Food Chemistry **28** (1988) 1–16

Effect of Ripening Temperatures on Proteolysis and Lipolysis in the Outer and Inner Regions of Ras-Type Cheese Made by Various Salting Methods

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(Received 1 May 1987; revised version received 3 July 1987; accepted 3 July 1987)

ABSTRACT

Ras-type cheese was manufactured using different salting techniques including addition of salt to the curd at a level of 2% plus surface dry salting for 7 days, immersion in brine (20% NaCl) for 4 days or immersion in brine for 2 days plus surface dry salting for 5 days. Cheeses were matured at 7, 11, 15 or 20°C throughout the entire ripening period.

Cheeses were organoleptically examined. Proteolysis and lipolysis in the outer and inner layers of the same cheese were assessed.

Manufacture, without salt addition to the curd and brining or brine/dry salting, produced good quality cheese and accelerated proteolysis and lipolysis to a degree dependent on ripening temperature. Degradation of both αS_1 *- and β-casein and production of free fatty acids were more rapid and extensive in the inner regions of the cheese than in the outer portion of the same cheese.*

High ripening temperatures enhanced the development of cheese flavour and this was associated with a marked reduction in the ripening period. Ripening at 20°C led to seepage of whey and fat from the cheese. Storage of

cheese at 15°C appears to be suitable for maturing good quality Ras-type cheese in 8 weeks and it can be recommended for accelerating the ripening of this, and probably similar, cheese varieties.

INTRODUCTION

The ideal ripening temperature should be the maximum possible at which no impairment of cheese quality occurs, i.e. the temperature at which the greatest acceleration in ripening can be achieved while maintaining an acceptably low risk of producing an atypical product. Law (1979) noted that among several manufacturing parameters, ripening temperature was the most important factor in determining flavour intensity; cheese matured at 13°C for 6 months had a stronger flavour than corresponding cheese ripened at 6°C for 9 months. The level of soluble nitrogen in Scottish cheddar cheese was greater when the product was matured at 13°C compared with 10°C and the maturation temperature had a highly significant effect on the cheese characteristics; the best quality was associated with a curing temperature of 10°C (Ridha *et al.*, 1984). Attempts to increase flavour in low fat Gouda cheese showed that flavour balance was easily imparted; at 16°C proteolysis was accelerated more than lipolysis and the cheese developed the bitter defect (Law, 1983). Accelerated ripening of Edam cheese by the application of high temperatures has been reported to cause microbiologically-induced texture and flavour spoilage due to *Clostridium tryobutyricum*, propionobacteria and heterofermentative *Lactobacilli* (Law, 1984). Although many techniques have been employed to accelerate cheese ripening, manipulation of storage temperature is at present one of the preferred methods (Law, 1983).

The level and method of salt addition is one of the most important factors influencing the ripening and quality of cheese. Excessive proteolysis, bitterness and other off-flavours are frequently associated with low salt levels while high salt levels retard maturation (Fox, 1975; Lelievre & Gilles, 1982).

Ras-cheese (National hard type in Egypt) is commonly salted in two stages, before and after pressing. In the first stage the heated curd is lightly salted in brine, while in the second stage, which continues for ~2 weeks after manufacture, dry salt is rubbed on the outside of the cheese. El-Gazzar *et al.* (1981) studied the effect of adding salt to either milk or curd at 1% on a milk weight basis, followed by dry surface salting of the pressed cheese. They reported that the quality of Ras cheese was improved when salt was added to the curd rather than to the cheese milk.

Little information is available on the manufacture of Ras cheese without

salt addition to the milk or curd before pressing or the effect of ripening temperature on the quality, proteolysis and lipolysis of Ras cheese. Therefore the present study was planned to evaluate the effect of storage at various temperatures on the ripening changes of Ras-type cheese made by some salting techniques. Attention was given to proteolysis and lipolysis in the outer and inner regions of the cheeses.

MATERIALS AND METHODS

Ras-type cheese was manufactured from pasteurized milk essentially by the method described by Abdel Tawab (1963) at the Department of Food and Dairy Chemistry, University College, Cork, Ireland. The following manufacturing conditions were used:

(1) 1% mixed starter culture (0·5% *L. casei* 155 + 0·5% *L. bulgaricus* 1006). Starter cultures were obtained from the National Collection of Dairy Organisms, National Institute for Research in Dairying, Shinfield, Reading.
(2) When the pH of the milk had reached 6·5, commercial standard strength Hansen's rennet was added at a rate of 30 ml/100 litres milk.
(3) The curd was cooked at 45°C until the pH of the whey reached 6·3.

