Research Paper

Effect of NaCl on Microbial Quality of Dairy Pack Food

Environment

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ABSTRACT   *Milk being a nutritious food for human being also provides an ideal environment for microbial growth since times immemorial, 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 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. Shrikhand was analyzed for pathogen and effect of NaCl on pathogens isolated from milk products. In this study it can be concluded that this milk products poses a serious health risks.*

INTRODUCTION:

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. 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.

MATERIAL AND METHODS:

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

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

1. Eosine Methylene Blue (EMB) for *Escherichia coli*. 2. Mannitol Salt Agar (MSA) for *Staphylococcus aureus.* 3. Wilson's and Blair (W&B) for *Salmonella typhi* Inoculated petriplates were incubated at 37⁰C for 24hrs. Identification of the pathogen on the basis of cultural characteristics, Gram staining, Biochemical test, Sugar fermentation test, Catalase 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 37⁰C 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 NaCl on microbial population:

Effect of NaCl on the growth of enteropathogens obtained from Shrikhand /Milk /Amrakhand /Ice Cream /Chocobar /Curd / Ghee /Milk Powder. Eight Nutrient agar plates with 2%, 4%, 6%. Each was prepared. The 0.1ml suspension of *Salmonella* obtained from Shrikhand/Milk/Amrakhand/Ice Cream/Chocobar/Curd/Ghee/Milk Powder were inoculated on the medium in plate by spread plate technique. The plates were incubated at various temperature from 7⁰C-77⁰C 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.*

RESULT:

Table1: Effect of NaCl concentration on growth of various pathogens obtained from shrikhand.

Sr. No.	Sample Name	Name of Pathogen	Incubation Temperature for 24hrs	CFU/g		
				2%NaCl	4% NaCl	6%NaCl
1	Shrikhand	Sa23	7^0C	NG	NG	NG
			17^0C	NG	NG	NG
			27^0C	NG	NG	NG
			37^0C	6.0×10^4	6.0×10^4	6.0×10^4
			47^0C	5.1×10^4	2.13×10	$1,80 \times 10^2$
			57^0C	NG	NG	NG
			67^0C	NG	NG	NG
			77^0C	NG	NG	NG
		St3	7^0C	NG	NG	NG
			17^0C	NG	NG	NG
			27^0C	NG	NG	NG
			37^0C	5.7×10^4	4.90×10^2	2.89×10^2
			47^0C	5.0×10^4	4.70×10^2	2.54×10^2
			57^0C	NG	NG	NG
			67^0C	NG	NG	NG
			77^0C	NG	NG	NG
		Sd4	7^0C	NG	NG	NG
			17^0C	NG	NG	NG
			27^0C	6.0×10^4	5.0×10^4	2.0×10^4
			37^0C	3.1×10^4	2.10×10^2	1.30×10^2
			47^0C	NG	NG	NG
			57^0C	NG	NG	NG
			67^0C	NG	NG	NG
			77^0C	NG	NG	NG
		Ec13	7^0C	NG	NG	NG
			17^0C	NG	NG	NG
			27^0C	0.43×10^2	0.30×10^2	0.21×10^2
			37^0C	4.63×10^2	4.5×10^2	0.06×10^2
			47^0C	0.93×10^2	0.43×10^2	0.12×10^2
			57^0C	NG	NG	NG
			67^0C	NG	NG	NG
			77^0C	NG	NG	NG

St=*Salmonella Typhi*, Ec=*Escherichia Coli*, Sa=*Staphylococcus aureus*, Sd=*Shigella dysentriae*, NG= No growth.

Table1.Count of various enteropathogens obtained from Shrikhand sample at different temperature and NaCl% is given in Table 28. It was found that cfu/ml of *Staphylococcus aureus* obtained from shrikhand sample was almost equal at 2%, 4%, 6% NaCl concentration when the organisms were incubated at 27^0C for 24hrs, When the organisms were incubated at 37^0C and 47^0C temperature for 24hrs, it was found that the maximum cfu/ml was obtained at 2% NaCl and the minimum cfu/ml was obtained at 6% NaCl concentration. There was no growth of *Staphylococcus aureus* on MSA agar plate at 2%, 4%, 6% NaCl concentration when incubated at 7^0C, 17^0C, 57^0C, 67^0C, 77^0C temperature.

It was found that cfu/ml of *Salmonella typhi* obtained from shrikhand sample was almost equal at 2%, 4%, 6% NaCl concentration when the organisms were incubated at 27^0C for 24hrs, When the organisms were incubated at 37^0C and 47^0C temperature for 24hrs, it was found that the maximum cfu/ml was obtained at 2% NaCl and the minimum cfu/ml was obtained at 6% NaCl. There was no growth of Salmonella typhi on W&B agar plate at 2%, 4%, 6% NaCl concentration when incubated at 7^0C, 17^0C, 57^0C, 67^0C, 77^0C temperature.

