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Effect of $_{P}$H on Microbial Quality of Dairy Pack Food

KEYWORDS: Dairy products, Microorganisms, Pathogens and pH

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ABSTRACT *Microorganisms have been playing both useful and harmful roles in human life and this has led to the need of studying these microscopic biological agents extensively. Milk being a nutritious food for human being also provides an ideal environment for microbial growth. Milk and milk products e.g. milk powder, milk, cheese, curd and ice cream etc. constitute important nutritional components for all age groups and also nutritive for pathogens. Study was carried out by using various microbiological techniques to isolate and identify pathogens. Samples of milk and milk product i.e. Amrakhand was analyzed for pathogen and effect of pH on pathogens isolated from milk products. In this study it can be concluded that this milk products poses a serious health risks.*

INTRODUCTION

In order to extend the shelf-life of milk for human consumption by preventing the growth of spoilage organisms as well as preventing the transmission of disease via milk, this highly nutritious, versatile food is usually pasteurized for a short time. The International Dairy Federation defines pasteurization as a process applied to a product with the aim of avoiding public health hazards arising from pathogenic microorganisms associated with such products by heat treatment which is consistent with Minimal chemical, physical and organoleptic changes in the product. Milk is pasteurized by heating at a temperature of about 63^0 C (145^0 F) for 30 min, rapidly cooling it, and then storing it at a temperature below 10^0C (50^0F). Pasteurization kills most, but not all bacteria in milk. The combination of time and temperature used for heat treatment of milk are however, designed to kill all pathogenic microorganisms (ICMSF, 1998; Edema and Akingbade, 2007). Acute diarrhea is a common cause of death in developing countries and the second most common cause of infant deaths worldwide (Victora*et. al.*, 2008).

The extent of contamination and subsequent microbial multiplication directly determine the microbiological quality of the product hence the major emphasis for combating the harmful effect of microorganisms in dairy products includes the following measures, avoiding or minimizing contamination of milk, refrigerated storage of raw milk before processing, avoiding prolonged storage of processed milk and milk products under favorable ambient conditions to control microbial multiplication.

Milk is supposed to constitute a complex ecosystem for various microorganisms including bacteria. Milk products like cheese and curd are widely consumed and marked and has enlisted in many parts of the world for many generations there is an increase demand by the consumer for high quality natural food free from artificial preservatives and contaminating microorganisms contaminations of milk and milk products with pathogenic bacteria is largely due to processing handling and unhygienic conditions (Priyanka singh et.al., 2000). Milk and milk products constitute important nutritional components for all age groups. Good quality milk meets the nutritional needs of the body better than any single food as it contains all the essential food constituents (Sharma and Joshi, 1992). As a result of the presence of these nutrients, milk is an excellent culture medium for many kinds of microorganisms (Henry and Newlander, 1997). A broad spectrum of microbial pathogens contaminates human food and water supplies and cause illness after they or their toxins are consumed. These include a variety of enteric bacteria, aerobes and anaerobes, viral pathogens and yeasts. During past decades microorganisms such as Staphylococcus spp. Salmonella spp. were reported as the most common food borne pathogens that are present in many foods and able to survive in milk and fermented milk products (Tekinsons and Ozdemir, 2006).

Pasteurization remains as essentials stage impossible to reduce the microbiological risk food and to prolong the preservability pasteurization does not impair the nutritional quality or milk fat, calcium and phosphorus. Independently of the situation of milk production in any area milk should not be consumable or used in dairy products without pasteurization. Pasteurization cannot guarantee the absence of pathogenic microorganisms when they are present in large numbers in raw milk or due to post-pasteurization contamination (Salmeron*et. al.*, 2002; Karmen torker and Godic torker, 2006).

Milk is a natural food that has no protection from external contamination and can be contaminated easily when it is separated from the cow (Rosenthal, 1991). Raw milk normally has a varied micro flora arising from a several sources such as the exterior surfaces of the animal and the surface of milk handling equipment such as milking machines, pipeline, and containers (Burton, 1980). Therefore, milk is susceptible to contamination by many pathogenic microorganisms which results in infection and threat to consumer's health additionally there is the potential that disease of cows such as tuberculosis, brucellosis, typhoid and listeriosis can be transmitted.

