

COMPOSITIONAL PROPERTIES AND RIPENING OF WHITE SOFT CHEESE MADE WITH PROBIOTIC CULTURES

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ABSTRACT: The effect of two probiotic starter culture, (*Bifidobacterium bifidum* and *Bifidobacterium breve*) as well as yoghurt culture containing *Streptococcus salivarius* subsp *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* on compositional properties and ripening of white soft cheese was investigated. The obtained results indicated that, there were no differences between soft cheese containing probiotic cultures and the control cheese in the chemical composition when fresh and during ripening except the acidity of the cheese which was relatively higher in the probiotic cheese than the control. With regard to the NPN, SN, AAN fractions and TVFA, it was noticed that their values were relatively higher in cheese made with probiotic bacteria compared with the control cheese. On the other hand, sensory evaluation of cheese showed no considerable difference in the scores gained for all cheeses.

So, it could be concluded that, adding bifidobacteria starter cultures instead of yoghurt starter to the cheese milk have the advantages of probiotic properties without undesirable effect on the cheese quality.

Key words: Proteolysis, lipolysis, soft cheese and probiotic cultures.

INTRODUCTION

In recent years, the consumption of probiotic foods has been increased especially fermented dairy products such as yoghurt and cheeses. Probiotic foods vary in level and type of probiotic bacteria. The most commonly used probiotics are

lactic acid producing bacteria from *Lactobacillus* and *Bifidobacteria* species (Gibson and Fuller, 1998). The consumption of probiotic foods could lead to a reduction in serum cholesterol levels, improve infant formula, inhibit pathogenic bacteria and make products more nutritious and more easily digestible by lactose intolerant people (Laroia and Martin, 1991).

Yoghurt culture micro-organisms, i.e. *S. salivarius* subsp. *theromphilus* and *L. delbrueckii* subsp. *bulgaricus*, could not survive after ingestion in the presence of acid in the stomach and bile juice which released into the duodenum (Hoover, 1993). Yoghurt culture bacteria are not considered as probiotic bacteria and this may be a limiting factor for its use for antibiotic therapy and/ or other medical purpose (Tamime and Robinson, 1985).

It was reported that *L. acidophilus* and bifidobacteria are more active in the small and large intestine of human (Mitsuoka, 1990). It has been suggested that the consumption of minimum viable cell number of 6 to 10^9 cfu/day is necessary for developing beneficial effect in human body (Gilliland, 1989 and Sellars, 1991).

Incorporation of probiotic cultures has recently been extended to cheese varieties. Studies have demonstrated that bifidobacteria survive well in Cheddar and Gouda cheeses (Stanton *et al.*, 1998 and Fitzgerald *et al.*, 1999), Bifidous Karish cheese (Osman and Abbas, 1999), Tallaga cheese (El-Zayat and Osman, 2001) and Chihuahua cheese (Gutierrez-Ndez *et al.*, 2004).

The objectives of this work were to study using of two probiotic bacteria strains namely; *Bifidobacterium bifidum* and *Bifidobacterium breve* as a single starter culture or as a mixture culture with yoghurt starter culture in white soft cheese making. The effect of these starter cultures on the compositional and organoleptic qualities of the resultant soft cheese was investigated.

MATERIALS AND METHODS

Materials:

Fresh buffaloes' milk (5.5% fat and 8.75% SNF) was obtained from the local market and was used in the manufacture of soft cheese.

A commercial rennet powder was obtained from L. C. Company a/s Copenhagen, Denmark.

Bifidobacterium bifidum ATCC 15696 and *Bifidobacterium breve* ATCC 15700 (probiotic cultures) were obtained from Microbiological Resources Center (Cairo, MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. Yoghurt starter culture (1: 1 mixture of *Streptococcus salivarius* subsp. *therophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*) was obtained from Chr. Hansens's Laboratory, Copenhagen, Denmark.

