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Enzyme Accelerated Ripening of Spanish Hard Cheese*

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ABSTRACT

The acceleration effect of lipolytic, proteolytic, and glycolytic enzymes on the ripening of a Spanish hard cheese made according to Manchego cheese manufacturing methods was studied. The results indicated that proteolytic enzymes caused substantial alterations in the nitrogen fractions. The addition of lactase resulted in early hydrolysis of lactose as well as a noticeable increase in the amino acid nitrogen fraction. The free fatty acid content increased in cheeses treated with lipases. The cheeses treated with lactase received the best taste panel rating, and a slightly bitter taste was detected in the cheeses treated with protease.

INTRODUCTION

For a number of years, attempts have been made in a number of countries to accelerate cheese ripening, in an effort to reduce manufacturing costs (Fernández-García, 1986). One of the most promising methods employed is the addition of commercially available enzymes to milk or to curd. Cheddar cheese (Sood & Kosikowski, 1979; Law & Wigmore, 1982, 1983; Law & King, 1985) and some Egyptian cheeses (Ridha *et al.*, 1984; Farahat *et al.*, 1985; Omar, 1985) have been studied extensively, and attempts to accelerate

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ripening by adding enzymes have also been made for other cheeses, such as Edam (Pahkala *et al.*, 1985), Saint Paulin (Gripon *et al.*, 1982), and blue cheese (Jolly & Kosikowski, 1975), among others.

The results of such studies indicate that the effect of the enzyme addition is not exactly the same for different types of cheese, and hence the conclusions reached for a given cheese cannot be extrapolated to other cheeses.

In view of the growing importance of modern ultrafiltration methods in cheese manufacturing, the study of the behaviour of soluble cheese proteins in the presence of commonly used enzymes is exciting renewed interest. To our knowledge, there are, however, no studies of the effect of such enzymes on the soluble protein fraction of cheese.

Since no data are available on ripening acceleration of Spanish cheese, and since Manchego cheese is the most important cheese variety manufactured and consumed in Spain, it is of interest to establish the conditions for ripening acceleration by enzyme addition. In this work we study the acceleration effect, produced by adding several different enzymes, on the ripening of a hard cow's milk cheese made using traditional industrial manufacturing methods for Manchego cheese on a laboratory scale, prior to a further study on the ripening acceleration of Manchego cheese on an industrial scale.

In order to be able to assess the degree of acceleration undergone by the cheese, the alterations taking place in the protein fraction, lipid fraction, and carbohydrate fraction of the cheeses were examined, and cheese taste and texture were also evaluated.

MATERIAL AND METHODS

Enzymes

Palatase A 750 L, a lipase obtained from *Aspergillus niger*.
Lactozym 3000L, a β-galactosidase obtained from *Kluyveromyces fragilis*.
Neutrase 0·5 L, a neutral protease obtained from *Bacillus subtilis*.
All the enzymes were supplied by Novo IndustriA/S.

Cheeses

All the cheeses were manufactured on the same day from the same lot of pasteurised cow's milk at the Escuela Nacional de Industrias Lácteas (National Dairy Training Institute) in Madrid in accordance with traditional methods for the industrial production of Manchego cheese. Following pasteurization, the milk was divided into four batches numbered

1 through 4. Palatase (1 ml/20 l milk) was added to batch number 2 just before the rennet. Curd production in batches 1, 3, and 4 followed traditional methods. Batch 1 was used as the control batch. Lactozym (8 ml/3 kg curd) was added to the curd obtained from batch 3, while Neutrase (0·075 ml/kg curd) was added to the curd from batch 4. In order to get uniformity of enzyme addition to the curd, the enzyme was thoroughly pre-mixed with 300 g of curd and added to the curd batch immediately. Four cheeses of approx. 1 kg weight were prepared from each batch, and these were pressed and steeped in brine for 12 h. Ripening took place in a cold store at a temperature of 12°C and 85% humidity. Analyses were performed in duplicate (a) on removal from the brine; (b) after 15 days; (c) after 30 days and (d) after 45 days of ripening. A single cheese was assessed at each sampling age.

Chemical analyses

Dry matter (DM), total fats and NaCl content were determined following the official methods recommended by the International Dairy Federation (1958, 1969, 1972), respectively. Total nitrogen (TN), water-soluble nitrogen (WSN), non-casein nitrogen (NCN) soluble at pH 4·6, and non-protein nitrogen (NPN) soluble in 12% trichloroacetic acid were determined using the Kjeldahl method, with extraction of the WSN as per Kuchro & Fox (1982). Amino acid nitrogen (NH_2N) soluble in 2·5% sulphosalicylic acid (SSA) was determined by the ninhydrin method.

