STUDIES ON THE INFLUENCE OF FAT PERCENTAGE OF MILK ON NISIN ACTIVITY

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ABSTRACT

Bioassay was performed to study the influence of fat percentage of milk on nisin activity. Micrococcus luteus (MTCC2848) was used as an indicator organism. Standardized milk, toned milk, double toned milk and skim milk added with 50, 100, 200 and 300 IU nisin/ml were studied for the nisin activity during a chiller (4±1°c) storage period of 24 days. The initial nisin concentrations in standardized milk samples were 41.4±0.51, 86.0±0.71, 175.0±0.71 and 267.4±1.78 respectively. The values decreased significantly during storage to 3.6±0.51, 12.8±1.02, 39.6±3.33 and 97.8±3.80 respectively on 24th day. Toned milk samples had initial nisin concentrations of 42.0±0.71, 86.0±0.71, 174.0±0.70 and 266.2±1.56 respectively. The values decreased significantly during storage to 9.20±0.57, 18.60±1.50, 48.0 ±2.77 and 98.6±4.30 respectively on 24th day. The initial nisin concentrations in double toned milk samples were 44.0±0.71, 86.6±0.93, 175.4±1.08 and 266.4±1.50 respectively. They decreased progressively during storage and the values on 24th day were 11.0±0.55, 30.2±2.08, 48.4±3.76and105.4±4.96respectively.Theskim milk samples had initial nisin concentrations of 43.6±0.51, 87.6±0.75, 175.0±1.00 and 267.2±1.77 respectively. The values at 24th day of storage were 11.2±0.37, 31.0±2.88, 51.4±4.89 and 111.0±5.34 respectively. The rate of decline in nisin activity was higher in standardized milk followed by toned milk. In double toned and skim milk decrease in nisin activity was relatively slower. The results showed a clear negative relationship between fat percentage and nisin activity.

Keywords: Milk, Fat percentage, Nisin, Bio-Assay, Bacteriocins.

INTRODUCTION

Non-thermal and low thermal treatments are attracting interest of the food industry due to their capability of assuring the quality and safety of food. Among them, bacteriocins from lactic acid bacteria, such as Nisin, Pediocin PA-1, Lacticin 3147 and Enterocins may be potentially useful for the dairy industry. Utilization of Bacteriocins alone, or combined with other treatments, could represent a promising advance for the microbiological safety and maintenance of sensory properties in milk and milk products (Lopez and Belloso, 2008). Exploitation of bacteriocin such as nisin as a bio preservative is a newer approach to achieve extended shelf life in regions with inadequate refrigeration facilities. Nisin is produced by safe food grade bacteria and it is also having favourable properties such as good stability under conditions of processing and easy degradation in the human gastro-intestinal tract. This makes it an ideal bio-preservative for use in foods (Daeschel, 1989; Ray 1992). Nisin activity is reported to be influenced by the fat

content of the product (Jung *et al.,* 1992). Nisin has been recognized as a safe food preservative by the joint Food and Agriculture Organisation and World Health Organisation (FAO/WHO) expert committee which has allowed a level of 3.3 × 106 units/kg body weight and permitted an unconditional acceptable daily intake (ADI) to be set at 3.3 × 104 units/kg body weight (Thomas *et al.,* 2000). It is the only commercially produced bacteriocin and is used in more than 50 countries for the last 60 years (Danisco, 2002). Nisin was also approved by the FDA (1988) as GRAS (Generally recognized as safe).

