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Effects of heating milk and accelerating ripening of low fat Ras cheese on biogenic amines and free amino acids development

K.M.K. Kebary*, A.H. El-Sonbaty, R.M. Badawi

Department of Dairy Science and Technology, Faculty of Agriculture, Menofiya University, Shibin El-Kom, Egypt

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Abstract

Two experiments were conducted to study the effects of heating milk and, consequently, incorporation of whey proteins into cheese curd, fat content, accelerating cheese ripening by attenuated lactobacilli, species of bacteria and method of attenuation on formation of biogenic amines and liberation of free amino acids. Total biogenic amines and total free amino acids increased as ripening period progressed in all cheese treatments. Total biogenic amines and total free amino acids decreased as the fat content was decreased. Heating of 3% fat milk up to 70°C caused a significant ($p < 0.05$) increase in total biogenic amines and total free amino acid concentrations, while raising the temperature of heat treatment up to 75 and 80°C decreased them. However, heating of 2% fat milk up to 75°C caused a definite ($p < 0.05$) increase in total biogenic arnines and total free amino acids; conversely raising heat treatment temperature to 80°C decreased them. These results indicate that there is a positive correlation between total biogenic amines and total free amino acids; moisture and salt contents affected the formation of biogenic amines, while incorporation of whey proteins had no significant effect on biogenic amines development. In a second experiment, addition of attenuated lactobacilli as adjunct bacteria caused a pronounced ($p < 0.05$) increase in free amino acids and biogenic amines. Addition of either freeze- or heat-shocked *Lactobacillus helviticus* was more effective in promoting the build-up of biogenic amines and free amino acids in the resultant cheeses than in cheeses made with addition of freeze- or heat-shocked *Lactobacillus casei*, respectively. There was a positive correlation between free amino acids and biogenic amine contents. The type of bacteria and prolongation of ripening period significantly affected the development of biogenic amines. Tyramine was the highest biogenic amine followed by histamine, while spermidine was the lowest. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

Ras cheese is the most popular hard cheese in Egypt. Because of the health problems associated with fat, there has been substantial interest in the development of a new range of a dairy products which are similar to the existing product but in which the fat content is substantially reduced (Williams, 1985). The important problems in the manufacture of low fat cheese are the lack of the typical flavour and proper body and texture (Anderson and Mistry, 1994; Banks et al., 1989; Johnson and Chen, 1991; Simard, 1991). Incorporating whey proteins into cheese is a promising method for improving the quality of low fat cheese (Banks, 1990; Khader et al., 1995). Khader et al. (1995) found that heating of milk prior to cheese making improved the resultant low fat cheese, but it gained lower organoleptic scores than the control cheese. Therefore, there was a need to accelerate the ripening of this cheese which was made from heated milk to produce a cheese with characteristics equivalent to standard matured cheese. Attenuated bacterial cells offer a promising method to accelerate the ripening of cheese. Attenuated lactobacilli cells have been used to accelerate the ripening of many cheese varieties (Johnson et al., 1995; Trepanier et al., 1992a, b; Bartels et al., 1987; Ezzat and El-Shafei, 1991; Kebary et al., 1996b). Kebary et al. (1996b) found that adding attenuated lactobacilli as adjunct starter improved the quality of low fat Ras cheese.

Proteolysis of casein during cheese ripening leads to a steady increase of amino acids content. Some of these free amino acids can be subjected to decarboxylation reactions, which are catalysed by specific bacterial decarboxylases and resulting formation of amines. These amines are called biogenic amines because of important physiological effects they may induce in human and animals (Edwards and Sandine, 1981; Joosten and Stadhouders, 1987). Physiological effects of biogenic

* Corresponding author.

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amines such as hypertension, headache, fever, nausea, urticaria, gastric and intestinal ulcers have been reported by Lembke (1978), Edwards and Sandine (1981) and Joosten and Stadhouders (1987). The poisoning and undesirable physiological effects depend on individual susceptibility, simultaneous alcohol consumption, use of certain medicine and potentiation by other amines (Joosten and Stadhouders, 1987).

Darwish (1993) reported that cheese is an ideal environment for biogenic amines formation. Biogenic amines have been detected in several cheeses such as Swiss, Emmental, Cheddar, Gouda, Blue, Ras, Processed cheese and Hungarian hard cheese (Koehler and Eitenmiller, 1978; Edwards and Sandine, 1981; Antila et al., 1984; Mehanna et al., 1989; Tawfik et al., 1992; Darwish, 1993; Abd Alla et al., 1996; El-Sonbaty et al., 1998). Presence and accumulation of biogenic amines depends on many factors such as presence of specific bacteria (enterococci and lactobacilli) and enzymes, availability of substrate (free amino acids), presence of suitable cofactor, existence of a proper environment in the cheese (higher pH, higher moisture, higher temperature and lower salt content), type of cheese and ripening and storage period (Edwards and Sandine, 1981; Joosten, 1988; Degheidi et al., 1992; Darwish, 1993; Petridis and Steinhart, 1996a,b).

The objectives of this study were to investigate the effects of (1) incorporation of whey proteins into cheese by heating milk; (2) fat content and consequently moisture content of low fat Ras cheese; (3) addition of attenuated lactobacilli to accelerate cheese ripening; (4) species of added lactobacilli and (5) attenuation methods on formation and accumulation of biogenic amines in low fat Ras cheese.

