J. Food and Dairy Sci., Mansoura Univ., Vol. 3 (3): 185 - 192, 2012

IMPACT OF USING METABOLITES OF SOME LACTIC ACID BACTERIA ON PROPERTIES AND QUALITY OF UF WHITE SOFT CHEESE

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ABSTRACT

UF-soft cheese (low-salt) was made from cow's skim retentate and vegetable oils with using metabolites from *Bifidobacterium bifidium* (Bb-11) yoghurt starter, and *Lactobacillus acidophilus* (La-5) (Treatments A, B and C in order). Impact of the prepared metabolites on total bacterial count (TBC) and counts of yeasts and moulds (YMC) as well as composition and quality of cheese was followed during 30 days of cold storage. Insignificant differences were recorded in TBC between the control and treated cheese in spite of the control cheese had always relatively higher TBC. All the metabolites used were more effective against yeasts and moulds since the control cheese had higher YMC than the treated cheeses which showed insignificant differences between samples of 0.0 and 30 days old. The results showed also that, addition of the metabolites significantly increased the acidity and decreased the pH. TS and fat were not significantly affected by the applied treatments. On the other hand, addition of the metabolites didn't affect formol number, SN/TN and TVFA of cheese compared with the control. The attained results suggest that metabolites of lactic acid bacteria (LAB) especially that from treatment (C) could be used to achieve better shelf life and improved quality of UF-soft cheese.

INTRODUCTION

White soft cheese is the most important dairy product in Egypt. It is consumed daily as a part of the Egyptian diet. Tallaga cheese is an Egyptian unripened soft cheese made by rennet coagulation of pasteurized milk with adding low concentration of salt. The cheese must be stored under cooling and consumed within two weeks of production. Moreover, UF-soft cheese was introduced to the Egyptian market in the last two decades and especially that contains low-salt content becomes part of the health full diet of the consumer. Presence of whey proteins in such cheese is of great importance for the human health (Sukkar and Bounous, 2004 and El-Dien *et al.*, 2010).

Such soft cheese especially that contains low salt content is usually suffering from poor keeping quality that may be due in many occasions to the traditional filling under non-aspetic conditions. In this respect, presence of food borne pathogenic microorganisms was previously reported in some Egyptian studies (Abou-Dawood *et al.*, 2005 ; Abou-Donia, 2007 and El-Kholy *et al.*, 2008).

On the other hand, lactic acid bacteria (LAB) are industrially important organisms recognized for their fermentative ability, their therapeutic and antibacterial properties as well as their nutritional benefits (Gilliand, 1990). These roles are chiefly ascribed to their metabolic activity. Among the different LAB the genera Lactobacillus, *Lactococcus leuconstoc* and *Pediococcus* are known for their ability to produce a wide variety of inhibitory

substances which inhibit undesirable pathogenic organisms (Lindgren and Dobrogosz, 1990 and Martinez *et al.*, 1995). These substances include lactic acid and other organic acids (acetic, formic and propionic) oxygen metabolites (e.g. H_2O_2), diacetyl, low molecular weight proteinaceous products including bacteriocins and other substances endowed with antibiotic activity (Piard and Desmazeaud, 1991 & 1992).

The present study was an attempt to apply the technology of ultrafiltration and using metabolites produced by *Bifidobacteria bifideum* (Bb-11), *Lactobacillus acidophilus* (La-5) and yoghurt starter in the production of low-salt UF-soft cheese. Role of the prepared metabolites as antimicrobial agent was strongly taken into consideration.

MATERIALS AND METHODS

Cultures activation:

Commercially lyophilized cultures of *Bifidobacterium* (Bb-11), *Lactobacillus acidophilus* (La-5) and yoghurt starter (Yc-380) that consisting of *Lactobacillus delbruckii* subsp. bulgaricus (Lb. bulgaricus) and *Streptococcus thermophilus* (*Str. thermophilus*) were obtained from Chr. Hansen laboratories, Copenhagen, Denmark. The starter cultures were activated in sterile skim milk (10% w/v) and incubated at 37°C for 18 h.

Preparation of the metabolites:

Flasks of sterile skim milk were incoulated with 2% of individual active lactic acid cultures and supplemented with 0.03% of yeast extract and 1% glucose, then incubated at proper temperature previously mentioned (37°C) for 48 h. The coagulum was then stirred up and filtered through Whatman No. 42 filter paper. The clear filtrate was concentrated at temperatures ranging from 40 to 55°C for about 1/10 of the original volume. The metabolites were sterilized by autoclaving at 121°C for 5 min (Girgis *et al.*, 2003).

