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Microstructure and Chemical Changes in Domiati Cheese made from Ultrafiltered Milk

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ABSTRACT

Cheese of good quality was made from ultrafiltered cow's milk and compared with that made by a conventional method. Domiati cheese made by ultrafiltration had a uniform and closed texture, good appearance and better organoleptic properties, and the breakdown of protein, the accumulation of free amino acids and the liberation of Volatile Free Fatty Acids were faster than in cheese made by the conventional method.

The pattern of total amino acids was nearly the same and slightly decreased during ripening; glutamic acid, aspartic acid, leucine and lysine represented about half of the total amino acid concentration in both cheeses.

The microstructure of the protein matrix in Domiati cheese consists of fused casein micelles in homogeneous masses. There were similarities in the appearance of the cheese matrix between the cheese made by the conventional method and that made from ultrafiltered milk when young and during ripening.

INTRODUCTION

Concentration of milk by ultrafiltration saves 80% of the quantity of rennet usually needed for the preparation of a given weight of cheese and increases cheese yield (Maubois & Mocquot, 1975).

The technology of ultrafiltration was first used on a large scale in the production of white soft cheese (Feta cheese) by concentrating a whole milk to 38%–40% dry matter (Albertsen *et al.*, 1983).

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Egyptian white soft cheese (Domiati cheese) was made from buffalo's ultrafiltered milk by Abd El Salam *et al.* (1981).

The quality and yield of Domiati and Feta cheeses made by ultrafiltration were studied by Mahmoud & Kosikowski (1979), and the quality and microstructure of white soft cheese (Solan cheese) from ultrafiltered milk was studied by Omar *et al.* (1986).

Therefore, the present work was carried out to investigate any effect of the ultrafiltration technique on chemical changes and microstructure of Domiati cheese during ripening.

MATERIALS AND METHODS

Cheese making

Domiati cheese was made from cow's milk standardized to 3% fat. The milk was divided into two aliquots: the first aliquot was concentrated four-fold by ultrafiltration at a pressure of 0·10 to 0·15 MPa and a temperature of 50°C using an Amicon DC 30 E ultrafilter equipped with HIOPIO membranes. The retentate was placed in plastic containers and stored for future use.

Four grams of NaCl and 3 g rennet/100 kg retentate were added. The second aliquot was made into Domiati cheese by the conventional method (Fahmi & Sharara, 1950), including the addition of NaCl (to 10%) to milk before renneting. The young cheeses were pickled in salted whey (14% NaCl) at room temperature (20–25°C) for 4 months. Three replicates were made and cheese samples were analyzed when young and after 2 and 4 months.

Amino acid composition

Total amino acids were determined as follows: acid hydrolysis was carried out according to Moore & Stein (1963). Cheese samples were hydrolyzed in 6N NCl at 110°C for 36 h and the acid was evaporated at a reduced pressure at 40°C. The hydrolyzate was dissolved in 0·5 ml of warm water and the solution was brought to pH 6·5 using 0·5 ml of a 0·2M sodium phosphate buffer, pH 6·5. The neutral solution was left to stand unstoppered for 4 h to oxidize any cysteine present into cystine and was then brought to pH 2·2 by addition of freshly prepared 0·2N sodium citrate buffer, pH 2·2.

Free amino acids were determined as follows: 10 g of the cheese were dissolved in 90 ml of a 0·5M Tris–sodium citrate solution. The solution was then heated to 75°C in a water bath and homogenized at 10 000 rpm for 3 min using a laboratory homogenizer. The samples were deproteinized with 5% sulfosalicylic acid and were filtered. The filtrate was adjusted to pH 2·0

by the addition of 5N NaOH and to pH 2·2 using a freshly prepared 0·2N sodium citrate buffer, pH 2·2; the solutions were then refiltered (Mondino *et al.*, 1972).

Total and free amino acid contents were determined in 0·8 ml aliquots of the filtrates using a JLC/6AH JEOL/Japan amino acid analyzer.

Free Fatty Acid composition

Sodium soaps of the Free Fatty Acids were released from cheese by the method of Kuzdzal & Kuzdzal-Savoie (1966). Volatile (C_2 to C_8) Fatty Acids were prepared as described by Roos *et al.* (1963). The methyl esters of C_{10} to C_{18} Free Fatty Acids were prepared by the method of Kuzdzal-Savoie & Kuzdzal (1967). Free Fatty Acids were separated on a Pye Unicam Series 104 gas–liquid chromatograph using a 1·5 m glass column, inner diameter 3 mm, packed with 10% dimethylglycol succinate on Chromosorb A W/80/100, with 2% H_3PO_4 added. The carrier gas was argon, at 40 ml/min, the column was at 150°C, and the detector at 250°C.

