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Brevibacterium linens AS AN ADJUNCT STARTER CULTURE IN LOW FAT RAS CHEESE

BY

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ABSTRACT

The aim of this study was to improve the quality of low fat Ras cheese *via* the application of adjunct starter culture.

Ras cheeses were made from cow's milk standardized to 1.0, 1.5, 2.0, 2.5 and 4.0% fat content. The fat level (4%) corresponds to the control. All milk batches were divided into two portions. The first portion of all batches was manufactured into Ras cheese using yoghurt starter culture. *Brevibacterium linens* was further added to cheese milks at the level of 0.5% in the second portion.

The obtained results reveal that, both contents of the dry matter (DM) and protein (total nitrogen, TN×6.38) of cheese increased as the fat content of cheesemilk decreased. The use *B. linens* was associated with increasing in the DM, protein, fat/DM contents of cheese, those also raised gradually along maturation period for 24 weeks. The water soluble nitrogen, non protein nitrogen, soluble tyrosine, soluble tryptophan, total volatile fatty acids and titratable acidty (TA) contents of cheese decreased as the fat content of cheesemilk was reduced, but they were raised when *B. linens* was used. While pH value was not affected either by lowering the fat content, or using *B. linens*, which did not significantly influence the TA% of cheese. The counts of total bacteria, lactic acid bacteria, proteolytic bacteria and lipolytic bacteria declined as the fat content decreased. On the contrary, those bacterial groups were higher when *B. linens* was applied. In all cases the bacterial counts were proportionally increased during maturation period until the 12th week and then gradually decreased by prolonging the ripening period theremore. Chalky appearance and crumbly texture were obviously noticed with the excessive reduction in the fat content more than 2.5%. The sensory quality, however, improved in the presence of *B. linens* even in the cheese made from milk containing 1%fat.

Finally, a palatable low fat Ras cheese could successfully made from low fat milk using *B. linens* as an adjunct starter culture.

Key words: adjunct starter culture, *Brevibacterium linens,* ripening indices, bacterial counts, sensory evaluation.

INTRODUCTION

The value of milk fat has been put in doubt on grounds of health considerations (Schelhaas, 1989). Milk fat has been identified as hypercholesterolemic because it contains cholesterol and is primarily saturated (Ney, 1991). Moreover high fat intake is associated with increased risk for obesity, some types of cancer, liver and heart diseases (Williams, 1985 and Akoh, 1998). Besides, because of the awareness of adverse effects of excessive dietary fat intake, many consumers are modifying their dietary habits (Badawi, 1998 and O'Connor and O' Brien 2000).

Technically, milk fat plays several important functions in cheesemaking affecting cheese firmness, adhesiveness, mouthfeel and also an important flavour ingredient whose partial or total removal causes major sensory problems (Mann, 2000 and O'Connor and O' Brien 2000) . Besides its significant partnership in the formation of the products consistency, the buttery flavour associated with milk fat contributes to the richness of cheese flavour and it plays crucial roles in developing the flavour perception, flavour stability, flavour generation and the overall sensation of dairy products (Anderson and Mistry, 1994; Giese, 1996; de Rose, 1997; Akoh, 1998 and Hussein, 2000). Whereas, the majority of flavour components are dissolved to some extent in the lipid phases of food, fat governs to release the flavour slowly in the mouth and to result in a pleasant aftertaste (Labell, 1991 and Ohmes, 1998). That means, as the fat provides mouthfeel and richness, it serves also as a reservoir of flavour. Thereby, removing any significant amount of fat (above 25%) from a product changes the flavour profile, in dairy products. (Furia and Bellanca, 1990; Hatchwell, 1994 and Ohmes *et al.*, 1998).

Some technical trials depending on the modification of some processing steps of cheesemaking for some varieties of semihard or hard cheeses other than Ras cheese were described by Olson and Johnson (1990), Rosenberg (1992) and Davide (1994). Locally, certain attempts were carried out for improving the quality of low fat Ras cheese involving the heat treatment of cheesemilk, application of fat replacers or the utilization of attenuated starter culture (El-Neshawy *et al*, 1986; Mahmoud, 1995; Khader *et al*, 1995; Kebary *et al*, 1996; Salem, 1998; Hussein, 2000 and Khalil, 2003). However, *Brevibacterium linens* was successfully used as adjunct starter culture in the manufacture of reduced fat Edam and Cheddar cheeses for improving their qualities (Broadbent *et al.*, 1997; Ummadi and Weimer, 2001 and Tungjaroeonchai *et al.*, 2001). For that in view, the present research was conducted to study the effect of the utilization of such strains as adjunct culture on the properties of low fat Ras cheese.