Cheese making:

The prepared retentate from UF of fresh cow's skim milk was mixed with palm oil and palm kernel oil (1:1) to attend 18% total fat content whereas skim milk powder was used to standardize the TS content to be 34%. The final mixture was homogenized and pasteurized at 70°C for 15 sec. After cooling to 45°C, sodium chloride (2.5%) and calcium chloride (0.02%) were added with gentle mixing.

The mixture was divided into 4 portions, one portion was served as a control and the others were achieved pH at 6.6 by adding metabolites produced from *Bifidobacterium bifidum* (A), yoghurt starter (B) and *Lactobacillus acidophilus* (C). To every portion, water diluted calf rennet was added and rapidly mixed. The mixtures were immediately filled into 100 g plastic cubs, incubated at 40°C until complete coagulation and then stored under refrigeration for 30 days.

Microbiological analysis:

Cheese samples were analyzed for total bacterial count and counts of yeasts and moulds. Total bacteria was counted on nutrient agar medium

as described by American Public Health Association (APHA, 1992). Yeast and moulds were counted on potato dextrose agar (PDA) medium (APHA, 1992).

Chemical analysis:

Cheese samples were analyzed when fresh and after 15 and 30 days for acidity and pH (Ling, 1963). Total solids content was determined according to the British Standard Institution (BSI, 1952). Gerber's method was followed for fat determination (Ling, 1963). Procedure of Tawab and Hofi (1966) was followed for determination of formol number. Total nitrogen (TN), non protein nitrogen (NPN) and soluble nitrogen (SN) were measured by semi-micro Kjaldahl procedure as give by Ling (1963) whereas total volatile fatty acids (TVFA's) content expressed as ml 0.1 N NaOH/100 g cheese was determined by direct distillation method according to Kosikowski (1978).

Sensor evaluation:

The sensory evaluation was assessed according to the scoring card recommended by Naguib *et al.* (1974), given the following points for the different properties: flavour (60 points), body and texture (30 points), saltiness (5 points) and appearance (5 points).

Statistical analysis:

Analysis of variance and Duncan's test as well as average and standard error were carried out using a SPSS computer program (SPSS, 1999).

RESULTS AND DISCUSSION

Table (1) shows total bacterial count (TBC) and count of yeasts moulds (YMC) as log cfu/g cheese as affected by using different metabolites A, B and C as well as the untreated cheese (the control). Both A and B treatments had relatively lower TBC that were insignificantly differed from those of the control and C treatment (P>0.05). This was true at the beginning and during cold storage period. However, the control samples was suffering from increasing TBC during storage with the highest rate compared to the treated samples.

Table (1): Total bacterial count (TBC) and count of yeasts and moulds (YMC) as log cfu/g of UF-soft cheese made from milk retentate treated with metabolites. (Mean±SE of 3 replicates)*.

Item	Storage period (day)	Treatments**			
		Control	A	B	C
TBC	0	5.51±0.21^{aB}	5.12±0.03^{aB}	5.27±0.11^{aB}	5.54±0.06^{aB}
	15	7.59±0.25^{aA}	7.47±0.09^{aA}	7.52±0.006^{aA}	7.72±0.02^{aA}
	30	8.12±0.13^{aA}	7.22±0.005^{aA}	7.20±0.005^{aA}	7.21±0.05^{aA}
YMC	0	3.08±0.12^{aB}	2.99±0.04^{abC}	2.65±0.05^{bC}	2.63±0.15^{bB}
	15	6.09±0.007^{aA}	5.02±0.03^{bB}	5.21±0.003^{bB}	5.71±0.14^{aA}
	30	6.31±0.13^{aA}	6.22±0.005^{bA}	6.20±0.005^{bA}	6.21±0.005^{bA}

*** Means with unlike small and capital letters within row and column respectively are significantly different (P<0.05)**

**** A= metabolite from *Bifidobacterium bifidum*, B= metabolite from yoghurt starter, C= metabolite from *Lactobacillus acidophilus*.**

The recorded TBC values as log cfu/g were 5.51 and 8.12 at the beginning and the end of storage period of the control cheese, respectively, whereas those of treatment A were 5.12 and 7.22, of treatment B were 5.27 and 7.2 and for treatment C were 5.54 and 7.21 in order. In all cases, such increase in TBC was significant.

