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PREVALENCE OF ESCHERICHIA COLI AND EFFECT OF DIFFERENT CONCENTRATIONS OF NISIN ON ITS VIABILITY IN CREAM

(With 3 Tables and 2 Figures)

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مدى تواجد ميكروب الأيشيريشياكولاى وتأثير تركيزات مختلفة من النيسين على حيوية هذا الميكروب في القشدة

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تم جمع ٦٠ عينة عشوائية من القشدة (بواقع ٢٠ عينة لكل من القشدة الخام والمخفوقة والمبسترة) من أماكن مختلفة بمدينة أسيوط وذلك لفحصهم ومعرفة تواجد بعض الميكروبات الخاصة. وقد أظهرت النتائج تواجد الميكروبات العصوية القولونية، الفيكال كوليفورم، الأيشيريشياكولاى بالنسب المئوية التالية: ٨٠، ٧٠، ٧٠% في عينات القشدة الخام (٢٥% دهن) على الترتيب. كما كانت نفس الميكروبات -سابقة الذكر- ملوثة لعينات القشدة المخفوقة (٣٠% دهن) بالنسب المئوية الآتية: ٥٥، ٥٥، ٤٥% على التوالى. بينما لم يمكن عزل أى من الميكروبات السالفة في جميع عينات القشدة المبسترة (٢٥% دهن). وفيما يختص بعينات القشدة الخام المفحوصة، فقد كانت معظم العينات الموجبة، (٥٠، ٧٨,٦، ٦٤,٣%) ملوثة بميكروبات عصوية قولونية، فيكال كوليفورم، أيشيريشياكولاى بالإعداد التالية: أكثر من 10^{3}، $10^{3}-10$، $10^{2}-10$/جم على الترتيب. أما بالنسبة لعينات القشدة المخفوقة، فقد احتوت غالبية العينات الموجبة (٦٣,٦، ٥٤,٥، ٦٦,٧%) على الميكروبات سالفة الذكر- بالأعداد الآتية: أكثر من 10^{3}، $10^{3}-10$، $10^{2}-10$/جم على التوالى. كما تناول البحث- دراسة تأثير التركيزات المختلفة من النيسين على حيوية ميكروب الأيشيريشياكولاى في القشدة المخزونة عند درجة حرارة الثلاجة (٤±١م). لذلك تم حقن القشدة معمليا بـ ١,١ × 10^{7} خلية/جم من ميكروب الأيشيريشياكولاى عند بداية التجربة- وقد لوحظ انخفاض في أعداد هذا الميكروب تدريجيا حتى اختفي ولم يتم كشفه بعد ١٣، ١١، ٩، ٧ أيام من التخزين لعينات القشدة المحتوية على تركيزات النيسين التالية: ٢، ٤، ٦، ٨ جزء في المليون/ جرام قشدة على الترتيب. على النقيض من ذلك- فقد تبين أن هناك زيادة مستمرة لهذا الميكروب حتى وصل إلى ٨ × 10^{9} خلية/جم بنهاية اليوم الحادى عشر من التخزين في القشدة الخالية من النيسين. كما أظهرت النتائج أيضا- أنه يوجد انخفاضات

مستمرة في أعداد البكتريا الهوائية الكلية بعينات القشدة المدعمة بتركيزات مختلفة من النيسين أثناء فترة التخزين. وقد استنتج من هذه الدراسة أن إضافة ٦، ٨ جزء في المليون من النيسين/جرام قشدة مع تخزين هذه القشدة عند درجة حرارة الثلاجة لهما أثر مثبط على ميكروب الأيشيريشياكولاى وكذلك على البكتريا الهوائية الكلية. وقد تمت مناقشة الأهمية الصحية لوجود تلك الميكروبات وبالأخص- ميكروب الأيشيريشيا كولاى في القشدة وكذلك الطرق الواجب اتباعها للحد من هذه الميكروبات الخطيرة، كما بين البحث الدور الرائد للنيسين الذي يمكن أضافته للقشدة.