MATERIALS AND METHODS

Materials

Fresh cow's milk was obtained from the herd of the dairy cattle at Faculty of Agriculture, Ain Shams University. A commercial yoghurt starter cultures (*Streptococcus thermophilus* + *Lactobacillus delbruckii ssp. bulgaricus* was obtained from Wiesby GmbH and Co. KG. Niebull, Germany. *Brevibacterium*

linens (ATCC 9172) was obtained from Egypt Microbial Culture Collection (EMCC) at Microbiological Resources Center, Cairo (MIRCEN), Faculty of Agriculture, Ain Shams University. Calf rennet powder (Ha-La) was obtained from CHR- Hansen's Lab. Denmark. Fine cooking salt produced by El-Nasr Saline's Co. was obtained from the local market. Plastic coat (CESKA, 0.05% Natamaycin – yellow) produced by CSK Food Enrichment, B. V., Holland was obtained from the Egyptian Office for Trading and Agencies (*eta*), Cairo, Egypt.

Experimental procedure

The manufacture procedure described by Hofi *et al.* (1970) and enacted by EOSQC (2001) was applied for Ras cheesemaking from low fat cow's milk containing 1.0, 1.5, 2.0, or 2.5% fat. Cheese milks were firstly heat treated at (72°C/15sec) and cooled immediately to 35°C, at which the milks were inoculated with 1% of activated yoghurt starter culture for milk ripening through 30 min. Then, every treatment was divided into two portions. The first one was further inoculated with 0.5% *Brevibacterium linens* as adjunct culture, while the second portion was made without adjunct culture and corresponding as controls for the analogous fat contents of the first portion. Moreover, a general control Ras cheese (full cream) was made from cow's milk containing 4.0% fat using the foregoing manufacturing procedure but without adjunct starter culture. Milks were renneted using predissolved rennet powder at the level of 3.0g/100 L. milk. Cheese was sampled for analyses when fresh and during ripening for 2, 4, 8, 12, 16, 20 and 24 weeks at 12±1°C and 85% relative humidity. Three replicates were done for every treatment.

Analytical methods

Dry matter (DM) content was determined according to AOAC (1998). Fat (F), total nitrogen (TN), water soluble nitrogen (WSN), non protein nitrogen (NPN), and titratable acidity (TA) contents were determined according to Ling (1963). The pH value was measured using Lab. pH–meter, Hanna model 8417 digital pH meter. Soluble tyrosine and soluble tryptophan contents of cheese were determined spectrophotometrically according to the method of Vakaleris and Price (1959). Total volatile fatty acids (TVFA) were determined by the distillation method described by Kosikowski (1977).

The total bacterial count (TBC) was enumerated according to Houghtby *et al.* (1993) using media Plate Count Agar at 32C for 48h. Lactic acid bacterial count (LABC) was enumerated by the conventional dilution pouring plate as described by De Man *et al.* (1960) using De Man Rogosa Sharp agar media at 30°C for 2-3 days. Proteolytic bacterial count (PBC) was examined using skim milk agar as described by Frank *et al.* (1993) at 30°C for 72h. Lipolytic bacterial count (LBC) was determined according to Harrigan (1998) using Victoria Blue butter fat Agar (VBBA). All plates were incubated at 30C for 7days.

Cheese samples from the beginning of the 8th week of ripening were organoleptically judged by panel members of experts at the Food Science Department, Faculty of Agriculture, Ain Shams University. Organoleptic properties of cheese samples were carried out using the figures of El-Koussy (1966), which were 50 points for flavour, 40 points for body and texture and 10 points for general appearance.

The obtained data were statistically analyzed according to statistical analysis system user's Guide, (SAS, 1996).

RESULTS AND DISCUSSION

1. Gross composition

Data in Tables (1-3) reveal that, the contents of dry matter (DM) and protein (TN×6.38) of cheese increased proportionally as the fat content of starting cheesemilk decreased and /or as the ripening period prolonged ($p < 0.001$), i.e. the relatively high level of fat allowed the resultant Ras cheese to hold more moisture and *vice versa*. Similar findings were reported by El- Neshawy *et al.* (1986); Mahmoud (1995); Khader *et al.* (1995); Kebary *et al.* (1996); Badawi (1998) and Hussein (2000). These results could be attributed to that the fat present in milk, reduced the whey syneresis from cheese curd due to the elimination of whey from it through its thinnest capillaries in which the fat globules are situated. That, because of the great number of globules, which hindered the whey flow (Dimov and Mineva 1962). On the other hand, Marshall (1982) and Storry *et al.* (1983) reported that, increasing fat content might increase the number of interstices within the network which are accupied by fat globules thus leading to increased impediment of whey drainage. The use of *B. linens* did not lead to any significant differences ($P > 0.05$) in the dry matter content of low fat Ras cheese. Similar findings were reported by Osman (2003). However, the increase rate in both content of protein and F /DM of cheese during ripening was higher in the case of using *B. linens* in cheesemaking (Tables, 1-3). These apparent increases could be ascribed to the volatile components, those formed during cheese ripening, and rather when *B. linens* was used, being able to evaporate through the determination of dry matter content in oven at 105°C for about 4h.and hence to alter the F/DM ratio in the direction of the former.

