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Microstructure and Chemical Changes in Twarog Cheese made from Ultrafiltrated Milk and from Lactose-Hydrolysed Milk

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ABSTRACT

Twarog cheese was manufactured from fresh milk, lactose-hydrolysed milk and retentate. Microstructure, chemical composition, protein breakdown, amino acid distribution and organoleptic properties were studied during ripening.

Inoculation of the milk with β-galactosidase, before cheese processing, reduced the clotting time, enhanced the protein breakdown and enhanced amino acid accumulation during ripening. Concentrating the milk, by ultrafiltration, prolonged the coagulation time and resulted in a cheese with greater amounts of moisture, fat, total nitrogen and higher pH than other cheeses.

Essential free amino acids are considered to constitute more than half of the total free amino acids of Twarog cheese and glutamic acid, leucine and phenylalanine are the major free amino acids in the ripened cheese.

Quality of the cheese was improved either by ultrafiltration or by lactose hydrolysis and the acceptable flavour was more pronounced in β-galactosidase-treated cheese.

Microstructure of the protein in young cheeses does not differ in all treatments. The disintegration and fusion of casein advanced during ripening in the outer protein matrix more than internally. The appearance of the cheese matrix was similar in both control and ultrafiltrated cheeses while the casein in β-galactosidase-treated cheese fused faster than in other treatments.

INTRODUCTION

Twarog cheese is one of the most popular white soft cheeses in Poland. It is an acid precipitation curd from cow's milk which can be whole, partially or completely skimmed. It has a high moisture content and its fat content ranges from 1% to 50% dry matter; it has a smooth buttery consistency and a mildly acidic flavour. It represents about 40% of the total cheese of Poland (Pijanowski, 1974).

Acid clotting cheeses are manufactured from ultrafiltrated milk or lactose-hydrolysed milk to improve their quality (Thompson & Guricsek, 1974; Covacevich & Kosikowski, 1978; Gungerish, 1981; Prokopek, 1984; El-Shibiny *et al.*, 1984; Omar, 1985). The microstructure of some acid coagulation cheeses has been studied by various workers (Glaser *et al.*, 1980; Knoop & Buchheim, 1980; Kaláb *et al.*, 1981; Omar, 1985; Abd El-Salam & Omar, 1985).

The objective of the current study is to compare the microstructures and qualities of Twarog cheese made from ultrafiltrated milk and from lactose-hydrolysed milk with that of control cheese.

MATERIALS AND METHODS

Cheese making

Twarog cheese was made from cow's milk standardised to 3% fat and pasteurised at 74°C for 15 s. The milk was divided into three portions; the first was concentrated by ultrafiltration on an Amicon DC 30 E ultrafilter to factor 4 using H10P10 membranes at a pressure of 0·1–0·15 MPa and 50°C; the retentate was put into plastic containers and held for further use. The second was treated with a β-galactosidase preparation from *Saccharomyces lactis* (Maxilact 26700 ONPG/g, Gist Brocades, Holland), 0·2 g/litre, for 1 h at room temperature before processing. The third portion of milk was used in cheese-making by the

traditional method as described by Pijanowski (1974). Five per cent of lactic acid starter (a mixture of *S. cremories*, *S. lactis* and *S. diacetilactis*) was added to the milk and the retentate then incubated at 40°C to complete coagulation. The curd was cut into 3–4 mm cubes and heated to 38–40°C during 30 min. The heated cubes were drained, scooped into cheese cloth, pressed and 3% sodium chloride was sprinkled onto the surface of the resultant cheese which was stored in plastic containers in a refrigerator. Cheese samples were analysed when fresh and after 1 and 2 weeks.

Electron microscopy

Samples for transmission electron microscopy (TEM) were prepared according to the methods of Shimmin & Hill (1964) and Wooding (1973) as follows.

Cheese slices, 1–1·5 mm thick, were fixed for 30 min in 5% aqueous glutaraldehyde solution, followed by 1 h in 1% OsO_4 solution in 0·1M phosphate buffer at pH 7·0, and for 1 h in 5% uranyl acetate solution. The specimens were successively washed with propylene oxide for 10 min and mixtures of propylene oxide and Epon 812 (3:1) for 2 h, 1:1 for 12 h and in a 1:3 mixture for another 12 h. The specimens were embedded in Epon in sealed gelatin capsules and kept at 60°C to harden. Ultrathin sections were made by a Reichert-Ultramicrotom and examined in a TESLA BS 500 electron microscope with an accelerating voltage of 80 kV.

