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Proteolytic Characterization of Kariesh Cheese Made from Lactose-Hydrolyzed Milk

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ABSTRACT

Differences in chemical and free amino acid compositions, microstructure and sensory quality during pickling were found between Kariesh cheese made from lactose-hydrolyzed milk and a control.

Sodium caseinate and essential free amino acids are considered to be the proteinaceous and free amino acids components of this type of cheese. Differences in flavour between the two cheese types are assumed to be due to differences in free amino acid contents during pickling. The microstructure of the ripened experimental cheese was more homogeneous than that of the control cheese.

Protein degradation, free amino acid concentration and fusion of casein micelles in the cheese protein matrix were increased, and the organoleptic quality of Kariesh cheese was improved, when β-galactosidase was added to the cheese milk before processing.

INTRODUCTION

Kariesh cheese, popular in Egypt, is a skim milk white soft cheese coagulated by the culture set method, either by natural fermentation or by starter cultures. It is characterized by an agreeable mildly acid flavour and high moisture and low fat contents. It is consumed either fresh or after pickling (El-Gendy, 1983).

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The microstructure of a number of lactic culture set cheeses has been studied—for example, cottage cheese (Glaser *et al.*, 1979, 1980), Harzer cheese (Knoop & Buchheim, 1980), cream cheese (Kalab *et al.*, 1981) and Kariesh cheese (Abd El-Salam & Omar, 1985).

The set time required for making acid coagulation cheese has been reduced—and its sensory properties improved—by using a β-galactosidase preparation, for example, during the making of cottage and Quark cheeses (Thompson & Brower, 1974, 1976; Thompson and Guricsek, 1974; Weaver & Kroger, 1978).

This work was therefore carried out to study the properties of Kariesh cheese made from milk treated with β-galactosidase compared with those of traditional cheese.

MATERIALS AND METHODS

Cheese making

Kariesh cheese was made from cow's milk pasteurized at 90 °C for 15 s. Half the milk was used as a control, the other half being 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. Five per cent of lactic acid starter (a mixture of *S. cremoris*, *S. lactis* and *S. diacetolactis*) was added to the cheese milk and incubated at 30 °C until there was complete coagulation. The curd was scooped into cheese cloth; 5% sodium chloride was added between curd layers and left to drain overnight at room temperature. The cheese was stored in a pickle solution (14% NaCl in whey) for 2 months. Cheese samples were taken after 1 day (Young) and after 1 and 2 months.

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 to 1·5 mm thick, were fixed for 30 min in 5% aqueous glutaraldehyde solution, followed by 1 h in 1% OsO_4 solution in 0·1 M phosphate buffer at pH 7·0 and 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 1:3 for another 12 h. The specimens were embedded in Epon in sealed gelatin capsules and kept at 60 °C to harden. Ultra-thin sections were made by means of a Reichert-Ultramicrotom and examined in a TESLA BS 500 electron microscope with an accelerating voltage of 80 kV.

Specimens of Kariesh cheese were prepared for scanning electron microscopy (SEM) as described by Glaser *et al.* (1979). Samples were dropped into vials containing 4% glutaraldehyde solutions in 0·1M phosphate buffer (pH 7) and held overnight at 4 °C. Secondary fixation was in 1% OsO_4 in phosphate buffer for 4 h. The specimens were dehydrated in a graded series of alcohol concentrations, 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.

Free amino acid composition

Ten grams of cheese were dissolved in 90 ml of 0·5M tris-sodium citrate solution. The mixture was 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 by 5% sulphosalicyclic 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). Free amino acids were determined in 0·8 ml of the filtrate using an amino acid analyzer (JLC/6AH Firma JEAL/JAPAN).

Chemical composition of cheese

The cheese was analysed when young—and after 1 and 2 months—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 expressed as percentage of total nitrogen content in the cheese. All samples were subjected to sensory evaluation using 40 points for body and texture and 60 points for flavour. The results were statistically analysed according to the methods of Steel & Torrie (1960).

RESULTS AND DISCUSSION

Chemical composition

The data presented in Table 1 show the changes in the chemical composition of Kariesh cheese during pickling. pH values range from 4·5 to 4·9, i.e. around the isoelectric point. The rapid coagulation and lowered pH of cheese made from lactose-hydrolyzed milk occur because β-galactosidase accelerates acid development by starter culture and increases the acidity of the product (Hassan *et al.*, 1983). The moisture content of the control cheese (74·6%) is less than that of the experimental cheese (78·2%), possibly because of the replacement of lactose by monosaccharides, which increases free water and decreases non-solvent water (Thompson & Brower, 1976). A similar result was obtained by Gooda *et al.* (1983).

