Microstructure and Firmness of Processed Cheese Manufactured from Cheddar Cheese and Skim Milk Powder Cheese Base

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MICROSTRUCTURE AND FIRMNESS OF PROCESSED CHEESE MANUFACTURED FROM CHEDDAR CHEESE AND SKIM MILK POWDER CHEESE BASE

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Abstract

Processed cheese (10 different types) was made from Cheddar cheese and a *cheese base* produced from reconstituted skim milk powder by blending and melting with commercial emulsifying salts at 90°C. In one experiment, the cheese base was subjected to accelerated cheese ripening by added enzyme. The finished products had 50.1-53.5% total solids, 18.2-19.3% protein, 47.4-49.7% fat in dry matter, and 2.7-3.0% salt in water; pH was 5.3-5.4 after three months of storage at 10° and 30°C.

The experimental cheeses were markedly firmer than the control cheeses. All processed cheeses exhibited a similar pattern of firmness whereby the samples stored at 10°C were firmer than the fresh cheeses and the cheeses stored at 30°C were firmest. Only blends containing a large proportion of a cheese base treated with added enzyme were crumbly and were not satisfactory.

Electron microscopy revealed differences in the structures of the raw materials and the processed cheeses. The cheese base, to which a protease was added, had an open structure compared to a compact structure of the untreated cheese base. The microstructures of all the finished processed cheeses stored at 10°C were similar to each other. Storage of these cheeses for 3 months at 30°C resulted in the development of irregularly shaped fat particles, but differences in their dimensions were statistically not significant.

Introduction

Different types of processed cheese have been manufactured successfully on a large scale in Europe and the United States of America since the beginning of this century. Meyer (1973) has provided an excellent historical background of the development of processed cheese, and recently various aspects of the manufacture of processed cheese have been reviewed by many authors (Kosikowski, 1977; Thomas, 1977, Carić et al., 1985; Carić and Kaláb, 1987). In brief, the product is primarily made by blending natural cheeses (young, mature, or different types) in the presence of water, colouring matter, emulsifying salts and other dairy ingredients, and then heating and agitating to produce a homogenous mixture.

Such products have gained consumer acceptability over the years. They may be classified into three different types which are referred to as block variety, slices, and cheese spread. In certain countries, e.g., the United States of America and Canada, the reliance on natural Cheddar cheese for the production of processed cheese (i.e., block and slices) is apparent. Prolonged storage of Cheddar cheese is required to achieve the maturation process and this could be a disadvantage to the processed cheese manufacturer because of high costs.

Recently, Ernstrom et al. (1980) have achieved the production of cheese base from whole milk which could be used in a processed blend. For example, a blend of 80% cheese base and 20% matured Cheddar cheese was suitable for the manufacture of processed cheese. A similar method for the production of cheese base in Europe and the United States was reported by Madsen and Bjerre (1981) and Rubin and Bjerre (1983a, 1983b) who recommended the use of similar proportions of cheese base and Cheddar cheese as those reported by Ernstrom et al. (1980) or lower, i.e., 50:50 ratio. Other patent applications for the production of cheese have been reported by Jameson and Sutherland (1986) and Moran et al. (1989).

The method of cheese base production could be briefly described as follows: (a) standardisation of the fat content in milk, (b) ultrafiltration (UF) of the milk to concentrate the protein, (c) diafiltration of the retentate to reduce the lactose content, (d) acidification of the concentrate (*i.e.*, addition of starter culture) to lower the level of calcium content in the casein micelles, and (e) vacuum evaporation to remove the excess moisture. The composition of the resulting product is similar to Cheddar cheese.

In Egypt, large volumes of processed cheese are imported every year. In 1984, the total cheese imports were 42,000 tonnes, of which 10,500 tonnes was processed cheese (IDF, 1986). In economic terms, the imported cheese was valued at 82 million US $. In order to reduce cheese imports, a collaborative programme of work was established two years ago between the West of Scotland College and University of Zagazig in Egypt for the development of cheese base production from reconstituted skim milk powder for local manufacture of processed cheese.

The purpose of this research work was to examine the effect of 2 cheese bases (one without and the other with added proteolytic enzyme to induce proteolysis) produced from reconstituted skim milk powder on the microstructure and firmness of block type processed cheese.

Materials and Methods

Materials

White Cheddar cheese (young, 5 months old, and mature, 11 months old) and medium heat skim milk powder (whey protein index - 4.5 mg N/g powder) were obtained from Scottish Pride Quality Dairy Foods Ltd., Galloway Creamery, Stranraer, Scotland.

Anhydrous milk fat (AMF) (Aberdeen and District Milk Marketing Board, Bucksburn, Aberdeen, Scotland) was added to all processed cheese blends except the control (Table 1) in order to maintain a constant fat content in the cheese; this ingredient was added to the melting cooker with the other additives.

Pure vacuum-dried sodium chloride (ICI Chlor-Chemicals, Cheshire, UK) was used in the manufacture of the cheese base and in the processed cheese formulations.

Production of cheese base

Cheese base was produced as reported by Younis (1989) and Tamime *et al.* (1990). In brief, this process may be described as follows:

Skim milk powder was reconstituted at 20% total solids using water at 50°C (total weight 1200 kg) followed by ultrafiltration (UF) (APV Baker Ltd., Preston, UK) to a 2-fold concentration at 50°C by removing 600 L of permeate. Water, equal to the volume of permeate removed, was added to the retentate and diafiltration of the milk was

carried out to a 2-fold concentration at 50°C by removing 600 L of permeate. The retentate was pasteurised at 72°C for 15 s using a plate heat exchanger (APV Baker Ltd., Crawley, UK), cooled in it to < 10°C, and stored overnight in a refrigerator.

On the following day, the retentate was warmed to 32°C and ripened with a multiple strain mesophilic cheese starter culture (*Streptococcus lactis* sub-sp. *lactis* and *Str. lactis* sub-sp. *cremoris* code MAO11C from Eurozyme Ltd., London, UK), which was added at 2 g/10 kg retentate until the pH reached 5.8 + 0.1. The fermented retentate was co-agulated in an ALCURD machine MK III (Alfa-Laval Eng., Ltd., Middlesex, UK) for 10-12 min using standard calf rennet (Chr. Hansen's Lab. Ltd., Reading, UK) added at 2.5 mL/10 kg retentate. The curd was delivered to an open top cheese vat, mixed gently with stainless steel forks until pH of the whey dropped to 5.6 ± 0.1, and the curd and whey were heated indirectly to 39°C in 15 min.

Cheese Base I. After draining the whey (*i.b.*, a very small volume was removed as compared with the conventional cheesemaking process), the curd was salted at 2.5% (w/w), mixed for 15 min, hooped into a 20 kg rectangular Cheddar cheese mould, pre-pressed for 1 h at an air line pressure of 0.27 MPa, (the pressure was acting on a 20 cm diameter cylinder) and pressed overnight at 0.59 MPa. On the following day, the pressed curd was divided into 4.5 - 5 kg blocks, placed in BB-1 bags (W.R. Grace Ltd., London, UK), vacuumed, heat-sealed, and shrunk in hot water at

Table 1.

