Food Chemistry **22** (1986) 83–94

Ripening Changes of Kashkaval Cheese Made from Cow's Milk

Mohamed M. Omar & Ali I. El-Zayat

Department of Food Science, Faculty of Agriculture,
University of Zagazig and Suez Canal, Egypt

(Received: 12 March, 1986)

ABSTRACT

Kashkaval cheese was made from cow's milk and examined for the changes in its microstructure and chemical composition during ripening.

The percentages of fat, protein, soluble nitrogen, non-protein nitrogen, amino acid nitrogen and the total free fatty and amino acids increased during ripening.

The presence of glutamic acid, leucine, phenylalanine, valine and tyrosine at high concentration, and of butyric, caproic, caprylic and capric acids may contribute to the formation of Kashkaval cheese flavour. The small concentrations of acetic and propionic acids preclude any contribution to Kashkaval flavour.

In young cheese, casein aggregates lose their spherical shape due to the scalding and kneading processes and they form a fibrous network including cavities.

During ripening, dissociation and fusion processes occur in protein fibres to form a more homogeneous structure and interaction between layers of casein sheets increases to give a more compact structure.

INTRODUCTION

Kashkaval cheese is similar to Ras cheese, the common hard cheese of Egypt, in body, texture and flavour, and is produced in all countries of the Balkan peninsula. Kashkaval cheese is known by a number of related names; in Italy it is known as Caciocavallo, in Greece, as Kasseri cheese, and, in Egypt, the name Romy is commonly used (El-Erian *et al.*, 1976).

Food Chemistry 0308-8146/86/$03·50

The best quality Kashkaval cheese is produced from sheep's milk; however, many trials have produced good quality Kashkaval cheese from buffaloe's and/or cow's milk (El-Shabrawy, 1973). Changes in the electrophoretic patterns of protein of Kashkaval cheese during manufacture and ripening (El-Shabrawy, 1973) and in the sub-microscopic structure only during processing, by using transmission electron microscopy (Hofi *et al.*, 1977) have previously been studied.

In this work, the ripening changes of Kashkaval cheese made from cow's milk are followed by scanning electron microscopy and gas–liquid chromatography.

MATERIALS AND METHODS

Cheesemaking

Fresh cow's milk was supplied from Misr Milk and Food Company, Ismailia Dairy Plant, Egypt. Lactic acid starter culture (a mixture of 50% *Streptococcus lactis* and *S. cremoris* and 50% *S. thermophilus* and *Lactobacillus bulgaricus*) and Habo rennet (Chr, Hansen Laboratories, Denmark) were used in Kashkaval cheese making as the method described by Hofi *et al.* (1977). Cheese was made in three replicates, waxed and 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 0·5M tris-sodium citrate solution. The mixture was then heated to 75°C in a water bath and homogenised at 10 000 rpm for 3 min using a laboratory homogeniser. The samples were deproteinised by 5% sulphosalicylic acid and filtered. The filtrate was adjusted to pH 2 by the addition of 5N NaOH and to pH 2·2 using freshly prepared 0·2N sodium citrate buffer (pH 2·2) followed by filtration (Mondino *et al.*, 1972). Free amino acids were determined in 0·8 ml of the filtrate using an amino acid analyser (JLC/6AH) Firma JEAL/JAPAN).

Scanning electron microscopy (SEM)

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, mounted on aluminium stubs and silver paint, sputter coated with gold and examined under a JOEL-SI SEM with an accelerating voltage of 10 kV.

Free fatty acids 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 Ross *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.

