STUDIES ON THE ACCELERATED RIPENING OF CHEDDAR CHEESE

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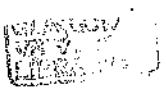

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DEDICATED TO

MY PARENTS,

SISTERS AND BROTHERS

WITH LOVE

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THESIS SUMMARY

The aim of this work was to evaluate the use of enzymes to accelerate the ripening of Cheddar cheese.

ADDITION OF PREPARATIONS OF β-D-GALACTOSIDASE TO THE CHEESE MILK

The ripening of Cheddar cheese produced from lactose-hydrolysed milk (e.g. up to 60% hydrolysis of the lactose), was only slightly accelerated even though one of the β-D-galactosidase enzymes used contained some proteolytic enzyme(s). The numbers of starter bacteria at the end of the cheesemaking process were higher in cheese made from enzyme-treated milk than from untreated control milk.

The level of soluble nitrogen gradually increases in Cheddar cheese as it becomes older. In these studies higher values for soluble nitrogen were observed in cheese made from lactose-hydrolysed milk compared with control cheese made from untreated milk after similar periods of ripening. This was true for both commercial lactase enzyme preparations - one of which was highly purified while the other contained substantial amounts of protease.

Cheddar cheese manufactured from the latter enzyme contained the highest level of soluble nitrogen throughout the ripening period and this could be associated with appreciable acceleration of the ripening of the cheese. More extensive hydrolysis of the casein fractions was also evident in 6-month old Cheddar cheese made from milk treated with the lactase containing protease compared with the control and also the cheese made from milk treated with highly purified lactase. Other desirable effects were achieved as the result of lactose hydrolysis of the cheese milk:

(i) Reduction in the cheesemaking time.

(ii) Greater judge preference for cheese manufactured from lactose-hydrolysed milk. This effect could be due to the slight increase in protein degradation of this cheese.

(iii) The increased level of glucose and galactose in the whey could be a desirable feature for further processing, i.e. in the production of a sweet syrup.

ADDITION OF A COMMERCIAL BRAND OF NEUTRAL PROTEINASE TO THE CHEESE CURD

Cheddar cheese was manufactured by using direct-to-vat inoculation of concentrated frozen mixed strains of mesophilic starter culture. In preliminary experiments the enzyme was provided by the manufacturer as a powdered chemical and added to the milled curd at a rate of 0.001, 0.002, 0.005 and 0.01% (w/w). Separate lots of cheese were ripened at 10° and 13°C, and the enzyme activity in the cheese as assessed by monitoring the level of soluble nitrogen, hydrolysis of casein and organoleptically. The extent of acceleration of Cheddar cheese ripening depended on the level of enzyme added to the curd; for example, experimental cheese to which 0.001 and 0.01% enzyme had been added, had characteristics at 2 months similar to those of the control cheese at 4 and 8 months respectively. In enzyme-treated cheese there was a greater liberation of the more soluble nitrogenous compounds, and gel electrophoresis showed a high reduction in β- and α_{s1}-caseins compared with the control. All the enzyme-treated cheese had defects in "body and texture" characteristics, and had mottled, weak body and bitter flavour. The extent of the defect was associated with the enzyme level. The changes brought about by the addition of the enzyme did not increase to any large extent after four to six months ripening. The effect of the higher ripening temperature was enhanced enzyme activity.

Follow up near-commercial scale trials the neutral proteinase was supplied by the manufacturer in the form of a coating on salt to enhance homogeneity of mixing with the curd.

The addition of Neutrase, 0.002, 0.003 and 0.005% (w/w) to the cheese curd increased the proteolytic activity, i.e. greater liberation of more soluble nitrogen and more extensive casein hydrolysis compared with the control made from untreated curd. The flavour intensity of cheese made from enzyme-treated curd was greater than from untreated curd but the following quality problems were observed:

- bitter and unacceptable flavour(s);
- open and crumbly texture;

- brittle and softer body cheese;
- discoloured or mottled.

The extent of these defects was related to the amount of enzyme added.

In order to overcome these defects in the experimental cheese, the enzymatic activity had to be stopped when the desirable flavour intensity in the cheese had been achieved.

Preliminary experiments have indicated that the use of Neutrase-treated curd which results in more rapid ripening and development of cheese flavour may have advantages in the production of processed cheese.

ABBREVIATIONS

DF	=	Degrees of Freedom
FDM	=	Fat in Dry Matter
MFFC	=	Moisture in Fat-Free-Cheese
MS	=	Mean of Sum of Squares
REP	=	Replicates
SED	=	Standard Error of Differences of Mean
SM	=	Salt in Moisture
SN	=	Soluble Nitrogen
TN	=	Total Nitrogen
VR	=	Variance Ratio
Leu	=	Leucine
Glu	=	Glutamic acid
Lys	=	Lysine
Val	=	Valine
Phe	=	Phenylalanine
Ser	=	Serine
Pro	=	Proline
Ala	=	Alanine
Ther	=	Thereonine
Gly	=	Glycine
Meth	=	Methionine
Asp	=	Aspartic acid
Iso	=	Isoleucine
Hist	=	Histidine
Arg	=	Arginine
Tyr	=	Tyrosine

CHAPTER ONE

STUDIES ON THE ACCELERATED RIPENING OF CHEDDAR CHEESE

1.1 INTRODUCTION

The origin of food production and preservation dates back some ten to fifteen thousand years ago (Jay, 1978; Pederson, 1979). Prior to that time the prehistoric man consumed food raw and the era is designated as a 'Food Gathering Period'.

Due to the natural composition of the majority of foods and the presence of enzymes and micro-organisms, these materials are highly perishable and subject to degradation. As man developed and learned how to domesticate animals, and to cultivate the land and to preserve food from times of plenty to periods of scarcity - an era known as a 'Food Production Period'.

The oldest methods of preserving food known to mankind are:

concentration, drying, fermentation and salting.

For example, cheese is a fermented dairy product which is partially concentrated and salted. Its origin could well be dated to the domestication of the cow, sheep, goat etc., or to the period of transition between 'Food Gathering' to 'Food Production'. However, Davis (1965) reported that both the Egyptian civilisation of 4000 BC and the Babylonian civilisation of 2000 BC were well advanced in husbandry methods and in the production of fermented food products including cheese.

The exact origin or method of manufacture of cheese is difficult to establish. However, it is safe to assume that in sub-tropical climatic conditions e.g. the Middle East, milk sours very rapidly in a few hours after milking due to the high ambient temperature and the presence of micro-organisms in the milk. These bacteria may have originated from the animal, the hands of the milker, the surfaces of utensils used to hold the milk or the environment. These organisms can produce two different types of fermentation. Firstly, the non-lactic fermentation, is brought about by micro-organisms other than lactic acid bacteria and

the product is normally stale, insipid or of bad taste when consumed. Secondly, a fermentation produced by the so-called lactic acid bacteria gives a more desirable product which is pleasant to eat.

Traditionally the containers used for carrying or storing milk were made from animal skins or stomachs. As the milk is left undisturbed, clotting of the milk may occur due to developed acidity as a result of bacterial activity and possibly due to the presence of clotting enzymes which originated from the stomachs. A soft coagulum is formed and some of the liquid phase of milk (whey) is absorbed into the skin or seeps through and is lost by evaporation. The coagulum is concentrated further by hand squeezing and sun drying. This dairy product was found to have better keeping quality compared to the original milk due to higher concentration of lactic acid, i.e. limiting or preventing the growth of bacteria producing severe taints etc. However, longer shelf life was achieved by preserving the concentrated curd in a salt solution (brine) which also improved its palatability. This fermented dairy product was later known as 'pickled cheese' which is still manufactured in parts of the Middle East.

As pickled cheese became popular in the Eastern Mediterranean region, its popularity spread to western countries, e.g. in some parts of Europe via tradesmen from the east. It is possible to suggest that as 'pickled cheese' became an acceptable dairy product in Europe, efforts were made in order to learn how to manufacture such a cheese on site or locally. With the establishment of dairies in Europe, i.e. in a comparatively colder climate than the Middle East, the preservation of cheese in brine was replaced by partial brining e.g. Dutch cheese variety or by using dry salting because the curd produced was much drier. It could be argued that because local manipulations in cheesemaking methods had taken place, the soft cheese has evolved into a new kind of cheese, i.e. the semi-hard cheese varieties.

Furthermore, the production of even drier curd cheese resulted in the production of different types e.g. hard pressed varieties which can be stored for a long period of time at ambient temperature. A typical example of such products is Cheddar cheese. It is estimated at the present that there are around 400 different types of cheese, and according to Davis (1965) they are broadly classified as:

a) Hard-pressed cheese

b) Semi-hard cheese

c) Soft cheese

d) Fresh cheese

Davis (1965) reported that Cheddar cheese was produced in the Somerset area, and visitors to the caves in that region used to buy such a cheese, which as a result became very popular in the United Kingdom. However, the best Cheddar cheese was produced in a town called Cheddar, and hence, it is possible that the name Cheddar was chosen for such a variety of cheese because of marketing aspects.

As Cheddar cheese became very popular in the U.K., this type of cheese also became popular in other parts of the world where there was a British dominance e.g. Australia, New Zealand, North America and Ireland.

At present Cheddar cheese is known by the country of origin, i.e. Scottish Cheddar, English Cheddar, New Zealand Cheddar, Australian Cheddar, Canadian Cheddar, Irish Cheddar, American Cheddar etc. The percentage of Cheddar cheese produced in the above mentioned countries as compared with the annual and total varieties of cheese, ranges from around 50% in Canada to 100% in New Zealand (see Table 1.1).

Cheddar cheese requires between 6-9 months curing time under controlled conditions, i.e. temperature at around 10°C. It is only with bandaged cheese that humidity is of importance. The longer maturation period the greater is the cost of production, and as energy becomes dearer every day, so does the price of cheese. Attempts have been made to shorten the maturation time thus reducing the cost of cheese manufacture. The process is referred to as 'Accelerated Ripening of Cheddar Cheese' (ARCC). However, it is fundamental that cheese produced by such methods must retain the same desirable characteristics as the traditional conventionally produced cheese; such as firm body, close texture, flavour profile etc. It is these aspects of the cheese characteristics that form the subject of the present study, and the factors that affect these aspects are reviewed below.

TABLE 1.1

Cheddar and total cheese production in some countries

Country	Year 1970			1975			1978		
	A	B	C	A	B	C	A	B	C
U.K.	130	97.7	75	240	163	67.92	207.4	149.4	72.03
Republic of Ireland	29			57	58	101.75	51	47.8	93.73
Canada	117.48	75.165	63.98	134	79.7	59.48	166.6	80.7	48.44
New Zealand	99.878	100.186	100.31	88.6	88.609	100.01	78.3	80.727	103.1
Australia	77.219			98.789	79.618	80.59	118.19	104.118	88.09

A = Total cheese production
B = Cheddar cheese production
} ,000 tonnes

C = Cheddar cheese production as a percentage of the total cheese production

EEC (1971 & 1976)

Anon (1980 a, b & c)

1.2 LITERATURE REVIEW

1.2.1 Factors Associated with Flavour Development in Cheddar Cheese

There are many factors which play an essential role in the development of flavour in cheese during the maturation period. Some of these factors are:

the changes and/or breakdown in the constituents of milk (fat, lactose, protein and minerals),

the additives used during cheese manufacture (starter culture, rennet and salt), and finally

the enzymes present in milk (natural or contaminant).

The various stages of Cheddar cheese manufacture are illustrated in Figures 1.1. The process is primarily dependent on the biological activity of the starter bacteria. For example, the starter utilise the milk sugar, lactose, as their energy source, and as a result lactic acid is produced (see Figures 1.2). Also the starter organism partially hydrolyse the proteins for their nitrogen requirement during multiplication and the overall protein breakdown is illustrated in Figure 1.2. Bacterial metabolism can also lead to the breakdown of the fat to yield various fatty acids which can also contribute towards the flavour in Cheddar cheese. Nevertheless, after the bacterial cell ceases to function, their enzymes remain active and continue to breakdown the various milk constituent.

Rennet which is used for the coagulation of milk, is a proteolytic enzyme. Hence during the ripening stage, these enzymes remain active and breakdown the proteins into smaller fractions (peptides and amino acids). In general the degree of protein hydrolysis affects the flavour and body (firmness) characteristics of Cheddar cheese. The reactions taking place during the maturation period of cheese is highly complexed and an attempt to review it in detail is made below.

1.2.1.1 Carbonyl compounds

Carbonyl compounds are volatile in nature and they can be classified as neutral and acidic compounds. The former carbonyl compounds e.g. (c=o group), aldehydes (-CH group), α- and β-unsaturated and aromatic aldehydes

FIGURE 1.1

Outline of the Cheddar cheese manufacture

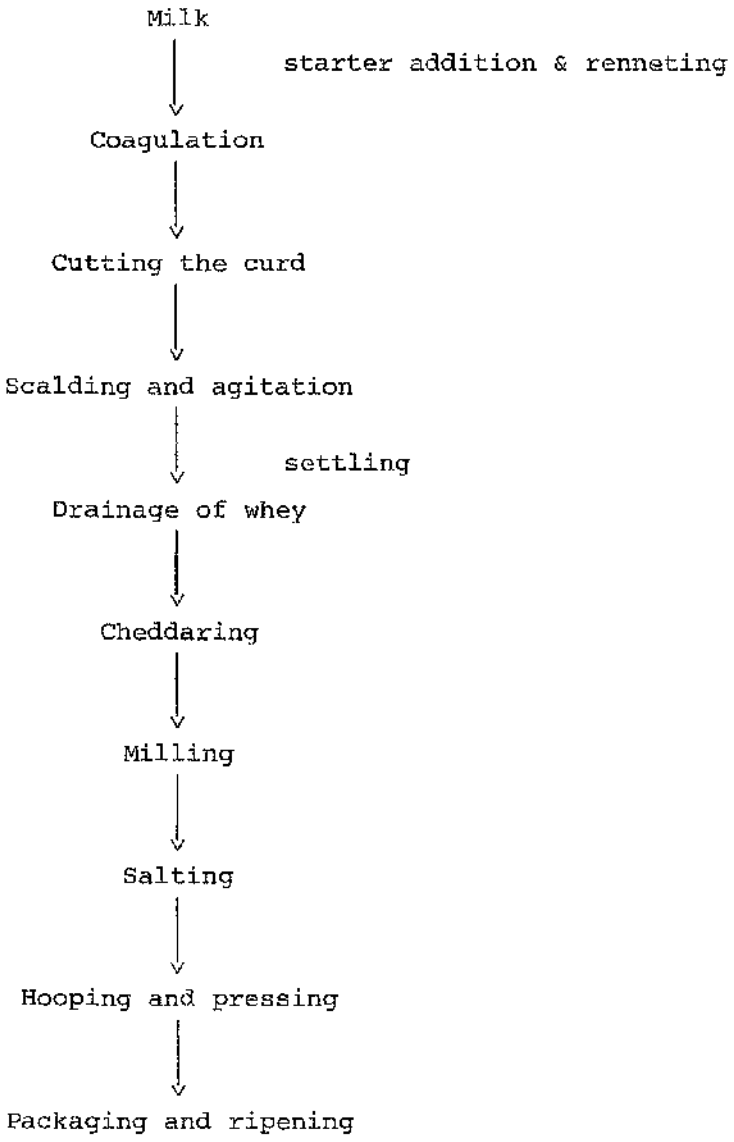
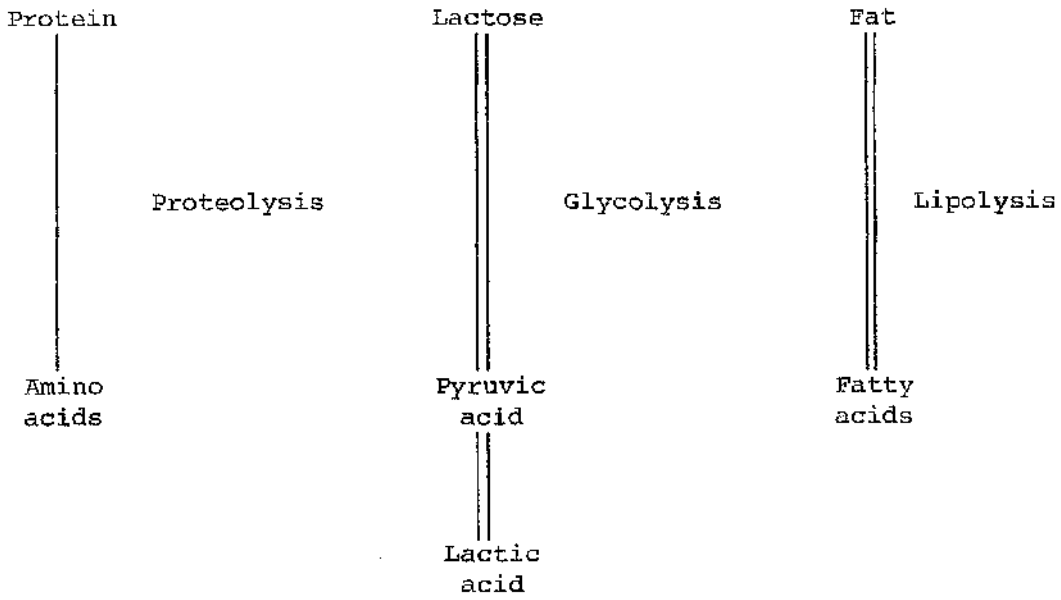


FIGURE 1.2

Simplified metabolic breakdown of milk components during the manufacture and ripening period of cheese



After: Harper & Kristoffersen (1956)

(C=C-CHO group), methyl ketones (CH_3-CO group), etc. ..... contribute to the "aromatic" flavour and aroma of Cheddar cheese.

The neutral carbonyl compounds are considered to be degradation products of the acidic components. For each neutral carbonyl compound produced in cheese is derived from an acidic precursor (Bassett & Harper 1956 and 1958) and the same workers associated the source of keto acid carbonyl compounds in Cheddar cheese from carbohydrates and citrate metabolism of the starter bacteria (see Figure 1.3).

While Wolin & Kosikowski (1959) and Wolin (1961) observed that some of the carbonyl compounds in matured cheese were derived from casein, Harvey & Walker (1960), suggested that a part from butan-2-one, and all the methyl ketones are produced during the ripening period by some type of decarboxylation reaction from the even numbered aliphatic fatty acids present in the milk fat.

The level of carbonyl compounds in Cheddar cheese can vary, and hence the flavour intensity of the cheese. Csiszar & Bakos (1941) concluded that the flavour of various types of cheese is related to the diacetyl content which ranged from 0 to 1 mg/100 g cheese.

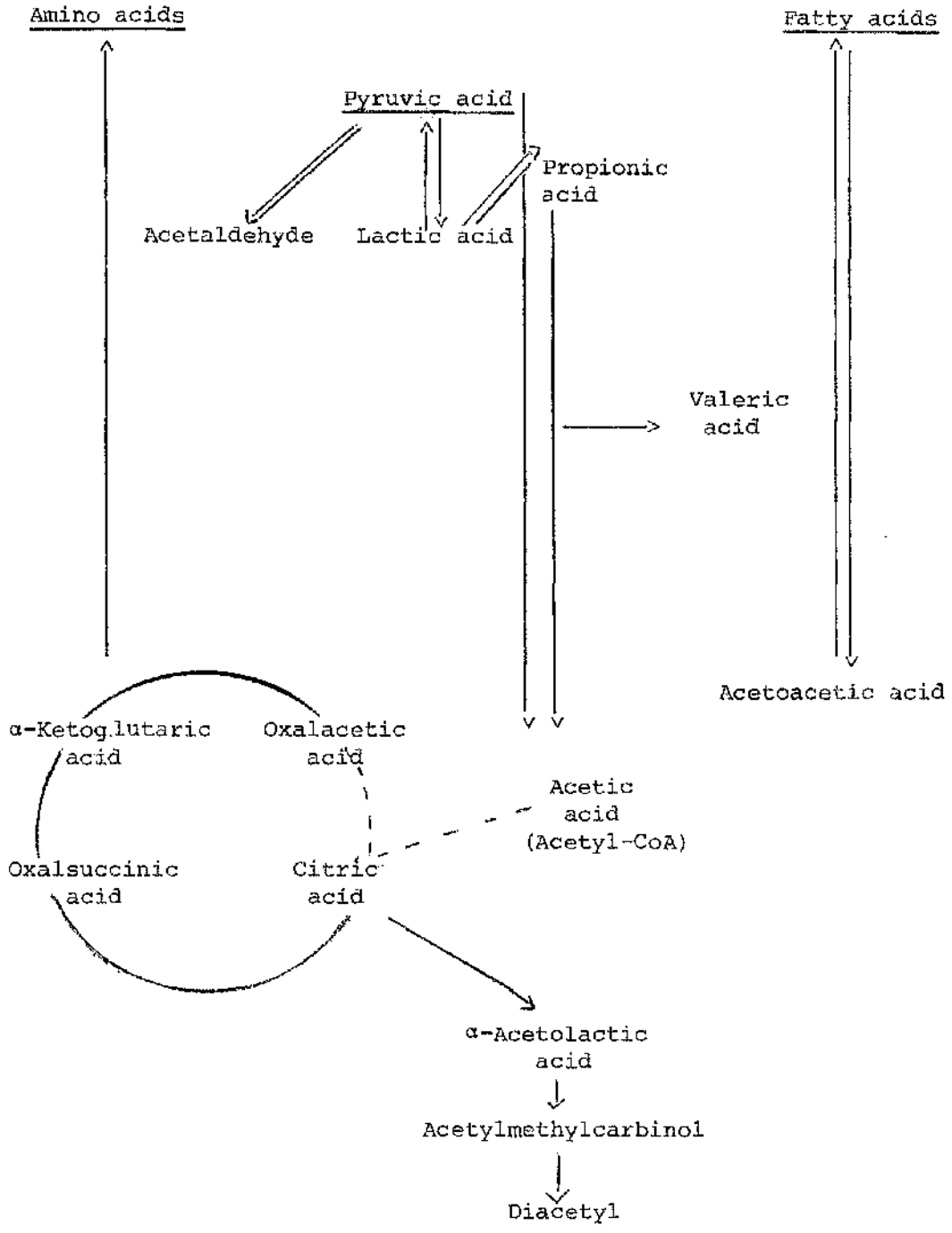
Calbert & Price (1949) examined the diacetyl content of 28 samples of Cheddar cheese, and recommended that an 'Excellent Flavour' cheese had diacetyl content of less than 0.05 mg/100 g cheese (Bassett & Harper, 1958). However, diacetyl content in cheese more than 0.05 mg/100 g cheese caused an off-flavour.

Vedamuthu, Sandine & Elliker (1966) detected diacetyl level in Cheddar cheese of between 1.27-7.4 ppm, and they concluded that high concentrations of diacetyl caused an off-flavour i.e. fruity. The high concentration of diacetyl in cheese was only observed when a Streptococcus lactis var. diacetylactis strain was included in a mixed strain starter culture. Keen & Walker (1974) found that diacetyl content in good flavoured Cheddar cheese after 5 months up to 1 year old was in small amounts (0.5-1.5 ppm). However, Calbert & Price (1948) suggested that small amounts (less than 5 ppm) of diacetyl was necessary for a typical flavour.

Basset & Harper (1958) concluded that a typical flavour in Cheddar cheese contains the following phenylhydrazones:

FIGURE 1.3

Possible biochemical reactions which take place during the ripening period of cheese



After: Harper & Kristoffersen (1956)

α-ketoglutaric acid (2+), oxalecetic acid (trace), pyruvic and isomer (4+), α-acetolactic (2+), diacetyl (trace), acetylmethyl - carbinol (trace) and acetone (trace).

Day, Bassett & Keeny (1960) reported the following concentration of carbonyl compounds (mg/kg cheese) present in Cheddar cheese:

2-tridecanone (2.10), 2-undecanone (1.43), 2-nonanone (0.68), 2-heptanone (0.82), 2-pentanone (0.37), 3-methylbutanol (trace), butanone (12.50), acetone (8.50), ethanol (7.50), propanol (2.60), methanol (1.00), and 3-methylthiopropanol (0.10),

and they concluded that although carbonyl compounds were detectable they had no role or influence in a typical Cheddar cheese flavour. Acetaldehyde, acetone, butanone-2 and pentoanone-2 were present in one day old cheese, while, heptanone-2, nonanone-2 and undecanone-2 were present in cheese 2-36 weeks old, and a typical Cheddar flavour was apparent between 8-12 weeks old and a more pronounced as the cheese matures (Harvey & Walker, 1960). Scarpellino (1961) found that butanone has been cited as a component of desirable Cheddar flavour, even if it was very low concentrations (trace - 0.24%).

