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EFFECT OF ELEVATED RIPENING TEMPERATURE ON THE PROPERTIES OF RAS CHEESE MADE FROM BUFFALOES' MILK

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ABSTRACT

In order to improve the organoleptic properties and the consumption acceptability of Ras cheese made from buffaloes' milk using different starters, cheese ripening was carried out in two successive period, the first at 25°C/30 days and the second at 12°C/60 days. Yoghurt starter was used in Ras cheese making either individually or mixed with mesophilic lactococci, *Lb. casei* or *Lb. helveticus*. Cheese chemical composition, ripening indices, bacterial counts and organoleptic properties throughout the ripening process were followed.

Buffaloes' Ras cheeses were ripened at elevated temperature had lower moisture content and higher fat and protein contents than that of control cheese (T2), and *Lb. helveticus* cheese (T6) had the most considerable effect on these parameters during ripening.

Treated cheeses were characterized by their higher numbers of different bacterial groups than the control cheese. Consequently, their titratable acidity (T.A), protein degradation (S.N) and total volatile fatty acids (T.V.F.A) were greater than that of control cheese. Using elevated ripening temperature in combination with the mixed culture of yoghurt starter + mesophilic lactococci or *Lb. casei* (T4 & T5) improved markedly organoleptic properties and flavour intensity and body of the resultant buffaloes' Ras cheese which gained the highest score. Therefore, these treatments can be recommended for manufacture of Ras cheese from buffalo milk.

INTRODUCTION

Hard cheese made from buffaloes' milk was reported to have slow ripening rate leading to a crumbly body accompanied with not fully developed flavour compared to cheese made from cows' milk (Gouda, 1976 and Waghmars & Gupta, 1987).

Number of attempts have been carried out to solve this problem. These included mixing both types of milk (Czulak, 1964); removing part of calcium contents (Anis, 1975); homogenization of milk (Abo El-Heba, 1976); adding sodium citrate to the milk (Teama *et al.*, 1979); modifying the manufacture technique (Jha and Singh, 1987); adding various enzymes (Abd-Rabo *et al.*, 1991); mixing cheese slurries with curd particles (Hofi *et al.*, 1991) and mixing buffaloes milk with butter milk (Abdel-Nabi *et al.*, 1994).

Ripening temperature was the most important factor that affect the properties of the resultant cheese. Elevation the temperature of ripening as a method for its acceleration is an attractive method since it is simple and it increases the microbial populations and activity and enhance the flavour development (Aston *et al.*, 1985). Furthermore, the rate of cheese ripening can be controlled successfully through adjustment of the ripening

temperature and good hygiene during production (Fryer, 1982). Also, it appears that initial ripening at elevated temperatures is more effective than increasing the temperature during ripening.

In their trials, Aston *et al.* (1983 and 1985) accelerated flavour production of Cheedar cheese by ripening it at 20°C for 4 wk, then 13°C for 28 wk or at 15°C for 32 wk. The use of 16°C for 120-d accelerated ripening of hygienic quality of raw ewes' milk Manchego cheese (Gaya *et al.*, 1990) and 30°C for 15 days, then 10°C for 75 days accelerated ripening of pasteurized cows' milk Ras cheese (Abd El-Fattah, 1999). Significantly, no adverse effect on cheese quality was noted for any treatments in these studies.

On the other hand, to improve the quality of Ras cheese, the use of various starter cultures represent a good potential to serve this purpose. Yoghurt starter was used as a matter of ease but not on proper selection. Consequently, many attempts were done to characterize a proper starter for this purpose namely Lb. helveticus or Lb. casei in combination with yoghurt starter which has been recommended by (Metwally *et al.*, 1984 and 1990) or mesophilic mixed lactic acid starter (El-Aassar *et al.*, 1999).

For these reasons it was thought that the use of elevated ripening temperature in combination with different starter cultures may produce further useful increases in the ripening rate and improve the properties of Ras cheese that was made from buffaloes' milk which contributes about 60% of the total milk production in Egypt. Therefore, the present work was planned to confirm this goal.

