Dept. of Food Hygiene,
Fac. of Vet. Med. Kafrelsheikh Univ., Egypt.

EFFECT OF LACTOBACILLUS ACIDOPHILUS ON SHELF-LIFE OF LOW SALT SOFT CHEESE

(With 4 Tables)

By

AZZA M.K. SOBEIH; EKBAL M.A. IBRAHIM* and HEND A. ELBARBARY*

* Dept. of Food Control, Fac. of Vet. Med. Benha Univ., Egypt.

(Received at 19/3/2011)

تأثير اللاكتوبسلس اسيدوفيلس على مدة صلاحية الجبن الطري منخفض الملح

عزة محمود كامل صبيح ، إقبال محمد عادل إبراهيم ، هند أحمد البربري

يعتبر اللاكتوبسلس اسيدوفيلس من البادئات الحيوية صديقة للإنسان حيث يستوطن الأمعاء ويمنع الإصابة بالعديد من الأمراض بالإضافة إلى تأثيره على منتجات الألبان كمادة حافظة طبيعية. أجرى هذا البحث لتصنيع جبن طرى منخفض الملح (3%NaCl) وإضافة اللاكتوبسلس اسيدوفيلس بتركيزي (1٪ و3٪Lb) ودراسة الخواص الحسية ، الكيميائية والميكروبيولوجية للجبن الناتج أثناء فترة الحفظ في الثلاجة. تم اخذ عينات في أول يوم بعد التصنيع ثم على فترات متساوية كل ثلاث أيام حتى فساد الجبن ظاهرياً. وجد أن الجبن المضاف إليه 3٪Lb أفضل من حيث التقييم الحسي واحتفظ بخواص حسية جيدة حتى اليوم 24 من الحفظ بالثلاجة بينما فسدت الجبن المضاف إليه 1٪Lb بعد 18 يوم والجبن الغير معامل بعد 15 يوم. ولقد تناقصت قيمة pH وتزايدت نسبة الحموضة في جميع العينات أثناء فترة الحفظ. وقد أوضحت النتائج أن العدد الكلى لبكتريا القولون والفطريات والخمائر اقل في الجبن المضاف إليه Lb عن الغير معامل، واستمرت هذه الميكروبات في التزايد في الجبن الغير معامل حتى نهاية الصلاحية حيث وصلت إلى 10x7,5 ^{4} ، 10x8,14 ^{3} ، 10x4,21 ^{3} خلية/ جم على التوالي. بينما كانت الزيادة فى الجبن المضاف إليه Lb في بداية فترة الحفظ ثم انخفضت تدريجياً. وسجلت 1٪Lb الأعداد 10x,93 ، 10x9,12 ^{2}و 10x5,25 ^{2} خلية/ جم (في اليوم 18) بينما سجلت 3٪Lb 10x2,3 ، 10x1,1 ^{2} و 10x6,96 ^{2}خلية/ جم (في اليوم 24) لكل من بكتريا القولون، الفطريات والخمائر على التوالي. دلت النتائج على خلو جميع العينات من ميكروب الايشريشا كولاى خلال فترة الحفظ وهو ما يتفق مع المواصفة القياسية المصرية. وبالنظر إلى اللاكتوبسلس اسيدوفيلس فقد استطاع البقاء حياً في كلتا المعاملتين وتزايد العدد تدريجياً وباستمرار حتى نهاية الصلاحية ووصل إلى 10x1,34 ^{8} و10x2,18 ^{9} في 1، 3٪Lb. ويستنتج من الدراسة أن إضافة 3٪ لاكتوبسلس اسيدوفيلس في تصنيع جبن طرى منخفض الملح (3٪) أفضل حيث تزايد العدد إلى أعلى من الموصى به

للحصول على الفوائد الصحية وكذلك امتدت فترة حفظ الجبن بالثلاجة عن الجبن الغير معامل وكذلك الجبن المعامل بنسبة 1٪Lb.

SUMMARY

Lactobacillus acidophilus is one of the most commonly used probiotics. This study aimed to produce low salt (3% Nacl) soft cheese with acceptable organoleptic characters and prolonged shelf-life using *Lb.acidophilus* (1 and 3%) as bio preservative. The obtained cheese (control, 1%Lb. and 3%Lb.) were kept in refrigerator, sampled fresh (zero time) and at 3days intervals till signs of spoilage were detected. Samples were examined organoleptically, chemically and microbiologically. Results showed that 3% Lb. cheese was superior to 1%Lb. and control cheese when fresh with an average organoleptic overall score of 95.96, 94.04 and 90.82, respectively. pH values at zero time for control, 1% Lb. and 3% Lb. cheese were 6.42, 6.33 and 6.17 then decreased at the end of shelf life (at 15th, 18th and 24th day)to 5.89, 5.65 and 5.43, respectively. Average coliforms count (MPN/g) was $3.6x10^1$, $1.1x10^1$ and $0.93x10^1$ at zero time then reached $7.5x10^4$, $0.93x10^1$ and $2.3x10^1$ at the end of shelf life for control, 1% Lb. and 3% Lb. cheese, respectively. While, *E.coli* was absent from all examined low salt soft cheese throughout the entire period. On storage, *Lb.acidophilus* was -sharply increased in their numbers-$1.34x10^8$ and $2.18x10^9$ cfu/g for 1% Lb. (at the 18th day) and 3% Lb. cheese (at the 24th day). Effect of *Lb. acidophilus* strain on mould and yeast count were highly significant (P<0.01). In conclusion, low salt soft cheese with 3% *Lb.acidophilus* culture had better organoleptic score, microbiological quality and prolonged shelf-life than control and 1% Lb cheese.

