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BIOCHEMICAL CHANGES IN NON FAT RAS CHEESE CURD AS AFFECTED BY SOME LACTIC ACID BACTERIA

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ABSTRACT

Ras cheese curd was produced from cow's skim milk and mixed buffaloes and cow's skim milk (1:1). The resultant curd was blended with5, 10 and 15g / 100g curd of mixed strains cultures of *Lactobacillus delbreukii ssp. bulgaricus* + *Lactobacillus casei* at the ratios of T I(1:1), T II (1:2) and T III (2:1). The curd was incubated at 37°C for 15 days.

The addition of mixed strains clearly enhanced the biochemical changes in all treated curds. The lactose in treated curds disappeared more rapidly than in untreated ones, particularly in T III during ripening. The total bacterial number or lactic acid bacteria were higher in fresh curd, followed by a decline during 7 and 15 days of curing, being more pronounced in curd containing 15% starter. The ripening indices values increased with the increase in percentage of added starter and with progressive incubation. The curd with 15% mixed starter (1:2) after 15 days gave the highest values for Formol and Shilovich ripening indices and SN/TN % values after 15 days of storage. The addition of mixed strains culture increased the tryptophane content of curd during ripening and these effect was more pronounced in curd containing high percentage of starter.

All the ripening indices of Ras cheese curd produced from cow's skim milk were higher than the corresponding values of curd from mixed buffaloes and cow's skim milk.

Keywords:cheese curd, Acceleration,*Lactobacillus delbreukii ssp bulgaricus, lactobacillus casei*

INTRODUCTION

Ras cheese is considered as one of the popular hard cheese type that received a great acceptance by the Egyptian consumers. Also it is used in the formulation of processed cheese produced in Egypt. Rapid development of flavour in Ras cheese can decrease the cost of processed cheese manufacture as the final product. Acceleration of Ras cheese ripening by using trace elements or enzymes, with the aim of stimulating the microbial population of the cheese offers rapid controlled approaches to the manufacture of high quality cheese flavouring material in short time(Hofi, *et al.*1973a,b; Abdel-Salam *et al.*, 1978, El-Soda *et al.*, 1985; Hofi *et al.*, 1991; El-Hofi and Ismail 2000;
Ding *et al.*2001; Soda, *et al.*2004 and Casalta, *et al.* 2005).

The addition of aged *Lb. helviticus* cell to Ras cheese caused an increase of protein breakdown and improved its organoleptic properties (Nassib, 1974;). The addition of probiotic cultures as *Lb. casei* culture ,*Lb. lactis* and *Lb. cremoris* produced positive sensory changes in relation to texture and flavour in low fat cheese (El-Sayed, *et al* ,1994; Mattila-Sandholm, *et al.* 2002; Kask, *et al.* 2003; Buriti, *et al.* 2005).

The present work was carried out to investigate some factors affecting ripening in Ras cheese curd. The curd was prepared using pasteurized cow's skim milk and mixed buffalo's and cow's skim milk. The factors are mainly aged curd, with starter *Lactobacillus delbreukii ssp. bulgaricus* + *Lactobacillus casei* in its optimum ratios.

MATERIAL AND METHODS

Cultures: *Lactobacillus delbruekii ssp. bulgaricus* + *Lactobacillus casei* were obtained from Hansen's laboratory Copenhagen, Denmark. Each strain culture was used after propogation in sterilized skim milk.

Ras cheese curd manufacture: Ras cheese curd was of made as described by Hofi *et al*. (1973 a) from pasteurized cow's skim milk and mixed skim buffaloes and cow's milk(1:1). The resultant curd was mixed with different starter mixtures as follows:

Control: The cheese curd without added starter.

Treatment I: The cheese curd was mixed with 5, 10, 15% of mixed starter culture (1:1) of *Lb.delbreukii ssp. bulgaricus* + *Lb. casei*).

