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Microstructure, Free Amino Acids and Free Fatty Acids in Ras Cheese

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ABSTRACT

The physical state of the protein in Ras cheese has been followed by TEM and SEM in parallel with the chemical changes during maturation. The microstructure of young Ras cheese, by TEM, consists of casein micelles joined to form an open network, with some clustering of the micelles that appear, by SEM, as thin fibres aggregated into cross-linked elongated strands. At higher magnification, the casein fibres appear folded to include cavities.

With further maturation to 4 months, the cheese matrix changed considerably by dissociation of the casein micelles to form a more homogeneous structure of sub-micelles, as seen by TEM, whilst SEM micrographs indicated that some protein fibres still existed.

The protein breakdown, the accumulation of free amino acids and the liberation of free fatty acids in Ras cheese increased during maturation by decomposition of paracasein and cheese fat.

Ras cheese is characterized as a compact body but can be viewed as an extensive inter-connection between the casein sheets in a cheese matrix and by a sharp flavour attributed to free glutamic acid and the presence of butyric, caprylic and caproic (free volatile) fatty acids.

INTRODUCTION

The common hard cheese of Egypt is Ras. It is similar to Caskawal cheese in body, texture and flavour and changes that occur in its chemical

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composition during ripening have been reported by Hofi *et al.* (1970) and by Omar & Ashour (1982). However, no information has been published on the microstructure of the protein of Ras cheese. Transmission electron microscopy (TEM) has been used to study the physical state of the protein in Cheddar (Hall & Creamer, 1972; Brooker *et al.*, 1975; Kalab, 1977), Cheshire (Hall & Creamer, 1972) and Provolone (Kalab, 1977); other workers have used scanning electron microscopy (SEM) to examine Cheddar (Hall & Creamer, 1972; Eino *et al.*, 1976; Kalab & Emmons, 1978), Emmental (Blanc *et al.*, 1979), Greyerzer (Rüegg *et al.*, 1980) and Grana cheese (Bottazzi & Bianchi, 1982).

In this work, changes in the microstructure of protein in Ras cheese have been followed by TEM and SEM.

MATERIALS AND METHODS

Cheesemaking

Ras cheese was made from pasteurized cow's milk as described by Hofi *et al.* (1970), in three replicates. The waxed cheese was ripened at 10–14 °C at 80–90 % humidity for 4 months.

Chemical analysis of cheese

The cheese was analysed when young and after 2 and 4 months for pH, moisture, fat 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.

Free amino acid composition

Ten grams of cheese were dissolved in 90 ml 0·5 M tris-sodium citrate solution. The mixture 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 by 5 % sulphosalicylic acid and filtered. The filtrate was adjusted to pH 2 by the addition of 5 N 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).

Electron microscopy

Samples for 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 and by a further hour in 5% uranyl acetate solution. The specimens were washed with propylene oxide for 10 min and were saturated with a mixture of propylene oxide and Epon 812 in the ratio 3:1 for 2 h, 1:1 for 12 h and 3:1 for 12 h. The specimens were sealed in capsules with pure Epon and kept at 60 °C until solidified. Ultra-thin sections were made by Reichert-Ultramicrotom and examined on a TESLA BS 500 electron microscope with an accelerating voltage of 80 kV.

Specimens for SEM were prepared as described by Glaser *et al.* (1979). Samples were collected and dropped into vials containing 4% glutaraldehyde solution 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 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 methods 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 AW/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.