Polyacrylamide gel electrophoresis (PAGE)

The WSN, NCN, and casein fractions were studied by means of PAGE, according to the method of Hillier (1976) for the whey ($T = 9{\cdot}4\%$, $C = 4{\cdot}2\%$) and the method of Ramos *et al.* (1977) for the casein fraction ($T = 7{\cdot}7\%$, $C = 2{\cdot}6\%$). Electrophoresis was carried out on 0·7 mm-thick plates, and the bands were stained with Comassie Blue 250G, following Blakesly & Boezi (1977).

High performance liquid chromatography (HPLC) analysis of free amino acids (FAA)

Determination of the FAA fraction soluble in SSA was carried out by HPLC, by derivatizing the amino acids using *o*-phthaldehyde. The derivatization reaction was performed automatically according to the method described by Fleury & Ashley (1983). The equipment employed consisted of two Waters M-6000 A pumps, an M-720 system controller, an

M-420 AC fluorimeter (340 ± 6 nm excitation filter, 425 nm emission long-pass-filter), an M-730 data module, a WISP 710 injector, a 10 μm Radial-Pak C_{18} column in an RCM-100 radial compression module, and a Bondapak C_{18}/Corasil guard column. Conditions of separation were as described in Ramos *et al.* (1987).

Gas–liquid chromatography (GLC) analysis of free fatty acids (FFA)

The FFA fraction was extracted according to the method of Martín-Hernández (1987, pers. comm.) for cheese. 10 g of cheese were homogenized in 10 ml of distilled water, 5 ml of ethyl ether, 0·2 ml of 25% sulphuric acid, and 1 ml of nonanoic acid (C_9) (5·49 mg/ml), used as an internal standard. The solution was centrifuged at 3000 rpm for 7–8 min, with the FFA being dissolved in the organic phase. The entire extraction process was performed at 0°C. Methylation and GLC separation of the FFA were as described by Martínez-Castro *et al.* (1986). The gas chromatograph was a Perkin-Elmer model F.990, equipped with a flame ionization detector and two stainless steel columns measuring 2 m × 3·2 mm packed with 4% DEGA (Diethyleneglycol Adipate) on Chromosorb g AW-DMCS, 80–100 mesh.

GLC analysis of carbohydrates

The sugars were extracted as per the modified method of Harvey *et al.* (1981). A 4-g cheese sample was homogenized in 20 ml of distilled water and 5 ml of phenyl-β-D-glucoside (1 mg/ml), used as an internal standard. The mixture was filtered through Whatman No. 1 paper. 5 ml of the resulting extract were diluted to 25 ml using methanol. The mixture was allowed to stand at room temperature for 1 h and then filtered through Whatman No. 42 paper. The filtrate was vacuum-dried at 38–40°C. 2 ml of anhydrous pyridine were added, and the mixture refluxed for 1·5 h. 100 μl of trimethylsilylimidazole were added to 200 μl of the pyridine solution. After 30 min at 65–70°C, the solution was cooled. 0·1 ml of hexane and then 0·2 ml of distilled water were added. 2 μl of the hexane solution were injected. Chromatographic conditions were those described by Olano *et al.* (1986). The GLC analyses were performed using a Sigma 3B gas chromatograph (Perkin-Elmer) equipped with a flame ionization detector, using a 3 m × 1·0 mm stainless steel column (Chrompack) packed with 2% OV-17 on non-silanized 120140 Volaspher A-2 (Merck).

Sensory analysis

Sensory analysis was carried out using a panel of nine trained tasters familiar with various types of Spanish cheeses, including Manchego cheese.

The tasters evaluated the quality of the taste, colour, body, and texture of the cheese. These parameters were combined under the concept of general acceptability, with a maximum score of 3 points. Tasters were also instructed to report any flavour (bitterness, rancidity) or texture defects detected.

RESULTS AND DISCUSSION

The overall composition of the cheeses is presented in Table 1. There were no appreciable differences between the control cheese and the cheeses to which enzymes had been added during the manufacturing process, except with regard to DM and NaCl contents, which were higher in batches 3 and 4. The

TABLE 1
pH, Protein, NaCl and Fat Contents as a Percentage of Dry Matter of Cheeses

Batch	*Dry matter*		*Protein* $\bar{x}$	*NaCl*		*Fat* $\bar{x}$	*pH*	
	1 day	*45 days*		*1 day*	*45 days*		*1 day*	*45 days*
1	44·65	51·88	37·5	2·21	2·80	54·10	5·50	5·64
2	45·86	52·08	38·3	2·31	2·90	53·76	5·74	5·41
3	49·18	56·25	38·6	2·41	3·69	51·25	5·53	5·77
4	50·21	60·40	38·1	2·36	3·20	53·11	5·58	5·49

1: Control; 2: added Palatase; 3: added Lactozym; 4: added Neutrase.

higher value of DM was due solely to more complete removal of the whey, partly in response to the need to properly blend the enzymes into the curds. The salt content of all cheeses was higher than that found in commercial Manchego cheese. This could be attributed to the small size of the cheeses (1 kg versus 2·5–3·0 kg of commercial cheeses). The salt in moisture content increased considerably after 45 days in batches 3 and 4 (only very matured Manchego cheeses have a salt in moisture content higher than 8% (Marcos *et al.*, 1985)).

