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VIABILITY OF *Escherichia coli* 0157 IN KAREISH CHEESE AND ZABADY AS AFFECTED BY *Bifidobacterium bifidum*

Saber, W. I. A. [1] and M. Y. Mehana [2]

[1]Microbiol. Dept., Soils, Water and Environ. Res. Institute, Agric. Res. Center, Giza, Egypt.

[2] Dairy Dept., Fac.. of Agric., Mansoura Univ., Mansoura, Egypt.

ABSTRACT

Sufficient volume of *E. coli* 0157 strain was added to milk used for Kareish cheese or Zabady to study the antibacterial activity of starter culture of both products and *Bifidobacterium bifidium* against *E. coli* 0157. The results showed that: *E. coli* was not counts decreased greatly during storage period of Kareish cheese at 5°C or/and at room temperature. *E. coli* was not detected after 15 days storage at 5°C and at room temperature. Pronounced reduction or disappearance of *E. coli* counts was observed in cheese stored at room temperature. The addition of probiotic bacteria to kareish cheese milk led to increase the disappearance of food-borne-pathogenic bacteria, *E. coli* was not detected after 7 days storage, this is in addition to its therapeutic and healthy benefits of bifidobacteria. More pronounced of acidity production was observed throughout the storage period, but in cheese stored at room temperature, higher increase of acidity was observed. Much more of acidity was developed in cheese treated with bifidobacteria. PH values also decreased sharply with the prolongation of storage period. Whereas this reduction of pH values was higher in cheese inoculated with probiotic bacteria.

The same trend in Zabady results was also observed, which *E. coli* began to lose its viability during the storage period at 5°C or/and at room temperature. Higher decrease of *E. coli* viability was observed when Zabady was stored at room temperature. Healthy and therapeutic benefits and greatest inhibition of *E. coli* were observed in Zabady milk inoculated with bifidobacteria. Acidity production was also pronounced with the prolongation of storage period. Greatest amount of acidity resulted in Zabady stored at room temperature especially, when inoculated with probiotic bacteria. pH values also decreased sharply with the increase of storage period, especially in Zabady treated with bifidobacteria.

Key words: Kareish cheese, Zabady, Viability, *E. coli*, *Bifidobacterium bifidium*.

INTRODUCTION

Kareish cheese is an acid coagulated skim milk product and considered as an important soft cheese type in Egypt. It constitutes a considerable part of the national daily diet of farmers and those suffering from cardioc disease in towns. This type of soft cheese is traditionally manufactured with mesophilic lactic acid bacteria. These bacteria may be able to inhibit other microorganisms by producing organic acids, hydrogen peroxide and other more specifiically inhibitory substances (Abd Rabo *et al*., 1991; Ibrahim, 1991; Shady *et al*., 2000 and Shalaby *et al*., 2001).

Even yoghurt, which has always been considered safe because of its intrinsic nature, was involved in a fatal infection with EHEC (Hudson *et al*., 1997). The occurrence of these such outbreaks might suggest that EHEC might be tolerant to acidic conditions, particularly at lower temperature

(Shalaby *et al*., 2001). However, Massa *et al*. (1997) stated that data about *E. coli* 0157 survival in yoghurt are lacking.

The use of *Bifidobacterium* in cultured or culture-containing milks received greater attention at the end of 1970S due to increased knowledge of the intestinal ecology of bifidobacteria and the improvements in anaerobic technology. More recent, world-wide interest in nutritional foods, especially in prebiotic compounds was reported. Other examples of bifidobacteria-amended foods include cottage cheese, sour cream, lite ice creams and orange juice (Reuter, 1990; Samona & Robinson, 1994 and Dechter & Hoover, 1998).

The beneficial roles of bifidobacteria in the human intestine have been reported. These microorganisms show antagonistic effects towards enteropathogenic bacteria. They inhibit the growth of *Staphylococcus aureus* and *Escherichia coli.* Because of the nutritional and therapeutic effects of bifidobacteria, many efforts have been devoted to incorporate the bifidobacteria in dairy products. Therefore, considerable attention has been sporadically directed on benefits derived from consumption of milk products containing bifidobacteria. (Hunger & Peiterson, 1992; Vijayendra & Gupta, 1992; Badawi & El-Sonbaty, 1997 and Shady *et al*., 2000).

