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Changes in organoleptic quality during ripening of cheese made from cows and soya milk blends, using microbial rennet

Meenakshi Rani* & N. S. Verma

Department of Food Science and Technology, G. B. Pant University of Agriculture and Technology, Pantnagar-263 145, India

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The organoleptic characteristics of cheese made from various cows and soya milk blends are directly related to the proportion of soya milk in the blend. The sensory attributes of cheese samples made from cows milk, using calf rennet, did not differ significantly from those of cows milk cheese made with microbial rennet. Soya cheese samples were prepared from different cows and soya milk blends and were evaluated for their sensory properties. Flavour and body texture scores of soya cheese decreased with increase in proportion of soya milk in the blend, whereas the color score increased with increase in the proportion of soya milk in the blend. However, during ripening, the sample made from a blend containing even 5% soya milk differed significantly from the control in respect of flavour, as well as in body and texture.

INTRODUCTION

Cheese is considered to be the best way of conserving milk (Davis, 1965). Among the cheese varieties, cheddar, a medium-hard cheese, is popular all over the world; it is the variety that is usually made in India due to its better keeping quality, consumer acceptability and mild flavour (Scott, 1979). In spite of significant increase in milk production in the country, fluid milk is not available to the common man, due to the enormous increase in population. With the increase in population and wide prevalence of protein malnutrition, attempts are being made to utilise protein from several unconventional sources (Liener, 1972). Soya beans, being very important nutritionally, are receiving priority in agricultural production. Therefore, milk made from soya beans could well be used to replace some part of the animal milk used in the production of cheddar-type cheese. Attempts at extending cheese with soy proteins have generally resulted in products of satisfactory quality (Hang & Jackson, 1976; Metwalli *et al.*, 1982*a*; Del Valle *et al.*, 1984).

Soya proteins are unique among plant proteins by virtue of their relatively high biological value and essential amino acid content. Therefore, the fortification of various milk products with soya bean could ameliorate the problem of milk availability (Kellort, 1971; Schroder *et al.*, 1973; Kumar & Angelo, 1983). Since the use of calf rennet is banned in the country — due to religious sentiments — microbial rennet is being used for cheese manufacture. Hence, a microbial rennet has been used in the present studies for making cheese from mixtures of cows milk and soya milk. Coagulation properties of cows and soya milk blends with microbial rennet were studied (Meenakshi Rani & Verma, 1994). Cheese thus obtained was ripened at 8 ± 1°C for 240 days and compared for sensory quality with that obtained by using calf rennet during ripening.

MATERIALS AND METHODS

Cows milk was obtained from the Livestock Research Centre of the University. The milk was not obtained from any particular breed of cow, but was representative of the mixed milks generally available in India. Mature soya beans of the variety 'PK-262' were obtained from the University Farm. Modilase, a double-strength microbial rennet produced from *Mucor miehei*, and calf rennet were obtained from Chr. Hansens Laboratory Inc. (Denmark), and from Australia, respectively. *Streptococcus lactis* was obtained from the National Dairy Research Institute, Karnal. A bulk culture was prepared by using skimmed cows milk.

*To whom correspondence should be addressed at: Department of Plantation Products and Flavour Technology, Central Food Technological Research Institute, Mysore-570 013, India.

Preparation of soya milk and cheese

Soya milk was prepared according to the method described by Nelson *et al.* (1976) (see Fig. 1). Cheese was prepared according to the method shown in Fig. 2. Each sample was made in triplicate in a 100-litre vat, and the volume of milk used each time was 30 litres. Standarized cows milk (casein/fat = 0·7) was pasteurised at 62°C for 30 min, and soya milk, heated to 100°C for 5 min, was added to it in different proportions (95:5, 90:10, 85:15, 80:20 and 75:25). Required amounts of bulk starter culture and coagulant were added to different blends of cows milk and soya milk at 30°C. The resulting curd was cut with cheese knives and scalded up to 37°C.