Nitrogen fraction

Ripening indices

Proteolysis in the cheeses was estimated on the basis of the nitrogen content of the various fractions (WSN, NCN, NPN and NH_2N).

Figure 1 represents the trends in WSN/TN over the ripening period. A higher WSN content was observed from day 1 in the cheese manufactured

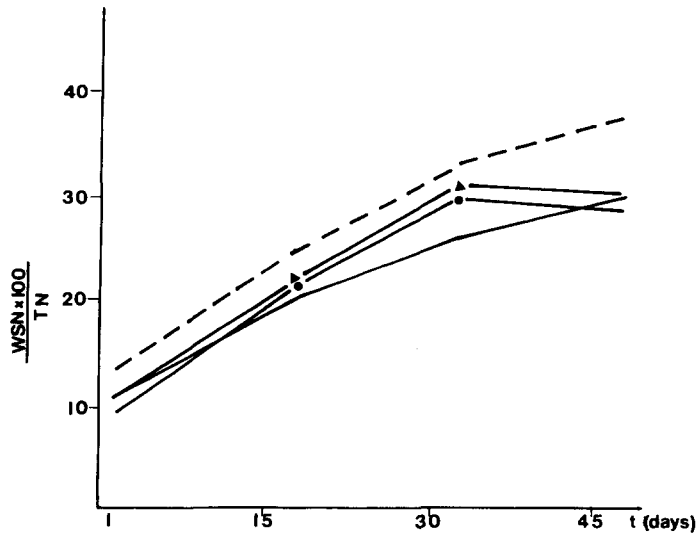

Fig. 1. Effect of enzymes on the water-soluble nitrogen (WSN) fraction during ripening: (——), control; (▲), Palatase; (●), Lactozym; (– – –), Neutrase.

with protease (batch 4). This difference increased, and by 45 days the WSN made up 36% of the TN in the cheeses in batch 4, compared with only 24% in the control cheeses (batch 1). These results are in agreement with those reported by other authors who added various proteolytic enzymes to Cheddar cheese (Kosikowski & Iwasaki, 1975; Sood & Kosikowski, 1979) and to Edam cheese (Pahkala *et al.*, 1985).

NCN was also assessed. NCN made up 80% of the WSN in the control cheeses (batch 1) and the cheeses made with Palatase (batch 2) and Lactozym (batch 3) and 90% of the WSN in the cheeses in batch 4. The differences between batches 1 and 4 were greater when NCN was taken, instead of WSN, as the index of proteolysis (Fig. 2).

Figure 3 shows the NPN/TN and NH_2N/TN values for cheeses made with enzyme additives, as percentages of the results obtained for the control batch. Increases in the NPN of up to 50% over the control cheeses were observed for batch 4, but the most notable difference between the enzyme-treated cheeses and the control cheeses was found for the FAA content. Although the differences remained rather high for this parameter, they did tend to decrease with time. In contrast, the difference in the NPN between batch 4 and the control batch increased with time. Law & Wigmore (1982) found much lower increases in the NPN and NH_2N contents while using identical enzyme concentrations in the production of Cheddar cheese. Pahkala *et al.* (1985) also reported smaller differences for Edam cheese, though they did not indicate the Neutrase concentration employed. Taking into account that the high value of salt in moisture found in batch 4

contributed to a decrease of the starter activity, it can be concluded that the presence of neutrase considerably increased the rate of proteolysis.

The addition of Lactozym did not affect the WSN, or NPN contents of the cheese, but differences were found for NH_2N (Fig. 3). Farahat *et al.* (1985) detected increases in the different nitrogen fractions (NPN, WSN, and NH_2N) when Lactozym was added. Other authors (Marschke & Dulley, 1978; Weaver & Krager, 1978; Gooda *et al.*, 1983) also reported increases in the soluble nitrogen fractions when Maxilact (a β-galactosidase obtained

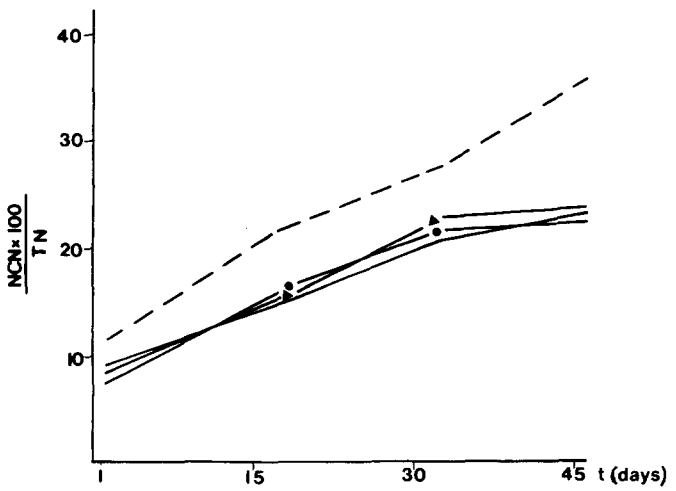

Fig. 2. Effect of enzymes on the non-casein nitrogen (NCN) fraction during ripening: (——), control; (▲), Palatase; (●), Lactozym; (– – –), Neutrase.

from *Kluyveromyces lactis*) was added to Cheddar cheese. Such increases might have been due to the presence of protease contamination detected in the Maxilact (Marschke *et al.*, 1980; Olano *et al.*, 1983).

