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Occurrence of Disease Causing Organisms Including Bacteriophages in Indigenous Fermented Milk Products

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Abstract: The objective of this investigation is to focus on such microbial changes, which are a consequence of unhygienic practices during production, incomplete fermentation and poor storage conditions. In India different fermented products are prepared from milk. These products are mostly intended primarily to conserve the nutritional values of milk. However, since most of these products are flavored and sweetened, these are mostly consumed during festival seasons. Like milk these products not only provide nutrients, but also are excellent media for the growth of microorganisms. Microbial growth is seen in the results, which involves successive changes with enteric or food related factors leading to incidence of food poisoning and deterioration of product. Isolation of coliphages is an indication of presence of other phages which could be aetiology of certain improper fermentation due to lack of proper inoculum.

Key words: Coliphages, dahi, lassi, shrikhand, total bacterial count, yeast mould count

INTRODUCTION

Fermented milk products not only are consumed directly but often form starting materials from which other dairy products are also manufactured (Thapa, 2000). In countries like ours, naturally controlled fermentation is observed in curdling of milk to make 'Dahi'. One of the primary objectives of making such products was to preserve the nutritive values of milk from spoilage due to growth of harmful microorganisms (Harper *et al*., 2008) some of which were even pathogens (Durham, 2006). Contamination by disease causing microorganisms can occur at any point in the food-handling sequence. Infections like septic sore throat, scarlet fever and food poisoning has been traced to the consumption of milk products. Human being and animals both are responsible for the contamination of milk with streptococci (Yadav *et al*., 1993). The detection and control of pathogens and food spoilage microorganisms are important parts of food microbiology (Fahey *et al*., 2006). Some of these like the bacteriophages also cause diseases to the other beneficial microbes too, like phages those destroy the gram positive lactic acid bacteria which are critical to the production of fermented milk products such as dahi while others carry variety of virulence factors that make their bacterial hosts potent human pathogens; this seems to be the case for major human pathogens such as *Micrococcus aureus* and *E.coli* (O'Flaherty *et al*., 2005) and (Reid *et al*., 2000). Coliphages have received increased attention and support in recent years as rapid and inexpensive indicators of fecal pollution and enteric pathogens (Lugoli *et al*., 2009). Not only this, presence of coliphages can also be inferred as possibility of presence of other phages especially those which infect important organisms like *Lactococcus* and *Lactobacillus* species.

One of important factor, that affects the presence, survival and growth of unwanted microorganisms in such products, is the pH of the product. It is known that a low pH favors the growth of yeast and molds. In neutral or alkaline pH foods bacteria are more dominant (Todar, 2000).

In this investigation emphasis is given not only on the presence of pathogens which are bacteria, yeast and molds but also on bacteriophages which can serve as indicator of the fermentation efficiency.

MATERIALS AND METHODS

These studies were conducted over a period of 24 months starting from April 2008 to April 2010 to record the seasonal variations.

Sampling of dairy products from market: Representative but random samples of different dairy products like dahi, shrikhand (various flavors) and lassi and fruit shrikhand were picked up from retail market according to (IS: 5404:1995).

The samples were tested for physical parameters like pH, temperature, color, odor and feel of consistency. These were also subjected to chemical analyses like:

Determination of total solids: Total solid contents of dahi, shrikhand, amrakhand and lassi were determined as

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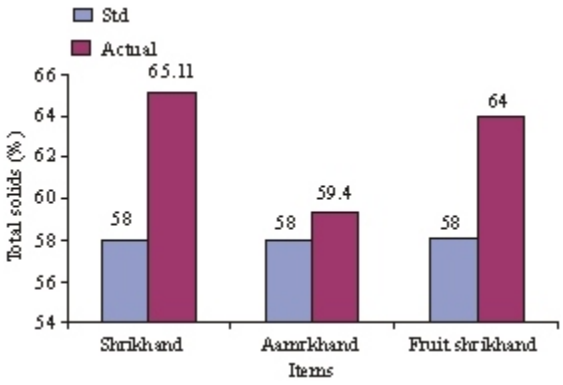

Fig. 1: Total solids of products examined. The standard values are as per PFA (1954, 2009)

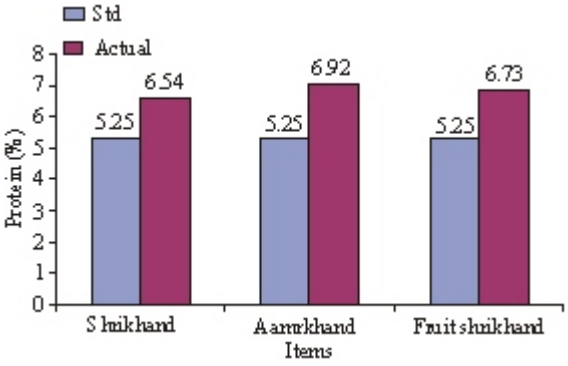

Fig. 2: The protein content of the different products. The standard values are as per PFA (1954, 2009)

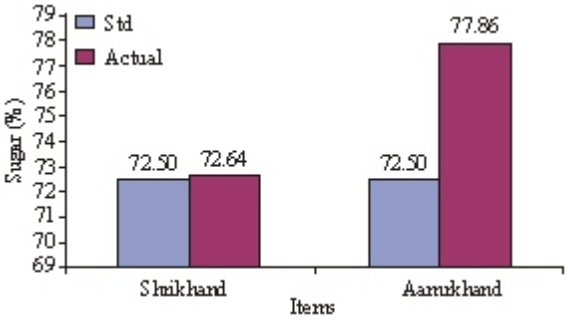

Fig. 3: Sucrose content of products. The standard values are as per PFA-1954 (2009)

per BIS (1989). The standard values were obtained from PFA (1954, 2009). The results are as shown in Fig. 1.

