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EFFECT OF SOME NONSTARTER LACTIC ACID BACTERIAL STRAINS ON THE QUALITY AND RIPENING CHANGES OF WHITE SOFT CHEESE

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ABSTRACT: Soft cheese was made from pasteurized mixed buffaloes and cows milk (1:1) inoculated with either nonstarter lactic acid bacterial cultures *(Pediococcus damansus and Micrococcus lutes)* or normal starter of *Lactococcus lactis* subsp *lactis*. The gross chemical composition, casein breakdown, fat hydrolysis and sensory evaluation of both fresh and ripened cheeses at 1, 2 and 3 months were investigated. Results showed that addition of nonstarter lactic acid bacterial cultures to cheese milk had no marked effect on the moisture, salt and fat contents of all treated cheeses when fresh and during cheese ripening, while cheese made with nonstarter cultures had slightly higher acidity than the control cheese. Soft cheeses made with the addition of nonstarter cultures ripened faster than control cheese. The soluble nitrogenous compounds increased at a greater rate in cheese treated with added *Lactococcus lactis, Pediococcus damansus and Micrococcus lutes* (1:1:1). Evalution of free fatty acids during ripening was also investigated and compared with that obtained for control cheese. Both control and experimental soft cheeses underwent slight lipolysis during ripening, resulting in the production of mostly medium-and long-chain fatty acids during ripening. In addition, nonstarter cultures treated cheeses contained higher concentrations of total free fatty acids than the control cheese. The organoleptic properties of cheese made with added mixed nonstarter lactic acid bacterial cultures at a proportion of (1:1:1) gave a product with a taste and texture comparable to control cheese.

Key words: Soft cheese, Gross composition, Soluble nitrogen compounds, Free fatty acids, Organoleptic properties.

INTRODUCTION

Soft cheese is a popular variety produced in Egypt. Ripening of cheeses is governed by many different factors. Proteolysis and lipolysis are two major biochemical processes in the phenomenon of cheese maturation, which involves a variety of chemical, physical, and microbiological changes under controlled environmental conditions.

Proteolysis of cheeses, in general, is influenced by several factors including plasmin, chymosin, protease from starter and non-starter lactic acid bacteria (NSLAB) The role of NSLAB in ripening has not yet been resolved satisfactory, although inclusion of adjunct cultures of some strains of NSLAB or use of raw milk during cheese manufacturing increases the level of free amino acids, peptides and free fatty acids, which leads to enhanced flavour intensity and accelerates cheese ripening (McSweeney and Sousa, 2000). Todiseco et al. (1981) showed that the bacterial extra cellular proteolytic enzymes accelerated the degradation of α- and β-casein, while the extra cellular lipase were able to hydrolyse milk triglycerides. Therefore, Micrococci can play an important role in the development of taste and flavour characteristics during ripening of soft cheese.

Barthelemy and lablee (1983) found that addition of 50-190 units/L milk of a proteolytic enzyme extracted from *Micrococcus caseolyticus* before or during renneting accelerated the ripening of French soft cheese.

Bhowmik and Marth (1988) examined nine different strains of Micrococci for theirin-tracellular proteiase and amino-peptidase activity and found that the highest intracellular pro-teinase activity appeared in *Micrococcus sp.* LL3 isolated from Cheddar cheese.

Hassan and Abo-Zeid (1988) showed that white soft cheese containing cell free extract of *Micrococcus* had similar gross composition and higher ripening indices than the control-cheese and gained also the best sensory scores.

Pediococci and Micrococci are rarely used as starters for the production of cheese. Nevertheless, Pediococci are considered important to some food fermentation industries.

Pediococci and Micrococci constitute a major proportion of the secondary flora of much raw and pasteurized milk of cheese varieties. (Bhowmik and Marth, 1990). Some *Micrococcus* and *Pediococcus spp.* strains have been

used to accelerate cheese ripening (Bhowmik and Marth, 1990). They contribute to flavour devel-opment and texture changes owing to their high proteolytic and lipolytic activities.

Lee and Joo (1993) confirmed the potential utilization of *Micrococcus sp.*LL3 as an agent in the acceleration of Cheddar cheese ripening.