Methyl ketones can be detected in Cheddar cheese and it may or may not affect the overall flavour. Law, Castonon & Sharpe (1976) observed that an average level of methyl ketones may be 0.25 μg/10 g ketones. Such a level is increased by the activity of microflora of the curd, i.e. starter culture or can be decreased by reducing the per cent of fat in the milk.

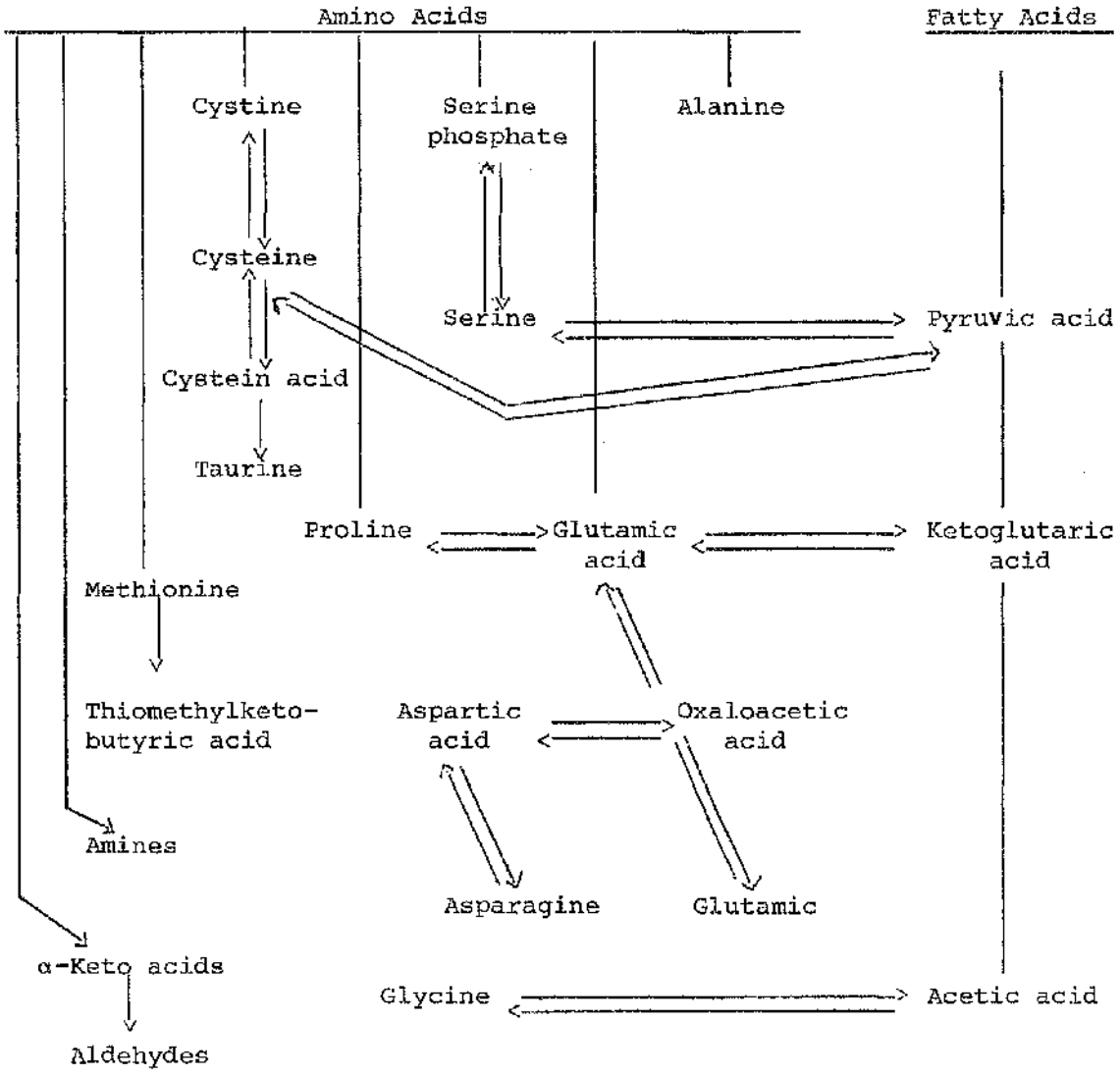
It is safe to conclude from the above information that the typical flavour of Cheddar cheese is associated with a complex mixture of carbonyl compounds, and care must be given to obtain the correct component balance.

1.2.1.2 Nitrogenous compounds

The breakdown of protein in milk (mainly casein) to smaller units i.e. the peptides, dipeptides or amino acids can also contribute towards the flavour of Cheddar cheese. The hydrolysis of protein is carried out by action of proteolytic enzymes which originate from starter culture and the rennet enzyme (Peterson, Johnson & Price, 1948). Figure 1.4 illustrates some possible metabolic pathways which lead towards the

FIGURE 1.4

Metabolic pathways and products of amino acid catabolism in cheese



After: Harper & Kristoffersen (1956)

proteolysis of milk proteins, and an example of amino acid content in Cheddar cheese is illustrated in Table 1.2.

A little accumulated data is available on this subject. In the early 1950s (see Table 1.2), lysine was detected at an early age and increased steadily during the ripening period (Bullock & Irvine 1956), Histidine was not present (Kosikowski (1951) and did not appear until after 170 days of maturation and stayed constant at a relatively low concentration (Bullock & Irvine 1956). Arginine is particularly important, because of its repulsive, unpleasant, bitter-sweet taste, and has been held responsible for abnormal flavour development in cheese (Schormüller, 1968).

Other free amino acids such as:

alanine, aspartic acid, phenylalanine, serine and therionine,

have been shown to increase throughout one year of ripening before levelling off (Bullock & Irvine 1956). However, Kosikowski (1951) reported that therionine increases by a small amount. The other amino acids which increased continuously in concentration throughout the maturation are:

glutamic acid, valine, methionine, isoleucine and leucine,

(Bullock & Irvine 1956; and Kosikowski 1951).

Keeny & Day (1957), reported that methional was an important compound in Cheddar cheese flavour and it is considered to be a derivative of methionine. Proline, which is the main flavour component in Swiss cheese (Harper & Kristofferson, 1956) was not detected in Cheddar cheese until after 180 days of ripening (Bullock & Irvine, 1956), and was not detected in ripened cheese at all (Kosikowski, 1951). Glycine has been found in all cheeses ripened more than 5 weeks (Bullock & Irvine, 1956), and it increases slowly during maturation and small amounts have been found after 180 days of ripening (Kosikowski, 1951).

It has been reported that the relative proportions of amino acids in Cheddar cheese serum during ripening are different from those in casein:

a) Of the total amino acids of Cheddar cheese serum lysine (12.00%) and arginine (9.05%) from the total amino acids, which were (8.50%) and (4.20%) in casein.

TABLE 1.2

Spectrum of amino acid content of Cheddar cheese made from raw and pasteurised milk

Amino acid	mg/g cheese	
	Raw milk	Pasteurised milk
Alanine	0.20-4.20	0.06-12.50
Arginine	none-2.70	trace-11.70
Asparagine	none-trace	none-6.60
Aspartic acid	0.08-5.90	trace-13.20
Glutamic acid	1.50-19.40	1.10-26.40
Glycine	none-0.36	none-20.90
Histidine	1.20-2.40	none-1.80
Leucine	1.90-10.30	1.00-21.70
Lysine	0.91-10.10	0.21-10.60
Methionine	0.20->6.60	trace-8.40
Phenylalanine	1.10-4.90	0.54-15.90
Serine	trace-2.80	none-5.50
Therionine	trace-5.30	trace-24.20
Valine	0.80-8.00	0.60-15.90
Tyrosine	0.20-5.10	0.34-8.40
Proline	none-1.06	none-trace

Adapted from Kosikowski (1951), Bullock & Irvine (1956) and Kristoffersen & Gould (1960)

b) Cheese serum contained smaller quantities of proline (4.40%) while it was (13.10%) of total amino acids in casein.

c) Presence of ornithine (3.30%) which is absent from casein, could be synthetically produced by micro-organisms.

The addition of a mixture of amino acids normally present in casein at the rate of 0.5 g/2.5 lb curd, showed that histidine produced a definitely inferior cheese having a pronounced unnatural flavour due to the amino acids itself (Baker & Nelson, 1948). The addition of glycine, methionine, tyrosine, serine, glutamic acid, arginine, aspartic acid and valine tended to increase the desirable flavour level in the test cheese.

Other nitrogenous compounds (amines and their related compounds) might play an important part in Cheddar cheese flavour. These compounds are::

γ-amino butyric acid, tryramine, cadaverine, urea, putrescine, histamine and tryptamine,

which are related to the activity of bacterial enzyme system. According to Silverman & Kosikowski (1956) the following reactions indicate their origin:

Glutamic acid	⟶	γ-amino butyric acid + CO_2
Tyrosine	⟶	Tyramine + CO_2
Lysine	⟶	Cadaverine + CO_2
Arginine	⟶	Urea + Ornithine + Putrescine + CO_2
Histidine	⟶	Histamine + CO_2
Tryptophane	⟶	Tryptamine + CO_2

According to Dahlberg & Kosikowski (1948), the amount of tyramine in American Cheddar cheese varied between 25 to 2330 γ/g and they concluded that the higher the tyramine content in Cheddar cheese the higher the flavour intensity in the cheese.

The tyramine content in Cheddar cheese is dependent on the type or strain of starter culture used, and the temperature during maturation of the cheese (Dahlberg & Kosikowski, 1949). For example

a) After 6 months ripening at 40, 50 and 60°F, the tyramine content of cheese made with commercial lactic starter was only 3, 12 and 17 γ/g. The cheese lacked good Cheddar characteristics.

b) The use of Streptococcus faecalis alone as a starter culture during cheese making produced the following content of tyramine in cheese after 6 months ripening at 40, 50 and 60°F was 18, 108 and 315 γ/g; the flavour intensity was characterised as mild, medium and medium plus, respectively.

c) The use of both commercial lactic and Strep. faecalis starters produced the greatest content of tyramine in cheese. Storing the cheese for the same duration and temperature conditions mentioned above the amount of tyramine was 85, 428 and 1172 γ/g, and the flavours of the cheese were mild, medium and sharp. The sharpest intensity was obtained after 4 months.

Silverman & Kosikwski (1955) found that the total tyrosine in cheese was between 69 γ/g at 4 days old to 3583 γ/g dry weight in cheese ripened for 6 months, and they also noted a steady increase as the cheese became old. The tyrosine content in cheese at 6 months old was found to decrease by 6% which was due to hydrolysis or to decarboxylation to free tyramine. The conversion of tyrosine into tyramine was rapid as tyrosine became available.

Silverman & Kosikowski (1956) suggested that although amino-butyric acid is not amine, it was found that this compound formed like the other amines by decarboxylation reaction, and its amount varied widely between the cheese made from raw milk (0.0-14.0 γ/5 ml cheese filtrate) and from pasteurised milk (0.0-3.0 γ/5 ml cheese filtrate). Its absence was referred to the typical flavour of Cheddar cheese. In the same study a significant difference was found in the amount of cadaverine & putrescine in cheese made from raw milk (1.0-3.0 γ/5 ml cheese filtrate) and pasteurised milk (0.2-3.6 γ/5 ml cheese filtrate) but they could not determine a relationship between the concentration of these compounds and unclean flavour.

1.2.1.3 Sulphur containing compounds

Sulphural compounds such as hydrogen sulphide (H_2S), methanethiol (CH_3SH), methyl mercaptan and dimethyl sulphide originate from some sulphur - containing amino acids, e.g. cystine and methionine. These compounds play an important part in Cheddar cheese flavour, because of its distinct flavour enhancement and its proven importance to the flavour

in milk and other food products (Walker, 1959).

Barnicoat (1950) found that the concentration of sulphydryl compounds in New Zealand Cheddar cheese greater than 35 µg/g caused discolouration and off-flavour in cheese.

According to Kristoffersen & Nelson (1955), the amounts of hydrogen sulphide in Cheddar cheese increased as the cheese ripening (10 months old) up to a level of 19 µg/g. They reported that few strains of Lactobacillus casei were present in the cheese which were able to form hydrogen sulphide.

Walker (1959) suggested that the level of the individual sulphur compounds was not important when evaluating the flavour of New Zealand Cheddar cheese, but the combined total of all these compounds had to be in the right proportion in order to give a characteristic flavour.

Kristoffersen & Gould (1960) reported higher levels of hydrogen sulphide (H_2S) in raw milk cheese (2.3-3.7 mM/100 g when at ½ to 1½ months period and 2.3 mM/100 g when 12 months old) than in pasteurised milk cheese (1.2 and 1.6 mM/100 g after corresponding period of ripening. Lawrence (1963) found the concentration of H_2S in Cheddar cheese was 52 µg/100 g of cheese, and reached a maximum at 60 µg/100 g in cheese after 1-4 months old. The level of H_2S remained constant while the flavour intensity of the cheese increased which indicates that the contribution of H_2S to flavour does not give a direct flavour effect, but might be that the H_2S combines with other ripening compounds responsible for the typical flavours. Law & Sharpe (1978) concluded that the formation of CH_3SH in Cheddar cheese might be brought about by non-enzymic reaction involving low molecular weight compounds produced by the lactic starter culture.

Manning & Robinson (1973), Manning (1974) and Manning, Chapman & Hosking (1976) observed the following effect of sulphur compounds on the flavour of Cheddar cheese: firstly hydrogen sulphide was slightly significant, secondly methanethiol was significant up to 19 µg/5 ml (head space analysis), and above this level flavour defects are pronounced; and thirdly dimethyl sulphide was insignificant in its effect. These findings were confirmed by Manning & Price (1977); Manning (19 1979a, 1979b); Manning & Moore (1979) and Manning et al. (1983) who reported th hydrogen sulphide affected slightly the aroma of Cheddar cheese, while the thic

compounds, such as CH_3SH, significantly contributed towards the typical aroma of Cheddar cheese.

1.2.1.4 Fatty acids

The chemical changes involved in the ripening of Cheddar cheese are primarily a decomposition of the fats with the formation of free fatty acids (FFA) by microbial enzymes contained in cheese. The enzymes responsible for the degredation of fat are the lipases. These enzymes are active in Cheddar cheese after 30 to 50 days then their activity tends to decrease (Kannan & Basu, 1951). The possible pathways of fat hydrolysis and its resultant products in cheese are illustrated in Figure 1.5.

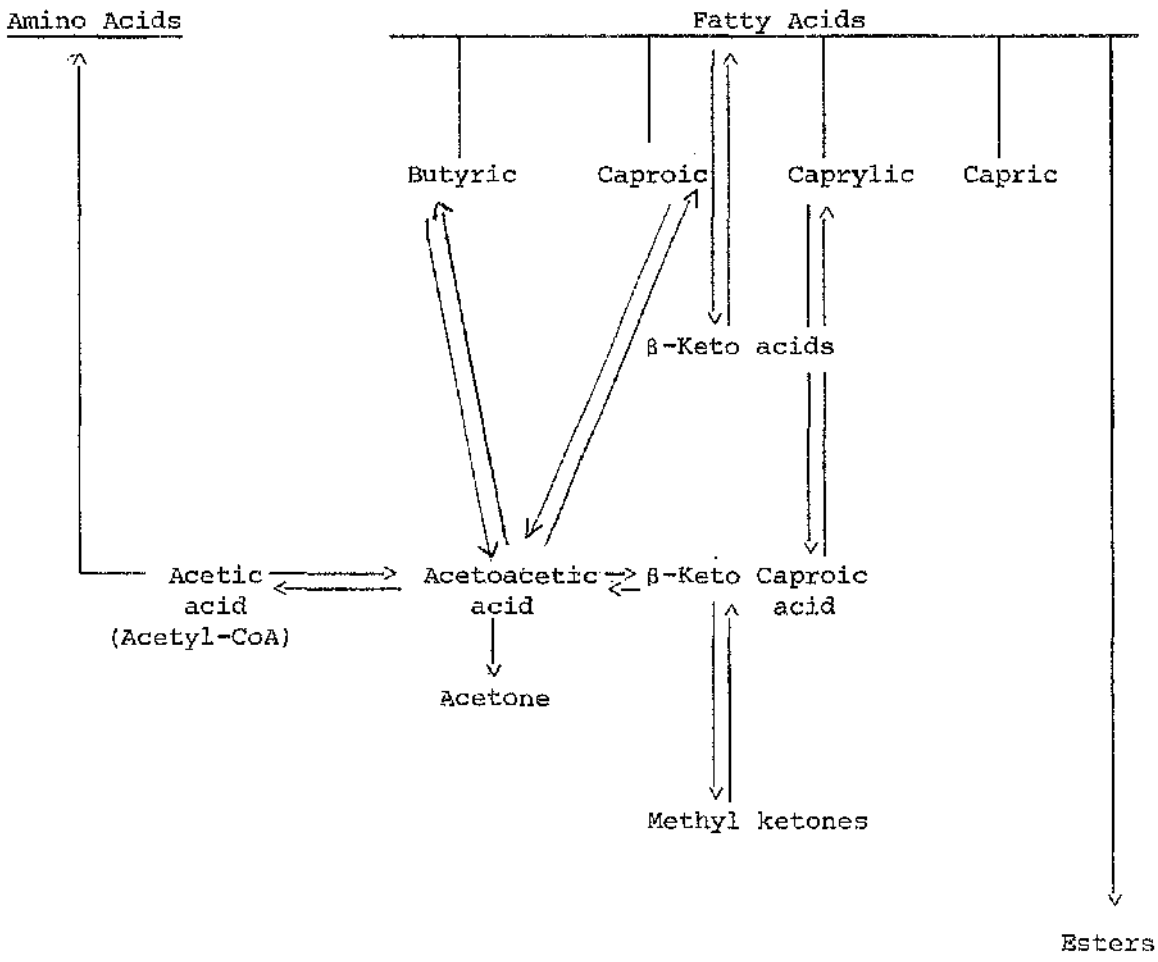
Peterson, Johnson & Price (1949) studied the level of free fatty acids during ripening of Cheddar cheese and found that intracellular bacterial lipases are responsible for the liberation of free n-butyric and acetic acids (low concentration in 420 days old cheese), but higher levels were present in cheese made from raw milk. Sheuring & Tuckey (1947) noticed that the hydrolysis of fat decreased during the maturation period of cheese made from pasteurised milk and they reported that the volatile acidity of the cheese reached a maximum when the cheese was 90 days old, and thereafter it gradually decreased. Ohren & Tuckey (1969) suggested that a typical Cheddar cheese flavour was a balance between even number carbon free fatty acids i.e. C_{10}, C_{12}, C_{14} and such a balance was dependent on the fat percentage and the microbiological quality of the milk. However, Reiter *et al*. (1969) manufactured cheese under aseptic conditions from aseptically drawn milk from the cow's udder and the FFA content in cheese was dependent on the following aspects:

1) Lipolytic activity of the starter culture;
2) Initial FFA in milk;
3) Chemical composition of the milk and in particular the type of fat (effect of winter feed *v/s* grazing);
4) Lipases naturally present in milk.

Dixon, deMan & Wood (1969) described how fatty acids are produced during the ripening of cheese, both by the hydrolysis of milk fat and during fermentation. The rate of flavour production in cheese from pasteurised milk was lower than from raw milk, and in some cases rancid flavour developed. They found out that the rancid samples had high butyric and

FIGURE 1.5

Metabolic pathways and products of fatty acids catabolism in cheese



After: Harper & Kristoffersen (1956)

caproic acids content and a low acetic acid content. Jaunzems (1976) concluded from his studies on fatty acids composition of Cheddar and Dutch type cheeses, that the contents of low molecular weight volatile fatty acids (C_6-C_{10}) were 6.6% for the former type of cheese and 5.98% in Dutch cheese. The polyunsaturated fatty acids ranged from 0.7 to 4.0%.

1.2.2 Methods used for Accelerated Ripening Cheddar Cheese (ARCC)

Many different approaches have been used to ACCR, and these can be divided into two major categories: firstly, the methods which are directly dependent on the cheese starter culture activity and their enzymes through:

a) an increase in the microbial population in the cheese;

b) the use of mutant strains; and

c) the use of Cheddar curd slurry.

Secondly, the methods which are dependent on the addition of enzymes (in the presence of milk coagulant) which do not originate from the lactic starter cultures. Examples of the latter methods are:

a) the use of proteinases enzyme(s);

b) the use of peptidases enzyme(s); and

c) the use of lipases enzyme(s).

1.2.2.1 Methods related to starter cultures

A. Increase of microbial population

The starter culture bacteria play an important role during the manufacture and maturation of Cheddar cheese. Their primary function is the production of lactic acid which is essential in the following aspects:

(i) to control the harmful micro-organisms in milk intended for Cheddar cheese production;

(ii) the rate of acid production must be carefully regulated during successive stages of cheesemaking.

During the ripening period of the milk the production of lactic acid helps to release the bound calcium ions (Ca^{++}) and they become readily available for the rennet which result in successful coagulation of the

milk (Ernstrom, 1965). Foster, et al. (1957) described further acid productio beyond that formed up to the time of coagulation stage is necessary for the expulsion of moisture (or whey) from the curd (during maximum scald and the stirring period) and at later stages during the matting and cheddaring which helps to control the body and texture characteristics of the cheese. However, the presence of acid also imparts some flavour characteristics of Cheddar cheese, usually described as the pleasant acid taste and sharpness (Foster et al., 1957).

The second important function of the starter is to develop some of the flavour characteristics of Cheddar cheese (Vedamuthu, Sandine & Elliker, 1966) Hence, the role of the starter culture is manifested in two ways, by direct or by indirect contribution to the flavour. Marth (1963), and Reiter et al., (1966) suggested that the direct contribution is due to the following:

(i) compounds (such as lactic, acetic and propionic acids) formed by the metabolism of lactose;

(ii) carbonyl compounds (acetaldehyde, acetone and diacetyl) or their precursors such as pyruvic acid; and

(iii) certain miscellaneous compounds such as, alcohol, fatty acid and their esters.

The indirect contribution of the starter culture towards the production of flavour could be associated with the following concepts.

(i) the breakdown of protein which imparts a distinctive mellowness of the flavour and helps to solubilise part of the protein (Marth, 1963; Reiter et al., 1966);

(ii) the starter helps to control the level of undesirable organisms in the cheese (Vedamuthu & Reinbold, 1967);

(iii) the proteolytic activity of the starter culture.

The latter aspect is of great importance. Desmazeaud et al.(1976) reported proteinases and peptidases activity in lactic streptococci while Law, Sharpe & Reiter (1974) and Exterkat (1975) reported dipeptidases activity in strep. cremoris. Since lactic streptococci do not possess high proteolytic activity, Law (1978) suggested that by increasing the population of starter bacteria in cheese the formation of protein breakdown products would be accelerated and these would affect the rate of flavour formation.

(B) Lysozyme treated starter cells

In this approach ordinary cheese starter culture was treated with lysozyme in the absence of salt, and such a culture is known as Lysozyme Treated Starter Cells (LTSC). According to Law, Castanon & Sharpe (1976b) ordinary cheese starter was supplemented with LTSC during the manufacture of Cheddar cheese and the viable count increased by approximately 2 to 8 times and the added LTSC did not produce any acid during cheese making. The LTSC were lysed when added to salted curd, and lysis was detected by release of cell-free deoxyribonucleic acid (DNA) and the latter activity was detected by the use of an intracellular enzyme marker. Concentration of the free amino acids in matured experimental cheeses was increased by three times compared with the control. Cheddar flavour intensity did not increase in cheese produced from starter with added LTSC, and there was no development of Cheddar flavour in cheese produced by direct acidification with added LTSC. Law, Castanon & Sharpe (1976b concluded that intracellular starter enzymes did not play a direct part in the formation of Cheddar flavour, but could be a produce precursor or source of Cheddar flavour compounds. Law (1978) mentioned that successful acceleration of typical Cheddar cheese flavour was not dependent on the starter culture and/or their enzyme(s) concentration, but on the formation of some other compounds. Manning, Chapman & Hosking (197 Manning & Price (1977),Manning (1978,1979a,1979b), Manning & Moore (1979) and Manning (1983), suggested that the flavour intensity of the cheese is related to the concentration of methanthiol (CH_3SH), which is essential to Cheddar cheese flavour or aroma.

However, Law & Sharpe (1978) found out that methanthiol is not produced by the starter culture or any other members of the cheese microflora. The exact mechanism of the formation of methanthiol is not well established. It appears that its formation is the result of chemical reaction involving amino acids containing sulphur compounds.