Three salting techniques were used and the resultant cheeses were ripened at different temperatures as follows. Experiment 1: Cheese was made with salt addition (2%) to the curd plus dry salting for 7 days; the cheeses were then vacuum packed and ripened at 7, 11, 15 or 20°C. Experiment 2: Cheese was manufactured without salt addition to the curd and the cheeses salted by either of two techniques: brining for 4 days or brining for 2 days plus dry-salting for 5 days. The cheeses were then vacuum-packed and ripened at 7, 11 or 15°C. Experiments were conducted in triplicate.

Sampling of cheese for analysis

A cheese from the first experiment was taken for analysis at various times up to 6 months of ripening, while in the second experiment a cheese was taken for analysis at various times up to 16 weeks. In experiment 2, samples were taken from outer and inner regions of the cheeses.

Chemical analysis

Cheese samples were analysed for moisture (IDF, 1982), salt (Fox, 1983), total N (IDF, 1964) and pH (Aly, 1987). The water-soluble N was prepared as

described by Kuchroo & Fox (1982). The nitrogen content of a sample (10 ml) of the extract was determined and the remainder was freeze-dried for analysis by gel electrophoresis.

Total free amino acids were determined as described by Jarrett *et al.* (1982). The total concentration of free fatty acids (FFA) was determined according to the method of Bynum *et al.* (1984).

Stacking gel electrophoresis (SGE)

SGE was used to follow gross changes of αS_1- and β-casein of cheese samples and that of water-soluble N (WSN) extract during ripening. Electrophoresis was performed in a vertical cell (Shandon Southern Products Ltd, Runcorn, Cheshire, Great Britain) using the method of Andrews (1983). Cheese samples were prepared by the method of Ledford *et al.* (1966). The gel was pre-run at 280 V for 20 min. 30 μl samples were layered on the slots, the power supply was set at 280 V and electrophoresis continued until the marker dye front reached the membrane at cell bottom. The buffers, gel composition and operation are described by Aly (1987).

Sensory evaluation

Cheese samples were organoleptically examined by individuals experienced in the sensory evaluation procedures at University College, Cork, Ireland.

RESULTS

Organoleptic assessment of the cheeses

Organoleptic evaluation of the cheeses at different stages of ripening revealed a significant influence of ripening temperature on their flavour quality, body and texture. Cheeses matured at high storage temperatures, i.e., 15 or 20°C, had good quality and matured faster than cheeses held at the lower temperatures, i.e. 7 or 11°C. Cheeses manufactured without salt addition to the curd had better flavour, body and texture than the cheeses to which salt was added at the curd stage. However, no differences in flavour, body and texture were observed between the brine-salted cheeses and cheeses which were brine/dry-salted.

pH changes

From the results presented in Fig. 1(a) and (b) it can be seen that the pH of Ras cheese made by different salting methods was somewhat higher in cheese

ripened at the higher temperature than at the lower temperature. Similar results for Manchego cheese were reported by Nunez *et al.* (1985).

The results in Fig. 1b (for experiment 2) show that the pH values of the inner samples of cheese were higher than those of the outer samples from the same cheese.

Gross chemical composition

Compositional data are summarized in Table 1.

(*a*) *Moisture content*

The results indicate that the moisture contents of all cheeses decreased as ripening progressed (Table 1); the results also show that the moisture contents of the cheeses decreased as ripening temperature was increased.

The moisture contents of the inner samples of cheese were higher than

TABLE 1
Gross Chemical Composition of Ras Cheese as Affected by Salting Methods and Ripening Temperatures

Salting method		*Ripening periods (weeks)*	*Ripening temperature (°C)*	*Moisture (%)*	*Salt in moisture (%)*	*Fat (DM%)*	*Total N (DM%)*
Salt addition		0	—	43·4	3·1	47·7	6·3
to curd + dry		16	7	35·7	9·1	49·7	6·4
salting		16	11	35·1	9·5	50·1	6·4
		16	15	35·0	9·7	50·0	6·4
		16	20	34·0	10·1	48·5	6·5
Brine salted		0	—	45·0	—	51·8	6·4
	A[a]	16	7	39·1	7·9	52·6	6·3
		16	11	38·9	8·0	52·3	6·4
		16	15	37·7	8·2	53·0	6·5
	B[a]	16	7	40·4	7·3	52·0	6·4
		16	11	40·8	7·4	52·4	6·4
		16	15	39·3	8·3	51·9	6·5
Brine/dry		0	—	45·0	—	51·8	6·4
salting	A	16	7	34·0	9·5	51·5	6·3
		16	11	34·5	9·2	51·2	6·4
		16	15	33·6	9·8	51·9	6·5
	B	16	7	37·8	9·6	51·5	6·4
		16	11	38·1	10·0	52·5	6·1
		16	15	36·7	10·4	52·1	6·3

[a] A and B indicate outer and inner regions of cheese, respectively.

those in the outer samples of the same cheeses and this observation was more marked in the brine/dry-salted cheeses than in the brine-salted cheeses.