It was found that cfu/ml of *Shigelladysentriae* obtained from shrikhand sample was almost equal at 2%, 4%, 6% NaCl concentration when the organisms were incubated at 27^0C for 24hrs, When the organisms were incubated at 37^0C temperature for 24hrs, it was found that the maximum cfu/ml was obtained at 2% NaCl and the minimum cfu/ml was obtained at 6% NaCl. There was no growth of *Shigelladysentriae* on SSA agar plate at 2%, 4%, 6% NaCl. Concentration when incubated at 7^0C, 17^0C, 47^0C, 57^0C, 67^0C, 77^0C temperature.

It was found that cfu/ml of *Escherichia coli* obtained from shrikh and sample was almost equal at 2%, 4%, 6% NaCl concentration when the organisms were incubated at 27^0C for 24hrs, When the organisms were incubated at 37^0C and 47^0C temperature for 24hrs, it was found that the maximum cfu/ml was obtained at 2% NaCl and the minimum cfu/ml was obtained at 6% NaCl concentration. There was no growth of *Escherichia coli* on EMB agar plate at 2%, 4%, 6% NaCl concentration when incubated at 7^0C, 17^0C, 57^0C, 67^0C, 77^0C temperature.

DISCUSSION:
Effect of NaCl on growth of pathogens:
It was found that cfu/ml of *Staphylococcus aureus* obtained from milk and milk products sample was equal at 2%, 4%, 6% NaCl concentration when the plates were incubated at 27^0C for 24 hrs, when the same organisms was incubated at 37^0C and 47^0C temperature for 24 hrs it was found that the maximum cfu/ml was obtained at 2% NaCl and the minimum cfu/ml was obtained at 6% NaCl concentration. Effects of NaCl of shrikhand, milk, milk powder, amrakhand, ice cream, curd are presented in Table 1. CFU/ml of *Salmonella typhi* obtained from milk and milk products sample was equal at 2%,4%,6% NaCl concentration when the organisms were incubated at 27^0C for 24hrs, when the plates were incubated at 37^0C and 47^0C temperature for 24 hrs it was found that the maximum cfu/ml was obtained at 2% NaCl and the minimum cfu/ml was obtained at 6% NaCl. CFU/ml of *Shigella dysentriae* obtained from milk and milk products sample was equal at 2%,4%,6% NaCl concentration when the organisms were incubated at 37^0C for 24hrs, when the plates were incubated at 37^0C temperature for 24hrs it was found that the maximum cfu/ml was obtained at 2% NaCl and the minimum cfu/ml was obtained at 6% NaCl. CFU/ml of *E. coli* obtained from milk and milk products sample was equal at 2%, 4%, 6% NaCl concentration when the organisms were incubated at 27^0C, 37^0C and 47^0C incubation temperature after 24hrs, it was found that the maximum cfu/ml was obtained at 2% NaCl concentration and the minimum cfu/ml was obtained at 6% NaCl concentration.

Zaika, (2002) reported that NaCl had a major effect on growth; bacterial survival was affected to a lesser extent. Lag times decreased with increasing NaCl levels; however, the effect on D-values and T4D values was less pronounced. The Growth did not occur and bacterial populations gradually declined in media of pH 4 while NaCl had a major effect on growth, bacterial survival was affected to a lesser extent. The microbiological qualities of packed and unpacked ice cream are shown in the above study, the microbiological load of the industrially produced and packed ice cream samples were lower compared to the findings of (Bostan and Akin, 2002). They could not isolate *Escherichia coli, Staphylococcus aureus, and Salmonella spp.* Joshi *et. al.,* (2004) investigated the microbiological quality of 72 ice creams from 12 different factories in Kathmandu. TAMB, coliform bacteria, and Staphylococcus spp. varied between 1.52x108 cfu/ml, 1.3x107 cfu/ml and 2x104 cfu/ml, respectively. He isolated only one *Salmonella spp.* from the samples tested. M-E-Elahi*et. al.,*

(2002) reported higher TAMB and coliform counts in packed ice cream than that found in this study. TAMB and coliform counts ranged between 2.8 x 103 and 5.6 x 104 cfu/ml and between 4 and 42 cfu/ml, respectively, in their study. Kivanc et.al., (1994); Erol *et.al.,* (1998); Warke *et.al.,* (2000); Baek *et. al.,* (2000); Kanbakan *et.al.,* (2004) isolated Listeria monocytogenes from 6.1% of samples (El-Sharef *et. al.,*2006) from 6% of samples and Warke *et. al.,*(2000) isolated Listeria monocytogenes from 100% of their samples. Erol *et. al.,* (1998); Korel*et. al.,* (2005); El-Sharef *et. al.,* (2006) reported isolation rates of *Salmonella spp.*as 6%, 4.54%, and 14%, respectively. A major cause of failure of processing and packaging systems is the development of biofilms on equipment surfaces (Sperber, and Doyle, 2009).