SUMMARY

Sixty random samples of cream (20 each of raw, whipped and pasteurized) were collected from different localities in Assiut City to be examined for coliforms, fecal coliforms and *E.coli*. The obtained results showed that the concerned microorganisms were detected in 80, 70 and 70% of the examined raw cream (25% fat) samples, respectively, and such bacteria existed in 55, 55 and 45% of the examined whipped cream (30% fat) samples, respectively. While, these bacteria could not be isolated from all the examined pasteurized cream (25% fat) samples. Regarding raw cream samples, most of the positive samples (50, 78.6 and 64.3%) had coliforms, fecal coliforms and *E.coli* in numbers of $>10^3$, $10\text{-}10^3$ and $10\text{-}10^2$/g, respectively. Concerning whipped cream samples, the majority of the positive samples (63.6, 54.5 and 66.7%) had these bacteria in counts of $>10^3$, $10\text{-}10^2$ and $10\text{-}10^2$/g, respectively. On the other hand, the effect of different concentrations of nisin on viability of *E.coli* in cream, stored at refrigerator temperature (4 ± 1°C) was studied. Cream inoculated with *E.coli* at density of 1.1×10^7 cfu/g showed decrease in counts and undetectable numbers of *E.coli* were observed after 13,11,9 and 7 days of storage in cream samples supplemented with 2,4,6 and 8 ppm of nisin/g cream, respectively. In contrast, counts of viable *E.coli* increased gradually during storage of control cream, reaching 8×10^9 cfu/g by the end of the eleventh day. In other words, a continuous reduction in the aerobic plate counts in cream samples containing various concentrations of nisin during the days of storage was noticed. It is concluded that nisin in concentrations of 6 and 8 ppm/g cream with refrigerator temperature had a great effect against *E.coli* and aerobic bacteria in cream. The public health hazards and preventive measures were discussed.

Keywords: *E.coli, Nisin, Cream.*

INTRODUCTION

Cream is one of the perishable dairy products, which has a high moisture content and enjoys only a limited shelf-life without any evidence of spoilage. It is used as direct consumption and in the manufacture of butter or ice cream. Likewise, cream is added as a major ingredient to a large number of commercial food products including canned soup, dried bakery mixes, desserts, etc. Pasteurization of cream extends its shelf-life to some extent (Robinson, 1994). Currently in Egypt, pasteurized cream usually has a shelf-life of two to three weeks when stored at refrigerator temperature. Pasteurized cream is one of the slow moving goods in the Egyptian markets, so the recommended shelf-life is considered short and may constitute an economic problem due to its spoilage on shelves (Abdou et al., 2001).

Coliform organisms including fecal coliforms and *E. coli* have probably received more attention than most other groups of bacteria occuring in cream owing to their importance as indicators species in routine analysis to ascertain the quality of this product. Coliforms are originated primarily from the lower intestine of man and/or animals. Cream contaminated directly or indirectly with fecal material may theoretically contain one or more of enteric pathogens and thus can be potentially hazardous to consumers. Such bacteria comprise a considerable proportion of the foodborne pathogens as a whole and most cases of food poisoning are attributed to these bacteria. Also, Davis (1983) stated that sewage contamination was the usual cause of food poisoning from cream and such incidents are not rare. *E.coli* has been incriminated in cases of diarrheal disease in adults and infants and it is a common cause of travelers' diarrhea in many countries which affects a large percentage of the one quarter billion people who annually travel across the world's countries. In recent years, certain serovars of *E.coli* are associated with outbreaks of haemorrhagic colitis and subsequent development of serious complications including thrombotic thrombocytopaenic purpura and the haemolytic uraemic syndrome which occurs more commonly in infants and young children, as it is the major cause of renal failure in childhood (Riley et al., 1983; Karmali, 1989; Tarr, 1994; Slutsker et al., 1997 and Law, 2000). In other words, the growth of *E.coli* in cream can lead to defects in texture and flavour.

Food preservation is designed to enhance or protect food safety while maintaining or improving product quality. It aims at inactivating or inhibiting the growth of undesirable microorganisms. Many

preservation processes are available to food processors, including thermal processing, refrigeration, addition of chemical preservatives as nisin, or a combination of several of these methods. Nisin is an antimicrobial biopeptide produced by *Lactococcus lactis subsp. lactis*, a bacterium that occurs naturally in milk. It has been used effectively as a preservative in milk products such as cream, cheese and others (Phillips et al., 1983; Hurst and Hoover, 1993 and Bender and Bender, 1995). In 1969 the Joint FAO/WHO Expert Committee on Food Additives gave nisin international acceptance (WHO, 1969), and up to 400 units of nisin/g food is usually recommended for food preservation, that is, about 10 ppm (Hurst and Hoover, 1993). The literatures on the effect of nisin on different microorganisms, specially Gram-positive bacteria (Ray, 1992; Dean and Zottola, 1996 and Beard et al., 1999) showed a variable sensitivity of the tested organisms towards nisin.