Methods:

White soft cheese manufacture:

Fresh milk was heated to 85° C for 15 sec, then cooled to 38° C. NaCl was added at a rate of 2.5% to the cheese milk and mixed well. The cheese milk was divided, under aseptic conditions, into five equal portions. The first portion was inoculated with 1.5% yoghurt starter culture and served as control. Cheese milk in the other four portions, yoghurt culture was substituted with *Bifidobacterium bifidum* or *Bifidobacterium breve* at a level of 50 or 100%. Rennet was added at a level of 3 gm rennet powder per 100 kg milk. After complete coagulation, each curd was cut into 500 gm weight cubs,

warped up in light plain paper then transferred to polyethylene pouches. Polyethylene pouches were sealed and stored refrigerated (5-7°C) up to 21 days. Cheese samples were tested when fresh, then after 7, 14 and 21 days of storage period for chemical and microbiological examination. Also, organoleptic properties were evaluated.

Chemical analysis of white soft cheese:

Moisture, titratable acidity, fat, protein and salt (NaCl) contents were determined according to Ling (1963). Proteolysis and lipolysis of white soft cheese samples were determined as described by Gripon *et al.* (1975) and Kosikowski (1978), respectively.

Bifidobacterial count:

Bifidobacteria were enumerated according to Dave and Shah (1996) using modified MRS agar supplemented with 0.05% L-cystein and 0.3% Lithium chloride. The plates were anaerobically incubated at 37° C for 48 hrs.

Organoleptic properties of white soft cheese:

The organoleptic properties of white soft cheese samples were

examined by five staff members of Food Sci. Dept., Fac. of Agric., Zagazig Univ., as described by El-Koussy *et al.*, (1970).

RESULTS AND DISCUSSION

1- The gross composition:

The gross chemical composition of soft cheese made with yoghurt and probiotic starter cultures are given in Table 1. There was no considerable difference between the control and experimental cheese except relatively high acidity of cheese made with probiotic starter culture than that of yoghurt starter. These results have been observed either when fresh or during storage period up to 21 days.

Also, it is clear from these results that acidity, fat, protein and salt content of white soft cheese in all treatments and the control calculated on dry matter base have been increased with the advance of the storage periods up to 21 days. On the other hand, the cheese moisture contents of all treatments as well as the control cheese have been decline with the advance of the storage period up to 21 days. This decline in the moisture content may be responsible for the

relative increase of the other gross constituents of the stored cheese over those of the fresh cheese. Misic and Petrovic (1972) and Daigle *et al.* (1999), reported similar results in this respect.

2- Ripening indices of white soft cheese:

White soft cheese ripening had been assessed by determination of soluble (S.N), non (NPN) and amino acid nitrogen (AAN) contents of the cheese and total volatile fatty acids (TVFA). The results given in Table 2 show that substituting yoghurt starter culture with probiotic culture of *Bif. bifidum* or *Bif. breve* up to 100% increased the NPN fraction percentage in the resultant cheese particularly at the end of the storage period (21 days). This increase ranged from 8% to 48.46% than the control when fresh and stored cheese, respectively. However, no considerable differences were noticed between *Bif. bifidum* and *Bif. breve* strains in this respect.

With regard to SN and AAN fractions it is clear that its percentage in white soft cheese exhibited the same tendency as NPN fractions with substituting yoghurt with probiotic starter

cultures. Both of those two nitrogen fractions have been increased in the produced white soft cheese with using probiotic starter culture of *Bif. bifidum* or *Bif. breve* than those made with yoghurt starter cultures. These results are in agreement with previous investigations (Mistic and Petrovic, 1972; Gomea and Malacata, 1998 and Gardiner *et al.*, 1999a). They reported that probiotic bacterial strains contributed significantly to cheese ripening through the formation of low molecular mass peptides and amino acids which were detected in cheese made with added probiotic strains.

It is well established that NPN, SN and AAN fractions contain small molecules of proteins, peptides and free amino acids which are commonly used as a cheese ripening indices (Grappin *et al.* 1985).

