, Belgium, pp: 101-109.

Lee, Y.K. and S.F. Wong, 1993. Stability of lactic acid bacteria in fermented milk. In: Lactic acid bacteria. In: Salminen, S. and A. Von Wright (Eds.), Marcel Dekker Inc., New York, pp: 103-114.

Lejeune, J.T. and P.J. Rajala-Schultz, 2009. Unpasteurized milk: A continued public health threat. Clin. Infect. Dis., 48: 93-100.

Lennie, T.A., 2001. Influence of market forces on nutraceutical research: Role of the academic researcher. Nutrition, 17: 423-424.

Leonard, S.S., D. Cutler, M. Ding, V. Vallyathan, V. Casrranova and X. Shi, 2002. Antioxidant properties of fruit and vegetable juices: More to the story than ascorbic acid. Ann. Clin. Lab. Sci., 32(2): 193-200.

Lindermark-Mansson, H. and B. Akesson, 2000. Antioxidative factors in milk. Br. J. Nutr., 84: s103-s110.

Liu, Q., A. Raina, M. Smith, L. Sayre and G. Perry, 2003. Hydroxynoneal, toxic carbonyls and Alzheimer disease. Molec. Aspect. Med., 24: 305-313.

Maduka, H.C.C., 2008. Effects of time course administration of bergenin an isolate of Sacoglottis gabonensis: Nigerian alcoholic beverage additive on lipid peroxidation and natural antioxidant defences during normal biological oxidation reaction in weaning rats in vivo. Niger. J. Botany, 21(1): 109-121.

Mahami, T., S. Odonkor, M. Yaro and A. Adu-Gyamfi, 2011. Prevalence of antibiotic resistant bacteria in milk sold in Accra. Int. Res. J. Microbiol., 2(4): 126-132.

Mohamed, N.N.I. and I.E.M. El-Zubeir, 2007. Evaluation of the hygienic quality of market milk of Khartoum State (Sudan). Int. J. Dairy Sci., 2: 33-41.

Mukhtar, H. and N. Ahmad, 2000. Tea polyphones: Prevention of cancer and optimizing health. Am. J. Clin. Nutr., 71: 1698s-1702s.

Muraoka, W.C., C. Gay, D. Knowles and M. Borucki, 2003. Prevalence of *Listeria monocytogens* in bulk tank milk of Pacific Northwest. J. Food Protect., 66: 1413-1419.

Murinda, S.E., L.T. Nguyen, S.J. Ivey, B.E. Gillespie, R.A. Almeida, F.A. Draughon and S.P. Oliver, 2002a. Molecular characterization of *Salmonella spp*. Isolated from bulk tank milk and cull dairy cow fecal samples. J. Food Protect., 65: 1100-1105.

Murinda, S.E., L.T. Nguyen, S.J. Ivey, B.E. Gillespie, R.A. Almeida and S.P. Oliver, 2002b. Prevalence and molecular characterization of *Escherichia coli* 0157: H7 in bulk tank milk and fecal samples from cull cows: A 12-month survey of dairy farms in East Tennessee. J. Food Protect., 65: 752-759.

Murinda, S.E., L.T. Nguyen, H.M. Nam, R.A. Almeida, S.J. Headrick and S.P. Oliver, 2004b. Detection of sorbitol-negative and sorbitol-positive Shiga toxin-producing *Escherichia Coli*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Salmonella spp*. in dairy environmental samples. Foodborne Pathog. Dis., 1: 97-104.

Natelson, S., 1961. Yoghurt Processing. In: Charles, T. (Ed.), Microtechnique of Clinical Chemistry. 2nd Edn., Spring Field, pp: 121.

Nettleton, J.A., L.M. Steffen, W. Palmas, G.L. Burke and D.R.J. Jacobs, 2008. Associations between microalbuminuria and animal foods, plant foods and dietary patterns in the multiethnic study of atherosclerosis. Am. J. Clin. Nutr., 87: 1825-1836.

Okonkwo, O.I., 2011. Microbial analysis and safety evaluation of Nono: A fermented milk product consumed in most parts of Northern Nigeria. Int. J. Dairy Sci., 6(3): 181-189.

Okpalugo, J., K. Ibrahim, K.S. Izebe and U.S. Inyang, 2008. Aspects of microbial quality of some milk products in Abuja, Nigeria. Trop. J. Pharm. Res., 7(4): 1169-1178.

Oliver, S.P., B.M. Jayarao and R.A. Almeida, 2005. Foodborne pathogens in milk and the dairy farm environment: foodsafety and public health implications. Foodborne Pathog. Dis., 2(2): 115-129.

Oliver, S.P., K.J. Boor, S.C. Murphy and S.E. Murinda, 2009. Food safety hazards associated with consumption of raw milk. Foodborne Pathog. Dis., 6(7): 793-806.