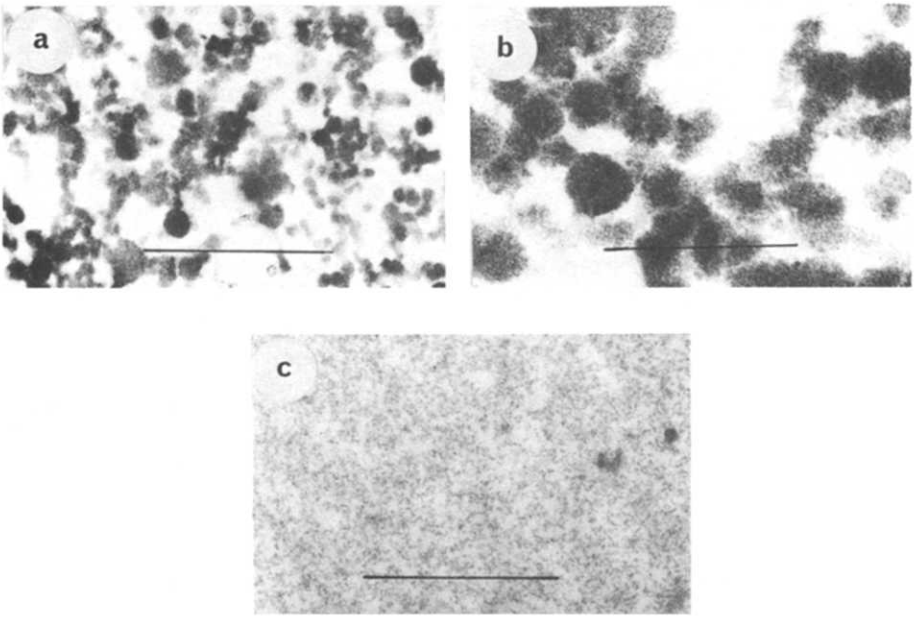

Fig. 1. TEM micrographs of Ras cheese during ripening. a—Young cheese. b—Two months matured cheese. c—Four months matured cheese. The bar indicates 1·0 μm.

RESULTS AND DISCUSSION

Electron microscopy

The microstructure of young Ras cheese is shown in the TEM micrograph, Fig. 1a. It consists of casein micelles joined to form an open network, with some clustering of the micelles. An SEM micrograph of the same sample at lower magnification (×1000) (Fig. 2a) shows thin fibres aggregated into cross-linked strands and assemblages that are elongated rather than spherical. At higher magnification, Fig. 2b, the casein fibres appear folded to include cavities, as described by Kalab & Emmons (1978) for Cheddar cheese; they explained texture development in terms of interfibrillar bonding or fusion of the casein, with consequent increase of strength.

During maturation to 2 months the casein micelles continued to aggregate and coalesce (Fig. 1b) with fusion of the micelles increasing. The extent to which this coalescence has occurred may be seen in Fig. 3a at lower magnification, the holes being due to fat which has been removed during specimen preparation. Similar cavities resulting from removal of

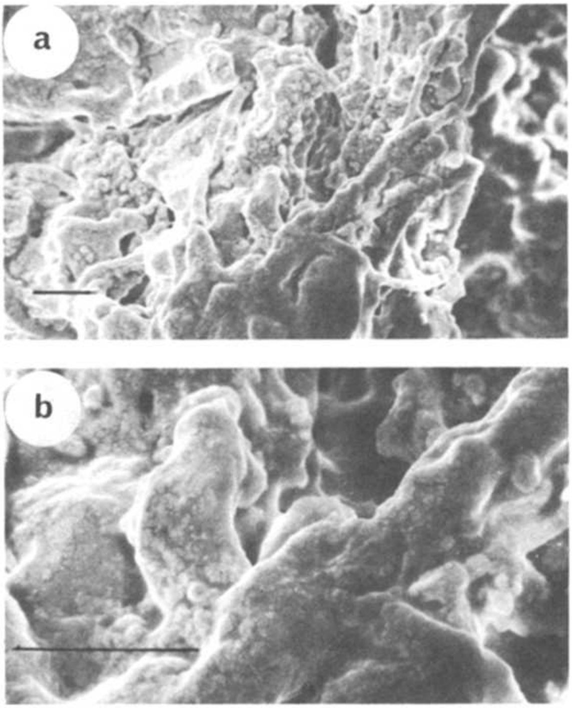

Fig. 2. SEM micrographs of young Ras cheese. a—Protein fibres at lower magnification 1000 ×. b—Detail of protein fibres by higher magnification 3000 ×. Bar indicates 10 μm.

fat were seen in Emmental (Blanc *et al.*, 1979) and Greyerzer (Rüegg *et al.*, 1980). Figure 3b indicates that, at 2 months, the protein fibres had become elliptical, around the fat globules; fusion of casein fibres into a coarser structure was also observed in Cheddar (Eino *et al.*, 1976).