Key words: *Lactobacillus acidophilus, low salt, soft cheese, shelf-life.*

INTRODUCTION

Lactobacillus acidophilus is known as probiotics or friendly bacteria (Ljungh and Wadstrom, 2006). Probiotics are mono or mixed culture of viable, defined micro-organisms with sufficient numbers that beneficially affect the host health through altering the intestinal microflora by implantation or colonization (Fuller, 1994; Schrezenmeir and de VrEse, 2001). The probiotic culture of lactic acid bacteria (LAB) had antagonistic actions against many intestinal and food borne pathogens. Different mechanisms of action such as organic acid, bacteriocins and others seem to be involved in this antibacterial activity (Millette *et al*., 2007)

Lactobacillus acidophilus occurs naturally in the human and animal gastrointestinal tract and has the ability to implant in the intestine and restore the normal intestinal flora, ferments lactose into lactic acid which responsible for low pH and more acidic media which attributed to its therapeutic role in prevention and treatment of many intestinal diseases (Gilliland, 1979; Sandine, 1979; Kandler and Weiss 1986).

Many health benefits have been documented for use of certain *Lb. acidophilus* strains as a dietary adjunct including; pathogens interference, immune stimulant, alleviation the symptoms of lactose intolerance people, reduction of serum cholesterol level and blood pressure also decrease incidence and duration of diarrhea and common infectious diseases as rhinopharyngitis (Montes *et al.,* 1995; Anderson and Gilliland, 1999; Chou and Weimer, 1999; Parodi, 1999; Guillemard *et al.*, 2010). Moreover, it was recorded that the much lower incidence of colon cancer in northern people was associated with significant and periodical consumption of fermented foods containing probiotics (Lidbeck *et al.,* 1991; Mc Intosh, 1996). Hence, the concept of functional food has known as food or food ingredient with positive effect on host health beyond its nutritive value (Huggett and Verschuren, 1996).

Therapeutically, *Lb. acidophilus* is considered the most potential probiotics (Klantschitsh *et al.,* 1996) and there is increasing evidence that the regular consumption of foods containing specific strains of lactobacilli as probiotic cultures has beneficial effect on the functioning of the human intestine (Fooks *et al.*, 1999; Mattila-Sandholm *et al.,* 1999; Ouwehand *et al.,* 1999). The most popular vehicle for incorporation of *Lb. acidophilus* into diet is fermented milk products as soft cheese.

Although sodium chloride is an important ingredient for cheese manufacture which exerts a major influence on its composition, microflora, ripening, texture, flavor and quality (Salem and AbeId, 1997), but high sodium chloride intake has been claimed to be a major contributor to development of hypertension and cardiovascular diseases, therefore low levels of sodium chloride intake is highly recommended for all consumers (El-Abd *et al.*, 2003; Drake *et al.,* 2011). As well as, high salt content used can limit growth of starter organisms and that other salt tolerant flora may be responsible for the developed acidity.

Manufacture of soft cheese by using reconstituted dried skim milk is aiming to improve body and texture character and nutritional values of cheese by raising its total solid content (Abou-Donia, 1991; El-Sheikh *et al.,* 2001). Furthermore, the protein content of cheese increased by lowering its fat content and as a result cheese becomes of high nutritive value (Chen *et al.,* 1991 and Zommar, 2000). Moreover, *Lb. acidophilus* DSMZ 2552 can grow well in skim milk at pH up to 4.37 (Metwally *et al.,* 2005).

This study aimed to produce low salt soft cheese (3% NaCl) with acceptable organoleptic characters and prolonged shelf-life by using *Lb. acidophilus* as bio preservative and assessment of organoleptic, chemical and microbiological characteristics of manufactured cheese.

MATERIALS and METHODS

1. Culture activation:

Lyophilized single strain culture of *Lactobacillus acidophilus* (Lb.) DSMZ 20079 was obtained from Cairo-MIRCEN, Faculty of Agriculture, Ain-Shams University, Cairo, Egypt.