2. Materials and methods

2.1. Bacterial strains and culture

Lactococcus lactis CH-1 was obtained from Chr. Hansen's laboratory (Horshohn, Denmark) and this bacterium was used as a starter culture. *Lactobacillus helveticus* CNRZ 53 and *Lactobacillus casei* NIH 334 which were added to cheese as adjunct starters were provided by Prof M. El-Soda (Dairy Science Department, Faculty of Agriculture, Alexandria University, Egypt). Preparation of freeze- and heat-shocked *Lactobacillus helveticus* CNRZ 53 and *Lactobacillus casei* NIH 334 were carried out as described by Hantira (1991).

2.2. Cheese making

Bulk fresh cow's milk was obtained from the herd of Tokh Tanbisha farm, Menofiya University, Shibin El-Kom, Egypt. Two experiments were conducted as described by Khader et al. (1995) and Kebary et al. (1996b) to study the effect of heating milk and accelerated ripening on biogenic amines formation in Ras cheese. Treatment of milk could be summarized as illustrated in Figs. 1 and 2. Heated milk was cooled immediately after reaching the specific temperature to renneting temperature with cold water. Ras cheese was made from variously

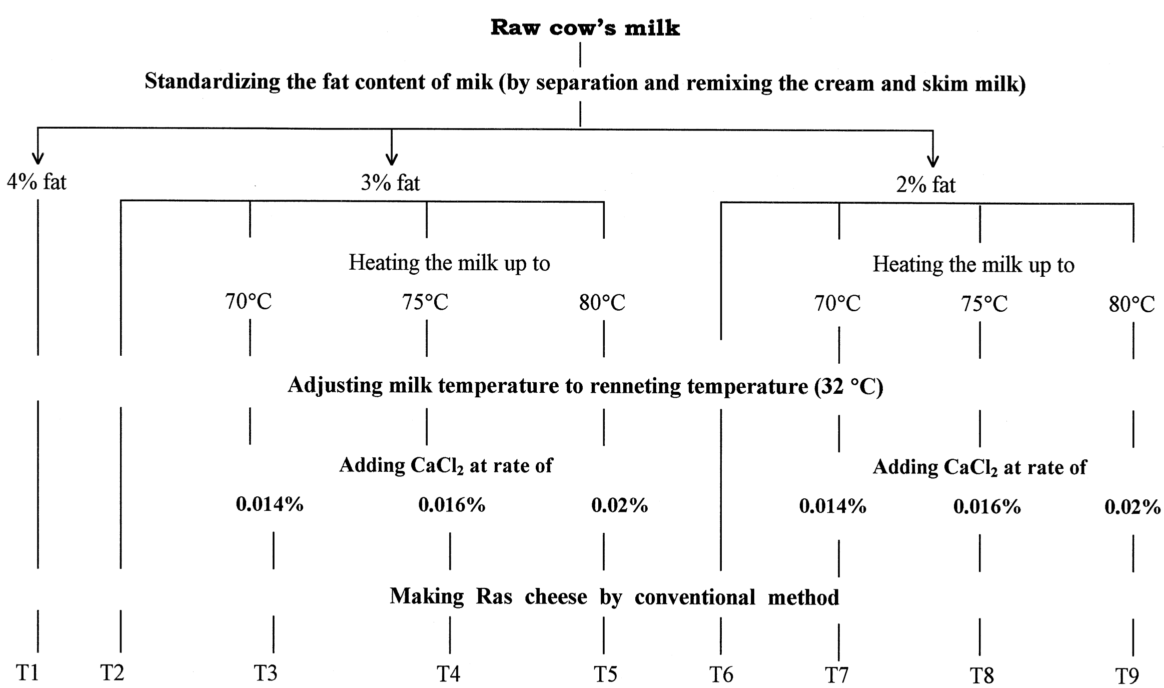

Fig. 1. Treatments of milk for low-fat Ras cheese making.

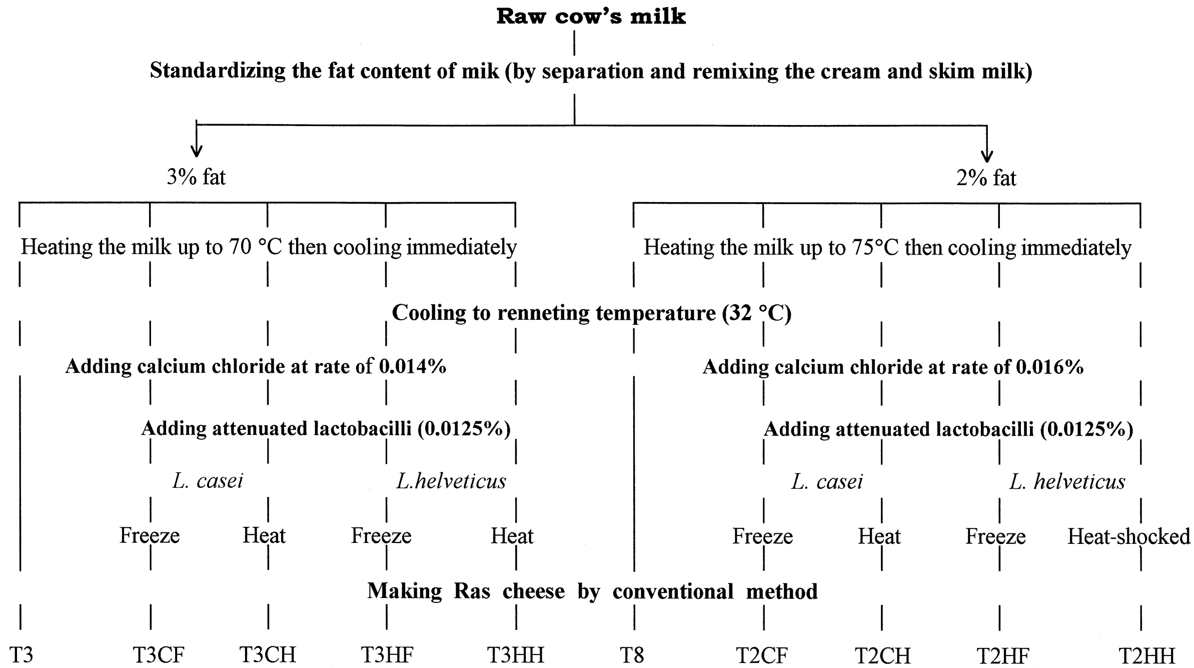

Fig. 2. Treatments of milk for accelerated low-fat Ras cheese making.