Chemical composition of cheese

The cheese was analyzed for pH, moisture, fat and total nitrogen contents according to the AOAC methods (Horwitz *et al.*, 1970) when fresh (young) and again after 2 and 4 months. Proteolysis in the cheese was determined from the concentrations of water-soluble nitrogen substances (Sode Mogensen, 1948), non-protein nitrogen (Schober *et al.*, 1961) and amino acid nitrogen (Garnier, 1962). Nitrogen content was determined by the Kjeldahl method and the results were expressed as the percentage of the total nitrogen content in the cheese.

All samples were subjected to sensory evaluation using a scale with 50 points for flavour, 30 points for body and texture and 20 points for finish and appearance (Bruncke, 1968). The results were analyzed statistically according to Steel & Torrie (1960).

Electron microscopy

Cheese samples were prepared for electron microscopy by application of the freeze-fracturing technique according to Buchheim (1982) and Prokopek *et al.* (1976). Small pieces (1 to 2 mm^3) of cheese were mounted on specimen holders, using glycerol as an intermediate layer for increasing mechanical stability. The specimens were quickly frozen by immersion into melting Freon 22 (−160°C) and stored under liquid nitrogen. Freeze-fracturing was carried out in a BALZERS BA 360 M unit at object temperature of −120°C.

TABLE 1
Scoring of Cheese Made from Fresh Milk after Four Months of Ripening

Treatment	*No.*	*Finish and appearance (20)*	*Body and texture (30)*			*Flavour (50)*		*Comments*	*Total (100)*
			Holes (5)	*Colour (10)*	*Consistency (15)*	*Aroma (25)*	*Taste (25)*		
Conventional method	1	18	4	9	13	20	19	Creamy, soft, salty, porous	83
	2	18	5	9	14	21	21	Creamy, salty, pure	88
	3	18	4	9	13	19	20	Acid, creamy, porous	83
$\bar{X}$		18	4·3	9	13·3	20	20		84·7
Ultrafiltration method	1	20	5	10	15	22	23	Smooth, pure, slightly salty	95
	2	20	5	10	15	21	23	Creamy, pure, smooth	94
	3	20	5	10	14	22	24	Pure, smooth, distinctly salty	95
$\bar{X}$		20	5	10	14·7	21·7	23·3		94·7
T ratio					2·8		5·0		5·9

For replication, the freshly freeze-cleaved surface was immediately shadowed with 2 nm platinum/carbon under an angle of 45° and further stabilized by 20 nm of pure carbon. The replicas were floated onto distilled water and then transferred to 5% sodium hypochlorite (undiluted bleach) for approximately 2 h and passed again to distilled water. Fat was removed by a short treatment in pure acetone. Electron microscopy was carried out with a Siemens ELMISKOP I at 80 kV.

RESULTS AND DISCUSSION

Results, presented in Table 1, show significant differences ($P < 0{\cdot}1$) in sensory evaluation. Cheese made from ultrafiltered (UF) milk had a high sensoric quality, good flavour, smooth body and texture and the appearance was improved as compared with cheese made by the conventional method. These results suggest that biochemical changes occurred more quickly in the former cheese than in the latter throughout the ripening period.

Results of chemical analyses are summarized in Table 2. They show variations in moisture, pH, fat and total nitrogen contents in young and

TABLE 2
Chemical Analysis of Domiati Cheese made from Fresh Milk

Index	*Conventional method*			*Ultrafiltration method*		
	Age of cheese					
	Young	*2 months*	*4 months*	*Young*	*2 months*	*4 months*
Moisture (%)	61·76	55·69	52·43	69·62	58·76	54·71
Fat in dry matter (DM %)	44·46	42·88	39·94	55·95	48·46	44·15
Total nitrogen (TN) in DM (%)	7·87	7·29	7·06	10·59	8·39	8·01
Soluble N (% of TN)	5·46	17·63	29·86	7·33	20·56	34·92
Non-protein N (% of TN)	2·49	8·89	15·46	2·26	9·43	19·37
Amino acid N (% of TN)	0·94	2·49	5·76	1·14	3·62	7·86
Free amino acids (mg/100 g cheese)	72·0	192	464	86·7	257	609
Volatile Fatty Acids (C_2–C_{10}) (μg per gram of cheese)	8·45	22·2	52·2	11·2	35·8	76·1
Non-Volatile Fatty Acids (more than C_{10} μg per gram of cheese)	216	343	482	229	383	498
pH	5·12	4·96	4·92	6·23	5·85	5·70

matured cheese. Domiati cheese made by ultrafiltration retained more moisture, fat and nitrogen substances than cheese made by the conventional method. This would be attributed to its higher retention of these contents as reported by Maubois & Mocquot (1975), Renner & Ömeroglu (1981) and Omar *et al.* (1986).