Therefore, this study was carried out to investigate the viability of *Escherichia coli* 0157 in Kareish cheese and Zabady made from buffaloe's skim milk (0.5-0.7% fat) as affected with lactic acid bacteria as a starter and probiotic bacteria (bifidobacteria).

MATERIALS AND METHODS

Kareish cheese and Zabady milk:

Skim buffaloe's milk (0.5-0.7% fat) was obtained from Misr Milk and Food Company, Mansoura, Egypt.

Starter cultures and Bifidobacteria:

Cultures of *Streptococcus sulivarius* subsp. *thermophilus*, *Lactobacillus delbreuckii* subsp. *bulgaricus* and *Bifidobacterium bifidum* were used, which obtained from Dairy Dept., Fac. of Agric., Mansoura Univ., Mansoura, Egypt. Tested cultures were maintained in trypticase soya broth (TSB) and stored at 4°C until use.

Starter cultures were propagated daily in 10% sterilized skim milk at 121°C for 5 min. 0.1 ml of propagated culture was added to 10 ml of skim milk and incubated at 37°C for 18 hours before use. Skim milk supplemented with 0.05% L-cysteine-HCl was used for *B. bifidum* growth (Sadek *et al*., 2000).

***E. coli* 0157 strain:**

This strain was obtained from Dairy Dept., Fac, of Agric., Mansoura Univ., Mansoura, Egypt.

Media:

Sorbitol MacConkey agar (SMA), trypticase soya agar (TSA) broth (TSB) and modified EC broth with novobiocin (MEC+n) was secured from Oxoid.

Preparation of *E. coli* cell suspension:

E. coli culture was incubated over night at 35°C in TSA. Cells were harvested and resuspended in phosphate buffer solution (pH 7.2) and the bacterial concentrations were adjusted according to the proper op+tical density (Shalaby *et al*., 2001), which yielded desired cell count. Cell suspension was added to both Zabady and Kareish cheese milk so as to reach the proper inoculum.

***E. coli* count:**

Standard plate count was performed to detect *E. coli* counts in cheese and Zabady milk, and the resultant stored cheese and Zabady by using SMA medium, which incubated at 35°C for 48 hrs after streaked onto SMA plates and suspected colonies were counted (Shalaby *et al*., 2001).

Cheese and Zabady manufacture:

Cheese and Zabady were manufactured by the convenient method of processing under careful closed area. A 1:1 mixture of *S. salivarius* subsp. *thermophilus* and *L. delbreuckii* subsp. *bulgaricus* was used as starter, for cheese and Zabady at 3% and 2%, respectively.

Titratable acidity and pH values:

The titratable acidity and pH values were estimated as given by Ling (1963).

RESULTS AND DISCUSSION

It is well known that lactic acid bacteria, especially *Lactobacillus acidophilus* and *Bifidobacterium* sp. have been shown to be both preventive and therapeutic in controlling intestinal infections. Therefore, data presented in Tables (1, 2 and 3) revealed the effective inhibition of lactic acid bacteria (starter) and *Bifidobacterium bifidum* against *E. coli* presented in Kareish cheese milk. Data show that a gradual increase in the amount of acidity was observed with progressive storage period. While, the pH values were decreased consequently during this period. It is also clear that, during storage period the development of acidity was less rapid in Kareish cheese stored at 5°C than that stored at room temperature. Also, the results in Table (3) showed that the addition of *Bifidobacterium bifidum* to the milk used for making Kareish cheese enhanced the progressive development of acidity, and decrease of pH values was observed in cheese stored at 5°C or at room temperature. This might be due to this bacteria produced β–galactosidase or other enzymes which can ferment milk ingredients and produce acidity.

Additionally, obtained data also show that when resultant cheese was stored at room temperature, *E. coli* rapidly decreased in numbers.