(C) The use of mutant strains

By means of chemicals and x- and α-rays, mutants of lactobacilli spp. with increased proteolytic activity have been developed in India (Singh & Ranganathan, 1977a, 1977b, 1978 and 1979), and in Russia (Dilanyan, Makaryan & Sarkisyan, 1970) The latter investigators used a starter culture of lactic streptococci and some x-ray mutants to produce Armenian cheese, and found that the content of free amino acids

in cheese was higher than in cheese made with normal starter. The same approach was used by Dulley, Brooks & Grieve (1978) to produce mutants of Strep. lactis (strain C_2) and Strep. cremoris (strain E_8) for use as cheese starter. During cheesemaking the increase in strain C_2 was much slower than strain E_8 and their addition resulted in accelerated cheese ripening and less bitterness development compared with the control cheese.

(D) Heat-shocked starter cells (HSSC)

An alternative method, which could be used to accelerate cheese ripening, is to subject the starter culture to a thermal shock heat treatment. In principle, the objective of such an approach is to manipulate the enzymatic activity of the starter culture by destroying the enzyme activity involved in lactose hydrolysis while the other enzymes, e.g. for fat and protein hydrolysis were partially affected for most of the part, unaltered. Consequently, cheese could be manufactured by using an ordinary starter culture fortified with HSSC without affecting the rate of acid development during production, but increasing the number of viable cells in the cheese.

Petterson & Sjostrom (1975) heated streptococci and lactobacilli for 15 s at 59 and 60°C to reduce their ability of producing lactic acid. This treatment lowered their capacity for proteolysis by 10-30% measured in skim milk. They used these bacteria as starter culture in Swedish semi-hard cheese production. The numbers of starter bacteria in the experimental cheese was increased by 4-5 times more than in the control cheese. The trichloroaetic acid (TCA) and phosphotungstic acid (PTA) soluble nitrogen contents in cheese containing heat-shocked cells were increased up to 60% more than the control cheeses without affecting the pH, fat and moisture content of one day old cheese despite the high number of starter culture. Organoleptically, the experimental cheese showed greater score (4-6) compared with control cheese (3.2). Somkuti, Thompson & Flanagan (1979) subjected Strep. cremoris (American type culture collection - ATCC 14356) to heat shocking at 60 and 64°C for 15-16 s which reduced the protease activity of the starter by 15 and 30% respectively as measured in casein hydrolysis. Using a mixture of heat-shocked cells and direct-to-vat as starter culture to produce American Cheddar cheese of reduced fat (20-22%) in increasing the proteolytic and lipolytic activity in the experimental cheese compared with the control

(produced by using direct-to-vat only) throughout four months of ripening.

When the cheese was four months old the 5% TCA soluble materials was 2.85% in the experimental and 2.64% in the control cheese while free fatty acids were 1,521 µg/g in the experimental cheese and 968 µg/g in control cheese. Such increases in the soluble nitrogen and free fatty acids contributed to accelerate ripening of Cheddar cheese.

(E) Addition of aged lactic cultures to the cheese milk

The addition of aged cells of starter culture (i.e. inactive and/or dead cells) to cheese milk is yet another approach which has been used to accelerate ripening of cheese. Little information is available concerning this method, but according to Shchedusknov & D'yachenko (1974) inactivated starter culture containing dead cells of lactobacilli was added to a mesophilic lactic starter culture for the production of Cheddar cheese. The cheese curd was claimed to be enriched with increased proteolytic enzymes which accelerated the cheese ripening. The quality of the cheese was acceptable but slightly "spicy" in taste.

(F) Lactose hydrolysis

One of the methods, which has been used to accelerate the ripening process in Cheddar cheese, is the addition of a β-D-galactosidase preparation to the milk prior to the cheesemaking process. This enzyme hydrolyses the milk sugar, lactose, to yield glucose and galactose, and according to Gilliland, Speck & Woodard (1972) lactose hydrolysed milk stimulated the growth of lactic streptococci, possibly because of the presence of a readily available source of energy, i.e. glucose, in the growth medium. Such reported observations have encouraged researchers in different laboratories to study the quality of Cheddar cheese manufactured from lactose-hydrolysed milk (LHM). The majority have reported acceleration in the ripening process of Cheddar cheese (Woodard & Kosikowski, 1975; Thompson & Brower, 1974 & 1976; Anon, 1977; Marschke & Dulley, 1978; Gooda et al., 1981). However, Mulholland, O'Brian & Phelan (1976) and Cardwell & Prombutara (1976) could not achieve the same effect (incidentally the commercial brand name identifying the enzyme preparation used by Mulholland, O'Brian & Phelan (1976) was not mentioned).

Weaver & Kroger (1978) reported accelerated ripening of Cheddar cheese

produced from lactose-hydrolysed milk and they suggested that the increased proteolytic activity (through protease in the cheese) was achieved as a result of the increased number of cells of the starter culture. However, Hemme, Vassal & Auclair (1978) suggested that the enhanced proteolytic activity in cheese made from milk treated with one commercial brand of lactase was due to the presence of small amounts of proteolytic enzymes in the β-D-galactosidase preparation. A similar finding was reported by Marschke et al. (1980), and they concluded the following points:

(i) pasteurisation of lactose-hydrolysed milk inactivated the commercial preparation of β-D-galactosidase, but the proteolytic enzyme(s) in the material survived the treatment;

(ii) two strains of cheese starter cultures (Strep. cremoris ML & E_8) were not stimulated, but with strains Strep. cremoris AM_2 & ED_6 their growth was inhibited;

(iii) slight acceleration in the ripening of Cheddar cheese was observed when the milk was treated with inactivated (by heating), β-D-galactosidase, and the acceleration was due to survival of the proteolytic enzyme(s).

1.2.2.2 Methods related to the treatment of the cheese curd

(A) Cheddar curd slurry

The use of a Cheddar curd slurry is a process where accelerated cheese ripening could be achieved. In this approach different methods were developed (see Table 1.3), but in principle the technique(s) is aimed primarily at increasing the following factors in the cheese curd: firstly, the number of lactic starter bacteria and their enzymes, and secondly, miscellaneous compounds which constitute a typical mature Cheddar cheese flavour.

(B) Addition of enzymes

The ripening process of Cheddar cheese is directly dependent on the degree of hydrolysis of the proteins, fats and the remaining lactose in the curd as a result of enzymatic activity which originates from the rennet or other coagulants, natural or microbial enzymes in milk that survived the heat treatment and enzymes from the lactic starter cultures.

TABLE 1.3

Summary of different methods used for the production of curd slurry

Preparation of slurries	Comments	References
1. Blend unpressed Cheddar curd (24 hours old) with 5.2% sterile solution of common salt (NaCl) in some instances the following compounds are added: 10-100 ppm of reduced glutathione (GSH), 100 ppm porcine lipase, and the slurry stored at 30°C for 9 days.	The development of Cheddar cheese flavour have been achieved in a few days rather than months in the liquid cheese, but off-flavours have been detected.	Kristoffersen, Mikolajcik & Gould (1967) and Harper & Kristoffersen (1970)
2. Prepare slurry as described by Krostoffersen, Mikolajcik & Gould (1967) but using a salt concentration between 1-4.7% and a storage temperature of 15, 22, 30 and 35°C; different additives have been used such as: GSH, sodium citrate, mengeneze, riboflavin or cobalt stored up to 7 days.	Storage temperature at 30 and 35°C and the other additives contributed to typical Cheddar cheese flavour development	Singh & Kristoffersen (1970)
3. Slurry prepared from curd which has been produced by direct acidification of milk; starter culture and the materials including diacetyl are added to the slurry which is then held at 30°C, stored up to 7 days.	Acceptable Cheddar cheese flavour has been produced in a 'short' time	Singh & Kristoffersen (1972)
4. A base slurry similar to the type reported by Kristoffersen, Mikolajcik & Gould (1967) is prepared. Different combinations of compounds are added. These additives are an aqueous solution of rennet, GSH, lipolytic enzymes. Oxygen is pumped into the head space.	Acceptable Cheddar cheese flavour was obtained and the author recommended this type of material for use in processed cheese.	Sutherland (1975)

cont'd.....

Table 1.3 cont'd.....

Preparation of slurries	Comments	References
5. Neutral protease, lipase and/or neutral protease-peptidase (microbial or animal derived) are added to the cheese slurry.	The addition of enzymes helped to produce cheese flavour very quickly, but bitterness was associated with microbial acid protease.	Kosikowski & Iwasaki (1975)
6. A slurry is prepared by mixing 2 parts of fresh salted curd with 1 part of 5% sterile solution of sodium chloride and/or 0.3% potassium sorbate. The slurry is added at 30°C for 7 days; and finally the slurry is added to cheddared curd before hooping.	Accelerated cheese ripening was achieved but moisture content of cheese increased by 2%; (the addition of a freeze-dried slurry minimized such problems). The addition of sorbate reduced detectable off-flavours in the cheese.	Dulley (1976)

However, the biochemical processes during the maturation of cheese are highly complexed, and flavour and aroma and body and texture of Cheddar cheese are directly influenced by the degree of enzymatic activity.

The addition of enzymes such as proteinases and/or lipases have been used to accelerate the ripening process in different cheeses. Means of accelerating the ripening of Cheddar cheese have been studied extensively since the early 1970s by scientists in different parts of the world. The addition of proteinase enzymes has been most widely studied and in this case the enzyme is mainly added to the curd after milling.

Nakanishi and Itoh (1973 and 1974) achieved a high degree of proteolysis in cheese after the addition of a protease enzyme (from Aspergillus spp.) to the preformed curd. Kosikowski & Iwasaki (1975) reported an acceleration in the formation of free volatile acids, soluble nitrogen and flavour production in Cheddar cheese by the addition of a mixture of commercial proteases and lipases at the rate of 2.5 g per 5.9 kg of curd. After one month storage at 20°C the enzyme-treated cheese had developed a higher flavour intensity compared to the control. While no bitterness was detected in the enzyme treated cheese, but it had a distinct rancid flavour. Maturation for a longer time at higher temperatures resulted in 'burnt' and more pronounced rancid flavour development and the cheese also became bitter. Such cheese was used for processing, and the mix consisted of using 2 months old 'natural' Cheddar cheese (at a rate of 60 to 90%) along with 1 month old enzyme treated cheese (at a rate of 10 to 40%). In another trial Jolly & Kosikowski (1978), added preparations of animal and microbial lipase, which were contaminated with protease and Penicillium roqueforti spores to the cheese curd. Proteolysis was inhibited at the earlier stages of ripening (as measured as soluble N and free amino acids) which could be due to excessive lipolysis. After two months ripening, the cheese, which contained microbial lipase, had higher levels of free amino acids compared with cheese made from animal lipase or the control. From such results the authors concluded that proteases were permanently inhibited in the animal lipase, but not in microbial lipases. In the microbial enzymes the protease activity was dependent on the pH which became more suitable to the enzyme activity as the ripening of the cheese progressed. Sood & Kosikowski (1979) added different combinations and

concentrations of the following enzymes:

fungal protease (31000 Miles), 0.005%
plus fungal lipase (MY Meito) 0.00005 to 0.0002%;

or fungal protease (p-53 Rohm & Haas), 0.0035%
plus fungal lipase (MY Meito), 0.00005 to 0.0002%,

to Cheddar cheese curd and stored it at 10°C for 3 months. The cheese had a good quality, more acceptable and had a medium sharp flavour as assessed by the authors. The contents of soluble nitrogen, and volatile fatty acids were higher compared with the control cheeses. The authors concluded that the addition of microbial proteases increased the degradation of casein, especially β-casein and α-S_1 and the production of more free amino acids, which accelerated cheese ripening. Law & Wigmore (1982 and 1983) and Law (1981) developed a simple and effective method to accelerate the formation of English Cheddar cheese flavour by using neutral proteinase from Bacillus subtilis. The enzyme was added to the curd during the salting stage, and during the maturation process the action of proteolysis was evident on the β-casein fraction followed by α-S_1 casein. The products of hydrolysis were an increase in trichloroacetic acid (TCA)-soluble nitrogen and sulphosalicylic acid (SSA)-soluble nitrogen. The flavour of 2 months old cheese was similar to an ordinary "natural" Cheddar cheese at 4 months old. The texture characteristics assessed by the panelists showed that enzyme-treated cheese was more greasy, crumbly, less elastic and weak in body as compared with the control cheeses. Some physical properties of the cheese (i.e. brittleness and hardness) were measured by using the Instron machine, and the enzyme-treated cheese was brittle and soft. Such characteristics (brittle and soft) were influenced by the rate of enzyme used. The addition of neutral proteinase at a rate of 0.001% (w/w), did not affect the physical properties of the enzyme treated cheese and the experimental cheese was almost similar compared with the control.

In another set of experiments Law & Wigmore (1982) and Law (1981) evaluated the use of acid proteinase extracted from fungi for the production of accelerated Cheddar cheese. The cheese at 2 months old showed extensive hydrolysis of casein fractions which affected adversely the texture characteristics of the cheese. Furthermore, the addition of this enzyme produced an extremely bitter flavour even at low level of concentration.

A different proteinase enzyme preparation (intracellular exopeptidase and extracellular endopeptidase from Pseudomonas fluorescens) was patented by Malkki et al. (1976) and Malkki & Nikkila (1977) for addition to milk for accelerated cheese ripening. Addition of these enzymes to milk released between 60 to 100% of the amino acid present in milk, produced low level of tryptamine and tyramine, and potentially-harmful histamine. The manufacture of Cheddar cheese from milk treated with these enzymes increased the soluble nitrogen, peptide nitrogen and amino acid contents compared to the control.

I.2.3 Compare and Evaluate the Different Methods of Accelerated Cheese Ripening

From the above literature review it can be observed that different methods have been proposed to accelerate the ripening process of Cheddar cheese. However, it must be emphasised at this stage that some of these treatments used resulted in cheese which was either bitter, or was affected by the development or presence of off-flavour(s) or noticeable defects in the body and texture characteristics of the cheese. Furthermore, some of the accelerated ripening techniques, e.g. the slurry system, are only suitable for the production of processed cheese.

There is, of course, a degree of overlap between the different methods used for ARCC and by far the most favoured methods are the use of β-D-galactosidase and/or the addition of proteinase enzymes. Some controversy surrounds the treatment of milk with lactase enzyme for accelerated ripened cheese. The investigations described below were undertaken to re-examine and re-evaluate some of the published techniques:

a) study the influence of β-D-galactosidase preparation(s) on the quality and rate of ripening of Cheddar cheese;

b) study the influence of proteinase enzyme for ARCC.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MILK ANALYSIS

2.1.1 Determination of Fat

Throughout the cheesemaking experiments fat in milk was determined according to B.S. 696 - Part 2 : 1969 (British Standards Institution, 1969).

2.1.2 Determination of Total Nitrogen

The determination of total nitrogen (expressed as percentage protein) was made by the micro-Kjeldhal method of the Association of Official Agricultural Chemists (AOAC) (1965). Instead of mercuric oxide, Kjeldhal copper catalyst tablets supplied by BDH Chemicals Ltd., U.K. were used as a catalyst. A standard solution of 0.02 N hydrochloric acid (HCl) was used as a receiver, and the excess acid was titrated with 0.02 N of sodium hydroxide (NaOH).

2.1.3 Determination of Carbohydrate

Glucose, galactose, lactose and oligosaccharides in milk were determined using gas liquid chromatography (GLC) according to the method of Sweeley et al. (1963) and based on the modification of Olling (1972) and Tamime (1977) (see Figure 2.1). The specification of the column used for the GLC analysis was the same as that recommended by Sweeley et al. (loc. cit.), and is as follows:

(i) length: 1.5 m of pyrex glass;

(ii) diameter (internal): 3.5 mm;

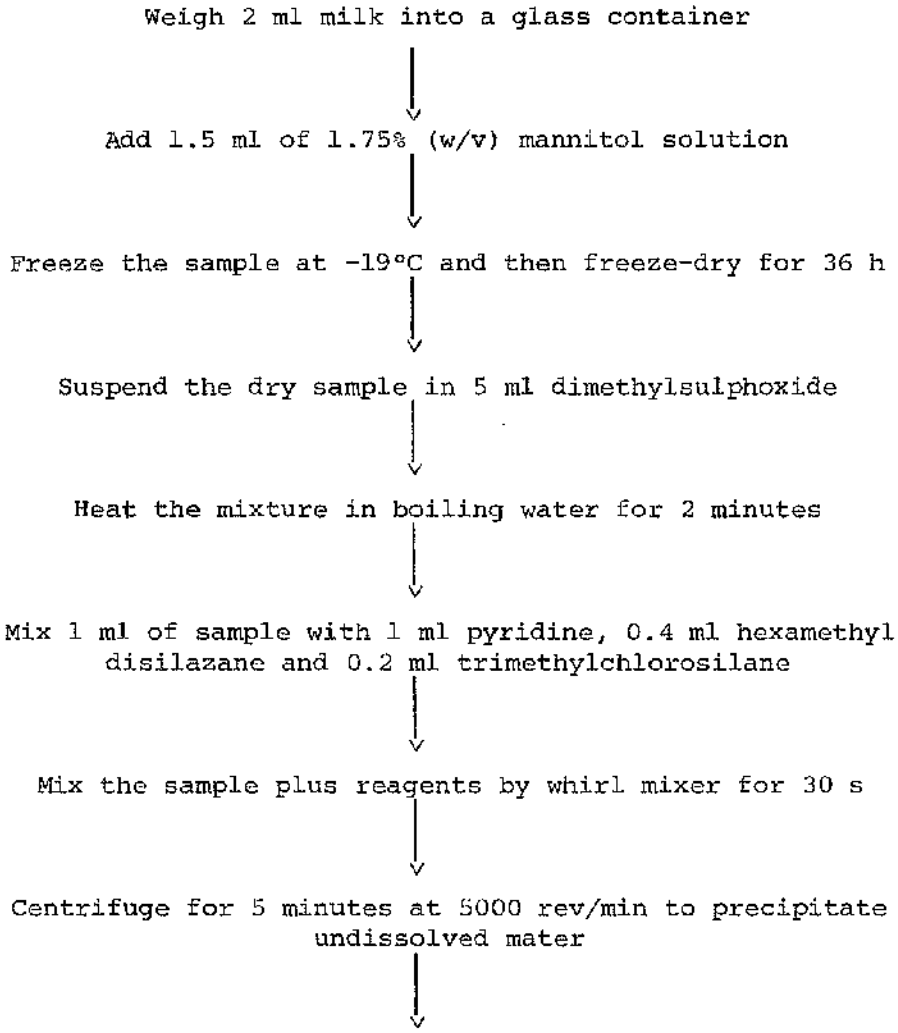
(iii) packed with 1% (w/w) SE 52.

The chromatograph used was the 104 series manufactured by PYE Unicam Ltd., U.K.

The 'silylated' samples were measured using a micro-syringe (10 µl - series CG-130 manufactured by Precision Sampling Corp., P.O. Box 15119, Baton Rouge, Louisiana 70815, U.S.A.).

FIGURE 2.1

Silylation procedure for the milk and whey samples



(all the reagents were supplied by BDH Chemicals Ltd., Poole, Dorset, U.K.)

a) nitrogen gas as a carrier, flow rate 22 ml/minute;

b) nitrogen and hydrogen, flow rate 50ml/minute;

c) iso-therm temperature 170°C for 15-16 minutes then increasing to 250°C at a rate of 16°C/minute.

2.1.4 Determination of Total Solids

The International Dairy Federation standard method 21 (IDF/FIL, 1962) was used to determine the total solids in milk by drying the sample at 102°C in a hot air oven supplied by Townson and Mercer Ltd., Croydon, U.K.

2.1.5 Determination of Solids not Fat

The method described in B.S. 734 - Part 2 : 1973 (British Standards Institution, 1973) was used to determine the milk density. The solids not fat in milk was calculated using a calculator supplied by Astell Laboratory Service Co. Ltd., London, U.K.

2.1.6 Determination of Titratable Acidity

Titratable acidity of milk was determined using 10 ml sample with 1 ml of 0.5% (w/v) solution of phenolphthalein as an indicator according to the method of B.S. - 1741 : 1963 (British Standards Institution, 1963). The milk plus indicator is titrated with N9 NaOH until the end point (faint pinkish colour) is reached. The volume of NaOH used is divided by 10 to express the acidity as per cent lactic acid.

2.1.7 Determination of Hydrogen Ion Concentration (pH)

A pH meter Model Pye 290 fitted with a combined glass electrode (Activion Glass Ltd., York Street, Cambridge, U.K.) was used to measure the hydrogen ion concentration in milk. The pH meter was adjusted with buffer solutions of pH4 and pH7 to standardize the equipment before use.

2.1.8 Determination of Acid Soluble Tyrosine

Acid soluble tyrosine in milk was determined using the colourimetric method to measure the partial protein hydrolysis which is based on the reaction between the free protein and Folin and Ciocalteu's phenol reagent to form a blue colour. The method of Hull (1947) was used and

5 ml of milk was pipetted into a dry test tube and mixed with 1 ml distilled water and 10 ml of 0.72 N trichloroacetic acid. The sample and reagents were agitated vigorously to mix the content thoroughly, and then allowed to stand for 10 min followed by filtration through Whatman No. 1 filter paper. A 10 ml solution of sodium carbonate-sodium tetraphosphate [75 g of anhydrous sodium carbonate is mixed with 10 g of sodium tetraphosphate (BDH Chemicals Ltd., Poole, Dorset U.K.) and made up to 500 ml in a volumetric flask with distilled water] was added to 5 ml filtrate in 50 ml conical flask followed by addition of 3 ml phenol reagent. The mixture is shaken continuously while adding the phenol reagent and 5 min is allowed before taking the reading at 650 λ using a Spectronic 20 (supplied by Bausch and Lomb Inc., Rochester, NY, U.S.A.). Acid soluble tyrosine was then calculated from a standard tyrosine curve prepared by adding stock solution of tyrosine which was prepared as follows:

> dissolve 100 mg of tyrosine (supplied by Sigma London Chemical Co. Ltd., Dorset, U.K.) in distilled water and make up to 500 ml in a volumetric flask; deliver different quantities of the stock solution into a series of test tubes and diluted with distilled water up to 6 ml followed by the addition of 10 ml of 0.72 N trichloroacetic acid.

Filtration of the standard solutions was not necessary, and the tyrosine content was measured colorimetrically at 650 λ in a Spectronic 20 and plotted on semi-log graph paper.

2.1.9 Determination of Freezing Point Depression (FPD)

Extraneous water in milk was determined using the Advanced Milk Cryoscope Model 4L supplied by Advanced Instrument Inc., Massachusetts, U.S.A. which measures the freezing point depression (FPD) of milk. This instrument meets the requirements specified by AOAC (1965).

2.1.10 Determination of Viable Starter Count

Colony forming units (CFU) of lactic starter culture in the milk direct after the addition of starter culture was enumerated using M17 and PLGYG agar media according to the method of Terzaghi & Sandine (1975) and Mullan, Daly & Fox (1981). The main difference between these agars is that the former agar contain lactose and the PLGYG agar contains glucose

as a source of energy. Serial ten-fold dilutions of milk were prepared using sterile quarter-strength Ringer's solution and 1 ml amounts of the dilutions were plated in accordance with the pour plate technique. The plates were incubated at 30°C for 72 h according to B.S.- 4285: 1968 and Supple. No. 1 : 1978 (British Standards Institution, 1968 and 1978).

2.1.11 Determination of Antibiotics Residues

The method of Galesloot & Hassing (1962) as modified by Crawford & Galloway (1964) was used for the detection or presence of antibiotics in milk. This test detects antibiotics or other inhibitory substances at levels of 0.01 to 0.02 International Units (IU) of penicillin per ml of milk. A small disc (6 mm diameter) of filter paper is dipped into the milk sample and placed on the surface of an agar medium contained in a petri dish and inoculated with a sensitive test organism Bacillus stearothermophilus var. calidolactis, the plate was then incubated at 55°C for 2½ h. Normal growth of the test bacteria is rapid and by the end of the incubation period has caused the medium to become cloudy. Antibiotics or other inhibitory substances, if present in the milk sample, pass out into the agar medium round the disc and prevent the growth of the test organism. Inhibition of the test bacteria results in the formation of a circular clear zone round the disc. The size of this clear zone is related to the type and concentration of the antibiotic or other inhibitory material present in the milk.