Moisture in cheese was determined gravimetrically (MIF, 1959), protein, pH and total solids were determined by standard methods (ISI, 1961). Fat, ash and titratable acidity of cheese samples were determined by methods recommended by AOAC (1975). The soluble protein was determined by the method described by Kosikowski (1966). The free fatty acids (μmol/g fat) were estimated as percentage oleic acid, as per the method described by Rama Murthy and Narayanan (1974). The requirement of clotting enzyme was determined as suggested by Davis (1965).

Sensory evaluation

The samples of cheese made from cows:soya milk blends and drawn at intervals of one month were evaluated for sensory characteristics, by an untrained laboratory panel consisting of 10 members. The panellists were requested to record their judgement on the prescribed proforma provided with a gross rating scale. The data thus obtained were analysed statistically, using two way analysis of variance as described by Snedecor and Cochran (1967). Least significant differences among treatments were determined using Duncan's multiple range test.

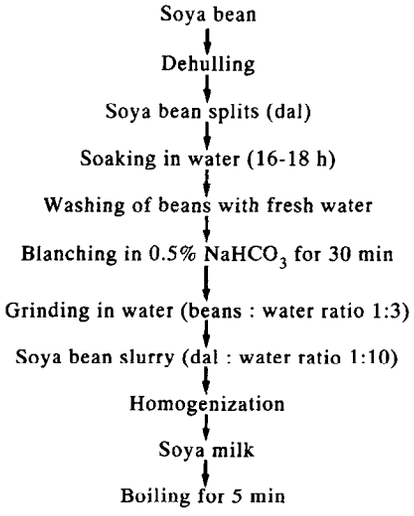

Fig. 1. Flow diagram for manufacturing soya milk.

RESULTS AND DISCUSSION

Table 1 shows that the moisture in green soy cheese ranged from 40·60 to 49·05%. The moisture content increased as the proportion of soya milk was increased in the blends. Increase in percentage moisture could be attributed to the hydrophilic nature of soy proteins (Noyes, 1969; Aworh *et al.*, 1987). Fat content in green soy cheese ranged from 44·30 to 50·17%, and the protein content from 31·20 to 39·75%. The highest contents of fat and protein were observed in green soya cheese made from a blend containing 95% cows milk, and the lowest fat and protein contents were found in a cheese made with a blend having 75% cows milk. Salt content, however, was highest (3·47%) in the cheese made with a blend containing 75% cows milk, and lowest (2·44%) in the cheese made with a blend having 95% cows milk. The protein, fat and salt contents on a dry weight basis of cheese made with pure cows milk did not show any change during ripening. But a decrease was observed in the protein and fat content in cheese made with blends of cows and soy milks (El-Sokkary & Hassan, 1952; Tewari, 1982; Jha, 1984; Reddy *et al.*, 1984; Hwang *et al.*, 1987); on the other hand, there was