Concerning YMC, it is clear from Table (1) that at the beginning of storage period, the control cheese had the highest YMC ($P<0.05$) being 3.08 that was not differed significantly from that of A treatment (2.99) but significantly higher than those of B (2.65) and C (2.63) treatments. Relatively higher YMC was recorded for 15 and 30 days old control cheese, but at the end of storage period the control cheese still had the highest significant YMC, whereas the differences in this respect due to the applied treatments were insignificant. In all cases, YMC gradually increased during storage.

The present results suggest that the used metabolites had impact on decreasing the numbers of bacteria and yeasts and moulds in UF soft cheese and this effect was more pronounced during cold storage of the resultant cheese.

The present trend of results agrees in general with the articles and studies given in the literature towards that goal (Lindgren and Dobrogosz, 1990 and Martinez *et al.*, 1995). El-Ziney (1999) demonstrated in details the role of LAB in producing bio preservatives such as acetic acid, lactic acid, bacteriocins and reuterin which have great impact on controlling food-borne pathogens in different models and application in foods. The antimicrobial effect of *Bifidobacterium bifidum* (Treatment A) agrees with the results given by El-Kholy *et al.* (2005) who studied impact of *B. bifidum* metabolite with or without activation with *P. thoenii* P-127 on inhibition of many pathogenic organisms as well as yeasts and moulds.

Presence of yeasts and moulds also in Kareish cheese and Domiati cheese was previously reported by Effat *et al.* (2001) and El-Kholy *et al.* (2005). The prementioned authors tried also to prevent their occurrence in cheese by means of using LAB metabolites. Aseptic filling of UF-soft cheese seems to be of great importance in this respect.

Table (2) reveals acidity, pH, total solids (TS) and fat content of UF-soft cheese. The control cheese had significant low acidity values when fresh and during the storage period as compared to the treated cheese samples.

At the beginning of storage period, cheese from treatment C had the highest acidity ($P<0.05$), whereas the differences between acidity of cheese from treatments A and B were insignificant ($P>0.05$). In the 15 and 30 days old cheese, the control cheese still had the lowest acidity, whereas the differences in this respect due to the different metabolites used were insignificant ($P>0.05$). Such data could be attributed to the acidic nature of the prepared metabolites since it was reported in the literature that it contains lactic acid and some other organic acids like acetic, formic and propionic acids as well as some another compounds (Piard and Desmazeaud, 1991, 1992).

The opposite trend was observed with respect to pH values which showed the highest values in the fresh control cheese (6.97) that significantly decreased during storage ($P<0.05$). Some differences in pH of the fresh

treated cheese being 6.76, 6.74 and 6.55 for A, B and C treatments that decreased to be 6.12, 6.35 and 6.15 at the end of storage period, respectively.

Table (2): Chemical composition of UF-soft cheese made from milk retentate treated with different metabolites. (Mean±SE of 3 replicates)*.

Item	Storage period (day)	Treatments			
		Control	A	B	C
Acidity (%)	0	0.17±0.01^{cA}	0.23±0.01^{bcB}	0.23±0.005^{bB}	0.33±0.02^{aA}
	15	0.20±0.05^{bA}	0.28±0.02^{aB}	0.26±0.00^{aA}	0.30±0.01^{aA}
	30	0.24±0.03^{bA}	0.36±0.005^{aA}	0.27±0.06^{aA}	0.36±0.02^{aA}
pH	0	6.97±0.01^{aA}	6.76±0.03^{bA}	6.74±0.03^{bcA}	6.55±0.09^{cA}
	15	6.80±0.01^{aB}	6.62±0.03^{bA}	6.47±0.01^{cB}	6.31±0.02^{dAB}
	30	5.51±0.02^{aC}	6.12±0.06^{bB}	6.35±0.01^{cC}	6.15±0.03^{cB}
TS (%)	0	34.32±0.19^{aB}	34.69±0.53^{aB}	35.48±0.95^{aB}	34.52±0.51^{aB}
	15	37.91±0.05^{aA}	37.62±0.09^{aA}	37.23±0.17^{aA}	36.34±0.07^{aA}
	30	38.07±0.35^{aA}	37.92±1.20^{aA}	38.53±0.83^{aA}	38.92±0.31^{aA}
Fat (%)	0	18.25±0.00^{aC}	18.00±0.25^{aB}	18.25±0.01^{aC}	18.25±0.25^{aB}
	15	21.50±0.50^{aB}	21.00±1.00^{aA}	21.50±0.50^{aAB}	22.00±0.01^{aA}
	30	22.50±0.50^{aA}	23.20±0.01^{aA}	24.00±0.01^{aA}	24.00±0.50^{aA}