Treatment II: The cheese curd was mixed with 5, 10, 15% of mixed starter culture (1:2) of *Lb.delbreukii ssp. bulgaricus* + *Lb. casei*).

Treatment III: The cheese curd was mixed with 5, 10, 15% of mixed starter culture (2:1) of *Lb.delbreukii ssp. bulgaricus+Lb. casei*).

Three replicates of each treatments were carried out . The preparations were incubated at 37°C for 15 days.

Methods of analysis: Curds of all treatments were analyzed for total solids, total nitrogen, soluble nitrogen, lactose contents and pH according to A.O.A.C. (1990). Soluble tryptophane(mg/100gm) measured according to Vakaleris and Price (1959). Formol and Shilovich ripening indices were determined as described by Abd El-Tawab and Hofi (1966). Total counts and lactic acid bacteria counts were carried out according to American Public Health Association (1978).

RESULTS AND DISCUSSION

Data presented in Tables (1-3) illustrate that the total solids contents of fresh curd from different treatments decreased as the percentage of added starter increased. This can be attributed to the low solids content in the starter culture. The curd from mixed buffaloe's and cow's milk contained slightly higher total solids than that from cow's milk in the different treatments. The same trend was mentioned by Hefnawy (1977),Hofi *et al.*, (1991); El-Battawy (1992) and Hassan *et al.* (1996). The total solids content in all treatments were sharply increased during the ripening period. This might be due to the more curd contraction and whey exudations. Also, this increase was most marked after 7 days, particularly in T III (15%).

The pH of Ras cheese curd decreased slightly as the percentage of added starter increased. This may be attributed to the higher acidity of starter culture compared to curd used in the blend. However, the high buffer capacity

of the curd allow for a slight change in pH as the added starter increased. In all trials, the pH of Ras cheese curd decreased with extended ripening and the rate this decrease was slightly greater in the early stages of ripening, but slightly increased in T III(15%) at the end period. Such increase, in pH observed during progressive ripening period, could be attributed to formation of some basic compound from protein breakdown.

In the same Tables, it is obvious that the TN/DM% decreased as the percentage of added starter increase. Also the curd prepared from mixed buffaloes and cow's skim milk contained higher TN/DM than that prepared from cow's skim milk in all treatments. During curing, the trials, TN/DM %sharply increased as ripening progressed. This may be due to the high loss of moisture during ripening as an effect of great rate of acidity, which led its increase the shrinkage process of curd. Thereafter, 15 days of curing, the increase in TN/DM % values was slightly less than that obtained after 7 days of ripening in all treatments. This slight decrease can be attributed to protein degradation and the formation of water soluble compounds.

Also, the changes in lactose content during ripening of Ras cheese curd from various treatments are shown in Tables (1-3). Lactose of treated Ras cheese curd disappeared more rapidly than that of control curd, which may be due to the rapid fermentation process by the high percent of culture used. The same trend of lactose changes was reported by El-Shibiny *et al.*, (1991).

Table 1: Composition of fresh non fat Ras cheese curd as affected by some lactic acid bacteria

	T.S %		pH		T.N/DM %		Lactose %	
	C	M	C	M	C	M	C	M
Control	35.30	36.00	5.54	5.52	13.11	14.11	1.65	1.70
Treatment I								
5 %	34.00	35.09	5.42	5.40	12.86	13.54	1.57	1.60
10 %	33.80	34.59	5.36	5.34	12.46	13.86	1.50	1.55
15 %	33.00	33.99	5.30	5.28	12.10	13.23	1.45	1.50
Treatment II								
5 %	34.85	35.20	5.44	5.42	12.81	13.52	1.54	1.57
10 %	33.65	34.48	5.38	5.36	12.41	13.72	1.47	1.52
15 %	32.75	33.84	5.30	5.30	12.05	13.06	1.42	1.47
Treatment III								
5 %	34.65	35.34	5.43	5.41	12.84	13.62	1.55	1.58
10 %	33.72	34.52	5.38	5.35	12.44	13.88	1.48	1.53
15 %	33.00	33.91	5.31	5.29	12.08	13.27	1.43	1.48