Adding Palatase (batch 2) brought about only a slight increase in NH_2N, which might be indicative of a low level of activity by protease contaminants. El Neshawy *et al.* (1983) reported increases in the WSN and NH_2N when treating milk with two different lipases in the manufacture of Domiati cheese, possibly due to higher levels of protease contamination in the enzyme preparations used.

The results obtained suggest that acceleration of the proteolysis, which plays an important role in the cheese ripening process, did, in fact, take place when Neutrase was added. Thus, the cheeses made with Neutrase exhibited the same NCN content after just 15 days as the control cheeses did after 45 days. The addition of Lactozym and Palatase only accelerated the freeing of amino acids.

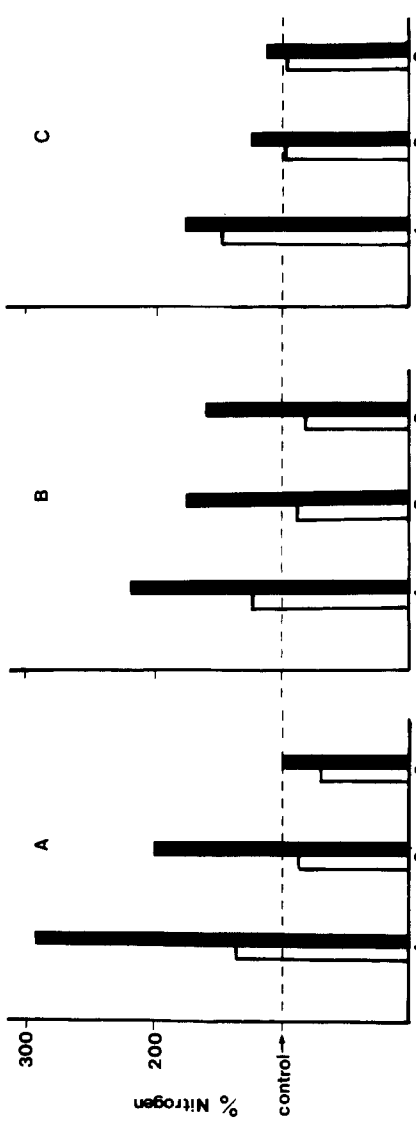

Fig. 3. Comparison of non-protein nitrogen/total nitrogen (NPN/TN) (□), and amino acid nitrogen/total nitrogen (NH_2N/TN) (■), in enzyme-treated cheeses as percentages of the values for the control cheeses. (2) Palatase; (3) Lactozym; (4) Neutrase; (A) 1-day-old cheese; (B) 30-day-old cheese; (C) 45-day-old cheese.

HPLC analysis of FAA

Table 2 presents the results obtained by HPLC analysis of the fractions from the four cheese batches soluble in 2·5% SSA after 30 and 45 days of ripening. The contents of each of the 18 amino acids and two amines analyzed tended to increase with ripening time, the increase being most pronounced in batch 4. Sood & Kosikowski (1979) and Law & Wigmore (1982) reported differences between Cheddar cheeses made with proteolytic enzymes and control cheeses, but the differences were not as marked as those we have found in Manchego cheese. Increases of more than 100% were found between the cheeses in batch 4 after 30 days and the control cheeses for the following amino acids: methionine, tyrosine, valine, phenylalanine, and leucine, and for the amine histamine.

The increases for arginine, tryptophan, isoleucine, and lysine were even greater. These appreciable differences continued in the cheeses after 45 days of ripening, with increases also taking place in three other amino acids,

TABLE 2

Free Amino Acid Contents (mg/100 g dry matter) of Cheeses after 30 and 45 Days of Ripening