MATERIALS AND METHODS

Materials:

Whole fresh cows' and buffaloes' milk were obtained from the herd of the Faculty of Agriculture, Cairo University.

Cheese plastic coating of HALA PLAST Lb type YL and pure lyophilized Direct Vat Set (DVS) cultures of yoghurt starter YC-281 (*Lactobacillus delbreuckii subsp. bulgaricus* and *Streptococcus thermophilus*, 1:1), mesophilic lactococci 0-114 (*Lactococcus lactis subsp. lactis* and *Lactococcus lactis subsp. cremoris*), *Lactobacillus casei* 01 and *Lactobacillus helveticus* B-20 were obtained from Chr. Hansen's Laboratories, Copenhagen, Denmark. The DVS cultures were intended for direct inoculation to the process milk at 50u/ton (≈5 g/100kg).

All media used were obtained from Oxoid Division of Oxoid LTD, London.

Experimental procedure:

Six treatments of Ras cheese were undertaken of 20 kg milk (0.7 casein/fat ratio) each, and they were manufactured into Ras cheese following the procedure of *Hofi et al. (1970)* with some modification in the starter used. The first treatment was made from cows' milk with yoghurt starter which served as a control (T1). The other five treatments were made from buffaloes' milk using different starter cultures as follows:-

T2: Yoghurt starter which served as a control.
T3: Yoghurt starter.
T4: Yoghurt starter + mesophilic lactococci (1:1).
T5: Yoghurt starter + *Lb. casei* (1:1).
T6: Yoghurt starter + *Lb. helveticus* (1:1).

After salting, cheese was coated by plastic coat contain natamycin as antifungal agent and ripened at 85% R.H. The first and second treatments (T1 & T2) were ripened at 12°C for 90 days, while the other treatments were ripened at 25°C for 30 days, then 12°C for 60 days.

Experiments were carried out under good sanitary conditions in all steps and in triplicats for each treatment.

Methods of analysis:

Samples were periodically analyzed chemically for total solids (T.S), titratable acidity (T.A), fat, total nitrogen (T.N), soluble nitrogen (S.N) and salt as described by *Ling (1963)* and for total volatile fatty acids (T.V.F.A) as suggested by *Kosikowski (1982)*. They were also microbiologically analyzed for total bacterial count (T.C), lactobacilli, streptococci, proteolytic bacteria, lipolytic bacteria, coliforms and moulds & yeasts according to the methods described by the American Public Health Association *(A.P.H.A., 1992)*. Cheese samples were also organoleptically judged by 10 panel members of experts at the Dairy Department, Faculty of Agriculture, Cairo University. The cheese was judged for flavour (50), body and texture (40) and general appearance (10).

RESULTS AND DISCUSSION

Chemical composition:

As shown in Table (1), the buffaloes' Ras cheese (T2) had lower moisture content than cows' Ras cheese (T1). This could be due to the difference in the casein micellar composition and high ratio of ca^{++} content in buffaloes' milk *(Abdel-Salam et al., 1978)* which led to expel more whey from cheese curd.

As for fat and protein content, the buffaloes' cheeses were characterized by its higher fat and protein contents when compared with the cows' cheese (T1). While, the changes in the T.A, T.V.F.A and S.N/T.N was faster in cows' cheese than buffaloes' cheeses.

Composition of Ras cheese made from buffaloes' milk with different types of starter and ripened at 25°C for 30 days, then at 12°C for 60 days is reported in Table (1). As can be observed, the moisture content of all samples decreased during ripening and the rate of decrease was higher in treated cheeses than that of the control (T2). This decrease was due to the increase in ripening temperature. On the other hand, cheese made with *Lb. helveticus* (T6) had lower moisture content than other treatments when fresh and during ripening. This decrease might be attributed to expulsion of more moisture with increase acidity attained by *Lb. helveticus* as shown in Fig (1).