The Lyophilized culture of *Lb. acidophilus* was firstly propagated into MRS broth and incubated at 37°C for 24h for three successive transfers. Then the strain was sub cultured into reconstituted sterile skim-milk powder and incubated at 37°C for 24h in order to further activate the bacterial strain and increase its number to the suitable probiotic dose (10^7cfu/g). This Lb. *acidophilus* culture was inoculated during the manufacture of soft cheese.

2. Cheese manufacture:

Low salt soft cheese was manufactured as described by Mehanna and Rashed (1990); El-Sheikh *et al.* (2001) with slight modification. Reconstituted skim milk powder (<1.25% fat,< 32% protein and >53% lactose) was used for manufacture of cheese with 3% NaCl.

The bulk volume was divided into 3 groups, the first was regarded as control, the second and third were inoculated by 1% *Lb. acidophilus* culture (1% Lb.) and 3% *Lb. acidophilus* culture (3% Lb.). The three groups were kept at 42°C till proper curd was obtained, then the curd was kept to drain for 18h in a previously sterilized stainless steel frames lined with cheese cloth.

The obtained cheese with their respective whey were packaged in pre-sterilized aluminum cups and tightly covered with aluminum foil paper then kept at refrigerator. Cheeses were sampled fresh (zero time) and at equal intervals of 3 days till the sings of spoilage were detected.

The experiment was repeated in triplicates and average results for each group (control, 1% Lb. and 3% Lb. cheese) were recorded.

3. Cheese analysis:

3.1. Organoleptic evaluation:

Cheese samples were examined for appearance (10 points), body and texture (60 points) and for flavor (30 points) and the overall score was100 points according to Bodyfelt *et al.* (1988).

3.2. Chemical examination

Cheese samples were examined for titratable acidity (T.A%) and pH according to Pearson (1984).

3.3. Microbiological examination

Cheese samples were homogenized with sodium citrate (2%) and tenth fold serial dilutions were prepared as described by BSI (1984). The prepared samples were examined for total coliforms count "MPN" (APHA, 1992); *Lactobacilli count* (Dave and Shah, 1996); *E.coli count* (APHA, 1992) as well as mould and yeast count (Koburger and Farahat, 1975).

4. Statistical analysis:

Data obtained were statistically analyzed by ANOVA test according to Clarke and Kempson, (1997).

RESULTS

Table 1: Influence of *Lb. acidophilus* on organoleptic characteristics of low salt soft cheese

Storage time	Average Appearance (10)			Body texture (60)			Flavor (30)			Overall score (100)		
	Control	1% Lb.	3% Lb.*	Control	1% Lb.	3% Lb.*	Control	1% Lb.	3% Lb.*	Control	1% Lb.	3% Lb.*
Zero time**	9.17	9.40	9.63	54.49	56.71	57.86	27.16	27.93	28.47	90.82	94.04	95.96
3 days	8.26	8.92	9.26	46.14	53.38	56.62	25.21	28.05	27.85	79.61	90.35	93.73
6 days	8.01	8.83	9.07	40.35	51.91	54.79	24.10	27.80	27.53	72.46	88.54	91.39
9 days	7.45	8.58	8.79	37.51	49.67	53.28	21.93	26.28	27.11	66.89	84.53	89.18
12 days	5.94	8.15	8.36	36.25	47.80	52.01	20.17	25.54	26.73	62.36	81.49	87.10
15 days	5.06	7.69	8.10	32.74	46.27	51.96	17.03	24.40	26.47	54.83	78.36	86.53
18 days	S	7.22	7.89	S	44.65	49.95	S	23.63	25.36	S	75.50	83.18
21 days		S	7.54		S	49.43		S	25.02		S	81.99
24 days			7.18			47.19			24.50			78.87
27 days			S			S			S			S

* = Significant differences (P< 0.05) ** = High significant differences (P< 0.01)
S = spoiled

Table 2: Influence of *Lb. acidophilus* on acidity of the examined samples of low salt soft cheese

Storage time	Average pH			Average titratable acidity % (T.A %)		
	Control	1% Lb.	3% Lb.*	Control	1% Lb.	3% Lb.*
Zero time**	6.42	6.33	6.17	0.25	0.27	0.28
3 days	6.36	6.20	6.05	0.26	0.30	0.33
6 days	6.25	6.07	6.00	0.28	0.32	0.35
9 days	6.09	5.93	5.76	0.32	0.33	0.36
12 days	6.06	5.88	5.71	0.32	0.37	0.40
15 days	5.89	5.79	5.64	0.34	0.39	0.41
18 days	S	5.65	5.55	S	0.42	0.44
21 days		S	5.48		S	0.48
24 days			5.43			0.51
27 days			S			S

* = Significant differences ($P< 0.05$) ** = High significant differences ($P< 0.01$) S = spoiled

Table 3: Influence of *Lb. acidophilus* on bacteriological aspect of the examined samples of low salt soft cheese