Formulations (%) of major ingredients in processed cheese blends

Processed Cheese	Cheddar M*	Y*	Cheese Base I	II	AMF	Added Water
Control	19.7	59.2	-	-	-	18.1
Exptl.	-	-	42.2	14.1	26.5	14.3
Blend A	20.5	-	41.8	-	19.9	14.8
B	20.0	40.0	13.6	-	6.4	17.1
C	-	59.7	13.6	-	6.3	17.5
D	13.2	59.4	4.5	-	2.1	17.9
A_1	20.5	-	-	41.6	19.6	15.3
B_1	20.0	39.9	-	13.6	6.4	17.2
C_1	-	59.7	-	13.6	6.3	17.5
D_1	13.2	59.3	-	4.5	2.1	18.0

* M: mature; Y: young; Cheese Base I: with no added enzyme; Cheese Base II: with added Savorase-A; AMF: anhydrous milk fat. Sodium chloride, Nisin and emulsifying salts were added to each blend at 0.44%, 0.01%, and 2.50%, respectively.

85ºC. The cheese base was stored at 10ºC until required for processing.

Cheese Base II. The drained curd was salted with 1.13% (w/w) of salt and mixed with a dried enzyme preparation from *Str. lactis* sub-sp. *lactis* (Savorase-A, marketed by Imperial Biotechnology Ltd., London, UK) at a rate of 1.65% (w/w) (the enzyme preparation was a cell-free extract and contained 50% w/w sodium chloride), and mixed for 15 min. The salted curd was handled afterwards as described above for cheese base I. According to the supplier, the Savorase-A activity was expressed as 5.3 flavour amino peptidase (FLAP) units/g.

Production of Processed Cheese

Ten different formulations of processed cheese (Table 1) were manufactured as follows: The cheese ingredients were cut into small cubes by hand and blended in a Stephan cooker, type UMM SK40E (A. Stephan u. Söhne, GmbH & Co., Hamelin, West Germany) for 1 min at 750 rpm. Different amounts of AMF, sodium chloride, and water were added to each blend including 1.4 g Nisin (Alpin and Barrett Ltd., Wiltshire, UK) which was used as a preservative, and the mixture was blended for 1 min at 750 rpm. Joha SE, C, and T (Fibrisol Services Ltd., London, UK) commercial emulsifying salts were used at a ratio of 1.25 : 1.0 : 0.75 at the total level of 3% (w/w). They were blended with the other ingredients for 1 min at 1500 rpm. Direct steam injection was used to heat and melt the cheese ingredients and blending continued at 1500 rpm until the temperature reached 85-90ºC. Vacuum was applied for 1 min to remove air bubbles and stirring was continued for 2 min. The melted cheese was hot-filled in cartons lined with commercial pouches (Pukkafilm, DRG Flexible Packaging Ltd., Bristol, UK), sealed, and cooled in a refrigerator for 3 days at 5ºC; the cheese samples were then stored for 6 months at 10º and 30ºC. The mass of each batch was 13.6 kg.

The pouches (185 x 380 mm) had the moisture vapour transmission rate of 0.8 - 1.0 g/m²/24 h at 25ºC and 75% relative humidity and the oxygen permeability rate of 20 cm³/m²/24 h at 20ºC and 0% relative humidity.

Chemical Analysis

Total solids, fat, salt, phosphorus (total), and pH were determined according to British Standards Institution methods (BSI, 1969 and 1976). Calcium and soluble nitrogen were determined by the methods described by Pearce (1977) and Kosikowski (1977, p. 572), respectively.

Casein hydrolysis was determined using polyacrylamide gel electrophoresis according to the method reported by Ridha *et al.* (1984).

Firmness Analysis

A Stevens LFRA Texture Analyser (C. Stevens & Son Ltd., Hertfordshire, UK) equipped with a type TA26/TFE-

105-524Y probe was used to assess firmness of the processed cheese. The U-shaped probe, with a wire connection, 0.33 mm in diameter, penetrated the samples into a depth of 15 mm at a rate of 0.5 mm/s. The chart recorder was operated at 500 mV and 30 mm/min chart speed. The results were expressed in newtons (N).

The firmness measurements were carried out on processed cheese samples that had been tempered at 7ºC for 3 days and cut into blocks of 50 x 20 x 20 mm.

Microscopic Analysis

Processed cheese was sampled by cutting a slice about 10 mm thick which was then sectioned into columns 10 mm wide and 25 mm long. Sample columns were fixed in a 2.8% aqueous glutaraldehyde solution and mailed to Ottawa for electron microscopy (Allan-Wojtas, 1984). After arrival, the samples were cut into prisms, 1 x 1 x 15 mm, for scanning electron microscopy (SEM) and into cubes, ~0.6 mm on a side, for transmission electron microscopy (TEM), and placed into a fresh glutaraldehyde solution for 2 h.

For SEM, the cheese prisms, fixed with glutaraldehyde, were washed with water and subsequently dehydrated in a graded (20, 40, 60, 80, 96, and 100%) ethanol series. The prisms impregnated with absolute ethanol were defatted by extraction using 3 changes of chloroform, returned into ethanol, frozen in Freon 12 cooled to its freezing point with liquid nitrogen, and placed in liquid nitrogen, where they were fractured. The fragments were critical point-dried from carbon dioxide, mounted on aluminium SEM stubs, sputter-coated with gold, and examined at 20 kV in an ISI DS-130 scanning electron microscope equipped with an external oscilloscope (Bond and Kaláb, 1988). Micrographs were taken on 35 mm film.

For TEM, the 0.6 mm cubes were washed with a 0.05 M veronal-acetate buffer (pH 6.75) and were postfixed for 2 h with a 2% osmium tetroxide solution in the same veronal-acetate buffer. Then, the cubes were embedded in Spurr's low-viscosity embedding medium (J. B. EM Service, Inc., Pointe Claire, Dorval, Quebec), and sectioned. The sections (approx 90 nm thick) were stained with uranyl acetate and lead citrate solutions (Reynolds, 1963) and examined in a Philips EM-300 transmission electron microscope operated at 60 kV. Micrographs were taken on 35 mm film.

Digital Image Analysis.

TEM micrographs of 9 μm x 10 μm areas, taken at a 20,000x magnification, were evaluated using a Kontron IBAS image analyser (Carl Zeiss Canada, Don Mills, Ontario, Canada) for the distribution of fat globule section diameters; in the case of irregularly shaped fat particles, their section areas were measured and the results expressed as the diameters of circles with equivalent areas.

Results and Discussion

Chemical Composition

Ten different blends of processed cheese under study were made from young and mature Cheddar cheese and cheese base I or II, or a combination of both; the formulations are presented in Table 1. In blends A and B, the young Cheddar cheese was replaced by cheese base I at different proportions, and in blends C and D, the mature Cheddar cheese was replaced by the same type of cheese base. A similar approach was used for blends A_1 to D_1 where cheese base II was used to replace young or mature Cheddar cheese.

Studies of processed cheese samples prepared according to the formulations shown in Table 1 were carried out in order to: (a) establish the feasibility of using a cheese base made from reconstituted skim milk powder in the manufacture of processed cheese, (b) compare the results obtained with the recommendations for various amounts of a whole-milk cheese base in processed cheese by other authors (Ernstrom et al., 1980; Madsen and Bjerre, 1981; Rubin and Bjerre, 1983a, 1983b), and (c) establish appropriate proportions of the developed cheese bases I and II so that they could be recommended for the production of block type processed cheese suitable for the Egyptian market.

