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Microbiological Analysis of Pathogenic Organisms in Indigenous Fermented Milk Products

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Abstract: Milk is one of the most affordable source of many nutritions like proteins and vitamins. Many people do not like to consume milk in its liquid form. Therefore, there are several products which are made out milk called as Dairy Products, which preserve the nutritive values of milk and makes it easily acceptable to consumers. One such class of products, is the fermented dairy products. In India these are named in vernacular language as Dahi (lactic fermented and curdled milk), Shrikhand with different flavours prepared from hung curd, sugar and real fruit pulps, lassi which is nothing but a sort of butter milk with slightly high milk solid content and sweetened with sugar. However a large volume of these products are produced in unorganized sector, unbranded, with little precautions of food safety and quality. This investigation is a factual documentation of such a finding and suggestions as to the methods needed to improve the safety and quality.

Key words: Dahi, shrikhand, lassi, micrococcus, escherichia and milk

INTRODUCTION

Food is the matter of health and a matter of trust also. Traditionally for several centuries man has adopted fermentation as a means of food preservation and also found them to have nutritional and therapeutic advantages. Fermented milk products constitute a vital component of the human diet in many regions of the world. In the Indian sub-continent such products are also classified as "indigenous milk products" like dahi (curd), lassi, shrikhand etc. which are prominent in peoples diet. Indigenous milk products refer exclusively to dairy products of a particular region or country. In the present context, these are products of Indian origin.The significance of milk in human nutrition in now well established as it is considered as the best, ideal and complete food for all age groups. Milk and dairy products are an excellent source of calcium, phosphorus and magnesium. These minerals in optimum ratio are present in milk and are required for optimum growth and maintenance of bones (Aneja *et al.*, 2002). However, in spite of this, milk can also serve not only as a potential vehicle for transmission of some pathogens but also allows these organisms to grow, multiply and produce toxins. A variety of pathogenic organisms may gain access in to milk and milk products from different sources and cause different types of food born illnesses. Milk and milk products may carry toxic metabolites of different organisms growing in it. Ingestion of such products, contaminated with these metabolites, cause food poisoning. On the other hand the ingestion of viable pathogenic bacteria along with the food product leads to food borne infection. Some time these organisms undergo lysis in the gastrointestinal tract and liberate toxic substances from inside the cells which are detrimental to the health of the consumers (Aneja *et al.*, 2002). Recent development regarding Quality and safety management systems such as ISO and Hazard Analysis Critical Control Point (HACCP) has reduced such incidences. Indian Standard Institution in their "Specification for fermented milk products" (IS: 7035, 1973) has clearly defined the acceptable quality of such products, which would be safe for consumption.

This study has been attempted to carry out a survey of some local suppliers of such products within an area of radius of 100 km.

MATERIALS AND METHODS

This studies were conducted over a period of 24 months to see the difference in the quality in different seasons. The period of study was June 2007 to May 2009.

Sampling of dairy products from market: Representative samples of different dairy products like Dahi, Shrikhand (various flavors) and lassi were picked up from retail market at random depending on the volume of stock with the retailer. If required, composite samples were made, of each product from each retailer, in the laboratory. The samples were then subjected to different tests which were physical like pH, temperature, color, odor and feel of consistency. These were the subjected to chemical tests like

Estimation of fats: The fat content of the products were estimated by Gerber method (IS: 1224, 1977) (Fig. 1).

Estimation of Moisture content: The moisture content of all the products were estimated (IS: 11623, 1986) (Fig. 2).

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Estimation of Titrable Acidity: The titrable acidity of all samples are detected as per (IS: 11766, 1986), which is the average results of 12 samples of each products (Fig. 3).

Microbiological analysis: The samples were homogenized in a blender using sterile physiological saline (0.85% NaCl solution).

Coliform count: The supernatant of the above mentioned suspension was diluted 1:10 and was directly plated on Violet Red Bile Agar (IS: 5404, 1995). The inoculated plates of this medium were incubated 37ºC for 48 h (Fig. 4). In case of suspected colonies, the samples were then enriched in Brilliant Green Lactose Bile Broth (BGLB) having dehydrated ox bile-2%, lactose-1%, peptone-1% and 1.3 mL of 1.0 % solution of brilliant green in water, final pH 7.2. The tubes were incubated at 37ºC for 48 h. This growth in these tubes were then inoculated on Hicrome *E. coli* agar (Hi Media Ltd., Bombay). The plates were incubated at 30ºC for 4 h and then at 44ºC for 18 h (Table 1).

***Micrococcus aureus*:** Since milk is very good nutrient for *Micrococcus aureus*, the supernatant of the homogenized samples were subjected to detection of this microorganism, which is a potent exotoxin producer (Draft ISO/DIS 6888, 1983). The isolates were checked for coagulase positive nature using defibrinated plasma (Table 2).