Scanning electron microscopy (SEM) of Twarog cheese was carried out according to the method of Glaser *et al.* (1979). Cheese slices (1–1·5 mm thick) were fixed successively in 4% glutaraldehyde in 0·1M phosphate buffer (pH 7·0) overnight at 4°C and then in 1% OsO_4 in phosphate buffer for 4 h. The specimens were dehydrated in a graded series of alcohol solutions and dried in a critical point drier, fractured, mounted on aluminium stubs and silver paint, sputter-coated with gold and examined under a JEOL-SI SEM with an accelerating voltage of 10 kV.

Amino acid composition

Total amino acids

The acid hydrolysis was performed according to Moore & Stein (1963). Samples were prepared for analysis in 6N HCl at 110°C for 36 h,

followed by evaporation of the acid under vacuum at 40°C. The hydrolysate was dissolved in 0·5 ml of warm water and the solution was brought to pH 6·5 by the addition of 0·5 ml of 0·2M sodium phosphate buffer of pH 6·5. The neutral solution was allowed to stand unstoppered for 4 h to oxidise any cysteine to cystine and was then brought to pH 2·2 by the addition of freshly prepared 0·2N sodium citrate buffer (pH 2·2).

Free amino acids

Ten grams of cheese were dissolved in 90 ml 0·5M tris-sodium citrate solution. The mixture was then heated to 75°C in a water bath and homogenised at 10 000 rpm for 3 min using a laboratory homogeniser. The samples were deproteinised with 5% sulphosalicylic acid and filtered. The filtrate was adjusted to pH 2 by the addition of 5N NaOH and to pH 2·2 using freshly prepared 0·2N sodium citrate buffer (pH 2·2) followed by filtration (Mondino *et al.*, 1972).

Total and free amino acid contents were determined in 0·8 ml of the filtrate using an amino acid analyser (JLC/6AH JEAL/Japan).

Chemical composition of cheese

The cheese was analysed when young and after 1 and 2 weeks for pH, moisture and total nitrogen according to the AOAC methods (Horwitz *et al.*, 1970).

The protein breakdown of cheese was measured as water-soluble nitrogen (Sode Mogensen, 1948), non-protein nitrogen (Schober *et al.*, 1961) and amino acid nitrogen (Garnier, 1962). The nitrogen in each fraction was determined by the Kjeldahl method and the results were expressed as percentage of total nitrogen content in the cheese. All samples were subjected to sensory evaluation using 40 points for body and texture (uniform creamy colour, smooth outside and meaty inside) and 60 points for flavour (slightly acid, delicate flavour and aroma of a good lactic starter). The results were statistically analysed according to Steel & Torrie (1960).

RESULTS AND DISCUSSION

Analytical results obtained for the chemical composition and protein breakdown of Twarog cheese are given in Table 1. It was remarkable

that the clotting time of retentate was about 3 h longer than that of the control cheese (about 5 h) due to the higher protein concentration, while the treatment with β-galactosidase reduced the clotting time of milk to 3·5 h due to the higher growth of starter bacteria (Erik, 1983; Gooda *et al.*, 1983).

Table 1 shows the difference in the initial pH, moisture, fat and total nitrogen between young control cheese and experimental cheeses. Cheese made from ultrafiltrated milk retained fat, nitrogenous substances and moisture more than other cheeses, whereas no differences were significant in the chemical composition of control and β-galactosidase-treated cheese.

TABLE 1
Chemical Analysis of Twarog Cheese (Average of Three Treatments)

Cheese made from	*Age of cheese*	*pH*	*Moisture (%)*	*Fat % of DM*	*(TN) Total nitrogen (%)*	*Soluble N of TN (%)*	*(NPN) Non-protein N of TN (%)*	*Amino acid nitrogen of TN (%)*
Fresh milk (control)	Young	4·5	75·29	42·2	2·52	12·2	5·35	1·65
	1 week	4·6	70·48	33·9	2·85	18·9	7·13	1·98
	2 weeks	4·9	68·98	36·2	3·20	24·7	9·67	2·18
β-galactosidase-treated milk	Young	4·6	72·87	40·6	2·58	20·1	8·86	3·87
	1 week	4·9	70·12	36·3	3·05	26·4	15·6	8·76
	2 weeks	4·9	69·65	33·6	3·12	32·7	26·2	14·9
Ultrafiltrated milk	Young	5·1	78·15	47·4	3·25	23·1	4·57	2·12
	1 week	4·9	75·98	53·1	3·34	27·1	9·16	3·32
	2 weeks	5·2	72·65	43·9	3·39	33·5	18·6	6·43