Estimation of protein: The protein content of the products was determined as per BIS (1989). The standard values were obtained from PFA (1954, 2009). The results are described in Fig. 2.

Estimation of sucrose: The Sucrose content of the products was estimated as per BIS (1989). The standard

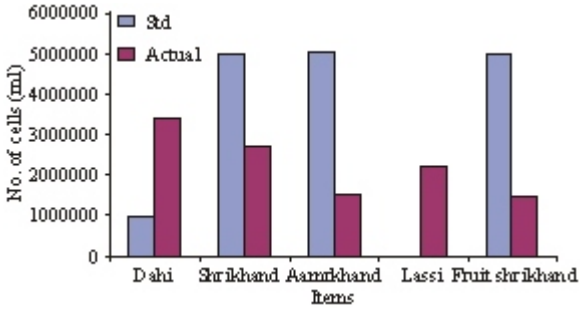

Fig. 4: Total Plate Count of the product. The standard values are as per PFA (1954, 2009)

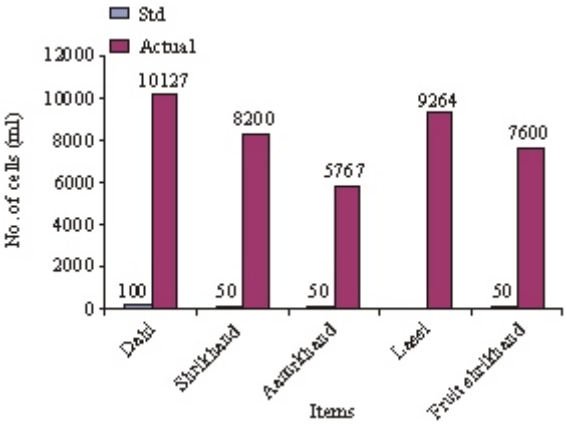

Fig. 5: Yeast and mould count of the product. The standard values are as per PFA (1954, 2009)

values were obtained from PFA (1954, 2009). The results are described in Fig. 3.

Microbiological analysis: The samples, which were collected under aseptic conditions, were homogenized in a blender using sterile physiological saline (0.85% NaCl solution).

Total plate count: Detection of total plate count was carried out as per IS: 5402 (1995). The standard values were obtained from PFA (1954, 2009). The results are described in Fig. 4.

Yeast and mold count: Detection of yeast and mould count were carried out as per IS: 5403 (1999). The standard values were obtained from PFA (1954, 2009). The results are described in Fig. 5.

Coliphages Isolation: The isolation of coliphages is carried out as a two-step enrichment procedure as per methods to detect and genotype coliphages in water and shellfish (US EPA 1601).

RESULTS AND DISCUSSION

It can be seen from the Fig. 1 that in amrakhand and fruit shrikhand total solid were higher than standard value due to addition of pulp and fruits in comparison with that of shrikhand.

Protein content of shrikhand, amrakhand as well as fruit shrikhand were found to be higher than the standards as evidenced from Fig. 2. The reasons for such high protein content are unknown.

It can be seen from Fig. 3 that the sucrose content of shrikhand was observed to be slightly lower than standard but in case of amrakhand it may be very high than the standards. It is due to addition of mango pulp, which contains sucrose as well as fructose.

It can be seen from Fig. 4 that the total plate count of Shrikhand and amrakhand are less than standard it is due to sugar, which acts as a preservative and inhibit the bacterial growth and the total plate count of dahi is above the standard inspite of lactic acid present. Lassi is not included in PFA (1954, 2009). Therefore there is no standard for lassi.

It evident from Fig. 5 that yeast mould count of dahi is above the standard as these organisms can grow at a low pH and in low water activity (a_w) created by high sugar concentration.

CONCLUSION

The authors have earlier pointed that since most of the traditionally fermented dairy products in India are manufactured in the unorganized sector, safety is often compromised with investment and profit, leading to contamination of these products with pathogens belonging to the genera *Salmonella, Shigella, Listeria and Escherichia* (Kumbhar *et al*., 2009). In this survey it has been observed that besides these there are other microbes-both eukaryotes (yeast and molds) and prokaryotes (bacteria) present in these products. These might have come in large numbers, as a contaminant during manufacturing or may have multiplied in the product during storage. The factors that affect their number are:

- Poor sanitation and hygiene during manufacturing. This is especially so during the dry seasons when there are acute water scarcity in many regions in our country, where even safe potable water is not available for drinking, leave aside proper cleaning of utensils used in manufacturing of these products
- Incomplete fermentation leading to lesser amount of production of substances like lactic acid
- improper packaging and finally
- Improper distribution and storage during its shelf life. Of these 2 and 4 plays very important role as incomplete fermentation could be due to starters which are not defined and often various coliforms (which are heterofermentative lactic acid producers) carry out the fermentation which is far below the standard specified limits

This problem gets further aggravated as these coliforms are often contaminated with coliphages, which can drastically reduce the bacterial population leading to poor lactic fermentation and thus allowing other organisms to grow. Presence of such coliphages has been frequently observed in such contaminated milk products. Coliphages can be considered as indicator phages, as under such unhygienic conditions, other phages can also get access in the manufacturing process leading to reduction of number of lactic microorganisms like *Lactobacillus* and *Lactococcus* spp.

Upon scrutinizing the results it can be seen that the yeast and mold population are very high above the standard values, where as bacterial count (as seen from TPC) is well below the standard values except in curd (Fig. 5). Again this is due to the fact that the other products like shrikhand, amrakhand and lassi contain high percentage of sugar and sugar is added in products in amrakhand at levels far higher than that specified (Fig. 3) which lowers the water activity (a_w) and under such condition only yeast and mold can thrive.

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