MATERIALS AND METHODS:

Collection of dairy products i,e. shrikhand sample were directly transported to the laboratory in ice box. They were stored in refrigerator and analyzed within 24 hours. Isolation of the pathogens i.e. *Salmonella, Staphylococcus, Escherichia coli* from dairy samples on selective media petriplates.

Microbiological analysis:

A portion (1 g or 1 ml) from each sample was taken aseptically and diluted in 9 ml sterile distilled water the diluted sample was streak inoculated on sterile selective media as given below

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1. Eosine Methylene Blue (EMB) for Escherichia coli
2. Mannitol Salt Agar (MSA) for *Staphylococcus aureus*
3. Wilson's and Blair (W and B) for *Salmonella typhi*Inoculated petriplates were incubated at 37^{0}C for 24hrs. Identification of the pathogen on the basis of cultural characteristics, Gram staining, Biochemical test, Sugar fermentation test, Catalse test, Oxidase test, Urease test and Coagulase test.

Identification of pathogens:
1. Cultural characteristics, gram nature and colour of colonies were noted.
2. Biochemical examination colonies from each petriplate were picked, subcultured, incubated at 37^{0}C and then identified by the various biochemical tests.

Biochemical tests were performed to confirm *Escherichia coli, Staphylococcus aureus and Salmonella typhi* using Catalase test, Indole test, Methyl red test, Voges prousker test, Urease production, Citrate utilization test and glucose, lactose, mannitol, sucrose sugar fermentation test.

Effect of pH:
Effect of pH on the growth of enteropathogens obtained from amrakhand. 4 Nutrient agar plates with pH5, pH6, pH7, pH8. Each was prepared. The 0.1ml suspension of *Salmonella* obtained from Shrikhand was inoculated on the medium in plate by spread plate technique. The plates were incubated at various temperature from 7^{0}C-77^{0}C as given in table. The plates were incubated for 24hrs at the end on incubation period. The colony count on the petriplate was noted. The same procedure was adopted for *Staphylococcus aureus, Escherichia coli, Shigella dysentriae.*

RESULTS:
Table1. Showed the growth of isolated bacterial pathogens from amrakhand sample at different pH viz.5.0, 6.0, 7.0 and 8.0. The inoculated broth tubes at each pH were incubated at different temperature viz.7^{0}C, 17^{0}C, 27^{0}C, 37^{0}C, 47^{0}C, 570C, 67^{0}C and 77^{0}C. The growth in the tube was compared with control tube. *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* and *Shigelladysentriae* have shown growth at pH 5 and at 37^{0}C incubation temperature\ at pH 6 and at 37^{0}C incubation temperature, also at pH 6 and 47^{0}C incubation temperature. Further it was observed that *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* have shown growth at pH6 and 47^{0}C incubation temperature.

It was also found that *Salmonella typhi, Staphylococcus aureus*, *Escherichia coli* and *Shigelladysentriae* have shown growth at pH 7 and at 370C incubation temperature and 27^{0}C incubation temperature at pH 7. *Staphylococcus aureus, Escherichia coli and Shigelladysenteriae*have shown growth at pH 7.0 and 47^{0}C incubation temperature and *Salmonella typhi* have shown growth at pH 7 and 57^{0}C incubation temperature. Thus it is clear from the table that at favorable pH7. There was no growth when incubated at 7^{0}C, 17^{0}C, 27^{0}C, 37^{0}C, 47^{0}C, 57^{0}C, 67^{0}C and 77^{0}C temperature at pH 8.0.