(*b*) *Salt-in-moisture* (*S/M*)

The S/M levels for all cheeses increased with the progress of ripening and all cheeses also showed increases in S/M as ripening temperature was increased (Table 1); similar results for Manchego cheese were reported by Nunez *et al.* (1985).

As shown in Table 1, the S/M levels in the outer samples of cheese were markedly higher than those in the inner samples from the same cheeses until 4 weeks of ripening for brine-salted cheese or 8 weeks for brine/dry-salted cheese. Diffusion of salt from the surface to the interior of the cheese led to an approximate equilibration of S/M throughout the cheeses in the latter half of ripening.

(*c*) *Fat content*

The results indicate that the fat contents, on a dry basis, of all cheeses increased slightly throughout ripening (Table 1); also, the cheeses ripened at high temperatures had higher fat contents than those ripened at low temperature, except the cheese ripened at 20°C.

The outer regions of the cheeses have higher fat contents than the inner regions of the same cheese until the second week, after which no marked differences were observed (Table 1).

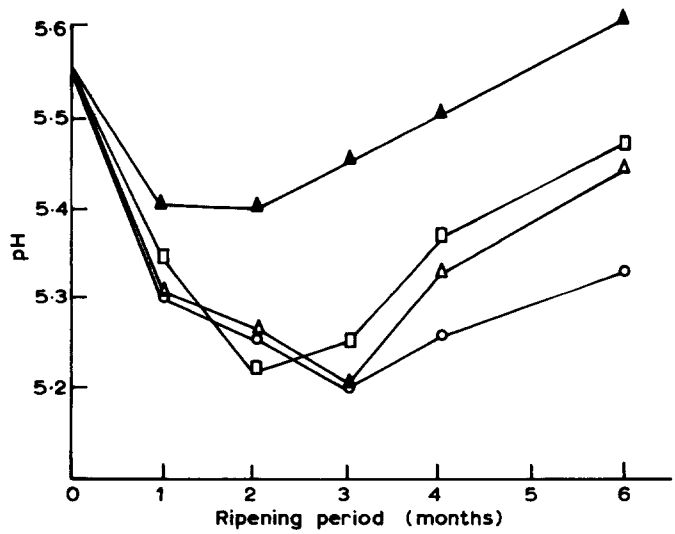

Fig. 1(a). pH Profile of Ras cheese made with salt addition to curd, followed by dry salting, as affected by ripening at 7°C (○), 11°C (△), 15°C (□), or 20°C (▲) (experiment 1).

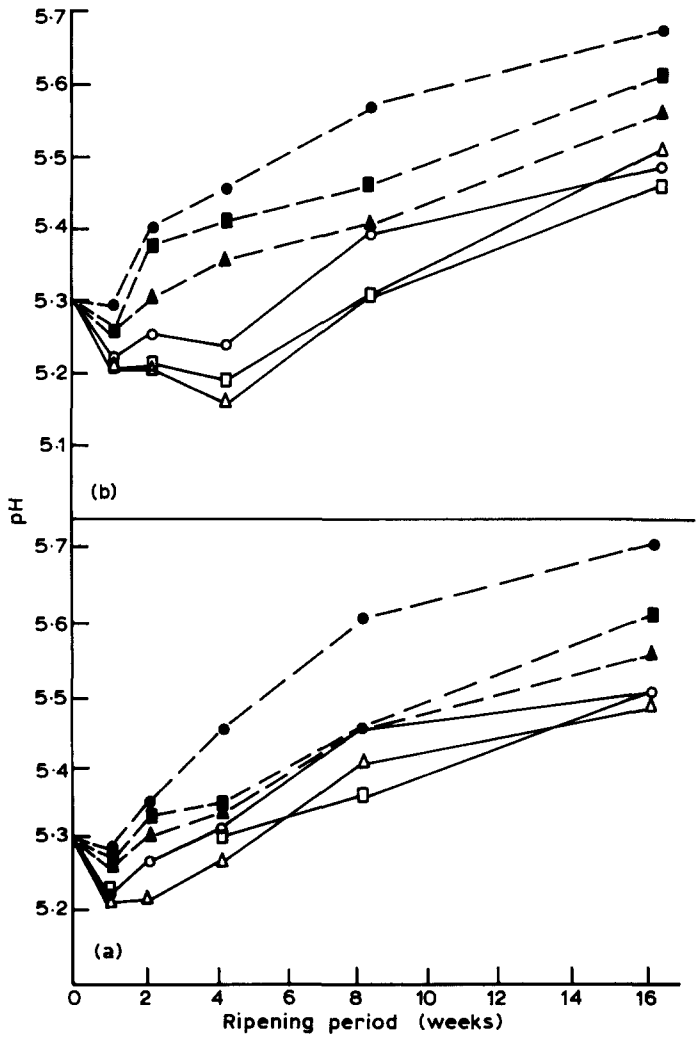

Fig. 1(b). pH profile of Ras cheese made using brine (A) or a combination of brine/dry salting (B), as affected by ripening at 7°C, inner (▲), outer (△); 11°C, inner (■), outer (□); or 15°C, inner (●), outer (○), (experiment 2).