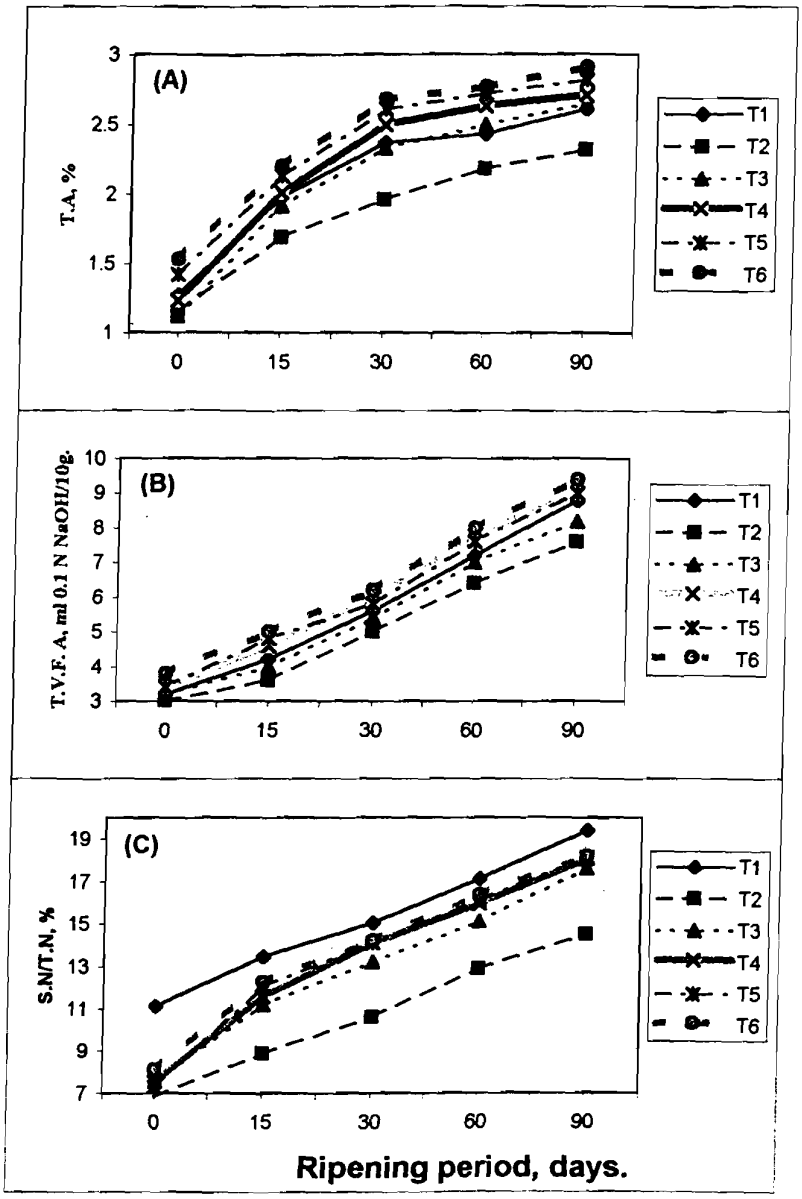

Fig. (1): Changes in T.A [A], T.V.F.A [B] and S.N/ T.N [C] of Ras cheese made from buffaloes' milk with different starters and ripened at high temperature during initial ripening period.

Concerning fat, protein and salt/water of treated cheese compared with the control, there were gradual increases by advanced ripening period and the ratios depended on the rate of moisture decrease. This effect on moisture contents also led to the higher percentages of fat and protein in treated cheese than that of the control (T2).

Table (1): Effect of high temperature during initial ripening period on the chemical composition of Ras cheese made from buffaloe's milk with different starters.