Table 1: Effect of pH on growth of microbial populations obtained from Amrakhand

Sr. No.	Sample Name	Name of Pathogen	Incubation Temperature for24hrs	CFU/g at pH 5.0	at pH 6.0	at pH 7.0
4	Amrakhand	Sa15	7^{0}C	NG	NG	NG
			17^{0}C	NG	NG	NG
			27^{0}C	0.86×10^2	3.45×10^2	5.9×10^4
			37^{0}C	6.5×10^4	6.4×10^4	7.2×10^4
			47^{0}C	4.6×10^4	4.7×10^4	5.2×10^4
			57^{0}C	NG	NG	NG
			67^{0}C	NG	NG	NG
			77^{0}C	NG	NG	NG
		St4	7^{0}C	NG	NG	NG
			17^{0}C	NG	NG	NG
			27^{0}C	1.22×10^2	2.31×10^2	4.9×10^4
			37^{0}C	5.8×10^4	1.6×10^4	6.7×10^4
			47^{0}C	0.4×10^4	5.8×10^4	1.6×10^4
			57^{0}C	0.21×10^2	0.64×10^2	0.9×10^4
			67^{0}C	NG	NG	NG
			77^{0}C	NG	NG	NG
		Sd4	7^{0}C	NG	NG	NG
			17^{0}C	NG	NG	NG
			27^{0}C	8.43×10^2	20.21×10^2	41.26×10^4
			37^{0}C	4×10^4	6.2×10^4	47.40×10^4
			47^{0}C	NG	NG	NG
			57^{0}C	NG	NG	NG
			67^{0}C	NG	NG	NG
			77^{0}C	NG	NG	NG
		Ec2	7^{0}C	NG	NG	NG
			17^{0}C	NG	NG	NG
			27^{0}C	0.80×10^2	3.6×10^2	8.83×10^2
			37^{0}C	9.76×10^2	6.76×10^2	7.0×10^2
			47^{0}C	0.40×10^2	0.80×10^2	5.43×10^2
			57^{0}C	NG	NG	NG
			67^{0}C	NG	NG	NG
			77^{0}C	NG	NG	NG

St=*Salmonella Typhi*,Ec=*Escherichia Coli*,Sa=*Staphylococcus aureus*,Sd=*Shigella dysentriae*,EC=*Escherichia coli*,N-G=No growth,

DISCUSSION:
Effect of pH on growth of pathogens:
It was found that cfu/ml of *S. aureus* obtained from milk and milk products sample was almost equal at pH 5.0, 6.0, 7.0.at 27^{0}C after 24hrs incubation; it was found that the maximum cfu/ml was obtained at pH 7 at 37^{0}C and the minimum cfu/ml was obtained at pH 5.0 at 47^{0}C. CFU/ml of *S. typhi* obtained from milk and milk products sample was almost equal at pH 5.0, 6.0, 7.0 at 27^{0}C after 24 hrs, it was found that the maximum cfu/ml was obtained at pH 7 at 37^{0}C and the minimum cfu/ml was obtained at pH 5.0 at 57^{0}C. CFU/ml of S. dysentry obtained from milk and milk products sample was almost equal at pH 5.0, 6.0, 7.0 at 270C after 24 hrs incubation, it was found that the maximum cfu/ml was obtained at pH 7 at 370C and the minimum cfu/ml was obtained at pH 5.0 at 470C. CFU/ml of *E. coli* obtained from milk and milk products sample was equal at pH 5.0, 6.0, 7.0. When the organisms were incubated at 27^{0}C after 24 hrs it was found that the maximum cfu/ml was obtained at pH 7 and the minimum cfu/ml was obtained at pH 5.0 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 favours the growth of yeast and molds. In neutral or alkaline pH foods bacteria are more dominant (Todar, 2000). The most important factor controlling Salmonella spp. during the manufacture of cheddar cheese is the acid production by the starter culture, a pH of 5.0 at the time of press-

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ing is sufficient to cause the death of Salmonellae (Keceli, 1997). The pH range at which various groups of bacteria have been shown to grow is from less than 3 to greater than 9. The pH range of an individual species can be quite narrow and may differ greatly from other species. Fermentation acids may affect bacterial growth independently of pH. Milk pH is affected by temperature, generally decreasing with increasing temperature, due to changes in dissociation of ionizable groups. Lipolysis can also decrease the pH of milk, due to hydrolysis of esters, especially phosphoric esters. Heating milk causes a decrease in its pH, and at temperatures <800C, milk pH decreases in linear fashion with increasing temperature (Ma and Barbano, 2003). Comparison of maximum growth rates among microbes at various pH shows that ranking of growth rate between individual species can be pH dependent. The results suggest that pH can be a significant factor determining competition among bacteria (Hlide M. Ostlie, 2004; Russell *et.al.,* 1979). The shift in pH also failed to inhibit growth (Maria *et. al*., 2001).

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