Salmonella: Detection of *Salmonella* were carried out as per the prescribed method of (IS: 5887, 1999).

***Shigella*:** The presence or absence of *Shigella* spp. was carried out as per (IS: 5887, 1999).

***Listeria monocytogenes*:** The detection of *Listeria monocytogenes* was done as per (ISO 10560, 1999).

RESULTS AND DISCUSSION

It can be seen from the Fig. 1 that only in case for shrikhand and fruit shrikhand, there is a large unjustifiable deviation from the standard, which often does more harm than good.

It is only in case of shrikhand and amrakhand, that titrabele acidity is below standard which might pose not only a threat of spoilage by microorganisms, but allow food poisoning organisms like *Micrococcus aureus* to grow and make the product hazardous. (Fig. 3)

It may be noted from the Fig. 2 that Moisture contents of the products mentioned are well within limits. However, since there are no standards for Dahi and Lassi, these parameters were not checked as there is no means of comparison.

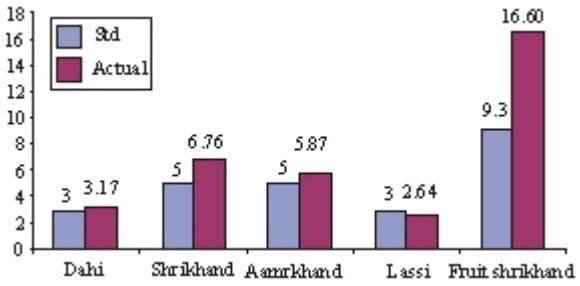

Fig. 1: Fat content of the products examined. The standard values are as per PFA-1954 (2009)

Fig. 2: Moisture of the different products. The standard values shown in figure are as per PFA-1954 (2009)

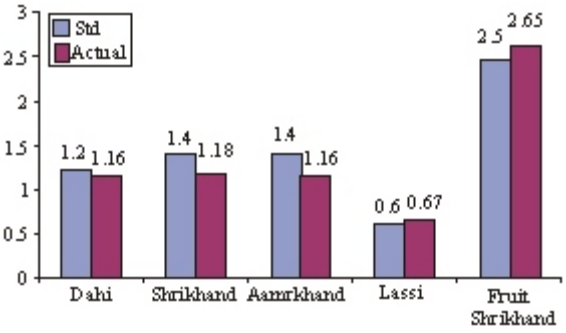

Fig. 3: Titrable acidity of the different products. The standards as shown in the figure are as per PFA-1954 (2009)

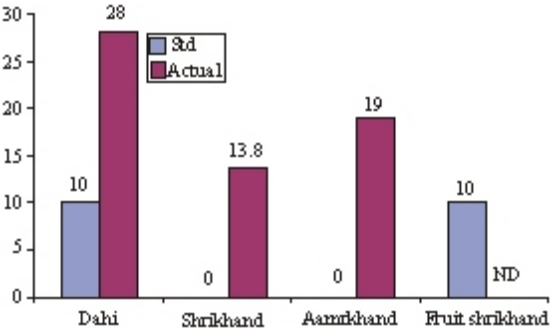

Fig. 4: Coliform count of different products. The standards shown above are as per PFA-1954 (2009)

Table 1: Microbiological analysis of Different Dahi samples

S. No.	Body & Texture	Ent *E. coli* /gm	Salmonella /gm	Shigella/gm	Micrococcus aureus /gm	*Listeria monocytogens* /gm
1.	Loose	ND	ND	ND	ND	ND
2.	Firm	Present	ND	ND	ND	ND
3.	Loose	ND	ND	ND	ND	ND
4.	Loose	ND	ND	ND	ND	ND
5.	Loose	ND	ND	ND	Present	ND
6.	Loose	Present	ND	ND	Present	ND
7.	Loose	Present	ND	ND	ND	ND
8.	Firm	Present	ND	ND	ND	ND
9.	Loose	ND	ND	ND	ND	ND
10.	Firm	Present	ND	ND	ND	ND
11.	Firm	ND	ND	ND	ND	ND
12.	Loose	ND	ND	ND	ND	ND

ND: Not Detected

Table 2: Microbiological analysis of Shrikhand samples

S. No.	Ent *E. coli* /gm	*Salmonella* /gm	Shigella /gm	*Micrococcus aureus* /gm	*Listeria monocytogenes* /gm
1.	ND	ND	ND	ND	ND
2.	ND	ND	ND	Present	ND
3.	Present	ND	ND	ND	ND
4.	ND	ND	ND	ND	ND
5.	Present	ND	ND	ND	ND
6.	ND	ND	ND	ND	ND
7.	ND	ND	ND	ND	ND
8.	ND	ND	ND	ND	ND
9.	Present	ND	ND	Present	ND
10.	ND	ND	ND	ND	ND
11.	Present	ND	ND	ND	ND
12.	ND	ND	ND	ND	ND