	1		*2*		*3*		*4*	
	30 days	*45 days*	*30 days*	*45 days*	*30 days*	*45 days*	*30 days*	*45 days*
Asp	17·6	17·7	17·7	16·8	16·4	19·6	16·1	18·3
Glu	69·0	83·9	61·7	87·1	80·9	119	73·5	116
Asn	27·7	33·2	26·1	33·9	37·8	60·0	34·4	43·9
Ser	11·1	8·81	8·12	11·0	9·64	13·0	12·4	19·8
β-Ala	5·72	3·87	4·15	4·88	4·49	5·33	7·16	7·95
α-Ala	7·09	7·84	6·44	9·64	8·75	13·1	10·1	17·5
γ-Aba	5·80	4·68	3·50	5·20	4·62	4·62	5·80	7·53
Tyr	8·68	6·94	29·9	35·2	21·6	22·4	20·7	15·4
α-Aba	—	0·73	0·73	1·77	0·87	1·05	1·59	2·83
Arg	27·3	42·0	59·7	61·2	80·1	105	81·8	85·1
Met	7·29	9·16	9·05	15·8	10·7	14·8	16·5	22·5
Val	31·5	47·2	58·5	76·1	57·0	91·5	81·4	107
Trp	5·09	7·52	5·87	7·85	4·86	7·40	24·2	37·7
Phe	42·4	46·0	53·5	63·5	54·8	72·2	90·2	111
Ile	7·25	9·19	10·4	13·7	16·9	16·0	25·3	35·9
Leu	67·1	85·1	92·0	115	101	144	140	195
Orn	9·52	8·15	7·60	10·5	9·68	12·1	13·5	18·3
Lys	11·7	11·5	18·9	20·2	21·1	31·5	31·0	31·6
Histam	16·8	16·6	18·9	—	17·7	18·5	28·5	29·4
Tyram	15·6	22·6	—	—	12·5	26·6	8·50	28·2
Total	394	473	493	589·72	571	798	723	951

1: Control; 2: added Palatase; 3: added Lactozym; 4: added Neutrase.

serine, α-alanine, and ornithine. The only amino acid for which no difference was recorded was aspartic acid. The relative percentages of the amino acids were affected slightly by treatment with Neutrase, since they did not all undergo the same increase.

Significant increases compared to the control cheeses were found for the cheeses to which Lactozym had been added, for all the amino acids analyzed except for aspartic acid, γ-aminobutyric acid (GABA), α-aminobutyric acid, tryptophan, and the amine histamine. FAA levels in these cheeses tended to remain below those in batch 4. However, in general, the relative percentages of the amino acids were closer to those for the control cheeses than was the case for batch 4. Similar results have been reported by other authors when employing various commercial brands of β-galactosidases (Weaver & Krager, 1978; Gooda *et al.*, 1983; Farahat *et al.*, 1985; Omar, 1985).

Higher FAA contents were recorded for arginine, methionine, valine, leucine, lysine, and particularly tyrosine after 30 and 45 days of ripening in the cheeses in batch 2, with respect to the control cheeses. The tyrosine content in these cheeses was higher, not only than that in the control cheeses, but also than that in the cheeses in the other two batches, possibly because of contamination of the preparation with proteolytic enzymes, as mentioned earlier. The relative percentages of the amino acids were affected to a lesser extent than in the case of the other enzyme treatments.

Thus, we detected considerable increases in the FAA content in all the cheese batches to which enzymes were added, the most appreciable increase being for batch 4. The FAA content in the control cheeses after 45 days was comparable to the FAA content in the cheeses in batch 2 after 30 days and to that in the cheeses in batch 4 after approximately 20–25 days and lower than that in batch 3 after 30 days of ripening.

The results obtained with the ninhydrin method were two to ten times higher than those for the sum of all the different amino acids analyzed by HPLC. This might have been caused by peptides capable of reacting with the ninhydrin but which go undetected in HPLC analysis.

PAGE analysis of the protein fraction

Figure 4 presents a photograph of the plate electrophoresis of caseins from the cheese batches over the ripening period. The plate shows an appreciable breakdown (approximately 40%) of β-casein in batch 4, and a slight breakdown (approximately 10%) in the remaining lots. The region of the β-caseins from the cheeses in batch 4 contained two well-defined bands, with a further two bands (which we have designated *Y* and *Y'*), much stronger than for the control cheeses, in the region of the γ-caseins. Law & Wigmore (1982) and Law & King (1985) also observed an increase in the number and size of