2.2 CHEESEMAKING SYSTEM

2.2.1 Production of Cheese

The cheese (control and experimental) was produced on a time sequence basis as illustrated in Figure 2.2. Details of different treatments of the milk and the experimental cheese are in 2.2.5.2 and 2.2.6.2.

2.2.2 Starter Culture

Three different strains of mesophilic cheese starter cultures (coded 850, 870 and 890) were used for the production of cheese, and they were obtained from Chr. Hansen's Laboratory Ltd., Reading, UK. These cultures were of mixed-strains type, concentrated, frozen in liquid nitrogen, and were suitable for direct-to-vat inoculation (DVI) (32 g starter to 205 l, or 64 g to 410 l, or 352 g to 2255 l of milk).

FIGURE 2.2

Flow diagram of cheesemaking process

Day 1 — Reception, storage and/or treatment* of the milk

↓

Day 2 — Heat treatment of the milk at 72°C for 15 s using plate heat exchanger (supplied by APV Co. Ltd., Crawley, UK)

↓

Cool the milk to 30°C and inoculate with starter culture

↓

Ripen the milk at 30°C for 20 min

↓

Add the rennet at a rate of 28.4 ml/114 l of milk and allow 45-50 min for the milk to coagulate

↓

Cut the coagulum (cutting time approximately 2-3 min or 15 min depending on the capacity of the cheese vat)

↓

Heat the whey and curd indirectly from 30°C to 39°C (maximum scald period is 45 min)

↓

Stir the curd and whey for 45 min

↓

Settle the curd for 5 min and drain off the whey when the acidity of the whey from the pressed curd is 0.18% lactic acid

↓

Start the cheddaring process until the acidity rises to 0.6% lactic acid

↓

Mill the curd in a Cheddar cheese chip mill (Wincanton Engineering Ltd., Dorset, UK)

↓

Salt** the curd at a rate of 2.5% (estimated w/w), and mix for 15 min

↓

Hoop 20 kg salted curd in a rectangular mould and press overnight at an air line pressure of 85 $lb.f/in^2$, acting on an 8 in (20 cm) diameter cylinder

↓

Day 3 — Cut the block of cheese in four sections each weighing around 4.75 - 5 kg and pack in a nylon/polythene laminated pouch, evacuate and seal.

↓

Transfer the cheese to the curing room and mature at 10°C or other temperature depending on the experiment

Footnote:
* Treatment of the milk with lactase enzyme
** Treatment of milled curd with neutral proteinase enzyme

Preliminary experiments had shown that this amount of inoculum was sufficient for the appropriate rate of acid production during cheese-making.

2.2.3 Coagulant

Standard calf rennet obtained from Chr. Hansen's Laboratory Ltd., Reading, UK was used as the coagulant. This enzyme was supplied in a liquid form and contained sodium chloride, rennet enzyme, propylene glycol, sodium benzoate, caramel colour and flavour. It was stored in a dark room at 10°C until used. The rennet consisted of two enzymes (chymosin and pepsin), and according to the supplier they were approximately at a ratio of 80:20 respectively.

2.2.4 Salt

Pure dried vacuum salt supplied by Imperial Chemical Industries Ltd., Cheshire, UK, which meets the characteristics contained in B.S. 998 : 1971 (British Standards Institution, 1971) was added to the milled curd at a rate of 2.5% (estimated w/w), and mixed thoroughly for 15 min.

2.2.5 Lactose Hydrolyzed Milk

2.2.5.1 Lactase enzyme (β-D-galactosidase) (EC 3.2.1.23) from Kluyveromyces lactis

Two lactase enzyme preparations (Maxilact brand) were obtained from Gist Brocades NV, Delft, Holland. The preparation coded E_1 in this study was reported to have a specific activity of 5200 Natural Lactase Units (NLU)/g and 77 Natural Protease Units (NPU)/g. The second preparation coded E_2 in this study had a reported activity of 2500 NLU/g and 15000 NPU/g. Enzymes E_1 and E_2 were obtained in a liquid and freeze-dried form respectively.

2.2.5.2 Treatment of milk

Cold raw milk was delivered by road tanker and it composed of the milk from several farms. The milk at the College Dairy was divided into two portions, adjusted to 4°C and stored in two identical refrigerated farm milk tanks (supplied by Dairy Supply Co. Ltd., Park Royal, London, UK). The first portion was not treated, and was used as a control, while the second portion was treated with the lactase enzyme. Both

lots of milk were agitated mechanically for 2 min once every two hours. The duration of hydrolysis was 18 h at 4°C for both types of enzymes.

2.2.6 Addition of Neutral Proteinase

2.2.6.2 Specification of the enzyme

Two neutral proteinases (EC 3.4.24.4) from Bacillus subtilus known as Neutrase was obtained from Nova Enzyme Products Ltd., Windsor, UK, in a dried form. The first preparation (coded N1 in this study) was reported to have a specific activity of 1.5 G Anson Unit (AU)/g, and it was mixed with the salt prior to its addition to the milled curd. The second preparation (coded N2 in this study) had a specific activity of 0.001 AU/g, and this enzyme was also provided by the manufacturer in a dried form but coated on salt granules. Enzyme N2 was also mixed with salt before applying it on the milled curd, but the quantity of added salt, i.e. vacuum dried, was reduced in order to compensate for the salt carried on the enzyme.

2.2.6.3 Maturation conditions of the N1 and N2 treated cheeses

The experimental N1 treated cheese and the control were stored at 10 and 13°C for up to 12 months; however, the cheese (control and enzyme N2 treated) were ripened at 10°C only for 4 months.

2.3 CHEESE ANALYSIS

2.3.1 Determination of Fat

Fat was determined according to B.S. 696 - Part 2:1969 (British Standards Institution, 1969).

2.3.2 Determination of Moisture

Moisture was determined according to B.S. 770· 1963 (British Standard Institution, 1963), and using aluminium foil containers supplied by A.R. Brodie Ltd., Glasgow, UK.

2.3.3 Determination of Salt

Salt percentage in cheese was determined according to the method of B.S. 770 - Part 4 : 1976 (British Standards Institution, 1976).which was based on the principle of the reaction between the sodium chloride and

silver nitrate in hot acid to form silver chloride. The difference between the titration of the excess silver nitrate with potassium thiocyanate and the blank was taken to calculate the salt percentage in the cheese sample, e.g. 1 ml of 0.05 N potassium thiocyanate ≙ 0.00292 g salt.

2.3.4 Determination of Hydrogen Ion Concentration (pH)

A Silverson mixer was used to mix 10 g grated cheese with 10 ml distilled water in small plastic containers until a fine paste was obtained and the pH was measured electrometrically as mentioned in Section 2.1.7.

2.3.5 Determination of Total Nitrogen

The method of Vakaleris & Price (1959) as described by Al-Obaidi (1980) was used to determine the total nitrogen content in cheese expressed as percentage of protein. Grated cheese (5 g) was mixed with 20 ml of 0.5 M solution of sodium citrate and 40 ml distilled water which were mixed using a Silverson mixer. Transfer quantitatively. The mixture was transferred into a volumetric flask and made up to 100 ml with distilled water. The total nitrogen in this extract was then determined by the micro-Kjeldhal method referred to above (Section 2.1.2).

2.3.6 Determination of Soluble Nitrogen

Sodium citrate cheese extract was prepared as described in Section 2.3.5, and 50 ml was mixed with 5 ml of 1.41 N hydrochloric acid (HCl) and 7.5 ml of distilled water (the final pH of the mixture should be 4.4 ±0.05). The mixture was filtered using Whatman No. 42 filter paper and the soluble nitrogen was determined by the micro-Kjeldhal described in Section 2.1.2.

2.3.7 Determination of free amino acids

Cheese samples were prepared according to the method of Weaver & Kroger (1978) and was modified as follows:

> grated cheese (1 g) was placed in 50 ml plastic test tubes and mixed with 25 ml of 1% aqueous solution of picric acid. The sample and reagent were mixed by whirl mixer for 3 min before centrifugation at 3000 rev/min. The supernatant was eluted through a prepared 10 cm resin bed, type Dowex 2 x 8 200 (CL), and the remaining sample was washed twice with 10 ml 0.02 N HCl which

was later eluted into the resin bed.

Before use the resin bed was prepared as follows:

wash the resin in a beaker with 1 N HCl;

decant the reagent and place resin into the column;

wash again with 1 N HCl;

finally rinse twice with 20 ml distilled water.

The cheese sample, i.e. freeze-dried, was dissolved in citrate buffer before injecting 1 ml into a Jeol JLC-5AH automatic amino acid analyser supplied by Jeol (UK) Ltd., London, England.

2.3.8 Polyacrylamide Gel Electrophoresis (PAGE)

Electrophoresis is a method that utilizes charge differences for the separation and purification of protein (Haschemyer & Haschemyer, 1973). Polyacrylamide gel electrophoresis was used in the identification and comparison of casein breakdown subsequent to hydrolysis during cheese ripening. The gel used was prepared by dissolving 8 g of cyanogum 41 (a commercial mixture of acrylamide and N,N' - methylenebisacrylamide, supplied by BDH Chemicals Ltd., Poole, Dorset, UK) in 100 ml of tricitrate buffer which was made as follows:

4.59 g tris (hydroxymethyl) methylamine + 0.53 g citric acid + 270.270 g urea were dissolved in distilled water and made up to 1 litre.

To the cyanogum and tris buffer, 0.2 ml of dimethylamine propionitride (DMAPN), 0.15 g of ammonium persulphate and 150 µl of mercaptoethanol were added and the solution was mixed very gently and deareated and poured into a model 220 vertical slab electrophoretic cell supplied by Bio Rad Laboratories Ltd., Herfordshire, UK. Care must be taken to exclude all air bubbles, and the cell left until polymerisation was completed (overnight). Samples were prepared by weighing 100 mg of grated cheese into a centrifuge tube with 0.1 ml distilled water and 3 ml of gel buffer. The mixture was then warmed to 42°C and stirred for 4 minutes to disperse the cheese particles. The tube was then centrifuged at 3000 rev/min for 10 minutes.

After the samples had been centrifuged, 'Pasteur' pipettes were used to transfer the supernatant from the cheese samples to a test tube and

20 µl of mercaptoethanol was then added.

To run the gel, the cell was placed in the electrophoresis tank which contained buffer solution made up as follows:

> 18.5 g boric acid and 2 g of sodium hydroxide were dissolved in 600 ml of distilled water. The pH was then adjusted to 8.6 and the volume was made up to 1 litre with distilled water.

When the fast moving bands - indicated by a blue line - had travelled more than 10 cm, the current was stopped and the gel was removed from the cell and stained either by immersing overnight in a solution of 0.1 per cent (w/v) Coomassie blue R (kenaucid blue R' - BDH Chemicals Ltd.) in 10 per cent acetic acid (v/v). Destaining the gel was carried out by immersing the gel in a tank containing 5 per cent (v/v) acetic acid solution and applying electrical current of 12 volts (0.6 - 1.0 AMP) through the acid for 4 h using a battery charger, ('Popular', supplied by F.C. Heayberd and Co. Ltd., London, UK). The acid solution was changed once during destaining. When the gel became clear, it was preserved by vacuum sealing in a nylon polythene laminate pouch and photographed.

Identification of the separated bands in the gel was done after scanning the gel using 2202 Ultro Scan Laser Densitometer supplied by LKB Instruments Ltd., Croydon, UK. With the laser densitometer there was no need to chop the gel as there is a path where the gel (off 16 track in the author's work) and the laser beam is programmable to go through each track subsequently. The laser beam is very narrow so giving better isolation for casein fractions than the ordinary scanner. Due to the last fact there was slight variation in the scanning trace if the position at which the laser goes through within the same band is changed. So it was necessary to make sure that the laser beam was positioned to go through the middle of each band or to take more than one reading for each band. It was also possible to scan the gels at different absorbance range for the same gel. The necessity for this depends on whether the minor or the major bands are of interest to the user.

The following conditions were used with the laser densitometer:

Scan speed	50 mm/min
Start position	Mostly from the stacking gel

End position	The end of all peaks
Absorbance range	0.5 OD some gels were scanned at 1.0 OD (The absorbance range specifies the optical density range which is represented by the full scale on the chart paper.)
Integration factor	1.0 The integration factor determines the length of the gel, which is scanned during a full-scale deflection of the integration pen according to the following equation: $L = K \times (IF) \times (AR)/a$ where L = Length (mm) for full scale deflection K = constant, 2.5 IF = entered integration factor AR = entered absorbance range A = actual absorbance being measured.

The laser densitometer was connected to a 3390 A integrator supplied by Hewlett-Packard Ltd., Analytical Instrumentation Group, Berkshire, UK.

The following conditions were used for integrating:

ATT 27	= 10 (this step is performed to set the height scale for plotting peaks)
PK WD value	= 0.04 (this value tells the integrator the kind of peaks to be expected, matching its response to width of peaks to be detected and measured)
THRSH integrator value	= 1.0 (THRSH's function is to set a discrimination level for the integrator to ignore signal changes to be regarded as noise)
CHT SP	= 6.0 (chart speed in cm/min)
AR REJ	= 0 (area rejected).

Under these conditions for the laser densitometer and the integrator the peaks obtained were within the limit at which the intensity and light bands can be determined. However, some very faint bands could not be detected as peaks because they had smaller area than the limit used for the integrator.

2.3.9 Rheological Assessment

Cheese samples (15 mm cubes) were obtained for the rheological measurements using the Instron Compression Tester (Food Testing Instrument, Table Model 1140 Instron Ltd., High Wycombe, UK).

Crosshead speed and diameter were set at 50 mm/min and 35 mm/min respectively. The cheese samples were compressed to 80% of their original length and the curves obtained for the cheese samples were used to calculate the brittleness and hardness characteristics. Brittleness of the cheese was calculated from the area of the cruve between the start and the inflexion point of the graph, and the hardness was equivalent to the total area recorded.

2.3.10 Determination of Colony Forming Unit (CFU) in the Curd

Unsalted cheese curd was obtained immediately after milling, and 10 g were homogenised with 90 ml of sterile 2% (w/v) tri-sodium citrate in a Colworth Stomacher for 5 min. CFU in an unsalted curd was determined using the improved medium M17 agar and PLGYG agar according to the methods of Terzaghi & Sandine (1975) and Mullan, Daly & Fox (1981) as described in Section 2.1.10.

2.4 WHEY ANALYSIS

Whey samples were analysed for fat, total nitrogen, carbohydrates, total solids, pH and freezing point depression (FPD) according to the methods described in Sections 2.1.1, 2.1.2, 2.1.3, 2.1.4, 2.1.7 and 2.1.9 respectively.

CHAPTER THREE

PRODUCTION, ANALYSIS AND EVALUATION OF CHEDDAR CHEESE MANUFACTURED FROM LACTOSE HYDROLYSED MILK

3.1 INTRODUCTION

As discussed above, the literature suggests that controversy still exists regarding the exact role of β-D-galactosidase during the manufacture and accelerated ripening of "natural" Cheddar cheese. Some research workers in different laboratories have reported accelerated ripening of Cheddar cheese (Woodward & Kosikowski, 1975; Thompson & Brower, 1974; Thompson & Brower, 1976; Anon, 1977; Marschke & Dulley, 1978 and Gooda et al., 1981) while others could not achieve the same effect (Mulholland, O'Brian & Phelan, 1976; Cardwell & Prombutara, 1976 and Marschke et al., 1980). In principle, the stimulation of the lactic acid bacteria in LHM during cheesemaking was attributed to the availability of glucose and galactose in milk rather than lactose alone (Gilliland, Speck & Woodard, 1974). However, more recently the enhanced activity of the starter cultures in LHM was reported as being due to the presence of proteolytic enzyme(s).

The primary objective of the work discussed in this chapter was to examine the production of Cheddar cheese from lactose-hydrolysed milk (using two β-D-galactosidase preparations of different lactase and protease activities), which might influence the process of ripening of natural Cheddar cheese.

3.2 MATERIALS AND METHODS

3.2.1 Hydrolysis of Milk (Enzymes)

The milk was treated with two different β-D-galactosidase preparations as described in 2.2.5.1 and 2.2.5.2.

3.2.2. Analysis of Milk

The milk was analysed for fat, total nitrogen, carbohydrates, total solids, solids not fat, titrable acidity, pH, acid soluble tyrosine, FPD and antibiotics as described in 2.1.1, 2.1.2, 2.1.3, 2.1.4, 2.1.5, 2.1.7, 2.1.8, 2.1.9 and 2.1.11 respectively.

3.2.3 Production of Cheese

The cheesemaking system followed in this experiment was as described in Figure 2.2.

3.2.4 Cheese Ingredients

The cheese ingredients or additives such as starter culture, coagulant and salt which were used in this experiment is mentioned in 2.2.2, 2.2.3 and 2.2.4.

3.2.5 Chemical Analysis of Cheese

The fat, moisture, salt, pH, total nitrogen, soluble nitrogen and casein hydrolysis in cheese were determined as described in 2.3.1, 2.3.2, 2.3.3, 2.3.4, 2.3.5, 2.3.6 and 2.3.8 respectively.

3.2.6 Organoleptic Assessment

For this present study the cheese were examined when 2, 4 and 6 months old by the official grader from the Company of Scottish Cheesemakers Ltd., and 8 panelists from the Department of Dairy Technology. Scores were awarded for

(i) flavour and aroma

and (ii) body and texture.

Colour and finish characteristics were uniform and not affected by the treatments.

3.3 RESULTS AND DISCUSSION

3.3.1 Activity of Starter Cultures in LHM

Reconstituted skim milk (11% total solids) was used to monitor the growth of the direct to-vat-inoculation (DVI) starter culture (850, 870 890) during 6 h of incubation at 30°C. The milk was divided into 3 portions, two treated with either Maxilact E_1 or E_2 for 18 h at 4°C, while the other portion was left untreated (as a control). After 18 h of lactose hydrolysis, the milk was pasteurized at 63°C for 30 min and then cooled to 30°C in a warm water bath, then the starter was added and the milk, and the starter were agitated vigorously every 30 min. The acidity of the curd made from LHM was higher than the curd made from untreated milk especially with the use of Maxilact (brand) E_2 (see Table 3.1).

TABLE 3.1

The acidity, pH and the colony forming unit (CFU) of the direct to-vat-inoculation (DVI) starter culture in reconstituted skim milk and after 6 h of incubation at 30°C

Starter culture \ Treatment*		On inoculation			After 6 h of incubation		
		Titrable acidity (% lactic acid)	pH	CFU/ml^{-5}	Titrable acidity (% lactic acid)	pH	CFU/ml^{-7}
850	Control	0.17	6.59	64	0.63	5.21	80
	Maxilact E_1	0.18	6.58	102	0.65	5.18	93
	Maxilact E_2	0.17	6.57	42	0.65	5.11	166
870	Control	0.16	6.62	254	0.60	5.22	75
	Maxilact E_1	0.16	6.61	282	0.61	5.17	94
	Maxilact E_2	0.16	6.62	243	0.61	5.15	79
890	Control	0.16	6.62	172	0.62	5.31	55
	Maxilact E_1	0.16	6.62	151	0.63	5.15	68
	Maxilact E_2	0.16	6.62	99	0.65	5.10	100

*Average of two trials

The rate of Maxilact E_1 addition = 0.078 g/l

The rate of Maxilact E_2 addition = 0.54 g/l

Table 3.1 also illustrated the growth rate/activity of the DVI starter culture in the curd which could be summarised as follows:

(i) strain 850 showed increased activity of 16.25% with Maxilact E_1 and 107.5% with Maxilact (brand) E_2 as compared with the control;

(ii) strain 870 showed increased activity of 25.33 and 5.33% with the use of Maxilact (brand) E_1 and E_2 respectively as compared with the control;

(iii) strain 890 showed increased activity of 23.63 and 81.81% with the use of Maxilact (brand) E_1 and E_2 respectively as compared with the control.

The stimulation of the starter culture in the curd made from LHM could be attributed to the presence of glucose and galactose in the LHM. This finding agreed with the conclusion of Gilliland, Speck & Woodard (1974), and/or to the presence of protease in both enzymes (E_1 and E_2), e.g. 77 and 15000 NPU/g respectively.

3.3.2 The Effect of Lactose Hydrolysis on the Chemical Composition of Milk

Six trials of cheesemaking (control and experimental) were carried out using direct to-vat-inoculation (DVI) starter cultures (850, 870 and 890) in this present study, and the effect of β-D-galactosidase (E_1 and E_2) on the chemical composition of the milk as compared with the untreated milk is illustrated in Table 3.2. The extent of lactose hydrolysis in raw milk after 18 h at 4°C was directly dependent on the activity of the enzyme used and on the duration of hydrolysis and temperature at which the reaction took place. For example, 28.54 and 43.60% hydrolysis of lactose in milk were achieved by using 0.078 g/l of β-D-galactosidase E_1 (5200 NLU/g and 77 NPU/g). However, a larger amount of enzyme E_2, i.e. 0.54 g/l was required to hydrolyse 57.62 and 61.19% of lactose because the specific lactase activity of E_2 enzyme was less (2500 NLU/g) although the proteolytic activity was greater (15000 NPU/g). The major changes that had occurred in the milk constituents could be summarised as:

(i) The extent of lactose hydrolysis in milk was governed by the amount of enzyme added and/or its activity (as discussed in the previous paragraph). The products of hydrolysis

TABLE 3.2

Chemical composition of milk before and after hydrolysis with β-D-galactosidase enzymes (E_1 and E_2) for 18 hours at 4°C

Treatment / Starter culture		Before hydrolysis					Mono- and di-saccharide present in milk after hydrolysis								Degree of hydrolysis (%)	Fat (%)	Protein (%)	SNF (%)	TS (%)
		Tritable acidity (%lactic acid)	pH	FPD	Tyrosine (mg/5ml) aliquot	Lactose (%)	Tritable acidity (% lactic acid)	pH	FPD	Tyrosine (mg/5ml) aliquot	Glucose (%)	Galactose (%)	Lactose (%)	Oligo-saccharide (%) *					
850	C	0.16	6.70	0.543	0.019	4.73	0.18	6.67	0.543	0.019	-	-	4.70**	-	-	3.95	3.24	8.85	12.77
	E_1	0.16	6.70	0.543	0.019	4.73	0.18	6.66	0.602	0.020	0.35	0.32	3.71	0.35	28.54				
870	C	0.15	6.69	0.539	0.017	4.74	0.15	6.72	0.539	0.017	-	-	4.74	-	-	4.00	3.21	8.85	12.72
	E_1	0.15	6.69	0.539	0.017	4.74	0.15	6.69	0.608	0.018	0.49	0.37	3.57	0.31	28.90				
890	C	0.16	6.67	0.539	0.020	4.84	0.16	6.71	0.539	0.020	-	-	4.80	-	-	4.00	3.30	8.85	12.83
	E_1	0.16	6.67	0.539	0.020	4.84	0.16	6.69	0.612	0.021	0.84	0.75	2.73	0.52	43.60				
850	C	0.16	6.67	0.535	0.028	4.53	0.16	6.62	0.536	0.028	-	-	4.53	-	-	3.50	3.23	8.92	12.42
	E_2	0.16	6.67	0.535	0.028	4.53	0.16	6.60	0.676	0.033	0.97	0.72	1.92	0.92	57.62				
870	C	0.16	6.67	0.540	0.022	4.20	0.16	6.64	0.534	0.022	-	-	4.20	-	-	3.60	3.25	8.99	12.46
	E_2	0.16	6.67	0.540	0.022	4.20	0.16	6.61	0.674	0.026	1.00	0.65	1.63	0.92	61.19				
890	C	0.16	6.69	0.528	0.021	4.21	0.16	6.72	0.530	0.022	-	-	4.21	-	-	3.70	3.35	8.89	12.43
	E_2	0.16	6.69	0.528	0.021	4.21	0.16	6.69	0.666	0.029	0.82	0.57	1.81	1.01	57.01				

Footnote: E_1 and E_2 are different β-D-galactosidase preparations.