treated milk according to Abdel-Tawab (1963) with some modifications as follows: the milk temperature was adjusted to renneting temperature (32°C), calcium chloride was added at the rate of 0.014, 0.016 and 0.020% to milks heated to 70, 75 and 80°C (cooled immediately), respectively. Then 1.0% of starter culture of *Lactococcus lactis* CH. 1 was added. The milk was ripened until acidity developed to 0.19%. Rennet was added at the rate of 30 ml 100 kg^{-1} milk. The milk was thoroughly mixed and left to curdle. Thereafter, the curd was cut vertically and horizontally into cubes using 0.5 inch knives. The curd was stirred and heated gradually to 45°C in 15 min, and held at this temperature until the whey acidity reached 0.14%. About one-third of the whey was drained off and commercial salt was added at the rate of 2%. After 15 min, the whey was completely drained off and the curd was cooled. The curd was then molded and pressed for 24 h. The cheese was turned over every day and rubbed with dry salt for one week. then coated with paraffin wax. Cheese made from heated milk (first experiment) was ripened for 180 days, while accelerated ripening cheese (second experiment) was ripened for 90 days at 15 ± 2°C and 75–80% relative humidity. Each experiment was carried out twice. Sample designations are shown in Figs. 1 and 2.

2.3. Chemical analysis

Cheeses from the first experiment were sampled at the beginning, 90 and 180 days, while accelerated ripening cheeses were sampled at the beginning, and after 45 and 90 days of ripening period. Free amino acids were determined according to Weaver and Kroger (1978) using an amino acid analyzer (Beckman Amino Acid Analyzer 119 CL, Spinco Division of Beckman Instruments, Inc., Palo Alto, California). The method of Miller (1967) was used to determine tryptophan, in extracted free amino acids solution. Biogenic amines were determined at Dr. F. Örsi's lab (Department of Biochemistry and Food Technology, Technical University, Budapest, Hungary) according to the method of Simon-Sarkadi and Hodosi (1995) using an automatic amino acid analyzer (A minochrom. II OE 914, Hungary). Moisture, salt, fat and total nitrogen content were determined according to Ling (1963).

2.4. Statistical analysis

Factorial design 2 factors×2 replicates was used to analyze the data for each effect and Duncan's test was used to make the multiple comparisons (Steel and Torrie, 1980). Significant differences were determined at the $p < 0.05$ level.

3. Results and discussion

The total and individual free amino acids liberated during ripening of low fat Ras cheese made from heated milk are presented in Tables 2–4. Concentrations of individual free amino acids showed wide variations among cheese treatments (Kebary et al., 1996a). Total free amino acids increased ($p \leq 0.05$) throughout the ripening period (Tables 2–4). These results are in agreement with those reported by Badawi (1987); Aly (1990); El-Shibiny et al. (1991), Ezzat and El-Shafei (1991), Khader et al. (1995) and Kebary et al. (1996b), who found that soluble nitrogen increased during ripening of Ras cheese. Heat treatment of milk, prior to cheese making, to a specific temperature significantly increased ($p \leq 0.05$) the formation of free amino acids then decreased it (Tables 2–4). Cheese made from 3% fat milk and heated to 70°C had the highest amount of free amino acids followed by treatments 2, 4 and 5. A higher concentration of free amino acids in T3 (heated) compared with T2 suggests that heat treatment of milk may create favourable conditions for the growth of proteolytic bacteria and/or activity of proteolytic enzymes (Table 1) (Weber and Ramet, 1983). However, raising the temperature up to 75°C (T4) and to 80°C (T5) caused a significant ($p < 0.05$) reduction in their free amino acids content which might be due to the destruction of indigenous milk proteases and proteolytic bacteria and increasing acidity as a result of growth of lactic acid bacteria which suppress the growth of proteolytic bacteria. Total free amino acids of cheese made from 2% fat milk increased significantly ($p \leq 0.05$) by raising the temperature of heat treatments up to 75°C, which could be attributed to the improvement of bacterial growth conditions (Table 1), especially increasing water activity (Weber and Ramet, 1983). Conversely raising the temperature of heat treatments to 80°C (T9) caused a definite decrease ($p < 0.05$) in total free amino acids content (Tables 2–4), which might be due to the pronounced destruction of indigenous milk proteases, proteolytic bacteria and the formation of whey protein–casein complexes. Lowering the fat content of cheese milk caused a marked decrease ($p < 0.05$) in total free amino acids of the resultant cheese (Tables 2–4). These results might be because cheese made from low-fat milk had lower moisture and higher salt contents (Table 1) (lower water activity), which might suppress the growth of proteolytic bacteria and the activity of proteolytic enzymes (Banwart, 1981). Similar trends for soluble nitrogen, tyrosine and tryptophan were reported by Khader et al. (1995). Concentration of tyrosine, tryptophan, histidine and lysine, as affected by heat treatment, fat content or ripening period, followed similar trends of total free amino acids (Tables 2–4), while specific free amino acids showed wide variations.