Table (1): Dry matter content of low fat Ras cheese during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0		2.5		2.0		1.5		1.0
		C	B	C	B	C	B	C	B
0	56.53	59.41	59.96	60.30	60.27	61.52	61.56	62.65	65.67
2	57.67	60.5	60.34	61.37	61.30	62.44	62.43	63.12	63.20
4	58.72	61.47	61.42	62.63	62.24	63.47	63.61	64.03	64.51
8	60.70	63.92	63.82	64.13	64.02	65.21	65.24	65.80	65.98
12	63.24	65.75	65.35	66.43	66.19	67.57	67.17	68.82	67.85
16	64.88	67.29	67.24	68.43	68.45	68.92	68.03	69.53	69.56
20	65.7	68.45	68.34	69.00	69.18	69.44	69.16	70.24	69.99
24	66.86	69.13	69.16	69.85	69.78	70.76	69.64	71.24	71.00

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

Table (2): Fat / dry matter content of low fat Ras cheese during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
0	47.40	34.68	34.40	23.88	23.81	18.47	18.39	12.32	11.80
2	48.93	35.88	36.29	24.71	25.04	19.17	19.22	12.88	12.66
4	49.42	36.32	36.99	25.17	25.34	19.59	19.24	13.19	12.94
8	49.78	36.37	37.29	25.29	26.55	20.30	20.67	13.66	14.08
12	50.00	36.55	37.48	25.40	26.11	21.06	21.26	14.13	14.93
16	50.29	36.84	36.88	25.95	26.60	21.35	22.07	14.29	14.50
20	50.32	36.93	37.60	25.97	26.55	21.63	22.00	14.44	14.70
24	50.53	37.45	37.59	26.13	26.94	21.81	22.31	14.53	14.59

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

Table (3): Total nitrogen × 6.38 content of low fat Ras cheese during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
0	22.33	28.26	28.20	30.30	30.18	32.35	32.22	34.38	34.26
2	24.18	30.62	30.69	32.86	32.79	32.02	35.02	37.26	37.45
4	25.77	32.62	32.79	35.02	34.90	37.32	37.64	39.74	39.94
8	26.28	33.30	33.37	35.73	35.66	38.02	38.15	40.51	40.64
12	27.00	34.19	34.39	36.67	36.68	39.04	39.17	41.66	41.85
16	27.43	34.71	34.89	37.26	37.20	39.68	39.88	42.30	42.55
20	27.88	35.22	35.35	37.83	37.89	40.32	40.57	42.94	43.19
24	28.33	35.73	35.79	38.41	38.47	40.96	41.09	43.58	43.70

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

2. Ripening indices

A reduction rate in all ripening indices, expressed as WSN/TN, NPN/TN, soluble tyrosine, soluble tryptophan, TVFA contents (Tables 4-8) of Ras cheese was recorded among the descending levels of the fat content ($p < 0.001$). This indicates, that reducing the fat content of cheese milk led to delay the proteolysis rate and hence a decrease in the WSN, NPN, soluble tyrosine and soluble tryptophan released during cheese ripening ($p<0.001$). These findings are in agreement with those reported by El- Neshawy *et al.* (1986); Mahmoud (1995); Khader *et al.* (1995); Kebary *et al.* (1996); Badawi (1998); Hussein (2000) and Khalil (2003). Moreover, Ohran and Tucky (1969) reported that, a fat in dry matter content in the cheese of more than 50% is required for development of true Cheddar flavour. While, data showed that, the increased rate of foregoing ripening indices were higher in the presence of *B. linens* in cheese ($p < 0.001$).

Similar observations were found by Ades and Cone (1969) in a semi soft surface - ripened cheese (Trappist – type); Leclercq-Perlat *et al.* (2000) in soft smear cheese; Ummadi and Weimer (2001) in reduced fat Cheddar cheese and Osman (2003) in full cream Ras cheese. Neither the lowering of the fat content nor the employing of *B. linens* led to any significant differences in the reduction rate caused in the pH values during cheese ripening ($p>0.05$). While the increment trend of TA% of cheese decreased as the fat content reduced and was not affected by the application of *B. linens* as adjunct starter culture in cheesemaking (Tables, 9-10). Likewise, Osman (2003) reported that, the addition of *B. linens* in Ras cheese had no significant effect on the acidity content.