C is control (no added enzyme)

*calculated by difference assuming the weight remains approximately constant

**assuming no change in the control

Figure 3.1: Standard curve for the relation between freezing point depression (FPD) and the percentage of lactose hydrolysis

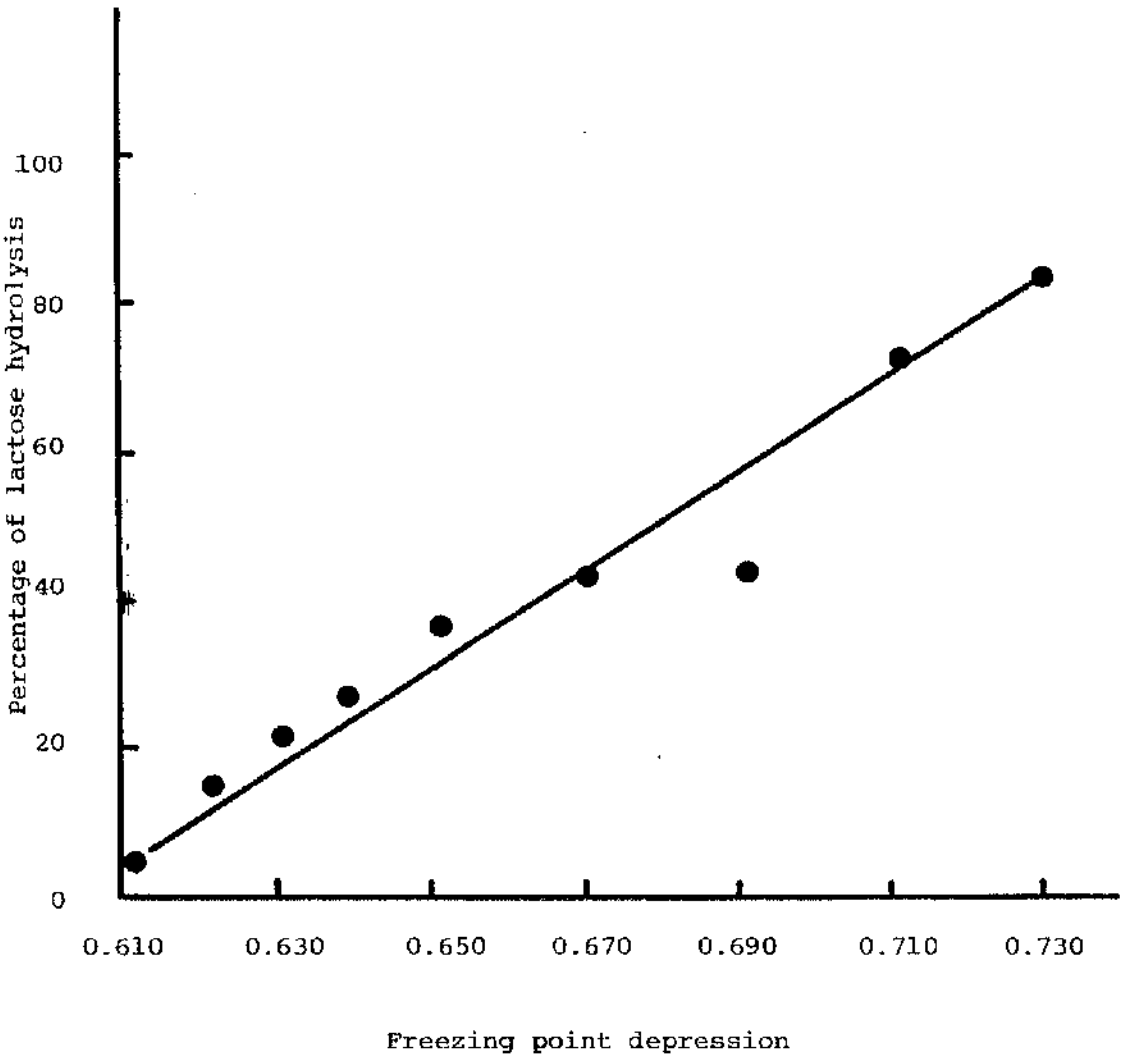


TABLE 3.3

Time sequence during cheesemaking

Starter culture \ Treatment		Ripening (min)	Renneting (min)	Renneting to milling (h.min)	Total process time (h.min)
850	C	25	47	5.44	6.09
	E_1	20	45	5.28	5.48*
870	C	21	53	4.28	4.49
	E_1	20	52	4.28	4.48
890	C	20	55	4.50	5.10
	E_1	20	48	4.55	5.15
850	C	20	51	5.31	5.51
	E_2	20	44	4.59	5.19*
870	C	20	51	4.41	5.01
	E_2	20	48	3.38	3.58*
890	C	20	46	5.14	5.36
	E_2	20	45	4.33	4.53*

Footnote: E_1 and E_2 are different β-D-galactosidase preparations
C is control with no added enzyme
* shortened processing time compared with the control

LHM) from ripening to milling using DVI starter cultures (850, 870 and 890). It can be observed that as the extent of lactose hydrolysis is progressively increased from 28.5 to 61.2%, the shorter the processing time. The cheesemaking trials from LHM (E_1) had similar processing time with the exception of the first trial, i.e. starter 850 (see Table 3.3) where prolonged processing time was observed. This could be attributed to low cell content of the starter culture (see Table 3.4). However, the shortest Cheddar cheesemaking time (from renneting to milling, i.e. 3 h 38 min) was observed in 61.2% LHM (E_2).

The hydrolysis of lactose in milk results in the liberation of monosaccharides (glucose and galactose), the viable count of the starter culture bacteria was monitored in milk after the addition of the direct to-vat-inoculation, and in the milled curd prior to salting, and Table 3.4 illustrates the activities of three different strains of mixed-cheese starter cultures. Slight variation in the initial viable count of the starter cultures in milk was observed in M 17 and PLGYG media, and the reason could be attributed to the fact that each agar medium contained different types of energy source, i.e. lactose in M 17 and glucose in PLGYG. The concentrated frozen cultures were pre-packed by the manufacturer in approximately 32 g portions. If the counts of each batch of experimental cheese was compared with its control, the overall trend of the growth rate/activity of the starter cultures in the curd prior to salting could be summarised as follows:

(i) strains 850 and 870 showed a reduced activity (40-75% in CFU/g in LHM (28-29%) as compared with the control);

(ii) strain 890 showed improved activity in LHM to 43.6%;

(iii) strains 850, 870 and 890 showed stimulation in their activity between 13-38% in LHM with over 57% hydrolysis.

The variable effect, i.e. inhibition/stimulation, of the starter cultures during the manufacture of Cheddar cheese from untreated milk and LHM could not be attributed only to the degree of lactose hydrolysis mentioned above since both enzymes E_1 and E_2 possess some proteolytic activity, e.g. 77 and 15000 NPU/g respectively. During the hydrolysis treatment of the milk, the acid soluble tyrosine in milk increased by 0.001 and 0.006 mg/5 ml aliquot (see Table 3.1) when treated with enzymes E_1 and E_2 respectively. The latter enzyme demonstrated a proteolytic activity

TABLE 3.4

The viable count of starter bacteria in cheese milk and in curd prior to salting

Treatment / Starter culture		After addition to milk (CFU/ml^{-5})		In curd prior to salting (CFU/g^{-1})		Activity	Degree of lactose hydrolysis in cheese milk (%)
		M17 medium	PLGYG medium	M17 medium	PLGYG medium		
850	C	36	31	86	109	Inhibition	28.54
	E_1	40	36	35	27		
870	C	189	217	580	495	Inhibition	28.90
	E_1	188	195	345	260		
890	C	151	104	78	102	Slight stimulation	43.60
	E_1	114	125	102	105		
850	C	107	77	15	12	Stimulation	57.62
	E_2	110	73	20	19		
870	C	485	485	650	800	Stimulation	61.19
	E_2^2	370	365	875	925		
890	C	174	169	181	199	Stimulation	57.01
	E_2	270	247	296	247		

Footnote: E_1 and E_2 are different β-D-galactosidase preparations

C is control

Single sample plated in duplicate and average calculated in each medium

in which stimulation of the starter is the result; a view which was also reported by Hemme, Vassal & Auclair (1978) with yoghurt strains. Furthermore, the enhanced activity of the starter cultures increased their viable count during cheesemaking (Table 3.4), and as a result the cheesemaking process has been reduced in time (see Table 3.3) and in particular when the milk was treated with Maxilact (brand) E_2. The same effect has also been observed by Marschke & Dulley (1978).

3.3.4 Relationship between Lactose Hydrolysis and Accelerated Cheddar Cheese Ripening

The chemical composition of 6 batches of Cheddar cheese (control and experimental E_1 and E_2) is shown in Tables 3.5 and 3.6. It can be observed that the moisture content and fat in dry matter (FDM) of these cheese ranged between 34.39-38.10% and 51.44-54.94% respectively. However, in this present study the high moisture content was mainly experienced in LHM cheese due to enhanced activity of the starter culture, i.e. fast acid development during the cheddaring stages especially in milk where the extent of lactose hydrolysis was in excess of 57%. Nevertheless, it can be observed from Tables 3.5 and 3.6 that all the Cheddar cheese produced complies with existing legal standards in the United Kingdom, i.e. maximum 39% moisture and minimum 48% FDM.

Slight variation in the chemical composition of these cheeses was observed during the maturation period up to 6 months old which could be attributed primarily to experimental error (i.e. sampling procedure), slight dehydration (through the packaging material) and salt diffusion.

The variation in the chemical composition of the cheese during ripening could be summarised as follows:

A) Fat, protein and salt content change which could be attributed to improper salt distribution and the variation of moisture.

B) The pH of the cheese depends on the activity of the starter culture, other microflora, enzymes and the amount of lactose in cheese. The small variation observed in the initial value of the pH between the control and the experimental cheeses which could be due to the high production of lactic acid by the starter culture during the cheese production especially with enzyme E_2 (high level of lactose hydrolysis and stimulation of starter culture (see

TABLE 3.5

The chemical composition (%) of Cheddar cheese (control and LHM-E_1) during the ripening period at 10°C

Starter culture	Age of cheese (days)	Treatment	Moisture	Fat	FDM	MFFC	Salt	SM	pH	TN	SN	SN/TN
850	1	C	34.39	33.75	51.44	51.91	1.83	5.30	5.10	22.98	1.06	4.61
		E_1	35.05	34.75	53.50	53.72	1.72	4.90	5.16	22.69	1.05	4.63
	60	C	35.35	33.60	52.00	53.20	1.58	4.47	5.03	23.19	3.19	13.76
		E_1	34.82	34.90	53.50	53.50	1.70	4.88	5.17	22.74	3.10	13.68
	120	C	34.99	34.30	52.76	53.26	1.65	4.72	4.92	22.79	3.96	17.36
		E_1	34.46	34.30	52.41	52.45	1.71	4.96	5.04	22.58	4.19	18.56
	180	C	35.14	34.50	53.19	52.12	1.72	4.89	4.94	23.17	4.92	21.23
		E_1	34.46	34.60	52.78	52.69	1.69	4.90	4.99	23.25	5.44	23.44
870	1	C	35.03	34.00	52.33	53.08	1.77	5.00	5.00	22.27	1.16	5.21
		E_1	34.94	34.50	53.03	53.34	1.67	4.80	5.04	22.91	1.10	4.80
	60	C	35.66	34.40	53.50	54.40	1.63	4.57	4.95	22.24	3.25	14.61
		E_1	35.52	34.30	53.20	54.41	1.76	4.95	5.03	22.87	3.13	13.69
	120	C	35.34	34.85	53.90	54.24	1.43	4.05	4.91	22.29	4.18	18.75
		E_1	35.61	34.30	53.27	54.20	1.44	4.04	4.84	23.08	4.07	17.63
	180	C	35.81	35.00	54.33	55.09	1.51	4.22	4.89	23.02	4.85	21.07
		E_1	35.81	34.00	52.97	54.26	1.50	4.19	4.88	23.24	4.75	20.35
890	1	C	35.65	34.00	52.12	52.64	1.81	4.68	5.12	21.65	1.15	5.31
		E_1	35.91	34.50	52.16	53.94	1.75	4.51	5.10	21.39	1.23	5.75
	60	C	35.84	34.90	54.40	55.10	1.69	4.72	5.09	21.77	3.17	14.56
		E_1	36.02	34.10	53.30	54.70	1.51	4.19	5.07	21.12	3.30	15.63
	120	C	35.43	34.30	53.12	53.93	1.56	4.40	5.10	23.03	3.96	17.98
		E_1	35.62	35.40	54.98	55.14	1.50	4.21	4.96	22.30	4.24	19.01
	180	C	35.50	34.70	53.80	54.36	1.49	4.20	5.12	22.60	4.86	21.49
		E_1	35.93	35.50	55.41	55.71	1.57	4.37	5.00	22.46	4.92	21.91

Footnote: C = control (no enzyme added) E_1 = Maxilact (brand) enzyme (5200 NLU/g and 77 NPU/g)

TABLE 3.6

The chemical composition (%) of Cheddar cheese (Control and LHM-E_2) during the ripening period at 10°C

Starter culture	Age of cheese (days)	Treatment	Moisture	Fat	FDM	MFFC	Salt	SM	pH	TN	SN	SN/TN
850	1	C	35.87	33.00	51.46	53.54	1.50	5.18	5.12	23.94	1.03	4.13
		E_2	37.38	33.00	52.70	55.79	1.56	4.17	5.01	23.35	1.04	4.45
	60	C	35.95	33.00	51.52	53.66	1.71	4.76	5.05	23.95	3.36	14.02
		E_2	36.84	33.00	52.41	55.06	1.58	4.29	5.02	22.11	3.90	17.64
	120	C	34.95	33.00	50.88	52.24	1.43	4.09	5.09	24.13	3.39	14.05
		E_2	36.67	33.10	52.27	54.81	1.68	4.58	5.07	23.87	3.66	15.33
	180	C	35.06	33.50	51.59	52.72	1.44	4.11	5.25	23.25	5.05	21.72
		E_2	36.48	33.40	52.58	54.77	1.73	4.74	5.18	23.11	5.32	23.02
870	1	C	37.20	34.50	54.94	56.79	1.67	4.49	5.07	23.58	1.03	4.19
		E_2	38.10	33.00	53.31	56.87	1.70	4.46	4.97	23.73	1.08	4.55
	60	C	36.73	34.50	53.74	55.65	1.63	4.44	5.01	23.65	3.47	14.67
		E_2	37.27	33.00	53.40	56.05	1.49	4.00	4.84	22.19	3.91	17.62
	120	C	36.34	34.00	53.02	54.85	1.60	4.40	4.92	23.40	3.50	14.96
		E_2	36.99	33.50	52.69	55.37	1.62	4.38	4.72	23.27	4.57	19.64
	180	C	36.26	34.50	54.13	55.39	1.59	4.38	5.13	23.58	5.37	22.77
		E_2	36.97	34.00	53.94	56.02	1.66	4.49	5.08	22.81	6.21	27.22
890	1	C	36.47	33.00	51.94	54.43	1.51	4.14	5.16	23.54	0.91	3.87
		E_2	37.55	33.00	52.84	56.04	1.55	4.13	5.06	22.81	0.97	4.25
	60	C	35.94	33.00	50.73	53.24	1.65	4.59	5.02	23.51	3.51	14.93
		E_2	36.49	33.00	51.80	54.38	1.56	4.28	4.97	22.91	3.53	15.41
	120	C	35.45	32.50	51.28	52.99	1.66	4.68	4.94	24.05	3.99	16.59
		E_2	35.81	32.90	51.57	53.53	1.66	4.64	4.81	23.84	4.26	17.87
	180	C	35.23	33.30	51.41	52.82	1.63	4.63	5.31	23.84	5.20	22.14
		E_2	35.79	33.10	51.55	53.50	1.71	4.67	5.25	24.22	5.90	24.36

Footnote: C = control (no enzyme added) E_2 = Maxilact (brand) enzyme (2500 NLU/g and 15000 NPU/g)

Table 3.5). In most cases the pH values decreased during the early stage of ripening (up to 4 months) which might be due to the increased production of lactic acid by the starter culture and possibly other acids (propionic and acetic acids) by the cheese microflora (non-starter bacteria). The increase of the pH value after six months of ripening with an exception of cheese produced from starter culture 850 (control and experimental cheese) and control cheese produced from starter culture 870 could be attributed to the increased activity of the enzymes e.g. proteinases which results in the production of different compounds (e.g. ketones, aldehydes, amines, peptides and volatile compounds) which leads to increased pH value (Kosikowski, 1977).

C) Protein hydrolysis:

The development of soluble nitrogen and hydrolysis of casein in cheese is sometimes used as an index of the extent of the ripening process, and the changes in the soluble nitrogen and casein fractions were as follows:

1) Soluble nitrogen:

Figure 3.2 illustrates the level of soluble nitrogen present in Cheddar cheeses manufactured from untreated milk and LHM. It can be observed that the soluble nitrogen value progressively increased during the maturation period and the level was higher in all the cheeses treated with Maxilact E_1 or E_2 as compared with the control. However, the latter enzyme, i.e. E_2 which had more proteolytic activity as compared with E_1, was associated with the higher levels of soluble nitrogen in the cheese which possibly was due to:

a) high number of the starter culture and its activity (confirmed with the reporting of Marschke & Dulley, 1978);

b) the high level of protease in β-D-galactosidase;

c) both factors mentioned above.

2) Casein hydrolysis:

The protein content of cheese is mainly casein, which consists of the following different fractions in the following proportions of the total milk casein: α-casein (40%); β-casein (35%), κ-casein (15%) and the minor caseins which represent 10% (McKenzie, 1970). The

Legend to:

Figure 3.2: The rate of proteolysis in Cheddar cheese manufactured from milk and LHM up to six months old

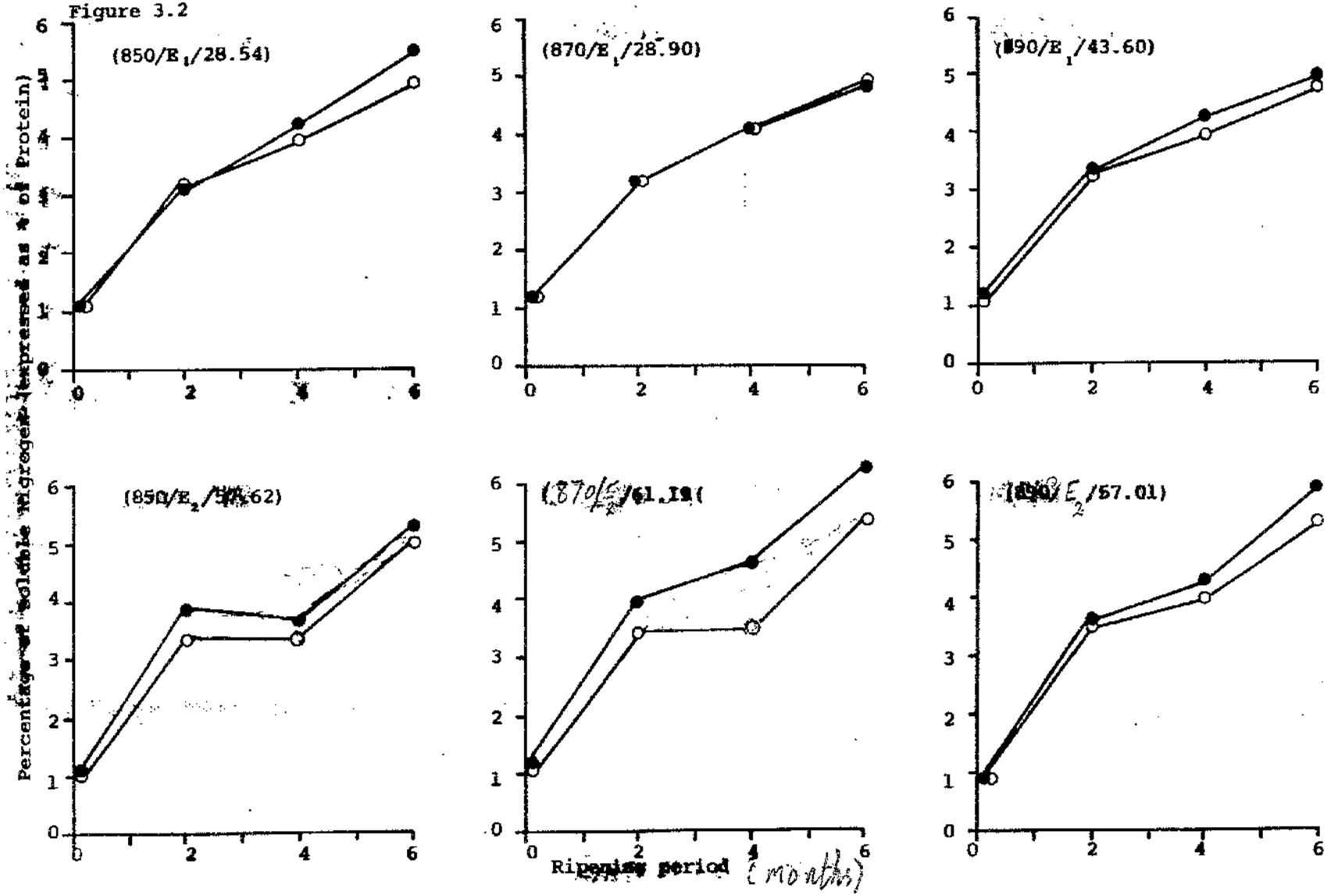
Data in parentheses represents:

Starter culture code/Maxilact enzyme/extent of hydrolysis %

● ß-D-galactosidase (E_1 or E_2) treated milk

○ control (untreated milk)

Figure 3.2


(850/E1/28.54)
(870/E1/28.90)
(890/E1/43.60)
(850/E2/57.62)
(870/E2/61.19)
(890/E2/57.01)
Percentage of soluble nitrogen (expressed as % of Protein)
Ripening period (months)

rennet coagulant, hydrolysis 50% of κ-casein to para-κ-caseinate and macropeptides. The majority of the latter fraction is lost in the whey, but the fate of the remaining macropeptides in the cheese is still unknown (O'Keeffe, Fox & Daly, 1977). The hydrolysis of κ-casein, which is the protective layer of the casein micelle, will expose other fractions and make them more susceptible to proteolytic activity which is controlled by the following factors:

(i) the activity of milk enzymes that survived the heat treatment, starter culture and rennet,

(ii) cheese conditions such as pH, water activity and solute concentration, and

(iii) the nature of casein fractions and their susceptibility to proteolysis.

PAGE method was used to study quantitatively the changes that occurred in the different casein fractions during 6 months of ripening. This method, which gives a high resolution for molecule fractionation of the casein, separates the molecules on the basis of their size, confirmation and net charges (Al-Obaidi, 1980).

PAGE gels were used to study the extent of hydrolysis of casein fractions in cheese (control and experimental) during 6 months maturation period and the gels were scanned at $A_{0.5}$ OD. Many peaks were observed in each electogram, and these peaks could be divided into the following mobility bands:

(i) slow mobility
(ii) β-casein
(iii) α_s-casein.

The extent of hydrolysis of casein during the ripening period is illustrated in Tables 3.7 and 3.8 and the overall pattern of casein degredation could be summarised as follows:

1. Slow mobility bands

The number of peaks in this mobility zone ranged between 2 and 8, and more peaks were observed in LHM (E_1 and E_2) Cheddar cheese. The size of peaks in the experimental cheeses were apparently larger as compared with the control which could be attributed to the proteolytic activity of the β-D-galactosidase used (Law & Wigmore,

TABLE 3.7

The extent of casein hydrolysis (expressed as a percentage of total area) in Cheddar cheese made from milk hydrolysed before manufacture with Maxilact (brand) E_1

Treatment / Starter culture		Curing period (months)	Slow mobility								ß-casein					α_s-casein									
			Fraction number							E_1-c*	Fraction number				E_1-c*	Fraction number									E_1-c*
			1	2	3	4	5	6	7		1	2	3	4		1	2	3	4	5	6	7	8	9	
850 (28.45%)	C	2	0.25	0.82	2.46	1.40	4.65			+1.59	0.90	40.20	0.56		-2.81	3.09	12.79	21.46	5.05	3.83	0.25	0.38	1.38		+1.78
	E_1		1.37	2.77	0.33	1.10	5.60					38.66	0.19			2.69	12.52	21.09	9.39	0.58	0.37	0.57	2.80		
	C	4	0.26	6.72						+1.61		30.74	0.32		-0.11	14.25	9.91	20.24	7.34	5.08	0.10	3.99	0.22	0.83	-1.50
	E_1		0.29	8.30								30.95				9.94	8.01	35.37	2.32	3.86	0.96				
	C	6	6.65	3.30	4.71	4.20				+0.14	1.91	27.30			-7.09	11.71	12.91	23.79	0.28	1.13	2.11				+6.98
	E_1		3.63	5.81	2.36	0.22	3.63	3.35			1.49	20.25	0.38			10.34	11.35	33.12	0.20	0.43	1.31	2.16			
870 (28.90%)	C	2	9.91	1.52	4.61					-3.86	0.86	33.38	0.37	0.52	-4.19	3.49	12.57	30.20	0.60	0.71	0.41	0.87			+8.05
	E_1		3.21	3.18	1.43	4.36					0.92	29.22	0.80			8.11	12.79	30.61	0.58	0.66	0.46	3.69			
	C	4	0.14	5.10						+2.59		28.84	0.35		+0.34	14.41	11.84	21.81	9.11	5.33	2.19	0.90			-2.94
	E_1		7.83									28.63	0.90			14.07	11.06	19.90	7.72	5.59	3.20	0.27	0.84		
	C	6	1.33	8.24	3.50	0.50	0.70	1.67	0.68	-2.23	0.17	23.35			-0.25	17.26	16.22	15.10	9.06	0.16	1.10	1.01			+2.27
	E_1		0.18	3.60	3.99	3.03	3.76					22.66	0.61			13.36	11.76	17.41	9.24	6.75	0.66	3.00			
890 (43.60%)	C	2	3.95	1.35	4.83					+1.15	0.84	30.39	0.23	0.64	-1.12	8.60	12.82	32.12	0.37	0.58	3.28				-0.03
	E_1		4.18	1.76	5.34							30.29	0.69			7.94	12.68	32.35	0.55	0.11	0.49	3.62			
	C	4	6.60							+3.16		35.58			-9.47	9.12	6.83	28.19	8.72	0.33	2.54	2.09			+6.31
	E_1		1.65	8.11								26.11				24.56	0.39	33.56	5.35	0.27					
	C	6	8.09	0.95	3.42	2.77				+0.78		22.34	0.60		+0.05	12.82	12.74	17.10	9.92	6.57	0.56	0.65	0.38	1.11	-0.26
	E_1		7.96	1.66	3.37	3.02						22.39	0.60			12.79	12.85	17.16	9.91	6.58	0.53	0.63	0.26	0.88	

Footnote: Figures in paranthesis represent degree of lactose hydrolysis in milk.