Samples of each type of processed cheese were analysed when fresh and then again after 3 months of storage at 10° and 30°C. Chemical composition of these cheeses is presented in Table 2 and data in greater detail have been published elsewhere (Younis, 1989). The total solids contents ranged between 50.1 and 53.5% and the fat contents in the dry matter ranged between 47.4 and 49.5% and, thus, met the Egyptian specifications for full-fat processed cheese, i.e., maximum 50% moisture and minimum 45% fat in dry matter (Egyptian Standards, 1970).

The level of sodium chloride (1.7%) and pH (5.4) were acceptable for block type processed cheese (Meyer, 1973; Kosikowski, 1977; Thomas, 1977). The calcium and phosphorus contents in Cheddar cheese and cheese bases I and II are shown in Table 3. It can be observed that the calcium content in the cheese bases was higher than in Cheddar cheese and the cheese bases contained approximately half the amount of phosphorus present in Cheddar cheese.

The firmness of the 10 various types of processed cheese (Figs. 1A and 1B) was affected by a number of factors such as:

(a) Type of cheese used: the firmness of the experimental processed cheese, which was made totally from cheese bases I and II, was 2.5 times higher than that of the control samples. (b) The addition of cheese base: blends

Table 2.

Chemical composition (%) of various processed cheese blends after storage for 3 months at 10°C and 30°C

Procd. Cheese	Temp.* °C	Total Solids	FDM*	Protein	SM*	pH
Control	10.0	50.5	48.3	18.5	3.5	5.4
	30.0	50.8	49.7	18.4	3.4	5.4
Exptl.	10.0	51.9	47.4	18.9	2.9	5.3
	30.0	51.7	48.4	19.3	2.7	5.4
Blend A	10.0	50.8	48.3	18.6	2.9	5.3
	30.0	50.6	48.8	19.2	3.0	5.4
B	10.0	52.7	48.0	19.0	3.7	5.4
	30.0	52.8	48.9	19.3	3.6	5.4
C	10.0	52.9	48.0	19.0	3.4	5.4
	30.0	53.5	48.6	19.3	3.5	5.4
D	10.0	52.3	48.0	19.0	3.5	5.4
	30.0	52.4	49.2	19.0	3.5	5.4
A_1	10.0	50.1	49.5	19.1	2.8	5.3
	30.0	52.4	49.1	19.1	2.7	5.3
B_1	10.0	50.2	48.4	18.5	3.3	5.3
	30.0	50.4	48.8	18.3	3.1	5.3
C_1	10.0	50.9	49.2	18.6	3.3	5.3
	30.0	51.4	48.9	18.8	3.3	5.3
D_1	10.0	50.4	48.0	18.2	3.6	5.4
	30.0	50.7	49.3	18.3	3.5	5.4

* Temp.: Storage temperature; FDM: fat in dry matter; SM: salt in moisture; for details refer to Table 1. Results are the average of two duplicates from the same sample.

Table 3.

Calcium and phosphorus contents (%) in cheese base and Cheddar cheese

Product	Ca	P
Cheese Base I*	1.02	0.45
Cheese Base II*	1.02	0.38
Cheddar cheese (Y)*	0.78	0.66
Cheddar cheese (M)*	0.82	0.64

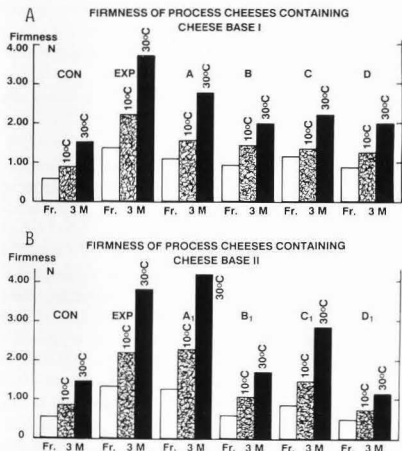*I, II, Y, M: see Table 1 for details. Ca: calcium; P: phosphorus.

Fig. 1. *Firmness of processed cheeses made with varying amounts of cheese base I (A) or cheese base II (B) as compared with the control and experimental processed cheeses.*

CON: Control; EXP: Experimental; Fr. Fresh; 3M: Storage for 3 months; A, B, C, D, A_1, B_1, C_1 and D_1: Corresponding processed cheese blends; for details refer to Table 1.

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containing the largest proportion of cheese base I or II were the firmest but a reduction in firmness was observed as the levels of cheese bases I or II were reduced in blends A-D and A_1-D_1, respectively. (*c*) Duration and temperature of storage: the firmness of all the processed cheeses markedly increased during storage and the cheeses became firmest by storage at 30°C. (*d*) Change in pH: all the cheeses exhibited a slight drop in pH from 5.5-5.6 when fresh to 5.3-5.4 after 3 months of storage. This change could be due to the presence of emulsifying salts and may have influenced some interactions that had increased the firmness of the cheese; it is known that as pH is decreased towards the isoelectric point of casein, the texture of processed cheese may become crumbly (Carić *et al.*, 1985).

The firmness of the various blends of processed cheese, including the control and experimental samples, found after 3 months of storage at 10° and 30°C, could be attributed to the interactions between the cheese proteins and the emulsifying salts. According to Carić *et al.* (1985), emulsifying salts are used in process cheese to remove calcium from the protein system, hydrate, peptise, swell, solubilise, and disperse the proteins, emulsify the fat and

stabilise the emulsion, control pH and stabilise it, and contribute to the formation of an appropriate structure of the processed cheese after cooling.

Concerning the functions of the emulsifying salts listed above, it may be assumed that the differences in firmness of the process cheeses under study were related to a number of factors such as protein-protein interactions and/or reactions of proteins with the SE, C, and T commercial Joha emulsifying salts.

Proteolysis

Electrophoretic patterns of cheese bases I and II and young and mature Cheddar cheeses are shown in Fig. 2A. It may be observed that α- and β-casein in cheese base I remained intact after two weeks. In cheese base II, which contained added Savorase-A, β-casein was extensively hydrolysed after 2 weeks whereas α-casein was hydrolysed to a lesser degree. Tamime *et al.* (1990) reported that the extent of protein hydrolysis (expressed as glycine equivalent in mg/mL of the supernatant) in 2 weeks old cheese base II and mature Cheddar cheese was 400 and 490, respectively, and the soluble nitrogen content of the same cheeses was 1.06 and 1.02%, respectively. Since the maturity index of natural cheeses is measured indirectly by the level of glycine equivalent and soluble nitrogen contents, it may be concluded on this basis that the extent of proteolysis in cheese base II was similar to that in a mature Cheddar cheese (Law, 1987).