(d) Total nitrogen (TN)

The trends for TN, on a dry weight basis, for all cheeses were similar to those for fat content.

Rate of ripening

Water-soluble nitrogenous compounds (WSN)

The levels of WSN, as a percentage of TN, for all cheeses throughout

ripening are shown in Figs 2(a) and (b). The levels of WSN in all cheeses increased as the cheeses aged. The results also indicate that the levels of WSN increased as the storage temperature was increased. Figure 2(b) also shows that the levels of WSN in the inner regions were higher than those in the outer layers of the same cheese.

Accumulation of WSN in cheese made with salt addition to the curd followed by dry salting was found to be slower than in cheese made using brine or a combination of brine/dry salting. However, very small differences in the levels of WSN could be observed between brine salted and brine/dry salted cheeses.

Characterization of proteolysis by stacking gel electrophoresis (SGE)

Figures 3(a), (b), (c) show the electrophoretograms of cheese samples at different stages of ripening. The following aspects can be observed:

(a) β-Casein was more resistant to hydrolysis than α_{s1}-casein which was rapidly degraded during ripening, as shown by stacking gel electrophoresis.
(b) Proteolysis of α_{s1}- and β-caseins was more rapid and extensive as the maturing temperature was increased.
(c) Degradation of both α_{s1}- and β-caseins was more rapid and extensive in the inner regions of the cheese than in outer portions of the same cheese.
(d) Very little difference in the levels of proteolysis of α_{s1}- and β-caseins was observed between brine-salted and brine/dry-salted cheeses, but

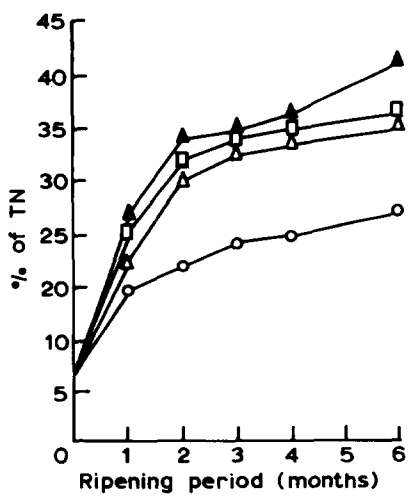

Fig. 2(a). Water-soluble N in Ras cheese made with salt addition to curd, followed by dry salting, as affected by ripening temperature at 7°C (○), 11°C (△), 15°C (□) or 20°C (▲).

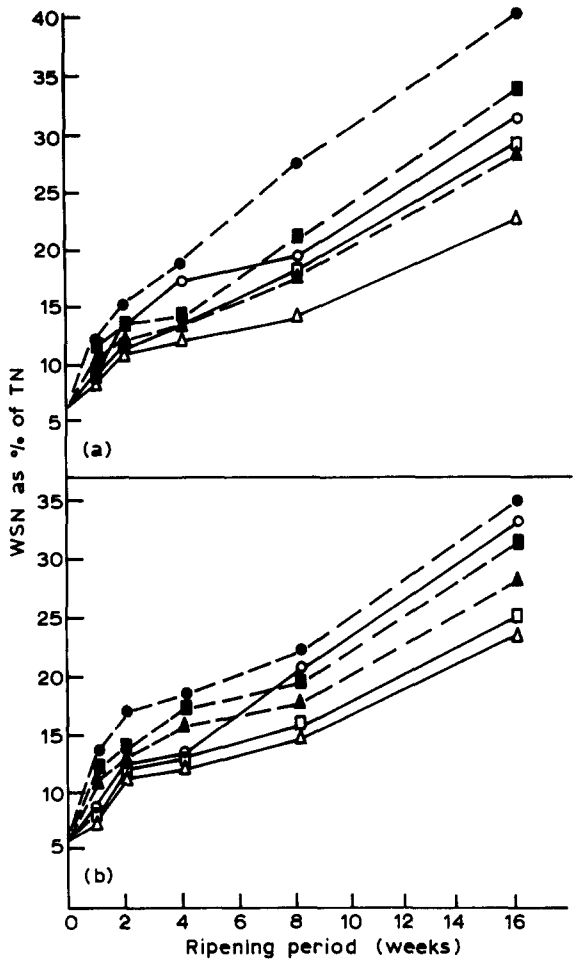

Fig. 2(b). Water-soluble N in Ras cheese made using brine (A) or a combination of brine/dry salting (B) as affected by ripening at 7°C, inner (▲), outer (△); 11°C, inner (■), outer (□); or 15°C, inner (●), outer (○).

there was a difference in the extent of proteolysis of both α_{s1}- and β-caseins in cheese made with salt addition to the curd and that made without salt addition to the curd.