C: Cow's skim milk M: mixed buffalo's and Cow's skim milk

Table 2: Composition of 7 days old non fat Ras cheese curd as affected by some lactic acid bacteria

	T.S %		pH		T.N/DM %		Lactose %	
	C	M	C	M	C	M	C	M
Control	37.62	37.39	5.42	5.40	13.38	14.58	1.43	1.48
Treatment I								
5 %	38.55	38.79	4.88	4.86	13.88	14.55	1.04	1.59
10 %	36.14	40.65	4.69	4.65	13.62	14.95	0.97	1.00
15 %	38.33	41.74	4.50	4.55	14.05	15.25	0.74	0.95
Treatment II								
5 %	37.37	38.64	4.89	4.88	13.83	14.50	1.08	0.78
10 %	36.75	40.50	4.44	4.69	13.44	14.92	0.92	0.98
15 %	38.02	41.60	4.55	4.59	14.15	15.20	0.72	0.75
Treatment III								
5 %	37.23	38.72	4.90	4.87	13.85	14.52	1.06	1.00
10 %	34.23	40.58	4.71	4.67	13.86	14.90	0.95	0.98
15 %	38.67	41.66	4.52	4.58	14.25	15.22	0.70	0.75

C: Cow's skim milk M: mixed buffalo's and Cow's skim milk

Table 3: Composition of 15 days old non fat Ras cheese curd as affected by some lactic acid bacteria

	T.S %		pH		T.N/DM %		Lactose %	
	C	M	C	M	C	M	C	M
Control	41.43	39.73	5.37	5.34	13.72	14.77	1.12	1.17
Treatment I								
5 %	41.33	41.60	4.50	4.45	14.07	14.90	0.55	0.60
10 %	40.63	43.92	4.82	4.80	14.27	15.20	-	-
15 %	41.63	44.70	5.00	4.90	14.97	15.30	-	-
Treatment II								
5 %	42.63	41.45	4.45	4.42	14.62	14.85	0.50	0.55
10 %	39.95	43.77	4.89	4.82	14.22	15.15	-	-
15 %	41.53	45.07	5.10	4.88	14.90	15.25	-	-
Treatment III								
5 %	40.00	41.60	4.43	4.40	14.65	14.88	-	-
10 %	41.41	43.85	4.86	4.81	14.24	15.18	-	-
15 %	41.29	44.63	5.15	5.00	14.94	15.28	-	-

C: Cow's skim milk M: mixed buffalo's and Cow's skim milk

Tables (4 and 5) illustrate that total and lactic acid bacteria counts were higher in all treated Ras cheese curd than control curd, then followed by a decline after 7 and 15 days of curing. These changes were more pronounced in Ras cheese curd produced from 10 and 15% starter added after 15 days of ripening period. The high number of lactic acid bacteria in treated curds, can be attributed to the added starter in these treatments. This increase would accelerate the Ras cheese curd ripening as evidenced by the increase in soluble nitrogen, tryptophane content and the values of ripening indices. These results agree with those reported by El-Sayed, *et al.*, (1994).

Table 4: Total count bacteria non fat Ras cheese curd as affected by some lactic acid bacteria