*** See legend to Table (1) for details.**

It seems from the data given in Table (3) that in the fresh cheese (at the beginning of storage) the values of formol number and SN/TN were insignificantly differed (P>0.05) due to the applied treatments in spite of presence of some differences in this respect. This finding was continued only for formol number during storage of cheese, whereas SN/TN values were significantly less in 30 days old cheese from treatments A and B.

Table (3): Impact of adding different metabolites on the proteolysis and lipolysis during cold storage of UF-soft cheese.(Mean±SE of 3 replicates)*.

Item	Storage period (day)	Treatments			
		Control	A	B	C
Formal number	0	51.0±1.00^{aA}	51.0±1.00^{aB}	54.0±4.01^{aA}	51.0±1.02^{aA}
	15	51.0±1.01^{aA}	55.0±1.05^{aA}	55.0±3.03^{aA}	52.0±2.05^{aA}
	30	53.0±1.06^{aA}	55.0±1.09^{aA}	54.0±1.08^{aA}	53.0±1.00^{aA}
SN/TN (%)	0	30.86±0.06^{aC}	30.82±0.02^{aC}	30.82±0.01^{aC}	30.80±0.01^{aA}
	15	31.28±0.06^{aB}	31.04±0.06^{bB}	31.06±0.06^{bB}	31.32±0.05^{aA}
	30	32.02±0.01^{bA}	31.51±0.01^{bA}	31.57±0.05^{cA}	32.31±0.01^{aA}
NPN/TN (%)	0	5.30±0.02^{bC}	5.46±0.06^{bB}	6.12±0.08^{aA}	6.12±0.09^{aA}
	15	6.12±0.08^{abB}	5.82±0.11^{bAB}	6.13±0.09^{bA}	6.18±0.04^{aA}
	30	6.92±0.03^{aA}	6.13±0.05^{bA}	6.14±0.07^{bA}	6.13±0.07^{bA}
TVFA**	0	1.70±0.01^{aA}	1.60±0.20^{aA}	1.65±0.05^{aA}	1.70±0.01^{aA}
	15	1.70±0.01^{aA}	1.60±0.20^{aA}	1.80±0.01^{aA}	1.70±0.01^{aA}
	30	1.75±0.15^{aA}	1.90±0.01^{aA}	1.90±0.01^{aA}	1.70±0.01^{aA}

*** See legend to Table (1) for details.**
**** TVFA expressed as ml 0.1 N NaOH/100 g cheese.**

The good correlation between both formol number and the SN content was previously reported by Tawab and Hofi (1966), whereas the higher values of SN/TN in all cheeses (more than 30%) is mainly due to

incorporation of whey proteins in the cheese matrix as a result of applying UF in the preparation of the retentate used in cheese making.

Concerning NPN/TN, it seems from Table (3) that in the fresh cheese the differences between the control and A treatment were insignificant and were significantly lower than those of B and C treatments (6.12%), whereas at the end of storage the control cheese had significant higher value (6.92%) compared to those from treatments A, B and C being 6.13, 6.14 and 6.13% (P>0.05) in order.

Such changes in rate of proteolysis was accompanied with insignificant rates of lipolysis that due to the treatments applied or along the storage period (Table 3).

Sensory evaluation of the resultant cheese (Table 4) revealed that the flavour attributes were the best in cheese from (C) treatment since at the beginning and the end of storage period treatment (C) received the highest scores for flavour followed by those for treatment (B). Such trend of results were insignificantly affected by advancing storage period.

Table (4): Sensory evaluation of UF soft cheese as affected by the different metabolites used. (Average±SE of 15 determination from 3 replicates)*.

