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Comparative Studies of Casein Breakdown in Cheddar Cheese Manufactured from Lactose-Hydrolysed Milk

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ABSTRACT

Scottish Cheddar cheese (12 trials) was produced from full-fat milk and from the same milk treated with different preparations of β-D-galatosidase. Appreciable hydrolysis of the casein fractions was evident in 6-month old Cheddar cheese using lactose hydrolysing enzyme containing a high level of natural protease. Lactose hydrolysis of milk up to 60% slightly accelerated the ripening process of Cheddar cheese, but greater judge preference of the enzyme-treated cheese was reported by the taste panelists as compared with the control.

Different methods have been used to accelerate the ripening process of Cheddar cheese, and Law (*16*) has recently compiled an updated review. For example, some of these methods include addition of protease and/or lipase enzymes of animal and microbial sources (*14,17,23*), addition of Cheddar cheese slurry (*6,15,29*), use of lysozyme-treated cells (*18*), addition of aged cells of mesophilic lactic acid bacteria to the cheese milk (*26*), use of starter cultures subjected to thermal shock (*27*), use of mutant strains of starter cultures (*7,8,9*), and/or addition of β-D-galactosidase to the milk (*5,10,20,31,32*). The objective of the latter five methods was primarily to increase the level of starter culture in the cheese milk, and thus increasing their metabolic activity during the maturation period.

The literature suggests that accelerated ripening of 'natural' Cheddar cheese could be achieved mainly by addition of enzymes, e.g. protease and/or lipase, but some controversy still exists regarding the exact role of and potential for the use of β-D-galactosidase during the manufacture of Cheddar cheese. Some research workers in different laboratories have reported accelerated Cheddar cheese ripening (*3,20,35*) by using the enzyme while others could not achieve the same effect (*5,22*). As a result of lactose hydrolysis in milk (LHM), the viable count of the starter culture is increased, but such enhanced microbial activity in LHM for cheesemaking or yogurt (*12,34*) was attributed to the presence of low levels of proteolytic enzyme(s) in the β-D-galactosidase used.

This study investigated the effect of using different lactose hydrolysing enzymes containing different levels of protease on the quality and rate of maturation of Cheddar cheese.

MATERIALS AND METHODS

Manufacture of cheese

Scottish Cheddar cheese (12 cheesemaking trials) was manufactured in 205-L vats, and Fig. 1 illustrates the processing schedule on a time basis and the enzymatic treatment of the milk.

Cheese starter culture (three different strains coded 850, 870 and 890) were used to produce cheese, and they were obtained from Chr. Hansen's Laboratory Ltd., Reading, U.K. The cultures were of mixed-strain type, stored in liquid nitrogen, and were used for direct-to-vat inoculation (DVI) at a rate of 32 g/205 L of milk. The viable count of these starter cultures ranged between $2\text{-}3.7 \times 10^{11}$ Colony Forming Unit (CFU)/g.

Standard calf rennet (supplied by Chr. Hansen's Laboratory Ltd., Reading, U.K) was used as a coagulant, and, according to the supplier, it consisted of approximately 80% chymosin and 20% pepsin. The coagulant was used at a rate of 28.4 ml/114 L of milk.

Pure dried vacuum salt (supplied by Imperial Chemical Industries Ltd., Cheshire, UK) was used and mixed thoroughly with milled curd for 15 min.

Two β-D-galactosidase preparations (Maxilact brand supplied by Gist Brocades NV, Delft, Holland) from *Kluyveromyces lactis* were used. The specifications of these enzymes were: – first preparation (coded E_1) was in liquid form and contained 5200 Natural Lactase Units (NLU)/g and 77 Natural Protease Units (NPU)/g and second preparation (coded E_2) was in freeze-dried form and had an activity of 2500 NLU/g and 15000 NPU/g. The extent of lactose hydrolysis in milk was determined by gas-liquid chromatography (*24,28,30*) in a 104 chromatograph manufactured by PYE Unicam Ltd., Cambridge, UK.

Chemical analysis of cheese

Soluble nitrogen filtrate was prepared and analysed according to the procedures of AOAC, Hull and Vakaleris and Price (*4,13,33*). Casein hydrolysis was determined using polyacrylamide gel electrophoresis (PAGE) (*2,11*), and the gels were scanned using an 2202 Ultro Scan Laser Densitometer (LKB Instruments Ltd., Croydon, UK).