Cheese fermentation involves the degradation of protein by the action of rennet and microbial enzymes (Chandan *et al.*, 1982). Proteolysis progressed more rapidly in this cheese than in the control. Moisture, pH, fat and total nitrogen (TN) contents of the cheeses decreased over the 4-month period under study whereas the ratios of soluble nitrogen/TN, non-protein nitrogen/TN, and amino acid nitrogen/TN increased in all cheese samples (Table 2).

The amino acid composition of the Domiati cheese samples under study is presented in Table 3. Both cheeses contained sixteen free amino acids.

The initially free amino acid pattern in young cheese differed in two respects; first was the absence of histidine and arginine and, secondly, the higher concentration of free amino acids in UF milk cheese than in the

TABLE 3
Free Amino Acid Composition of Domiati Cheese made from Fresh Milk (mg/100 g cheese)

Amino acid	*Conventional method*			*Ultrafiltration method*		
	Age of cheese					
	Young	*2 months*	*4 months*	*Young*	*2 months*	*4 months*
Lysine	3·80	12·8	46·43	7·98	21·4	66·2
Histidine	0·90	1·95	26·3	—	11·0	33·0
Arginine	7·71	13·41	28·36	—	10·20	31·80
Aspartic acid	2·73	12·1	18·9	2·32	10·9	21·0
Threonine	1·50	3·95	11·8	3·89	15·3	32·9
Serine	3·48	14·3	23·62	4·40	13·8	27·3
Glutamic acid	12·8	35·5	86·8	14·6	44·8	95·0
Proline	9·89	21·8	59·2	12·8	35·3	77·9
Glycine	2·35	4·02	5·20	1·60	8·53	9·06
Alanine	5·60	8·42	19·2	5·06	6·36	25·2
Valine	2·59	8·19	23·2	4·72	13·3	33·2
Methionine	1·09	3·89	6·88	1·19	5·41	5·82
Isoleucine	4·15	5·20	18·69	6·86	11·0	32·5
Leucine	10·2	23·4	48·2	11·9	27·6	62·2
Tyrosine	1·04	11·5	12·7	3·40	9·98	19·5
Phenylalanine	2·09	11·5	28·1	6·04	12·1	36·2
Total	72·0	192	464	86·7	257	609

control cheese. The total concentrations of free amino acids were 86·7 and 72·0 mg/100 g in the young cheeses and these values increased to 609 and 464 mg/100 g in the 4-month old UF milk cheese and control cheese, respectively. These results confirm the greater breakdown of proteins in UF milk cheese. Also, free glutamic acid, proline, lysine and leucine, which are mainly responsible for the formation of cheese flavour, were found at higher concentration in UF milk cheese. Differences in the free amino acid contents were related to differences in the cheese flavour (Omar *et al.*, 1986).

The pattern of total amino acids was nearly the same (Table 4) and slightly decreased during the ripening period with no significant differences between the two treatments. Glutamic acid, leucine, aspartic acid and lysine represented about half of the total amino acid concentration in Domiati cheese. This result is in agreement with that reported by Buruiana & Farag (1983) and Omar *et al.* (1986) in their study on white soft cheese, Telema and Solan cheeses, respectively.

Data in Table 5 summarize the distribution of Free Fatty Acids in Domiati cheese. In the two young cheeses (UF milk cheese and control) 23 and 20 Free Fatty Acids at total concentrations of 241 and 225 μg/g of

TABLE 4
Total Amino Acid Composition of Domiati Cheese made from Fresh Milk (μmol/g cheese)