	Cow's skim milk			Mixed skim milk		
	Fresh	7 days	15 days	Fresh	7 days	15 days
Control	$8.8x10^9$	$4.1x10^{10}$	$4.2x10^{10}$	$7x10^9$	$6.5x10^{10}$	$2.1x10^{11}$
Treatment I						
5 %	$1.7 x10^9$	$2.2x10^{10}$	$2.5x10^8$	$2.1x10^{10}$	$2.7x10^{10}$	$7.0x10^7$
10 %	$7 .3x10^9$	$3.9x10^{10}$	$7.4x10^8$	$1.7x10^{10}$	$4.0x10^{10}$	$2.6x10^9$
15 %	$2.6x10^{10}$	$6.9x10^{10}$	$2.0x10^9$	$2.1x10^{10}$	$2.1x10^{10}$	$1.7x10^9$
Treatment II						
5 %	$5.6x10^8$	$1.2x10^{10}$	$1.2x10^8$	$1.3x10^{10}$	$8.0x10^{10}$	$1.6x10^8$
10 %	$1.4x10^{10}$	9.6x109	$1.4x10^8$	$2.5x10^{10}$	$6.3x10^9$	$1.2x10^8$
15 %	$1.7x10^{10}$	$1.3x10^{10}$	$1.4x10^8$	$3.2x10^{10}$	$2.0x10^9$	$1.1x10^8$
Treatment III						
5 %	$1.4x10^{10}$	$2.6x10^9$	$1.1x10^8$	$1.4x10^{10}$	$1.2x10^9$	$8.4x10^8$
10 %	$1.1x10^{10}$	$1.9x10^9$	$1.3x10^8$	$2.3x10^9$	$1.0x10^9$	$1.5x10^8$
15 %	$1.4x10^{10}$	$1.3x10^9$	$9.0x10^7$	$7.3x10^9$	$1.0x10^9$	$2.0x10^8$

Table 5: The count of lactic acid bacteria non fat Ras cheese curd during ripening

	Cow's skim milk			Mixed skim milk		
	Fresh	7 days	15 days	Fresh	7 days	15 days
Control	$2.1x10^{10}$	$2.1x10^{10}$	$2.6 x10^{10}$	$4.7x10^9$	$2.3x10^{10}$	$1.8x10^{10}$
			Treatment I			
5 %	$7.6x10^9$	$1.8x10^{10}$	$9.0x10^7$	$1.2x10^{10}$	$1.5x10^{10}$	$5.0x10^8$
10 %	$7.0x10^9$	$2.9x10^{10}$	$8.0x10^7$	$1.2x10^{10}$	$3.1x10^{10}$	$2.1x10^8$
15 %	$1.3x10^{10}$	$5.2x10^{10}$	$1.6x10^8$	$7.3x10^9$	$1.2x10^{10}$	$4.0x10^8$
			Treatment II			
5 %	$4.6x10^9$	$6.0x10^9$	$1.2x10^8$	$9.0x10^9$	$3.9x10^9$	$1.0x10^8$
10 %	$9.0x10^9$	$2.6x10^9$	$1.4x10^8$	$2.3x10^{10}$	$4.0x10^9$	$1.2x10^8$
15 %	$1.4x10^{10}$	$5.6x10^9$	$1.2x10^8$	$2.8x10^{10}$	$1.0x10^9$	$1.0x10^8$
			Treatment III			
5 %	$6.3x10^9$	$1.6x10^9$	$1.9x10^9$	$1.3x10^{10}$	$1.6x10^8$	$7.0x10^7$
10 %	$1.1x10^{10}$	$1.1x10^9$	$1.2x10^8$	$3.0x10^9$	$1.2x10^8$	$9.0x10^7$
15 %	$1.2x10^{10}$	$1.5x10^9$	$9.0x10^7$	$6.0x10^9$	$1.0x10^9$	$9.0x10^7$

Tables (6-8) shows that the values of ripening indices increased with the increase of the starter amounts and progressive ripening period. Formol ripening index and Shilovich ripening values of Ras cheese curd containing 15% starter were higher than those of the other treatments at the different stage of ripening This increase may be due to the higher protein degradation in the former treatment. On the other hand, ripening indices values of skim cow's milk curd were higher than the corresponding values for mixed skim milk. The same trend was observed by Abd El-Salam *et al.*, (1979)and El-Sayed & Abbas (1992).The degree of ripening of Ras cheese curd expressed as SN/TN % is given in Tables (6-8). The calculated ratio of SN/TN % was higher in all treated curds than control curd. The SN/TN % of Ras cheese curd were increased continuously as the ripening period advanced and this