Protein breakdown indices of cheese (Table 1) indicate a significant increase in the ratio of soluble nitrogen/total nitrogen, non-protein nitrogen/total nitrogen and amino acid nitrogen/total nitrogen in experimental cheese, both when young and during pickling. This increase is attributed to residual proteolytic activity in the β-galactosidase preparation (Marschke *et al.*, 1980). Gooda *et al.* (1983) found that the more intense proteolysis in lactose-hydrolyzed cheese is related to the increased production of starter peptidases.

TABLE 1
Chemical Composition of Kariesh Cheese
(Average of three treatments)

Cheese	*Age of cheese*	*pH*	*Moisture* (%)	*(TN) Total nitrogen* (%)	*Soluble N of TN* (%)	*(NPN) Non-protein N of TN* (%)	*Amino acid nitrogen of TN* (%)
Control	Young	4·9	74·6	3·02	8·70	2·56	1·76
	1 month	4·7	67·7	3·22	10·3	5·56	2·87
	2 months	4·6	66·8	3·34	21·4	9·92	5·01
Treated with	Young	4·7	78·2	2·97	11·8	4·32	2·23
β-galactosidase	1 month	4·6	72·3	3·11	15·0	9·65	4·98
	2 months	4·5	69·5	3·23	27·7	16·1	8·71

Free amino acid composition

Breakdown of protein in cheese during the ripening process leads to the accumulation of free amino acids and the specific organoleptic properties of cheese. Thus, development of cheese flavour is influenced by the nature and quantity of free amino acids.

The pattern of free amino acids in control and experimental cheese is nearly the same (Tables 2 and 3). Thirteen free amino acids have been identified in young cheese and sixteen in pickled cheese. Leucine, phenylalanine, isoleucine, methionine, lysine and valine were the major free amino acids in young control cheese. Cheese made from milk treated with β-galactosidase contained more leucine, phenylalanine, methionine, valine, isoleucine and serine.

Significant differences ($p < 0.05$) in free amino acid concentrations

TABLE 2
Free Amino Acid Composition of Kariesh Cheese (Milligrams per 100 g of Cheese)

Amino acid	*Age of cheese*					
	Young		*1 month*		*2 months*	
	mg/ 100 g	*Per cent of total*	*mg/ 100 g*	*Per cent of total*	*mg/ 100 g*	*Per cent of total*
Lysine	5·49	8·79	11·3	7·47	82·6	15·9
Histidine	0·78	1·25	4·07	2·68	23·8	4·60
Arginine	—	—	0·99	0·65	26·9	5·20
Aspartic acid	—	—	1·53	1·01	9·86	1·91
Threonine	—	—	2·32	1·53	14·6	2·84
Serine	2·14	3·43	3·25	2·14	11·8	2·29
Glutamic acid	1·07	1·71	12·2	8·01	39·1	7·56
Proline	0·94	1·50	0·27	1·49	30·6	5·91
Glycine	1·20	1·92	3·24	2·14	4·65	0·90
Alanine	2·82	4·51	7·75	5·11	22·7	4·40
Valine	5·22	8·36	13·1	8·64	42·6	8·24
Methionine	6·58	10·6	11·0	7·23	26·6	5·15
Isoleucine	7·68	12·3	14·6	9·65	31·6	6·10
Leucine	18·1	28·8	39·9	26·2	90·4	17·5
Tyrosine	2·11	3·38	8·54	5·63	30·1	5·83
Phenylalanone	8·24	13·3	15·7	10·4	29·0	5·60
Total	62·55		151·8		517·1	

TABLE 3
Free Amino Acid Composition of Kariesh Cheese Made from β-Galactosidase-Treated Milk (Milligrams per 100 g of Cheese)