Grading and organoleptic assessment

The cheeses were graded at intervals by an official grader of the Company of Scottish Cheesemakers Ltd. and seven panelists of the Department of Dairy Technology. The following points scale was used: flavor and aroma - 45, body and texture - 40, color - 5; and finish and appearance - 10. It was found that the points awarded for color, and finish and appearance were identical for all samples and so these results were excluded from statistical analysis.

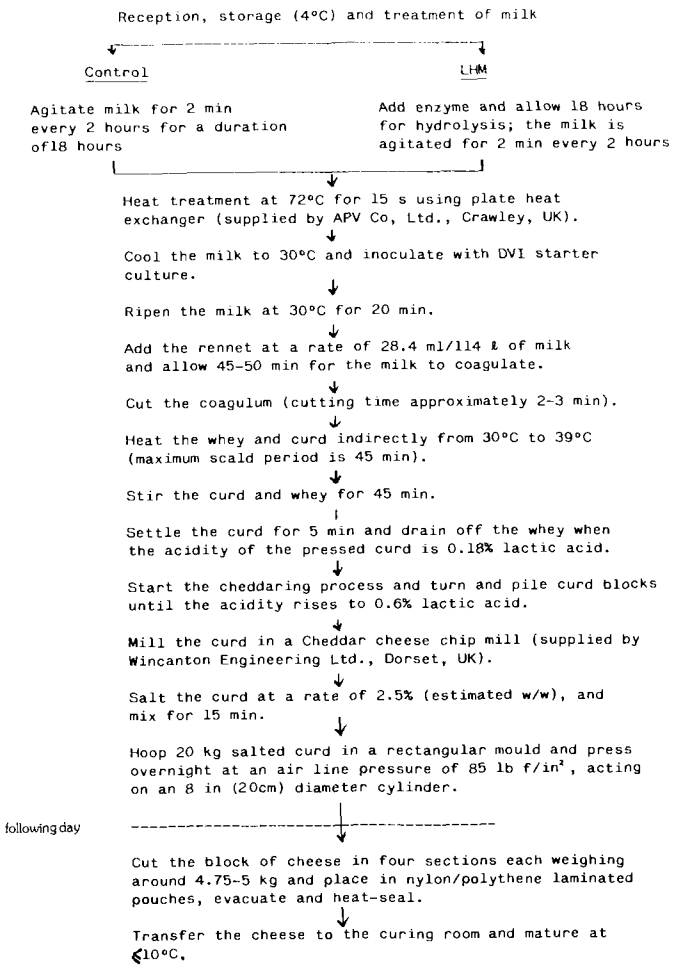

Figure 1. *Flow diagram of milk treatment and cheesemaking process.*

RESULTS AND DISCUSSION

The quality of Cheddar cheese manufactured from whole milk and LHM was evaluated and reported elsewhere (25), and in brief the chemical composition of 1-d-old cheese is summarized in Table 1.

Typical Scottish Cheddar cheese contains 35-36% moisture, but in this present study the high moisture content was mainly experienced in LHM cheese which was primarily due to enhanced activity of the starter culture, i.e. fast acid development during the cheddaring stage, where the extent of lactose hydrolysis was in excess of 57%. However, it can be observed from the above chemical composition that all the cheese produced complied with existing legal standards in the United Kingdom.

The extent of protein or casein hydrolysis in cheese is used as a ripening index during the maturation period, and Table 2 confirms such aspect. The level of soluble nitrogen (SN) gradually increases in Cheddar cheese (control and experimental) as it becomes older. Greater values for SN could be observed in LHM cheese (E_1 and E_2), but the latter enzyme released the highest level of SN, e.g. 5.32-6.21%. The enhanced activity in the cheese (E_2) is mainly attributed to the presence of contaminated protease enzyme (15000 NPU/g), which could have survived the pasteurization treatment of milk and to the increased number of viable count of the starter culture.

PAGE gels were used to study the extent of hydrolysis of casein fractions in cheese (control and experimental) during a 6-month maturation period, and the gels were scanned at $A_{0.5}$ OD. Many peaks were observed in each electrogram, and these peaks could be divided into the following mobility bands: (*a*) slow mobility, (*b*) β casein, (*c*) α_s casein.

The extent of hydrolysis of casein during the maturation period is illustrated in Tables 3 and 4, and the overall pattern of casein degradation could be summarized as follows.