The roles of the non-starter microbial flora in soft cheese varieties are not very well understood. Therefore, the present work was carried out to evaluate the effect of some non starter lactic acid bacterial strains on the quality and ripening changes of white soft cheese.

MATERIALS AND METHODS

Materials:

Milk:

Raw buffaloes and cows milk were obtained from the Dairy Farm of Agricultural Secondary School at Diarb Negm, Sharkia Governorate.

The average content of raw mixed buffaloes and cows milk (1:1) used in this study was: 0.17% acidity, 4.0 % of fat and 12.5% total solids.

Rennet:

A rennet powder(1:100000), Halla was obtained from L.C. Glad Company A/S Copenhagen, Denmark.

Non starter lactic acid bacterial cultures:

Pediococcus domanosus strain DSM 20331, *Micrococcus lutes* strain ATTCC 1246 and *Lactobacillus casei* were obtained from The Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. The culture were activated before being used singly or in combination with *Lactobacillus casei* at the proportion of (1:1:1).

Cheese Making:

Five experiments were conducted on mixed buffaloe milk and cows milk. Raw mixed milk was heat treated to72°C for 15 s, and cooled to 37°C, then divided into four equal parts: The first part was inoculated with *Lactococcus. Lactis ssp. Lactis* at a rate of 1% and served as a control. The second part was treated with *Lactobacillus casei (A)* at a rate of 1% The third and fourth parts were treated with 1% of *Pediococcus damanosus (B), Micrococcus lutes (C),* and the fifth part was inoculated with *Lactobacillus casei, Pediococcus damanosus* and *Micrococcus*

lutes at the proportion of (1:1:1) respectively. The rennet was added to each part of milk and the coagulum was obtained after 30 min. The cheese making process was completed as described by Fahmi and sharara (1950).

Chemical analysis:

White soft cheese samples were analysed when fresh, then after 1 and 2 months, for moisture, fat, salt and titratable acidity as described by Ling (1963).

Determination of proteolysis:

Proteolysis of soft cheese samples were determined by the methods described by Gripon et al., (1975). Nitrogen content of the samples were determined by Kjeldahl method. Nitrogen content in the various fractions was expressed as percentages of total N. Water-soluble nitrogen (WSN) was extracted as follows: 10g cheese sam-ple were homogenized with 50 ml distilled water (40°C), and held 40°C for 1 h. Then, the homogenate was centrifuged at 3000 g for 30 min. The extract was cooled to 4°C, filtered, and used for determination of nitrogen content. Trichloroacetic acid (TCA)-soluble N was prepared by mixing a 25ml volume of WS extract with an equal volume of 24% TCA (w/v). The mixture was left to stand 2 h and filtered with Whatman no. 40 paper. Then, nitrogen content was determined. PTA-soluble N was prepared from the WS extract. Then, 10 ml of WS extract were taken and 7 ml 3.95 M H2SO4 and 3 ml 33% (w/v). phosphotungstic acid were added. The mixture was held at 4 C for 12 h, then filtered through Whatman no. 40 paper, and after that, nitrogen content of the filtrate was determined.

Determination of free fatty acids:

Free fatty acids were Gas Chromatographically determined at the Department of Food Chemistry, Technical University, Berlin, Germany.

Free fatty acids were isolated from soft cheese samples mixed with pelargonic acid (20mg) as an internal standard.The sodium soaps of the free fatty acids were prepared according to the method of Kuzdzal and Kuzdzal Savoie (1966). Methyl esters of the free fatty acids were prepared as described Kuzdzal Savoie & Kuzdzal (1967). Methyl esters were separated in a pye Unicam series 104 gas chromatograph (pye Unicam, Cambridge, Great Britain) equiped with a dual flame ionisation detector. Column 3.6m long and of 2 mm inner diameter were used with 80-100 mesh silnised Chromosorb

W carrier, coated with 10% polyethylene glycol adipate as a stationary phase. Temerature programming at a rate of 5°C/min was applied in the range of 130-180°C. The temperature of the injection port was 200°C and that of the detector, 300°C. Carrier gas flow (He) was adjusted to 35 ml/min. Chart speed was 5 mm/min. Peak areas were calculated by multiplying the peak height at the maximum by the wedth of the peak at half its height. Results were expressed as mg/100g of cheese.