This work was undertaken to throw light on the incidence and counts of coliforms, fecal coliforms and *E.coli* in raw, whipped and pasteurized cream available in Assiut City, as well as to study the effect of adding different concentrations of nisin on *E.coli* growth in cream during storage at refrigerator temperature.

MATERIAL and METHODS

I- Prevalence of coliforms, fecal coliforms and *E.coli* in cream.

- Collection of samples:

Sixty random samples of cream (20 each of raw, whipped and pasteurized cream) collected from different localities in Assiut City were transferred to the laboratory with a minimum of delay to be examined for the concerned microorganisms, after Storch's test according to Lampert (1975) and Gerber method for fat % according to A.P.H.A. (1992).

- Preparation of samples:

Samples were prepared following the technique described by A.P.H.A. (1992).

- Enumeration of microorganisms:

MPN/g cream was adopted according to A.O.A.C. (1975).

- Identification of *E.coli*:

The technique recommended by Krieg and Holt (1986) was used based on morphological and biochemical characters of *E. coli*.

II- Effect of different concentrations of nisin on viability of *E.coli* in cream, stored at refrigerator temperature (4±1°C):

- Organism:

Culture of *E.coli* used in this study was obtained from the previously examined cream samples.

- Experimental procedure:

Pasteurized cream samples, which proved bacteriologically to be free from *E.coli*, were inoculated with a suspension of 24 hours incubation *E.coli*. The initial count was to be 1.1 x 10^7 cells/g. Then the inoculated cream sample was divided into 5 parts. Control, (nisin free); A,B,C and D containing 2,4,6 and 8 ppm nisin /g cream, respectively.

Control samples together with the inoculated ones were kept in refrigerator temperature (4 ± 1°C) for studying the survival of *E.coli* on Sorbitol MacConkey agar (A.P.H.A., 1992) and aerobic plate count. Examinations were done at time zero, then after one and three days, then periodically every two days till 15 days storage at refrigerator temperature.

Table 1: Incidence of coliforms, fecal coliforms and *E.coli* in the examined cream samples.

Type of cream	Positive samples					
	Coliforms		Fecal coliforms		*E.coli*	
	No./20	%	No./20	%	No./20	%
Raw	16	80	14	70	14	70
Whipped	11	55	11	55	9	45
Pasteurized	0	0	0	0	0	0

Table 2: Frequency distribution of positive raw cream samples based on their coliforms, fecal coliforms and *E.coli* counts (MPN/g).

Counts / g	Positive samples					
	Coliforms		Fecal coliforms		*E.coli*	
	No./16	%	No./14	%	No./14	%
<10	1	6.3	0	0.0	1	7.1
$10-10^2$	4	25.0	6	42.9	9	64.3
10^2-10^3	3	18.8	5	35.7	1	7.1
$>10^3$	8	50.0	3	21.4	3	21.4
Total	16	100	14	100	14	100

Table 3: Frequency distribution of positive whipped cream samples based on their coliforms, fecal coliforms and *E.coli* counts (MPN/g).

Counts / g	Positive samples					
	Coliforms		Fecal coliforms		*E.coli*	
	No./11	%	No./11	%	No./9	%
<10	0	0.0	2	18.2	2	22.2
$10-10^2$	4	36.4	6	54.5	6	66.7
10^2-10^3	0	0.0	0	0.0	0	0.0
$>10^3$	7	63.6	3	27.3	1	11.1
Total	11	100	11	100	9	100

RESULTS

All the results obtained are recorded in Tables 1-3 and Figures 1&2.

DISCUSSION

The summarized results in Tables 1 and 2 pinpoint that 80% of the examined raw cream (25% fat) samples were contaminated by coliforms. The majority of the examined samples (50%) had counts $>10^3$ coliforms/g. Raw cream proved to have fecal coliforms in a percentage of 70% of the examined samples. The highest frequency distribution (78.6%) lies within the range of $10-10^2$/g. While, *E.coli* could be isolated from 70% of the examined raw cream samples. Most of positive samples (64.3%) had counts of $10-10^2$/g. 21.4% of the positive samples contained *E.coli* in numbers of over 10^3/g. The rest of positive samples were equally distributed among <10 and 10^2-10^3/g (7.1% each).