Storage time	Average coliforms count (MPN/g)			Average *Lb. acidophilus* count (cfu/g)		*E. coli* count (cfu/g)		
	Control	1% Lb.	3% Lb.**	1% Lb.	3% Lb.**	Control	1% Lb.	3% Lb.
Zero time**	$3.6x10^1$	$1.1x10^1$	$0.93x10^1$	$6.72x10^6$	$2.43x10^7$	0	0	0
3 days	$9.3x10^1$	$0.3x10^1$	$0.3x10^1$	$8.91x10^6$	$3.96x10^7$	0	0	0
6 days	$5.5x10^2$	$2..5x10^2$	$1.02x10^2$	$2.52x10^7$	$6.13x10^7$	0	0	0
9 days	$8.4x10^2$	$3.93x10^2$	$1.67x10^2$	$7.18x10^7$	$9.54x10^7$	0	0	0
12 days	$4.1x10^3$	$5.2x10^2$	$2.78x10^2$	$7.64x10^7$	$2.01x10^8$	0	0	0
15 days	$7.5x10^4$	$3.5x10^2$	$2.15x10^1$	$8.99x10^7$	$2.65x10^8$	0	0	0
18 days	S	$0.93x10^1$	$1.48x10^1$	$1.34x10^8$	$4.30x10^8$	S	0	0
21 days		S	$1.72x10^1$	S	$5.71x10^9$		S	0
24 days			$2.3x10^1$		$2.18x10^9$			0
27 days			S		S			S

** = High significant differences ($P< 0.01$) S= spoiled

Table 4: Influence of *Lb. acidophilus* on mycological aspect of the examined samples of low salt soft cheese

Storage time	Average mould count (cfu/g)			Average yeast count (cfu/g)		
	Control	1% Lb.	3% Lb.**	Control	1% Lb.	3% Lb.**
Zero time	<10	<10	<10	$2.90x10^1$	$6.30x10^1$	$1.50x10^1$
3 days	<10	<10	<10	$5.70x10^1$	$8.20x10^1$	$5.20x10^1$
6 days	$1.0x10^1$	<10	<10	$9.20x10^1$	$9.40x10^1$	$7.10x10^1$
9 days	$4.50x10^2$	$1.50x10^1$	$5.20x10^1$	$3.22x10^2$	$2.17x10^2$	$1.18x10^2$
12 days	$7.26x10^2$	$5.10x10^1$	$1.60x10^1$	$9.47x10^2$	$7.25x10^1$	$1.06x10^2$
15 days	$8.14x10^3$	$4.96x10^2$	$1.0x10^1$	$4.21x10^3$	$8.30x10^1$	$9.70x10^1$
18 days	S	$9.12x10^2$	$1.50x10^1$	S	$5.25x10^2$	$6.80x10^1$
21 days		S	$2.20x10^1$		S	$3.41x10^2$
24 days			$1.10x10^2$			$6.96x10^2$
27 days			S			S

** = High significant differences (P< 0.01) S= spoiled

DISCUSSION

Table 1 showed the organoleptic evaluation of the manufactured cheese samples. In general, cheese inoculated with 3% *Lb. acidophilus* was superior to 1% Lb. and control cheese samples when fresh with an average organoleptic overall score of 95.96, 94.04 and 90.82, respectively. As well as, this superiority continued till 24 days of refrigerated storage with an average overall score of 78.87 for 3% *Lb.* cheese (Table 1). Such variation was significant at p<0.05. Nearly similar scores were recorded by El-Shibiny *et al.* (2005). While higher organoleptic scores were recorded by El-Zayat and Osman (2001).

Addition of LAB starter culture was recorded to improve the quality as well as the organoleptic characteristic of fresh, soft and Domiati cheese (Effat, 2000; Mehanna *et al.*, 2002; Shin *et al.*, 2004; Dpesic and JOvanovic, 2005; Dabiza, 2008).

El-Shibiny *et al.* (2005) found that probiotic soft cheese with 2% salt was superior than control cheese and this continued till the 20th day of storage. As well as, AbdAlla *et al.* (2008) stated that probiotic Ras cheese get higher score than traditional control Ras cheese.

Concerning chemical indices, pH values of the examined low salt soft cheese samples (control, 1% Lb. and 3% Lb.) were 6.42, 6.33 and 6.17, respectively at zero time and decreased to 5.89, 5.65 and 5.43 at the end of shelf-life (at 15^{th}, 18^{th} and 24^{th} days of storage), respectively (Table 2).

Lower pH values were recorded by El-Zayat and Osman (2001); EL-ABD *et al.* (2003) and El-Shibiny *et al.* (2005).

The relatively high pH values at zero time of cheese manufacture may be attributed to the time of drainage as the retention of calcium phosphate increased within the curd matrix, which act as a buffering agent against the developed acidity of cheese (Johnson *et al.*, 1998).