Table (4): Water soluble nitrogen (WSN) / TN content of low fat Ras cheese during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
0	6.9	5.12	5.42	4.69	4.86	4.14	4.36	3.71	3.72
2	7.12	5.41	9.98	4.85	9.14	4.19	8.19	3.76	7.50
4	13.86	10.54	14.01	9.47	12.9	8.20	10.68	7.38	9.58
8	17.47	13.21	22.17	11.78	20.04	10.40	18.06	9.29	16.01
12	24.11	18.28	23.56	16.34	21.22	14.21	18.72	12.55	16.92
16	25.34	19.11	24.86	17.12	22.30	14.95	20.84	13.27	17.84
20	32.72	24.63	30.81	22.09	28.40	19.30	26.44	17.08	24.02
24	34.45	25.71	32.92	22.75	30.71	20.56	28.27	18.30	26.97

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

Table (5): Non protein nitrogen (NPN) / TN content of low fat Ras cheese during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
0	5.14	3.99	4.28	3.64	3.82	3.27	3.40	3.00	3.13
2	6.33	4.89	6.15	4.52	5.48	4.00	4.76	3.59	4.35
4	7.67	5.85	7.80	5.28	6.73	4.61	6.06	4.17	5.52
8	9.22	7.08	10.23	6.42	9.51	5.70	8.72	5.03	8.05
12	11.34	8.76	13.42	7.82	11.48	7.02	10.93	6.12	10.12
16	12.62	9.82	15.69	9.04	14.19	8.23	12.38	7.33	11.48
20	13.67	10.87	16.94	10.09	16.11	9.27	14.61	8.38	13.72
24	14.71	11.92	17.85	11.13	17.00	10.32	15.82	9.44	14.24

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

Table (6): Soluble tyrosine content of low fat Ras cheese (mg /100g cheese) during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
0	30.6	29.9	30.21	29.3	30.00	28.65	29.50	28.00	28.21
2	46.85	45.34	58.18	44.43	56.67	43.45	53.79	42.46	52.30
4	64.86	62.77	90.74	61.51	87.48	60.15	81.12	58.78	78.75
8	81.51	78.88	128.03	77.30	114.93	75.59	100.94	73.86	97.95
12	109.38	105.87	136.05	103.75	130.55	101.45	120.47	99.12	115.38
16	132.25	127.8	150.82	125.44	142.46	122.66	137.19	119.84	133.43
20	141.52	136.76	159.13	134.00	148.90	131.26	143.16	128.24	140.14
24	156.73	151.46	175.58	148.40	167.32	145.37	160.53	142.03	150.95

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

Table (7): Soluble tryptophan content of low fat Ras cheese (mg /100g cheese) during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
0	20.60	19.51	20.00	18.65	19.23	17.50	17.95	17.22	17.52
2	26.04	24.00	45.51	23.49	43.45	22.10	40.06	20.84	38.91
4	40.59	37.41	66.34	36.61	62.25	34.44	95.30	32.48	55.34
8	63.40	58.43	96.46	57.18	92.58	53.79	85.19	50.73	63.13
12	84.54	77.91	103.42	76.25	99.50	71.73	94.98	67.65	71.91
16	96.98	89.36	111.93	87.47	107.24	82.28	100.05	77.60	87.37
20	104.38	96.18	117.35	94.15	113.00	88.56	110.41	83.52	96.21
24	111.05	102.32	126.67	100.16	120.81	94.22	117.51	88.85	100.50

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

Table (8): Total volatile fatty Acids (TVFA) content of low fat Ras cheese (ml 0.1N NaOH /100 g cheese) during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
0	16.29	14.61	14.30	14.06	13.91	13.13	13.14	12.20	12.72
2	31.70	28.43	43.15	27.36	40.08	25.55	39.33	23.74	37.40
4	41.59	37.30	58.80	35.90	57.17	33.52	54.69	31.06	50.12
8	56.85	50.99	67.35	49.07	94.58	45.82	60.33	42.46	58.17
12	74.11	66.47	82.00	63.97	79.43	59.73	74.19	55.35	70.81
16	87.42	78.41	103.41	75.46	99.55	70.46	95.43	65.29	90.22
20	100.46	90.11	113.62	86.72	109.23	80.97	100.47	79.03	97.36
24	105.68	94.79	120.31	91.23	115.73	85.18	108.22	83.14	101.30

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

Table (9): Titratable acidity (TA)percent of low fat Ras cheese during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
0	0.87	0.75	0.89	0.65	0.79	0.56	0.70	0.46	0.95
2	1.39	1.24	1.38	1.15	1.27	1.03	1.19	0.90	1.15
4	1.47	1.32	1.42	1.22	1.38	1.19	1.27	0.96	1.20
8	1.52	1.47	1.57	1.30	1.45	1.26	1.36	1.10	1.25
12	1.73	1.58	1.69	1.40	1.51	1.25	1.39	1.18	1.30
16	1.81	1.66	1.78	1.57	1.68	1.32	1.46	1.24	1.36
20	2.05	1.80	1.93	1.61	1.76	1.45	1.59	1.37	1.43
24	2.23	2.08	2.5	1.88	1.95	1.61	1.77	1.53	1.60

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

Table (10): pH value of low fat Ras cheese during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
0	5.52	5.59	5.48	5.61	5.57	5.68	5.59	5.74	5.63
2	5.54	5.51	5.40	5.56	5.49	5.60	5.51	5.66	5.58
4	5.34	5.42	5.35	5.50	5.36	5.52	5.39	5.58	5.43
8	5.24	5.30	5.21	5.36	5.25	5.38	5.27	5.30	5.32
12	5.17	5.19	5.09	5.23	5.17	5.25	5.18	5.27	5.20
16	5.03	5.10	5.00	5.14	5.06	5.16	5.08	5.22	5.10
20	4.94	5.01	4.79	5.05	4.82	5.07	4.95	5.13	5.03
24	4.82	4.89	4.71	5.00	4.75	5.03	4.84	5.11	4.95