Amino acid	*Conventional method*			*Ultrafiltration method*		
	Age of cheese					
	Young	*2 months*	*4 months*	*Young*	*2 months*	*4 months*
Lys	110	99·2	88·8	119	106	107
His	38·1	32·7	29·6	38·0	36·9	36·1
NH_3	110	108	93·4	110	120	128
Arg	39·3	33·9	28·9	35·5	36·7	38·6
Asp	136	124	95·9	143	127	117
Thr	65·9	63·4	56·6	71·6	66·8	64·9
Ser	90·6	91·4	73·5	97·0	93·0	92·2
Glu	296	269	242	312	301	294
Ala	53·7	59·3	43·8	71·0	58·9	50·1
Gly	42·4	38·2	40·9	54·3	49·0	39·4
Val	80·4	65·3	63·3	85·1	86·4	81·4
Ile	83·3	73·6	65·5	83·0	80·5	77·7
Leu	125	119	109	158	134	129
Tyr	61·3	52·9	53·4	68·5	64·9	64·1
Phe	58·7	59·4	57·9	75·0	66·6	67·4
Total	1 389	1 291	1 143	1 510	1 428	1 387

TABLE 5
Free Fatty Acid Composition of Domiati Cheese made from Fresh Milk (μg/g cheese)

Fatty acid	*Conventional method*			*Ultrafiltration method*		
	Age of cheese					
	Young	*2 months*	*4 months*	*Young*	*2 months*	*4 months*
C_2	2·68	5·25	8·40	3·20	9·67	18·1
C_3	0·22	1·04	3·18	1·42	5·06	9·21
C_4	0·42	0·92	2·30	0·41	1·30	3·25
iso-C_5	—	0·05	0·64	0·07	—	0·05
C_5	0·05	0·05	0·09	0·11	0·04	0·18
C_6	1·54	2·85	4·74	1·35	3·20	5·01
C_8	1·22	2·78	10·08	1·35	3·37	12·58
C_{10}	2·32	9·28	22·8	3·33	13·2	27·7
$C_{10:1}$	3·43	2·39	2·60	0·91	1·75	4·26
C_{12}	13·8	22·7	37·5	9·55	12·1	20·4
$C_{12:1}$	3·11	2·40	1·82	1·91	1·88	1·38
iso-C_{14}	1·47	1·02	0·50	1·68	0·92	0·48
C_{14}	40·9	56·7	82·2	45·4	66·1	83·4
$C_{14:1}$	9·58	8·15	8·82	7·90	9·21	12·4
C_{15}	6·57	4·56	4·64	3·38	5·86	9·20
iso-C_{16}	1·55	0·91	0·89	0·89	2·95	1·29
C_{16}	80·2	110	140	85·7	120	134
$C_{16:1}$	4·40	8·86	9·72	4·95	12·5	22·2
C_{17}	—	2·42	2·37	1·28	4·15	4·23
$C_{17:1}$	—	2·06	0·86	—	6·37	1·70
C_{18}	17·5	42·7	78·5	16·9	52·0	84·4
$C_{18:1}$	31·4	62·4	102	42·2	72·4	106
$C_{18:2}$	2·41	11·8	8·03	3·88	10·4	7·60
$C_{18:3}$	—	4·24	3·45	2·65	4·78	4·75
Total	225	365	534	241	419	574

cheese, respectively, were formed. With time, these values increased to 24 Free Fatty Acids at total concentrations of 574 and 534 μg/g in the 4-month-old cheeses. It seems that the release of Free Volatile Fatty Acids (C_2–C_{10}) was significantly different ($P < 0·1$) while this was not the case for Free Non-volatile Fatty Acids (C_{12}–$C_{18:3}$). Kosikowski & Mocquot (1958) reported that the Free Volatile Fatty Acids are important in the formation of cheese flavour. The higher flavour scores in UF milk cheese (Table 1) could be attributed to increased concentrations of Free Amino Acids and Free Volatile Fatty Acids.