Casein fractions in the processed cheeses under study are shown in Fig. 2B (fresh samples), Fig. 2C (3-month storage at 10°C), and Fig. 2D (3-month storage at 30°C). The following phenomena have been observed:

(*a*) The two main bands of α-casein in the experimental processed cheese made solely from cheese bases I and II appear to be intact in contrast with the control processed cheese where α-casein has been extensively hydrolyzed. It is probable that firmness of the experimental cheese (Fig. 1A) could have been associated with reduced hydrolysis of α-casein and the presence of a greater amount of β-casein which has originated from cheese base I (Fig. 2A). (*b*) The concentrations of the β-casein fraction in the eight processed cheese blends examined when fresh and after storage for 3 months at 10° and 30°C, appear to be similar. (*c*) In processed cheese blends A, B, C, and D, there was a progressive reduction in the intensity of the α-casein band which could have affected the firmness of these cheeses (Fig. 1A). A similar pattern may also be observed in blends A_1, B_1, C_1, and D_1. The increased number of fractions beyond α-casein could also play a role in the protein-protein interactions in relation to the firmness of the products. As shown in Fig 1B, the firmness of blend A_1 was the highest (4.263 N in the sample stored for 3 months at 30°C).
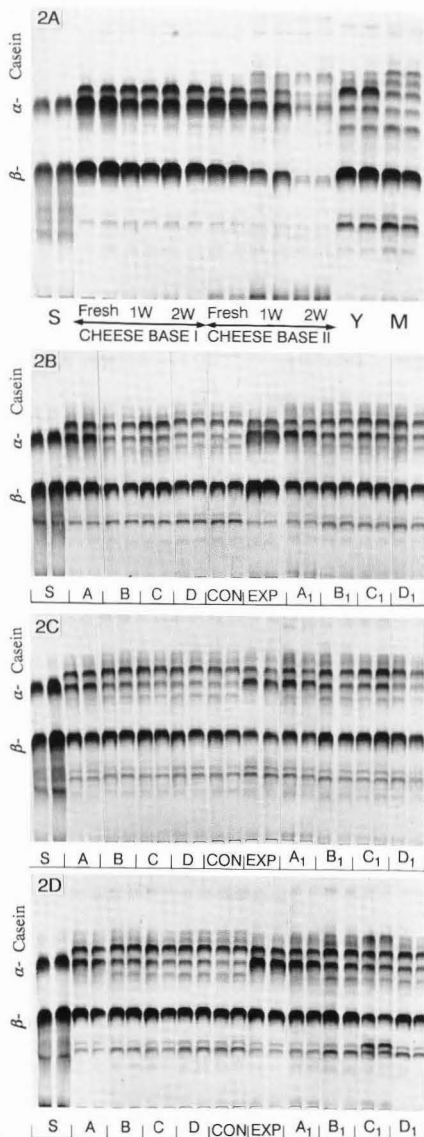
Fig. 2. *Polyacrylamide gel electrophoresis patterns of the cheese bases and Cheddar cheese (A) and processed cheese blends analysed when fresh (B) and after storage for 3 months at 10°C (C) and at 30°C (D).*

S: *casein standard; W: week; Y: young Cheddar cheese; M: mature Cheddar cheese; for letters A to D1 refer to Table 1.*

It would be difficult to conclude that the firmness of the processed cheese was attributed only to the degree of casein hydrolysis and the possible interactions of the casein fractions. Probably, whey proteins also play a role. A yield of protein greater than would be expected from a normal cheese-making process was obtained when a cheese base was made from ultrafiltered milk (Ernstrom *et al.*, 1980). This increased yield has been attributed to the retention of greater amounts of whey proteins in the cheese base. It is assumed that during the melting stage of the cheese ingredients in the processing kettle at temperatures above 70°C, β-lactoglobulin unfolds due to denaturation (de Wit, 1985) and readily reacts with κ-casein as a result of disulphide interchange (Walstra and Jenness, 1984) This may explain the consistent difference in firmness between the experimental and control processed cheeses. It was also observed that as the amounts of cheese bases I or II in the processed cheese blend were reduced, and, thus, the amount of β-lactoglobulin was decreased, the products became softer (Fig. 1).

Effect of Emulsifying Salts

Another factor, which may affect firmness of processed cheese, is the type of the emulsifying salt used. The exact composition of the SE, C, and T emulsifying salts is not known, but according to the supplier in the UK (M. Nightingale - personal communication), the main components are sodium polyphosphates. Table 4 provides some specifications of the emulsifying salts used. According to the review by Carić *et al.* (1985), all condensed polyphosphates hydrolyze partially during cheese melting and the hydrolysis continues in the processed cheese during storage

Table 4.
Some specifications of Joha emulsifying salts

Type	Ion-Ex-change	pH Change	Creaming	P-polymer (%)			
				P_1	P_2	P_3	P_4-P_{10}
SE	XXX	(+) 0.2-0.4	XX	-	50	Tr	45
C	XXX	(-) 0.4-0.6	0	-	5	-	95
T	X	(+) 1.0-1.5	0	95	5	-	-

O: nil; X: slight; XX: medium; XXX: strong; Tr: trace.
P: phosphate polymer.

Firmness of all the processed cheese samples under study exhibited a similar pattern. They became firmer after 3 months of storage and the firmness was higher in samples stored at 30°C than in samples stored at 10°C. It is possible that the higher firmness found in samples stored at 30°C may be attributed to a more extensive hydrolysis of the polyphosphates at this temperature as compared to 10°C. This could lead to a calcium-induced interaction among the proteins and result in a harder product.

Lee et al. (1979, 1986) concluded that the nonsedimentable nitrogen (possibly soluble nitrogen) in the supernatant obtained by centrifugation of an aqueous extract of processed cheese was markedly increased when the amount of the emulsifying salts (Joha S4, K, and T) was increased from 0.42 to 3.33%. These results may confirm the effect of emulsifying salts on the peptisation of proteins during the manufacture of processed cheese. The soluble nitrogen content in all the processed cheese blends is shown in Table 5. Using an analytical method different from that used by Lee et al. (1979), the following observations were made: (a) all the processed cheese blends stored at 30°C for 3 months contained slightly higher concentrations of soluble nitrogen than the freshly made processed cheeses, and (b) all processed cheese samples stored at 10°C for 3 months had lower soluble nitrogen contents. The former finding confirms that a more extensive hydrolysis of condensed polyphosphates takes place in processed cheeses

during storage, particularly at a higher temperature. This effect may be considered similar to the effect of an increased amount of emulsifying salt in the processed cheese blend as reported by Lee et al. (1986).

Microstructure

Electron microscopy showed that there were marked differences in the microstructure of the raw materials used to produce the processed cheese samples. Cheddar cheese, which was made from full-fat nonhomogenised milk, consisted of a protein matrix in which large fat globules and their clusters were dispersed (Fig. 3) in agreement with the findings of other authors (Green et al., 1981). Because fat was extracted with chloroform from Cheddar cheese samples destined for the SEM examination, void spaces indicate in the micrographs the initial presence of the fat particles and their aggregates in the samples. Residues of the fat globule membranes may usually be seen in the void

Table 5.
Soluble nitrogen content (%) in various types of processed cheese

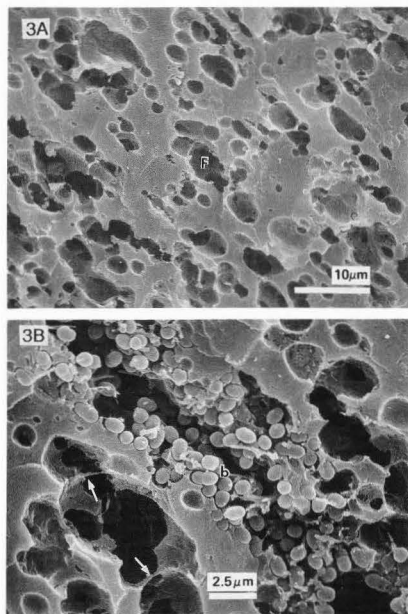| Processed Cheese	Fresh	Duration of Storage 3 Months	
		10°C	30°C
Control	0.538	0.494	0.576
Experimental	0.342	0.269	0.392
Blend A	0.392	0.341	0.398
B	0.498	0.443	0.531
C	0.441	0.414	0.459
D	0.539	0.474	0.550
A_1	0.553	0.469	0.571
B_1	0.576	0.480	0.575
C_1	0.469	0.460	0.478
D_1	0.541	0.479	0.560