Slow mobility bands

The number of fractions in this mobility zone ranged between 2 and 8, and more fractions were observed in LHM (E_1 and E_2) Cheddar cheese. Differences in the size of these fractions in the experimental cheeses as compared with the control were evident, which could be attributed to the proteolytic activity of β-D-galactosidase used or the enhanced enzymatic activity that originated from the starter culture. However, the overall trend is shown in Tables 3 and 4.

Cheeses (experimental and control) at 4 months old showed the least number of fractions on the gel electrogram, and it is most likely that the casein fraction(s) that fall within such mobility band was readily hydrolysed as compared with α_s casein. The products of hydrolysis could be amino acids, peptides and/or soluble nitrogen (see Table 2).

The observed increase in the number of fractions in the slow mobility zone in all the cheese (at 6 months)

TABLE 1. *Chemical composition of cheese[a] manufactured from milk and LHM.*

Treatment	Moisture (%)	Fat (%)	Fat in dry matter (%)	Moisture in fat free cheese (%)	Salt (%)	pH
C[b]	34.78	33.92	52.00	52.62	1.80	5.07
E^c_1	35.02	34.58	53.21	53.53	1.71	5.10
C	36.53	33.50	52.78	54.92	1.56	5.11
E^d_2	37.68	33.00	52.95	56.93	1.60	5.01

[a]Age of cheese, 1-d-old, and the above figures represent average of 3 trials.
[b]C is control.
[c]E_1 - β-D-galactosidase and its specific activity was 5200 NLU/g and 77 NPU/g.
[d]E_2 - β-D-galactosidase and its specific activity was 2500 NLU/g and 15000 NPU/g.

TABLE 2. *Extent of protein hydrolysis (% SN) during maturation of Cheddar cheese manufactured from whole milk and LHM.*

Starter culture	Treatment[a]	Age of Cheese (SN)[b] One day	2 months	4 months	6 months	Extent of lactose hydrolysis in milk (%)
850	C	1.06	3.19	3.96	4.92	–
	E_1	1.05	3.10	4.19	5.44	28.54
870	C	1.16	3.25	4.18	4.85	–
	E_1	1.10	3.13	4.07	4.75	28.90
890	C	1.15	3.17	3.96	4.86	–
	E_1	1.23	3.30	4.24	4.92	43.60
850	C	1.03	3.36	3.39	5.05	–
	E_2	1.04	3.90	3.66	5.32	57.62
870	C	1.03	3.47	3.50	5.37	–
	E_2	1.08	3.91	4.57	6.21	61.19
890	C	0.91	3.51	3.99	5.29	–
	E_2	0.97	3.53	4.26	5.90	57.01

[a]E_1 and E_2 are different β-D-galactosidase preparations, C is control cheese.
[b]SN soluble protein nitrogen.

could possibly originated from β casein hydrolysis to release nitrogeneous compounds that have bands appearing in the slow mobility zone of the electro gels.

β *Casein*

The number of bands/peaks of β casein in the untreated and LHM Cheddar cheese ranged from 1 to 4, and it can be observed that β casein was progressively reduced as the cheese becomes older. The degree of β casein hydrolysis was greater in the experimental cheese as compared with the control. (See Tables 3 and 4).

The major band of the β casein which was readily hydrolysed was number 2 (Tables 3 and 4), and degradation of such bands contributed to the apparent increase of other bands in this mobility and/or slow mobility zones. The control and experimental cheeses in nine out of 12 trials showed a progressive decrease in the level of total β casein during the maturation period up to six months old. In the remaining three trials, a different trend was found in the β casein hydrolysis. For example, in experimental (E_1) cheese made from starter culture 870, the extent of hydrolysis at 4 months was less than that of the control at the same age. A similar trend was observed for the experimental cheese (E_1 and E_2) made with starter cultures 890 and 870 at 6 months old (see Tables 3 and 4). The reason(s) for such pattern of casein hydrolysis is not well established.

α_s *Casein*

The highest number of bands, i.e. 9, was observed in this mobility zone for all types of cheese. It is apparent that the major hydrolysis was of bands number 3 and 2 in cheeses treated with E_1 and E_2, respectively. The reason(s) of such a pattern is not well established, but the effect of α_s caein hydrolysis in the cheese aged 2 and 4 months had an ultimate effect of the number and size of other bands in this mobility zone. However, an apparent reduction in α_s casein in cheese at 6 months old was observed which could be due to hydrolysis, and the extent of hydrolysis was greater in the enzyme treated cheese.