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

3. Microbiological properties

Data in Tables (11-14) indicate that the reduction in the fat content of Ras cheesemilk was associated with proportional reduction in the counts enumerated in the resultant cheese of TBC, LABC, PBC and LBC. All bacterial counts were increased by prolonging the cheese ripening period until the 12th week then declined gradually uptill the end of the experimental period (24weeks). The statistical analysis confirmed that, the adjunct starter culture of *B. linens* promoted the growing of all monitored bacterial kinds. Similar observations were reported by Mahmoud (1995) and Salem (1998).

Table (11): Total viable bacterial counts (CFU $\times 10^6$/g) of low fat Ras cheese during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
0	3.1	2.7	2.9	2.6	2.7	2.3	2.5	2.2	2.4
2	4.15	3.47	4.7	3.18	4.2	2.81	3.7	2.69	3.00
4	5.2	4.24	5.6	3.75	5.4	3.31	4.6	3.17	4.1
8	5.9	5.58	6.7	5.00	6.1	4.41	5.9	4.22	5.3
12	8.4	7.11	8.3	6.45	7.5	5.68	6.7	5.44	6.2
16	7.5	6.11	8.00	5.75	6.9	5.06	6.1	4.84	5.3
20	5.35	4.99	5.8	4.26	5.00	3.74	4.2	3.58	3.9
24	3.6	2.59	3.9	2.17	3.5	1.90	3.00	1.82	2.7

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

Table (12): Lactic acid bacteria counts (CFU $\times 10^6$/g) of low fat Ras cheese during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
0	1.86	1.51	1.50	1.41	1.43	1.11	1.5	1.01	1.00
2	2.52	1.94	1.95	1.72	1.70	1.35	1.35	1.21	1.22
4	3.17	2.37	2.34	2.03	2.06	1.60	1.61	1.40	1.41
8	3.59	3.11	3.1	2.70	2.72	2.10	2.15	1.80	1.83
12	5.11	3.96	3.93	3.47	3.41	2.70	2.72	2.30	2.31
16	4.50	3.96	3.90	3.09	3.08	2.40	2.43	2.05	2.09
20	3.21	2.77	2.77	2.28	2.29	1.77	1.77	1.51	1.52
24	2.16	1.43	1.45	1.16	1.17	0.90	0.94	0.76	0.76

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

Table (13): Proteolytic bacterial counts (CFU $\times 10^3$/g) of low fat Ras cheese during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
0	1.25	1.0	1.1	0.8	0.7	0.5	0.6	0.4	0.5
2	1.59	1.30	1.9	1.10	1.6	0.65	1.3	0.55	1.00
4	1.92	1.60	2.3	1.40	2.00	0.8	1.8	0.7	1.6
8	2.68	2.30	3.1	2.01	2.8	1.15	2.6	1.00	2.3
12	4.02	3.49	3.9	2.18	3.5	1.25	3.0	1.08	2.8
16	3.35	2.80	3.2	1.65	2.4	0.85	2.1	0.82	2.00
20	1.91	1.52	1.9	1.04	1.4	0.6	1.2	0.5	1.1
24	0.66	0.42	1.00	0.26	0.9	0.15	0.8	0.12	0.6

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

Table (14): Lipolytic bacterial counts (CFU $\times 10^3$ /g) of low fat Ras cheese during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2		1.5		1	
		C	B	C	B	C	B	C	B
0	0.35	0.27	0.3	0.25	0.24	0.15	0.16	0.13	0.14
2	0.92	0.72	0.9	0.62	0.8	0.52	0.7	0.49	0.7
4	1.48	1.17	1.3	1.06	1.1	0.9	0.95	0.84	0.93
8	2.09	1.91	2.1	1.71	1.9	1.17	1.6	1.09	1.5
12	3.47	2.88	3.0	2.44	2.8	1.92	2.4	1.89	2.3
16	1.73	1.63	2.1	1.47	2.00	0.82	1.8	0.73	1.6
20	0.69	0.28	1.0	0.49	0.9	0.72	0.7	0.24	0.6
24	0.39	0.18	0.8	0.3	0.7	0.10	0.5	0.19	0.4