Low salted cheese (3% and 5%) with added mesophilic starter showed higher acidity than control without starter cheeses either when fresh or throughout storage for 60 days and this is due to the action of starter culture (Kehagias *et al.,* 1995 and El-Abd *et al.,* 2003). The increase in titratable acidity controlled the rate of bacterial growth as it acts as bactericidal agent (El-Abd *et al.,* 2003).

As well as, results in Table 2 revealed that cheese samples with *lactobacillus acidophilus* culture showed slightly higher acidity than the control ones. These results agree with El-ShibinY *et al.* (2005) and Dabiza (2008). On the day of manufacture the average T.A% were 0.25, 0.27 and 0.28 for control, 1% Lb and 3% Lb. cheese, respectively (Table 2).

During storage, the T.A% of all cheese samples were increased as the storage period progressed, while the pH values showed an opposite trend. These results agreed with those recorded by El-Sissi (1996) and El-Abd *et al.* (2003). After, the 15^{th}, 18^{th} and 24^{th} day of refrigerated storage, the T.A% reach 0.34, 0.42 and 0.51 for control, 1% Lb. and 3% Lb. cheese, respectively (Table 2). Higher results were recorded by El-Zayat and Osman (2001) and El-Shibiny *et al.* (2005). It was recorded that T.A% of cheese was greatly affected by salt level and the level of starter culture (El-Abd *et al.,* 2003).

Regarding coliforms, Table 3 showed the effect of using *Lb. acidophilus* in manufacture of low salt soft cheese on coliforms count. The mean coliforms count was $3.6x10^1$, $1.1x10^1$ and $0.93x10^1$ MPN/g at zero time then reached to $7.5x10^4$, $0.93x10^1$ and $2.3x10^1$ MPN/g at the 15^{th}, 18^{th} and 24^{th} of refrigerated storage for control, 1% Lb. and 3% Lb. cheese, respectively. In spite of coliforms count in 3% Lb. cheese were higher than the EOSQ (2005) that stipulate less than 10 cfu/gm but it is much lower than counts in control cheese.

Rheem *et al.* (2002) and El-Abd *et al.* (2003) recorded that low coliforms count in low salt Domiati cheese is possibly due to the high acidity and production of other antimicrobial substances by action of LAB culture**.** Furthermore**,** the preserving effect of LAB are due to production of wide range of antimicrobial metabolites as organic acids, diacetyl, hydrogen peroxide and bacteriocin which have the advantage in competition with other microorganisms including pathogens and other harmful (Oyetayo *et al.,* 2003; marteinez Bveno *et al.,* 2007)

The extended shelf-life of low salt soft cheese with 3% *Lb. acidophilus* up to the 24th day of refrigerated storage with restricted and relatively low coliforms may be due to the suppressive effect of several antimicrobial metabolites produced by the added *Lb. acidophilus* on coliforms.

On the other hand, *E.coli* were absent from all examined low salt soft cheese (control, 1% and 3% Lb. cheese samples) throughout the entire period (Table 3). This result came in accordance with EOSQ (2005) for cold stored soft cheese, that it must be free from *E.coli.*

The antimicrobial activity of lactobacilli is associated with the production and synergistic activity of organic acids and hydrogen peroxide, whereas their antagonistic activity against gram-negative and gram-positive bacteria is dependent on the fermentation group of lactobacilli (Annuk *et al.,* 2003).

Table 3 revealed that the mean values of lactobacilli count at zero time were $6.72 x 10^{6}$ and $2.43 x 10^{7}$ cfu/g for 1% Lb. and 3% Lb. cheese, respectively. On storage, *Lb. acidophilus* were sharply increased in their numbers and reached $1.34 x 10^{8}$ cfu/g for 1% Lb. cheese at the 18th day and $2.18 x 10^{9}$ cfu/g for 3% Lb. cheese at the 24th day of refrigerated storage (Table 3).

This viable *Lb. acidophilus* count met the requirements for successful probiotic functional foods that should contain at least 10^{7} cfu/g or ml at the time of consumption to promote their healthy benefits (IDF, 1988; Ishibashi and Shimamura, 1993, El-Shibiny *et al.,* 2005 and Marcatti *et al.,* 2009).

The extend shelf-life of 3% Lb. cheese is probably due to rapid development of titratable acidity in cheese manufactured with added Lb. *acidophilus* starter culture, compared with less acid development in control cheese samples. Survival of *Lb. acidophilus* could be attributed to its ability to grow at low pH as acid tolerant organisms. These results are nearly agreed with El-Zayat and Osman (2001) and El-Abd *et al.* (2003). The degree of survival and activity of *Lb. acidophilus* depend on salt content and the level of acidity in soft cheese (Mehanna *et al.,* 2002).