The presence of this high level of fecal coliforms contamination represents a public health risk due to the possible presence and transmission of pathogens such as enteropathogenic *Escherichia coli*, Hepatitis A virus, poliomyelitis virus, while *Entamoebahistolytica* may also be present in the ice cream (Arias and Windrantz, 2000).

REFERENCE

Arias, M. L. and P. Windrantz (2000): Evaluation of the bacteriological quality of ice cream sold at San Jose, Costa Rica. Alan, 50. | Baek, S. Y., S. Y. Lim, D. H. Lee, K. H. Min, and C. M. Kim (2000): Incidence and characterization of Listeria Monocytogenes from domestic and imported foods in Korea. Journal of Food Protection, 63: 186–189. | Bostan, K., and B. Akin (2002): A study on the microbiological quality of industrial ice cream, Turk. Vet. Anim. Sci. 26:623–629. | Burton, H. (1980): Microbiological aspects of pasteurized milk Bulletin of the International Dairy Federation, No.200, chapter III.pp.9-14. | El-Sharef, N., K. S. Ghenghesh, Y. S. Abognah, S. O. Gnan, and A. Rahouma (2006): 'Bacteriological quality of ice cream in Tripoli- Libya', Food Control, 17, pp. 637–641. Comparison of the Microbiological Quality of Packed and Unpacked Ice Creams Sold in Bursa, Turkey 102. | Erol, I., O. Kuplulu, B. Siriken, and T. H. Celik (1998): Determination of microbiological quality of ice cream belong to various patisserie in Ankara', Turk. Vet. Anim. Sci., 22:345–352. | Henry, A and I. Newlander (1997): Milk constituents In. Chemistry and Testing of dairy products 5th ed, John Wiley and Sons Inc, New York. pp 269-273. | Kanbakan, U., A. H. Con, and A. Ayar (2004): Determination of microbiological contamination sources during ice cream production in Denizli', Food Control, 15:463–470. | Karmengodictarker and Slavicagolctarker (2006): The presences of some pathogens, microorganisms, yeast and moulds in cheese samples produced at small dairy processing plant. ActaagriculturaeSlovenica, 1, 37:51. | Kivanc, M., M. Yamac, and B. Kunduhoglu (1994): The microbiological analysis of ice cream sold in Eskisehir city' Food, 14:317–322. | Korel, F., S. Omeroglu and G. Tan (2005): The evaluation of quality of packaged an unpackaged ice creams sold in retail markets of Manisa, J. Agric. Fac. Harran Univ., 9:11–18. | M-E-Elahi, A. T. M., S. Habib, M. M. Rahman, G. I. Rahman and M. J. U. Bhuiyan (2002): Sanitary quality of commercially produced ice cream sold in the retail stores', Pakistan J. Nutr., 1: 93–94. | Priyankasingh and Alkaprakash (2008): Isolation of Escherichia coli, Staphylococcus aureus and Listeria monocytogenes from milk products sold under market condition at agra region ActaagriculturaeSlovenica, 92(november 2008)1, 83–88. | Salmeron, J., C. De Vega, F. J. Perez-Elortondo, M. Albisu, L. J. R. Barron (2002): Effect of pasteurization and seasonal variations in the microflora of ewe's milk for cheesemaking. Food Microbiol, 19, 2–3, 167–174. | Sharma, D. K. and D. V. Joshi (1992): Bacteriological quality of milk and milk products with special reference to Salmonella and its public health significance. Journal of Science and Technology 22: 100 – 103. | Sperber, W. H. and M. P. Doyle (2009): Compendium of the Microbiological Spoilage 41of Foods and Beverages, Food Microbiology and Food Safety: 41-67. | Tekinsen, K. K and Z. Ozdemir (2006): Prevalence of food borne pathogens in Turkish van otlu (Herb) cheese. Food Control, 17:707-711. | Warke, R., A. Kamat, M. Kamat, and P. Thomas (2000): 'Incidence of pathogenic psychrotrophs in ice creams sold in some retail outlets in Mumbai, India' Food Control, 11:77–83. | Zaika L. L. (2002): The effect of NaCl on survival of Shigella flexneri in broth as affected by temperature and pH. J Food Prot ; 65(5):774-9. |