Item	Storage period (day)	Treatments			
		Control	A	B	C
Flavour (60)	0	45.00±0.63^{aA}	45.00±0.63^{aA}	48.00±0.63^{aA}	49.66±0.98^{aAB}
	15	45.33±0.33^{bA}	46.33±0.66^{bA}	49.33±0.33^{abA}	53.33±0.66^{aA}
	30	46.33±0.69^{bA}	46.33±0.66^{bA}	49.33±0.66^{bA}	55.66±0.66^{aA}
Body & texture (30)	0	24.00±0.63^{bB}	25.26±0.66^{abA}	28.33±0.88^{aA}	30.00±0.00^{aA}
	15	25.66±0.33^{bA}	25.22±0.33^{bA}	27.86±0.33^{aA}	30.00±0.00^{aA}
	30	26.66±0.33^{bA}	25.00±0.00^{bA}	28.00±0.66^{aA}	30.00±0.00^{aA}
Saltiness (5)	0	4.22±0.00^{aB}	4.40±0.00^{aB}	4.40±0.03^{aB}	4.66±0.33^{aA}
	15	4.40±0.00^{aB}	4.66±0.33^{bB}	4.66±0.33^{aA}	4.80±0.16^{aA}
	30	5.00±0.00^{aA}	5.00±0.00^{aA}	5.00±0.00^{aA}	5.00±0.00^{aA}
Appearance (5)	0	4.50±0.33^{aA}	4.60±0.16^{aA}	4.66±0.16^{aA}	4.80±0.33^{aA}
	15	4.66±0.33^{aA}	4.66±0.33^{aA}	4.80±0.30^{aA}	5.00±0.00^{aA}
	30	4.60±0.00^{aA}	4.66±0.33^{aA}	5.00±0.00^{aA}	5.00±0.00^{aA}

*** See legend to Table (1) for details.**

Concerning body and texture, the control cheese samples were always had the lowest scores, whereas cheese from treatments (B) and (C)ranked the highest significant scores (P<0.05). The score given for treatment (C) was not differed (30 out of 30 points) during storage.

Saltiness taste was not considered as flavour defect in the resultant cheese since the scores were always higher than 4 out of 5 points and at the end of storage period all cheese samples ranked the maximum attainable score (Table 4).

In spite of the general appearance was not affected by the applied treatments or by advancing storage period, cheese from (C) had always the highest scores.

In conclusion, better keeping quality for UF-soft cheese (low salt content) could be achieved by adding different metabolites from some lactic acid bacteria. The metabolite from *Lactobacillus acidophilus* gave the best results in this respect.

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تـأثير نـواتج التمثيـل الغذائى لـبعض سـلالات بكتريـا حـامض اللاكتيـك علـى خـواص وجودة الجبن الأبيض الطرى المحضر بالترشيح الفائق

منال على نعيم

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أهتمت الدراسة بتصنيع جبن أبيض طرى منخفض المحتوى من الملح وذلك بإستخدام مركز اللبن الفرز المحضر بطريقة الترشيح الفائق مع دراسة تـأثير نـواتج التمثيل الغذائى لـبعض البكتريـا التابعـة لبكتريـا حـامض اللاكتيـك وهـى بكتريـا *Bifidobacterium bifidium* (Bb-11) yoghurt starter, and *Lactobacillus acidophilus* (La-5). وأوضـحت النتـائج المتحصل عليها أن نواتج التمثيل الغذائى لم يكن لها تـأثيراً معنوياً على الاعداد الكلية للبكتريـا فـى الجبن الطـازج أو المخـزن لمدة ٣٠ يومـاً فـى الثلاجـة ، ولكن كانـت الاعداد أقل نسبيا فـى الجبن المعامل مقارنة بجبن المقارنة فى حين كان لنواتج التمثيل الغذائى تـأثيرا أكثر وضـوحاً على خفض أعداد الفطريات والخمائر أمـا حموضـة الجبن والرقم الايدروجينى لهـا فقد تـأثر معنويـا بالمعـاملات المذكورة حيث زادت ارقام الحموضة وانخفضت قيم الرقم الهيدروجينى وذلك بسبب الطبيعة ذات التأثير الحامضـى لنواتج التمثيل الغذائى المضـافة فـى حين لـم يتـأثر معدل التحليل البروتينـى (رقم الفورمـول وقيم النتـروجين الـذائب الـى النتـروجين الكلـى) ولا معدل التحلل الـدهنى (المحتـوى مـن الاحماض الدهنية الكلية الطيارة). هذا ويمكن تصنيع الجبن الأبيض الطـرى ذو المحتوى المنخفض من الملح بإضافة نواتج التمثيل الغذائى للبكتريـا المذكورة وخاصـة المعاملـة الثالثـة الـى مركز اللبن الفرز المستخدم فى الصناعة والمحضر بطريقة الترشيح الفائق.

قام بتحكيم البحث

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