The above observed patterns of the casein hydrolysis confirm other reports (*1,2,19*), and it is possible to suggest the following: (*a*) β caein is progressively hydrolysed in the cheese and the degraded products contribute to the increased level of SN or free amino acids (FAA) in the cheese, and possibly increasing the level of casein fractions in the slow mobility zone in 6 months old cheese; (*b*) hydrolysis of casein in the slow mobility zone (4 months old cheese) and α_s casein (6 months cheese) may contribute to the SN and FAA pool in Cheddar cheese; (*c*) the possible products of casein hydrolysis during the maturation period of Cheddar cheese are illustrated schematically in Figure 2.

The grading of Cheddar cheese in Scotland is carried out after 8 weeks of maturation by an official grader from the Company of Scottish Cheesemakers Ltd. Cheese in Scotland is graded into one of the following categories: 'choicest', 'first grade', 'first grade early sale', 'graded' and 'no stamp'. In this present study, only one cheese was awarded 'first grade' and the rest were 'graded'. Such grading results were obtained because the cheese was weak in body as result of high moisture and rough texture characteristics.

The organoleptic assessment of the cheese at 2, 4 and 6 months old was carried out by 8 panelists as well as the official grader. The data of cheese characteristics was analysed statistically by using the 'mean difference' between the control and LHM cheese taking into account the type of β-D-galactosidase treatment (E_1 and E_2), the starter culture (850, 870 or 890) and the age of the cheese (2, 4 and 6 months). Table 5 summarizes these results. It can be observed that the grader and all the panelists preferred the experimental cheese (LHM) to the control, i.e. for flavor and aroma, and seven out of eight panelists indicated a preference for body and texture char-

TABLE 3. *Extent of casein hydrolysis (expressed as % of total area) in Cheddar cheese made from milk hydrolysed before manufacture with Maxilact E_1.*

Starter culture	Treatment	Curing period (months)	Slow mobility								β-Casein					α_s-Casein									
			Fraction number							E_1-C*	Fraction number				E_1-C*	Fraction number									E_1-C*
			1	2	3	4	5	6	7		1	2	3	4		1	2	3	4	5	6	7	8	9	
850	C[b]	2	0.25	0.82	2.46	1.40	4.65			+1.59	0.90	40.20	0.56		−2.81	3.09	12.79	21.46	5.05	3.83	0.25	0.38	1.38		+1.78
(28.45%)	E_1[c]		1.37	2.77	0.33	1.10	5.60					38.66	0.19			2.69	12.52	21.09	9.39	0.58	0.37	0.57	2.80		
	C	4	0.26	6.72						+1.61		30.74	0.32		−0.11	14.25	9.91	20.24	7.34	5.08	0.10	3.99	0.22	0.83	−1.50
	E_1		0.29	8.30								30.95				9.94	8.01	35.37	2.32	3.86	0.96				
	C	6	6.65	3.30	4.71	4.20				+0.14	1.91	27.30			−7.09	11.71	12.91	23.79	0.28	1.13	2.11				+6.98
	E_1		3.63	5.81	2.36	0.22	3.63	3.35			1.49	20.25	0.38			10.34	11.35	33.12	0.20	0.43	1.31	2.16			
870	C	2	9.91	1.52	4.61					−3.86	0.86	33.38	0.37	0.52	−4.19	3.49	12.57	30.20	0.60	0.71	0.41	0.87			+8.05
(28.90%)	E_1		3.21	3.18	1.43	4.36					0.92	29.22	0.80			8.11	12.79	30.61	0.58	0.66	0.46	3.69			
	C	4	0.14	5.10						+2.59		28.84	0.35		+0.34	14.41	11.84	21.81	9.11	5.33	2.19	0.90			−2.94
	E_1		7.83									28.63	0.90			14.07	11.06	19.90	7.72	5.59	3.20	0.27	0.84		
	C	6	1.33	8.24	3.50	0.50	0.70	1.67	0.68	−2.23	0.17	23.35			−0.25	17.26	16.22	15.10	9.06	0.16	1.10	1.01			+2.27
	E_1		0.18	3.60	3.99	3.03	3.76					22.66	0.61			13.36	11.76	17.41	9.24	6.75	0.66	3.00			
890	C	2	3.95	1.35	4.83					+1.15	0.84	30.39	0.23	0.64	−1.12	8.60	12.82	32.12	0.37	0.58	3.28				−0.03
(43.60%)	E_1		4.18	1.76	5.34							30.29	0.69			7.94	12.68	32.35	0.55	0.11	0.49	3.62			
	C	4	6.60							+3.16		35.58			−9.47	9.12	6.83	28.19	8.72	0.33	2.54	2.09			+6.31
	E_1		1.65	8.11								26.11				24.56	0.39	33.56	5.35	0.27					
	C	6	8.09	0.95	3.42	2.77				+0.78		22.34	0.60		+0.05	12.82	12.74	17.10	9.92	6.57	0.56	0.65	0.38	1.11	−0.26
	E_1		7.96	1.66	3.37	3.02						22.39	0.60			12.79	12.85	17.16	9.91	6.58	0.53	0.63	0.26	0.88	