C - Control

E_1 - ß-D-galactosidase and its specific activity was 5200 NLU/g and 77 NPU/g

*Differences between the total area of the experiment and control cheese

TABLE 3.8

The extent of casein hydrolysis (expressed as a percentage of total area) in Cheddar cheese made from milk hydrolysed before manufacture with Maxilact (brand) E_2

Treatment / Starter culture		Curing period (months	Slow mobility									β-casein					α_s-casein									
			Fraction number								E_2-C*	Fraction number				E_2-C*	Fraction number									E_2-C*
			1	2	3	4	5	6	7	8		1	2	3	4		1	2	3	4	5	6	7	8	9	
850 (57.62%)	C	2	2.74	2.07	7.64						+1.72		35.76	0.26		-5.65	2.48	14.87	30.81	0.20	0.65	2.52				+3.93
	E_2		3.17	0.12	2.53	8.35						0.52	28.92	0.93			5.94	16.95	30.67	0.26	0.62	1.02				
	C	4	0.49	4.95							+5.47		32.39			-7.84	12.78	7.06	27.00	11.37	2.96	1.01				+2.35
	E_2		2.82	0.59	7.50								23.91	0.64			11.69	10.37	25.56	12.99	0.82	2.60	0.10	0.40		
	C	6	0.56	4.56	0.20	1.35	9.13				+5.52	1.12	27.88	1.53		-4.14	13.38	7.49	31.68	1.13						-1.38
	E_2		6.56	1.69	11.85	1.22							25.34	1.05			11.55	6.87	32.08	1.00						
870 (61.19%)	C	2	3.35	2.16	7.12	1.68					-4.18	0.54	41.75	0.47	1.63	-4.50	8.48	15.53	12.93	2.38	0.39	0.72	0.88			+8.19
	E_2		1.40	2.53	0.26	1.15	4.79					0.77	38.73	0.31			2.69	12.46	20.51	9.74	0.54	0.42	0.50	3.10		
	C	4	5.83								-0.74		30.49	0.62		-1.38	15.00	11.07	20.63	8.11	5.36	1.88	1.00			+2.12
	E_2		0.24	4.85									28.74	1.00			14.72	11.38	20.98	8.08	6.56	0.42	2.53	0.50		
	C	6	4.26	1.30	6.51	0.71					+5.28		27.50	0.32	0.96	+0.57	13.23	10.41	10.79	21.18	2.84					-5.84
	E_2		4.86	3.97	0.56	0.19	1.30	2.32	0.23	4.60		1.11	27.11	0.32	0.81		12.08	9.55	10.81	19.37	0.11	0.13	0.56			
890 (57.01%)	C	2	7.58	1.73	5.10						-1.21	0.99	31.30	0.81		-2.85	3.26	11.97	32.19	0.65	0.64	0.85	0.47	3.16		+3.36
	E_2		3.50	3.27	1.33	4.22	0.88						27.24	0.01	3.00		8.24	12.74	30.69	0.55	0.65	0.58	3.09			
	C	4	5.35								+2.19		30.77			-7.64	12.32	5.47	28.86	9.70	2.96	3.30	1.27			+5.46
	E_2		1.49	6.05									20.27	2.86			17.14	13.92	14.01	6.55	9.18	0.15	3.21	2.82	0.36	
	C	6	3.45	1.71	10.21	1.35					-2.42		28.50	0.26	0.80	-1.52	12.79	8.58	32.34							+3.95
	E_2		0.51	1.25	11.17	1.37							26.95	1.09			12.86	8.30	32.53	0.27	1.03	0.90	1.77			

Footnote: Figures in paranthesis represent degree of lactose hydrolysis in milk

C - control

E_2 - β-D-galactosidase and its specific activity was 2500 NLU/g and 15000 NPU/g

*Differences between the total area of the experiment and control cheese

1982), or the "enhanced" enzymatic activity that originated from the starter culture.

Cheeses (experimental and control) at 4 months old showed the least number of peaks on the gel electogram, and it is most likely that the casein fraction(s) that fall within such mobility band was readily hydrolysed as compared with α_s-casein. The products of hydrolysis could be amino acids, peptides and/or soluble nitrogen.

The observed increase in the number of peaks in the slow mobility zone in all the cheese (at 6 months) could possibly originate from β-casein hydrolysis to release nitrogenous compounds that have bands appearing in the slow mobility zone (Marcos et al., 1979).

2. β-Casein

The number of bands/peaks of β-casein in the untreated and LHM Cheddar cheese ranged from 1 to 4, and it can be observed that β-casein was progressively reduced as the cheese becomes older. The degree of β-casein hydrolysis was greater in the experimental cheese compared with the control.

The major band of the β-casein which was readily hydrolysed was the peak number 2 (Tables 3.7 and 3.8), and the degradation of such band contributed to the apparent increase of other bands in this mobility and/or slow mobility zones.

3. αs-Casein

The highest number of bands, i.e. 9, were observed in this mobility zone for all types of cheese. It is apparent that the major hydrolysis were of bands number 3 and 2 in cheeses treated with E_1 and E_2 respectively. The reason(s) of such pattern is not well established but the effect of α_s-casein hydrolysis in all the cheeses up to 4 months old had an ultimate effect on the number and size of other bands in this mobility zone.

From the above observed patterns of the casein hydrolysis it is possible to suggest the following:

a) β-casein is progressively hydrolysed in the cheese and the degraded products contribute to the increased level of SN or

free amino acids (FAA) in the cheese, and possibly increasing the level of slow mobility bands casein in 4 and 6 months old cheese respectively.

b) The hydrolysis of the slow mobility casein (4 months cheese) and α_s-casein (6 months cheese) may contribute to the SN and FAA pool in Cheddar cheese.

3.3.5 Quality Assessment of Cheddar Cheese made from Whole Milk and LHM

The cheeses were assessed and evaluated organoleptically at 2, 4 and 6 months old in accordance with the grading scheme of the Company of Scottish Cheesemakers Ltd. The data of the official grader and 8 panelists were analysed statistically by using the mean differences between the control and LHM cheese where the cheese characteristics were evaluated separately against each enzyme treatment (E_1 or E_2), the starter culture (850, 870 and 890) and the age of cheese (2, 4 or 6 months).

The statistical analysis are shown in Table 3.9 and it can be observed that the panelists preferred the experimental cheese to the control. However, statistical results of the individual grader mean differences of the taste panelists also favoured the enzyme-treated cheese, i.e. flavour and aroma (6 graders out of 9). From the statistical analysis it could be concluded the effect of the following aspects on the flavour and aroma:

(i) Maturation period: although the panelists preferred Cheddar cheese (control) at 2 months old, the overall preference was for the experimental cheese (E_1 and E_2) at 4 and 6 months old as compared with the control cheese (no significant preference at any level);

(ii) The enzymes: the panelists preferred the cheese manufactured from LHM to the control, but greater preference was for cheese made with enzyme E_2. This could be associated with proteolytic activity of these enzymes (no significant effect);

(iii) Starter culture: starter bacteria plays a major role in cheesemaking, and the panelists gave a high rating to the

TABLE 3.9

Average scores and statistical analysis (mean difference) of the nine taste panelists' assessment of the cheese made from whole and LHM milk

Starter culture	Age (months)	Flavour and aroma						Body and texture					
		C	E_1	D	C	E_2	D	C	E_1	D	C	E_2	D
850	2	37.060	37.250	-0.190	35.750	37.560	-1.81	34.000	33.310	40.690	32.250	31.750	+0.500
	4	29.685	28.185	+1.500	27.560	26.060	+1.500	26.000	26.375	-0.375	24.375	26.125	-1.75
	6	28.375	30.125	-1.750	24.370	27.255	-2.885	26.125	26.750	-0.625	23.560	26.060	-2.500
870	2	34.935	35.750	-0.815	32.685	31.560	+1.125	32.750	31.875	+0.875	30.935	30.000	+0.935
	4	23.375	24.750	-.1.375	21.875	24.935	-3.060	27.750	23.875	+3.875	24.750	26.750	-2.000
	6	24.185	20.250	+3.935	20.470	21.605	-1.135	25.000	23.875	+1.125	17.435	23.465	-6.030
890	2	36.560	37.310	-0.750	35.875	36.685	-0.810	31.685	30.000	+1.685	32.375	31.875	+0.500
	4	22.185	30.250	-8.060	26.560	28.250	-1.690	21.875	24.250	-2.375	26.750	27.625	-0.875
	6	27.000	29.375	-2.375	26.665	28.670	-2.005	24.375	26.750	-2.375	23.630	23.855	-0.225
Average		29.262	30.360	-1.098	27.979	29.176	-1.197	27.729	27.451	+0.278	26.229	27.501	-1.272

Footnoote: E_1 and E_2 are different β-D-galactosidase preparations.

850, 870 and 890 are codes for starter culture.

C is control.

D is the mean difference, (+ve) and (-ve) figures illustrates panelists' preference of the control or the experimental cheese respectively.

The characteristics of colour and finish and appearance of the cheese (control or experimental) were similar and were not affected; hence they were not included in the above data.

control cheese made with starter cultures 850 and 870. However, starter culture 890 of the experimental cheeses was greatly preferred by the panelists as compared with the other starter cultures (significant at level of 5%).

The statistical analysis for the body and texture (Table 3.9) could be summarised as follows taking into consideration the same factors mentioned in case of the flavour and aroma:

(i) Maturation period: LHM cheese was preferred by the panelists and greater preference was for cheese at 4 months old;

(ii) The ensymes: the panelists preferred the cheese made from LHM and greater preference to enzyme E_2 which could be due to its highly proteolytic activity;

(iii) Starter culture: cheese manufactured with starter culture 870 was highly preferred by the panelists especially the experimental cheese (E_1 and E_2).

It could be observed from the analysis of variance that the characteristic, i.e. body and texture, of all the cheeses insignificant at any level when the above factors were evaluated.

3.3.6 Conclusion

It is evident from the present study that the hydrolysis of lactose in milk up to about 60% did not accelerate the ripening process of Cheddar cheese even though one of the enzymes (E_2) contained proteolytic activity. It was found, however, that the following other desirable effects were achieved as the result of lactose hydrolysis of the cheese milk:

(i) reduction in the processing time;

(ii) greater judge preference for cheese manufactured from LHM. This effect could be due to the slight increase in protein degradation of this cheese;

(iii) the number of starter bacteria are higher in cheese manufactured from enzyme-treated milk;

(iv) the increased level of glucose and galactose in the whey could be a desirable feature for further processing, i.e. in the production of syrup (see Table 3.10);

(v) although, with the cheese from the enzyme-treated milk the hydrolysis of protein (liberation of SN and degradation of casein fractions) was slightly more as compared with the control cheese, but such effect could not be termed as accelerated cheese ripening, because such cheese at 2-4 months old must be comparable to naturally matured cheese of 6-8 months old.

TABLE 3.10

Composition of Cheese whey (from LHM and control milk) at drainage time

Starter culture \ Treatment		pH	FPD	Fat (%)	Protein (%)	glucose (%)	Galactose (%)	Lactose (%)	TS (%)
850	C	5.86	0.564	0.20	0.91	-	-	4.26	6.95
	E_1	5.92	0.621	0.20	0.89	0.27	0.21	3.47	6.97
870	C	5.56	0.607	0.25	0.82	-	-	4.38	7.01
	E_1	5.73	0.655	0.20	0.83	0.41	0.35	3.03	6.93
890	C	6.18	0.556	0.25	0.79	-	-	4.52	6.95
	E_1	6.18	0.641	0.25	0.78	0.57	0.47	2.92	6.96
850	C	6.21	0.551	0.20	0.88	-	-	4.57	6.92
	E_2	6.11	0.695	0.20	0.92	0.96	0.83	1.95	6.98
870	C	6.15	0.553	0.20	0.92	-	-	4.47	7.02
	E_2	5.65	0.704	0.25	0.91	0.81	0.83	1.74	7.08
890	C	6.18	0.553	0.20	0.74	-	-	4.39	6.96
	E_2	6.15	0.681	0.20	0.76	0.97	0.80	1.87	6.95

Footnote: E_1 and E_2 are different β-D-galactosidase preparations.

C is control.

TS is total solids.

CHAPTER FOUR

Production, analysis and evaluation of Cheddar cheese with added neutral proteinase

4.1 Introduction

Different microbial enzyme preparations were added to Cheddar cheese curd in order to accelerate the ripening process, i.e. flavour, body and texture qualities. Proteinases and/or lipases have been reported to be the main enzymes used to accelerate Cheddar cheese ripening (Kosikowski & Iwasaki,1975; Sood & Kosikowski, 1979; Law, 1981 and Law & Wigmore, 1982). Law & Wigmore (1982) have used bacterial neutral proteinase and fungal acid proteinase to accelerate the ripening of English Cheddar cheese, concluded that a 20% increase in the extent of proteolysis of Cheddar cheese (2 months old) had been achieved by the addition of neutral proteinase and the level of flavour intensity had been accelerated by the equivalent of an additional ripening time of 2 months. The Neutrase treated cheese had stronger flavour, softer body and was more brittle than the untreated cheese, and was not detected in the cheese when the enzyme was added at low concentration. However, by increasing the amount of neutral proteinase in the cheese, enhanced proteolysis was observed and the cheese became more bitter, softer, very crumbly and brittle.

The use of fungal acid proteinase even at low concentrations produced cheese with the following defects:

> bitter flavour, soft body, crumbly and brittle compared with untreated cheese (Law & Wigmore, 1982).

However, the same workers attributed the faults in accelerated ripening of Cheddar cheese treated with acid proteinase to the lack of typical flavour development in the cheese and intense bitterness due to the high proteolytic activity of the enzyme. The latter fault, i.e. bitter taste, could be associated with the greater specifity of the acid proteinase to cleave the bonds near the aromatic and hydrophobic side chains in the protein molecule to produce more bitter peptides (Law & Wigmore, 1982).

It can be observed from the relevant information reviewed in Chapter One

that the work of Law and co-workers at the NIRD in the United Kingdom that ARCC (natural Cheddar cheese) could be produced by the addition of proteinase enzymes. The use of acid proteinase tends to produce cheese which is not acceptable, e.g. very bitter even at low concentrations, and hence it was decided to evaluate the efficacy of a food grade neutral proteinase enzyme on the quality of ARCC. In view of the complexity of the planned experiments, this chapter will be divided into two sections.

SECTION 1

In this section Cheddar cheese was produced by using starter cultures 850 and 890 as illustrated in Chapter 3. However, starter culture 870 was excluded from the present study because the judgement of three professional graders suggested that such starter causes bitterness in cheese (control and LHM) compared with starter cultures 850 and 890.

Neutrase was added to the curd at the following rates:

0.001, 0.002, 0.005 and 0.01% (w/w)

In an attempt to find the suitable level of enzyme which could be recommended for the production of an acceptable ARCC. Since the temperature during the ripening period could influence the rate of cheese ripening, two temperatures 10° and 13°C, were chosen to evaluate the effect of temperature on the activity of the enzyme used.

SECTION 2

In this section Cheddar cheese was produced by using starter culture 850. In the judgement of three professional graders, this starter produced least defects in cheese (ARCC and control) compared with starter culture 890. In order to overcome the day to day variation in the quality of milk (chemical and microbiological) Cheddar cheese was produced in a 2255 litre vat, and Neutrase (i.e. coated on salt NaCl granules) was added at the following rates:

0.002, 0.003 and 0.005% (w/w)

in order to evaluate the quality of ARCC.

SECTION 1

4.2 Materials and Methods

4.2.1 Neutral Proteinase

The Neutrase (NI) used had a reported specific acitivity of 1.5 g Anson Unit (AU)/g as described in 2.2.6.2 and it was mixed with the salt prior to its addition to the milled curd at the rate of 0.001, 0.002, 0.005 and 0.01% (w/w) of the curd.

4.2.2 Manufacture of Cheese

The cheesemaking system followed in this experiment was as described in Figure 2.2.

4.2.3 Cheese Ingredients

The cheese ingredients or additives such as starter culture, coagulant and salt which were used in this experiment were as mentioned in 2.2.2, 2.2.3 and 2.2.4.

4.2.4 Ripening Tmperature

The cheese was ripened at 10 and 13°C for 12 months.

4.2.5 Chemical Analysis of the Cheese

The fat, moisture, salt, pH, total nitrogen, soluble nitrogen and casein hydrolysis in cheese were determined as described in 2.3.1, 2.3.2, 2.3.4, 2.3.5, 2.3.6 and 2.3.8.

4.2.6 Organoleptic Assessment

The cheese was assessed organoleptically by eight panelists from the Dairy Technology Department (WSAC) and the official grader of the Company of Scottish Cheesemakers Ltd., Glasgow, UK. The scores were awarded on an eleven-point hedonic scale ranging from 'Like Extremely' (10) to 'Dislike Extremely' (0). The cheese qualities evaluated by the panelists were:

taste, odour or smell, bitterness, firmness, openness and smoothness.

4.3 Results and Discussion

4.3.1 Effect of Neutrase addition on the Chemical Composition of one-day-old cheese

Cheddar cheese (16 trials) was produced as illustrated in Figure 2.2 and the amounts of Neutrase enzyme added to the milled curd were 0.001, 0.002, 0.005 and 0.01% (w/w) respectively.

The chemical composition of one-day-old cheese (control and Neutrase treated) is summarised in Table 4.1, and it can be observed that all the cheeses comply with existing legal standards in the United Kingdom (e.g. maximum 39% moisture and minimum 48% fat in dry matter). Slight variation in the composition of cheese (control and experimental) was observed, i.e.:

	Moisture	Fat	Protein	Salt	pH
Control	35.48-36.43	32.20-33.70	22.55-24.83	1.49-1.76	5.22-5.32
Experimental	33.90-35.97	32.60-33.70	22.74-24.93	1.43-1.60	5.22-5.32

In the present study, the moisture content in the cheese averaged 35.4% which is a typical feature in Scottish Cheddar cheese and such cheese requires a long ripening period; hence, the efficacy of accelerated ripening could be properly evaluated by mixing different levels of Neutrase to the milled curd.

The pH measurements of these one-day old cheeses were rather high, but from preliminary experiments it was observed that the DVI starter cultures maintain acid development during the early stages of the ripening period and if the cheddared curd was milled above 0.6% lactic acid, the cheese tended to be acidic.

4.3.2 Effect of Neutrase addition on the pH development in the cheese

Table 4.2 illustrates the pH measurement of Cheddar cheese made from curd with and without added Neutrase at the following intervals:

2, 4, 6 and 12 months.

All the cheeses at 2 months of age had the lowest pH measurements due to the starter activity in cheese and the pH level reached the highest level after 12 months ripening. The pH ranges in cheeses over the ripening period were as follows:

(a) Control (10°C) - 5.04 - 5.67

(b) Control (13°C) - 5.03 - 6.49

(c) Experimental (10°C) - 5.10 - 5.99

(d) Experimental (13°C) - 5.09 - 6.66

TABLE 4.1

Chemical composition of one day old Cheddar cheese made from curd with and without added Neutrase

Starter culture	Treatment	Moisture %	Fat %	FDM %	MFFC %	TN %	SN %	Salt %	SM %	pH
850	C	35.56	33.70	52.30	53.63	22.68	0.93	1.65	4.64	5.26
	(0.001)	35.30	33.10	51.16	52.77	23.24	0.83	1.43	4.05	5.27
	C	35.61	33.10	51.41	53.23	24.57	0.94	1.66	4.66	5.22
	(0.002)	33.90	33.70	50.98	51.13	24.71	1.04	1.56	4.60	5.22
	C	35.48	33.60	52.08	53.43	22.62	1.01	1.69	4.76	5.27
	(0.005)	34.72	33.20	50.86	51.08	23.32	1.05	1.60	4.61	5.32
	C	35.85	33.10	51.60	53.59	24.03	1.01	1.63	4.55	5.26
	(0.01)	33.97	33.60	50.89	51.16	24.33	1.07	1.58	4.65	5.24
890	C	36.43	32.20	50.73	53.73	23.68	0.97	1.71	4.69	5.25
	(0.001)	35.97	32.60	50.91	53.37	22.74	1.02	1.55	4.31	5.32
	C	35.83	32.70	50.96	53.24	24.49	1.07	1.71	4.77	5.30
	(0.002)	35.65	33.20	51.59	52.58	24.93	1.03	1.51	4.24	5.28
	C	35.98	33.20	51.86	53.86	22.55	0.97	1.76	4.89	5.27
	(0.005)	35.17	33.10	51.06	52.57	23.87	0.99	1.53	4.35	5.31
	C	36.24	32.70	51.29	53.85	24.83	1.01	1.49	4.11	5.32
	(0.01)	35.56	32.70	50.74	52.83	24.59	1.06	1.49	4.19	5.23

Footnote: Figures in parenthesis represent rate (% w/w) of Neutrase enzyme added

C = the control (untreated) cheese

TABLE 4.2

The hydrogen ion concentration (pH) during the ripening of Cheddar cheese made from curd with and without added Neutrase

Treatment	Ripening temperature (°C)	Starter culture 850				Starter culture 890			
		Ripening period (months)				Ripening period (months)			
		2	4	6	12	2	4	6	12
C	10	5.07	5.11	5.15	5.51	5.08	5.15	5.20	5.54
(0.001)		5.15	5.14	5.24	5.67	5.16	5.12	5.25	5.62
C	13	5.09	5.11	5.20	6.43	5.11	5.17	5.11	6.19
(0.001)		5.09	5.29	5.51	6.32	5.18	5.21	5.28	6.32
C	10	5.17	5.12	5.15	5.67	5.11	5.13	5.10	5.47
(0.002)		5.18	5.21	5.31	5.86	5.20	5.21	5.27	5.93
C	13	5.12	5.03	5.13	6.49	5.12	5.20	5.23	6.26
(0.002)		5.16	5.36	5.48	6.53	5.24	5.27	5.33	6.48
C	10	5.04	5.13	5.16	5.45	5.13	5.10	5.15	5.56
(0.005)		5.16	5.25	5.28	5.99	5.16	5.20	5.41	5.78
C	13	5.10	5.07	5.03	6.31	5.19	5.17	5.19	6.20
(0.005)		5.19	5.30	5.61	6.47	5.20	5.23	5.43	6.61
C	10	5.13	5.16	5.18	5.55	5.13	5.20	5.18	5.63
(0.01)		5.10	5.28	5.37	5.81	5.29	5.30	5.41	5.93
C	13	5.08	5.11	5.09	6.26	5.17	5.15	5.08	6.31
(0.01)		5.15	5.27	5.50	6.62	5.30	5.30	5.42	6.66

Footnote: C = control
Figures in parenthesis represent amount (% w/w) of Neutrase added

cont'd.....