Amino acid	*Age of cheese*					
	Young		*1 month*		*2 months*	
	mg/ 100 g	*Per cent of total*	*mg/ 100 g*	*Per cent of total*	*mg/ 100 g*	*Per cent of total*
Lysine	8·75	7·96	21·3	8·54	113	13·0
Histidine	0·60	0·62	4·12	1·66	30·6	3·51
Arginine	—	—	1·00	0·40	41·6	4·78
Aspartic acid	—	—	2·59	1·03	14·2	1·63
Threonine	7·43	6·76	8·10	3·25	28·3	3·25
Serine	8·23	7·48	9·17	3·68	35·5	4·07
Glutamic acid	3·16	2·87	21·5	8·65	104	11·9
Proline	—	—	3·96	1·59	34·2	3·93
Glycine	2·11	1·92	8·50	3·41	9·70	1·11
Alanine	5·13	4·66	10·2	4·11	33·4	3·83
Valine	12·0	10·9	18·1	7·28	56·0	6·43
Methionine	12·0	10·9	17·9	7·22	47·8	5·48
Isoleucine	9·60	8·73	21·6	8·68	48·1	5·52
Leucine	22·5	20·4	51·9	20·9	146	16·7
Tyrosine	3·79	3·44	22·5	9·04	72·6	8·33
Phenylalanine	14·7	13·3	26·4	10·6	56·6	6·50
Total	110		248·8		871·6	

were found in experimental cheeses compared with the control, even after 1 month's pickling; this increase was attributed to increased production of starter bacterial enzymes by the β-galactosidase preparation (Gooda *et al.*, 1983).

As ripening progressed, the concentration of free amino acids increased from 62·6 mg per 100 g of cheese in young control cheese to 152 mg per 100 g and 517 mg per 100 g of cheese after 1 and 2 months' pickling, respectively. The concentrations of free amino acids in the experimental cheese were 110 mg, 249 mg and 872 mg per 100 g at the same maturations.

The liberation of free amino acids in Kariesh cheese, either experimental or control, increased 2·4 times and 3·5 times after 1 and 2 months' pickling, respectively.

TABLE 4
Essential Free Amino Acids of Kariesh Cheese (Milligrams per 100 g of Cheese)

Amino acid	*Control cheese*			*Cheese made from β-galactosidase-treated milk*		
	Age of cheese					
	Young	*1 month*	*2 months*	*Young*	*1 month*	*2 months*
Lysine	5·49	11·3	82·6	8·75	21·3	113
Methionine	6·58	11·0	26·6	12·0	17·9	47·8
Threonine	—	2·32	14·7	7·43	8·1	28·3
Isoleucine	7·68	14·6	31·6	9·60	21·5	48·1
Leucine	18·1	39·9	90·4	22·5	51·9	146
Phenylalanine	8·42	15·7	29·0	14·7	26·4	56·6
Valine	5·22	13·1	42·6	12·0	18·1	56·0
Total	52·49	107·9	317·5	87·08	165·3	495·8

Furthermore, this study has indicated that Kariesh cheese is very rich in essential free amino acids and that treatment of milk with β-galactosidase before processing increased the concentration of essential amino acids about 60% above that of traditional cheese (Table 4).

Undoubtedly, amino acid concentration plays a part in flavour; therefore, it can be concluded that leucine, lysine, valine and glutamic acid in traditional Kariesh cheese and leucine, lysine, glutamic acid and tyrosine in experimental cheese may be related to the agreeable flavour.

Electron microscopy

In the manufacture of Kariesh cheese, milk is coagulated by lowering its pH by lactic acid starter bacteria; enzymes then bring about a degree of proteolysis and changes in the physical structure of the product.

Figure 1 illustrates the microstructure of Kariesh cheese by TEM. Control and experimental 1 day-old cheese are composed of distributed casein micelles in the form of clusters and chains, 1 μm in diameter, similar to that observed by Abd El-Salam & Omar (1985) in Kariesh cheese. Fat was found in small globules incorporated in the protein clusters (Fig. 1(a1)), resembling the small fat globules in cream cheese reported by Kaláb *et al.* (1981).

The physical structure of Kariesh cheese changed during ripening (Fig.