RESULTS AND DISCUSSION

Gross composition

The fat and total nitrogen, as well as soluble nitrogen, contents of Kashkaval cheese increased during ripening as a result of moisture loss; the non-protein nitrogen and the amino acid nitrogen contents increased

TABLE 1
Chemical Analysis of Kashkaval Cheese (Average of Two 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	5·22	50·02	21·1	3·35	8·46	5·52	0·16
2 months	5·43	39·59	24·2	3·76	19·5	9·11	0·93
4 months	5·65	38·82	26·3	3·95	22·9	11·3	2·73

as a result of protein degradation (Table 1). These results are in agreement with those of Amer *et al.* (1979). Also, the pH value increased by 0·4 units after 4 months of ripening, which is the same value as that obtained by Buruiana & Zeidan (1982).

Free amino acids composition

Table 2 shows the analysis of the free amino acids of Kashkaval cheese when young and after storage periods of 2 and 4 months. Sixteen free amino acids have been identified in 2- and 4-month old cheese while, in young cheese, both histidine and arginine were absent. El-Shabrawy (1973) could not identify either histidine or arginine in young Kashkaval cheese.

As a characteristic feature of the ripening process, the total free amino acids increased from 59·4 mg/100 g young cheese to 130 and 238 mg/100 g at 2 and 4 months, respectively. The main free amino acids were glutamic acid, leucine, phenylalanine and tyrosine (in order), while histidine, serine, glycine and threonine were present in small amounts. Buruiana & Zeidan (1982) mentioned that the free amino acids increased during the ripening of Kashkaval cheese made from cow's milk and histidine, glycine and arginine were present in small amounts. Also, the major free amino acids in Kashkaval cheese are similar to those in Emmental, Gruyere (Antila & Antila, 1968) and Ras (Omar, 1984) cheeses.

During ripening, the distribution pattern of free amino acids changed, the concentration of glutamic acid, serine, aspartic acid, threonine, proline, alanine and lysine increased to a higher level in 4-months old cheese than in young cheese; the concentrations of valine, methionine, isoleucine, leucine and tyrosine in 4-month old cheese were lower than

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

Amino acid	*Age of cheese*					
	Young		*2 months*		*4 months*	
	(mg/100 g cheese	*(Per cent of total)*	*(mg/100 g cheese)*	*(Per cent of total)*	*(mg/100 g cheese)*	*(Per cent of total)*
Lysine	2·92	4·92	5·91	4·66	12·61	5·31
Histidine	—	—	1·79	1·38	6·22	2·62
Arginine	—	—	2·12	1·63	8·03	3·38
Aspartic acid	3·25	5·48	8·68	6·69	18·25	7·69
Threonine	1·76	2·96	4·93	3·80	10·94	4·60
Serine	1·01	1·70	2·73	2·10	4·17	1·76
Glutamic acid	8·94	15·1	26·4	20·4	40·1	16·90
Proline	3·48	5·86	9·16	7·06	17·37	7·31
Glycine	1·39	2·34	3·07	2·37	6·77	2·85
Alanine	3·53	5·94	6·53	5·03	19·11	8·05
Valine	6·48	10·92	9·92	7·65	10·43	4·39
Methionine	3·27	5·51	4·53	3·49	6·65	2·80
Isoleucine	3·76	6·33	6·36	4·90	8·15	3·43
Leucine	8·19	13·8	13·2	10·2	26·0	11·0
Tyrosine	4·21	7·11	8·15	6·28	13·09	5·52
Phenylalanine	7·17	12·1	16·2	12·5	29·5	12·4
Total	59·4		130		238	

those of young cheese. Buruaiana & Zeidan (1982) found that lysine increased during the ripening of Kashkaval cheese.

The above changes in levels of free amino acids during the maturation process can be considered a result of the continuous development process of free amino acids and other components of cheese, whereas the quantities of some free amino acids decreased after reaching the maximum (such as serine and glutamic acid in 2 and 4 months) as a result of secondary decomposition which occurs in the later stage of cheese ripening.

Therefore, the increasing amount of total free amino acids in 4-months old cheese, as well as the presence of glutamic acid, leucine, phenylalanine, valine and tyrosine at higher concentrations, may be correlated with the characteristic flavour of Kashkaval cheese.