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

4- Organoleptic quality

Sensory scores listed in Table (15) show that, the cheese appearance was negatively influenced by lowering the fat content than 2.5%, but improved from the beginning of the 12th week of ripening period. The mean defect observed in this respect was the chalky appearance, which fluctuated in its degree from "slight" to "observed" gradually, in order, according to the reduction rate in the fat content. Likewise the body and texture of cheese was also relatively harmed by reducing the fat content. The crumbly texture was the most pronounced defect in this respect. The cheese consistency was enhanced i.e. the crumbly defect had relatively trended to disappear by prolonging the ripening period. Similarly, cheese palatability was also negatively influenced by the reduction in the fat content. Gradually pronounced flatness was the chief defect observed in cheese to be associated with the decrease in the fat content. However, gradual increase in the flavour score of Ras cheese was recorded being positively depending on the cheese age. The trends of the organoleptic results whether of the effect of fat content or of ripening period are in agreement with those found by El- Neshawy *et al.* (1986); Mahmoud (1995); Khader *et al.* (1995); Kebary *et al.* (1996); Taha (1997); Badawi (1998); Hussein (2000) and Khalil (2003). The use of *B. linens* as adjunct starter culture improved significantly ($p<0.001$) all sensory criteria tested whether separately or as a total especially at the lower fat levels, so that the removing of 75% of the milk fat (to be 1% in the cheese milk) led to loss only 15% of the total sensory score (i.e. it was gained 85 out of 100 degree as a total) of resultant Ras cheese when *B. linens* was used compared with 67% of that made from milk containing the same level of the fat content but without adjunct starter culture. Similar findings were reported for reduced fat Cheddar cheese by Broadbent *et al.* (1997) and EL- Soda *et al.* (2000).

Finally, the foregoing results led evidently to conclude that, a palatable low fat Ras cheese could successfully be made from low fat milk with the use of *B. linens* as an adjunct starter culture.

Table (15): Organoleptic scores of low fat Ras cheese during ripening period (RP) as a function of the use of *Brevibacterium linens* as an adjunct starter culture

RP per week	Fat content of cheese milk %								
	4.0	2.5		2.0		1.5		1.0	
		C	B	C	B	C	B	C	B
Appearance score (out of 10 points)									
8	10	9	9	8	8	7	7	7	7
12	10	10	10	9	9	8	8	8	8
16	10	10	10	9	9	8	8	8	8
20	10	10	10	9	9	8	8	8	8
24	10	10	10	9	9	8	8	8	8
Body and Texture scores (out of 40 point)									
8	35	34	35	33	33	27	27	25	25
12	36	35	36	35	35	28	29	26	26
16	37	38	38	37	37	29	30	26	27
20	38	38	38	37	37	30	31	26	27
24	39	38	38	37	37	31	31	26	27
Flavour scores (out of 50 points)									
8	45	42	48	41	48	30	48	29	48
12	50	50	50	45	50	31	50	30	50
16	50	50	50	50	50	32	50	31	50
20	50	50	50	50	50	33	50	32	50
24	50	50	50	50	50	34	50	33	50
Total scores (out of 100 points)									
8	90	85	92	82	89	64	82	61	80
12	96	95	96	89	95	67	87	64	84
16	97	98	98	96	96	69	88	65	85
20	98	98	98	96	96	71	89	66	85
24	99	98	98	96	96	73	89	67	85

C: Without adjunct cultures (corresponding as control of each certain fat level)
B: Made using *Brevibacterium linens* at the level of 0.5%

REFERENCES

Ades, G. L. and Cone, J. F. (1969): Proteolytic activity of *Brevibacterium linens* during ripening of Trappist-Type cheese. J. Dairy Sci. 52: 957- 961.

A.O.A.C., (1998): Association of Official Analytical Chemists. Official Methods of Analysis. Washington, D.C., USA.

Akoh, C. (1998): Fat replacers. Food Tech., 52: 47-53.

Anderson, D.L. and Mistry, V. V. (1994): Reduced fat Cheddar cheese from condensed milk. 2- Microstructure. J. Dairy Sci., 77: 7-15.

Badawi, R. M. (1998): Effect of fat mimetics on low-fat Ras cheese quality. Menofiya J. Agric. Res., 23: 1601-1618.

Broadbent, J.; Brennand, C.; Jonson, M.; Steele, J.; Strickland, M. and Weimer, B. (1997): Starter contribution to reduced fat Cheddar. Dairy Indust. Int. 62: 2, 35, 37, 39.

Davide, C. L. (1994): New direction in low-fat dairy products processing towards better health. The Philippine Agriculturist, 77:1-16.

De Man, J. C.; Regosa, M. and Sharpe, M. E. (1960): A medium for cultivation of lactobacilli. J. Appl. Bacteriol. 22: 130-133.

de Roos, K. B. (1997): How lipids influence food flavour. Food Tech., 51: 60-62.

Dimov, N. D. and Mineva, P. (1962): Some factors influencing the syneresis of fresh curd and the loss of dry matter in the whey of cows, buffaloes and sheeps milks. 16th Int. Dairy Congr. B, IV: 817- 822.

EL-Koussy, L. A. (1966): Studies on soft cheese manufactured from pasteurized milk. Ph. D. Thesis, Fac. of Agric., Ain- Shams Univ.Egypt.

EL-Neshawy, A.A.; Abdel Baky, A.A.; Rabie, A.M. and Ashour, M.M. (1986): An attempt to produce low fat Cephalotyre (Ras) cheese of acceptable quality. Food Chem, 22: 123-137.