With further maturation to 4 months, the cheese matrix changed considerably by dissociation of the casein micelles to form a more

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

Age of cheese	*pH*	*Moisture (%)*	*Fat (%)*	*(TN) Total nitrogen*	*Soluble N of TN (%)*	*(NPN) Non-protein N of TN (%)*	*Amino acid nitrogen of TN (%)*
Young	5·16	47·51	23·20	3·75	5·86	3·74	0·51
2 months	5·21	39·69	24·26	4·12	11·4	5·46	1·98
4 months	5·25	37·07	25·70	4·19	16·3	8·31	3·16

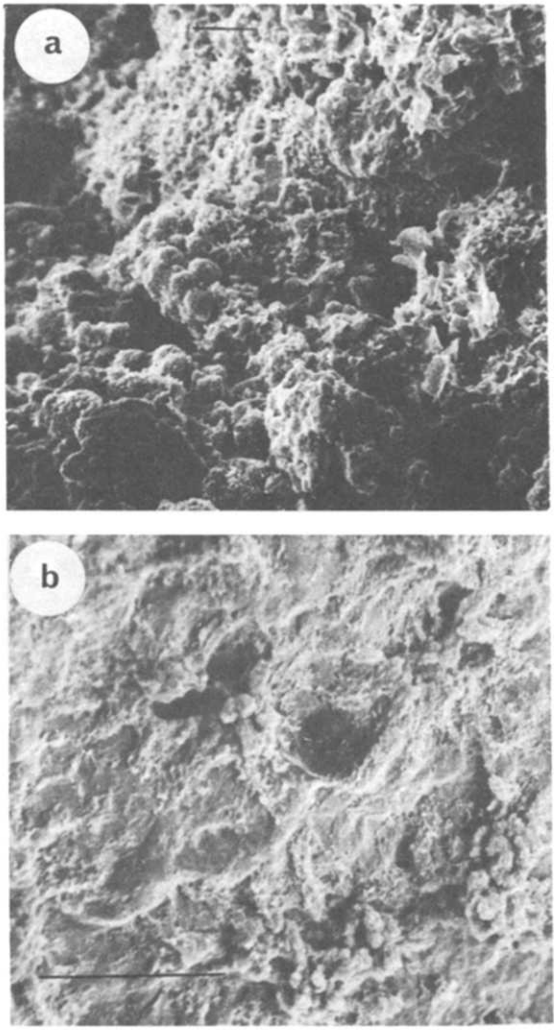

Fig. 3. SEM micrographs of two months matured Ras cheese. a—Fusion of the protein fibres into coarser structure with cavities of the cheese fat. b—Enlargement of protein matrix 3000×. Bar indicates 10 μm.

homogeneous structure of sub-micelles (Fig. 1c). The SEM (Fig. 4a) indicated that some protein fibres still existed at 4 months. Similar protein fibres were observed in matured Emmental cheese by Blanc *et al.* (1979) and in Grana cheese by Bottazzi & Bianchi (1982). Figure 4b indicates that, at 4 months, the casein sheets were compacted, extensively interconnected and warped so that the fat cavities could not be seen.