Free fatty acids composition

Table 3 shows the analysis of the free fatty acids of young, 2- and 4-month old Kashkaval cheese, and twenty-four free fatty acids can be identified.

The liberation of free fatty acids increased during ripening from 245 mg/kg young cheese to 785 and 1609 mg/kg cheese of 2- and 4-months old, respectively.

TABLE 3
Free Fatty Acids Composition of Kashkaval 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	0·04	0·02	0·74	0·09	0·31	0·02
C_3	0·02	0·01	0·66	0·08	0·43	0·03
C_4	0·39	0·16	4·85	0·62	5·61	0·35
iso-C_5	0·01	—	0·09	0·01	0·79	0·05
C_5	0·42	0·17	0·07	0·01	0·17	0·01
C_6	0·73	0·30	2·37	0·30	5·27	0·33
C_8	0·23	0·09	1·69	0·22	3·63	0·23
C_{10}	6·16	2·51	6·62	0·84	5·48	0·34
$C_{10:1}$	0·99	0·40	0·81	0·10	1·53	0·10
C_{12}	8·59	3·50	12·7	1·62	17·5	1·09
$C_{12:1}$	0·69	0·28	1·89	0·24	4·18	0·26
iso-C_{14}	0·48	0·20	1·68	0·21	3·98	0·25
C_{14}	24·1	9·83	81·9	10·4	168	10·4
$C_{14:1}$	4·86	1·98	14·4	1·84	23·6	1·47
C_{15}	3·48	1·42	13·62	1·73	26·74	1·66
iso-C_{16}	0·60	0·24	4·48	0·57	46·19	2·87
C_{16}	79·7	32·5	275	35·1	554	34·4
$C_{16:1}$	5·38	2·19	21·5	2·74	28·0	1·74
C_{17}	1·79	0·73	9·97	1·27	13·7	0·85
$C_{17:1}$	1·88	0·78	3·35	0·47	2·68	0·17
C_{18}	24·0	9·78	80·6	10·3	180	11·2
$C_{18:1}$	71·7	29·1	226	28·8	456	28·3
$C_{18:2}$	5·81	2·37	2·97	1·14	45·1	2·80
$C_{18:3}$	3·64	1·48	11·4	1·45	16·9	1·05
Total	245		785		1 609	

The main free fatty acids were, in order, C_{16}, $C_{18:1}$, C_{14} and C_{16}, while the more volatile fatty acids from C_2 to C_{10}, which are more responsible for the formation of cheese flavour (Kosikowski & Mocquot, 1958), were present in low concentrations.

In 4-month old Kashkaval cheese, acetic, propionic, valeric and isovaleric acids were present at very low concentrations as a result of the reduction in lactose content during cheese ripening (Hofi *et al.*, 1970) because of acetic acid, presumably originating from the activities of bacterial enzymes (Kosikowski & Mocquat, 1958). Therefore, the roles of C_2, C_4, C_5 and iso-C_5, in the formation of the characteristic flavour of Kashkaval cheese, are very limited, unlike Cheddar cheese in which acetic acid usually predominates. Baltadzhieva (1968) recorded the absence of acetic and propionic acids from Kefalotyri cheese.

On the other hand, the presence of high concentrations of butyric, caproic, caprylic and capric acids indicates their importance in the formation of Kashkaval cheese flavour. Omar (1984) attributed the flavour of Ras cheese, which resembles that of Kashkaval cheese, to the presence of high concentrations of butyric, caprylic and caproic acids.

During ripening, like the free amino acids, the absolute value of each free fatty acid increased; however, its percentage changed to become less than, equal to, and/or more than, that in young cheese. These changes in the distribution pattern of the free fatty acids can be explained by the continuous development processes which occurred during ripening, whereas the concentrations of some free fatty acids reached their maxima (as C_2, C_3, C_4 and $C_{16:1}$ acids of 2 month-old cheese), then decreased. These changes, together with other components, cause the characteristic flavour of Kashkaval cheese.