Yeasts and moulds play an important role in the spoilage of dairy products, primarily in fermented milks and cheese (Jakobsen and Narvhus, 1996; Welthagen and Viljoen, 1998). Low pH of fermented milk caused by the growth of LAB renders such foods as a good medium for the highly opportunistic fungi to proliferate and thrive leading to spoilage of such products (Batish *et al.,* 1993).

Table 4 declared that, at zero time the average mould count in all examined low salt soft cheese samples were <10 cfu/g and average yeast count for control, 1% Lb. and 3% cheese were $2.9x10^1$,$6.3x10^1$ and $1.5x10^1$ cfu/g. The effect of *Lb. acidophilus* strain on mould and yeast count were highly significant (at P<0.01), as their growth were restricted in 3% Lb. and 1% Lb. compared with control cheese. This is may be due to inhibitory effect of antifungal metabolites produced by *Lb. acidophilus* (Cassandra *et al.,* 2004)

Both mould and yeast were detected in acidophilus cheese after 18 days for 1% Lb. cheese & up to 24 days for 3% Lb. cheese with an average count (cfu/g) of $9.12x10^2$ & $1.1x10^2$ for mould and $3.25x10^2$ & $6.96x10^2$ for yeast. These results exceed the permissible limit (<10 cfu/g for mould and <400 cuf/g for yeast) suggested by Eosq (2005).

Many moulds find cheese an excellent medium for their growth and the cheese become undesirable with musty off-flavors (Abu Sree, 1997). While typical defects caused by yeasts are gas production, discoloration, change in the texture and yeasty flavors (Tudor and Board, 1993). Moreover, the potentially toxigenic species within the genera Penicillium, Aspergillus and Fusarium were detected in cheeses by Montagna *et al.* (2004).

In conclusion, low salt soft cheese (3% Nacl) with added *Lactobacillus acidophilus* culture at concentration of 3% had better organoleptic score, micrbiological quality and prolonged shelf-life (24 days) at refrigerated storage.

REFERENCES

Abdalla, E.A.M.; Aly, Soher, E.; Saleh, Y.; Mary, S. and Hathout, Amal, S. (2008): Probiotic Bacteria as tool to produce high quality and safe Ras cheese. Egypt. J. Dairy Sci., 36: 97-109.

Abou-Donia, S.A. (1991): Manufacture of Egyptian soft and pickled cheeses. In: Feta and related cheeses (Robinson, R.K. Tamime, A.Y.eds.,). Ellis Horwood ltd. New York.

Abu-Sree, Y.H. (1997): Effect of cheese processing and ripening of hard cheese on formation and stability of some mycotoxins. Ph.D. Thesis, Fac. of Agric., Cairo Univ.

Anderson, J. and Gilliland S. (1999): Effect of Fermented milk (yoghurt) containing *Lactobacillus acidophilus L.I* on serum cholesterol in hypercholesterolemic humans. J. Am Coll Nutr.18:43.

Annuk, H.; Scheptoca, J.; Kullisaar, T.; Songisepp, E.; Zilmer, M. and Milelsaar, M. (2003): Characterization of intestinal lactobacilli as putative probiotic candidates J. Appl. Microbiol., 94: 403.

APHA "American Public Health Association" (1992): Standard Methods for the Examination of Dairy Products. INC, 16th ed., New York.

Batish, V.K.; Grover, S. and Neelakantan, S. (1993): Improving shelf-life and safety of fermented milk products through genetically improved microorganisms. Ind. Dairyman. 45: 51.

Bodyfelt, F.W.; Tobias, J. and Trout, G.M. (1988): Sensory evaluation of cheese. In “The sensory evaluation of dairy products”. AVI, publ. by Van Nostrand Reinhold, New York, PP: 300-376.

BSI "British standards Institution" (1984): Microbiological examination of dairy purposes. BS4285: Section 2010.

Cassandra, De.M.; Annelies, I.J.; Leroya, Sofi, De.M.; Filip, E.; Win, S. and Vandammea, E.J. (2004): Potential of selected lactic acid bacteria to produce food compatible antifungal metabolites. Microbiological Research, 159: 339-346.

Chen, C.M.; Johnson, M.E. and Olson, N.F. (1991): Relationship between manufacturing practices and quality of reduced fat cheddar cheese. J. Dairy sci.74: 95.

Chou, L. and Weimer, B. (1999): Isolation and characterization of acid- and bile- tolerant isolates from strains of *Lactobacillus acidophilus* .J. Dairy Sci.; 82: 23.

Clarke, G.M. and Kempson, R.E. (1997): Introduction to the design and analysis of experiments. Arnold, a member of the Holder Headline Group, 1st ed., London,

Dabiza, Nadia, M.A. (2008): Production of soft cheese with *Lactobacillus acidophilus*. Egypt. J. Dairy Sci. 36: 63-71

Dave, R.I. and Shah, N.P. (1996): Evaluation of media for selective enumeration of *S. thermophilus, L. delbreukii ssp. Bulgaricus Lactobacillus acidophilus* and Bifidobacteria. J. Dairy sci., 79: 1529.