The electrophoretic patterns confirmed the data for the proportion of WSN during ripening.

Accumulation of total free amino acids

Figures 4(a) and (b) show the changes in free amino acids, as PTA-soluble amino N, in cheese samples taken during the ripening period. The results indicate that levels of free amino acids increased during ripening and

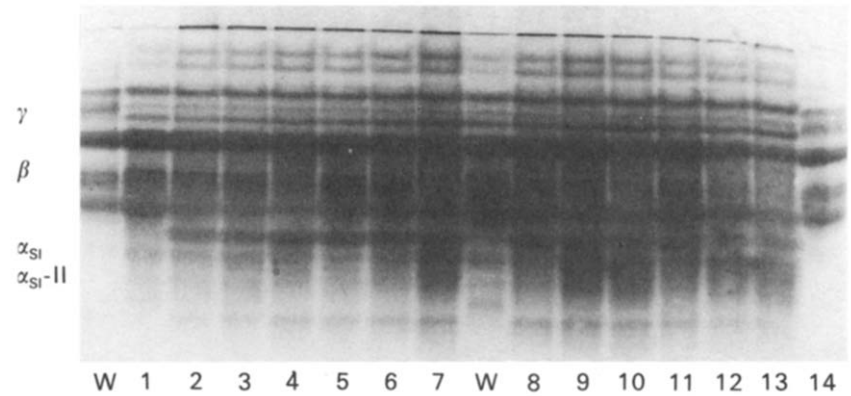

Fig. 3(a). Electrophoretograms of Ras cheese made with salt addition to curd, followed by dry salting, as affected by ripening temperatures, W: Na-caseinate; slots 1–8: zero time; slots 2–4; 7°C for 1, 3, 6 months, respectively; slots 5–7: 11°C for 1, 3, 6 months, respectively; slots 9–11: 15°C for 1, 3, 6 months, respectively; slots 12–14: 20°C for 1, 3, 6 months, respectively.

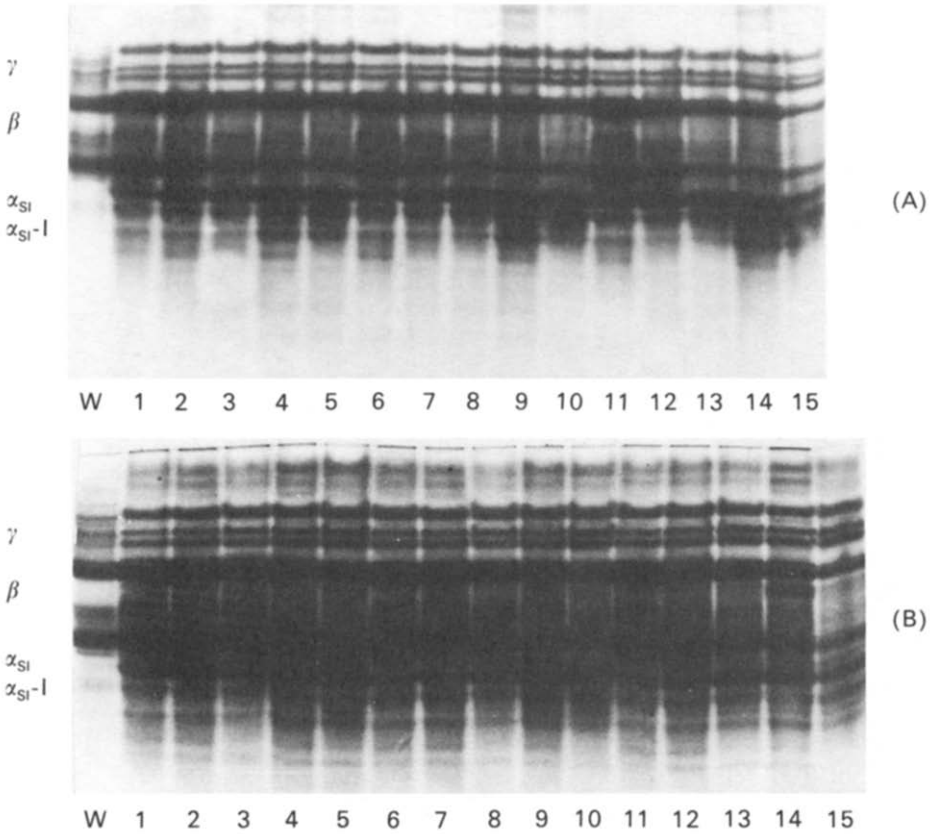

Fig. 3(b). Stacking gel electrophoresis of Ras cheese made using brine salting as affected by ripening temperature; inner samples (A), outer samples (B). W: Na-Caseinate; slots 1–5; 7°C for 0, 4, 8, 16 weeks, respectively; slots 6–10: 11°C for 0, 4, 8, 16 weeks, respectively; slots 11–15: 15°C for 0, 4, 8, 16 weeks, respectively.