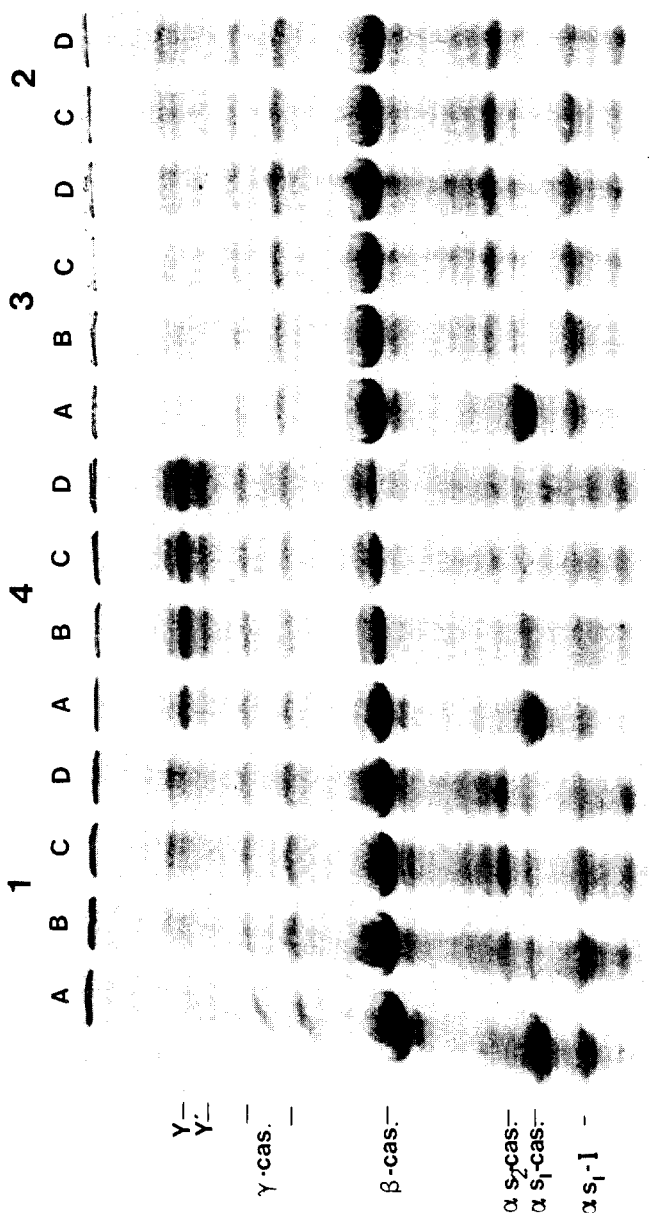

Fig. 4. Polyacrylamide gel electrophoresis of the casein fraction from batches 1 (control), 2 (Palatase), 3 (Lactozym), and 4 (Neutrase) at (A) 1 day, (B) 15 days, (C) 30 days, and (D) 45 days.

the bands in the region of the γ-caseins for cheeses treated with Neutrase and β-casein degradation of around 60%, as opposed to around 10% in the control batch, in Cheddar cheeses ripened for two and four months. Bands *Y* and *Y'* may be the degradation products of β-casein breakdown.

α_{s1}-casein and α_{s1}I peptide, regarded as a product of the action of the rennet on α_{s1}-casein, broke down rapidly and followed similar trends in all the lots. However, the α_{s1}-casein band for batch 4 was slightly stronger, whereas the α_{s1}I band practically disappeared. Batch 4 also exhibited a band between the α_{s1}-casein and α_{s1}I; this band was not visible in the other batches. Sood & Kosikowski (1979) described the near disappearance of both the α_{s1}-casein and α_{s1}I peptide bands from the electrophoretograms for 25-day-old Cheddar cheese made with protease from *Bacillus subtilis*. In contrast, Law & Wigmore (1982) reported similar degradation of α_{s1}-casein in both control cheeses and cheese made with Neutrase in an amount similar to the amount we used. Pahkala *et al.* (1985) also observed substantial breakdown of β-casein in Edam cheese treated with Neutrase, with no appreciable differences between the α_{s1}-casein in the control and treated cheeses.

PAGE analysis was also performed on the WSN and NCN fractions (Figs 5 and 6), and large differences between the two extracts were found. In particular, there were two very strong bands of lower electrophoretic mobility than α-lactalbumin and a third band, which we have designated X_1, located between α-lactalbumin and β-lactoglobulin B in the cheeses in batches 1, 2 and 3. These three bands showed up only faintly, or not at all, in the electrophoresis of the NCN. None of these three bands was present in either the WSN or the NCN from the cheeses in batch 4, and the differences between these two fractions in this batch were found to be minimal. This was not explainable by a low pH value for the WSN in the cheeses in batch 4, because the pH was practically the same in all the cheeses. The bands would appear to be degraded by the added protease. The four characteristic bands, serum albumin, α-lactalbumin, and β-lactoglobulins B and A, did not appear to undergo significant degradation over the ripening period and were found in all the batches. The cheeses made with Neutrase (batch 4) exhibited three well-defined bands, one of which was a doublet, of higher electrophoretic mobility than the β-lactoglobulins, which we have designated NX_1, NX_2 and NX_3. None of these was observed in the control cheeses or in the cheeses from the other batches. No pertinent data were found in the available literature, and thus these results could not be contrasted with the results reported by other authors.

No differences in the caseins or whey proteins were detected in the electrophoretograms for the cheeses in batches 1, 2 and 3.