The protein breakdown in this cheese resulted only from bacterial enzymes. Therefore, the stimulatory effect of the β-galactosidase preparation on the growth of proteolytic bacteria enhanced the protein breakdown of cheese (Marschke *et al.*, 1980; Abdu *et al.*, 1984; El Safty *et al.*, 1985; Omar, 1985). Moisture and fat (percentage of dry matter) were reduced over the period, while pH, total nitrogen (TN), soluble nitrogen (SN/TN), non-protein nitrogen (NPN/TN), and amino acid nitrogen/TN increased for all cheese samples (Table 1).

The distribution of the total amino acids revealed 14 amino acids

TABLE 2
Total Amino Acid Composition of Twarog Cheese (μmol/g Cheese)

Amino acid	*Cheese made from milk:*					
	Fresh		*Treated with β-galactosidase*		*Concentrated by ultrafiltration*	
	Age of cheese					
	Young	*2 weeks*	*Young*	*2 weeks*	*Young*	*2 weeks*
Lys.	40·6	34·5	57·6	37·6	62·6	62·2
His.	12·9	9·1	19·5	9·8	20·7	21·1
NH_3	56·3	98·4	74·2	174·0	135·0	141·0
Arg.	10·9	6·9	16·8	17·4	15·2	17·2
Asp.	77·0	61·5	62·1	63·5	68·8	65·6
Thr.	96·3	86·4	36·3	32·0	37·2	30·6
Ser.	52·1	50·3	50·0	48·8	51·9	48·1
Glu.	154·0	146·0	159·0	113·0	154·0	122·0
Ala.	24·4	26·1	36·5	20·4	14·6	33·2
Gly.	17·5	13·8	21·7	12·6	10·6	22·7
Val.	36·5	34·2	63·4	49·0	61·1	60·3
Ile.	26·2	21·3	40·4	44·9	48·9	40·1
Leu.	49·7	43·5	71·9	54·4	70·3	74·1
Tyr.	18·0	15·6	28·9	29·4	33·9	28·5
Phe.	21·0	19·2	35·0	27·9	31·8	35·9
Total	693·0	661·0	773·0	735·0	816·0	802·0

(Table 2). A slightly higher content of the total amino acids was found in ultrafiltrated cheese (about 17·8% and 5·61% more than that of control and β-galactosidase cheese, respectively). In time, the content of total amino acids slightly decreased without significant variation between the samples. Certain amino acids (glu., thr., asp. and leu.) represented about 50% of the total amino acids in control cheese, whilst glu., leu., val., and asp. in β-galactosidase treated cheese and glu., leu., asp. and val. in ultrafiltrated cheese constituted the same percentage.

Tables 3, 4 and 5 show the distribution of free amino acids in Twarog cheese. The pattern of the amino acids in young cheese revealed 11, 12 and 15 free amino acids with totals of 16·9, 24·5 and 44·8 mg/100 g cheese and these increased in time to 16 free amino acids, totalling 86·3,

TABLE 3
Free Amino Acid Composition of Twarog Cheese (mg/100 g Cheese)

Amino acid	*Age of cheese*					
	Young		*1 week*		*2 weeks*	
	mg/100 g	*Per cent of total*	*mg/100 g*	*Per cent of total*	*mg/100 g*	*Per cent of total*
Essential						
Lysine	1·49	8·83	0·81	1·38	3·46	4·01
Methionine	0·41	2·43	1·85	3·15	2·79	3·23
Threonine	1·39	8·24	3·96	6·75	5·19	6·02
Isoleucine	—	—	2·73	4·65	5·06	5·86
Leucine	1·61	9·54	7·60	12·9	11·4	13·2
Phenylalanine	—	—	5·81	9·90	6·93	8·03
Valine	3·36	19·9	6·58	11·2	7·42	8·60
Total	8·26	49·0	29·3	50·0	42·2	48·9
Non-essential						
Arginine	1·40	8·30	3·33	5·68	5·39	6·25
Histidine	—	—	0·66	1·12	1·73	2·01
Tyrosine	—	—	—	—	1·92	2·23
Serine	0·78	4·62	2·81	4·79	3·63	4·21
Glutamic acid	2·53	15·0	10·8	18·4	16·3	18·9
Aspartic acid	1·11	6·58	1·86	3·17	1·57	1·82
Glycine	1·36	8·06	2·73	4·65	3·97	4·60
Alanine	1·43	8·48	3·73	6·35	4·86	5·63
Proline	—	—	3·46	5·89	4·73	5·48
Total	8·61	51·0	29·4	50·0	44·1	41·1
Total FAA	16·9	100	58·7	100	86·3	100