Fig. 3. Microstructure of Cheddar cheese.
A: Void spaces in the protein matrix indicate the locations of fat globules (F) and their clusters in young Cheddar cheese. B: Mesophilic lactic streptococci (b); arrows point to residues of fat globule membranes which became noticeable after fat was extracted from the samples in preparation for SEM.
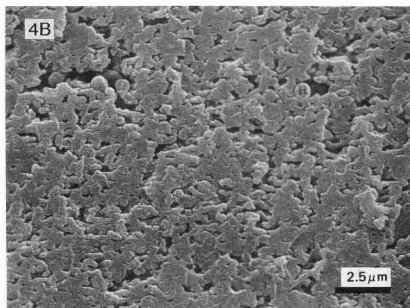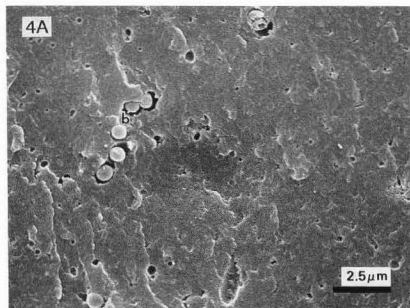
Fig. 4. Microstructure (SEM) of cheese bases I and II.
A: Cheese base I (free of added proteolytic enzyme) had a compact structure. b: Streptococci. B: Cheese base II (made

with added Savorase-A) had an open structure as the result of the proteolytic activity of the added protease.
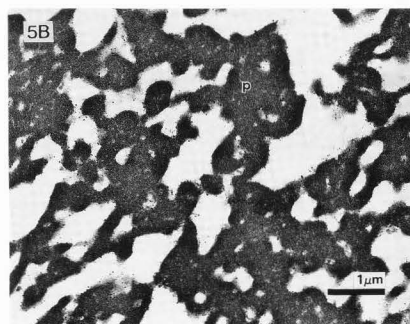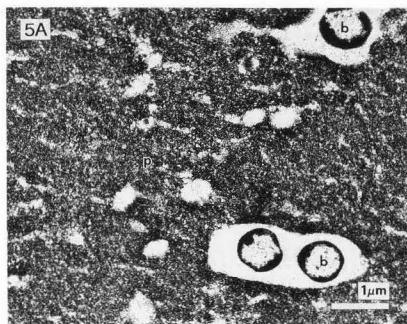

Fig. 5. Microstructure (TEM) of cheese bases I and II.
A: Cheese base I (free of added proteolytic enzyme) consist-ed of a compact protein matrix (p); b: Streptococci. B: Cheese

base II (made with added Savorase-A) had an open protein structure (p) as the result of the proteolytic activity of the added protease.

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spaces, particularly at a higher magnification (Fig. 3B). In addition, clusters of mesophilic lactic steptococci which originated from the starter culture were also present in the protein matrices (Fig. 3B) of the samples under study. There was a low incidence of calcium phosphate crystals in both Cheddar cheese varieties.

In contrast to Cheddar cheese, cheese bases I and II consisted of a protein matrix in which no fat was noticeable (Figs. 4 and 5). This was in agreement with the composition of the cheese bases which were made from reconstituted skim milk powder. There were differences between the structures of both cheese bases. Cheese base I was compact (Figs. 4A and 5A) but cheese base II was porous or open

(Figs. 4B and 5B) as the result of the treatment with Savorase-A which had been added in order to stimulate ac-celerated ripening in this cheese base.

Processing resulted in the development of structures in the control and experimental cheeses (Fig. 6) which differ-ed from the structures of the raw materials (Figs. 3, 4, and 5). As the result of emulsification, fat particles were reduc-ed in dimensions from those in excess of 1 μm in Cheddar cheese to 0.322 ± 0.105 μm (mean diameter $\pm$ standard de-viation of the fat particle sections) in processed cheese blend A_1, and to 0.362 ± 0.144 μm, 0.336 ± 0.127 μm, and 0.291 ± 0.082 μm in processed cheese blends B_1, C_1, and D_1, respectively. The emulsified fat particles were of globular
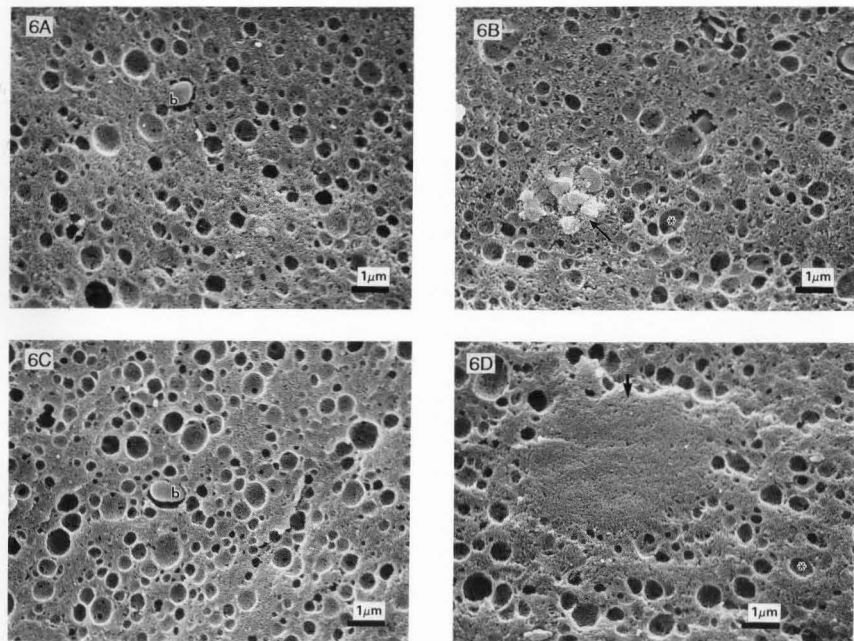
Fig. 6. *Control (A, B) and experimental (C, D) processed cheese samples stored for 3 months at 10°C (A, C) and 30°C (B, D). Fat particles (as indicated by the void spaces) were relatively* uniformly distributed in the protein matrix. In B and D, some fat particles were of irregular shapes (*). b: Streptococci; Small arrow points to crystalline inclusions; Large arrow points to a compact lump of cheese base.

shape in fresh processed cheese samples and were more or less uniformly distributed in their protein matrices.

Although the structures of the raw materials (Cheddar cheese, cheese base I, and cheese base II) used to make these processed cheeses considerably differed from each other, processing resulted in the development of structures in the processed cheese blends which were, in general, similar, irrespective of the proportions of the individual ingredients used. This similarity was particularly clearly evident in processed cheeses stored at 10°C (Figs. 6A and 6C). In all the processed cheeses stored at 30°C, the fat particles were of irregular shapes (Figs. 6C and D). Areas consisting of compact protein free of fat particles were occasionally seen in the experimental cheese (Fig. 6D) made with anhydrous milk fat and the cheese bases prepared from reconstituted skim milk. Either the cheese

base proteins were not yet properly peptised when processing was arrested, or a proper emulsification of the anhydrous milk fat was more difficult to achieve than the emulsification of fat present in the form of fat globules in natural cheeses such as Cheddar cheese in the control processed cheese made under similar conditions.