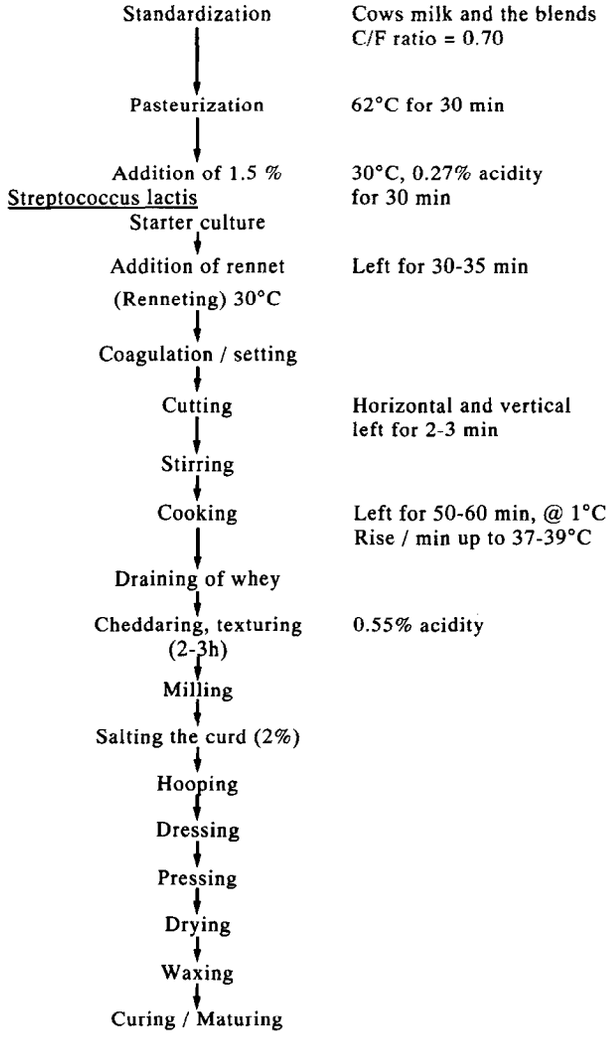

Fig. 2. Flow diagram for manufacturing cheese.

Table 1. Compositional changes[a] (dwb, %) of cheese made from different blends of cows:soya milk using microbial rennet