Parameter (%)	Ripening period (days)	Control		Treatments			
		T1	T2	T3	T4	T5	T6
Moisture	0	47.11	45.17	46.03	45.97	45.69	44.22
	15	43.42	42.21	41.19	41.08	40.94	39.13
	30	41.33	39.52	38.31	37.17	36.85	34.76
	60	38.82	37.88	35.72	34.11	33.43	32.25
	90	35.92	34.11	32.83	32.79	31.74	30.09
Fat	0	28.0	30.5	30.0	30.0	30.0	30.5
	15	29.5	32.5	32.0	32.0	33.0	33.0
	30	31.5	34.0	34.5	34.0	35.0	35.5
	60	33.0	35.5	36.0	35.5	36.5	37.0
	90	34.5	36.5	37.5	37.0	38.0	38.5
Total protein	0	22.90	24.24	24.44	24.50	25.33	25.14
	15	23.67	25.14	25.65	25.84	26.92	26.99
	30	25.84	27.63	27.94	28.01	28.90	28.71
	60	26.80	28.65	29.09	29.22	30.05	29.99
	90	27.63	29.41	30.05	30.05	30.94	31.20
Salt/water	0	3.95	4.01	3.76	3.74	3.81	4.03
	15	4.49	4.55	4.59	4.50	4.42	4.68
	30	5.01	5.14	5.24	5.33	5.16	5.41
	60	5.59	5.60	5.91	6.06	5.89	6.02
	90	6.35	6.60	6.58	6.53	6.46	6.75

- **T1 made from cows' milk and T2 made from buffaloes' milk and ripened at 12°C for 90 days.**
- **T3, T4, T5 and T6 made from buffaloes' milk and ripened at 25°C for 30 days, then 12°C for 60 days.**

Ripening development:

As can be observed from Fig. (1), the T.A was affected by the type of starter used whether when fresh or during ripening. The T. A of fresh cheese was highest in T5 and T6 being 1.42 and 1.53%, while it ranged between 1.12 - 1.27 in the rest of the samples. These results coincide with the ability of the various starters to produce lactic acid which is higher in the lactobacilli *spp.* than the other lactic acid bacteria *(Sneeth et al., 1986).* These results are partly in agreement with *Metwally et al. (1984)* and *El-Aassar et al. (1999).* Also, the rate of acid production was much faster in cheese ripened at higher temperature reaching higher values after 30 days than the buffaloes' control cheese (T2) after 90 days indicating the stimulatory action of increasing ripening temperature on the activity of the bacteria. Similar results were found with cows' Ras cheese *(Abd El-Fattah, 1999).* Regarding the total volatile fatty acids (T.V.F.A.), results given in Fig. (1) show that the cheese treated with *Lb. heveticus* (T6) started with high level of T.V.F.A. During ripening an obvious increase occurred in all treatments but at different rates. The cheese ripened at high temperature had higher T.V.F.A than that of control. This effect was more pronounced in T4, T5 and T6 than T3. This is obviously due to the initial high ripening temperature which enhanced the activity of lipolytic enzymes and bacteria and the effect of the starters used. Similar results were obtained by *Kosikowski and Iwasaki (1975),* who reported that FFA were 9.6% higher in 30-d Cheddar cheese ripened at 20°C than cheese ripened for the same time at 10°C.

Data given in Fig. (1) clearly indicate that the S.N/T.N markedly increased in all buffalo cheese samples as the storage period advanced. The S.N/T.N values were higher in buffalo cheese ripened at high temperature than the control (T2) throughout the ripening period. These results are partially in agreement with *Aston et al. (1983)* who found that in Cheddar cheese ripened for 30 d at 20°C, concentration of free amino acids (PTA-soluble N) was higher than in cheese ripened at 8°C. Also, it appears that the type of starter had a pronounced effect on the break down of protein. The S.N/T.N in samples contained mixed culture of yoghurt starter and different lactic cultures (T4, T5 and T6) was higher than corresponding sample with yoghurt starter only (T3). This is expected to be due to the variation in the proteolytic activity of the various starters used. These results are partially in agreement with *Metwally et al. (1990)* on buffalo Ras cheese.

Microbiological changes:

No growth of coliform and mould and yeast were observed throughout ripening as the processing and ripening in cheese were made under highly hygienic and sanitary conditions. Table (2) summarizes the effect of the elevated ripening temperature regime on the composition and microflora development of buffaloes' Ras cheese. It could be observed that there were small differences in the microbial groups of all cheese samples when fresh. As ripening progressed, the T.C decreased gradually in the control samples, while increased until the 30 days of ripening in cheese treatments and then slowed down until the end of ripening.

Table (2): Effect of high temperature during initial ripening period on the microbial contents of Ras cheese made from buffaloes' milk with different starters.