Table 4.2 (cont'd)

Source of variation	DF	MS	VR
C.TR.	1	0.836895	199.522***
Storage	3	5.814366	1386.189***
Enzyme	3	0.031382	7.482**
Starter	1	0.000038	0.009
Temperature	1	1.294037	308.508***
C.TR. X Storage	3	0.056963	13.580**
C.TR. X Enzyme	3	0.025715	6.131*
Storage X Enzyme	9	0.005681	1.354
C.TR. X Starter	1	0.001070	0.255
Storage X Starter	3	0.011103	2.647
Enzyme X Starter	3	0.010142	2.418
C.TR. X Temperature	1	0.012601	3.004
Storage X Temperature	3	0.931713	222.127***
Enzyme X Temperature	3	0.002384	0.568
Starter X Temperature	1	0.001582	0.377
C.TR. X Storage X Enzyme	9	0.005204	1.241
C.TR. X Storage X Starter	3	0.014542	3.467
C.TR. X Enzyme X Starter	3	0.003524	0.840
Storage X Enzyme X Starter	9	0.002804	0.669
C.TR. X Storage X Temperature	3	0.018703	4.459*
C.TR. X Enzyme X Temperature	3	0.002072	0.494
Storage X Enzyme X Temperature	9	0.002281	0.544
C.TR. X Starter X Temperature	1	0.005126	1.222
Storage X Starter X Temperature	3	0.005047	1.203
Enzyme X Starter X Temperature	3	0.003445	0.821
C.TR. X Storage X Enzyme X Starter	9	0.002638	0.629
C.TR. X Storage X Enzyme X Temperature	9	0.002771	0.661
C.TR. X Storage X Starter X Temperature	3	0.014557	3.470
C.TR. X Enzyme X Starter X Temperature	3	0.003522	0.840
Storage X Enzyme X Starter X Temperature	9	0.001962	0.468
Residual	9	0.004194	
Total	127	0.183043	

* Significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

cont'd.....

Table 4.2(cont'd)

Table	C.TR.	Storage	Enzyme	Starter	Temp.	C.TR. storage	C.TR. enzyme
REP	64	32	32	64	64	16	16
SED	0.01145	0.01619	0.01619	0.01145	0.01145	0.02290	0.02290

Table	Storage enzyme	C.TR. starter	Storage starter	Enzyme starter	C.TR. temp.	Storage temp.	Enzyme temp.
REP	8	32	16	16	32	16	16
SED	0.03238	0.016169	0.02290	0.02290	0.01619	0.02290	0.02290

Table	Starter temp.	C.TR. storage enzyme	C.TR. storage starter	C.TR. enzyme starter	Storage enzyme starter	C.TR. storage temp.	C.TR. enzyme temp.
REP	32	4	8	8	4	8	8
SED	0.01619	0.04580	0.03238	0.03238	0.04580	0.03238	0.03238

Table	Storage enzyme temp.	C.TR. starter temp.	Storage starter temp.	Enzyme starter temp.	C.TR. storage enzyme starter	C.TR. storage enzyme temp.	C.TR. storage starter temp.
REP	4	16	8	8	2	2	4
SED	0.04580	0.02290	0.03238	0.03238	0.06476	0.06476	0.04580

Table	C.TR. enzyme starter temp.	Storage enzyme starter temp.
REP	4	2
SED	0.04580	0.06476

Footnotes: Temp. = ripening temperature
Storage = ripening period
C.TR. = control versus treated.

Such change in pH values of cheese is normal during ripening, i.e. decrease after production followed by an increase which is due mainly to biochemical reactions taking place as the ripening period progresses. For example, the hydrolysis of protein helps to neutralise some of the hydrogen ion concentration present in the cheese and as a result the level of pH increases (Al-Obaidi, 1980). However, optimum biochemical activities can be observed in cheeses ripened at 13°C (control and experimental) compared with 10°C.

The following comclusions may be drawn from Table 4.2:

(i) The enzyme treated cheese had a higher pH value than the control and the difference was highly significant ($p < 0.001$). At 12 months of age the experimental cheese with 0.001% (w/w) Neutrase and 0.01 (w/w) Neutrase at 2 months old (starter culture 850), the control had higher pH than the experimental.

(ii) The pH value of the experimental cheese progressively increased in relation to: age of the cheese, temperature of ripening (e.g. 13°C) and the amount of enzyme added to the curd and it was highly significant ($p < 0.001$).

(iii) Other interactions, which were observed to effect the level of pH in cheese during ripening, were the following variants:

C.TR x amount of enzyme added to the curd and C.TR x ripening period x ripening temperature ($p < 0.05$), C.TR x ripening period and the ripening period x ripening temperature at $p < 0.01$.

The following could be concluded from the analysis of variance:

Firstly, the pH value of the Neutrase treated cheese increased dramatically compared with the control, and the increase was influence by the amount of enzyme added and the duration of ripening period. The enhanced protein breakdown in the experimental cheese might have increased the level of aldehydes, ketones and volatile flavour compounds, i.e. methanethiol, methanol,acetone, butanone, ethanol and 2-pentanone. The level of the above compounds have been shown to increase during the ripening period of Cheddar cheese (Manning, 1978) and affect the buffering system in the cheese, i.e. neutralising some of the hydrogen ions (Kosikowski, 1977).

Secondly, high pH values could be observed in the control and experimental cheeses ripened at 13°C compared with 10°C. The slight increase in the temperature during ripening, e.g. 3°C, had enhanced the enzymatic activity to hydrolyse more protein fractions which released more alkyl compounds.

4.3.3 The effect of Neutrase on the nitrogenous substances in cheese

4.3.3.1 Total nitrogen

The amount of total nitrogen (expressed as % of protein) present in Cheddar cheese made from curd with and without added Neutrase 'during 6 months of ripening) are illustrated in Table 4.3. The grand mean of total nitorgen in the cheese in relation to the different treatments could be summarised as follows:

	Control	Neutrase treated cheese		
C.TR	23.08%		22.70%	
Ripening period	2 months	4 months	6 months	
	22.84%	22.91%	22.93%	
Enzyme levels	0.001%(w/w)	0.002%(w/w)	0.005:(w/w)	0.01%(w/w)
	23.06%	22.68%	23.15%	22.67%
Starter culture	850		890	
	22.94%		22.84%	
Ripening temperature	10°C		13°C	
	22.81%		22.98%	

However, from the statistical analysis (see Table 4.3) and the grand mean figures mentioned above, the following aspects could be observed:

(i) The grand mean of the total nitrogen in the Neutrase treated cheese was less than that of the untreated cheese and it was significant at $p < 0.001$.

(ii) Although the amount of total nitrogen in the cheese varied during the ripening period, no significant effect was observed.

(iii) The level of added enzyme had an effect on the total nitrogen content in the cheese, but no trend could be observed, which could be attributed to the syneresis which took place in the

TABLE 4.3

Total nitrogen (calculated as protein and expressed as percentage) content of Cheddar cheese made from curd with and without added Neutrase

Treatment	Ripening temperature (°C)	Starter culture 850			Starter culture 890		
		Ripening period (months)			Ripening period (months)		
		2	4	6	2	4	6
C	10	22.92	22.87	22.79	22.84	22.87	22.98
(0.001)		23.73	23.89	23.77	21.89	21.88	21.69
C	13	23.52	23.59	23.83	22.80	22.93	22.84
(0.001)		23.25	23.33	23.38	23.26	23.31	23.22
C	10	22.80	22.93	23.35	21.97	22.13	22.37
(0.002)		23.00	23.12	23.43	21.28	21.41	21.42
C	13	23.88	22.98	22.72	22.54	22.57	22.62
(0.002)		23.85	23.87	23.65	22.55	22.62	22.34
C	10	22.91	23.02	23.15	23.62	23.66	23.81
(0.005)		22.99	23.11	23.48	23.35	23.53	23.38
C	13	23.16	23.32	23.43	23.54	23.41	23.11
(0.005)		22.57	22.73	22.60	22.58	22.47	22.77
C	10	23.58	23.55	23.10	23.40	23.47	23.64
(0.01)		20.90	21.20	21.16	22.49	22.33	22.59
C	13	22.67	22.68	22.47	23.62	23.63	23.37
(0.01)		21.15	21.37	21.53	23.31	23.19	23.67

Footnote: C = control
Figures in parenthesis represent amount (% w/w) of Neutrase added

cont'd.....

Table 4.3(cont'd)

Source of variation	DF	MS	VR
C.TR.	1	3.51133	206.317***
Storage	2	0.06397	3.759
Enzyme	3	1.51192	88.837***
Starter	1	0.26250	15.424**
Temperature	1	0.67670	39.761***
C.TR. X Storage	2	0.01002	0.589
C.TR. X Enzyme	3	1.96387	115.392***
Storage X Enzyme	6	0.00543	0.319
C.TR. X Starter	1	0.17002	9.990*
Storage X Starter	2	0.01602	0.941
Enzyme X Starter	3	5.72796	336.560***
C.TR. X Temperature	1	0.51626	30.334**
Storage X Temperature	2	0.03888	2.284
Enzyme X Temperature	3	0.92037	54.079***
Starter X Temperature	1	0.75260	44.221***
C.TR. Storage X Enzyme	6	0.04915	2.888
C.TR. X Storage X Starter	2	0.01306	0.768
C.TR. X Enzyme X Starter	3	1.37254	80.647***
Storage X Enzyme X Starter	6	0.02373	1.395
C.TR. X Storage X Temperature	2	0.01240	0.728
C.TR. X Enzyme X Temperature	3	0.79878	46.934***
Storage X Enzyme X Temperature	6	0.04821	2.833
C.TR. X Starter X Temperature	1	0.77041	45.268***
Storage X Starter X Temperature	2	0.00235	0.138
Enzyme X Starter X Temperature	3	0.42975	25.251***
C.TR. X Storage X Enzyme X Starter	6	0.01340	0.787
C.TR. X Storage X Enzyme X Temperature	6	0.00340	0.200
C.TR. X Storage X Starter X Temperature	2	0.06544	3.845
C.TR. X Enzyme X Starter X Temperature	3	0.70539	41.447***
Storage X Enzyme X Starter X Temperature	6	0.01984	1.166
Residual	6	0.01702	
Total	95	0.51028	

* Significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

cont'd.....

Table 4.3(cont'd)

Table	C.TR.	Storage	Enzyme	Starter	Temp.	C.TR. Storage	C.TR. Enzyme
REP	48	32	24	48	48	16	12
SED	0.0266	0.0326	0.0377	0.0266	0.0266	0.0461	0.0533

Table	Storage enzyme	C.TR. starter	Storage starter	Enzyme starter	C.TR. temp.	Storage temp.	Enzyme temp.
REP	8	24	16	12	24	16	12
SED	0.0652	0.0377	0.0461	0.0533	0.0377	0.0461	0.0533

Table	Starter temp.	C.TR. storage enzyme	C.TR. storage starter	C.TR. enzyme starter	Storage enzyme starter	C.TR. storage temp.	C.TR. enzyme temp.
REP	24	4	8	6	4	8	6
SED	0.0377	0.0922	0.0652	0.0753	0.0922	0.0652	0.0753

Table	Storage enzyme temp.	C.TR. starter temp.	Storage starter temp.	Enzyme starter temp.	C.TR. storage enzyme starter	C.TR. storage enzyme temp.	C.TR. storage starter temp.
REP	4	12	8	6	2	2	4
SED	0.0377	0.0922	0.0652	0.0753	0.0922	0.0652	0.0753

Table	C.TR. enzyme starter temp.	Storage enzyme starter temp.
REP	3	2
SED	0.1065	0.1305

Footnotes: Temp. = ripening temperature
Storage = ripening period
C.TR. = control versus treated.

experimental cheese.

(iv) The grand mean of the total nitrogen in the cheese made with starter culture 890 was slightly less compared with starter culture 850 ($p < 0.01$). This could be attributed to differences in the proteolytic activity of the starter culture strain.

(v) The cheese, which was ripened at 13°C had a higher grand mean of the total nitrogen content than that ripened at 10°C ($p < 0.01$).

(vi) Very strong interactions ($p < 0.01$) were found between: enzyme x starter culture, enzyme x ripening temperature, starter culture x ripening temperature, C.TR x enzyme x starter culture, C.TR x starter culture x ripening temperature, enzyme x starter culture x ripening temperature and significant interactions between C.TR x starter culture and C.TR x ripening temperature at $p < 0.05$ and 0.01 respectively. These interactions indicated the degree of effect of the above factors on the total nitrogen content in the cheese.

The variation in the total nitrogen content of the cheese could be associated with the high protein breakdown and syneresis that took place in the experimental cheese which affect the amount of the moisture and the protein in the cheese.

4.3.3.2 The extent of protein hydrolysis during the ripening of Cheddar cheese

The extent of protein hydrolysis in cheese during the ripening period is used as an index of the degree of ripening of the cheese. In this study the protein hydrolysis was monitored as:

firstly, the level or amount of soluble nitrogen present in the cheese, and

secondly, the extent of casein hydrolysis during 360 days of ripening.

(A) The level of soluble nitrogen in the cheese

Table 4.4 and Figure 4.1 illustrate the level of soluble nitrogen present is the cheese during 12 months of ripening period and the following can be observed:

TABLE 4.4

The extent of protein hydrolysis (calculated as % soluble nitrogen (SN) and expressed as % protein) during the ripening period of Cheddar cheese made from curd with and without added Neutrase

Treatment	Ripening temperature (°C)	Starter culture 850				Starter culture 890			
		Ripening period (months)				Ripening period (months)			
		2	4	6	12	2	4	6	12
C	10	3.40	5.04	5.24	5.93	3.42	4.85	5.03	6.37
(0.001)		3.77	6.11	7.23	7.66	4.42	6.36	6.38	7.43
C	13	3.58	5.74	6.54	6.97	4.23	5.41	5.59	6.56
(0.001)		4.15	7.11	8.24	8.40	4.88	6.80	7.02	8.21
C	10	3.07	4.97	5.07	6.28	3.19	4.92	5.57	6.04
(0.002)		4.85	8.00	7.29	8.15	5.01	7.87	7.92	8.34
C	13	3.53	5.57	5.88	6.94	3.48	5.23	6.14	6.92
(0.002)		5.42	9.06	8.77	8.85	5.81	7.89	8.13	8.69
C	10	3.07	4.67	5.11	6.18	3.14	4.80	4.91	6.01
(0.005)		7.59	10.05	9.35	8.78	7.09	9.92	9.43	9.19
C	13	3.42	5.37	5.91	6.63	3.56	5.26	5.61	7.09
(0.005)		8.49	10.72	10.39	9.70	8.86	10.48	10.07	10.16
C	10	3.02	4.45	4.72	6.36	3.16	4.81	5.06	6.30
(0.01)		9.42	11.25	10.72	9.82	9.53	10.99	10.75	9.79
C	13	3.32	5.00	5.72	6.58	3.63	5.17	5.62	6.95
(0.01)		10.03	11.34	10.99	10.10	10.15	11.41	11.24	9.58

Footnote: C = control

Figures in parenthesis represent amount (% w/w) of Neutrase added

cont'd.....

Table 4.4 (cont'd)

Source of variation	DF	MS	VR
C.TR.	1	358.75146	5329.117***
Storage	3	42.14816	626.095***
Enzyme	3	22.20874	329.902***
Starter	1	0.01221	0.181
Temperature	1	12.29459	182.632***
C.TR. X Storage	3	3.54738	52.695***
C.TR. X Enzyme	3	28.78769	427.630***
Storage X Enzyme	9	0.89070	13.231***
C.TR. X Starter	1	0.05908	0.878
Storage X Starter	3	0.24951	3.706
Enzyme X Starter	3	0.06507	0.967
C.TR. X Temperature	1	0.02231	0.331
Storage X Temperature	3	0.07303	1.085
Enzyme X Temperature	3	0.18309	2.720
Starter X Temperature	1	0.12563	1.866
C.TR. X Storage X Enzyme	9	1.24648	18.516***
C.TR. X Storage X Starter	3	0.03164	0.470
C.TR. X Enzyme X Starter	3	0.02170	0.322
Storage X Enzyme X Starter	9	0.19806	2.942
C.TR. X Storage X Temperature	3	0.08308	1.234
C.TR. X Enzyme X Temperature	3	0.08689	1.291
Storage X Enzyme X Temperature	9	0.03114	0.463
C.TR. X Starter X Temperature	1	0.01781	0.265
Storage X Starter X Temperature	3	0.16487	2.449
Enzyme X Starter X Temperature	3	0.09053	1.345
C.TR. X Storage X Enzyme X Starter	9	0.05151	0.765
C.TR. X Storage X Enzyme X Temperature	9	0.02921	0.434
C.TR. X Storage X Starter X Temperature	3	0.01482	0.220
C.TR. X Enzyme X Starter X Temperature	3	0.03468	0.515
Storage X Enzyme X Starter X Temperature	9	0.02277	0.338
Residual	9	0.06732	
Total	127	5.41328	

* Significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

cont'd.....

Table 4.4 (cont'd)

Table	C.TR.	Storage	Enzyme	Starter	Temp.	C.TR. Storage	C.TR Enzyme
REP	64	32	32	64	64	16	16
SED	0.0459	0.0649	0.0649	0.0459	0.0459	0.0917	0.0917

Table	Storage enzyme	C.TR. starter	Storage starter	Enzyme Starter	C.TR. temp.	Storage temp.	Enzyme temp.
REP	8	32	16	16	32	16	16
SED	0.1297	0.0649	0.0917	0.0917	0.0649	0.0917	0.0917

Table	Starter temp.	C.TR. storage enzyme	C.TR. storage starter	C.TR. enzyme starter	Storage enzyme starter	C.TR. storage temp.	C.TR. enzyme temp.
REP	32	4	8	8	4	8	8
SED	0.0649	0.1835	0.1297	0.1297	0.1835	0.1297	0.1297

Table	Storage enzyme temp.	C.TR. starter temp.	Storage starter temp.	Enzyme starter temp.	C.TR. storage enzyme starter	C.TR. storage enzyme temp.	C.TR. storage starter temp.
REP	4	16	8	8	2	2	4
SED	0.1835	0.0917	0.1297	0.1297	0.2595	0.2595	0.1835

Table	C.TR. enzyme starter temp.	Storage enzyme starter temp.
REP	4	2
SED	0.1835	0.2595

Footnotes: Temp. = ripening temperature.
Storage = ripening period.
C.TR. = control versus treated.

Legend to

Figure 4.2 Levels of soluble nitrogen present in Cheddar cheese made from curd with and without added Neutrase

Footnotes: Levels of Neutrase enzyme added were:

A - 0.001% w/w

B - 0.002% w/w

C - 0.005% w/w

D - 0.01% w/w

□ - Control cheese ripened at 10°C

■ - Control cheese ripened at 13°C

○ - Neutrase treated cheese ripened at 10°C

● - Neutrase treated cheese ripened at 13°C

Figure 4.2 (A) (cont'd)

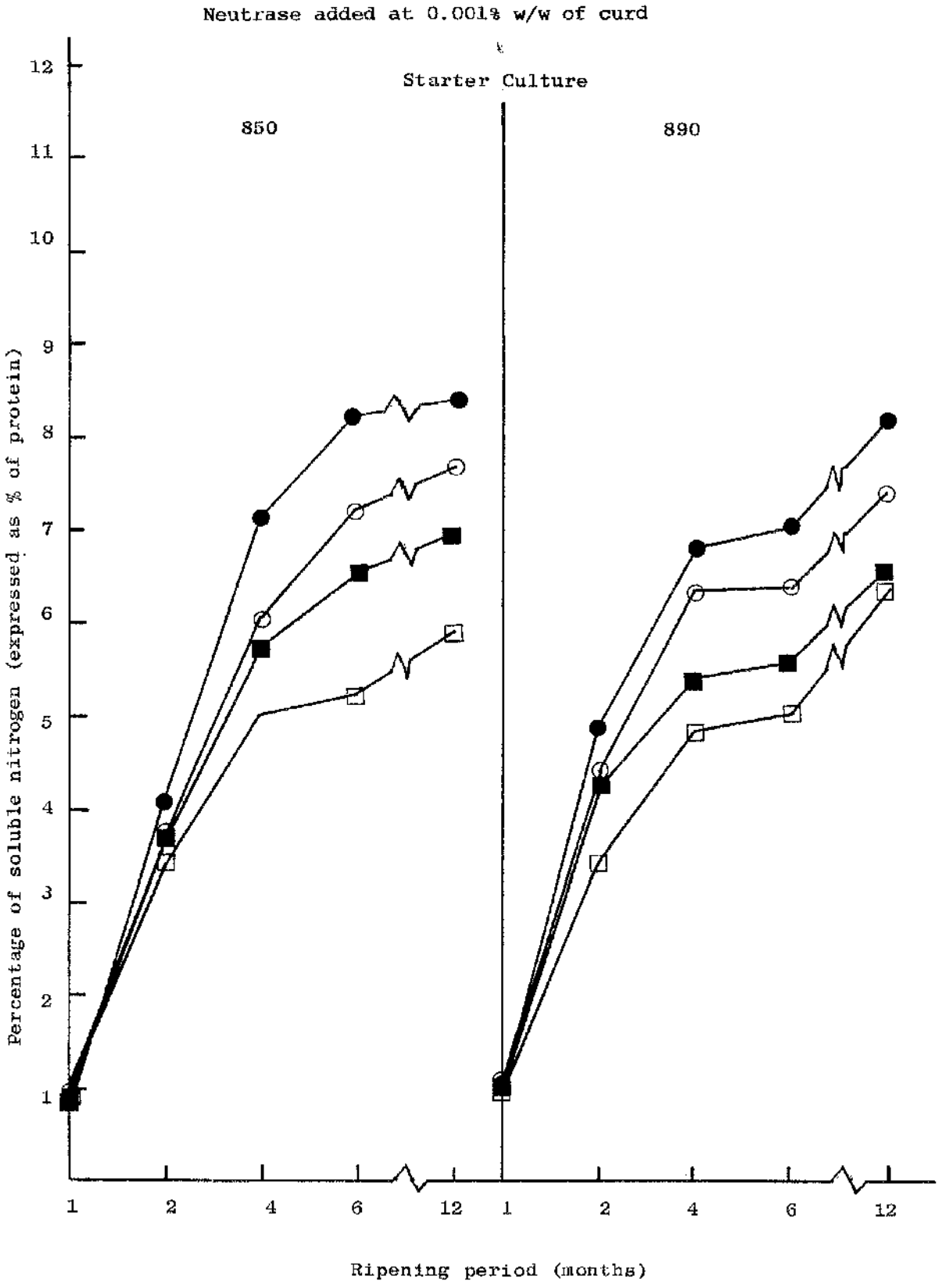


Figure 4.2 (b) (cont'd)