Differences between the structure of the experimental processed cheese samples stored at 10°C and 30°C are more noticeable in TEM micrographs (Figs. 7 and 8). The irregular shapes of the fat particles may be the result of coalescence of smaller fat particles and an incomplete restoration of the globular shapes. However, the irregular shapes of some fat particles, particularly those shown in Figs. 8B and 8F (arrows), seem to indicate that additional emulsification of the larger fat particles probably took place during storage at the higher temperature. Image
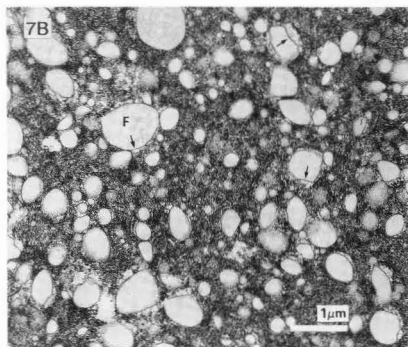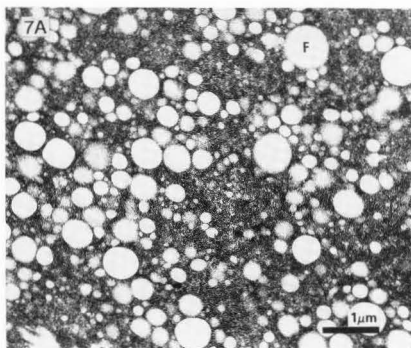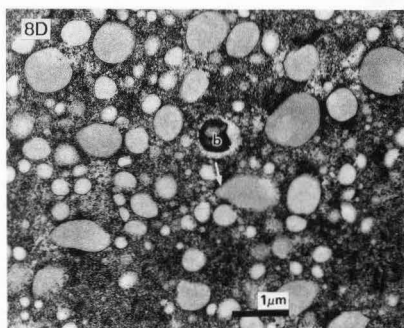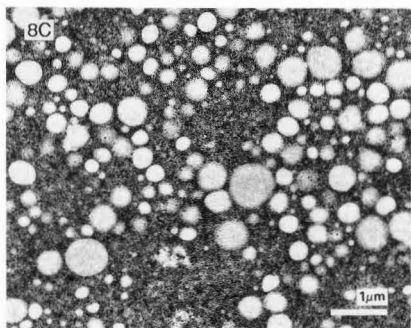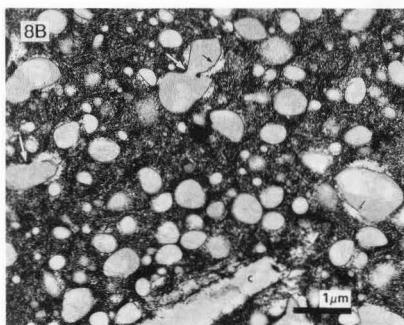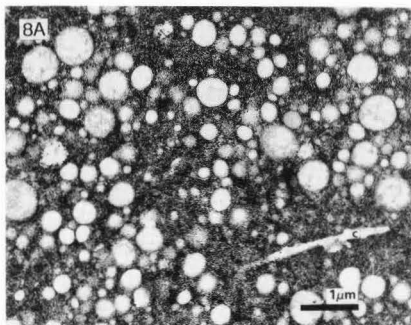
Fig. 7. TEM of experimental processed cheese stored for 3 months at 10°C (A) or 30°C (B). Deformation of fat globules (F) is evident in the product stored at 30°C; arrows point to newly developed fat globule membranes.
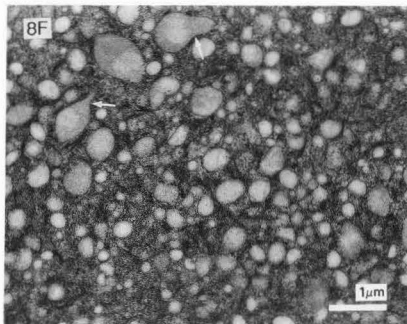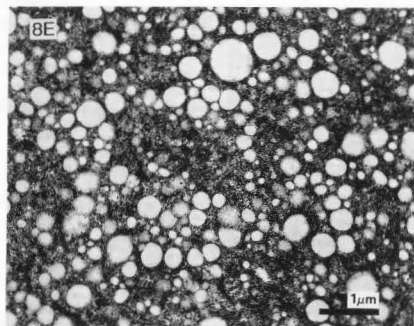
Fig. 8. TEM of processed cheese blends stored for 3 months at 10°C or 30°C.
Processed cheese blend A1 stored at 10°C (A) and at 30°C (B); blend B1 stored at 10°C (C) and at 30°C (D), and blend D stored at 10°C (E) and at 30°C (F). Light arrows point to fat particles of irregular shapes, dark arrows point to newly developed fat globule membranes. b: Bacterium; c: Crystals of melting salt.
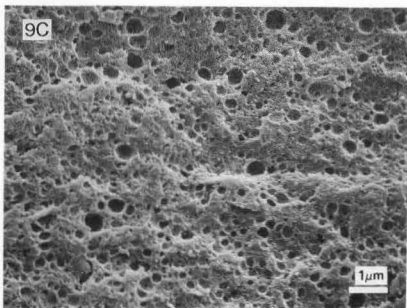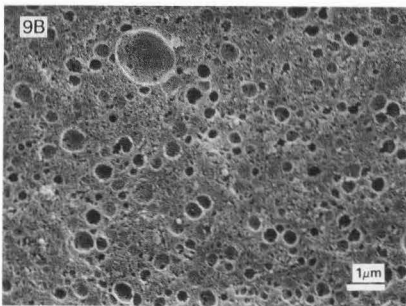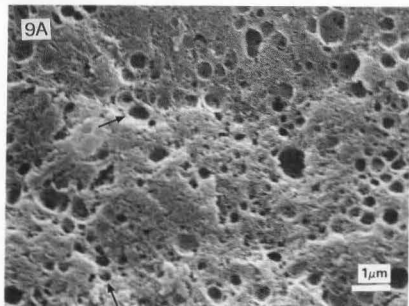

Fig. 9. SEM of processed cheese blend A (A), blend B (B), and blend C (C).
Granularity is well developed in A (arrows point to accumulated fat particles), is negligible in B, and is fine in C.

analysis (approximately 200 fat particles per field) failed to show any statistically significant differences between the dimensions of the fat globule sections in cheeses stored at 10°C and 30°C. Rather than an increase in the dimensions of the fat particles, which would be anticipated in the case of coalescence, there was a slight tendency toward the reduction in the fat particle dimensions in one half of the samples shown in Table 6.

Table 6.

Fat particle dimensions in various types of processed cheese stored for 3 months at 10°C or 30°C

Cheese	Diameters ($m\mu$) of fat particle sections*	
	10°C	30°C**
Control	0.303+0.095	0.309+0.098
Exp.	0.314+0.118	0.338+0.138
Blend A_1	0.322+0.105	0.358+0.145
B_1	0.362+0.144	0.351+0.133
C_1	0.336+0.127	0.325+0.126
D_1	0.291+0.082	0.282+0.080

*Approximately 200 fat particle sections were analysed with each processed cheese sample.
** Diameters calculated from mean areas of fat particle sections.