Table 1
Gross composition of fresh Ras cheese made from heat-treated milk

Cheese samples	Moisture (%)	Fat (%)	Total nitrogen (%)	Salt (%)
T_1[a]	47.26	24.23	2.51	1.77
T_2	44.52	21.65	3.14	1.81
T_3	46.38	20.76	3.55	1.79
T_4	47.43	20.23	3.76	1.74
T_5	50.13	20.67	3.91	1.62
T_6	43.23	18.20	3.87	1.85
T_7	45.35	17.35	4.21	1.81
T_8	46.11	16.32	4.35	1.76
T_9	48.93	14.65	4.61	1.70

Each value is the mean of two replications.
[a] See Fig. 1.

Table 2
Free amino acids of fresh low-fat Ras cheese (mg g^{-1} cheese)

Amino acids	Cheese treatments								
	T1[a]	T2	T3	T4	T5	T6	T7	T8	T9
Aspartic acid	1.31	1.03	1.00	0.95	1.02	1.17	1.05	1.11	0.93
Threonine	0.67	0.54	0.67	0.50	0.66	0.51	0.58	0.57	0.52
Serine	1.04	0.87	0.89	0.79	0.78	0.69	0.82	0.83	0.73
Glutamic	6.37	3.23	6.22	4.18	2.17	3.07	3.27	5.03	2.33
Proline	1.56	1.22	1.38	1.25	1.32	1.12	1.33	1.28	1.12
Glycine	0.40	0.34	0.37	0.37	0.39	0.44	0.34	0.43	0.38
Alanine	0.72	0.54	0.75	0.65	0.61	0.52	0.64	0.62	0.65
Cysteine	0.08	0.08	0.06	0.07	0.08	0.08	0.07	0.08	0.07
Valine	0.85	0.82	0.79	0.56	0.44	0.65	0.71	0.75	0.63
Methionine	0.46	0.39	0.38	0.40	0.39	0.40	0.35	0.41	0.36
Isoleucine	1.18	0.98	0.99	0.92	0.90	1.09	1.09	1.17	1.02
Leucine	1.42	1.19	1.05	1.08	1.00	0.98	1.18	1.22	1.10
Tyrosine	0.97	0.59	0.90	0.70	0.69	0.50	0.83	0.90	0.77
Phenylalanine	0.81	0.51	0.73	0.62	0.57	0.64	0.74	0.81	0.72
Histidine	0.80	0.60	0.84	0.74	0.47	0.53	0.70	0.84	0.65
Lysine	0.82	0.54	0.68	0.38	0.29	0.46	0.55	0.62	0.35
Tryptophan	0.41	0.33	0.39	0.27	0.21	0.25	0.28	0.35	0.31
Arginine	0.47	0.37	0.42	0.21	0.37	0.34	0.35	0.38	0.31
Total	**20.34**	**14.17**	**18.51**	**14.69**	**12.38**	**13.34**	**14.88**	**17.40**	**12.95**

Each value in the tables is the mean of two replications.
[a] See Fig. 1.

Table 3
Free amino acids of low-fat Ras cheese ripened for 90 days (mg g^{-1} cheese)

Amino acids	Cheese treatments								
	T1[a]	T2	T3	T4	T5	T6	T7	T8	T9
Aspartic acid	2.03	1.76	1.64	1.46	1.53	1.44	1.70	1.77	1.46
Threonine	0.98	0.83	0.98	0.81	0.97	0.84	0.88	0.83	0.77
Serine	1.57	1.40	1.34	1.16	1.21	1.11	1.30	1.20	1.04
Glutamic	7.03	4.23	6.87	4.00	4.00	4.87	5.01	5.96	4.08
Proline	2.41	1.93	2.16	1.87	2.00	1.77	1.90	1.96	1.66
Glycine	0.64	0.54	0.54	0.51	0.48	0.64	0.54	0.58	0.56
Alanine	1.11	0.91	1.07	0.97	0.93	0.80	1.00	0.97	0.97
Cysteine	0.13	0.11	0.08	0.13	0.11	0.11	0.10	0.13	0.10
Valine	1.14	1.24	1.13	0.93	0.63	0.93	1.13	1.07	0.91
Methionine	0.68	0.60	0.64	0.56	0.58	0.63	0.53	0.70	0.57
Isoleucine	1.76	1.48	1.57	1.38	1.38	1.46	1.54	1.67	1.54
Leucine	2.14	1.71	1.66	1.68	1.48	1.44	1.70	1.83	1.67
Tyrosine	1.41	0.96	1.43	1.24	1.20	0.98	1.20	1.36	1.14
Phenylalanine	1.21	0.81	1.11	1.07	0.86	0.91	1.10	1.14	1.11
Histidine	1.50	1.43	1.50	1.27	1.00	1.04	1.11	1.21	1.07
Lysine	1.10	0.71	0.83	0.58	0.56	0.64	0.68	0.76	0.51
Tryptophan	0.64	0.44	0.58	0.51	0.40	0.31	0.46	0.53	0.43
Arginine	0.74	0.64	0.67	0.54	0.53	0.54	0.53	0.51	0.44
Total	**28.22**	**21.73**	**25.80**	**20.67**	**19.85**	**20.46**	**22.41**	**24.18**	**20.03**

Each value in the tables is the mean of two replications.
[a] See Fig. 1.