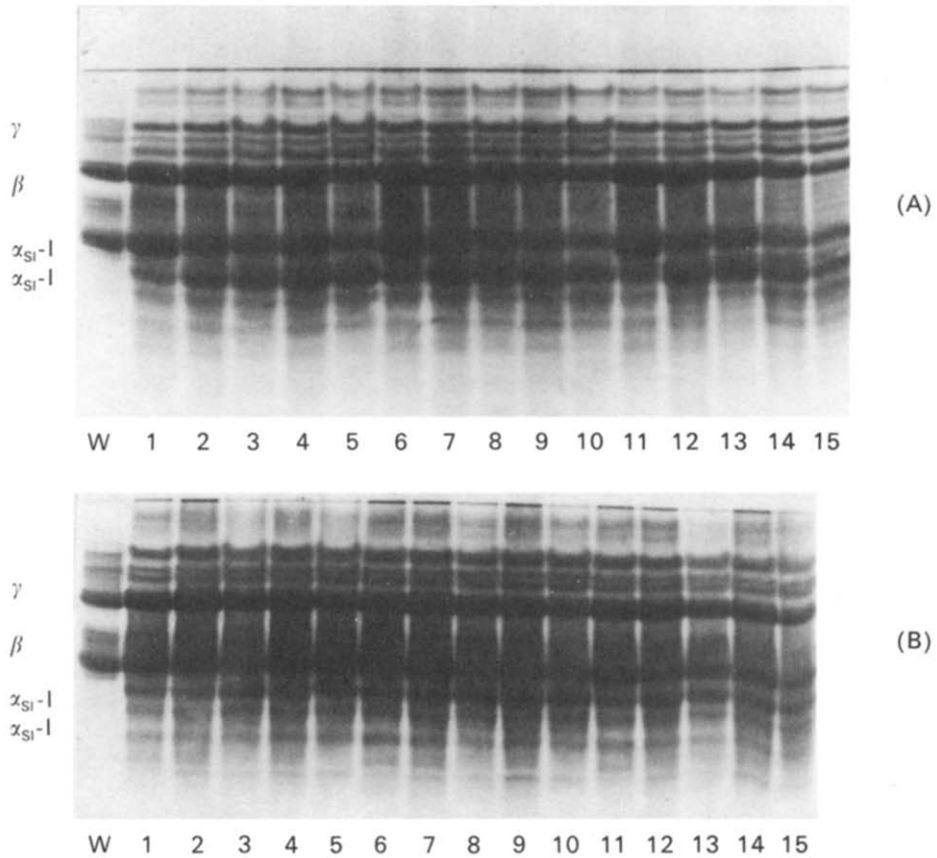

Fig. 3(c). Stacking gel electrophoretograms of Ras cheese made using brine/dry salting as affected by ripening temperature, inner samples (A), outer samples (B). W: Na-Caseinate;slots 1–5: 7°C for 0, 4, 8, 16 weeks, respectively; slots 6–10: 11°C for 0, 4, 8, 16 weeks, respectively; slots 11–15: 15°C for 0, 4, 8, 16 weeks, respectively.

increased faster in the cheeses matured at higher temperatures than in the cheeses ripened at lower temperatures at all stages of ripening; similar results were reported by Aston *et al.* (1983*a*,*b*), who showed that free amino acids, as PTA-soluble amino N, were higher in Cheddar cheeses matured at high temperature than at low temperature.

The changes in free amino acid levels in the inner and outer sections of the cheeses (Fig. 4(b)) showed that the rate of accumulation of free amino acids in the inner region was higher than in the outer layer of the same cheese at all sampling times.

Production of free fatty acids (FFA)

The FFA levels in all cheeses increased during ripening and were higher in

cheeses stored at higher temperatures than at low temperatures (Figs 5(a) and (b)). Similar results for Manchego cheese were reported by Nunez *et al.* (1985). The FFA levels in the inner regions of brine-salted or brine/dry-salted cheeses were higher than in the outer sections (Fig. 5(b)); the results also indicate that the FFA content of both inner and outer sections of the cheeses increased during the first week but then decreased between weeks 1 and 3. The FFA levels in all cheeses increased after 3 weeks until the end of ripening with a greater increase occurring in the inner than in the outer regions.

No marked differences were observed between brine-salted and brine/dry-salted cheeses.