Also it is evident from Table 2 that NPN, SN and AAN contents of the control cheese and treatments have been increased with the advance of the storage period up to 21 days. Kapac-Parkoceva *et al.* (1976); Chander *et al.* (1986); Fulco *et al.* (1990) and McSweeney Sousa (2000) reported very similar results in this respect.

With regard to the total volatile fatty acids (Table 3), it was found that replacing yoghurt starter culture partly or completely with probiotic culture, *Bif. bifidum* or *Bif. breve* in cheese milk ripening increased significantly the (TVFA) of the resultant product. There was no considerable difference between the two probiotic bacterial strains in this respect.

3- Bifidobacteria count:

As shown in Table 4, it is clear that the number of survival bifidobacteria in the cheese increased with the increase of the percentage of added bifidobacteria to the starter. Meanwhile, it was observed that this number decreased with the advance of the storage period up to 21 days but still over 10^6 cfu/g cheese. It ranged from $13-17 \times 10^6$ and $20-21 \times 10^6$ cfu/g cheese when 50% and 100% of the starter culture was *Bif. Bifidum* or *Bif. breve*, respectively. These results are very close to those obtained by other investigators (Osman and Abbass, 1999 & El-Zayat and Osman, 2001).

4- Organoleptic properties:

As shown in Table 5, sensory evaluation of soft cheese made

with or without probiotic culture showed no significant difference in the gained scores. Gardiner *et al.*, (1999 b) reported that sensory evaluation showed no significant difference in flavour, body and texture scores between control and experimental cheese made with probiotic bacteria.

So, it could be concluded that, adding bifidobacteria starter cultures instead of yoghurt starter to the cheese milk have the advantages of probiotic properties without undesirable effect on the cheese quality.

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Table (1): The effect of partial or total substitution of yoghurt starter culture with probiotic bacteria on the chemical composition of white soft cheese during storage up to 21 days at 5-7 °C.

Chemical composition	Storage period (days)	Control cheese	Cheese containing <i>Bifidobacterium bifidum</i>		Cheese containing <i>Bifidobacterium breve</i>	
			50%	100%	50%	100%
Moisture (%)	Fresh	67.39	63.40	66.66	66.96	68.18
	7	61.88	63.70	63.85	62.42	68.00
	14	60.82	61.34	62.14	60.85	64.90
	21	60.65	56.25	62.00	57.94	64.55
Acidity (%) as lactic acid	Fresh	0.20	0.50	0.40	0.65	0.40
	7	0.50	0.75	1.00	0.95	0.45
	14	0.60	0.90	1.05	1.05	0.55
	21	0.70	0.90	1.10	1.10	0.65
Fat (%)	Fresh	15.5	16.00	15.00	15.00	14.50
	7	17.00	17.00	17.00	18.00	15.00
	14	18.00	18.50	19.50	19.00	17.00
	21	19.50	22.00	20.00	21.00	18.00
Protein (%)	Fresh	19.97	23.35	22.01	22.20	23.09
	7	24.56	24.43	21.50	22.39	25.90
	14	25.07	24.88	27.62	25.45	26.54
	21	25.65	27.75	28.33	28.20	26.48
Salt (NaCl) (%)	Fresh	1.23	1.19	1.26	1.23	1.16
	7	1.23	1.18	1.29	1.29	1.18
	14	1.35	1.32	1.32	1.31	1.25
	21	1.40	1.40	1.35	1.43	1.29

Table (2): The effect of partial or total substitution of yoghurt starter culture with probiotic bacteria on the nitrogen fractions of white soft cheese during storage up to 21 days at 5-7 °C.