Organoleptic properties:

The organoleptic properties of soft cheese samples were examined by a test panel of five staff members, as described by El Koussy et al., (1970), with maximum score points of 10, 50, and 40 for appearance, flavor and body & texture, respectively.

RESULTS AND DISCUSSION

Gross Chemical Composition:

Table (1) shows the changes in the gross chemical composition of white soft cheese made from mixed pasteurised milk inoculated with some non-starter lactic acid bacteria during ripening. The results obtained showed a gradually decrease of moisture content and a slight increase in cheese acidity with increasing the ripening time. However, inoculation of che-ese milk with non lactic acid bacte-rial culture at a level of 1% had an insignificant effect on the moisture, fat and salt contents of the resultant cheese and contained slightly higher acidity. Misic and Petrovic (1972); Hassan and Abo-Zeid (1988) and Tayar (1995) obtained similar results in white soft cheese.

Ripening Indices:

Soft cheese ripening was assessed by the determination of WSN, NPN, and free fatty acids.

Soluble nitrogen compounds:

The changes in the WSN content of cheese samples during ripening period are presented in Table 2. The water soluble nitrogen (WSN) fraction contains small molecules of proteins (non-casein), peptides and free amino acids (Christensen et al., 1991) and is commonly used as an index of cheese ripening (Lopez-Fandino et al., 1994). In this experiment, the WSN content in all cheese samples showed a tendency to increase over the ripening period, and cheese with added NSLAB had higher WSN contents than the control cheese. This was more pronounced in treatment D containing 1% of *L. casei, Pediococcus damanosus and Micrococcus lutes (1:1:1).*

TCA-soluble nitrogen (TCA -SN) contains mainly small molecules of peptides (lower than 20 amino acid residue) and free amino acids. The TCA-SN contents of all cheese samples increased throughout the ripening period studied (Table2). However, cheese-containing NSLAB had higher lev-els of TCA-SN when compared with control cheese (Table 2). These differences between control and NSLAB containing cheeses were more pronounced at the end of cheese ripening.

In fraction of PTA-soluble nitrogen (AN), tri-di-peptides and free-amino acids are soluble state (Fialaire and Postaire, 1994). The changes in PTA-SN contents of soft cheese samples during ripening are given in Table 2. As could be seen from this table, contents of PTASN increased during ripening period in all cheeses. Nevertheless, higher PTASN contents were detected in cheese samples containing 1% of NSLAB than in the control cheese (Table2). In general aspect of prot-eolysis, many agents are involved in the ripening of cheese, such as rennet, indigenous milk enzymes, enzymes of starter bacteria, enzymes from yeasts and molds, and non-starter bacteria (Fox, 1989). The obtained results were in agreement with those obtained by Misic and Petrovic (1972).

Chander et al., (1986) and Fulco et al., (1990) also showed that the WSN contents of cheeses increased throughout the ripening period. Kapac-Parkoceva et. al, (1976) reported that the ripening indices of white cheeses increased during the ripening period.

Pediococcus pentosaceus proliferate in the maturing cheese (Chapman and Sharpe, 1981). Many of these nonstarter lactic acid bacteria (NSLAB) have complex proteolytic systems, which have been associated with the maturation process (Broome et al., 1990 and Trepanier et al., 1991). Also some *Micrococcus* and *Pediococcus spp.* strains have been used to accelerate cheese ripening (Bhowmik et al., 1990; Aly and Abdel Baky, 1993 and Mohedano et al., 1998).

Lipolysis:

The effect of the non starter lactic acid bacterial culture on lipolysis in the white soft cheeses is presented in Table 3.The degree of lipolysis in both control cheese and experimental cheeses increased during ripening, but lipolysis was more in NSLAB-added cheeses than the control. Especially cheese containing 1% of NSLAB and consisting of *L. casei, Pedicoccus*

damanosus and Micrococcus lutes (1:1:1) had the highest concentration of free fatty acids after 2 months of cheese ripening (Table3). Dolezalek and Plockova (1981) studied the effect of pure cultures of *Lactobacillus acidophilus, Bifidobacterium befidum* and *Ppediococcus acidilactici* and a mixture containing these 3 micrroorganisms in the proportion of (10:45:45) grown in reco-nstituted milk. They found that mixed culture containing *Pediococcus acidilactici* grown at 30 C produced more volatile the volatile fatty acids, diacetyl and acetoin.