Growth behavior of *E. coli* during manufacture and storage of Kareish cheese is shown in Tables (1, 2 and 3). This pathogenic organism decreased, to 10 x 10^4, 16 x 10^2, 4.5 x 10, 0.5 x 10 and 0.00 cfu/g in cheese milk and cheese stored at 5°C for 1, 7, 15 and 21 days, respectively. In addition, this organism could only be detected in a low count after 15 days in cheese stored at room temperature. Further, data of Table (3) show that count of *E. coli* was decreased greatly with the occurence of *Bifidobacterium bifidum*. The rate of the disappearance of *E. coli* during storage period of this cheese treatment was affected with bifidobacteria either stored at 5°C or at room temperature, especially at room temperature. This is may be due to its bacteriocidal activity or/and other antibacterial agents of *Bifidobacterium* against food pathogenic organisms such as *E. coli*. However, growth of *E. coli*, which occurred in stored Kareish cheese even with the unfavourable pH, might be attributed to the low salt content used in this type of cheese. Contrary to the other type of soft cheese such as Domiati cheese which contains a higher content of salt.

Table (1): Viability of *E. coli* in Kareish cheese made from milk inoculated with 10 x 10^4 cfu/ml and stored at 5°C.

Storage period (days)	*E. coli* counts	pH values	Acidity %
Cheese milk	10 x 10^4	6.70	0.17
1	16 x 10^2	4.35	0.97
7	4.5 x 10	4.00	1.17
15	0.5 x 10	3.95	1.35
21	ND	3.80	1.48
24	ND	3.75	1.56
35	ND	3.67	1.63

ND = Not detected.

Table (2): Viability of *E. coli* in Kareish cheese made from milk inoculated with 10 x 10^4 cfu/ml and stored at room temperature.

Storage period (days)	*E. coli* counts	pH values	Acidity %
Cheese milk	10 x 10^4	6.70	0.17
1	13 x 10^2	4.05	1.21
7	2.2 x 10	3.90	1.39
15	0.1 x 10	3.80	1.51
21	ND	3.70	1.62
24	ND	3.55	1.78

ND = Not detected.

It is well known that most of low salt Kareish cheese is consumed freshly and the maximum expiry date is only 15 days at refrigeration

temperature. Consequently, this type of cheese when infected with high counts of the pathogen, it could become health hazard and thus, it well be decreased greatly during storage period and the cheese sample become free from any pathogens in the presence of lactic acid bacteria especially *Bifidobacterium* for its prevention and therapeutic actions. Arocha, *et al*. (1992) and Massa *et al*. (1997) stated that the long term of the survival of enterohomorrhagic *E. coli* 0157 in cheese at different pH values may be explained by the acid-adaptive response. Shalaby *et al*. (2001) achieved similar results and findings.

Table (3): Viability of *E. coli* in Kareish cheese made from milk inoculated with 10 x 10^4 cfu/ml and affected with Bifidobacteria (5 x 10^7 cfu/ml) on MRS solution.

Storage period (days)	Stored at 5°C			Stored at room temperature		
	E. coli counts	pH values	Acidity %	*E. coli* counts	pH values	Acidity %
Cheese milk	10 x 10^4	6.70	0.17	10 x 10^4	6.70	0.17
1	11 x 10^2	4.21	1.05	7 x 10^2	3.99	1.25
7	2.7 x 10	3.92	1.22	0.5 x 10	3.82	1.39
15	ND	3.84	1.43	ND	3.65	1.54
21	ND	3.63	1.59	ND	3.55	1.71

ND = Not detected.

In order to study the viability of *E. coli* in Zabady, milk was inoculated with 10 x 10^4 cfu/ml and divided into four portions, one was stored at 5°C without inoculating with *Bifidobacterium bifidum* and the other was affected. Other two portions were stored at room temperature and one of them was inoculated with therapeutic bacteria (*Bifidobacterium bifidum*).