Blend[b] (cows:soya milk)	Ripening period (months)								
	0	1	2	3	4	5	6	7	8
Moisture									
100:00	38·30 ± 5·90	36·35 ± 6·38	36·25 ± 6·06	36·10 ± 6·04	35·90 ± 5·98	35·70 ± 5·95	35·60 ± 5·93	35·50 ± 5·90	35·40 ± 5·90
100:00	37·90 ± 6·33	37·00 ± 6·17	36·81 ± 6·14	36·52 ± 6·09	36·32 ± 6·05	36·13 ± 6·02	35·73 ± 5·92	35·53 ± 5·87	35·23 ± 5·87
95:5	40·60 ± 6·77	40·05 ± 6·68	39·95 ± 6·66	39·83 ± 6·64	39·64 ± 6·61	39·44 ± 6·57	39·24 ± 6·54	39·14 ± 6·49	38·94 ± 6·49
90:10	40·80 ± 6·80	40·50 ± 6·75	40·30 ± 6·72	40·11 ± 6·69	39·91 ± 6·65	39·69 ± 6·62	39·59 ± 6·60	39·39 ± 6·57	39·19 ± 6·53
85:15	41·55 ± 6·93	41·15 ± 6·86	41·06 ± 6·84	40·96 ± 6·83	40·76 ± 6·79	40·57 ± 6·76	40·37 ± 6·73	40·17 ± 6·67	40·03 ± 6·67
80:20	42·40 ± 7·07	42·40 ± 7·07	42·15 ± 7·03	42·05 ± 7·01	41·90 ± 6·85	41·65 ± 6·94	41·30 ± 6·86	41·15 ± 6·84	41·05 ± 6·84
75:25	49·05 ± 8·18	48·95 ± 8·16	48·75 ± 8·13	48·56 ± 8·09	48·15 ± 8·03	48·15 ± 8·03	48·05 ± 8·01	47·96 ± 8·00	47·76 ± 7·96
Protein									
100:00	38·82 ± 6·47	37·74 ± 6·29	37·74 ± 6·29	37·67 ± 6·28	37·57 ± 6·26	36·57 ± 6·10	37·47 ± 6·25	37·42 ± 6·24	37·37 ± 6·23
100:00	40·24 ± 6·80	39·76 ± 6·63	39·72 ± 6·62	39·56 ± 6·59	39·45 ± 6·58	39·33 ± 6·56	39·10 ± 6·52	38·99 ± 6·50	38·98 ± 6·50
95:5	39·75 ± 6·63	39·85 ± 6·64	39·48 ± 6·58	39·42 ± 6·57	39·31 ± 6·55	39·18 ± 6·53	39·91 ± 6·65	39·90 ± 6·65	38·96 ± 6·49
90:10	38·77 ± 6·46	38·66 ± 6·44	38·49 ± 6·42	38·50 ± 6·42	38·39 ± 6·40	38·25 ± 6·38	38·21 ± 6·37	38·10 ± 6·35	38·67 ± 6·45
85:15	37·00 ± 6·17	36·92 ± 6·15	36·90 ± 5·99	36·89 ± 6·15	36·79 ± 6·13	36·67 ± 6·11	36·57 ± 6·10	36·47 ± 5·97	36·40 ± 6·07
80:20	36·37 ± 6·06	36·30 ± 6·05	36·34 ± 5·06	36·29 ± 6·05	36·21 ± 6·10	36·11 ± 6·08	35·86 ± 5·98	35·79 ± 5·97	35·74 ± 5·96
75:25	31·20 ± 5·20	30·86 ± 5·14	30·79 ± 5·13	30·67 ± 5·11	30·53 ± 5·09	30·38 ± 5·06	30·33 ± 5·06	30·28 ± 5·88	30·15 ± 5·03
Fat									
100:00	51·09 ± 8·52	51·10 ± 8·30	50·09 ± 8·35	50·59 ± 8·43	51·09 ± 8·52	51·72 ± 8·62	51·94 ± 8·66	52·16 ± 8·69	52·03 ± 8·67
100:00	50·13 ± 8·36	50·16 ± 8·36	50·02 ± 8·34	50·19 ± 8·37	50·70 ± 8·45	51·17 ± 8·53	51·16 ± 8·53	51·34 ± 8·56	51·39 ± 8·57
95:5	50·17 ± 8·36	50·35 ± 8·39	50·36 ± 8·39	50·76 ± 8·46	50·70 ± 8·45	50·63 ± 8·44	50·66 ± 8·44	50·59 ± 8·43	50·70 ± 8·45
90:10	49·14 ± 8·19	49·03 ± 8·17	49·04 ± 8·17	49·04 ± 8·17	49·54 ± 8·26	49·88 ± 8·31	49·74 ± 8·29	49·73 ± 8·29	49·58 ± 8·26
85:15	48·93 ± 8·16	49·28 ± 8·21	49·54 ± 8·26	49·80 ± 8·30	49·97 ± 8·33	50·14 ± 8·36	50·31 ± 8·39	50·23 ± 8·37	50·19 ± 8·37
80:20	49·13 ± 8·19	49·48 ± 8·25	49·78 ± 8·30	49·87 ± 8·31	49·90 ± 8·32	49·70 ± 8·28	49·57 ± 8·26	49·48 ± 8·25	49·47 ± 8·25
75:25	44·30 ± 7·38	43·85 ± 7·31	43·95 ± 7·33	44·20 ± 7·37	44·41 ± 7·40	44·52 ± 7·42	44·62 ± 7·44	44·90 ± 7·48	44·93 ± 7·49
Salt content									
100:00	2·27 ± 0·38	2·23 ± 0·37	2·24 ± 0·37	2·25 ± 0·38	2·26 ± 0·38	2·28 ± 0·38	2·29 ± 0·38	2·28 ± 0·38	2·29 ± 0·38
100:00	2·25 ± 0·38	2·23 ± 0·37	2·25 ± 0·38	2·25 ± 0·38	2·27 ± 0·38	2·26 ± 0·38	2·27 ± 0·38	2·27 ± 0·38	2·28 ± 0·38
95:5	2·44 ± 0·41	2·46 ± 0·41	2·45 ± 0·41	2·46 ± 0·41	2·47 ± 0·42	2·48 ± 0·41	2·49 ± 0·42	2·50 ± 0·42	2·51 ± 0·42
90:10	2·48 ± 0·41	2·44 ± 0·42	2·49 ± 0·42	2·54 ± 0·42	2·53 ± 0·42	2·54 ± 0·42	2·55 ± 0·43	2·56 ± 0·43	2·57 ± 0·43
85:15	2·74 ± 0·46	2·74 ± 0·46	2·75 ± 0·46	2·74 ± 0·46	2·77 ± 0·46	2·78 ± 0·46	2·78 ± 0·46	2·77 ± 0·46	2·78 ± 0·46
80:20	3·00 ± 0·50	2·82 ± 0·47	2·83 ± 0·47	2·85 ± 0·48	2·84 ± 0·47	2·84 ± 0·47	2·84 ± 0·47	2·85 ± 0·48	2·85 ± 0·48
75:25	3·47 ± 0·58	3·35 ± 0·56	3·36 ± 0·56	3·36 ± 0·56	3·35 ± 0·56	3·36 ± 0·56	3·35 ± 0·56	3·36 ± 0·56	3·35 ± 0·56