EL-Soda, M.; Madkor, S. A. and Tong, P. S. (2000): Adjunct cultures: recent developments and potential significance to the cheese industry. J. Dairy Sci. 83: 609-619.

E.O.S.Q.C: Egyptian Organization for Standardization and Quality Control, (2001): Hard cheese Part 5: Ras cheese, Egyptian Standard (ES) 1007 / 2001.

Franc, J.F.; Christen, G.L. and Bullerman, L.B. (1993): Tests for groups of microorganisms. In: Standard Method for the Examination of Dairy products (16th Ed.), R. T. Marshal (Ed.), American Public Health Association, Washington, D. C., USA. pp. 213-246.

Furia, T.E. and Bellanca, N. (1990): "Fenarali's handbook of flavour ingredients". Vol. I and II, 2 nd Ed., CRC press, Inc., Boca Raton, Fla.

Giese, J. (1996): Fats, oils and fat replacers. Food Tech., 50: 78- 84.

Harrigan, W. F. (1998): Laboratory Method in Food Microbiology. Academic press. London. UK. pp. 111-112.

Hatchwell, L. C. (1994): Overcoming flavor challenges in low- frozen dessert. Food Tech., 48: 98-100, 102.

Hofi, A. A.; Youssef, E.H.; Ghoneim, M.A. and Tawab, G.A. (1970): Ripening changes in Cephalotyre (Ras) cheese manufactured from raw and pasteurized milk with special reference to flavor. J. Dairy Sci., 53: 1207 –1211.

Houghtby, G.A.; Maturin, L.J. and Koenig, E.K. (1993): Microbiological count methods. In: Standard Method for the Examination of Dairy products (16th Ed.),

Marshal, R. T. (Ed.), American Public Health Association, Washington, D. C., USA. pp. 213-246

Hussein, S. A. (2000): Ripening acceleration of low fat Ras cheese made by adding fat replacers. Minofiya J. Agric. Res., 25: 427-444.

Kebary, K.M.K.; Khader, A.E.; Zedan, A.N. and Mahmoud, S.F. (1996): Accelerated ripening of low fat cheese by attenuated Lactobacilli cells. Food Res. Int., 29: 705-713.

Khader, A.E.; Zedan, A.N.; Kebary, K.M.K. and Mahmoud, S.F. (1995): Quality of low fat Ras cheese made from heat treated milk. Proc. 6th Egypt. Conf. Dairy Sci & Tech., 184-216.

Khalil, R.E.M.A. (2003): Acceleration ripening of low fat Ras cheese. M.Sc.Thesis, Fac. of Agric., Suez Canal Univ., Egypt.

Kosikowski, F. V. (1977): Cheese and fermented milk foods.2nd Ed., publ. by. F. V Kosikowski and Assoc., Brooktondale, New York, USA. pp. 573-574.

Labell, F. (1991): Flavour designed for low-fat or no-fat frozen dessert. Food Process., 52: 94-97.

Leclercq-Perlat, M. N.; Oumer, A.; Buono, F.; Bergere, J. L.; Spinnler, H. E. and Corrieu, G. (2000): Behavior of *Brevibacterium linens* and *Debaryomyces hansenii* as ripening flora in controlled production of soft smear cheese from reconstituted milk: Protein Degradation. J. Dairy Sci., 83: 1674 – 1683.

Ling, E.R. (1963): A Text Book Dairy Chemistry Vol. 2. practical 3rd Ed., Chapman and Hall. Ltd London, UK.

Mahmoud, S. F. (1995): Studies on low fat cheese. M.SC. Thesis, Fac. of Agric. Menofiya Univ. Egypt.

Mann, E. (2000): Low fat cheese. Dairy Indust. Int., 65:19-20.

Marshall, R. J. (1982): An improved method for measurement of the syneresis of curd formed by rennet action on milk J. Dairy Res. 49: 329 – 336.

Ney, D.M. (1991): Potential for enhancing the nutritional properties of milk fat. J. Dairy Sci., 74: 4002-4012.

O'Connor, T.P. and O'Brien, N.M. (2000): Nutritional Aspects of Cheese In Fundamentals of Cheese Science, Ed., by Fox, P. F., Guinee, T. P., Cogan, T. M. and Mc Sweeney, P. L., An Aspen publ., Gaithersburg, Maryland, USA. pp. 504-512.

Ohmes, R.L.; Marshall, R.T. and Heymann, H. (1998): Sensory and physical properties of ice creams containing milk fat or fat replacers. J. Dairy Sci., 81: 1222-1228.

Ohran, J.A. and Tuckey, S.L. (1969): Relation of flavour development in Cheddar cheese to chemical changes in the fat of the cheese. J. Dairy Sci., 52: 589 - 609

Olson, N.F. and Johnson, M.E. (1990): Light cheese products: Characteristics and economics. Food Tech., 44: 93-96.