These results indicate that the caseins were, in fact, degraded more actively

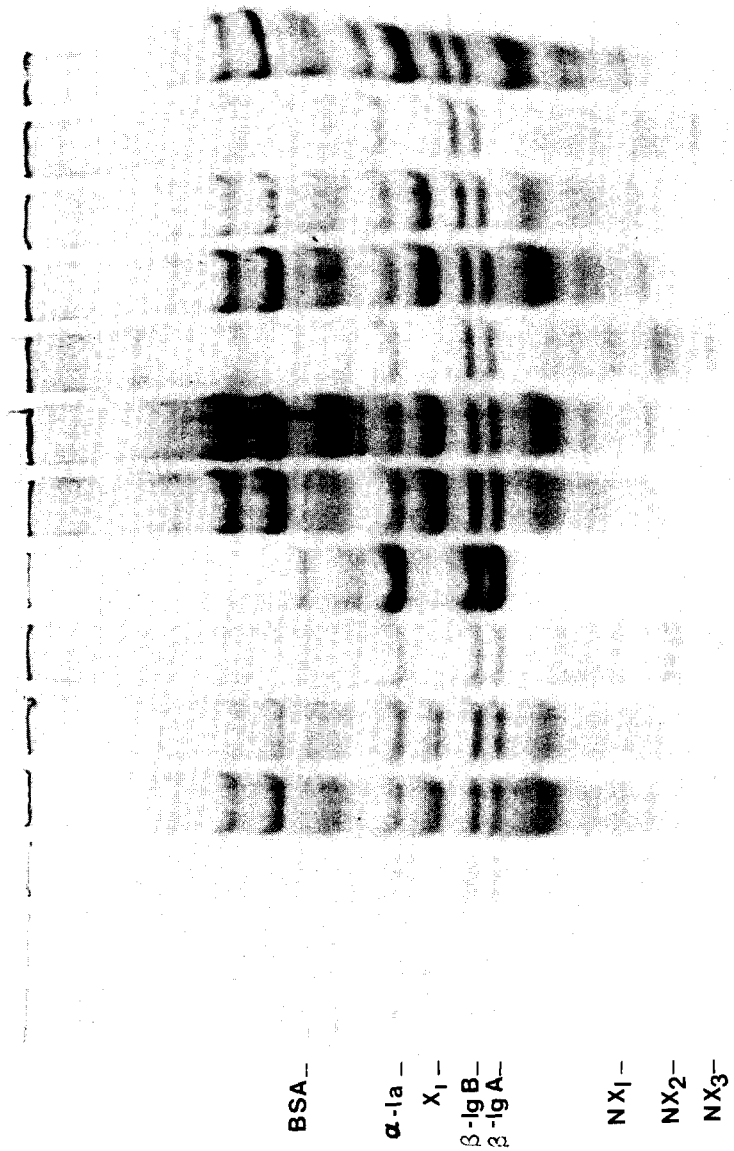

Fig. 5. Polyacrylamide gel electrophoresis of the water-soluble nitrogen fraction from batches 1 (control), 2 (Palatase), 3 (Lactozym), and 4 (Neutrase) at (A) 1 day, (B) 15 days, (C) 30 days, and (D) 45 days. (BSA), serum albumin; (α-la), α-lactoalbumin; (β-lg), β-lactoglobulin.