139 and 202 mg/100 g in 2-weeks old control, ultrafiltrated cheese and β-galactosidase-treated cheese, respectively. It seems that the treatment with β-galactosidase enhances ($P<0{\cdot}01$) the liberation of free amino acids. This could be attributed to the increased production of starter bacterial enzymes by this preparation, especially peptidases (Gooda *et al.*, 1983) and the effect of protein concentration in ultrafiltrated milk on the growth of bacteria (to reduce their count) (Erik, 1983). On the other hand, the essential amino acids in Twarog cheese account for about 48%, 54% and 61% of the total free amino acids of 2-weeks

TABLE 4
Free Amino Acid Composition of Twarog Cheese Made from Ultrafiltrated Milk (mg/100 g Cheese)

Amino acid	*Age of cheese*					
	Young		*1 week*		*2 weeks*	
	mg/100 g	*Per cent of total*	*mg/100 g*	*Per cent of total*	*mg/100 g*	*Per cent of total*
Essential						
Lysine	—	—	3·16	3·83	11·4	8·15
Methionine	1·38	5·63	3·76	4·56	7·56	5·42
Threonine	1·57	6·41	6·54	7·94	8·12	5·83
Isoleucine	—	—	5·17	6·27	11·9	8·51
Leucine	3·45	14·1	16·3	19·8	22·0	15·8
Phenylalanine	1·36	5·55	8·46	10·3	16·4	11·7
Valine	4·51	18·4	6·06	7·35	8·12	5·83
Total	12·3	50·1	49·5	60·0	85·4	61·3
Non-essential						
Arginine	0·95	3·88	2·17	2·63	3·16	2·27
Histidine	—	—	0·96	1·17	3·20	2·30
Tyrosine	—	—	1·12	1·36	2·49	3·71
Serine	0·38	1·55	2·63	3·19	5·17	11·0
Glutamic acid	4·63	18·9	8·36	10·2	19·3	13·9
Aspartic acid	0·51	2·08	2·38	2·89	5·86	4·20
Glycine	2·89	11·8	4·51	5·47	5·63	4·04
Alanine	1·76	7·18	3·86	4·68	5·22	3·75
Proline	1·12	4·57	6·93	8·41	3·96	2·84
Total	12·2	49·9	32·9	40·0	54·0	38·8
Total FAA	24·5	100	82·4	100	139	100

old control, β-galactosidase-treated cheese and ultrafiltrated cheese, respectively. This result is similar to that in Kariesh cheese (Omar, 1985).

Certain free amino acids (glu., leu., val. and phe.) were found in significant amounts in control cheese (above 50% of the total free amino acids). The corresponding value in β-galactosidase cheese was calculated from leu., glu., phe. and ser. and in ultrafiltrated milk cheese was from glu., leu., lys. and phe. The differences of free amino acids cause differences in cheese flavour.

TABLE 5
Free Amino Acid Composition of Twarog Cheese made from Lactose-Hydrolysed Milk (mg/100 g Cheese)

Amino acid	*Age of cheese*					
	Young		*1 week*		*2 weeks*	
	mg/100 g	*Per cent of total*	*mg/100 g*	*Per cent of total*	*mg/100 g*	*Per cent of total*
Essential						
Lysine	5·34	10·7	11·9	9·36	20·9	10·4
Methionine	2·11	4·24	5·61	4·41	7·06	3·50
Threonine	1·86	3·73	7·51	5·90	4·43	2·19
Isoleucine	2·83	5·68	9·05	7·11	18·9	9·38
Leucine	5·76	11·6	27·1	21·3	32·4	16·0
Phenylalanine	4·46	8·96	11·6	9·14	14·4	7·13
Valine	1·30	2·61	7·77	6·11	11·0	5·44
Total	23·7	47·5	80·6	63·3	109	54·0
Non-essential						
Arginine	—	—	1·43	1·12	6·18	3·06
Histidine	1·06	2·13	3·34	2·63	6·41	3·18
Tyrosine	1·20	2·41	3·76	2·96	9·43	4·67
Serine	1·86	3·74	3·21	2·52	4·96	2·46
Glutamic acid	8·78	17·6	14·4	11·2	33·4	16·5
Aspartic acid	2·92	5·86	3·47	2·73	5·95	2·95
Glycine	2·36	4·74	4·53	3·56	4·86	2·41
Alanine	4·22	8·48	6·09	4·79	9·72	4·82
Proline	3·73	7·49	6·46	5·08	11·9	5·88
Total	26·1	52·5	46·7	36·7	92·8	46·0
Total FAA	49·8	100	127	100	202	100