DISCUSSION AND CONCLUSION

From the above results, it is evident that the manufacture of Ras-type cheese without salt addition to the curd and brining or brine/dry salting produced good quality cheese and accelerated proteolysis and lipolysis in the cheese to a degree dependent on ripening temperature. No marked differences in quality were observed between brine-salted and brine/dry-salted cheese (no

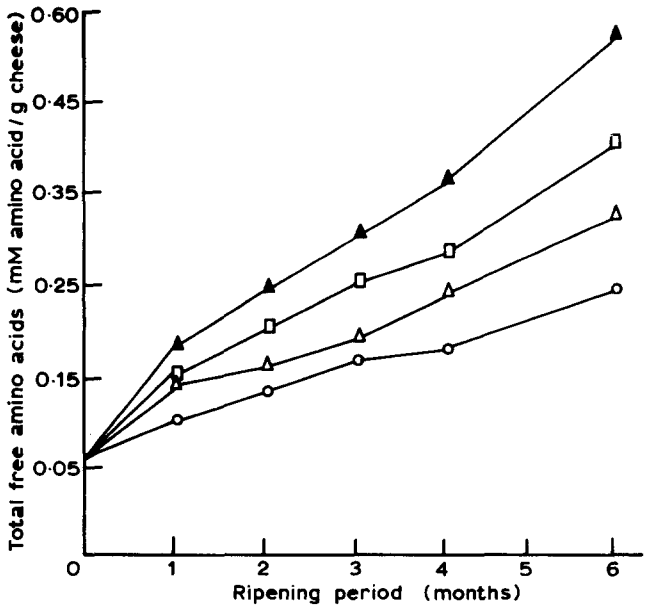

Fig. 4(a). Accumulation of total free amino acids in Ras cheese made with salt addition to curd followed by dry salting, as affected by ripening at 7°C (○), 11°C (△), 15°C (□), or 20°C (▲).

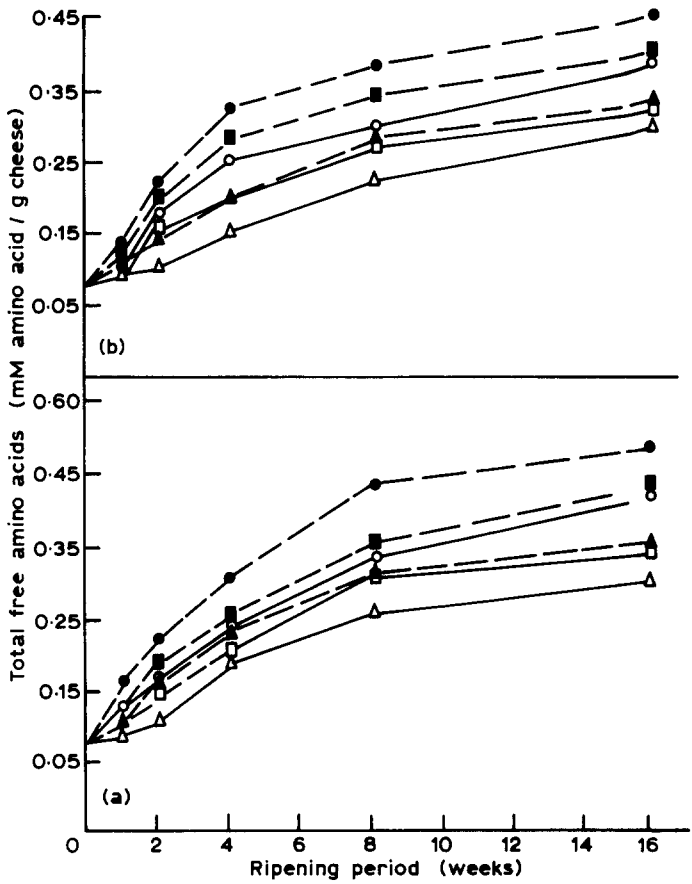

Fig. 4(b). Accumulation of total free amino acids in Ras cheese made using brine (A) or a combination of brine/dry salting (B), as affected by ripening at 7°C, inner (▲), outer (△); 1°C, inner (■), outer (□), or 15°C, inner (●), outer (○).

salt addition to curd) at any ripening temperature, but cheeses made with salt addition to the curd, followed by dry salting, were of lower quality and underwent less proteolysis and lipolysis than those that were brine-salted or brine/dry-salted only.

High storage temperatures produced large advancements in age and increases in mature flavour very early in the ripening period; cheeses stored at 15°C or 20°C showed the most marked flavour enhancement without off-flavours, although ripening at 20°C led to seepage of whey and fat from the cheese which is an extra cause of loss of weight and is, therefore, of some economic consequence. Moreover, such leakage of whey and fat invariably leads to faults, e.g. a rough, crumbly texture. A similar observation has been reported by several investigators (O'Keeffe *et al.*, 1979; Aston *et al.*, 1983*a,b*,

1985; Ridha *et al.* 1984) who showed that high maturation temperatures had highly significant effects on cheese ripening and quality.