During maturation the cheese became harder and firmer due to the

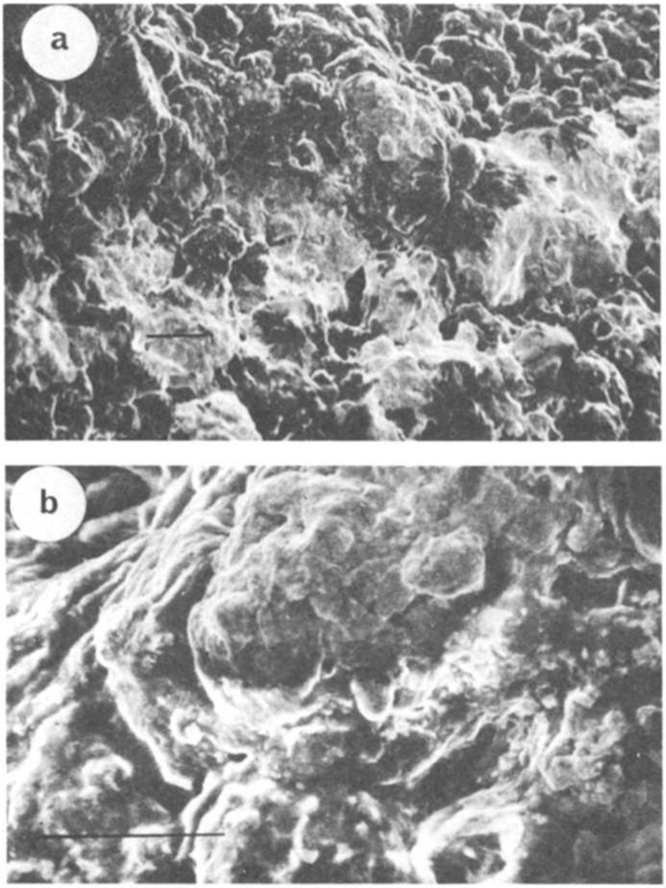

Fig. 4. SEM micrographs of four months matured Ras cheese. a—Compact structure and thin protein fibres (2000 ×). b—Interconnection of casein sheets (6000 ×). Bar indicates 5·0 μm.

increased compaction of the casein and loss of moisture (Table 1). pH, per cent fat and non-protein nitrogen increased during ripening because of enzymatic and bacterial activities, in agreement with the findings of Hofi *et al.* (1970) and Omar & Ashour (1982).

Free amino acid composition

During ripening, paracasein and the minor proteins are converted to simpler nitrogenous compounds—proteose, peptones, amino acids and ammonia—and flavour increases. Some free amino acids appeared within

TABLE 2
Free Amino Acid Composition of Ras Cheese (mg/100 g Cheese)

Amino acid	*Age of cheese*					
	Young		*2 months*		*4 months*	
	mg/100 g	*Per cent of total*	*mg/100 g*	*Per cent of total*	*mg/100 g*	*Per cent of total*
Lysine	5·43	8·20	14·6	9·25	15·9	4·51
Histidine	7·18	10·8	11·0	6·97	8·71	2·47
Arginine	3·68	5·56	8·05	5·15	27·9	7·89
Aspartic acid	2·21	3·34	4·82	3·09	1·81	0·51
Threonine	2·37	3·59	6·65	4·26	10·3	2·91
Serine	1·16	1·75	2·65	1·70	9·99	2·83
Glutamic acid	3·40	5·14	6·57	4·21	70·0	19·8
Proline	5·45	8·23	8·75	5·61	16·7	4·74
Glycine	2·36	3·57	6·75	4·33	8·33	2·36
Alanine	6·38	9·64	10·8	6·91	27·9	7·91
Valine	7·15	10·8	18·2	11·9	30·7	8·68
Methionine	3·22	4·86	10·8	6·94	25·3	7·15
Iso-leucine	1·56	2·36	8·05	5·15	26·1	7·38
Leucine	4·34	6·56	14·7	9·39	34·5	9·77
Tyrosine	1·61	2·43	1·89	1·21	7·53	2·13
Phenylalanine	8·69	13·1	21·8	14·0	31·5	8·94
Total	66·2		156		353	

a few hours of manufacture and others within weeks, as described by Webb & Johnson (1965). The concentration of free amino acids increased from 66·2 mg/100 g in young cheese to 156 mg/100 g at 2 months and 353 mg/100 g at 4 months (Table 2). The free amino acid composition of the cheese changed through maturation, the young cheese containing mainly phenylalanine, valine, histidine, alanine, proline and lysine whilst the 4 months cheese contained more glutamic acid, leucine, phenylalanine and valine and less aspartic acid, tyrosine, glycine, histidine and serine.

The major free amino acids in Ras cheese are similar to those in some hard type cheese such as Emmental, Gruyere (Antila & Antila, 1968) and Kashkwal (Buruiana & Zeidan, 1982) cheese.

The development of cheese flavour is influenced by the concentration of free amino acids and the higher percentage of glutamic acid in 4-month-old cheese is probably related to the sharp flavour in Ras cheese. The characteristic flavour intensity in Romano hard type cheese was also

TABLE 3
Free Fatty Acid Composition of Ras Cheese (mg/kg Cheese)