Table 6 shows the organoleptic properties of Twarog cheese. The quality of Twarog cheese was improved by using either ultrafiltrated or β-galactosidase-treated milk. The flavour and consistency of experimental cheese were preferred to the control one when young. Ultrafiltrated cheese had a compact and smooth body and texture with a mildly acidic flavour while lactose-hydrolysed cheese had a meaty consistency and better flavour.

The lower flavour scores of ultrafiltrated cheese could be attributed to the increased protein concentration which led to changes in the

TABLE 6
Quality of Twarog Cheese made from Ultrafiltrated Milk (I) and β-Galactosidase-Treated Milk (II) Compared with Control (III)

Treatment	*Number*	*Age of cheese*								
		Young			*1 week*			*2 weeks*		
		A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
I	1	38	40	78	39	44	83	39	45	84
	2	39	41	80	39	43	82	39	46	85
	3	37	39	76	39	42	81	39	44	83
	$\bar{x}$	38	40	78	39	43	82	39	45	84
II	1	33	43	76	35	46	81	37	49	86
	2	31	39	70	33	43	76	35	48	83
	3	32	41	73	34	46	80	36	47	83
	$\bar{x}$	32	41	73	34	45	79	36	48	84
iII	1	32	40	72	34	42	76	36	45	81
	2	30	34	64	32	37	69	33	39	72
	3	28	31	59	30	35	65	30	36	66
	$\bar{x}$	30	35	65	32	38	71	33	40	73

A, Body and texture and appearance (40). B, Flavour (60). C, Total (100).

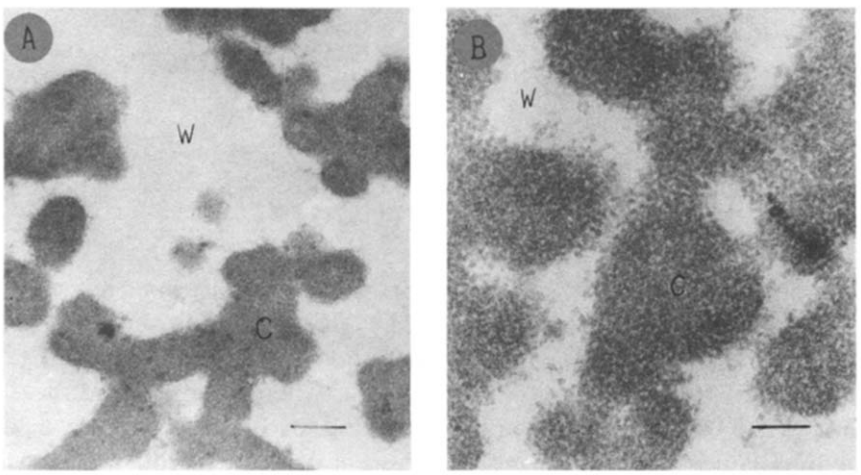


Fig. 1. SEM micrographs of young (A) and 2-weeks old (B) cheese. A, B, Control. A1, B1, Cheese treated with β-galactosidase. A2, B2, Cheese made by ultrafiltration. C, Casein. W, Whey. K, Whey capillary. Bar, 10 μm.