[a]Figures in parenthesis represent degree of lactose hydrolysis in milk.

[b]C - Control.

[c]E_1 - β-D-galactosidase and its specific activity was 5200 NLU/g and 77 NPU/g.

* Differences between the total area of the experiment and control cheeses.

TABLE 4. *Extent of casein hydrolysis (expressed as % of total area) in Cheddar cheese made from milk hydrolysed before manufacture with Maxilact E_2.*

Starter culture	Treatment	Curing period (months	Slow Mobility									β-Casein					α_s-Casein									
			Fraction number								E_2-C*	Fraction number				E_2-C*	Fraction number									E_2-C*
			1	2	3	4	5	6	7	8		1	2	3	4		1	2	3	4	5	6	7	8	9	
850	C[b]	2	2.74	2.07	7.64						+1.72		35.76	0.26		−5.65	2.48	14.87	30.81	0.20	0.65	2.52				+3.93
(57.62%)[a]	E_2[c]		3.17	0.12	2.53	8.35						0.52	28.92	0.93			5.94	16.95	30.67	0.26	0.62	1.02				
	C	4	0.49	4.95							+5.47		32.39			−7.84	12.78	7.06	27.00	11.37	2.96	1.01				+2.35
	E_2		2.82	0.59	7.50								23.91	0.64			11.69	10.37	25.56	12.99	0.82	2.60	0.10	0.40		
	C	6	0.56	4.56	0.20	1.35	9.13				+5.52	1.12	27.88	1.53		−4.14	13.38	7.49	31.68	1.13						−1.38
	E_2		6.56	1.69	11.85	1.22							25.34	1.05			11.55	6.87	32.08	1.80						
870	C	2	3.35	2.16	7.12	1.68					−4.18	0.54	41.75	0.47	1.63	−4.58	8.48	15.53	12.93	2.38	0.39	0.72	0.88			+8.19
(61.19%)	E_2		1.40	2.53	0.26	1.15	4.79					0.77	38.73	0.31			2.69	12.46	20.51	9.74	0.54	0.42	0.50	3.10		
	C	4	5.83								−0.74		30.49	0.62		−1.38	15.00	11.07	20.63	8.11	5.36	1.88	1.00			+2.12
	E_2		0.24	4.85									28.74	1.00			14.72	11.38	20.98	8.08	6.56	0.42	2.53	0.50		
	C	6	4.26	1.30	6.51	0.71					+5.28		27.50	0.32	0.96	+0.57	13.23	10.41	10.79	21.18	2.84					−5.84
	E_2		4.88	3.97	0.56	0.19	1.30	2.32	0.23	4.60		1.11	27.11	0.32	0.81		12.08	9.55	10.81	19.37	0.11	0.13	0.56			
890	C	2	7.58	1.73	5.10						−1.21	0.99	31.30	0.81		−2.85	3.26	11.97	32.19	0.65	0.64	0.85	0.47	3.16		+3.36
(57.01%)	E_2		3.50	3.27	1.33	4.22	0.88						27.24	0.01	3.00		8.24	12.74	30.69	0.55	0.65	0.58	3.09			
	C	4	5.35								+2.19		30.77			−7.64	12.32	5.47	28.86	9.70	2.96	3.30	1.27			+5.46
	E_2		1.49	6.05									20.27	2.86			17.14	13.92	14.01	8.55	9.18	0.15	3.21	2.82	0.36	
	C	6	3.45	1.71	10.21	1.35					−2.42		28.50	0.26	0.80	−1.52	12.79	8.58	32.34							+3.95
	E_2		0.51	1.25	11.17	1.37							26.95	1.09			12.86	8.30	32.53	0.27	1.03	0.90	1.77			

[a]Figures in parenthesis represent degree of lactose hydrolysis in milk.

[b]C - Control.