Development of biogenic amines in low fat cheese made by heating milk is shown in Table 5. Total biogenic amines increased significantly ($p \leq 0.05$) as ripening period progressed (Degheidi et al., 1992). Similar results were reported for other cheeses (Joosten, 1988; Darwish, 1993; Abd Alla et al., 1996 and Petridis and Steinhart, 1996a). Total biogenic amines of cheeses made from 3% fat milk increased significantly ($p < 0.05$) by heating the milk up to 70°C, while they decreased in cheeses made by raising heat treatments to 75 and 80°C respectively (Table 5). Moreover, heating the milk up to 75°C increased total biogenic amines content of cheese made from 2% fat milk; on the other hand, biogenic amines decreased in cheese made from milk heated to 80°C (Table 5). These results indicate that there is no effect of incorporation of whey proteins into cheese curd on the formation of biogenic amines, but the main effect of heat treatment may be due to the creation of favourable conditions for the growth of bacteria and/or enzyme activity. Concentration of total biogenic amines decreased significantly ($p < 0.05$) as the fat content of cheese milk was decreased (T1, T2 and T6) (Table 5), which might be due to the decrease of water activity (Table 1) (decreasing moisture and increasing salt content) as mentioned previously. These results suggest that there is a positive correlation between liberation of total free amino acids and development of total biogenic amines ($r = 0.97$). Also, moisture and salt content significantly affected the formation of biogenic amines. Changes in putrescine, cadaverine and tyramine concentration, as affected by milk fat content, heat treatment of milk, or ripening period, followed similar trends of total biogenic amines and total free amino acids. Tyramine, histamine, tryptamine, phenylethylamine, putrescine, cadaverine and spermine were detected in all cheese treatments at any age, while spermidine was not detected in some fresh cheeses. Tyramine was the highest biogenic amine in all cheese treatments, while spermidine was the lowest biogenic amine in cheese treatments. There are positive correlations among tyrosine, histidine, lysine and tyramine, histamine and cadaverine, respectively. However, other biogenic amines and free amino acids showed wide variations, which suggested that the presence of free amino acids is important for developing the biogenic amines in addition to the presence of bacteria that are capable of producing biogenic anunes (Edwards and Sandine, 1981; Joosten and Northolt, 1987).

Changes in total and individual free amino acids of Ras cheese made with added attenuated lactobacilli cells are presented in Tables 6–8. Concentrations of total free amino acids increased gradually ($p \leq 0.05$) in all cheese

Table 4
Free amino acids of low-fat Ras cheese ripened for 180 days (mg g^{-1} cheese)

Amino acids	Cheese treatments								
	T1[a]	T2	T3	T4	T5	T6	T7	T8	T9
Aspartic acid	2.86	2.48	2.36	2.14	2.26	2.30	2.40	2.52	2.12
Threonine	1.38	1.20	1.38	1.18	1.38	1.18	1.32	1.18	1.12
Serine	2.26	1.96	2.06	1.70	1.88	1.62	1.88	1.72	1.44
Glutamic	8.52	6.50	8.19	5.76	5.54	6.96	6.63	8.60	5.34
Proline	3.34	2.86	3.06	2.80	3.04	2.68	2.60	2.70	2.44
Glycine	0.92	0.80	0.78	0.86	0.72	0.92	0.80	0.82	0.74
Alanine	1.56	1.34	1.54	1.40	1.38	1.14	1.44	1.40	1.38
Cysteine	0.18	0.16	0.18	0.14	0.16	0.16	0.16	0.20	0.14
Valine	1.72	1.74	1.60	1.44	1.08	1.30	1.60	1.52	1.30
Methionine	0.98	0.96	0.94	0.80	0.84	0.94	0.76	1.00	0.84
Isoleucine	2.48	2.18	2.36	2.06	2.00	2.06	2.18	2.32	2.18
Leucine	3.02	2.62	2.44	2.38	2.16	2.20	2.46	2.64	2.34
Tyrosine	2.06	1.40	1.82	1.66	1.54	1.50	1.72	1.92	1.72
Phenylalanine	1.72	1.16	1.85	1.48	1.38	1.30	1.52	1.64	1.50
Histidine	1.80	1.54	1.90	1.82	1.56	1.46	1.67	1.78	1.58
Lysine	1.12	0.76	0.93	0.84	0.78	0.64	0.76	0.86	0.80
Tryptophan	0.94	0.82	0.94	0.78	0.84	0.72	0.78	0.94	0.90
Arginine	1.10	1.14	1.00	0.76	0.76	0.80	0.74	0.78	0.68
Total	**37.96**	**31.62**	**35.33**	**30.00**	**29.30**	**29.88**	**31.42**	**34.54**	**28.56**

Each value in the tables is the mean of two replications.
[a] See Fig. 1.

Table 5
Biogenic amines of low-fat Ras cheese (mg 100 g^{-1} cheese)