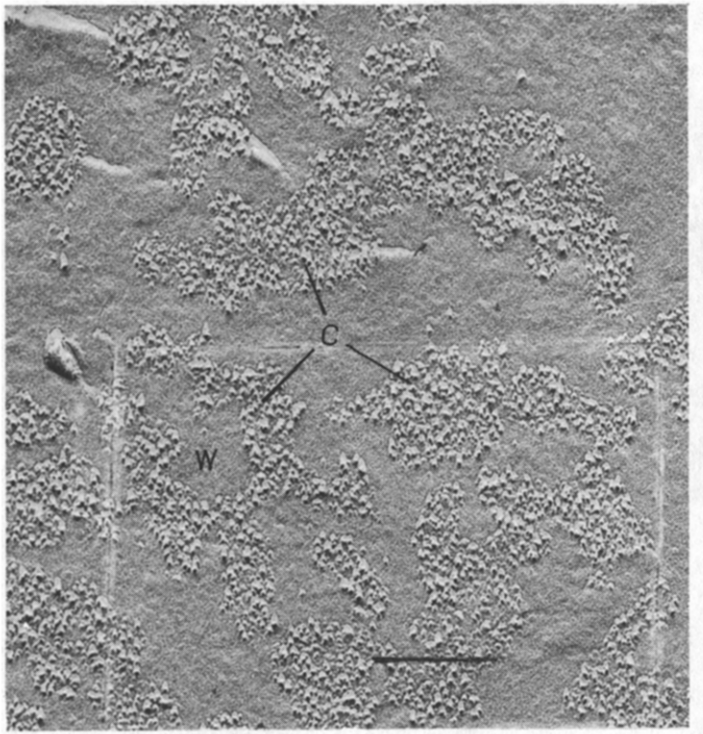

Fig. 1. Young Domiati cheese made by conventional method. The casein micelles (C) dispersed in separated bundles in the whey phase (W) formed a loose external surface. Bar, 0·5 μm.

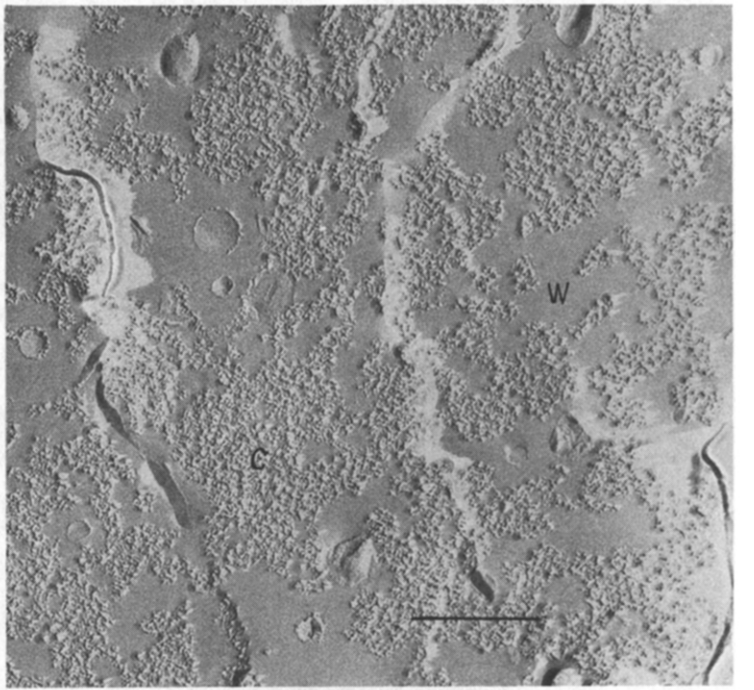

Fig. 2. Micrograph of young Domiati cheese made from ultrafiltered milk. An open network of casein granules coalesced or contiguous in aqueous phase. C: casein; W: whey; bar, 0·5 μm.