Fatty acid	*Age of cheese*					
	Young		*2 months*		*4 months*	
	mg/kg cheese	*Per cent of total*	*mg/kg cheese*	*Per cent of total*	*mg/kg cheese*	*Per cent of total*
C_2	0·696	0·33	2·32	0·60	0·600	0·06
C_3	0·084	0·04	1·05	0·27	0·450	0·05
C_4	1·47	0·69	1·42	0·37	4·49	0·48
iso-C_5	—	—	0·050	0·01	0·070	0·01
C_5	0·270	0·13	1·09	0·22	3·38	0·36
C_6	1·61	0·75	5·47	1·42	4·14	0·44
C_8	3·01	1·41	4·23	1·10	1·04	0·11
C_{10}	8·79	4·13	11·3	2·94	37·4	3·98
$C_{10:1}$	0·870	0·41	2·66	0·69	2·85	0·30
C_{12}	8·95	4·20	9·99	2·59	56·6	6·02
$C_{12:1}$	0·080	0·04	0·333	0·09	5·49	0·58
iso-C_{14}	1·40	0·66	0·666	0·17	5·94	0·63
C_{14}	23·4	11·0	53·0	13·8	126	13·4
$C_{14:1}$	5·89	2·77	7·99	2·08	51·5	5·48
C_{15}	3·89	1·83	7·32	1·90	35·7	3·30
iso-C_{16}	1·30	0·61	1·33	0·35	8·92	0·95
C_{16}	59·0	27·7	131	33·9	203	21·6
$C_{16:1}$	8·54	4·01	29·9	7·77	73·3	7·80
C_{17}	2·00	0·94	1·57	0·47	12·3	1·31
$C_{17:1}$	1·01	0·47	0·666	0·17	9·62	1·02
C_{18}	15·3	7·17	17·3	4·50	93·9	10·0
$C_{18:1}$	57·2	26·9	87·9	22·9	175	18·6
$C_{18:2}$	5·44	2·55	1·33	0·35	13·6	1·45
$C_{18:3}$	2·87	1·35	5·33	1·39	14·4	1·54
Total	213		385		940	

related to the concentrations of free glutamic acid and free butyric acid (Webb & Johnson, 1965).

Free fatty acids (FFA) composition

It is clear from the free fatty acids pattern in Table 3 that the liberation of fatty acids increased throughout the ripening period. Young cheese contained 213 mg/kg of FFA whilst their concentration was increased to 385 mg/kg at 2 months and to 940 mg/kg at 4 months. The main FFA's were the C_{16}, $C_{18:1}$ and C_{14} acids, but the flavour of the cheese was more probably due to the presence of smaller concentrations of the more volatile C_2–C_{10} acids, some of which are produced by fermentation of lactose (Kosikowski & Mocquot, 1958).

The smaller concentrations of acetic and propionic acids in the 4 month cheese (Table 3) may be due to a reduction in the lactose content of the cheese through maturation (Hofi *et al.*, 1970). Baltadzhieva (1968) reported the absence of acetic and propionic acids from Kefalotyri cheese, which resembles Ras cheese. It is therefore likely that neither acetic nor propionic acids are essential to the characteristic flavour of Ras cheese.

High concentrations of butyric, caprylic and caproic acids imply that they contribute to the sharp flavour of this cheese; this agrees with the finding of Baltadzhieva (1968) and Webb & Johnson (1965) who attributed the sharp note of cheese flavour to the presence of free butyric and caprylic acids.

REFERENCES

Antila, V. & Antila, M. (1968). Der gehalt des Finnischen Käses an frein amino säuren. *Milchwissenschaft*, **23**, 597–602.

Baltadzhieva, M. (1968). Free volatile fatty acids (C_2–C_{10}) in Kefalotyri cheese. *Dairy Sci. Abst.*, **30**, 2154.

Blanc, B., Rüegg, M., Baer, A., Casey, M. & Lukesch, A. (1979). Essais comparatifs dans le Fromage d'Emmental avec et sans fermentation secondaire. *Schweiz. Michw. Forsch.*, **8**, 27–36.

Bottazzi, B. & Bianchi, F. (1982). The microscopic crystalline inclusions in Grana cheese and their X-ray microanalysis. *Milchwissenschaft*, **37**, 577–80.

Brooker, B. E., Hobbs, D. G. & Turvey, A. (1975). Observation on the microscopic crystalline inclusions in Cheddar cheese. *J. Dairy Res.*, **42**, 341–8.

Buruiana, L. M. & Zeidan, A. N. (1982). Variation of free amino acids in Kashkawal 'DALIA' cheese during ripening. *Egyptian J. Dairy Sci.*, **10**, 209–13.