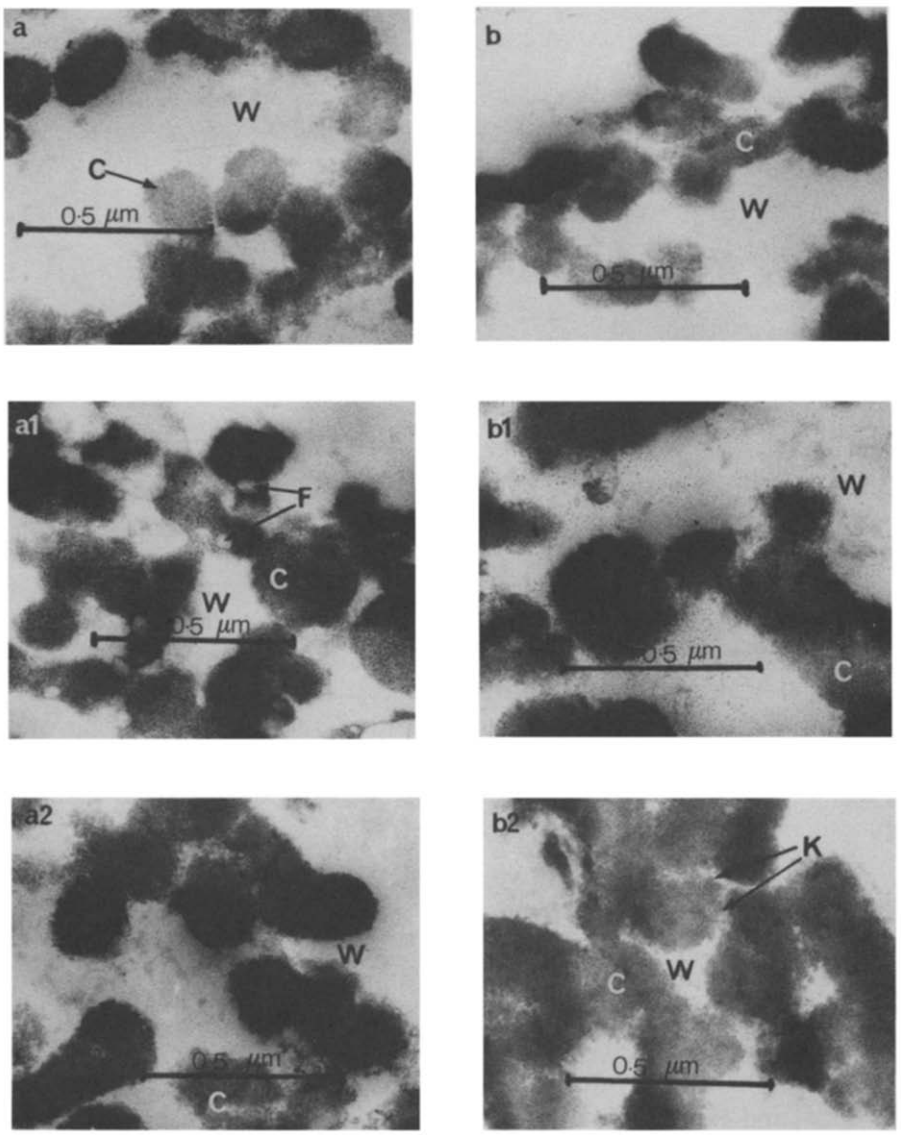

Fig. 1. TEM changes of the protein in Kariesh cheese matrix during pickling. (a, a1, a2) and (b, b1, b2): young, 1 month old and 2 months old cheese (control) and cheese treated with β-galactosidase, respectively. Casein micelles in the β-galactosidase cheese, tightly fused, form aggregates larger than in the control cheese. C = casein. F = fat. W = whey. K = whey capillary.