The data in Tables 1 and 3 reveal that coliforms existed in 55% of the examined whipped cream (30% fat) samples. 63.6% of the positive samples had counts more than 10^3/g. Fecal coliforms contaminated 55% of the examined samples in variable numbers. Most of the positive samples (54.5%) had fecal coliforms in numbers varied from $10-10^2$/g. However, *E.coli* could be detected in 45% of whipped cream samples, and the highest frequency distribution (66.7%) lay within the range of $10-10^2$/g. While, 22.2% of the positive samples had counts less than 10/g. Likewise, it is evident from the results presented

in Table 1, that coliforms, fecal coliforms and *E.coli* could not be isolated from all the examined pasteurized cream (25% fat) samples.

Higher incidence and counts of coliforms and fecal coliforms were obtained by El-Kosi (2001). However, the obtained results of *E.coli* agree to a certain extent with those reported by Nazem and El-Hawary (1997). Comparing the obtained results (Tables 2 and 3) with the suggestive standards for coliforms in cream, it is evident that 75% and 55% of the examined raw and whipped cream samples, respectively, did not comply with the suggestive standards of Campell and Marshall (1975), Davis (1983) and USDA (1988) that coliforms must not exceed 10/g. While, all of the examined samples of pasteurized cream comply with these standards (Table 1) and could be judged satisfactory. It is worthwhile to state that coliform organisms including fecal coliforms and *E.coli* contaminating cream samples could be attributed to poor quality raw milk, ineffective sanitizing practices, wrong choice of temperature of heat treatment and careless during handling, storage and distribution. Moreover, contamination of cream by coliforms beyond certain level should be considered a public health hazard as they may cause dreadful diarrhea disease (Robert et al., 1977). Likewise, occurrence of fecal coliforms in such product is a real indication of fecal pollution and possible existence of other enteric pathogens, besides the public health hazards of *E.coli* which have been emphasized by several investigators (Marier et al., 1973; Mossel, 1975; Riley et al., 1983 and Tarr, 1994).

The data illustrated in Figure 1 show a continuous reduction in the numbers of *E.coli* in cream samples containing different concentrations of nisin during the days of storage at refrigerator temperature (4 ± 1°C). A rapid reduction in counts of the organism occurred in cream containing 2 ppm of nisin/g cream by the end of 5th day to reach a minimum count of 1×10^2 cfu/g at the end of 11th day. *E.coli* sharply decreased in numbers from 1.1×10^7 cfu/g to undetectable number by the end of 11th day of storage of cream sample with 4 ppm of nisin/g cream. While, the counts of *E.coli* reduced in cream samples with 6 and 8 ppm of nisin/g cream during the days of storage and no viable *E.coli* could be detected in the samples by the end of 9th and 7th day, respectively. On the other hand, the numbers of *E.coli* increased gradually in nisin free cream sample (control), to achieve a count of 8×10^9 cfu/g by the end of 11th day of storage.

The results in Figure 2 verify that there was a steady decrease in numbers of aerobic bacteria during storage at refrigerator temperature

(4±1°C) of cream samples containing various concentrations of nisin. The numbers of bacteria diminished gradually to reach minimum counts of 3 x 10^4 and 1 x 10^3 cfu/g by the end of 13th day in cream samples containing 2 and 4 ppm of nisin/g cream, respectively. However, cream samples with 6 and 8 ppm of nisin/g cream achieved minimum levels of acrobic counts of 4 x 10^3 and 3 x 10^3 cfu/g, respectively, by the end of 7th day. In other words, in nisin free cream samples (control), the aerobic plate counts increased gradually during storage period to reach its maximum level of 5 x 10^{12} cfu/g by the end of 11th day.