Pangloli, P., Y. Dje, O. Ahmed, C.A. Doane, S.P. Oliver and F.A. Draughon, 2008. Seasonal incidence and molecular characterization of *Salmonella* from dairy cows, calves and farm environment. Foodborne Pathog. Dis., 5(1): 87-96.

Philanto, A., 2006. Antioxidative peptides derived from milk proteins. Int. Dairy J., 16: 1306-1314.

Prior, R.L., G. Cao, R.L. Prior and G. Cao, 2000. Analysis of botanicals and dietary supplements for antioxidant capacity: A review. JAOAC Int., 83: 950-956.

Roberts, A.J. and M. Wiedmann, 2003. Pathogen, host and environmental factors contributing to the pathogenesis of Listeriosis. Cell. Mol. Life Sci., 60: 904-918.

Roe, J.H. and C.A. Kuether, 1943. Estimation of ascorbic acid. J. Biol. Chem., 147: 3999.

Rohm, H., F. Eliskases-Lechner and M. Brauer, 1992. Diversity of yeasts in selected dairy products. J. Appl. Bacteriol., 72: 370-376.

Schlegelova, J., V. Babak, E. Klimova, J. Lukasova, P. Navratilova, A. Sustackova, I. Sediva and D. Rysanek, 2002. Prevalence of and resistance to anti-microbial drugs in selected microbial species isolated from bulk milk samples. J. Vet. Med. B., 49: 216-225.

Smooth, L.M. and M.D. Pierson, 1997. Indicator Microorganisms and Microbiological Criteria. In: Doyle, M.P., L.R. Beuchat and T.J. Montville (Eds.), Food Microbiology: Fundamentals and Frontiers. American Society for Microbiology, Washington DC., pp: 66-80.

Soomro, A.H., M.A. Arain, M. Khaskheli and B. Bhutto, 2002. Isolation of *Escherichia coli* from raw milk and milk products in relation to public health sold under market conditions at Tandojam, Pakistan. Pak. J. Nutr., 1: 151-152.

Sympoura, F., A. Cornu, P. Tournayre, T. Massouras, J.L. Berdague and B. Martin, 2009. Odor compounds in cheese made from the milk of cows supplemented with extruded linseed and alpha tocopherol. J. Dairy Sci., 92: 3040-3048.

Tietz, N., 1970. Yoghurt Processing. Fundamental of Clinical Chemistry. W.B. Saunders, Philadelphia, West Washington, pp: 173-176.

Tormo, H., C. Agabriel, C. Lopez, D.A.H. Lekhal and C. Roques, 2011. Relationship between the production conditions of goat's milk and the microbial profiles of milk. Int. J. Dairy Sci., 6: 13-28.

Umoh, V.J., A.A. Adesiyun and N.E. Gomwalk, 1990. The occurancce of staphylococcus aureus in fermented milk products (Fura and Manshanu) in Nigeria. Int. J. Food Microbiol., 10: 343-348.

Van Kessel, J.S., J.S. Karnes, L. Gorski, B.J. McCuskey and M.L. Perdue, 2004. Prevalence of *Salmonella*, *Listeria monocytogens* and fecal coliforms in bulk tank milk on U.S dairies. J. Dairy Sci., 87: 2822-2830.

Van Soestbergen, A.A. and H.L. Ching, 1969. Pour plates or streak plates? Appl. Microbiol., 18(6): 1092-1093.

Waak, E., W. Tham and M.L. Danielsson-Tham, 2002. Prevalence of *Listeria monocytogenes* strains isolated from raw milk in farm bulk tanks and dairy plants receiving tanks. Appl. Environ. Microbiol., 68: 3366-3370.

Wells, S.J., P.J. Fedorka-Cray, D.A. Dargatz, K. Ferris, and A. Green, 2001. Fecal shedding of Salmonella by dairy cows on farm and at cull cow markets. J Food Protection. 64:3-11.

Wills, D.E., 1987. Evaluation of Lipid Peroxidation in Lipids and Biomembranes. In: Snell, K. and Mullock, (Eds.), Biochemical Toxicology. B. Oxford IFL Press, Chapter 6, 127-152.

Wong, A.C., 1998. Biofilms in food processing environments. J. Dairy Sci., 81: 2765-2770.

Yusuf, S., S. Hawken, S. Ounpuu, T. Dans, A. Avezumi, F. Lenas, M. McQueen, A. Budaj, P. Pais, J. Varigos and L. Lisheng, 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): Case control study. Lancet. 364: 937-952.

Zall, R.R., 1981. Lactose utilization by bacteria. Dairy Microbiology. Applied Science Publishers Ltd., London, pp: 150-190.

Zulueta, A., A. Maurizi, A. Frigola, M.J. Esteve, R. Coli and G. Burini, 2009. Antioxidant capacity of cow milk, whey and deproteinized milk. Int. Dairy J., 19(6-7): 380-385.

Kirkova, M., E. Ivancheva and E. Russanov, 1995. Lipid peroxidation and antioxidant enzyme activity in aspirin-treated rats. General pharmacol., 26(3): 613-617.