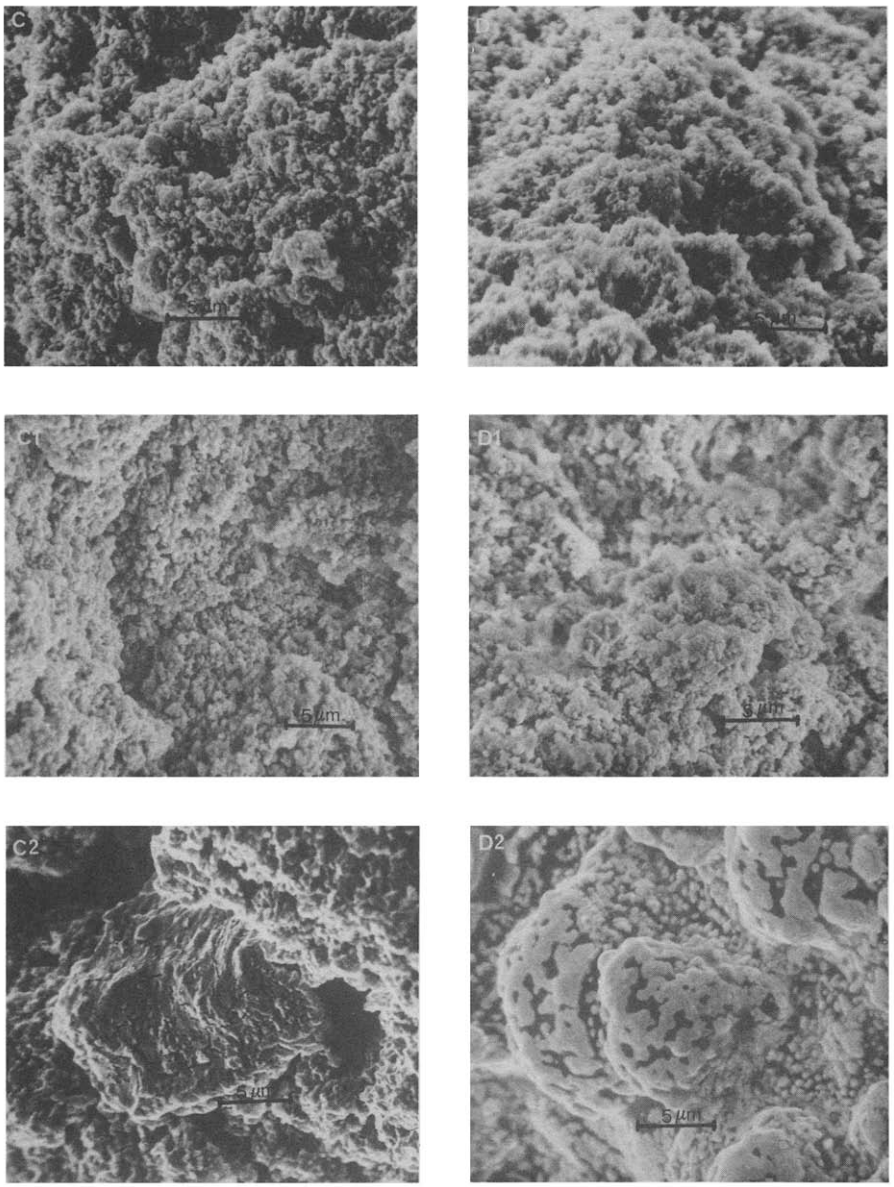

Fig. 2. SEM micrographs of control cheese (C, C1, C2) and β-galactosidase cheese (D, D1, D2). C and D: young cheese. C1 and D1: 1 month old cheese. C2 and D2: 2 months old cheese. Black area: whey phase pockets. White area: casein matrix. Protein matrix in β-galactosidase cheese (D2) is more uniform and homogeneous than in control cheese (C2).

1(a1) and (b1)). Disintegration of protein was more pronounced in experimental cheese (b1) than in control (a1).

Figure 1(a2) and (b2) indicates the extent of hydrolysis of casein in cheese ripened for 2 months. Casein micelles in experimental cheese, tightly fused, form large aggregates with homogeneous structures where the whey capillaries can be seen within the casein matrix (b2), as reported by Knoop & Buchheim (1980). The fusion of casein was lower in control cheese which had a loose structure (a2).

SEM micrographs in Fig. 2 confirm the TEM micrographs. It is clear that the protein matrix (white areas) is composed of casein micelles fused together; they form clusters and chains which interconnect to form a network filled with whey (black areas in Fig. 2(C) and (D)). The same structure was described by Glaser *et al.* (1980) in cottage cheese. The fusion of casein chains in experimental cheese (D1 and D2) was more complete than in the control, micelles agglomerating to such an extent that most of their individual identity was lost.

Sensory quality of cheese

A significant difference (from the control) in the organoleptic properties of Kariesh cheese was found in both the fresh and the pickled product (Table 5). High scores were obtained for the β-galactosidase cheese which had a good flavour and a smooth texture.

TABLE 5

Organoleptic Properties of Kariesh Cheese Made from Milk Treated with β-Galactosidase Compared with Control

	No.	*Age of cheese*								
		Young			*1 month*			*2 months*		
		A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
Control cheese	1	25	40	65	28	46	74	32	50	82
	2	24	33	57	26	38	64	28	41	69
	3	26	39	65	27	42	69	30	47	77
	$\bar{x}$	25	37·3	65·3	27	42	69	30	46	76
Treated cheese	1	29	43	72	32	49	81	30	56	86
	2	30	49	79	34	50	84	36	55	91
	3	31	42	73	35	48	83	35	57	92
	$\bar{x}$	30	44·7	74·7	33·7	49	82·7	33·7	56	89·7

A, Body and texture (40); *B*, flavour (60); *C*, total (100).

From the foregoing results it can be seen that the incorporation of a β-galactosidase preparation in cheese milk reduces the time the milk takes to coagulate, enhances breakdown of cheese protein during ripening, increases the accumulation of free amino acids and the fusion of the casein matrix, and improves the quality of Kariesh cheese.

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