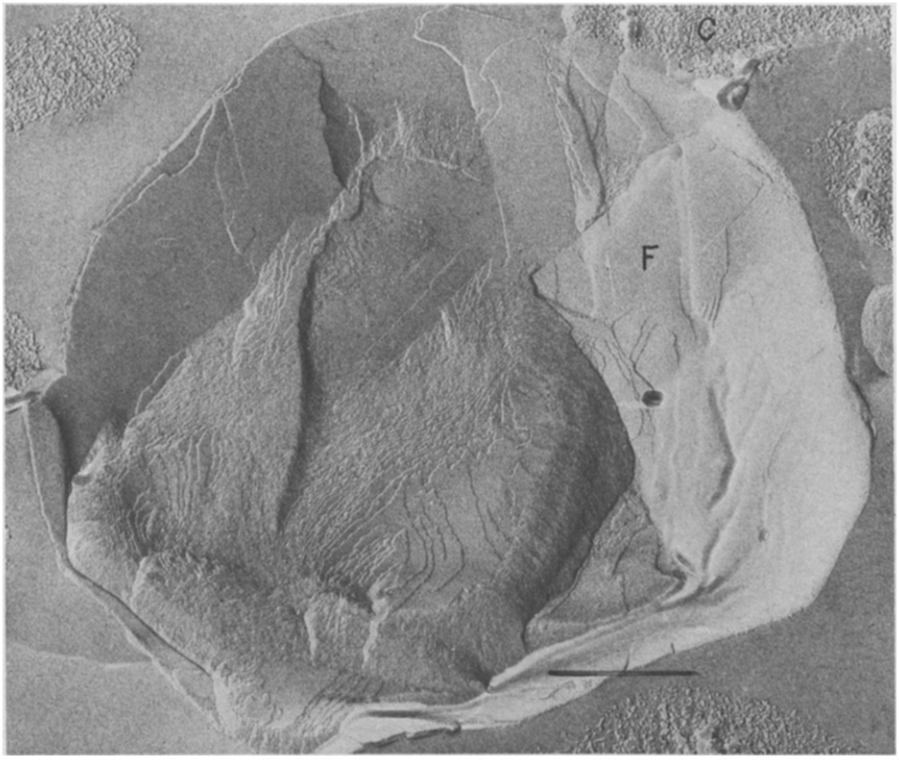

Fig. 3. Micrograph of fat globule in Domiati cheese made by conventional method, shows the interaction between casein micelles (C) and fat globule (F). bar, 0·5 μm.

These chemical changes in Domiati cheese made from ultrafiltered milk confirm the report of Mahmoud & Kosikowski (1979), Abd El-Salam *et al.* (1981), Renner & Ömeroglu (1981), and Omar *et al.* (1986).

The protein microstructures of the young cheeses did not differ from each other (Figs 1 and 2). The cheese network consists of a loose agglomerate of casein particles dispersed in separated bundles to form loose external surfaces. The fat globules were discrete and the aqueous phase concentrates at the interface (Figs 3 and 4).

Degradation of the cheese protein was observed during the ripening period on the surface area of the micrographs shown in Figs 5 and 6. The casein particles disintegrated into subunits, which then fused into a continuous protein matrix in which drainage channels contained the aqueous phase. There were no apparent differences in the fusion of the casein particles between control cheese and cheese made from ultrafiltered milk.

Fig. 4. Micrograph of fat globule of Domiati cheese made from ultrafiltered milk shows the adsorption of casein (C) at the surface of fat globule (F). Bar, 0·5 μm.

Fig. 5. Micrograph of four months matured Domiati cheese made by conventional method. Homogeneous casein bundles (C) fused together in greater masses with the whey phase (W). Bar, 0·5 μm.

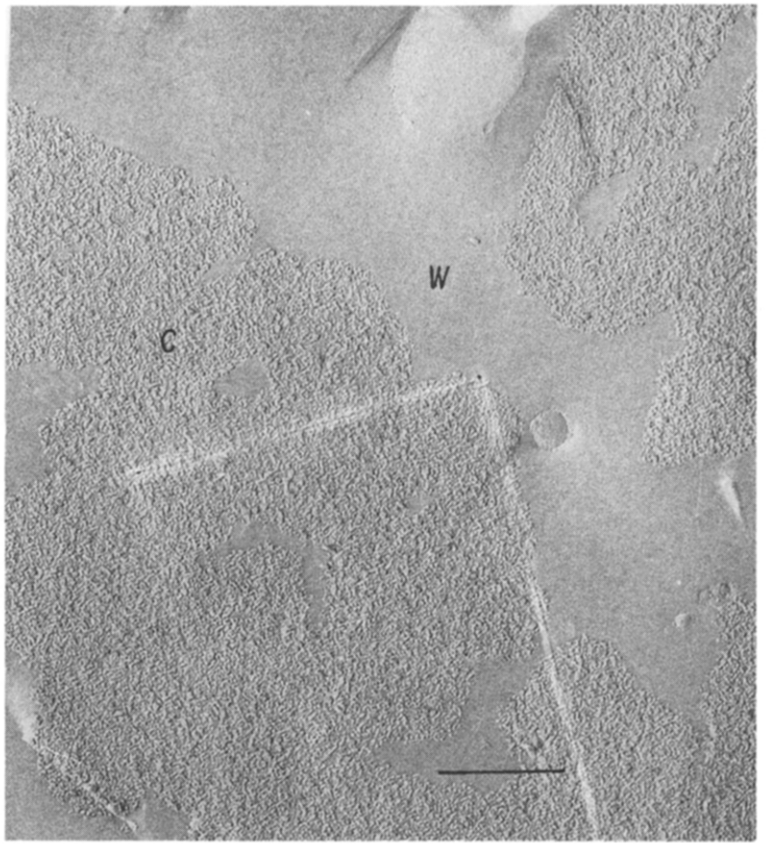

Fig. 6. Micrograph of four months matured Domiati cheese made from ultrafiltered milk. The cheese dough consists of a nearly continuous mass of single protein particles similar to that in cheese made by conventional methods. C: casein; W: whey; bar, 0·5 μm.

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