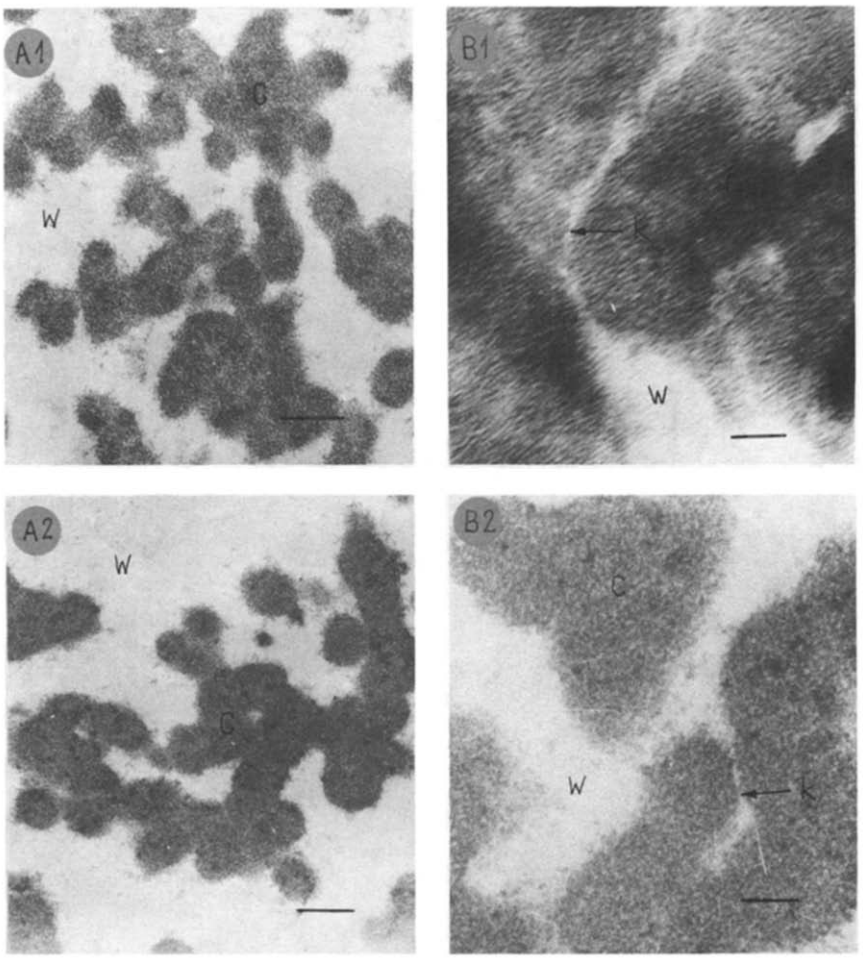


Fig. 1—*contd.*

proportions of acid- and aroma-forming bacteria (Erik, 1983). No differences were significant in the quality of the two experimental cheeses at 2 weeks old; the variation was significant ($P < 0{\cdot}01$) between them and the control cheese.

Figure 1 (A, A1 and A2) shows the loose network of aggregates of casein micelles. It consists of homogeneous masses in clusters and the spherical shape of casein micelles over $0{\cdot}5\,\mu$m in diameter can be seen. The protein substructure of young cheeses does not differ systematically