Microbial groups (CFU/g)	Ripening period (days)	Control		Treatments			
		T1	T2	T3	T4	T5	T6
Total bacterial count X 10^6	0	22	16	19	25	18	14
	15	13.5	6.7	24	29	25	17
	30	5.7	3.3	30	36	32	28
	60	4.8	2.2	17	25.3	21.7	16.2
	90	2.9	1.3	10.3	12.1	9.8	7.4
Streptococci X10^6	0	49	56	44	61	48	53
	15	38	42	33	45	36	34
	30	31	34	26	32	27	25
	60	27	29	19	23	19	17
	90	22	24	12	15	11	10
Lactobacilli X 10^7	0	35	29	34	31	40	37
	15	73	65	67	71	84	87
	30	130	125	128	133	144	139
	60	158	152	154	162	173	168
	90	123	120	129	135	136	131
Proteolytic bacteria X10^2	0	16	18	15	17	20	19
	15	25	22	32	30	36	33
	30	30	28	41	44	47	42
	60	19	18	23	21	23	20
	90	3.0	3.7	4.6	6.4	7.7	6.7
Lipolytic bacteria X10^2	0	10	8	11	9	10	12
	15	18	15	20	24	26	23
	30	26	27	33	31	29	33
	60	13	12	18	17	19	16
	90	1.2	1.0	2.5	2.4	2.3	2.8

- **T1 made from cows' milk and T2 made from buffaloes' milk and ripened at 12°C for 90 days.**
- **T3, T4, T5 and T6 made from buffaloes' milk and ripened at 25°C for 30 days, then 12°C for 60 days.**

As for the lactic acid bacteria, the maximum count for streptococci was attained in the fresh cheese and then decreased throughout the ripening period with higher rate in treated cheeses. On the other hand, the lactobacilli count increased in all treatments until 60 days with higher rate in treated cheeses then decreased after 90 days. A similar trend for lactobacilli growth throughout the ripening at elevated temperature of Cheddar cheese was reported by *Cromie et al. (1987).*

Concerning the proteolytic and lipolytic bacterial counts, data in the same Table show that they followed similar trend of changes during ripening, although the counts of proteolytic bacteria were generally higher than lipolytic bacteria. Both groups increased gradually in all the cheeses by ripening up to 30 days then decreased sharply at the end of ripening period. However, the treated cheese showed higher numbers of proteolytic and lipolytic bacteria than that of the control cheese throughout the ripening period. This indicates the stimulatory effect of elevated ripening temperature. These results are partly in agreement with *Abd El-Fattah (1999).*

Organoleptic properties:

Table (3) *shows* the average scores for the organoleptic properties of the control and treated cheeses throughout ripening. It appears from the presented data, the best organoleptic properties were found with cows' Ras cheese (control-T1) as it gained a total score of 91 at the end of ripening. Buffaloes' Ras cheeses ripened at high temperature (T3, T4, T5 and T6) were organoleptically better than the control buffaloes' cheese (T2) as they gained a total score of 80-88 as compared to 72 for the control. The treated cheeses falvour was acceptable, clean and well developed and having a good body and texture. This might be attributed to the contribution of elevated ripening temperature in releasing the responsible bacterial enzymes for protein decomposition and fat hydrolysis of cheese forming more flavour components. Moreover, T4 and T5 where yoghurt starter mixed with mesophilic lactococci or *Lb. casei* were used, recorded the highest score of 87 and 88, resp. These results are partially in agreement with *Puchades et al. (1989)* who reported that the *Lb. casei-added* Cheeder cheese was classified as having a "strong Cheddar cheese" flavour. However, the acidity was evident in the flavour of cheese made with *Lb. helviticus* and the body was slightly hard.

In conclusion, the use of elevated ripening temperature in combination with the mixed culture of yoghurt starter + mesophilic lactococci 0 -114 or + *Lb. casei*-01 proved to be of value for making Ras cheese from buffaloes' milk and. The body was smooth, the flavour was well developed. Not only that, but also, this method is simple, producing fully ripened product with good properties using the traditional ripening technique. Of course, this work is flexible and lend itself easy to manipulate both the temperature and its period to get the proper flavour. The success of this technique depends heavily on the strict sanitary conditions that should be followed throughout processing.

