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

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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 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
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; Symoura 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 retail 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 fecal 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 5º-55ºC 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 ºC 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 then washed off with distilled water. They were decolorized with acetone alcohol solution and washed immediately with distilled water. The smears were 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 acid solution). 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 acid solution). The smears 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:

\[
\text{Absorbance} \times 46
\]

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^{-3}mole/s. Results were expressed as mean±standard deviation and could be reproduced within±5%. The experiment was repeated three times.

Determination 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 diketoglutaric acid in a strong acid and the reaction of diketoglucinic acid with 2, 4-DNPH to form diphenyl hydrazine. The hydrazine dissolves in strong H_{2}SO_{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 malondialdehyde 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 prepared 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 Nørhhus, 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
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 Republic, 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).

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Malondialdehyde (mg/mL)</th>
<th>Malonaldehyde (mg/mL)</th>
<th>Ascorbic acid (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.93±0.05^b</td>
<td>2.80±0.10^b</td>
<td>1.50±0.30</td>
</tr>
<tr>
<td>B</td>
<td>2.50±1.60^a</td>
<td>3.50±0.80^a</td>
<td>0.90±0.10^a</td>
</tr>
<tr>
<td>C</td>
<td>1.40±0.70^a</td>
<td>5.50±0.10^a</td>
<td>1.20±0.15^a</td>
</tr>
<tr>
<td>D</td>
<td>1.20±0.15^a</td>
<td>4.50±0.70^a</td>
<td>1.40±0.10^a</td>
</tr>
<tr>
<td>E</td>
<td>1.40±0.10^a</td>
<td>4.50±0.70^a</td>
<td>1.40±0.10^a</td>
</tr>
<tr>
<td>F</td>
<td>1.40±0.10^a</td>
<td>4.50±0.70^a</td>
<td>1.40±0.10^a</td>
</tr>
</tbody>
</table>

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

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 aureus 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 malondialdehyde 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, Staphylococcus 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