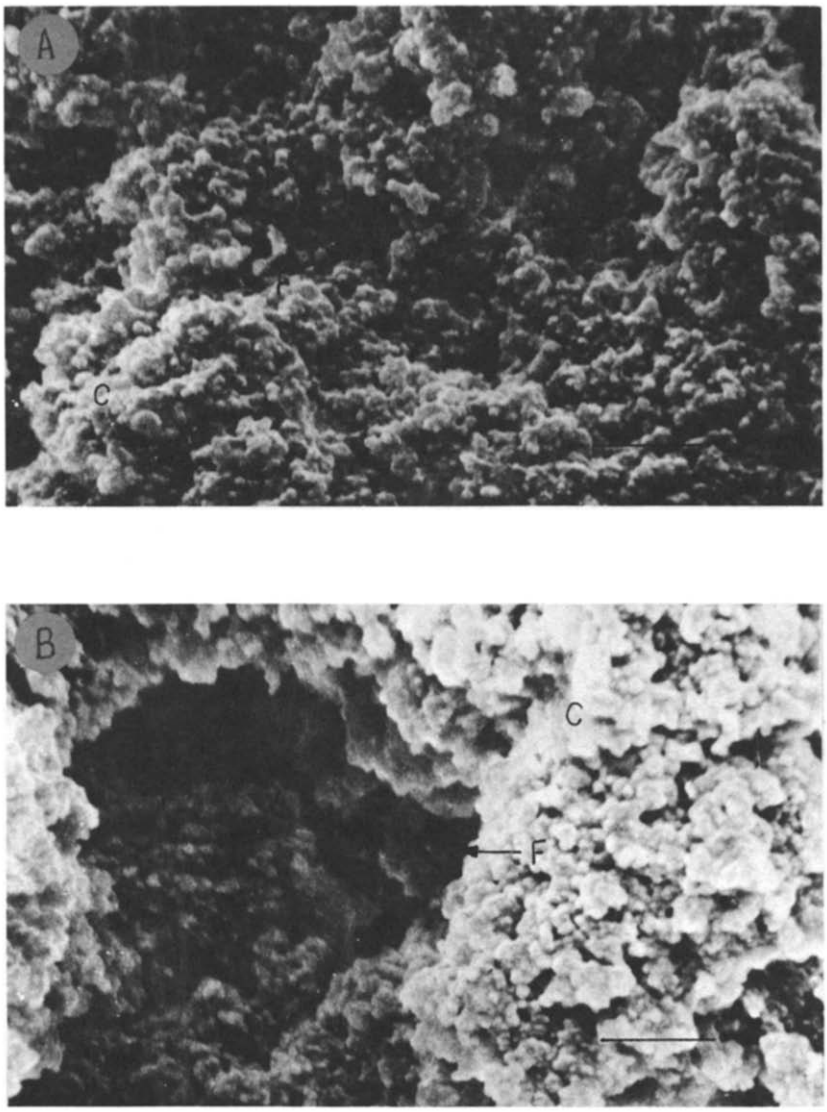


Fig. 2. SEM micrographs of young Twarog cheese. A, Control. B, Cheese treated with β-galactosidase preparation. C, Cheese made by ultrafiltration. C, Casein. F, Fat gap. Bar, 10 μm.

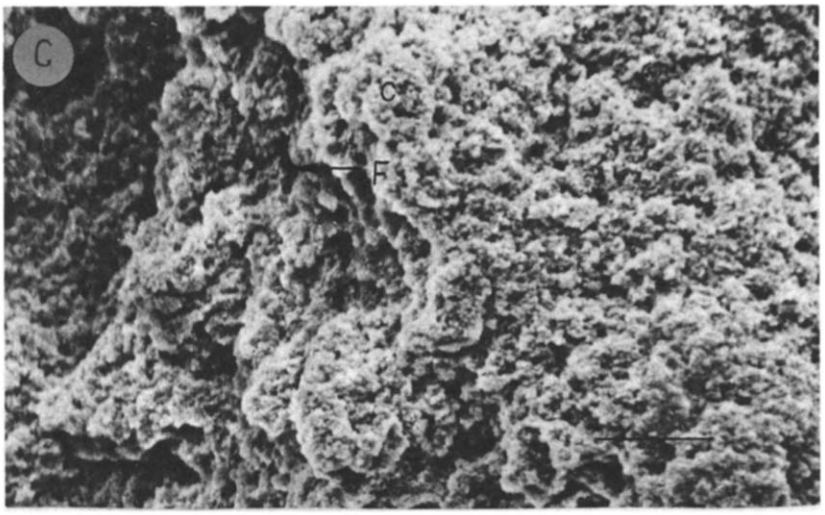
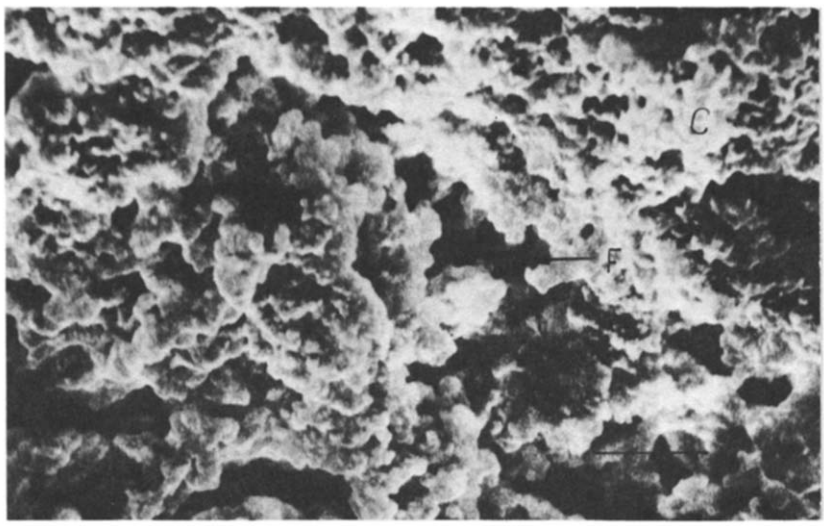