[a]Averaged data for triplicates of three batches.
[b]Calf and microbial rennets were used for clotting cows milk while microbial rennet alone was used for clotting cows milk : soya milk blends; dwb: dry weight basis.

Table 2. Changes in the mean scores for sensory attributes during ripening of cheese (at 8 ± 1°C) made from different blends of cows:soya milk using microbial rennet

Blend[a] (cows:soya milk)	Ripening period (months)								
	0	1	2	3	4	5	6	7	8
Flavour[b]									
100:00	31·00	32·80	35·30	35·50+	40·35+	40·00++	41·30++	43·15+++	41·60
100:00	31·50	31·80	32·90	34·90	39·90+	39·95+	41·05+	42·30	40·60
95:5	30·00	29·60	32·60	32·80	35·40	37·35	37·10	39·10	36·10
90:10	28·70	29·00	31·70	30·30	32·65	34·65	36·20	39·00	36·20
85:15	28·40	28·60	30·30	28·90	32·25	33·30	36·00	38·50	35·80
80:20	27·20	27·80	28·10	27·90	30·60	31·15#	35·80#	38·00#	35·60#
75:25	24·10	24·71	26·20	25·50#	30·35#	31·00-	34·10-	37·90–	35·10–
Body and texture[b]									
100:00	25·95	26·20	26·40	26·60	28·20*	28·45**	28·85***	29·25***	28·70
100:00	25·90	26·05	26·20	27·00	28·15**	28·30**	28·55**	28·75**	28·00**
95:5	23·05	23·20	23·70	24·00	26·30*	27·00**	27·00**	27·60**	27·10**
90:10	23·05	22·35	24·30	23·90	25·45	25·70	26·30**	27·90**	26·90*
85:15	23·10	23·30	23·60	23·80	25·40	24·67	26·10*	27·35**	26·60*
80:20	21·40	21·70	22·20	22·70	24·05	24·40	26·00*	27·15**	26·00*
75:25	21·00	20·70	20·90	21·00	23·95	24·10x	25·70x	25·90x	25·10x
Colour									
100:00	9·37	9·30	9·23	9·20	9·15	9·10	9·00	8·90	8·80
100:00	9·53	9·50	9·35	9·30	9·25	9·20	9·30	9·00	8·90
95:5	9·36	9·50	9·26	9·21	9·35	9·10	8·90	8·90	8·80
90:10	9·43	9·39	9·35	9·40	9·15	9·20	9·10	9·00	8·90
85:15	9·36	9·34	9·17	9·22	9·15	9·10	9·00	8·90	8·80
80:20	9·30	9·26	9·23	9·19	9·15	9·10	9·00	8·90	8·80
75:25	9·30	9·24	9·16	9·05	9·15	9·00	8·90	8·80	8·70

[a]Calf and microbial rennets were used for clotting cows milk, while microbial rennet alone was used for clotting cows:soya milk blends.
[b]Symbols: +, bitter; #, off-flavour; –, acidic, x; crumbly; *, close.

an increase in the salt content (Ridha *et al.*, 1984; Jha, 1984).