Pediococci *and* Micrococci constitute a major proportion of the secondary flora of many raw and pasteurized milk of cheese varieties(Bhowmik and Marth,1990).

Organoleptic properties:

(Table4): Shows the change in the organoleptic properties of soft cheese samples containining NSLAB during ripening.Results showed that addition od NSLAB to soft cheese milk improved the flavour intensity and body characteristics of soft cheese after 2 months of cheese ripening. These results could be explained on the basis that cheese containing these microorganisms had higher levels of both soluble nitrogen compounds and free fatty acids compared with control cheese (Table 2&3).

Lee et al., (1992) found that *Micrococcus* treated cheese had more intense flavour, less bitterness, less off flavours and a smother body & texture than the control cheese. Hassan and Abo-Zeid (1988) showed that white soft cheese containing cell free extract of *Micrococcus* had similar gross composition and higher ripening indices than the control and gained the best sensory scores. Moreover, Mohedano et al., (1998) also showed that flavour quality of Mancheago cheese was improved by adding Micrococcus cystein proteinase to cheese milk.

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Table (1): Changes in the gross chemical composition of soft cheese made with added some non lactic acid bacterial cultures during ripening.

Items %	Ripening period (months)	Treatments				
		Control	A	B	C	D
Moisture	Fresh	63.80	63.20	63.70	63.88	63.91
	1	60.46	60.37	60.75	61.53	60.60
	2	57.51	57.57	57.93	57.63	57.77
Fat	Fresh	15.60	15.40	15.50	15.60	15.40
	1	18.80	18.90	18.60	18.70	18.80
	2	19.90	19.70	19.30	19.60	19.70
Salt	Fresh	5.90	5.72	5.84	5.95	5.79
	1	5.19	6.05	5.89	6.05	5.32
	2	5.39	5.29	5.37	5.42	5.40
Acidity	Fresh	0.30	032	0.30	0.30	0.32
	1	1.10	1.30	1.40	1.42	1.60
	2	1.40	1.70	1.50	1.70	1.86

A: Lactobacillus casei ***B: Pediococcus damanosus***

C: Micrococcus lutes ***D:*** Mixed of all (1:1:1)

Table (2): Changes in some ripening indices of soft cheese made with added some non lactic acid bacterial cultures during ripening.

Nitrogen fractions (%)	Ripening period (Weeks)	Treatments				
		Control	A	B	C	D
SN/TN	Fresh	5.45	5.69	6.70	6.88	6.91
	2	8.35	8.31	7.78	9.22	9.75
	4	10.46	11.37	12.15	12.33	13.60
	6	13.79	14.85	15.29	15.57	16.78
	8	15.51	17.57	17.93	18.33	20.67
NPN/TN	Fresh	4.65	4.75	4.85	4.93	4.98
	2	5.94	5.97	6.29	6.59	6.91
	4	7.09	7.20	8.11	8.51	8.29
	6	9.55	9.73	.10.20	10.84	11.21
	8	10.34	10.63	11.93	12.21	12.80
AN/TN	Fresh	1.47	1.59	1.85	1.90	1.98
	2	2.82	2.90	2.99	3.20	3.70
	4	3.25	3.52	3.85	3.89	4.25
	6	4.21	4.52	4.95	5.24	5.89
	8	6.20	6.48	6.98	7.58	7.94

Table (3): Free fatty acids (mg/100g) of ripened soft cheese made with added some non lactic acid bacterial cultures.

Fatty acids (mg/100g)	Treatments				
	Control	A	B	C	D
C4	3.80	3.20	3.70	3.88	3.91
C6	20.00	22.00	16.75	18.33	19.60
C8	7.51	10.00	7.93	17.33	20.67
C10	23.60	25.00	23.50	23.60	23.40
C12	5.10	4.00	6.80	5.50	6.20
C14	72.20	69.40	70.25	72.30	79.10
C16	180.90	172.00	180.90	185.90	189.28
C18	32.19	37.00	36.70	34.25	36.32
C18:1	102.85	108.00	.106.37	106.42	110.25
C18:2	8.30	10.32	9.45	10.50	12.45
C18:3	1.40	1.60	1.72	1.77	1.85
Total	457.85	462.52	464.07	479.78	503.03

A: Lactobacillud casei ***B: Pediococcus damanosus***
C: *Micrococcus lutes* D: Mixed of all (1:1:1)

Table (4): Changes in organoleeptic properties of soft cheese made with added some non starter lactic acid bacterial cultures during ripening at room temp.