The decreasing percentage of *E.coli* and aerobic bacteria due to addition of 6 and 8 ppm of nisin/g cream were higher as compared with cream samples containing 2 and 4 ppm of nisin/g cream. Furthermore, the reduction rate of these bacteria in nisin treated sample as compared with control one (nisin free) was very higher (Figures 1 and 2). The inhibitory effect of nisin on various microorganisms was recorded by several workers (Hurst, 1981; Ray, 1992; Bender and Bender, 1995; Dean and Zottola, 1996; El-Hawary and Aman, 1998 and Beard et al., 1999). Generally, nisin is a bacteriocin or antimicrobial peptide produced by some strains of *Lactococcus lactis*. This peptide is reported to strongly inhibit the growth of a wide range of Gram-positive bacteria (Ray, 1992 and Beard et al., 1999). While, Hurst (1981) and others have indicated that some Gram-negative lactic acid bacteria show sensitivity to nisin. Addition of nisin extended the shelf life of cream at a concentration of 25 units per gram (Phillips et al., 1983). Moreover, Hurst and Hoover (1993) stated that up to 400 units/g food is usually recommended for food preservation, that is, about 10 ppm. Nisin is the only antibiotic permitted in Great Britain to preserve specified foods and it is useful to prolong storage life of cream, milk, cheese and other dairy products (Hurst and Hoover, 1993 and Bender and Bender, 1995).

On the other hand, *E.coli* include enteropathogenic *E.coli*, enterohaemorrhagic *E.coli*, enterotoxigenic *E.coli* and others. All these groups can induce both intestinal and extra-intestinal diseases (ICMSF, 1996). Certain serotypes of *E.coli* are associated with severe diarrhea in infants and young children, cholera like syndrome and shigella like illness. Recently, certain strains of *E.coli* have been implicated in some cases of haemorrhagic colitis and haemolytic uraemic syndrome, also more than 100 serotypes of *E.coli* can produce verotoxins which may produce a milder form of illness (Riley et al., 1983; Tarr, 1994 and Law, 2000). Furthermore, *E.coli* is considered one of the most common causes of food poisoning outbreaks allover the world (Marier et al.,

1973; Riley et al., 1983; Griffin, 1991; Tarr, 1994 and Reilly, 1996). In other words, *E.coli* in cream may grow and cause disagreeable changes leading to high economic losses.

In conclusion, the present investigations have shown that the existence of coliforms, fecal coliforms and *E.coli* in cream samples (specially, raw and whipped cream) is suggestive of unsanitary conditions or practices during production, processing or storage. Therefore, strict hygienic measures should be imposed to prevent contamination and consequently avoid additional outbreaks of foodborne illness caused by these bacteria. However, the pasteurized cream samples were satisfactory from the quality point of view. Moreover, it is clearly evident from the obtained results, that nisin in concentrations of 6 and 8 ppm/g cream with refrigerator temperature had a great effect against *E.coli* and aerobic bacteria in cream even if they were present in large numbers.

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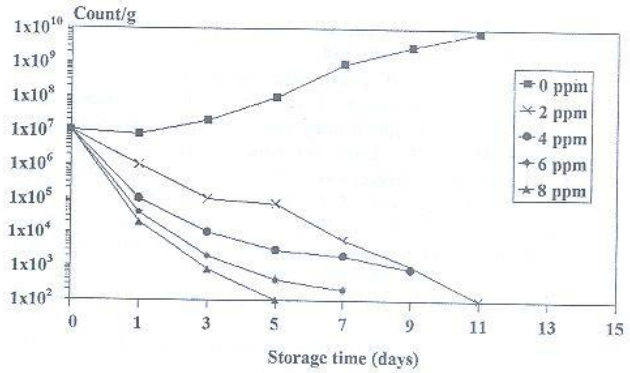

Figure 1. Effect of different concentrations of nisin on viability of E.coli in cream stored at refrigerator temperature (4±1°C) .

ppm = part per million of nisin / g cream.

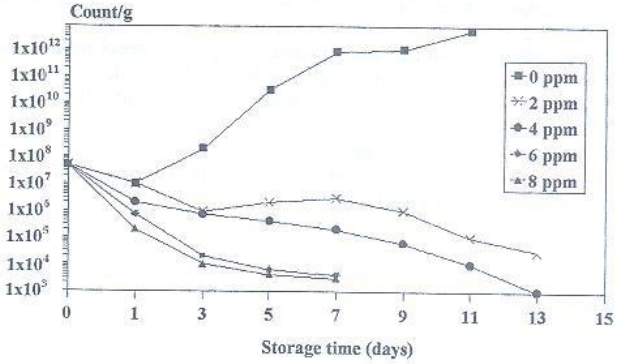

Figure 2 . Effect of different concentrations of nisin on aerobic plate count of cream stored at refrigerator temperature (4±1°C) .

ppm = part per million of nisin/ g cream.