increase was more pronounced as the percentage of starter added was increased. It was also of the interest to notice that curd from treatment. the T II at ratio (1:2) of *Lb. bulgaricus* + *Lb. casei* showed the highest SN/TN values at the end of curing period. This can be explained by the positive effect of mixed culture of *Lb. bulgaricus* + *Lb. casei* as a good source of proteolytic enzymes. The same trend of SN/TN % behaviour reported by Hofi, *et al.*, (1991), Gomaa, *et al.*,(1992), Shukry (1993), El-Tahra, et al (1994), Abeib (1996) and Hassan, *et al.*, (1996).

As given in Tables(6-8), addition the mixed cultures to Ras cheese curd increased the soluble tryptophane content during ripening period and its effect was more pronounced as the starter percentage was increased which results in more protein degradation. Curd from T II at ratio (1:2) gave the highest tryptophane values and followed T III by ratio (2:1) of tryptophane content after 15 days of ripening period. Somewhat similar results reported by Mohamed, *et al.* (1989), Abeid (1996) and Hassan, *et al.*, (1996).

From the foregoing results we can conclude that addition of mixed *Lb. delbreukii ssp. bulgaricus* + *Lb. casei* (1:2) to Ras cheese curd 15% gave a product with acceptable ripening quality and therefore, it can be recommended as an ingredient in processed cheese blends.

Table 6: Ripening indexes of fresh non fat Ras cheese curd as affected by some lactic acid bacteria

	Formol ripening index		Shilovich ripening index		S.N/T.N %		Tryptophane mg/100g	
	C	M	C	M	C	M	C	M
Control	17.00	15.00	19.00	16.00	1.98	1.75	21.35	20.53
Treatment I								
5 %	20.00	18.00	25.00	24.00	4.15	4.04	24.88	23.87
10 %	25.00	23.00	37.00	35.00	5.15	5.01	30.92	30.10
15 %	42.00	40.00	44.00	42.00	5.52	5.43	34.52	32.67
Treatment II								
5 %	24.00	20.00	33.00	29.00	5.25	5.20	28.43	27.37
10 %	31.00	27.00	42.00	39.00	5.63	5.50	34.67	33.51
15 %	51.00	47.00	52.00	47.00	5.98	5.89	39.62	38.52
Treatment III								
5 %	22.00	19.00	29.00	27.00	5.15	5.00	25.73	25.32
10 %	26.00	23.00	37.00	35.00	5.17	5.15	31.73	30.65
15 %	46.00	43.00	47.00	44.00	5.57	5.50	35.65	34.55

C: Cow's skim milk M: mixed buffalo's and Cow's skim milk

Table 7: Ripening indexes of 7 days old non fat Ras cheese curd as affected by some lactic acid bacteria

	Formol ripening index		Shilovich ripening index		S.N/T.N %		Tryptophane mg/100g	
	C	M	C	M	C	M	C	M
Control	55.0	55.0	58.0	58.0	5.53	5.53	30.87	30.83
Treatment I								
5 %	91.0	91.0	65.0	65.0	4.92	4.92	28.55	27.57
10 %	120.0	117.0	84.0	80.0	6.34	6.24	34.22	32.22
15 %	156.0	157.0	128.0	125.0	7.21	7.00	41.15	38.15
Treatment II								
5 %	102.0	102.0	77.0	77.0	6.83	6.83	31.67	30.56
10 %	130.0	123.0	92.0	88.0	7.66	7.54	38.42	35.45
15 %	165.0	163 0	136.0	130.0	8.42	7.99	41.66	40.61
Treatment III								
5 %	95.0	95.0	73.0	73.0	6.19	6.19	29.25	28.42
10 %	122.0	118.0	85.0	81.0	7.36	7.25	35.13	33.24
15 %	157.0	156.0	130.0	127.0	7.61	7.42	51.35	48.99