Another interesting phenomenon noticeable in the processed cheese stored at 30°C was the existence of membranes encasing fat particles (Figs. 7B and 8B). It has been assumed (Carić et al., 1985) that fat globule membranes are destroyed by cheese processing but it was not clear as to whether new membranes develop on the surface of the emulsified fat particles. In the processed cheeses stored at 30°C, such membranes became visible because of a light background provided by the presence of electron-translucent material around the fat particles. It is not possible to hypothesize at this stage as to whether the electron translucent areas beyond the perimeter of the fat particles contained fat or the aqueous phase.

Some differences observed in the microstructures of the cheeses under study could be attributed to the type of cheese used in the blend (e.g., Cheddar cheese and cheese base I or II, or a combination of both cheese bases) in addition to the storage temperature. Processed cheeses made from cheese base I and II consisted of compact protein matrices possibly due to the removal of calcium ions from the casein micelles under the sequestering or calcium-complexing action of the emulsifying salts. The main differences in the microstructures of these cheeses as compared with the control cheese may be summarised as follows:

(a) Blend A, where all the young Cheddar cheese had been replaced by fat-free cheese base I and an amount of anhydrous milk fat making it equivalent to the fat deficient in cheese base II had a structure which, following storage at 30°C, upon freeze-fracturing gave an impression of

granularity, as relatively compact protein areas were surrounded by areas rich in fat particles (Fig. 9A). The relatively high proportion of cheese base I in comparison with the proportion of mature Cheddar may also be a factor contributing to that granularity. (b) Blend B, which was made from young and mature Cheddar cheese and contained smaller proportions of cheese base I and anhydrous fat than blend A, had, when stored at 10°C, a less grainy structure (Fig. 9B) than blend A. (c) Blend C, in which all mature Cheddar cheese was replaced by cheese base I was macroscopically heterogeneous but the samples examined by SEM (Fig. 9C) and TEM showed finer granularity than blend A. The granular appearance was not noticeable in blend D where Cheddar cheese was only partially replaced. (d) In the remaining blends, i.e., A_1, B_1, C_1, and D_1, the microstructures were similar to the control and experimental cheeses, but blend A_1 had a crumbly and sticky texture as revealed by sensory evaluation, probably because it consisted of a high proportion of young Cheddar cheese and cheese base II in which proteolysis was induced by added Savorase-A.

Various salt crystals were observed in the protein matrices of the raw materials and the processed cheeses under study (Fig. 10). It would be difficult to suggest the exact nature of these crystals because of lack of data regarding the components of the Joha emulsifying salts. However, it may be assumed that these crystals were phosphate complexes, possibly calcium phosphate, because the Joha salts contain a large proportion of sodium phosphate. Crystal formation in processed cheese has been reported in the literature when a known type of emulsifying salts has been used (Brooker et al., 1975; Rayan et al., 1980; Kaláb, 1981; Carić et al., 1985; Brooker, 1987; Carić and Kaláb, 1987; Pommert et al., 1988; Savello et al., 1989) and such data could be used to help identify the crystals observed in the present study.

This study indicates that the microstructures of the different blends of processed cheese were affected by the type of cheese (Cheddar and/or cheese base) used in the blend. It is safe to conclude that the new method used for the production of cheese bases I and II from reconstituted skim milk powder is suitable for the manufacture of block type processed cheese, which in this study has been marked as the experimental batch.

In processed cheese blends containing different amounts of Cheddar cheese and cheese base I or II, however, some undesirable characteristics, e.g., crumbliness or grainy structure, were observed. These could be eliminated by altering the processing conditions, e.g., by using different types of emulsifying salts.

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References

Allan-Wojtas P. (1984). Transportation of fragile food specimens such as milk gels destined for electron microscopy. Food Microstruc. 3 (1), 93.

Bond EF, Kaláb M. (1988). An external oscilloscope improves photography with the ISI DS-130 scanning electron microscope. EMSA Bull. 18(1), 90-91.

Brooker BE. (1987). The crystallization of calcium phosphate at the surface of mould-ripened cheeses. Food Microstruc. 6, 25-33.

Brooker BE, Hobbs DG, Turvey A. (1975). Observation on the microscopic crystallinic inclusions in Cheddar cheese. J. Dairy Res. 42, 341-348.

BSI. (1969). BS:692 Part 2 Gerber method for the determination of fat in milk and milk products. British Standards Institution, London, UK.

BSI. (1976). BS:770 Part 1 to 5. The chemical analysis of cheese. British Standards Institution, London, UK.

Carić M, Kaláb M. (1987). Processed cheese products. In: Cheese: Chemistry, Physics and Microbiology - Major Cheese Groups, Vol. 2, PF Fox (ed.), Elsevier Applied Science Publishers Ltd., London, UK, 339-383.

Carić M, Gantar M, Kaláb M. (1985). Effects of emulsifying agents on the microstructure and other characteristics of process cheese - A review. Food Microstruc. 6, 297-312.

de Wit JN. (1985). Milk Proteins - Proceedings of the International Congress on Milk Protein 1984, TE Galesloot and BJ Tinbergen (eds.), Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, 183-195.

Egyptian Standards. (1970). Specifications of dairy products - MKM 999. Ministry of Agriculture, Cairo, Egypt, 3-5.

Ernstrom CA, Sutherland BJ, Jameson, GW. (1980). Cheese base for processing - A high yield production from whole milk by ultrafiltration. J. Dairy Sci. 63, 228-234.

Green ML, Turvey A, Hobbs DG. (1981). Development of structure and texture in Cheddar cheese. J. Dairy Res. 48, 343-355.

Heertje I, Boskamp MJ, van Kleef F, Gortemaker FH. (1981). The microstructure of processed cheese. Neth. Milk Dairy J. 35, 177-179.

IDF (1986). The World Market of Cheese - Doc 203. International Dairy Federation, Brussels, Belgium, 5-7.

Jameson GW, Sutherland BJ. (1986). Processing dairy products. Australian Patent No. 559 021.

Kaláb M. (1981). Electron microscopy of milk products: A review of techniques. Scanning Electron Microsc. 1981; III, 453-472.

Kaláb M, Yun J, Yiu SH. (1987). Textural properties and microstructure of process cheese food rework. Food Microstruc. 6(2), 181-192.

Kosikowski FV. (1977). Cheese and Fermented Milk Foods. Kosikowski and Associates, New York, USA, 382-406.

Law BA. (1987). Cheese: Chemistry, Physics and Microbiology - General Aspects, Vol. 1, PF Fox (ed.), Elsevier Applied Science Publishers Ltd., London, UK, 365-392.

Lee BO, Paquet D, Alais C. (1979). Etude biochimique de la fonte des fromages - I: Mesure de la peptisation. Le Lait 59, 589-596.

Lee BO, Paquet D, Alais C. (1986). Etude biochimique de la fonte des fromages - IV: Effet du type de sels de fonte et de la nature de la matière protéique sur la peptisation/utilisation d'un système model. Le Lait 66, 257-267.

Madsen RF, Bjerre P. (1981). Production of cheese base. North European Dairy J. 47, 135-139.

Meyer A. (1973). Processed Cheese Manufacture. Food Trade Press Ltd., London, U.K., 1st ed., 11-29.

Moran JW, Peedal JR, Trecker GW. (1989). Manufacture of curd and cheese from a milk retentate. US Patent No. 4 820 530.