MATERIALS AND METHODS

Fresh cow milk obtained from the cattle farm maintained at Indian Veterinary Research Institute, Izatnagar was used in all the experiments after suitable standardization. Nisaplin, a commercial preparation of nisin with an activity of 1000 IU/mg was obtained from Danisco International, Brabrand, Denmark. Nisin was added at the rate of 50, 100, 200 and 300 IU/ml at about one hour prior to pasteurization in order to facilitate thorough dispersion. After pasteurization these samples were stored at 4±1°C and checked for the activity of nisin during storage for a period of 24 days in order to assess the influence of fat percentage on the nisin activity. Residual nisin concentrations were determined during storage period in standardized milk, toned milk, double toned and skim milk added with 50, 100, 200 and 300 IU nisin/ml. Bioassay was carried out as per the method described by Wolf and Gibbons (1996) with suitable modifications. Micrococcus luteus (MTCC2848) obtained from Institute of Microbial Technology, Chandigarh, was used as indicator organism. Bacto-agar medium (Bacto-peptone -10g; Nacl-3g; yeast extract-1.5g; glucose-1g; Bacto-agar-10g and Disodium hydrogen Phosphate (Na2 HPO 4) -10g) with 1% of Tween 20 was used for bioassay. The indicator organism at 1% concentration was inoculated into medium and then dispensed into sterile petridishes. On solidification, test wells were bored into the agar by using an 8mm diameter metal tube. Standard nisin solutions were dispensed into the wells and plates were incubated at 30°C for 48 hours. Similarly, milk samples with added nisin were also dispensed into the wells and plates were incubated at 30°C for 48 hours. After incubation, zones of inhibition were measured to the nearest 0.1mm. Regression analysis was applied to estimate the residual nisin concentration during storage (Snedecor and Cochran, 1994).

RESULTS& DISCUSSION

The nisin concentrations (IU/ml) in standardized, toned, double toned and skim milk during storage are presented in table 1.

The initial nisin concentrations in standardized milk samples added with 50 and 100 IU nisin/ml were 41.4±0.51 and 86.0±0.71 respectively. The values decreased during storage to 3.6±0.51 and 12.8±1.02 respectively on 24th day. Samples added with 200 and 300 IU nisin/ml had initial concentrations of 175.0±0.71 and 267.4±1.78 respectively and the values decreased to 39.6±3.33 and 97.8±3.80 respectively at 24 days of storage. Toned milk samples added with 50 and 100 IU nisin/ml had initial nisin concentrations of 42.0±0.71 and 86.0±0.71 respectively at zero day of storage. The values decreased during storage to 9.20±0.57 and 18.60±1.50 respectively on 24th day. In samples with 200 and 300 IU nisin/ml the initial concentrations were 174.0± 0.70 and 266.2±1.56 respectively and the values significantly decreased to 48.0 ±2.77 and 98.6±4.30 respectively at 24 days of storage.

The initial nisin concentrations in double toned milk samples added with 50 and 100 IU nisin/ml were 44.0±0.71 and

Table.1: Mean ± SE Nisin concentration values (IU/ml) in different types of pasteurized milk during storage at 4±1 °C