Scanning electron microscopy

SEM micrographs of young Kashkaval cheese in Fig. 1(A), show that the casein aggregates lose their normal spherical shape and form a fibrous network. Also the curd alters its character changing from a loose structure to a fibrous striated material like that of Cheddar cheese (Hall & Creamer, 1972).

The SEM micrograph of the same sample at higher magnification, Fig. 1(B), shows that the casein fibres appear folded to include cavities which represent the sites of the fat globules during the dehydrating process, similar to that observed in Ras cheese (Omar, 1984).

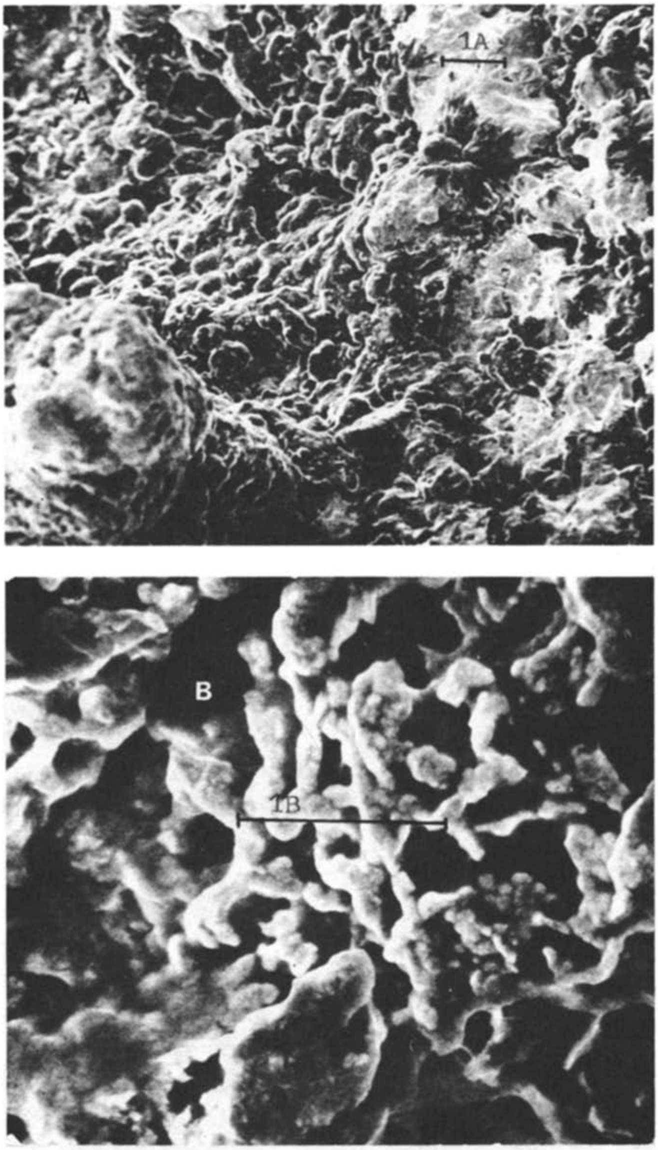

Fig. 1. SEM micrographs of young Kashkaval cheese. (Bar = 10 μm.) (A) Protein matrix consists of a cluster of fibres (original magnification × 1000). (B) Enlargement of protein fibres (original magnification × 3000).