The results obtained can be explained on the basis that high ripening temperatures enhanced both proteolysis, as indicated by the levels of N soluble in the various precipitants and by gel electrophoresis and lipolysis in cheese (Nunez *et al.*, 1985). The effect of high ripening temperatures on proteolysis in cheese is probably due to increased activities of rennet and the proteinases and peptidases from starter bacteria (Aston *et al.*, 1983*a*). Increases in the levels of free fatty acids at high temperatures could be due to increased activities of intracellular lipases from lactic acid bacteria (Peterson *et al.*, 1948). Increased lipolysis could be explained on the basis that high ripening temperature enhanced proteolysis and the formation of free amino acids which are considered to be precursors for certain FFA through specific metabolic pathways (Nakae & Elliot, 1965).

The differences in the extents of proteolysis and lipolysis between the inner and outer regions of the cheeses could be due to the observed differences in NaCl levels (Guinee and Fox, 1984; Hewedi & Fox, 1984).

Significant differences were observed between the pH values of cheeses ripened at the various temperatures, probably due to the influence of temperature on the extent of proteolysis and lipolysis in the cheeses (Nunez *et al.*, 1985).

In conclusion, the quality and rate of ripening of Ras-type cheese could be enhanced by ripening the cheese at high temperatures; 15°C appears to be a

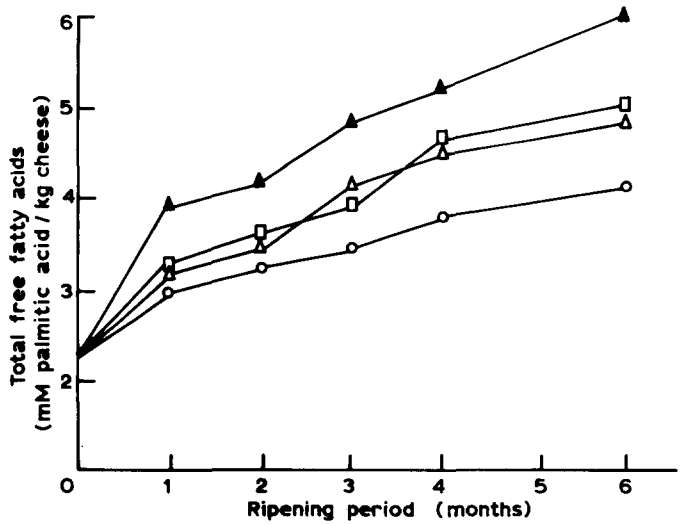

Fig. 5(a). Accumulation of total free fatty acids in Ras cheese made with salt addition to the curd, followed by dry salting, as affected by ripening at 7°C (○), 11°C (△), 15°C (□), or 20°C (▲).

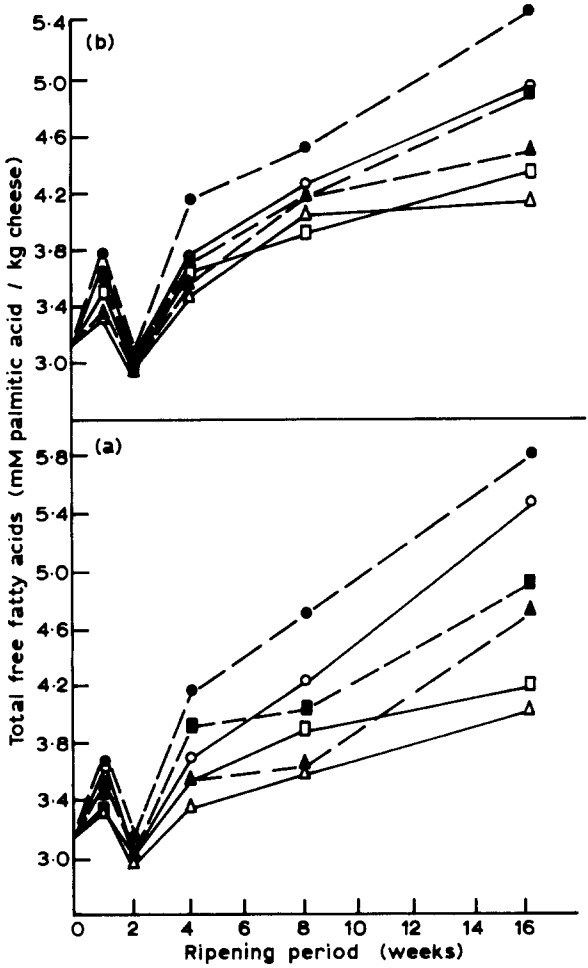

Fig. 5(b). Accumulation of total free fatty acids in Ras cheese made using brine (A), or combination brine/dry salting (B), as affected by ripening at 7°C, inner (▲), outer (△); 11°C, inner (■) outer (□); 15°C, inner (●), outer (○).

suitable temperature for maturing good quality Ras-type cheese in 8 weeks and it can be recommended for accelerating the ripening of this, and probably similar, cheese varieties.

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