Storage period (Days)	Nisin Concentration IU/ml				Days Mean
	50	100	200	300	
Standardized milk					
0	41.4±0.51[a]	86.0±0.71[a]	175.0±0.71[a]	267.4±1.78[a]	142.4±19.89[a]
4	40.8±0.37[a]	84.4±0.25[a]	173.0±0.71[a]	265.8±1.32[a]	141.0±19.82[a]
8	40.0±0.55[a]	81.2±0.37[b]	163.8±1.36[b]	260.8±1.16[b]	136.4±19.39[b]
12	33.4±0.68[b]	62.4±2.84[c]	140.8±1.16[c]	227.4±1.54[c]	116.0±17.31[c]
16	24.8±0.86[c]	43.0±1.79[d]	112.2±2.16[d]	193.6±2.66[d]	93.4±15.26[d]
20	12.2±0.86[d]	28.6±1.21[e]	75.0±1.84[e]	152.6±2.36[e]	67.1±12.52[e]
24	3.6±0.51[e]	12.8±1.02[f]	39.6±3.33[f]	97.8±3.80[f]	38.4±8.51[f]
Toned milk					
0	42.0±0.71[a]	86.0±0.71[a]	174.0±0.70[a]	266.2±1.56[a]	142.1±19.74[a]
4	41.8±0.58[a]	85.4±0.51[a]	172.0±0.55[a]	262.0±0.84[a]	140.3±19.38[a]
8	41.2±0.59[a]	81.8±0.66[b]	165.4±1.63[b]	253.8±1.16[b]	135.5±18.74[b]
12	34.8±1.02[b]	69.4±1.03[c]	143.8±1.85[c]	224.4±1.72[c]	118.1±16.73[c]
16	24.4±0.87[c]	53.0±0.71[d]	117.0±2.75[d]	188.8±2.67[d]	95.8±14.55[d]
20	16.8±0.58[d]	36.0±0.95[e]	76.4±2.73[e]	145.4±3.41[e]	68.6±11.3[e]
24	9.2±0.57[e]	18.6±1.50[f]	48.0±2.77[f]	98.6±4.30[f]	43.6±8.08 f
Double toned milk					
0	44.0±0.71[a]	86.6±0.93[a]	175.4±1.08[a]	266.4±1.50[a]	143.1±19.63[a]
4	43.0±0.70[a]	84.4±0.68[ab]	172.4±0.93[a]	263.6±1.12[a]	140.8±19.48[a]
8	41.4±0.60[a]	82.0±0.71[b]	168.0±0.95[b]	259.8±1.02[b]	137.8±19.27[b]
12	35.6±0.51[b]	70.8±0.86[c]	153.2±0.97[c]	228.6±1.72[c]	122.3±17.11[c]
16	29.8±0.37[c]	56.0±1.41[d]	125.4±2.09[e]	195.2±1.85[d]	101.8±14.72[d]
20	20.2±0.86[d]	43.8±1.36[e]	80.4±3.78[f]	152.6±2.75[e]	74.2±11.54[e]
24	11.0±0.55[e]	30.2±2.08[f]	48.4±3.76[g]	105.4±4.96[f]	48.7±8.23[f]
Skim milk					
0	43.6±0.51[a]	87.6±0.75[a]	175.0±1.00[a]	267.2±1.77[a]	143.4±19.67[a]
4	42.8±0.58[a]	87.4±0.68[a]	173.6±0.93[a]	266.8±1.93[a]	142.6±19.62[a]
8	42.6±0.51[a]	83.6±0.93[b]	166.0±1.58[b]	263.9±2.20[b]	139.0±19.43[b]
12	37.8±0.37[b]	72.4±0.92[c]	149.0±2.68[c]	232.7±0.86[c]	123.0±17.24[c]
16	30.8±0.36[c]	59.4±1.08[d]	121.0±4.30[d]	201.2±1.77[d]	103.1±15.03[d]
24	11.2±0.37[e]	31.0±2.88[f]	51.4±4.89[f]	111.0±5.34[f]	51.2±8.76[f]

- Means ±SE are averages of five replications
- Means with common superscripts in a column (alphabets) do not differ significantly (P< 0.01)

86.6±0.93 respectively. Then the values decreased progressively during storage and the concentrations on 24th day were 11.0±0.55 and 30.2±2.08 respectively. In samples added with 200 and 300 IU nisin/ml the initial values were 175.4±1.08 and 266.4±1.50 respectively and declined to 48.4±3.76 and 105.4±4.96 respectively at 24 days of storage. The skim milk samples had initial nisin concentrations of 43.6±0.51 and 87.6±0.75 respectively in samples added with 50 and 100 IU nisin/ml. Nisin concentrations progressively decreased during storage and the values at 24th day of storage were 11.2±0.37 and 31.0±2.88 respectively. Samples added with 200 and 300 IU nisin/ml had initial concentrations of 175.0±1.00 and 267.2±1.77 respectively and the values declined to 51.4±4.89 and 111.0±5.34 respectively at 24 days of storage.

The rate of decline in nisin activity was higher in standardized milk followed by toned milk. In double toned and skim milk the decrease in nisin activity was relatively slower. The results showed a clear negative relationship between fat percentage and nisin activity.

Several studies have shown that nisin activity is diminished in foods that contain high fat. Jung *et al.* (1992) reported a 33% decrease in initial nisin activity of skim milk and more than 88 % decrease in nisin activity of milk containing 12.9% fat. Nisin was more effective in controlling *Staphylococcus aureus* in skim milk than in whole milk and this is attributed to the effect of milk fat content (Jones, 1974). Cytoplasmic membrane of the microbes was the major target of nisin action. Possibly this was disrupted by nisin's interaction with phospholipid components (Henning *et al.*, 1986).

It appears that binding or adsorption of the polypeptide structure of nisin occurs with certain food components, which makes it inactive or unavailable to inhibit microbes. Exactly how lipids interact with nisin and affect its activity is not clearly understood, but this phenomenon warrants further investigation in order to optimize the effective use of nisin in food applications.

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