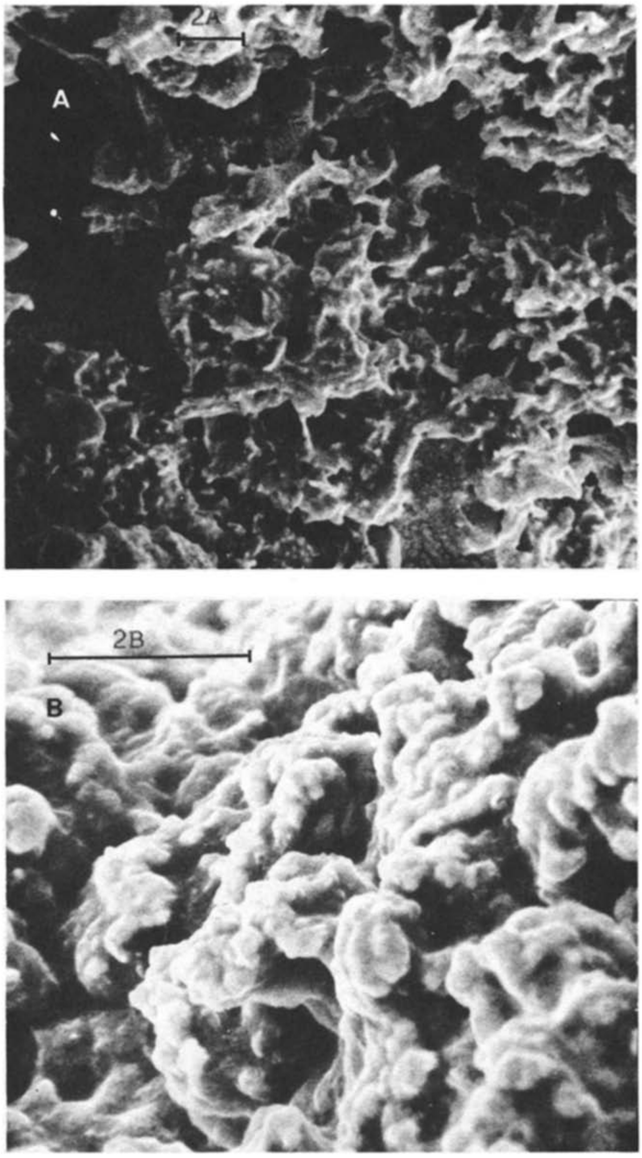

Fig. 2. SEM micrographs of 2-month matured Kashkaval cheese. (Bar = 10 μm.) (A) Disintegration of the protein fibres in area around the fat gaps (black area) (original magnification × 1000). (B) Clumps of protein matrix at large magnification (original magnification × 3000).