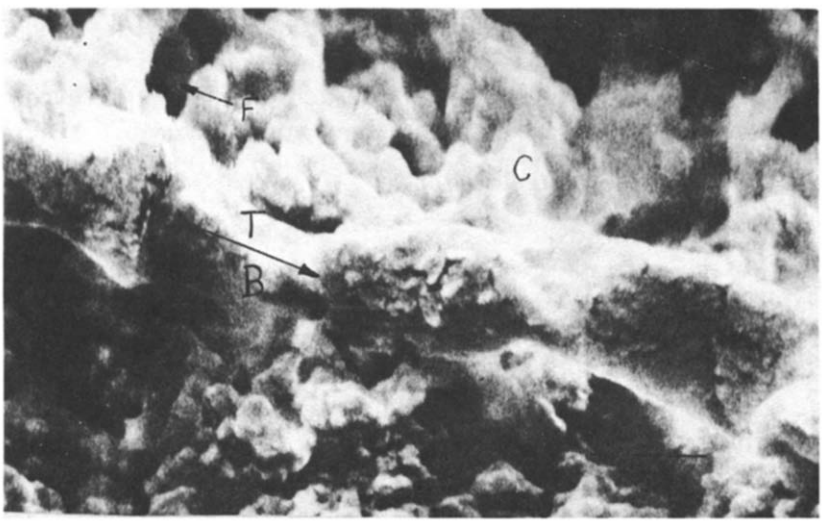
Fig. 2—*contd.*

and is quite similar to an acid-set curd reported by Glaser *et al.* (1980), Knoop & Buchheim (1980), Kaláb *et al.* (1981), Omar (1985) and Abd El-Salam & Omar (1985). During the ripening process, the protein mass disintegrates into subunits fused with extensive interaction inside the protein matrix, causing a continuous framework with drainage channels in the aqueous phase of this cheese. The fusion of disintegrated casein is not particularly conspicuous between the protein matrix of control cheese and ultrafiltrated cheese [Fig. 1(B and B2)], whereas the disintegrated casein in β-galactosidase-treated cheese spreads over the surface in larger areas [Fig. 1(B1)]. This is attributed to the effect of bacterial enzymes (Gooda *et al.*, 1983).

SEM micrographs shown in Fig. 2(A, B and C) illustrate no significant differences between the inner and outer parts of the protein matrix of young cheese. The cheese matrix appears in closely packed lumps (finely granulated mass), the granules forming a network with clearly visible fat gaps between the clusters. There was no consistent difference between the surfaces in structure or porosity except a compaction of casein micelles at the outside surface of β-galactosidase-treated cheese. The fusion of casein micelles in 2-weeks old cheese is illustrated in Fig. 3. It is clear that the fusion advances during ripening and is more extensive at the outer part of the protein matrix than the inner, as found by

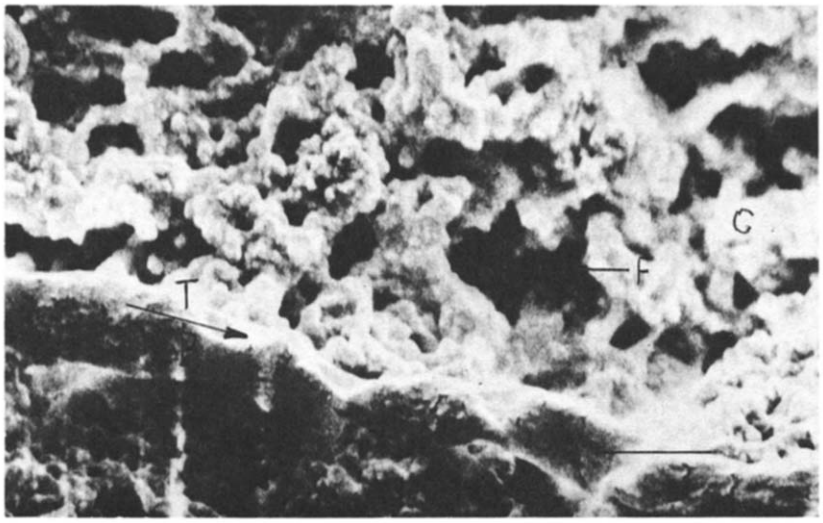


(a)



(b)

Fig. 3. SEM micrographs of 2-weeks old cheese fractured (at arrows) to show outside surface at top (T) and inside surface at bottom (B). (a) Control cheese; (b) cheese treated with β-galactosidase; (c) cheese made by ultrafiltration. C, Casein. F, fat gap. Fusion of casein at outside is more than at inside. Bar 10 μm.



(c)

Fig. 3—*contd.*

Knoop & Buchheim (1980) in acid-set curd. The disintegration of the casein occurs much faster in β-galactosidase-treated cheese, with more extensions of aggregated casein micelles, than in the other cheeses.

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