Results of Tables (4, 5 and 6) show that acidity values were progressively increased in all Zabady samples with the prolongation of storage period either in samples stored at 5°C or at room temperature. But an increment in acidity values were observed in Zabady samples stored at room temperature and samples treated with *Bifidobacterium bifidum*. At the same time, highest amount of acidity were observed with the bifido treated samples. However the pH values showed opposite trend to acidity in all treatments. Furthermore, it could be observed that all Zabady samples kept at room temperature were found to be rejected after 3 days of storage. Thereafter, all samples were characterized as spoiled and further deteriorations were occurred. Contrarily to storage at room temperature, shelf-life of resulted Zabady kept at 5°C was found to be long and retained acceptable flavor till the end of storage period (10 days). Similar results were observed by Sadek *et al*. (2000) and Shalaby *et al*. (2001).

Lactic acid bacteria have various mechanisms that inhibit other bacteria. These include, production of organic acids (reduction in pH), bacteriocins, hydrogen peroxide and ethanol and deplction of nutrients (Bodnaruk *et al*., 1998 and Shalaby *et al*. (2001). Regardings the viability of

E. coli in the resultant Zabady, Tables (4, 5 and 6) indicate that the target organism began to lose its viability during the time of Zabady preparation. Numbers of pathogen *E. coli* decreased from 10 X 10^4 to 18 x 10^2, 7 x 10^2, 4.6 x 10, 2 x 10 cfu/g in Zabady milk and the resultant Zabady after 1 (fresh samples), 2, 3 and 5 days of storage at 5°C, respectively. Higher reduction and disappearance of pathogenic organism were observed with treating zabady with bifidobacteria as well as storage at room temperature. These sharp decreases in the populations of target organism could be attributed to the higher production of organic acids and the resultant low pH values in samples stored at room temperature or/and the bacterocidal action of *Bifidobacterium* and its production of antimicrobial agents or substances which has inhibitory effect against wide range of food-borne pathogenic organisms. Therefore, the probiotic organisms are known to be involved in resistance to infectious diseases and are an important element in protection against infections due to *Salmonella*, *Shigella*, *Escherichia coli*, *Clostridium*, *Candida* and others (Ozbas & Aytac, 1995). Similar findings were observed by Dineen *et al.* (1998); Mack *et al.* (1999); Sadek *et al*. (2000) and Shalaby *et al*. (2001).

Table (4): Viability of *E. coli* in Zabady made from milk inoculated with 10 x 10^4 cfu/ml and stored at 5°C.

Storage period (days)	*E. coli* counts	pH values	Acidity %
Zabady milk	10 x 10^4	6.70	0.17
1	18 x 10^2	4.41	0.99
2	7.0 x 10	4.05	1.09
3	4.6 x 10	3.99	1.17
5	2.0 x 10	3.91	1.28
7	ND	3.78	1.33
10	ND	3.52	1.70

ND = Not detected.

Table (5): Viability of *E. coli* in Zabady made from milk inoculated with 10 x 10^4 cfu/ml and stored at room temperature.

Storage period (days)	*E. coli* counts	pH values	Acidity %
Zabady milk	10 x 10^4	6.70	0.17
1	4 x 10^2	3.95	1.25
2	0.5 x 10	3.50	1.68
3	ND	ND	ND
5	ND	ND	ND

ND = Not detected.

Table (6): Viability of *E. coli* in Zabady made from milk inoculated with 10 x 10^4 cfu/ml and affected with bifidobacteria (5 x 10^7 cfu/ml) on MRS solution.

Storage period (days)	Stored at 5°C			Stored at room temperature		
	E. coli counts	pH values	Acidity %	*E. coli* counts	pH values	Acidity %
Zabady milk	10 x 10^4	6.70	0.17	10 x 10^4	6.70	0.17
1	8 x 10^2	3.95	1.15	3 x 10	3.81	1.39
2	0.5 x 10	3.72	1.45	0.1 x 10	3.42	1.87
3	ND	3.50	1.79	ND	ND	ND
5	ND	ND	ND	ND	ND	ND

ND = Not detected.

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حيوية ميكروب الإيشريشيا كولى فى الجبن القريش والزبادى المعامل بميكروب البافيدوبكتريم بافيديم

وسام الدين اسماعيل على صابر[١] ومحمد يونس رياض مهنا[٢]

[١] قسم الميكروبيولوجيا – معهد الأراضى والمياه والبيئة – مركز البحوث الزراعية – الجيزة – مصر .