Biogenic amines	Cheese treatments								
	T1[a]	T2	T3	T4	T5	T6	T7	T8	T9
Fresh									
Putrescine	2.10	2.00	2.30	2.03	1.20	1.90	1.63	2.07	1.33
Cadaverine	1.67	1.00	1.10	0.83	0.90	1.07	1.10	1.37	0.93
Spermidine	0.00	0.10	0.03	0.13	0.07	0.00	0.07	0.17	0.10
Spermine	2.43	1.63	1.64	1.32	1.18	1.27	0.44	1.48	0.78
Tyramine	4.07	3.17	3.37	2.90	2.73	3.00	3.60	3.40	2.60
Histamine	2.90	2.90	3.03	3.30	2.83	2.37	3.87	2.70	2.10
Tryptamine	0.13	0.08	0.11	0.08	0.05	0.07	0.09	0.10	0.07
Phenylethylamine	0.07	0.06	0.08	0.03	0.04	0.06	0.07	0.09	0.05
Total	**13.37**	**10.94**	**11.66**	**10.62**	**9.00**	**9.74**	**10.87**	**11.38**	**7.96**
90 days									
Putrescine	2.80	2.70	2.80	2.64	1.28	2.28	2.44	2.92	1.76
Cadaverine	1.92	1.58	1.68	1.20	0.96	1.40	1.20	1.92	1.40
Spermidine	0.12	0.12	0.20	0.32	0.16	0.00	0.12	0.36	0.04
Spermine	2.44	1.73	2.36	1.61	1.79	1.70	1.85	2.29	0.90
Tyramine	6.20	4.34	5.36	4.40	4.84	4.28	4.52	5.24	3.84
Histamine	5.68	4.64	4.40	3.76	3.88	4.16	4.20	4.60	3.56
Tryptamine	0.27	0.19	0.21	0.17	0.12	0.13	0.15	0.14	0.18
Phenylethylamine	0.17	0.14	0.15	0.14	0.09	0.09	0.10	0.15	0.16
Total	**19.60**	**15.44**	**17.16**	**14.24**	**13.12**	**14.04**	**14.68**	**17.62**	**11.84**
180 days									
Putrescine	3.75	3.60	4.00	3.50	1.80	3.15	2.85	3.85	2.15
Cadaverine	2.45	1.80	2.30	1.40	1.35	1.70	2.05	2.80	1.85
Spermidine	0.25	0.15	0.10	0.45	0.30	0.05	0.25	0.65	0.20
Spermine	3.26	2.65	2.91	2.22	1.88	2.30	2.19	2.31	1.10
Tyramine	8.00	6.85	7.50	6.05	6.60	7.05	7.00	7.15	4.45
Histamine	7.45	6.00	6.25	5.35	5.00	5.60	6.20	5.05	5.00
Tryptamine	0.33	0.22	0.25	0.21	0.22	0.21	0.19	0.23	0.18
Phenylethylamine	0.26	0.18	0.19	0.17	0.15	0.14	0.17	0.21	0.22
Total	**25.75**	**21.45**	**23.50**	**19.35**	**17.30**	**20.20**	**20.90**	**22.25**	**15.15**

Each value in the tables is the mean of two replications.
[a] See Fig. 1.

treatments as ripening period progressed. Cheeses made with attenuated lactobacilli had higher ($p \leq 0.05$) concentrations of total free amino acids than cheeses made without lactobacilli (Tables 6–8). These results might be due to the presence of a wide range of proteolytic enzyme systems in lactobacilli (Aly, 1990; El-Soda et al., 1991; Ezzat and El-Shafei, 1991; Kanawija et al., 1993; Sasaki et al., 1995). Cheese made with either freeze-or heat-shocked *L. helveticus* had a higher ($p \leq 0.05$) concentration of total free amino acids than corresponding

Table 6
Effect of acceleration ripening on free amino acids of fresh low-fat Ras cheese (mg g^{-1} cheese)

Amino acids	Cheese treatments									
	T3[a]	T_3CH	T_3HH	T_3CF	T_3HF	T_8	T_2CH	T_2HH	T_2CF	T_2HF
Aspartic acid	1.00	1.15	1.29	1.42	1.26	1.11	1.30	1.35	1.47	1.55
Threonine	0.67	0.51	0.60	0.70	0.70	0.57	0.52	0.51	0.74	0.63
Serine	0.89	0.82	0.75	0.81	0.88	0.83	0.72	0.78	0.83	0.74
Glutamic	6.22	8.89	8.66	9.32	8.98	5.03	7.54	7.75	8.50	7.97
Proline	1.38	1.68	1.72	1.93	1.81	1.28	1.63	1.70	1.89	2.01
Glycine	0.37	0.24	0.30	0.34	0.35	0.43	0.32	0.29	0.34	0.36
Alanine	0.75	0.55	0.51	0.57	0.57	0.62	0.53	0.56	0.40	0.55
Cysteine	0.06	0.06	0.07	0.05	0.13	0.08	0.09	0.08	0.10	0.09
Valine	0.79	0.68	0.70	0.97	0.92	0.75	0.70	0.75	0.81	0.79
Methionine	0.38	0.32	0.36	0.39	0.46	0.41	0.37	0.35	0.42	0.40
Isoleucine	0.99	0.75	0.70	0.68	0.76	1.17	0.70	0.73	0.70	0.78
Leucine	1.05	1.21	1.36	1.17	1.40	1.22	1.35	1.34	1.15	1.45
Tyrosine	0.90	1.18	1.22	1.27	1.36	0.90	1.03	1.10	1.12	1.20
Phenylalanine	0.73	0.52	0.68	0.64	0.62	0.81	0.59	0.57	0.50	0.57
Histidine	0.84	0.90	0.93	0.97	1.01	0.84	0.74	0.76	0.75	0.82
Lysine	0.68	1.17	1.20	1.08	1.41	0.62	1.22	1.20	1.35	1.34
Tryptophan	0.39	0.25	0.51	0.46	0.57	0.35	0.33	0.40	0.47	0.49
Arginine	0.42	0.53	0.69	0.53	0.68	0.38	0.50	0.62	0.41	0.67
Total	**18.51**	**21.41**	**22.25**	**23.30**	**23.87**	**17.40**	**20.18**	**20.84**	**21.95**	**22.41**

Each value in the tables is the mean of two replications.
[a] See Fig. 2.