Changes in sensory characteristics during ripening of cheese are presented in Table 2. The values are the means of ten observations of the cheese samples presented to the panelists for the evaluation of flavour, body and texture, and colour. Soya cheese samples showed normal flavour in the range of 36–37, after 150 days of ripening at 8 ± 1°C. The soya cheese samples made from different blends of cows milk and soya milk (95:5, 90:10, 85:15, 80:20 and 75:25) exhibited maximum flavour scores of 39·1, 39·0, 38·5, 38·0 and 37·9, respectively, after a ripening period of 210 days.

The soya cheese sample, having 25% of soya milk, had an acidic flavour after 150 days of ripening. None of the soya cheese samples were criticised for bitter flavour from the very beginning. This could be attributed to the addition of soya milk which might have played a great role in the formation of non-bitter peptides. The acidic taste of the soya cheese sample having 25% soya milk may be due to the higher percentage of moisture in the cheese sample. These results are in conformity with the results reported by Metwalli *et al.* (1982*b*) and Barlow *et al.* (1989).

The soya cheese samples made from different blends of cows milk and soya milk showed normal body and texture (26) after 180 days of ripening. Thereafter, the scores for body and texture showed a slight improvement, reaching their maximum values after a ripening period of 210 days. However, the soya cheese sample made from a 75:25 blend of cows milk and soya milk showed no improvement in body and texture during later period of ripening and was criticised for crumbliness. The control cheese samples showed granule formation after 90 days of ripening, without much further improvement in body and texture. Aworh *et al.* (1987) also have reported that warankasi cheese, made from 20% soya milk, had a brittle texture; nevertheless, they found it acceptable for consumption. The colour of soya cheese samples made from different blends was on par with the colour of the control sample.

The results of statistical analysis (Tables 3–5) show the effect of ripening on cheese. The mean flavour score of all cheese samples made from various cows and soya milk blends was less than the mean flavour score of cheese made from cows milk alone, using either type of enzyme, and a significant difference at $P \sim 0{\cdot}01$ in the flavour was noted.

Cheese samples made from cows milk with calf and microbial rennets showed no significant flavour difference at $P \sim 0{\cdot}01$ during ripening. The cheese made from a blend containing 5% soya milk showed significant flavour difference when compared with cheeses made from remaining blends, for up to 150 days, in the present series of experiments. After 150 days no significant difference in cheese made from various blends could be detected on account of off-flavour development in all the cheese samples. No significant difference in flavor score was observed in cheese made from 80:20 and 75:25, cows: soya milk blends at any stage during ripening.

The mean score for body and texture for cheese made from cows milk, using calf or microbial rennets, differed significantly at $P \sim 0{\cdot}01$ with cheese made from various blends throughout the ripening period. The cheese made from a blend containing up to 15%

Table 3. Mean values for flavour during ripening (at 8 ± 1°C) of cheese made from the blends of cows:soya milk using microbial rennet

Ripening period (days)	Mean values[a]							CD[b]
	R_1 100:00	R_2 100:00	R_3 95:5	R_4 90:10	R_5 85:15	R_6 80:20	R_7 75:25	
0	31·00	31·50	30·00	28·70	28·40	27·20	24·10	4·02
30	32·80	31·80	29·60	29·00	28·60	27·80	24·70	1·61
60	35·30	32·90	32·60	31·70	30·30	28·10	25·20	3·88
90	35·50	34·40	32·80	30·30	28·90	27·90	25·50	3·19
120	40·35	39·90	35·40	32·65	32·25	30·60	30·35	1·87
150	40·00	39·95	37·35	34·65	33·30	31·15	31·00	1·27
180	41·30	41·05	37·10	36·20	36·00	35·80	34·10	2·89
210	43·15	42·30	39·10	39·00	38·50	38·00	37·90	1·49
240	41·60	40·60	36·40	36·20	35·80	35·60	35·10	1·38