[٢] قسم الألبان – كلية الزراعة – جامعة المنصورة – المنصورة – مصر .

أثناء تداول اللبن المعد لصناعة المنتجات اللبنية وأيضاً أثناء الخطوات التصنيعية وحتى أثناءتسويق المنتج اللبنى قد يتم تلوثه ببعض الميكروبات المرضية مثل الإيشيريشيا كولى والأستافيلوكوكس والسالمونيلا وغيرها من ميكروبات الغذاء المرضية ولما كان لهذه الميكروبات المرضية آثار ضارة بصحة الفرد وقد تسبب كوارث وبائية فى بعض الأحيان . ولذلك كان لابد من دراسة سلوكها الحيوى وإستخدام بعض العوامل التى تحد من تواجدها وبقاؤها فى المنتجات اللبنية حيث تم إستخدام البافيدوبكتيريا لما لها من تأثيرات صحية وعلاجية علاوة على تأثيرها المضاد للميكروبات الممرضة وذلك فى الجبن القريش والزبادى كمنتجات لبنية وغذائية لعدد وفير من الناس

بالإضافة إلى أهميتها كغذاء آمن وصحى وعلاجى للعديد من الحالات المرضية وبخاصة حالات القلب المرضية وقد بينت الدراسة النتائج التالية :-

1- تناقصت أعداد الإيشريشيا كولى فى الجبن القريش مع زيادة مدة التخزين وقد زاد هذا التناقص بالتخزين على درجة حرارة الغرفة وإن تعاظم هذا التناقص فى حيوية وأعداد هذا الميكروب المرضى مع وجود البافيدوباكتريا فى اللبن المعد لصناعة الجبن القريش لما لها من قدرة على إنتاج مواد حيوية مضادة لميكروبات الغذاء المرضية ومنها الإيشريشيا كولى .

2- زاد إنتاج الحموضة زيادة طردية مع مدة التخزين وبخاصة عند التخزين على درجة حرارة الغرفة وتعاظمت هذه الزيادة فى الحموضة أيضاً فى اللبن الملقح بالبافيدوباكتريم .

3- تناقصت قيم الـ pH فى الجبن القريش خلال فترة التخزين وإن كان لإضافة الميكروبات العلاجية أثر إيجابى كبير على تناقص درجات الـ pH مما يعكس الأهمية الصحية والعلاجية لهذه الميكروبات .

4- إضافة البافيدوباكتيريم إلى الزبادى كان لها أثر واضح فى إختفاء ميكروب الإيشريشيا كولى حيث إختفى هذا الميكروب المرضى بصورة أوضح عند تلقيح اللبن المعد لصناعة الزبادى بهذه البكتيريات الهامة صحياً وغذائياً وبخاصة عند التخزين على درجة حرارة الغرفة .

5- زادت حموضة الزبادى بزيادة مدة حفظه وإن كانت الزيادة فى الحموضة أكثر عند التخزين على درجة حرارة الغرفة وبخاصة فى اللبن الملقح بالفيدوبكتيريا ذات الأهمية الحيوية والصحية.

6- تناقصت قيم الـ pH للزبادى بزيادة مدة الحفظ وبخاصة عند التخزين على درجة حرارة الغرفة وإن كان هذا التناقص واضح وجلى فى الزبادى المضاف له البافيدوباكتيريم .

وفى النهاية هذه الدراسة تبين الأثر الصحى والعلاجى الهام لبكتيريا حمض اللاكتيك ودورها فى إختفاء ميكروبات الغذاء المرضية وبخاصة عند إعداد وصناعة منتجات معدة حيوياً بإضافة بكتيريا البافيدوباكتيريم لما لها من قدرة فائقة على إنتاج مضادات حيوية مثل البكتريوسين والأحماض العضوية وفوق أكسيد الهيدروجين وغيرها والتى لها دورها الحيوى صحياً وعلاجياً فى مثل هذه المنتجات اللبنية .