Table 7
Effect of acceleration ripening on free amino acids of low-fat Ras cheese ripened for 45 days (mg g^{-1} cheese)

Amino acids	Cheese treatments									
	T3[a]	T_3CH	T_3HH	T_3CF	T_3HF	T_8	T_2CH	T_2HH	T_2CF	T_2HF
Aspartic acid	1.20	1.67	2.14	2.31	2.06	1.24	1.77	1.89	1.83	2.30
Threonine	0.72	0.81	0.86	1.01	0.96	0.58	0.81	0.90	1.06	0.84
Serine	0.98	1.07	1.17	1.24	1.33	0.84	1.13	1.08	1.03	0.96
Glutamic	5.02	9.37	9.51	10.71	10.69	4.17	9.53	9.93	10.32	9.11
Proline	1.58	2.71	2.60	2.91	3.16	0.137	2.36	2.26	2.43	2.88
Glycine	0.39	0.41	0.50	0.43	0.51	0.41	0.39	0.43	0.54	0.53
Alanine	0.78	0.86	0.91	0.72	0.77	0.68	0.84	0.77	0.86	0.91
Cysteine	0.06	0.11	0.13	0.18	0.14	0.09	0.11	0.09	0.13	0.10
Valine	0.82	1.08	1.01	1.11	1.24	0.75	0.91	1.00	1.17	1.23
Methionine	0.47	0.64	0.54	0.57	0.73	0.70	0.54	0.44	0.48	0.56
Isoleucine	1.15	1.10	1.01	1.14	1.27	1.17	1.06	1.10	0.94	1.21
Leucine	1.21	2.17	1.83	1.90	2.17	1.28	1.81	2.00	1.93	2.17
Tyrosine	1.04	1.73	1.83	1.81	1.96	0.95	1.57	1.78	1.71	1.97
Phenylalanine	0.81	0.98	0.83	0.80	0.86	0.80	0.71	0.91	1.00	0.90
Histidine	1.10	1.36	1.47	1.44	1.56	0.85	1.06	1.10	1.20	1.24
Lysine	0.60	1.83	1.96	2.01	2.17	0.53	1.60	1.76	1.72	1.97
Tryptophan	0.42	0.60	0.68	0.76	0.84	0.37	0.63	0.68	0.76	0.86
Arginine	0.49	0.86	0.93	0.74	1.03	0.36	0.81	0.89	0.80	0.86
Total	**18.84**	**29.36**	**29.91**	**31.79**	**33.45**	**17.14**	**27.64**	**29.01**	**29.91**	**30.60**

Each value in the tables is the mean of two replications.
[a] See Fig. 2.

Table 8
Effect of acceleration ripening on free amino acids of low-fat Ras cheese ripened for 90 days (mg g^{-1} cheese)

Amino acids	Cheese treatments									
	T3[a]	T_3CH	T_3HH	T_3CF	T_3HF	T_8	T_2CH	T_2HH	T_2CF	T_2HF
Aspartic acid	1.64	2.68	2.98	3.14	2.80	1.77	2.58	2.76	2.84	3.18
Threonine	0.98	1.20	1.10	1.36	1.42	0.83	1.00	1.30	1.46	1.20
Serine	1.34	1.50	1.58	1.62	1.80	1.20	1.54	1.54	1.36	1.24
Glutamic	6.87	9.47	10.44	11.40	11.74	5.96	11.72	10.55	12.42	10.76
Proline	2.16	3.92	4.02	4.46	4.14	1.96	3.18	2.68	3.24	3.74
Glycine	0.54	0.62	0.70	0.74	0.82	0.58	0.60	0.70	0.84	0.80
Alanine	1.07	1.44	1.36	1.12	1.20	0.97	1.12	1.18	1.28	1.30
Cysteine	0.08	0.24	0.16	0.18	0.26	0.13	0.14	0.16	0.22	0.18
Valine	1.13	1.58	1.52	1.42	1.78	1.07	1.28	1.42	1.60	1.74
Methionine	0.64	0.80	0.74	0.98	1.00	0.70	0.84	0.70	0.78	0.90
Isoleucine	1.57	1.56	1.50	1.66	1.70	1.67	1.40	1.58	1.34	1.62
Leucine	1.66	3.14	2.84	2.56	2.78	1.83	2.58	2.74	2.82	2.78
Tyrosine	1.43	2.51	2.60	2.86	3.06	1.36	2.44	2.86	2.84	3.01
Phenylalanine	1.11	1.34	1.24	1.10	1.20	1.14	1.16	1.04	1.28	1.24
Histidine	1.50	2.00	2.10	2.26	2.34	1.21	1.72	1.76	1.84	2.12
Lysine	0.83	2.60	2.76	2.98	3.12	0.76	2.46	2.80	2.60	2.93
Tryptophan	0.58	0.96	0.98	1.12	1.22	0.53	0.90	1.03	1.02	1.16
Arginine	0.67	1.34	1.32	0.84	1.40	0.51	1.22	1.32	1.00	1.14
Total	**25.80**	**38.90**	**39.94**	**41.80**	**43.78**	**24.18**	**37.88**	**38.12**	**40.78**	**41.04**

Each value in the tables is the mean of two replications.
[a] See Fig. 2.