Storage period (days)	Characteristics	Control	*L.helvetics*	*P.damnosus*	*M.lutes*	Mixed
Fresh	Appearance (10)	8.0	8.0	8.0	8.0	8.0
	Body &Texyure (40)	32.8	32.4	32.4	32.4	32.6
	Flavour (50)	41.3	41.3	40.2	40.3	40.4
	Total (100)	82.1	81.7	80.6	80.7	81.0
15	Appearance (10)	7.9	7.8	7.6	7.6	7.7
	Body &Texyure (40)	33.8	33.7	33.5	33.6	33.6
	Flavour (50)	41.7	41.5	40.4	40.4	40.5
	Total (100)	83.4	83.0	81.5	81.6	81.8
30	Appearance (10)	7.0	7.0	7.0	7.0	7.0
	Body &Texyure (40)	34.4	34.4	34.4	34.4	34.4
	Flavour (50)	42.8	42.6	42.4	42.5	42.8
	Total (100)	84.2	84.3	83.8	83.9	84.2
45	Appearance (10)	7.0	7.0	7.0	7.0	7.0
	Body &Texyure (40)	34.8	34.8	34.4	34.4	34.8
	Flavour (50)	45.0	44.8	43.8	45.8	45.8
	Total (100)	86.8	86.6	85.2	85.2	87.6
60	Appearance (10)	7.0	7.0	7.0	7.0	7.0
	Body &Texyure (40)	35.2	35.0	35.0	35.0	35.4
	Flavour (50)	45.8	45.8	45.8	45.8	46.8
	Total (100)	88.0	87.8	87.8	87.8	89.2

تأثير بعض السلالات من بكتريا حمض اللاكتيك غير البادئات على جودة وتسوية الجبن الطرية

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فـــى هذا البحث صنعت الجبن الطرية من لبن مبستر خليط من لبن بقرى وجاموسى بنسبة ١ : ١ ، ولقح بسلالات من بكتريا غير بادئات حمض اللاكتيك وبادئات حمض اللاكتيك .

وأشـــارت النتائج أن إضافة سلالات من بكتريا غير بادئات حمض اللاكتيك إلى اللبن المعد لصناعة الجبن لم يكن لها تأثير على نسبة الرطوبة والملح والدهن على الجبن المعامل الطازج وخلال فترة التسوية ، بينما تميز هـــذا الجبـــن بزيادة طفيفة فى نسبة الحموضة وكانت التسوية فى الجبن المصنع بإضافة سلالات من بكتريا غير بادئـــات حمـــض اللاكتـــيك أسرع بالمقارنة بجبن المقارنة ، زاد معدل تكوين المركبات النيتروجينية الذائبة فى الجبـــن المعـــامل والمصـــنع بإضافة خليط من سلالات من بكتريا غير بادئات حمض اللاكتيك وبكتريا حمض اللاكتيك بنسبة (١ : ١ : ١) .

وقـــد تم دراسة ومقارنة تغير الأحماض الدهنية فى الجبن المعامل أثناء فترة التسوية بجبن المقارنة ، وقد وجد أن كل من الجبن المعامل وجبن المقارنة قد تعرض لحدوث تحلل فى الدهن وقد نتج عن ذلك إنتاج أحماض دهنية متوسطة وطويلة السلسلة أثناء فترة التسوية.

بالإضـــافة إلى ذلك فقد احتوى الجبن المصنع بإضافة سلالات من بكتريا غير بادئات حمض اللاكتيك على تركيزات مرتفعة من الأحماض الدهنية الكلية بالمقارنة بجبن المقارنة .

كمـــا أظهـــرت الخـــواص الحسية للجبن المصنع بإضافة خليط من سلالات من بكتريا غير بادئات حمض اللاكتيك وبكتريا حمض اللاكتيك بنسبة (١ : ١ : ١) بإمكانية الحصول على جبن ذو طعم ونكهة وقوام وتركيب يقارب جبن المقارنة .