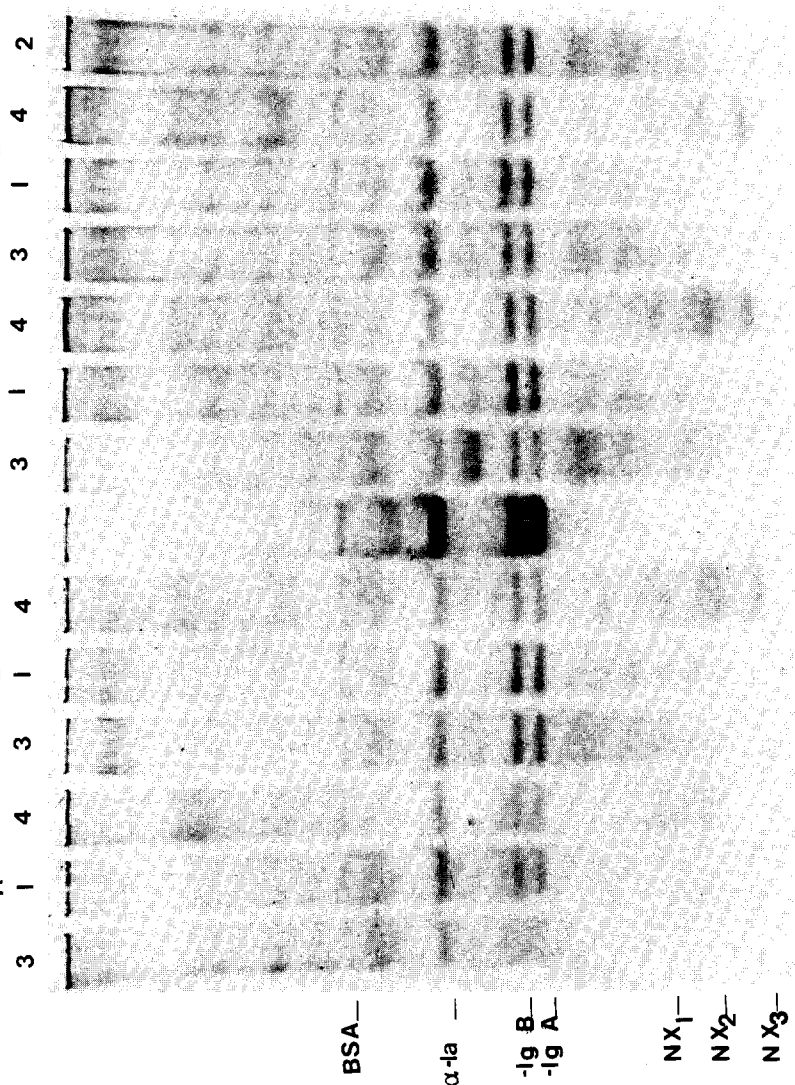

Fig. 6. Polyacrylamide gel electrophoresis of the noncasein nitrogen fraction from batches 1 (control), 2 (Palatase), 3 (Lactozym), and 4 (Neutrase) at (A) 1 day, (B) 15 days, (C) 30 days, and (D) 45 days. (BSA), serum albumin; (α-la), α-lactoalbumin; (β-lg), β-lactoglobulin.

in the cheeses in batch 4 than in the cheeses in the remaining lots. This was not the case for the whey proteins, the bands for which appeared to be only slightly fainter than for the control cheeses. The bands appearing in the electrophoresis of the whey proteins from the cheeses in batch 4, which were not found for the cheeses from the other lots, were probably the result of the action of Neutrase on the caseins.

Analysis of the lipid fraction

GLC analyses of FFA

The results of GLC analysis of the FFA fraction from the cheeses in batches 1, 2 and 4 are summarized in Table 3. As might be expected, the FFA level increased when Palatase was added to the cheese, although the difference in comparison with the control cheeses was not very large (around 20%). This increase was not the same for all the fatty acids. It was noted basically for such long-chain fatty acids as myristic, palmitic, stearic, and oleic acids. There were no significant differences between batches 3 and 4 and the control, which indicates a low contamination with lipases in the Lactozym and Neutrase preparations.

GLC analysis of the carbohydrate fraction

The lactose and galactose contents of the cheeses during ripening were also studied, and the results are set out in Table 4. The lactose content in 1-day-old cheese from batches 1 and 2 was high in comparison with the levels

TABLE 3

Free Fatty Acid Content (mg/100 g dry matter) of Cheeses after 1 and 45 Days of Ripening

Fatty acid	*1*		*2*		*4*	
	1 day	*45 days*	*1 day*	*45 days*	*1 day*	*45 days*
C_4	59·1	74·3	77·3	71·2	61·6	83·5
C_6	29·2	37·3	42·6	38·7	27·2	25·3
C_8	11·8	15·0	15·8	13·3	10·4	13·2
C_{10}	25·0	29·7	31·4	28·6	21·0	27·1
C_{12}	26·8	32·2	27·7	31·1	21·5	26·8
C_{14}	107	90·1	78·5	102	68·3	90·8
C_{16}	200	238	202	302	199	255
C_{18}	73·4	85·4	63·3	112	73·1	93·7
$C_{18}=$	181	220	183	283	190	241
Total	713	822	722	982	672	856

1: Control; 2: added Palatase; 4: added Neutrase.

TABLE 4
Carbohydrate Composition (mg/100 g dry matter) after 1, 15 and 30 Days of Ripening

Time (days)	*1*		*2*		*3*		*4*	
	Lactose	*Galactose*	*Lactose*	*Galactose*	*Lactose*	*Galactose*	*Lactose*	*Galactose*
1	1 194	96·0	1 584	98·0	261	578	271	45·8
15	16·1	4·10	55·6	4·50	15·4	23·1	6·80	1·80
30	6·77	2·00	34·4	1·60	—	—	—	—

1: Control; 2: added Palatase; 3: added Lactozym; 4: added Neutrase.

found for the other two batches. A low lactose content, coupled with a high galactose content, in 1-day-old cheese from batch 3 can be attributed to hydrolysis by β-galactosidase. Traces of glucose were also found in this batch. Low lactose and galactose contents in the cheeses in batch 4 may have been caused in part by the larger amount of whey removed or by accelerated growth of the starter microorganisms as a result of the presence of low molecular weight nitrogen compounds. The low glucose content detected, even in the cheeses in which the lactose had been hydrolyzed, was due to rapid glucose assimilation by the microorganisms.

Sensory analysis

Pearson χ^2 analysis was applied to the scores between 1 and 3 awarded by the tasters in accordance with the scale employed. Significant differences were found for the batch treated with Lactozym (batch 3).

Certain tasters reported that the cheeses in the batches made with Lactozym and Neutrase seemed somewhat riper (but not significantly so) than the cheeses in the other two batches.

The texture was appreciably better in the cheeses made with Palatase and Lactozym than in the control cheeses, while the cheeses made with Neutrase were found to be crumbly.

A slightly bitter taste was detected in the cheese treated with Neutrase.

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