Pearce KN. (1977). The complexometric determination of calcium in dairy products. N.Z. J. Dairy Sci. Technol. 12, 113-115.

Pommert JF, Klaebe A, Perie J, Lebugle A, Puech J. (1988). Observation and analysis of crystalline phases in processed cheese. J. Food Sci. 53, 1367-1369 and 1447.

Rayan AA, Kaláb M, Ernstrom CA. (1980). Microstructure and rheology of process cheese. Scanning Electron Microsc. 1980; III, 635-643.

Reynolds ES. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17, 208-212.

Ridha SH, Crawford RJM, Tamime AY. (1984). Comparative studies of casein breakdown in Cheddar

cheese manufactured from lactose-hydrolysed milk. J. Food Prot. 47, 381-387.

Rubin J, Bjerre P. (1983a). A process for preparing cheese-base. UK Patent No. GB 2 101 866 A.

Rubin J, Bjerre P. (1983b). A process for preparing cheese-base. US Patent No. 4 401 679.

Savello PA, Ernstrom CA, Kaláb M. (1989). Microstructure and meltability of model processed cheese made with rennet and acid casein. J. Dairy Sci. 72, 1-11.

Shimp LA. (1983) Basic knowledge simplifies choice. Dairy Field 166(10), 116-117.

Tamime AY, Younis MF, Davies G, Bradbury I, Dawood AH, Abdou SM. (1990). The quality of processed cheese made from reconstituted skim milk powder cheese base. Egyptian J. Dairy Sci. 1990 (in press).

Taneya S, Kimura T, Izutsu T, Buchheim W. (1980). The submicroscopic structure of processed cheese with different melting properties. Milchwissenschaft 35, 479-481.

Thomas MA. (1977). The Processed Cheese Industry - Bulletin D 44. Department of Agriculture, New South Wales, Australia, 3-49.

Walstra P, Jenness R. (1984). Dairy Chemistry and Physics, John Wiley and Sons Inc., New York, USA, 173-177.

Younis MF. (1989). Some studies on processed cheese. PhD Thesis. Zagazig University, Moshtohor, Egypt.

Discussion with Reviewers

D. Paquet: What effects had anhydrous milk fat on the texture or microstructure of processed cheese?

Authors: The overall impression was that in the processed cheese blends it had no evident effect on the texture or microstructure.

D. Paquet: What type of mix do you recommend for the manufacture of Egyptian processed cheese?

Authors: Practically most of the blends were suitable for the Egyptian market. However, in order to reduce cheese imports to Egypt, the experimental cheese, which was made totally from cheese bases I and II, may be used to manufacture processed cheese locally.

D.N. Holcomb: Can the authors provide additional information on the emulsifying salts used, e.g., whether phosphates or citrates were used?

Authors: You have asked a very interesting question because the properties of the processed cheese are influenced by the constituents of the emulsifying salts used, e.g., phosphates or citrates. However, Joha SE, C, and T are commercial formulations of emulsifying salts which are

widely used in the processed cheese industry. The exact formulation and the available technical data are, as a consequence, somewhat limited. The supplier had provided us with some information as shown in Table 4.

D.N. Holcomb: During the manufacture of cheese base, the reconstituted skim milk powder was ultrafiltered, diluted with water, and then diafiltered. It might be useful if the authors would explain why these two filtration steps were both necessary.

Authors: The two filtration steps were necessary for the following reasons: (a) the ultrafiltration was carried out primarily to concentrate the protein in milk and (b) the diafiltration process helped to reduce the level of lactose in the retentate.

D.N. Holcomb: Could the authors provide more detail of the staining procedure with uranyl acetate and lead citrate?

Authors: Thin sections were stained with a saturated uranyl acetate solution in methanol for 5 min, washed in methanol, stained with alkaline lead citrate (Reynolds, 1963) for 5 min, washed with 0.01 N NaOH and with water, and dried.

I. Heertje: Creaming time is considered to be an important variable in the production of processed cheese. No clear mention is made about the creaming time. Was it used to induce changes in the firmness? If so, were differences in microstructure observed?

Authors: It is a well established fact that creaming time can induce considerable changes in the firmness of processed cheese. In the present study, the creaming time was maintained at a constant period (i.e., using vacuum for 1 min at 85-90°C followed by stirring for 2 min) in order to minimise any variation between the processing of each blend.

I. Heertje: Can an explanation be offered for the considerable increase in firmness of the products after storage at 30°C? Is this reflected in the microstructure?

Authors: Differences in the firmness of fresh (soft) and stored (firm) cheeses cannot be explained on the basis of the micrographs obtained. The aggregation of fat particles and their fusion led to an increase in the fat particle dimensions. Large fat particles are usually found in soft processed cheeses (Shimp, 1983). Since the experimental cheese under study was firmer after storage at 30°C than the fresh cheese, we may assume that its high firmness is most probably associated with changes in the protein matrix.

Excessive heating is known to harden process cheese. It takes only several hours of heating at 82°C to severely thicken a processed cheese emulsion (Kaláb et al., 1987).

The change in firmness is accompanied by the development of electron-dense areas in the heated processed cheese, but it is not known whether such areas consist of chemically modified protein or whether the protein is compacted in these areas.

Thinner sections and a high-resolution electron microscope would be necessary to reveal differences (if any) in the microstructures of the protein matrices of the soft and firm samples. Cryofixation of the cheeses followed by replication of planes obtained by freeze-fracturing and examination of the replicas by TEM would probably be even more suitable.

I. Heertje: Is there an indication in the protein phase for the existence of string-like structures as observed by others in processed cheese?

Authors: String-like structures similar to those shown by Taneya et al. (1980), Heertje et al. (1981), and Carić and Kaláb (1987) were noticeable in the processed cheeses stored at 30°C (Fig. 11A); the incidence of minute electron-dense particles, assumed to be artefacts, would weaken any conclusions which we would attempt to draw on a possible relationship between the firmness of the processed cheeses and the ultrastructure of their protein matrices. The development of the artefacts has been quite difficult to avoid.

In addition to the string-like structures in the protein matrices, membranes were seen at a higher magnification in thin sections of process cheeses stored at 10°C to cover the emulsified fat globules (Fig. 11B).
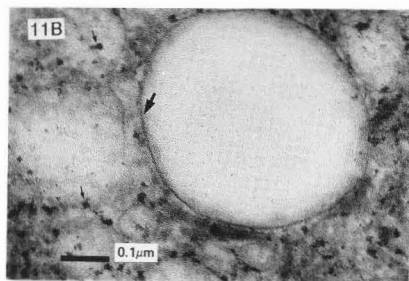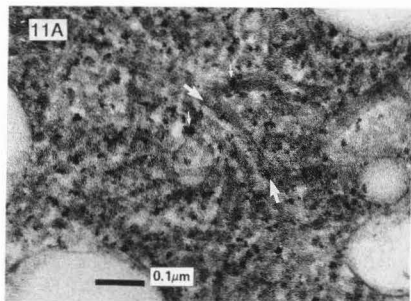

Fig. 11. High-magnification TEM of the A_1 processed cheese blend.
A: String-like structures (large light arrows) were present in the protein matrices of processed cheese blends stored at 30°C; sample A_1 is shown in this micrograph as an example.

B: Membranes (large arrows) covering emulsified fat globules were found in processed cheese blends stored at 10°C. Small arrows in A and B point to minute electron-dense particles considered to be artefacts.