Dpesic, M. and Jovanovic, L. (2005): Microbiology study of fresh white cheese (A Serbian Craft Variety). Appli- Ecol.s Environ. Res., 4: 129.

Drake, S.L.; Lopetcharat, K. and Drake, M.A. (2011): Salty taste in dairy foods. Can we reduce the salt? J. Dairy Sci., 94: 636-645.

Effat, B.A. (2000): Effect of using *lactobacillus reuteri* with other probiotic cultures on quality of Domiati cheese. Minufiya J. Agric. Res. 25: 445.

EL-Abd, M.M.; Abd EL- Fattah, A.M.; Osman, S.G. and Abdel EL-Kader, R.S. (2003): Effect of some lactic acid bacteria on the properties of low salt Domiati cheese Egypt. J. Dairy Sci., 31: 125-138.

EL-Sheikh, M.M.; Farrage, A.F.; Shahein, N.M. and EL-Shibiny, S. (2001): Low fat Domiati cheese with particulate whey protein concentrate (PWPC).Egypt. J. Dairy sci.29: 331-342.

EL-Shibiny, S.; Metwally, M.M.; Abd EL-Gani, S.; Abd EL-Fattah, A.M. and Okda, A.Y.M. (2005): Manufacture of some probiotic dairy products from ultra-filtered milk retentate. Egypt. J. Dairy Sci., 33: 215-227.

El-Sissi, M.G.M. (1996): Studies on Nasr cheese- A newly ripened white soft cheese. Egypt. J. Dairy Sci., 24: 235-246.

EL-Zayat, A.I. and Osman, M.M. (2001): The use of Probiotics in Tallaga cheese. Egypt. J. Dairy Sci., 29: 99-106.

EOSQ "Egyptian Organization for Standardization and Quality" (2005): ES: 1008-5/2005 Soft cheeses, part 5 (Cold stored cheese), ICS: 67.100. 30 Arab Republic of Egypt.

Fooks, L.J.; Fuller, R. and Gibson, G.R. (1999): Prebiotics, probiotics and human gut microbiology. Int. Dairy J. 9: 53.

Fuller, R. (1994): Probiotics.2: Applications and Practical Aspects. Chapman-and Hall. London

Gilliland, S.E. (1979): Beneficial interaction between certain microorganisms and humans: Candidate organisms for use as a dietary adjunct. J. Food Prot.42: 164.

Guillemard, E.; Tondu, F.; Lacoin, F. and Schrezenmeir, J. (2010): Consumption of fermented dairy product containing the probiotic *Lactobacillus casei* DN-114001 reduces the duration of respiratory infections in the elderly in a randomized controlled trial: Br. J. Nutr. 103 (1) 58-68

Huggett, A.C. and Verschuren, P.M. (1996): The safety assurance of functional foods. Nutrition Reviews 54: 5132-5140.

IDF "International dairy federation" (1988): Fermented milks: Science and Technology. Bulletin of the IDF No 227, Brussels

Ishibashi, N. and Shimamura, S. (1993): Bifidobacteria: research and development in Japan. J. Food Technol., 6: 126-135.

Jakobsen, M. and Narvhus, T. (1996): Yeasts and their possible beneficial and negative effects on the quality of dairy products. Int. Dairy J. 6: 755.

Johnson, M.E.; Steele, J.L.; Broad bent, J. and Weimer, B.C. (1998): Manufacture of Gouda cheese and flavor development in reduced –fat cheddar cheese. Aust. J. Dairy Technol.,1: 53-67.

Kandler, O. and Weiss, N. (1986): Genus Lactobacillus. In: Bergeys Manual of Systematic Bacteriology (Smeath, P.H. A.; N.S. Mair and shalpe, M.E. eds.,). Vol. 2. Williams and Wilkins, Baltimore, M.D., pp1209.

Kehagias, C.; Kolouris, S.; Samona, A.; Malliou, S. and Koumoutsos, G. (1995): Effect of various starters on the quality of cheese in brine. J. Food Microbiol, 12 (5): 413.

Klantschitsch, T.; Spillmann, M. and Puhan, Z. (1996): Lactobacillus reuteri: A newcomer in dairy technology. Lebensmittel Technol. 92:1

Koburger, J.A. and Farahat, B.Y. (1975): Fungi in foods. I.A comparison of media to enumerate yeasts and molds. J. Milk and Food Technol.38: 466-468.

Lidbeck, A.; Nord, C.E.; Gustaffson, J.A. and Raftel, J.J. (1991): Lactobacilli anti- carcinogenic activities and human intestinal microflora Eur. J. Cancer Prevention. 1:341-353.