Osman, M.M. (2003): Acceleration of the ripening and flavour development of Ras cheese using *Brevibacterium linens*. Egypt. J. Dairy Sci., 31: 159 – 172.

Rosenberg, M. (1992): Cheese: the toughest low fat challenge. Dairy Foods 39: 44,48.

Salem, E.L.M. (1998): Use of starters in the acceleration of low fat Ras cheese ripening. Ph.D. Thesis, Fac. of Agric. Cairo Univ. Egypt.

SAS, (1996): Statistical Analysis System. SAS user s Guid : Statistics SAS Inst. Inc. Ed., Cary, Nc. USA.

Schelhass, H. (1989): Dairying in the nineties. IDF Bulletin 243:36 Int. Dairy Federation, Belguim.

Storry, J.E.; Grandison, A.S.; Millared, D.; Owen, J.A. and Ford, G.D. (1983): Chemical composition and coagulating properties of renneted milks from different breeds and species of ruminant. J. Dairy Res. 50: 215 – 229.

Taha, S. H. (1997): An attempt to improve the quality of low fat Domitti and Ras cheese. Annals of Agric. Sci., Moshtohor, 35: 833-845.

Tungjaroenchai, W.; Drake, M.A. and White, C.H. (2001): Influence of adjunct cultures on ripening of reduced fat Edam cheeses. J. Dairy Sci., 84: 2117-2124.

Ummadi, M. and Weimer, B.C. (2001): Tryptophan catabolism in *Brevibacterium linens* as a potential cheese flavour adjunct. J. Dairy Sci., 84: 1773- 1782.

Vakaleris, D.G. and Price, W.V. (1959): A rapid spectrophotometric method for measuring cheese ripening. J. Dairy Sci. 42: 264 – 276.

Williams, S.R. (1985): Nutrition and Diet therapy. Times Mirror Mosluy, College Publ., St. Louis, Toronto and Santa Clara.

استخدام الـ *Brevibacterium linens* كبادئ مساعد في صناعة الجبن الرأس منخفضة الدهن

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تستهدف الدراسة إلى تحسين جودة الجبن الرأس منخفضة الدهن وذلك باستخدام بادئ مساعد.

ولإنتاج جبن رأس منخفضة الدهن استخدم لبن بقري يحتوي على ١، ١,٥ ، ٢ ،٢,٥، ٤% دهن (كنترول). ولقد تم تقسيم اللبن في كل نسبة دهن إلى قسمين في القسم الأول تم تصنيع جبن رأس تقليديا باستخدام بادئ الزبادي لتسوية اللبن بينما في القسم الثاني تم إضافة بادئ *Brevibacterium linens* كبادئ مساعد بنسبة ٥٠% بالإضافة لبادئ الزبادي التقليدي.

وقد أوضحت النتائج المتحصل عليها أن محتوي الجبن من المادة الجافة والبروتين يزداد بخفض نسبة الدهن في لبن الجبن. بينما أرتبط استخدام *Brevibacterium linens* بالزيادة في محتوى الجبن من المادة الجافة والدهن في المادة الجافة والبروتين والتي تزداد أيضا تدريجيا بتقدم فترة التسوية لمدة ٢٤ أسبوع. اتصفت الجبن منخفضة الدهن بانخفاض محتوي الجبن من النيتروجين الذائب والنيتروجين غير البروتيني منسوبين للنيتروجين الكلي وكذلك التيروزين والتربتوفان الذائبين والأحماض الدهنية الطيارة الكلية والحموضة وازداد معدل الانخفاض بخفض نسبة الدهن في لبن الجبن بينما إرتفع باستخدام *B. linens* في حين أنه لم تتأثر الـ pH سواء بخفض نسبة الدهن أو باستخدام *B. linens* والتي لم تؤثر معنويا أيضا علي معدل التطور في الحموضة في الجبن.انخفضت أعداد كلا من البكتريا الكلية وبكتريا حامض اللاكتيك والبكتيريا المحللة للبروتين والبكتريا المحللة للدهن بانخفاض الدهن في لبن الجبن وعلى العكس زادت باستخدام *B. linens* وفي جميع الحالات فقد زادت العداد البكتيرية بتقدم التسوية حتى الأسبوع الـ ١٢ ثم انخفضت تدريجيا بعد ذلك عند نهاية فترة التسوية (٢٤ أسبوع). ارتبط المظهر الجيري و التركيب المفرول بالانخفاض في نسبة الدهن الزائد عن ٢,٥%. في حين أنه قد تحسنت الجودة الحسية معنويا استخدام *B. linens* في صناعة الجبن من لبن خفضت فيه نسبة الدهن حتى ١%.

وأخيرا فانه يمكن التوصية بإمكانية تصنيع جبن رأس منخفض الدهن مستساغ الطعم باستخدام لبن بقري معدل المحتوى من الدهن حتى ١% مع ضرورة استخدام *B. linens* كبادئ مساعد مع بادئ اليوجهورت المعتاد.