[c]E_2 - β-D-galactosidase and its specific activity was 2500 NLU/g and 15000 NPU/g.

* Differences between the total area of the experiment and control cheese.

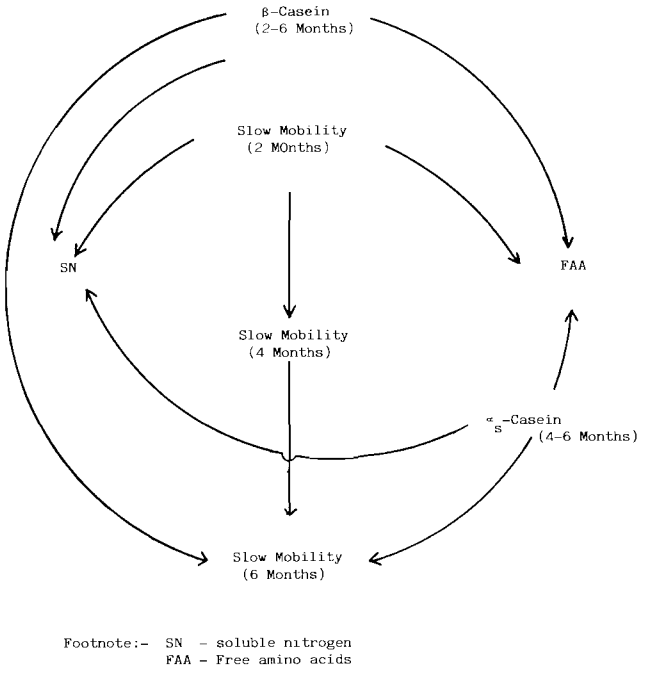

Figure 2. *Schematic illustrated showing degradation of casein fractions in cheese during the maturation period.*

acteristics for enzyme-treated cheese. From the same data (Table 5) it may be observed that the panelists showed a greater preference for the experimental cheese manufactured by using enzyme E_2. However, in judging the effect of the starter culture on the quality of Cheddar cheese (control and experimental), panelists had a preference for starters 890, 850 and 870 in this order.

CONCLUSION

It can be observed from our study that up to 60% hylysis of lactose was used in milk manufactured into Cheddar cheese and slight acceleration in the ripening process was achieved despite the fact that one enzyme preparation had a proteolytic activity of 15000 NPU/g as compared with the control cheese.

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TABLE 5. *Average scores and statistical analysis (mean difference) of the nine taste panelists' assessment of the cheese made from whole and LHM milk.*

Starter culture	Age (months)	Flavor and aroma						Body and texture					
		C[c]	E_1[a]	D[d]	C	E_2[a]	D	C	E_1	D	C	E_2	D
850[b]	2	37.060	37.250	−0.190	35.750	37.560	−1.81	34.000	33.310	+0.690	32.250	31.750	+0.500
	4	29.685	28.185	+1.500	27.560	26.060	+1.500	26.000	26.375	−0.375	24.375	26.125	−1.750
	6	28.375	30.125	−1.750	24.370	27.255	−2.885	26.125	26.750	−0.625	23.560	26.060	−2.500
870[b]	2	34.935	35.750	−0.815	32.685	31.560	+1.125	32.750	31.875	+0.875	30.935	30.000	+0.935
	4	23.375	24.750	−1.375	21.875	24.935	−3.060	27.750	23.875	+3.875	24.750	26.750	−2.000
	6	24.185	20.250	+3.935	20.470	21.605	−1.135	25.000	23.875	+1.125	17.435	23.465	−6.030
890[b]	2	36.560	37.310	−0.750	35.875	36.685	−0.810	31.685	30.000	+1.685	32.375	31.875	+0.500
	4	22.185	30.250	−8.060	26.560	28.250	−1.690	21.875	24.250	−2.375	26.750	27.625	−0.875
	6	27.000	29.375	−2.375	26.665	28.670	−2.005	24.375	26.750	−2.375	23.630	23.855	−0.225
Average		29.262	30.360	−1.098	27.979	29.176	−1.197	27.729	27.451	+0.278	26.229	27.501	−1.272

[a]E_1 and E_2 are different β-D-galactosidase preparations.
[b]850, 870 and 890 are codes for starter culture.
[c]C is control.
[d] − D is the mean difference, (+ ve) and (− ve) figures illustrates panelists' preference of the control or the experimental cheese respectively.
The characteristics of color and finish and appearance of the cheese (control or experimental) were similar and were not affected; hence they were not included in the above data.

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