Table (3): Effect of high temperature during initial ripening period on the organoleptic properties of Ras cheese made from buffaloes' milk with different starters.

Treatment	Ripening period (days)	Score points				Comments
		Appearance (10)	Body & Texture (40)	Flavour (50)	Total (100)	
T1	0	7	26	35	68	Clean flavour
	15	7	28	37	72	
	30	8	31	39	78	Developed flavour
	60	8	34	42	84	Proper body & texture
	90	9	37	45	91	Good flavour & smoothness
T2	0	5	22	27	54	Clean flavour
	15	5	23	28	56	
	30	6	25	31	62	Flat flavour & crumbly body
	60	6	28	33	67	
	90	7	30	35	72	Flat flavour & medium smoothness
T3	0	6	23	27	56	Clean flavour
	15	6	26	30	62	
	30	7	29	35	71	Developed flavour
	60	8	32	38	78	Proper body & texture
	90	9	34	40	83	Good flavour & smoothness
T4	0	6	23	28	57	Clean flavour
	15	6	26	30	62	Slight bitterness
	30	7	29	35	71	Developed flavour
	60	8	32	40	80	Proper body & texture
	90	9	35	43	87	Good flavour & smoothness
T5	0	6	23	28	57	Clean flavour
	15	6	25	32	63	Acid flavour
	30	7	29	36	72	Developed flavour
	60	8	32	41	81	Proper body & texture
	90	9	35	44	88	Good flavour & smoothness
T6	0	6	23	28	57	Clean flavour
	15	6	24	32	62	Acid flavour
	30	7	26	35	68	Mild-flavour
	60	7	29	38	74	Slight hard body
	90	8	32	40	80	Mild-flavour & slight hard body

- **T1 made from cows' milk and T2 made from buffaloes' milk and ripened at 12°C for 90 days.**
- **T3, T4, T5 and T6 made from buffaloes' milk and ripened at 25°C for 30 days, then 12°C for 60 days.**

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تأثير حرارة التسوية المرتفعة على خواص الجبن الراس المصنع من اللبن الجاموس

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استهدف هذا البحث تحسين الخواص الحسية للجبن الراس المصنع من لبن جاموسي وذلك باستخدام درجة حرارة عالية فى المرحلة الاولى من التسوية (٢٥ °م /٣٠ يوم) يتبعها استخدام درجة الحرارة المعتادة فى المرحلة التالية (١٢°م /٦٠يوم) مع استخدام مزارع بادئ مختلفة مكونة من بادئ الزبادي فقط أو من خليط منه مع الـ mesophilic lactococci أو *Lb. casei* أو *Lb. helveticus* وتم متابعة التركيب الكيماوي ومؤشرات التسوية واعداد البكتيريا والخواص الحسية أثناء عملية التسوية.

وقد أظهرت النتائج أن:

الجبن الراس الجاموسى المسوي على درجة الحرارة المرتفعة احتوى على نسبة رطوبة منخفضة ونسبة عالية من كل من الدهن والبروتين مقارنة بجبن المقارنة وكان ذلك اكثر وضوحا فى المعاملة السادسة (T6) التى استخدم فيها البادئ المكون من خليط من بادئ الزبادي مع *Lb. helveticus* وقد تميز الجبن المعامل بالحرارة العالية باحتوائه على أعداد عالية من المجاميع البكتيرية المختلفة وكذلك على درجة حموضة ونيتروجين ذائب وأحماض دهنية طيارة أعلى من جبن المقارنة.

وكان لاستخدام درجة حرارة عالية فى المرحلة الاولى من التسوية مع البادئ المكون من خليط بادئ الزبادي والـ mesophilic lactococci أو الـ *Lb. casei* تأثير واضح على تحسين الخواص الحسية وقوام ونكهة الجبن الراس المصنع من اللبن الجاموسى حيث حصلت هذه المعاملات (T5,T4) على أعلى درجات التحكيم الحسي.

وبناء على ذلك فان هذا البحث يوصى باستخدام هذه المعاملات لتصنيع الجبن الراس من اللبن الجاموسى.