[a]Calf and microbial rennets were used for clotting cows milk while microbial rennet alone was used for clotting cows:soy milk blends. The underlined treatments do not differ significantly at the 1% level of significance.
[b]Critical difference.

soya milk showed a significant difference for body and texture with cheese made from blends containing 20 and 25% soya milk throughout the ripening period. However, no significant difference was observed in the body and texture of cheese made from 80:20 and 75:25, cows:soya milk blends at any stage during ripening.

From the analysis it can be seen that the mean score for colour of cheese made from cows milk, using either enzyme, and cows and soya milk blends, using microbial rennet, did not differ significantly (at $P \sim 0{\cdot}01$) up to 150 days. Thereafter, at 180 days the mean score for colour of cheese made from cows milk and blend containing 10% soya milk using microbial rennet differed significantly (at $P \sim 0{\cdot}05$) from the colour score of cheese made from blend containing 5, 15, 20 and 25% soya milk. However, at 210 and 240 days these samples differed significantly with the samples containing 25% soya milk only at $P \sim 0{\cdot}01$.

Thus, the results of this investigation indicate that cows milk can be replaced to the extent of 15% with soya milk without affecting the sensory characteristics

Table 4. Mean values for body and texture during ripening of cheese made from the blends of cows:soya milk using microbial rennet

Ripening period (days)	Mean values[a]							CD[b]
	R_1 100:00	R_2 100:00	R_3 95:5	R_4 90:10	R_5 85:15	R_6 80:20	R_7 75:25	
0	25·95	25·90	23·05	23·05	23·10	21·40	21·00	1·73
30	26·20	26·05	R_5 23·30	R_3 23·20	R_4 22·35	21·70	20·70	0·97
60	26·20	26·20	R_4 24·30	R_3 23·70	23·60	22·20	20·90	1·83
90	R_2 27·00	R_1 26·60	24·00	23·90	23·80	22·70	21·00	2·08
120	28·20	28·15	26·30	25·45	25·40	24·05	23·95	1·24
150	28·45	28·30	27·00	25·70	24·67	24·40	24·10	1·44
180	28·85	28·55	27·40	26·30	26·10	26·00	25·70	1·38
210	29·25	28·75	R_4 27·90	R_3 27·60	27·35	27·15	25·90	1·08
240	R_1 28·90	R_1 28·70	27·10	26·90	26·60	26·00	25·10	1·71

[a]The underlined treatments do not differ significantly at the 1% level of significance. Calf and microbial rennets were used for clotting cows milk while microbial rennet alone was used for clotting cows:soya milk blends.
[b]Critical difference.

Table 5. Mean values for colour during ripening of cheese made from the blends of cows:soya milk using microbial rennet

Ripening period (days)	Mean values[a]							CD[b]
	R_1 100:00	R_2 100:00	R_3 95:5	R_4 90:10	R_5 85:15	R_6 80:20	R_7 75:25	
180	R_2 9·29	R_4 9·11	R_1 9·00	R_5 9·00	R_6 9·00	R_3 8·90	R_7 8·90	0·21
210	R_2 9·05	R_4 9·01	R_1 8·91	R_3 8·90	R_5 8·90	R_6 8·90	R_7 8·80	0·15
240	R_2 8·91	R_4 8·91	R_1 8·81	R_3 8·81	R_6 8·81	R_5 8·79	R_7 8·71	0·15

[a]The underlined treatments differ significantly at the 5% level of significant at 180 days at the 1% level of significance from 180 to 240 days. Calf and microbial rennets were used for clotting cows milk while microbial rennet alone was used for clotting cows:soya milk blends.
[b]Critical difference.

of cheese adversely. However, the quality of cheese is lowered significantly if there is more than 15% of soya milk in the blend.

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