Table 9
Biogenic amines of accelerated low-fat Ras cheese (mg 100 g^{-1} cheese)

Biogenic amines	Cheese treatments									
	T_3[a]	T_3CH	T_3HH	T_3CF	T_3HF	T_8	T_2CH	T_2HH	T_2CF	T_2HF
Fresh										
Putrescine	2.30	0.08	0.21	0.12	0.29	2.07	0.17	0.04	0.25	0.21
Cadaverine	1.10	0.12	0.50	0.17	0.37	1.37	0.25	0.37	0.46	0.62
Spermidine	0.03	0.08	0.08	0.08	0.21	0.17	0.08	0.04	0.21	0.33
Spermine	1.64	2.17	2.50	2.84	3.18	1.48	2.21	2.40	2.50	2.77
Tyramine	3.37	5.00	5.42	5.50	6.33	3.40	4.71	5.00	5.25	6.02
Histamine	3.03	3.17	3.54	4.21	3.75	2.70	3.37	3.79	3.79	4.12
Tryptamine	0.11	0.19	0.23	0.26	0.31	0.10	0.13	0.16	0.19	0.24
Phenylethylamine	0.08	0.14	0.19	0.23	0.33	0.09	0.08	0.15	0.18	0.23
Total	**11.66**	**10.95**	**12.67**	**13.41**	**14.77**	**11.38**	**11.00**	**11.95**	**12.83**	**14.54**
45 days										
Putrescine	2.18	0.25	0.30	0.25	0.05	2.19	0.05	0.20	0.35	0.40
Cadaverine	1.31	0.25	0.27	0.24	0.35	1.44	0.15	0.50	0.41	0.85
Spermidine	0.16	0.25	0.25	0.15	0.30	0.27	0.05	0.20	0.25	0.30
Spermine	1.84	4.66	4.81	4.96	5.25	1.71	4.54	4.70	4.95	6.00
Tyramine	4.18	7.85	7.95	8.15	8.50	3.93	6.80	7.10	7.15	8.03
Histamine	3.43	5.65	5.90	6.10	6.15	3.45	6.20	6.65	6.75	6.75
Tryptamine	0.16	0.23	0.29	0.34	0.39	0.11	0.17	0.21	0.24	0.29
Phenylethylamine	0.12	0.16	0.23	0.31	0.41	0.11	0.14	0.19	0.25	0.28
Total	**13.38**	**19.30**	**20.00**	**20.50**	**21.40**	**13.21**	**18.10**	**19.75**	**20.35**	**22.90**
90 days										
Putrescine	2.80	0.38	0.55	0.44	0.44	2.92	0.50	0.33	0.44	0.55
Cadaverine	1.68	0.22	0.67	0.44	0.67	1.92	0.22	0.55	1.00	0.83
Spermidine	0.20	0.50	0.33	0.28	0.44	0.36	0.22	0.39	0.33	0.44
Spermine	2.36	6.48	6.52	6.79	7.08	2.29	5.28	5.34	6.69	7.20
Tyramine	5.36	9.40	9.50	9.95	10.00	5.24	8.78	9.05	9.05	10.23
Histamine	4.40	8.02	8.43	8.37	9.24	4.60	8.55	8.61	8.89	9.02
Tryptamine	0.21	0.29	0.33	0.36	0.40	0.14	0.23	0.26	0.29	0.33
Phenylethylamine	0.15	0.23	0.26	0.25	0.33	0.15	0.21	0.25	0.30	0.32
Total	**17.16**	**25.52**	**26.59**	**26.88**	**28.60**	**17.62**	**23.99**	**24.78**	**26.99**	**28.92**

Each value in the tables is the mean of two replications.
[a] See Fig. 2.

cheese made with either freeze- or heat-shocked *L. casei*. These results are in agreement with those reported by Fery et al. (1986), Aly (1990) and Sasaki et al. (1995) who reported that *L. helveticus* was more effective in proteolysis than *L. casei*. However, addition of freeze-shocked lactobacilli caused a definite ($p \leq 0.05$) increase in total free annino acid in the resultant cheese compared with cheeses made with heat-shocked lactobacilli (Tables 6–8), which might be attributed to the lower destruction of proteolytic enzymes during freezing than during heating. Similar trends of total free amino acids were reported for soluble nitrogen, tyrosine and tryptophan (Kebary et al., 1996b). Changes in concentrations of tyrosine, lysine, histidine and tryptophan as affected by ripening period, addition of attenuated lactobacilli, method of attenuation and type of bacteria, followed similar trends of total free amino acids, while individual free amino acids showed wide variations among cheese treatments (Tables 6–8).

Formation of biogenic amines in Ras cheese made with attenuated lactobacilli is presented in Table 9. Total biogenic amines in all cheese treatments increased significantly ($p \leq 0.05$) during ripening (Degheidi et al., 1992). Cheeses made with attenuated lactobacilli had higher concentrations of total biogenic amines than cheese made without lactobacilli. Moreover, addition of either freeze- or heat-shocked *L. helveticus* was more effective in promoting the build up of biogenic amines than addition of freeze- or heat-shocked *L. casei* (Table 9). Cheese made with freeze-shocked lactobacilli contained higher amounts of total biogenic amines than corresponding cheese made with heat-shocked lactobacilli (Table 9). Tyramine, histamine, spermine and tryptamine followed similar trends of total biogenic amines, while other biogenic amines showed wide variations among cheese treatments. There is a good positive correlation between total free amino acids and total biogenic amines. These results indicate that, in addition to the importance of presence of free amino acids, the kind of bacteria is crucial for the formation of biogenic amines in cheese.

It can be concluded that heating of 3% fat milk up to 70°C and 2% fat milk up to 75°C causes a significant ($p \leq 0.05$) increase in concentration of biogenic amines and total free amino acids, while raising the temperature above that decreases the formation of biogenic amines and total free amino acids. Total biogenic amines and total free amino acids content decreases as the fat content of cheese milk is decreased. Addition of lactobacilli prior to cheese-making increases the formation of biogenic amines and free amino acids. However, addition of *L. helveticus* is more effective for increasing the formation of biogenic amines and liberation of free amino acids than *L. casei*. These results suggest that prolongation of ripening period and kind of bacteria are crucial factors affecting formation of biogenic amines.

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