Nitrogen fractions	Storage period (days)	Control cheese	Cheese containing <i>Bifidobacterium bifidum</i>		Cheese containing <i>Bifidobacterium breve</i>	
			50%	100%	50%	100%
TN (%)	Fresh	3.43	4.04	3.81	3.84	4.00
	7	4.24	4.24	4.84	3.88	4.50
	14	4.36	4.00	4.94	4.44	4.70
	21	4.50	4.90	5.10	4.97	4.77
NPN /TN (%)	Fresh	8.75	9.45	9.37	9.37	9.37
	7	9.07	9.55	9.60	9.58	9.72
	14	9.82	10.83	12.35	10.06	11.5
	21	10.67	11.21	12.99	11.05	12.89
SN/TN (%)	Fresh	13.75	13.00	15.40	13.62	15.40
	7	13.83	14.48	16.00	14.38	15.55
	14	14.74	16.26	17.92	16.09	16.97
	21	18.89	18.97	20.93	18.09	19.88
AAN/TN (%)	Fresh	3.10	3.31	3.31	3.30	3.30
	7	3.45	1.64	4.00	3.56	3.98
	14	3.68	3.74	5.24	3.62	4.73
	21	3.95	3.92	5.46	4.02	5.38

Table (3): The effect of partial or total substitution of yoghurt starter culture with probiotic bacteria on the total volatile fatty acids (TVFA) of white soft cheese during storage up to 21 days at 5-7 °C.

Storage period (days)	Control cheese	Cheese containing <i>Bifidobacterium bifidum</i>		Cheese containing <i>Bifidobacterium breve</i>	
		50%	100%	50%	100%
Fresh	9.80	10.20	11.60	10.40	11.20
7	13.20	13.40	15.40	13.40	14.40
14	15.60	16.00	16.80	15.80	16.40
21	18.40	18.60	19.80	18.60	19.00

Table (4): The survival of Bifidobacteria in white soft cheese during storage up to 21 days at 5 -7 ° C. (x 10⁶ cfu/g cheese).

Bifidobacteria stain	Bifidiobacteria in the starter	Storage period (days)			
		Fresh	7	14	21
<i>Bifidobacterium bifidum</i>	50%	80	71	20	17
	100%	98	89	25	20
<i>Bifidobacterium breve</i>	50%	60	26	23	13
	100%	112	39	32	21

Table (5): The effect of partial or total substitution of yoghurt starter culture with probiotic bacteria on the organoleptic properties (total scores) of white soft cheese during storage up to 21 days at 5 -7 ° C.

Storage period (days)	Control	Cheese containing <i>Bifidobacterium bifidum</i>		Cheese containing <i>Bifidobacterium breve</i>	
		50%	100%	50%	100%
Fresh	80.50	89.00	88.20	90.00	90.20
7	91.40	92.20	91.40	90.20	88.00
14	92.25	93.50	92.00	89.25	85.00
21	89.50	91.50	87.50	89.50	83.50

الخواص التركيبية وتسوية الجبن الأبيض الطرى المصنع
باستخدام البكتريا الحيوية

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فى هذا البحث تم دراسة تأثير سلالتين من البكتريا الحيوية *Bif. bifidum* و *Bif. breve* وأيضا مزرعة من باديئ اليوجورت المحتوية على ميكروبات *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus*. على الخواص التركيبية وتسوية الجبن الأبيض الطرى.

لوحظ عدم وجود اختلاف فى التركيب الكيماوى بين الجبن التى تحتوى على البكتريا الحيوية والجبن التى تحتوى على باديئ اليوجورت فيما عدا الحموضة حيث لوحظ ارتفاع نسبى فى حموضة الجبن المحتوى على البكتريا الحيوية.

بالنسبة لشقوق النيتروجين (النيتروجين الذائب، والنيتروجين الغير بروتينى ونيتروجين الأحماض الأمينية) وكذلك الأحماض الدهنية الطيارة وجد أن استخدام البكتريا الحيوية ادى الى ارتفاع هذه القيم مقارنة مع عينات الكونتروول.

إلا أن نتائج التحكيم الحسى أشارت إلى تقارب كبير بين درجات التحكيم التى حصلت عليها الجبن المحتوية على البكتريا الحيوية وجبن الكونتروول.

ومن ذلك يمكن القول أنه يمكن تصنيع جبن أبيض طرى حيوى بإضافة البكتريا الحيوية للبن المعد لصناعة الجبن بدلا من باديئ اليوجورت للاستفادة من مزاياها الصحية دون تأثير غير مرغوب على خواص الجودة للجبن.