C: Cow's skim milk M: mixed buffalo's and Cow's skim milk

Table 8: Ripening indexes of 15 days old non fat Ras cheese curd as affected by some lactic acid bacteria

	Formol ripening index		Shilovich ripening index		S.N/T.N %		Tryptophane mg/100g	
	C	M	C	M	C	M	C	M
Control	62.0	65.0	61.0	65.0	6.22	5.93	40.63	39.53
Treatment I								
5 %	143.0	140.0	105.0	100.0	5.43	5.37	38.62	35.61
10 %	175.0	172.0	140.0	135.0	7.14	6.94	40.99	39.98
15 %	203.0	200.0	157.0	155.0	7.88	7.67	60.00	55.00
Treatment II								
5 %	153.0	147.0	124.0	115.0	7.54	7.34	44.65	40.56
10 %	205.0	195.0	145.0	140.0	8.34	8.05	53.21	47.00
15 %	230.0	225.0	184.0	180.0	9.45	8.89	70.35	63.00
Treatment III								
5 %	144.0	140.0	115.0	109.0	6.98	6.83	39.55	37.44
10 %	200.0	190.0	140.0	137.0	7.23	7.71	42.63	40.65
15 %	220.0	215.0	175.0	173.0	8.25	7.93	65.00	60.00

C: Cow's skim milk M: mixed buffalo's and Cow's skim milk

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التغيرات البيوكيمائية في خثرة جبن الراس اللادهنية ومدى تأثرها ببعض بكتيريا حمض اللاكتيك

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تعتبر بكتيريا حمض اللاكتيك من أكثر الكائنات الحية الدقيقة نفعا للإنسان حيث نجدها ركنا أساسيا في صناعة العديد من الأغذية المتخمرة كما أنها المسئولة عن إنتاج الطعوم المختلفة في الأغذية التي تدخل في صناعتها.

تم إنتاج خثرة جبن الراس من لبن بقري فرز ومن خليط من اللبن البقري والجاموسي الفرز بنسبة (١:١) ، كما تم تحضير بادئ من خليط من سلالات بكتيريا حمض اللاكتيك:

Lactobacillus delbrukii ssp. bulgaricus + Lactobacillus casei

بنسب ١:١ للمعاملة الأولي و ٢:١ للمعاملة الثانية ونسبة ٢:١ للمعاملة الثالثة وتم إضافتها للخثرة بالنسب التالية ٥ ، ١٠ ، ١٥ جم من خليط البادئ /١٠٠ جم خثرة والتحضين على 37°C /١٥ يوم.

وقد أظهرت النتائج أن إضافة خليط البادئ قد حسن بوضوح التغيرات البيوكيمائية في كل المعاملات ، ولوحظ معدل اختفاء اللاكتوز في الخثرة المضاف لها البادئ كان أسرع من عينة المقارنة خاصة في المعاملة الثالثة (٢:١) ز كما أظهرت النتائج أن العدد الكلي للبكتيريا وكذلك عدد بكتيريا حمض اللاكتيك أعلا في الخثرة الطازجة وبعد التخزين لمدة ١٥ يوم خاصة المعاملات المضاف لها معدل ١٥ جم بادئ/١٠٠ جم خثرة.

كما لوحظ زيادة قيم دلائل التسوية مع زيادة نسبة البادئ المضافة ومع زيادة مدة التحضين، كما وجد أن المعاملة الثانية (١:٢) بنسبة ١٥% أعطت أعلا معدلات في قيم الفورمول والبروتين الذائب/البروتين الكلي ويظهر هذا التأثير بوضوح مع زيادة نسبة البادئ المضاف.

وأوضحت النتائج انه يمكن إضافة مخلوط البادئ *Lactobacillus delbreukii ssp. bulgaricus + lactobacillus casei* (١:٢) بنسبة ١٥% لخثرة الجبن الراس كانت ذات جودة مقبولة ودلائل تسوية جيدة ويوصي باستخدامها في مخاليط الجبن المطبوخة.