Ljungh, A. and Wadstrom, T. (2006): Lactic acid bacteria as probiotics. Curr. Issues Intest. Microbiol.7: 73.

Marcatti, B.; Habitante, A.M.Q.B.; Sobral, P.J.D.A. and Trindade, C.S.F. (2009): Minas-Type fresh cheese developed from buffalo milk with addition of L.acidophilus. Sci. Agric. (Piracicaba, Braz.) 66 (4): 481-485.

Maritinez Bveno, M.; Ananou, S.; Maqueda, M.; Galvez, A. and Valdivia, E. (2007): Bio preservation. an ecological approach to improve the safety and shelf life of food. Letters in Applied Microbiology. 475-486.

Mattila- Sandholm, T.; Jaana, M. and Maria, S. (1999): Lactic acid bacteria with health claims- interactions and interference with gastrointestinal flora. Int. Dairy J. 9:25.

Mc Intosh, G. (1996): Probiotics and colon- cancer prevention. Asia Pacific J. Clin. Nutr.5: 48-52.

Mehanna, A.S. and Rashed, M.A. (1990): An attempt to improve the keeping quality of Tallaga cheese by using milk treated with carbon dioxide. Egypt. J. Dairy Sci., 18: 377-388.

Mehanna, Nayra, S.H.; Sharaf, O.M.; Ibrahim, G.A. and Tawfik, N.F. (2002): Incorporation and viability of some probiotic bacteria in functional dairy food. 1-soft cheese. Egypt. J. Dairy sci.,30: 217.

Metwally, M.M.; EL-Shibiny, S.; Abd EL- Fattah, A.M.; Abd EL- Gani, S. and Okda, A.Y.M. (2005): The interaction between probiotic bacteria and their growth behavior in ultra filtrated milk retentate. J. Dairy Sci., 33: 143.

Millette, M.; Luguet, F.M. and Lacroix, M. (2007): In vitro growth control of selected pathogens by *Lactobacillus acidophilus*- and *Lactobacillus casei*- fermented milk. Letters in Appl. Microbiol. 44: 314-319.

Montagna, M.T.; Santacroce, M.P.; Spilotros, G.; Napoli, C.; Minervini, F.; Papa, A. and Dragoni, I. (2004): Investigation of fungal contamination in sheep and goat cheeses southern Italy. Mycopathologia, 158: 245.

Montes, R.G.; Bayless, T.M; Saacedra, J.M. and Perman, J.A. (1995): Effect of milk inoculated with *lactobacillus acidophilus* or a yoghurt starter culture in lactose- mal digesting children J. Dairy sci.78: 1657.

Ouwehand, A.C.; Prikka, V.K.; Colette, S. and Seppo, S. (1999): Probiotics: mechanisms and established effects. Int. Dairy J.9:43.

Oyetyo, V.O.; Adetuyi, F.C. and Akinyosoyr, F.A. (2003): Safety and protective effect of *Lactibacillus acidophilus* and *Lactibacillus casei* used as probiotic agent in vivo. African J. of Biotechnol., 2: 448- 452.

Parodi, P.W. (1999): The role of intestinal bacteria in the causation and prevention of cancer. Modulation by diet and probiotics. Aust. J. Dairy Tech. 54: 103.

Pearson, D. (1984): Chemical analysis of foods. 8th ed., Churchill Livingston, Edinburgh, London.

Rheem, S.; Sejong, O.H.; Han, K.H.; Imm, J.Y. and Kim, S. (2002): New response surface approach to optimize medium composition for production of bactericin by *lactobacillus acidophilus* ATCC 4356. J. Microbiol. Biotechnol.,12: 449.

Salem, A.S. and Abeid, A.M. (1997): Low sodium and cholesterol Domiati cheese. Egypt. J. Daily Sci., 25: 123.

Sandine, W.E. (1979): Roles of lactobacillus in the intestinal tract. J. Food prot. 42: 259.

Schrezenmeir, J. and De Vrese, M. (2001): Probiotics, prebiotics, and synbiotics-approaching a definition. Am. J. Clin. Nutr., 73(supplement), 3615-3645.

Shin, K.G.W.M.; Jean, K.G.B. and Leen, B.H. (2004): Purification and characterization of intracellular proteins from *Lactobacillus casei spp. Casei LL G*.J. Dairy Sci., 87: 7097.

Tudor, D.A. and Board, R.G. (1993): Food spoilage yeasts. In: the yeasts. vol.5: yeast technology, 2 nd Ed., (A.H. Rose and J.S. Harrison, Eds.,). Academic Press.

Welthagen, J.J. and Viljoen, B.C. (1998): Yeast Profile in Gouda cheese during processing and ripening. Int. J. Food Microbiol, 41: 185.

Zommar, O.A. (2000): Technological studies on mozzarella cheese. Ph. D. Thesis Ain- Shams. Univ.