ND: Not Detected

Table 3: Microbiological analysis of Shrikhand with mango pulp samples

Sr. No.	Ent *E. coli* /gm	Salmonella /gm	Shigella /gm	*Micrococcus aureus* /gm	*Listeria monocytogenes* /gm
1.	ND	ND	ND	ND	ND
2.	ND	ND	ND	ND	ND
3.	ND	ND	ND	ND	ND
4.	ND	ND	ND	ND	ND
5.	ND	ND	ND	ND	ND
6.	Present	ND	ND	Present	ND
7.	ND	ND	ND	ND	ND
8.	ND	ND	ND	ND	ND
9.	ND	ND	ND	Present	ND
10.	ND	ND	ND	ND	ND
11.	ND	ND	ND	ND	ND
12.	ND	ND	ND	ND	ND

ND: Not Detected

It is vivid from the results above that 3 out of 4 products shows high coliform count which is detrimental to the safety of the product, from public health point of view (Fig.4).

It can be seen from Table 1, that enteric *E. coli* was present in 5 out of 12 samples and *Micrococcus aureus* is present in 2 out of 12 samples. This means that chances of contracting an enteric disease through consumption of such curds is nearly 50%

Shrikhand is a sweetened product made out of hung curd, but the sugar is used as a flavouring agent rather than reducing water activity. However, there are certain spices like crushed cardamom seeds, the oleoresins of which would inhibit the growth of many microorganisms and that is why it is observed from Table 2, that 4 out of 12 samples which were really prepared in unhygienic conditions shows the presence of *E. coli* and 2 shows the presence of *Micrococcus aureus*. Therefore, this product has more chances of giving food poisoning cases as *Micrococcus aureus* is a potent exotoxin producer.

This is a product like shrikhand. The only difference is that it contains actual Alphonso mango pulp. Again the point that is to be noted from Table 3, is that, there is presence of *Micrococcus aureus* which is a potent exotoxin producer, capable of causing food poisoning.

In fruit shrikhand, pineapple, grape, apple and pomegranate was found to have been added shrikhand. Table 4 show that 2 samples were found to be safe as only one of it showed the presence of *E. coli*.

Table 4: Microbiological analysis of Fruit Shrikhand other than mango samples

Sr. No.	Ent *E.coli* /gm	Salmonella /gm	Shigella /gm	*Micrococcus aureus* /gm	*Listeria monocytogenes* /gm
1.	ND	ND	ND	ND	ND
2.	Present	ND	ND	ND	ND

ND: Not Detected

Table 5: Microbiological analysis of Lassi samples

Sr. No.	Ent *E.coli* /ml	Salmonella /ml	Shigella /ml	*Micrococcus aureus* /m	*Listeria monocytogenes* /ml
1.	Present	ND	ND	Present	ND
2.	Present	ND	ND	Present	ND
3.	Present	ND	ND	Present	ND
4.	Present	ND	ND	Present	ND
5.	Present	ND	ND	ND	ND
6.	ND	ND	ND	ND	ND
7.	ND	ND	ND	ND	ND
8.	ND	ND	ND	ND	ND
9.	ND	ND	ND	ND	ND
10.	Present	ND	ND	ND	ND
11.	ND	ND	ND	ND	ND
12.	Present	ND	ND	ND	ND

ND: Not Detected

This product is prepared from curd, which was mixed with water to get certain pulp density. There is no standard for the pulp density and due to this every manufacturer would add any amount of water they like as long as they the customers for their product. There is also presence of sugar in this product. Table 5 show that out of 12 samples there are about 7 samples containing enteric *E. coli* and 4 out of 12 contained *Micrococcus aureus*. Production of this is mostly in the unorganized sector and hence there is no brand that needs to be put nor there is any need for a definite retail outlay to sell this product. All this factors make the manufacturer free to add any water they like and these contaminants are from the water.

CONCLUSION

Most of the Indigenous fermented milk products are prepared by traditional methods in the unorganized industrial sector. Such methods often bring about contamination of various microorganisms including pathogens. To overcome these problems these traditional methods are automated in different organized sectors which should have helped to improve the microbiological quality of the products. However, due to high cost of capital investment, a sizeable portion of these products are still produced in the unorganized sectors, many of which do not even have the necessary licenses to do so and hence are not bound by any law of product safety. These are, more than often, not branded and the volume of sales are significantly high but localized regionally. This has resulted in poor microbiological quality with respect to presence of pathogens and spoilage organisms. Therefore, If the taste and flavors of these tradional flavors is to be maintained traditionally, the producers of these high risk products should be educated in the minimum requirements of good hygienic and sanitary conditions, good manufacture practices and good storage conditions which will reduce the risk of poor microbiological quality and increase the safety of the products.

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