Eino, M. E., Biggs, D. A., Irrive, D. M. & Stanley, D. W. (1976). A comparison of microstructure of Cheddar cheese curd manufactured with calf rennet, bovine pepsin and porcine pepsin. *J. Dairy Res.*, **43**, 113–15.

Garnier, J. (1962). *Etude cinétique de la proteolyse par la présure de la caseine Kappa*. Thése de dectorat, Faculté des sciences de l'Universite de Paris.

Glaser, J., Carroad, P. A. & Dunkley, W. L. (1979). Surface structure of cottage cheese curd by electron microscopy. *J. Dairy Sci.*, **62**, 1058–68.

Hall, D. M. & Creamer, L. K. (1972). A study of the sub-microscopic structure of Cheddar, Cheshire and Gouda cheese by electron microscopy. *New Zealand J. Dairy Sci. Technol.*, **7**, 95–102.

Hofi, A. A., Youssef, E. H., Ghoneim, M. A. & Tawab, G. A. (1970). Ripening changes in Cephalotyre 'Ras' cheese manufactured from raw and pasteurized milk with special reference to flavor. *J. Dairy Sci.*, **53**, 1207–11.

Horwitz, W., Chichilo, P. & Reynolds, H. (eds) (1970). *Official Methods of Analysis of the Association of Official Analytical Chemists* (13th edn.), Washington DC, 238–74.

Kalab, M. (1977). Milk gel structure. VI. Cheese texture and microstructure. *Milchwissenschaft*, **32**, 449–58.

Kalab, M. & Emmons, D. B. (1978). Milk gel structure. IX. Cheddar curd. *Milchwissenschaft*, **33**, 670–3.

Kosikowski, F. V. & Mocquot, G. (1958). *Advances in cheese technology*, FAO Agri. Studies No. 38. Food and Agri. Org. of the UN, Rome.

Kuzdzal, W. & Kuzdzal-Savoie, A. (1966). Etude compuree des acids gras non volatiles libres et esterfies dans les Fromages. *XVII. Int. Dairy Congr.*, D.335.

Kuzdzal-Savoie, A. and Kuzdzal, W. (1967). Les acids gras libres de Fromage. *Le Lait*, **47**, 9.

Mondino, A., Bonglovanni, G., Fumero, S. & Ross, L. (1972). An improved method of plasma deproteination with sulphosalicylic acid for determining amino acids and related compounds. *J. Chromatog.*, **74**, 255–63.

Omar, M. M. & Ashour, M. M. (1982). Studies in the use of recombined milk for the manufacture of Ras cheese. *Food Chem.*, **8**, 33–41.

Roos, J. B., Versnel, A. & Werdmuller, A. (1963). Die gaschromatographische Bestimmung der niederen Fettsäuren von Milchfett und deren Anwendung zum Nachweis von Fremdfetten. *Kieler Milch. Forsch.*, **15**, 515–26.

Rüegg, M., Moor, U. & Blanc, B. (1980). Veränderungen der Feinstruktur von Greyerzer Käse im verlauf der Reifung. Eine studie mit dem Raster-Electronenmikroskop. *Milchwissenschaft*, **35**, 329–35.

Schober, R., Niclaus, W. and Christ, W. (1961). Anwendung der 'Finger-Abdruck-Methode' auf die Kennzeichnung von Käsesorten durch ihre proteolytischen Inhaltsstoffe, *Milchwissenschaft*, **16**, 140–2.

Shimmin, P. D. & Hill, R. D. (1964). An electron microscope study of the intermolecular structure of casein micelles. *J. Dairy Res.*, **31**,121.

Sode Mogensen, M. T. (1948). *Determination of the degree of proteolytic decomposition in cheese with special reference to the formol titration.* Meddelande No. 21 fran Statens Mejeriförsök, Alnarp-Akarp. Sweden, 281–436.

Webb, B. & Johnson, A. (1965). *Fundamentals of dairy chemistry.* (2nd edn.), The AVI Publishing Company, Westport, Connecticut, 651–62.

Wooding, F. B. (1973). *Encyclopedia of microscopy and microtechnique.* (Gray, P. (ed.)), V.N.R. Comp. 330.