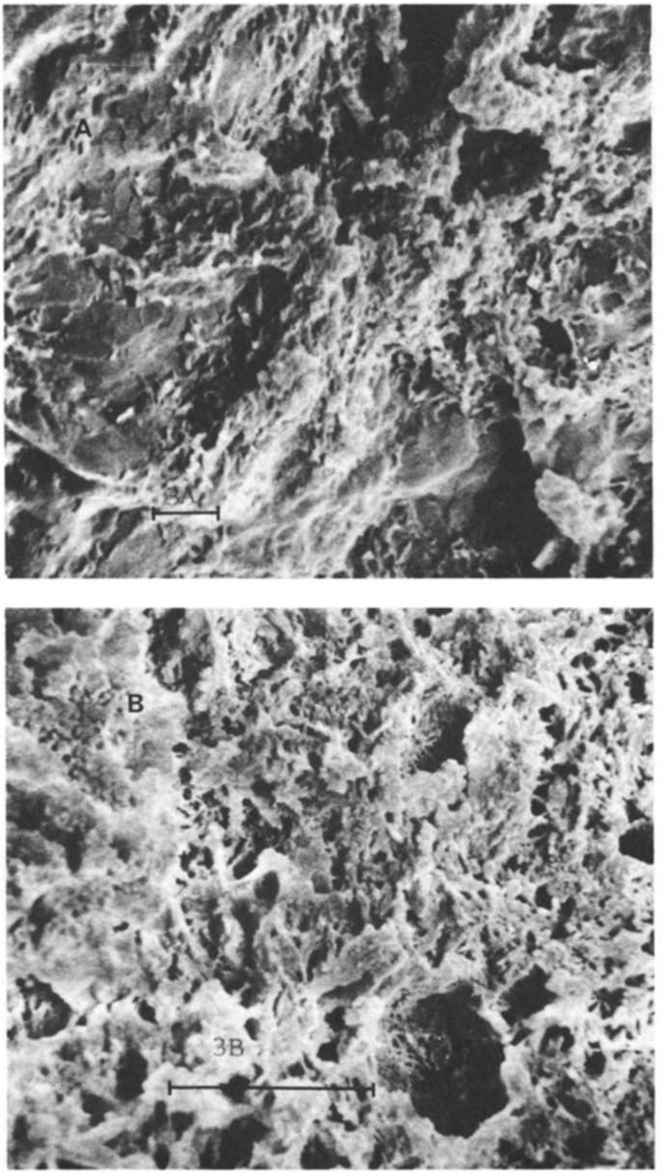

Fig. 3. SEM micrographs of 4-month matured Kashkaval cheese. (Bar = 10 μm.) (A) Degradation of protein matrix where some fibres and spherical casein micelles still appear (original magnification ×1000). (B) A homogeneous mass of matured cheese (original magnification ×3000).

The disintegration of casein fibres into partially homogenized structure mass is clearly seen in cheese matured for 2 months (Fig. 2(A)). However, some casein fibres are still found. This appears at the higher magnification, Fig. 2(B), as homogeneous casein fibres, clumps of protein, the interaction of casein sheets to form a compact surface; it also shows that the casein fibres have become elliptical around the fat globules.

With further maturation to 4 months (Fig. 3 (A and B)), the casein matrix undergoes more degradation and the presence of casein fibres or fat globules was rare, resembling that found in Cheddar cheese by Green *et al.* (1980).

REFERENCES

Abd-El-Salam, M. H. & El-Shibiny, S. (1973). An electron microscope study of the structure of Domiati cheese. *J. Dairy Res.*, **40**, 113–15.

Amer, S. N., Naghmoush, M. R. & Anis, S. M. K. (1979). Changes in the calcium paracaseinate phosphate complex during ripening of Kashkaval cheese as affected by the kind of milk. *Egyptian J. Dairy Sci.*, **7**, 25.

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

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

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

El-Erian, A. F., Nour, M. A. & Shalaby (1976). The bacterial flora of Caskawal and Ras cheese. *Egyptian J. Dairy Sci.*, **4**, 91–8.

El-Shabrawy, S. A. (1973). *Studies on Cashcaval cheese made from buffaloes' milk*, MSc Food Sci. Depart., Faculty of Agric., Ain Shams University, Cairo, Egypt.

Garnier, J. (1962). Etude cinetique de la proteolyse par la presure de la caseine kappa. These de doctorat, Faculte 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.

Green, Margaret, L., Turvey, Alan & Hobbs, David G. (1981). Development of structure and texture in Cheddar cheese. *J. Dairy Res.*, **48**, 343–55.

Hall, D. M. & Creamer, L. K. (1972). A study of the sub-microscopic structure of Cheddar, Cheshire and Gouda cheeses 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.

Hofi, A. A., El-Nimer, A. A., El-Shabrawy, S. A. & Abd-El-Salam, M. H. (1977). Changes in the sub-microscopic structure of Kashkaval cheese during manufacture. *Egyptian J. Dairy Sci.*, **5**, 25–30.

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

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

Kuzdzal, W. & Kuzdzal-Savoie, A. (1966). Etude compurée des acids gras non volatiles librés et éstérfiés dans les fromages. *XVII. Int. Dairy Congr.*, D.335.

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

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

Omar, M. M. (1984). Microstructure, free amino acids and free fatty acids in Ras cheese. *Food Chemistry*, **15**, 19–29.

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

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

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 Mejeriforsok, Alnarp-Akarp, Sweden, 281–436.