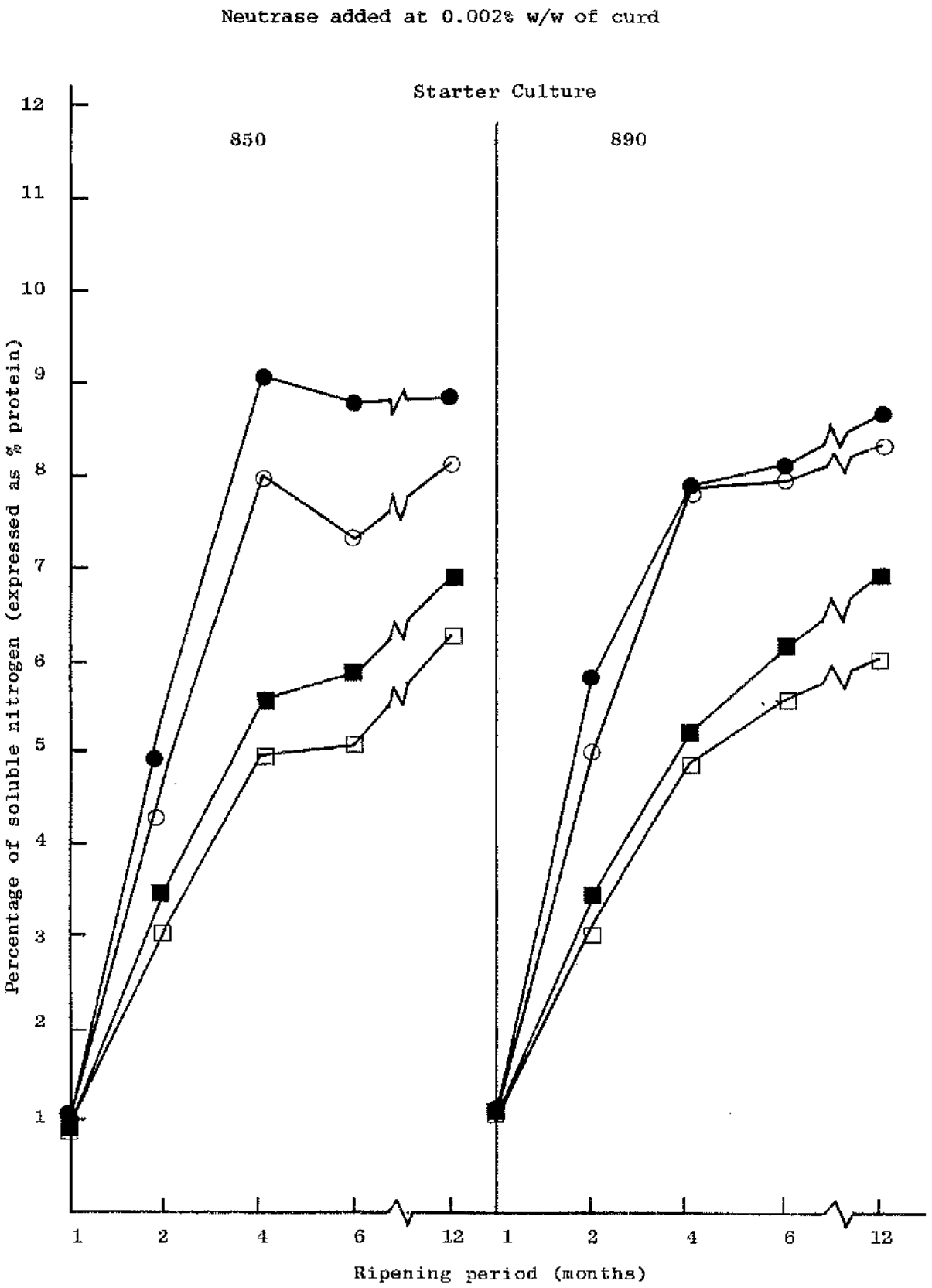


Figure 4.2 (C) (cont'd)

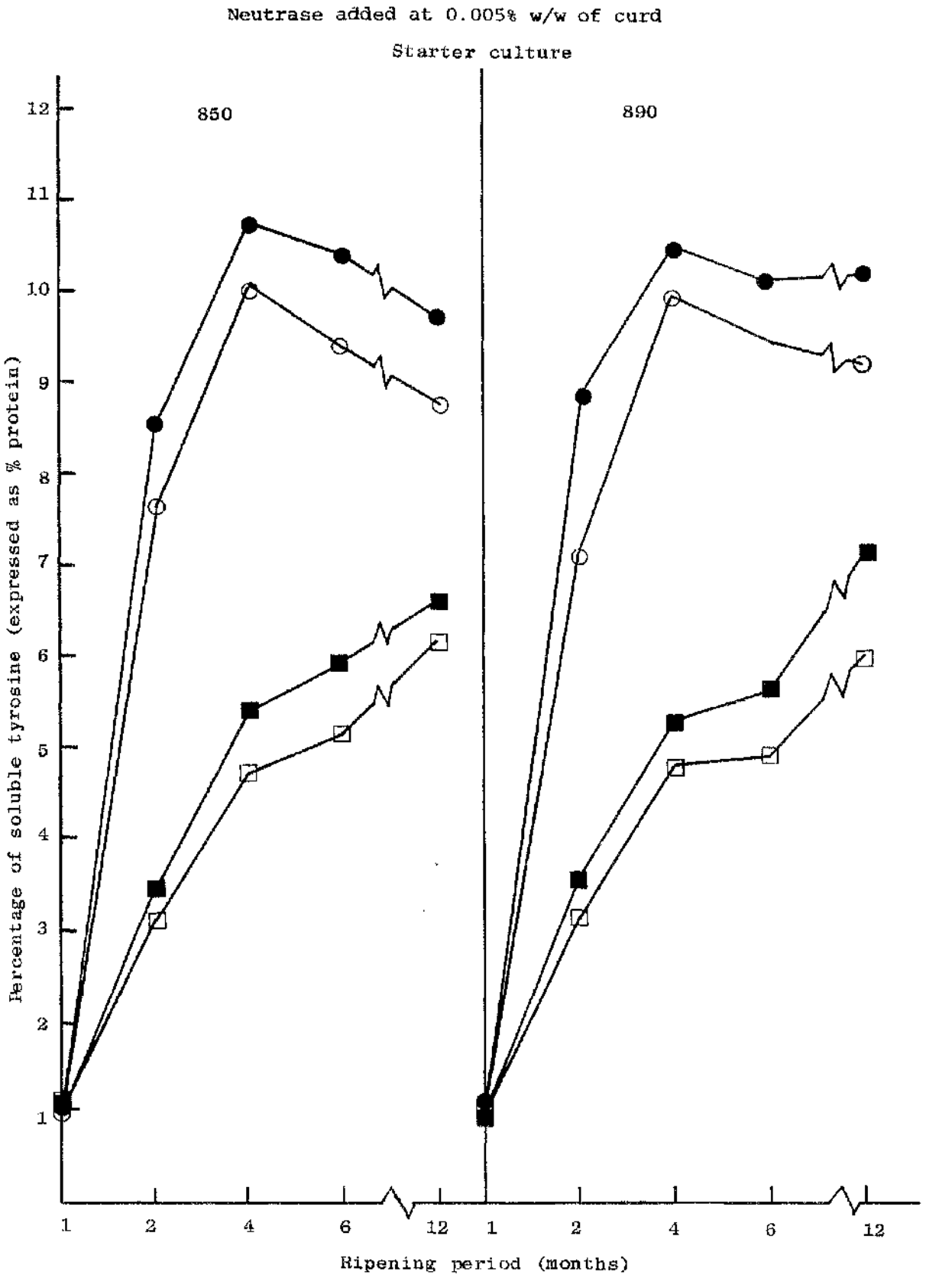
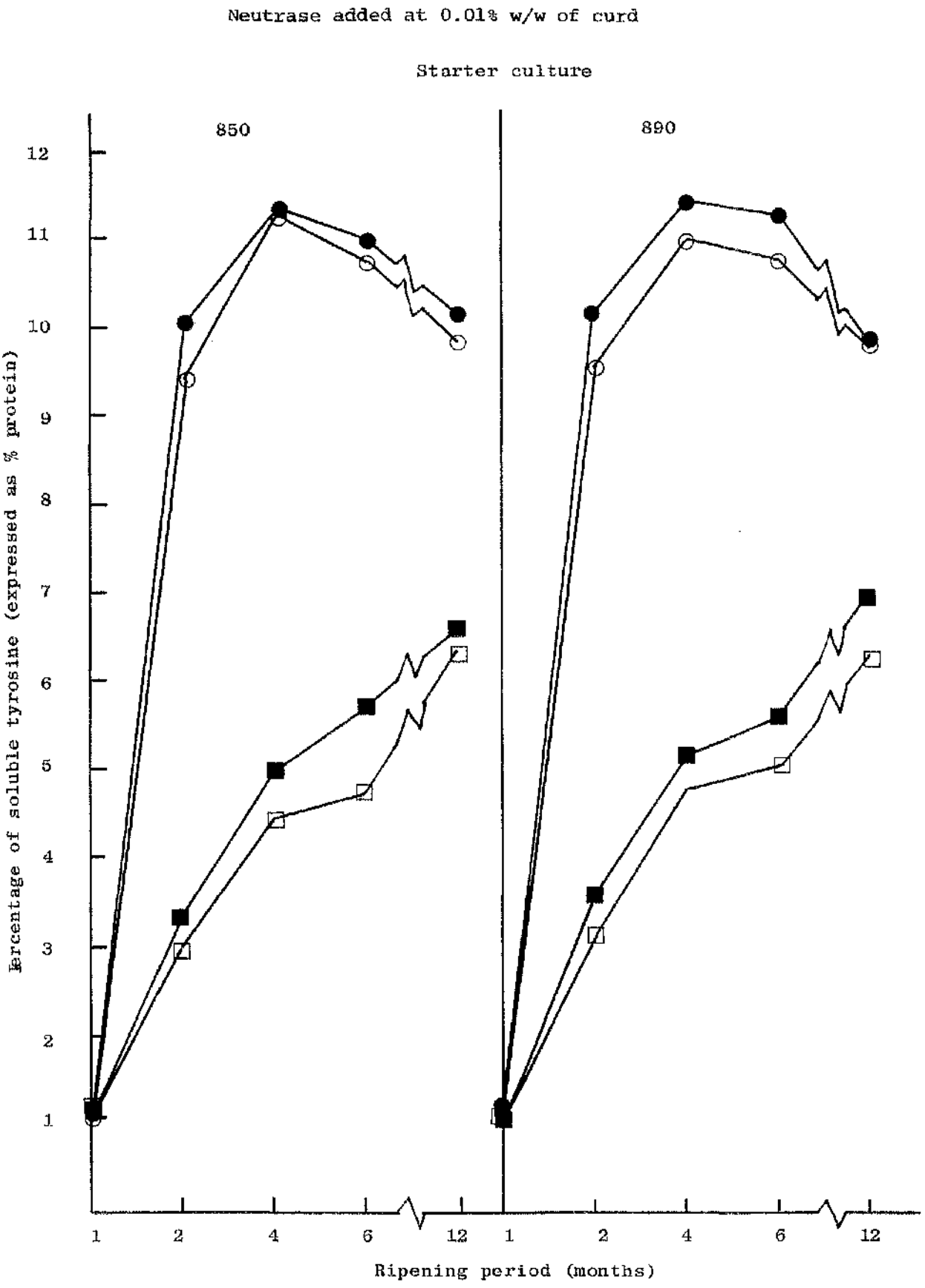


Figure 4.2 (D) (cont'd)



(i) The soluble nitrogen content in all the cheeses showed a similar trend where the level had increased as the cheese had matured ($p < 0.01$).

(ii) The enzyme treated cheese contained higher levels of soluble nitrogen compared with the control and the increase was highly significant ($p < 0.001$).

(iii) The amount of soluble nitrogen in the experimental cheese progressively increased in relation to the amount of Neutrase added to the curd. For example, the grand mean of soluble nitrogen content in cheese was dependent on the level of enzyme, i.e. rate of enzyme addition 0.001, 0.002, 0.005 and 0.01% (w/w) - soluble nitorgen 5.88, 6.34, 7.22 and 7.72% respectively.

(iv) The level of soluble nitrogen in all the cheeses was greater ($p < 0.001$) when the product was ripened at 13°C and the grand mean were:

Ripening temperature	10°C	13°C
Soluble nitrogen (%)	6.48	7.10

(v) Small increase in the amount of soluble nitrogen in the experimental cheese between 2-4 months was observed (for example, at 0.001 and 0.01% (w/w) enzyme concentrations and the increase after 2 months were 2.94 and 8.35 respectively compared with 2.34 and 1.83 after 4 months respectively), and this could be attributed to the self regulatory mechanism (i.e. reduced) that the enzyme possess at pH 5.2 (Law & Wigmore, 1982).

(vi) The reduction in the amount of soluble nitrogen in the experimental cheese after 6 and 12 months ripening periods could be attributed to the following factors:

firstly, the self regulating mechanism of the enzyme,

secondly, the utilisation by the cheese microflora (Manson, 1984), and

thirdly, the loss of some of the soluble nitrogen due to syneresis.

(vii) Very strong interactions ($p < 0.001$) were observed between: C.TR x ripening period, C.TR x enzyme, ripening period x enzyme and C.TR x ripening period x enzyme, which could indicate the

strong effect of the enzyme treatment and the ripening period on the liberation of soluble nitrogen in the cheese.

(B) Casein Hydrolysis

PAGE was used to study the extent of hydrolysis of casein fractions in the cheese during 360 days of ripening period, and the gels were scanned at 0.5 OD. Many peaks were observed in each electrogram and these peaks could be divided into the following mobility fractions:

(i) Slow mobility casein fractions which varied between 2-9 peaks.

(ii) β-casein fractions which varied between 1-4 peaks.

(iii) α_{-s}-casein fractions which varied between 1-13 peaks (see Appendices 1-8).

The extent of the casein(s) hydrolysis in the cheese (control and experimental) is illustrated in Tables 4.5 to 4.12, Figures 4.3 and 4.4, and Plates 4.1 to 4.9, and the overall pattern of casein degradation could be summarised as follows:

(i) Slow mobility casein fractions

The differences in the slow mobility casein fractions of Cheddar cheese made from curd with and without added Neutrase could be summarised as follows:

(a) The Neutrase treated cheese had a higher percentage of these fractions compared with the untreated cheese (60 out of 80 observations), which agreed with the early report of Law (1981) and Law & Wigmore (1982).

(b) Control and Neutrase treated cheese ripened at 13°C contained a higher percentage of slow mobility casein fractions compared with the cheese ripened at 10°C (22 and 20 out of 32 observations respectively).

(c) Cheddar cheese made from starter culture 850 contained a higher percentage of these fractions compared with starter culture 890 (49 out of 72 observations) which could possibly suggest that this culture (850) was more proteolytic than 890. A similar trend was observed when the soluble nitrogen content in the cheese was monitored.

(d) The percentage of these fractions progressivley increased during the ripening period, possibly originating from the β-casein fraction (Marcos et al., 1979, and Ridha, Crawford & Tamime, 1984).

However, some fluctuations in the number and the size of these fractions were observed during the ripening of the cheese which could be attributed to:

Firstly, the uneven distribution of Neutrase in the cheese, secondly, the experimental error of analysis, and thirdly, the hydrolysis of these fractions into free amino acids and/ or soluble nitrogen. Figure 4.5 illustrates the different ways in which the casein fractions were degraded.

TABLE 4.5

Total casein fractions in each mobility zone (expressed as a percentage of the area of the peaks of an electrogram) in Cheddar cheese made from curd with and without added Neutrase during 360 days of ripening

Ripening period (d)	Ripening temperature (°C)	Treat-ment	TS	E-C	Tβ	E-C	$T\alpha_s$	E-C
1	10	C	7.288	8.322	41.065	-7.934	51.647	-0.388
		E	15.610		33.131		51.259	
	13	C	7.288	8.322	41.065	-7.934	51.647	-0.388
		E	15.610		33.131		51.259	
60	10	C	16.543	7.057	23.528	0.576	59.930	-7.634
		E	23.600		24.104		52.296	
	13	C	22.071	-2.935	25.416	-4.069	52.515	7.004
		E	19.136		21.347		59.519	
120	10	C	17.862	3.956	19.704	-2.008	62.435	-1.868
		E	21.818		17.616		60.567	
	13	C	17.315	2.423	16.036	-4.006	66.659	1.573
		E	19.738		12.030		68.232	
180	10	C	22.408	2.023	20.600	-1.234	56.992	-0.788
		E	24.431		19.366		56.204	
	13	C	28.113	12.647	22.862	-9.473	49.024	-3.172
		E	40.760		13.389		45.852	
360	10	C	11.884	27.612	20.596	-6.752	68.022	-21.364
		E	39.496		13.844		46.658	
	13	C	24.323	-9.586	25.186	3.847	50.401	
		E	14.737		29.033		56.231	5.830

C = control
E = Neutrase enzyme treated cheese
Neutrase enzyme added at a rate of 0.001 % (w/w) starter culture used (850)
TS = total slow mobility casein fractions
Tβ = total β-casein fractions
$T\alpha_s$ = total α_s-casein fractions

TABLE 4.6

Total casein fractions in each mobility zone (expressed as a percentage of the area of the peaks of an electrogram) in Cheddar cheese made from curd with and without added Neutrase during 360 days of ripening

Ripening period (d)	Ripening temperature (°C)	Treat-ment	TS	E-C	Tβ	E-C	$T\alpha_s$	E-C
1	10	C	9.618	4.910	31.330	0.228	59.054	-5.139
		E	14.528		31.558		53.915	
	13	C	9.618	4.910	31.330	0.228	59.054	-5.139
		E	14.528		31.558		53.915	
60	10	C	18.372	2.097	23.546	-4.654	58.082	2.556
		E	20.469		18.892		60.638	
	13	C	15.110	8.301	27.485	-6.810	57.404	-1.489
		E	23.411		20.675		55.915	
120	10	C	20.709	-0.368	20.322	2.476	58.969	-2.108
		E	20.341		22.798		56.861	
	13	C	17.263	2.468	22.489	-9.717	60.250	7.244
		E	19.731		12.772		67.494	
180	10	C	26.953	-9.925	15.992	-4.913	57.055	14.840
		E	17.028		11.079		71.895	
	13	C	29.080	2.371	15.124	-9.667	55.797	7.122
		E	31.451		5.457		62.919	
360	10	C	14.877	27.408	23.882	-10.229	60.791	-16.729
		E	42.285		13.653		44.062	
	13	C	18.520	24.289	25.256	-6.299	56.224	-17.999
		E	42.809		18.957		38.225	

C = control
E = Neutrase enzyme treated cheese
Neutrase enzyme added at a rate of 0.002% (w/w) starter culture used (850)
TS = total slow mobility casein fractions
Tβ = total β-casein fractions
$T\alpha_s$ = total α_s-casein fractions

TABLE 4.7

Total casein fractions in each mobility zone (expressed as a percentage of the area of the peaks of an electrogram) in Cheddar cheese made from curd with and without added Neutrase during 360 days of ripening

Ripening period (d)	Ripening temperature (°C)	Treat-ment	TS	E-C	Tβ	E-C	$T\alpha_s$	E-C
1	10	C	14.014	-3.560	32.903	1.482	53.082	2.078
		E	10.454		34.385		55.160	
	13	C	14.014	-3.560	32.903	1.482	53.082	2.078
		E	10.454		34.385		55.160	
60	10	C	10.595	18.145	28.089	-12.130	61.319	-6.018
		E	28.740		15.959		55.301	
	13	C	19.329	5.472	25.706	-7.419	54.966	1.945
		E	24.801		18.287		56.911	
120	10	C	18.247	1.399	19.832	-2.450	61.922	1.050
		E	19.646		17.382		62.972	
	13	C	17.282	6.001	20.453	-15.611	62.266	9.609
		E	23.283		4.842		71.875	
180	10	C	28.580	8.181	13.764	-6.634	57.658	-1.548
		E	36.761		7.130		56.110	
	13	C	32.711	0.646	19.998	-15.130	47.280	14.496
		E	33.357		4.868		61.776	
360	10	C	16.711	36.054	19.036	-11.360	64.254	-24.690
		E	52.765		7.676		39.564	
	13	C	15.466	48.533	24.809	-14.189	59.726	-34.343
		E	63.999		10.620		25.383	

C = control
E = Neutrase enzyme treated cheese
Neutrase enzyme added at a rate of 0.005% (w/w)
starter culture used (850)
TS = total slow mobility casein fraction
Tβ = total -casein fractions
$T\alpha_s$ = total α_s-casein fractions

TABLE 4.8

Total casein fractions in each mobility zone (expressed as a percentage of the area of the peaks of an electrogram) in Cheddar cheese made from curd with and without added Neutrase during 360 days of ripening

Ripening period (d)	Ripening temperature (°C)	Treat-ment	TS	E-C	Tβ	E-C	$T\alpha_s$	E-C
1	10	C	10.218	2.851	37.965	-2.808	51.815	-0.041
		E	13.069		35.157		51.774	
	13	C	10.218	2.851	37.965	-2.808	51.815	-0.041
		E	13.069		35.157		51.774	
60	10	C	10.018	17.222	25.953	-12.916	64.03	-4.307
		E	27.240		13.037		59.723	
	13	C	13.354	16.664	24.901	-18.742	61.745	1.762
		E	30.018		6.159		63.507	
120	10	C	19.312	5.435	23.601	-14.535	57.087	9.101
		E	24.747		9.066		66.188	
	13	C	19.802	2.762	23.662	-16.155	56.535	13.394
		E	22.564		7.507		69.929	
180	10	C	25.664	6.208	19.446	-7.436	53.808	2.311
		E	31.872		12.010		56.119	
	13	C	17.994	21.192	21.020	-18.116	60.906	-2.997
		E	39.186		2.904		57.909	
360	10	C	8.454	40.501	19.674	-12.586	71.870	-27.912
		E	48.955		7.088		43.958	
	13	C	17.442	31.370	27.714	-22.770	54.846	-8.601
		E	48.812		4.944		46.245	

C = control
E = Neutrase enzyme treated cheese
Neutrase enzyme added at a rate of 0.01% (w/w) starter culture used (850)
TS = total slow mobility casein fractions
Tβ = total β-casein fractions
$T\alpha_s$ = total α_s-casein fractions

TABLE 4.9

Total casein fractions in each mobility zone (expressed as a percentage of the area of the peaks of an electrogram) in Cheddar cheese made from curd with and without added Neutrase during 360 days of ripening

Ripening period (d)	Ripening temperature (°C)	Treatment	TS	E-C	T^{β}	E-C	T^{α_s}	E-C
1	10	C	22.275	-1.104	35.041	0.472	42.030	1.287
		E	21.171		35.513		43.317	
	13	C	22.275	-1.104	35.041	0.472	42.030	1.287
		E	21.171		35.513		43.317	
60	10	C	11.903	-2.208	24.556	-0.900	63.486	3.164
		E	9.695		23.656		66.650	
	13	C	16.829	3.103	30.739	-2.909	52.432	-0.193
		E	19.932		27.830		52.239	
120	10	C	12.912	-0.632	26.599	-2.662	60.483	3.690
		E	12.280		23.937		63.785	
	13	C	13.214	1.381	20.691	-2.095	66.095	0.715
		E	14.595		18.596		66.810	
180	10	C	22.023	-1.800	19.971	4.985	58.007	-3.185
		E	20.223		24.956		54.822	
	13	C	15.283	8.333	25.088	-10.973	59.629	2.639
		E	23.616		14.115		62.268	
360	10	C	15.232	11.739	19.171	-1.380	65.597	-7.652
		E	26.971		17.791		57.945	
	13	C	20.464	10.816	23.977	0.484	55.559	-11.300
		E	31.280		24.461		44.259	

C = control
E = Neutrase enzyme treated cheese
Neutrase enzyme added at a rate of 0.001% (w/w)
starter culture used (890)
TS = total slow mobility casein fractions
$T\beta$ = total β-casein fractions
$T\alpha_s$ = total α_s-casein fractions

TABLE 4.10

Total casein fractions in each mobility zone (expressed as a percentage of the area of the peaks of an electrogram) in Cheddar cheese made from curd with and without added Neutrase during 360 days of ripening

Ripening period (d)	Ripening temperature (°C)	Treat-ment	TS	E-C	Tβ	E-C	$T\alpha_s$	E-C
1	10	C	16.549	3.991	32.074	-0.399	51.201	-3.415
		E	20.546		31.675		47.786	
	13	C	16.549	3.991	32.074	-0.399	51.201	-3.415
		E	20.540		31.675		47.786	
60	10	C	6.352	10.435	30.426	-10.611	63.221	0.117
		E	16.787		19.815		63.398	
	13	C	21.731	-2.378	28.006	-3.046	50.264	5.424
		E	19.353		24.960		55.688	
120	10	C	11.105	-1.795	24.128	-5.671	64.767	7.468
		E	9.310		18.457		72.235	
	13	C	12.061	-0.297	22.111	-7.537	65.830	7.833
		E	11.764		14.574		73.663	
180	10	C	20.191	9.772	21.173	-6.649	58.636	-3.123
		E	29.963		14.524		55.513	
	13	C	26.843	3.539	16.796	-6.512	56.362	2.973
		E	30.382		10.284		59.335	
360	10	C	34.258	21.986	14.534	-9.934	51.212	-12.055
		E	56.244		4.600		39.157	
	13	C	35.380	13.863	9.759	0.264	54.861	-14.126
		E	49.243		10.023		40.735	

C = control

E = Neutrase enzyme treated cheese
Neutrase enzyme added at a rate of 0.002% (w/w) starter culture used (890)

TS = total slow mobility casein fractions

Tβ = total -casein fractions

$T\alpha_s$ = total $_s$-casein fractions

TABLE 4.11

Total casein fractions in each mobility zone (expressed as a percentage of the area of the peaks of an electrogram) in Cheddar cheese made from curd with and without added Neutrase during 360 days of ripening

Ripening period (d)	Ripening temperature (°C)	Treat-ment	TS	E-C	Tβ	E-C	$T\alpha_s$	E-C
1	10	C	14.322	2.964	40.572	-6.567	45.106	3.013
		E	17.286		34.005		48.119	
	13	C	14.322	2.964	40.572	-6.567	45.106	3.013
		E	17.286		34.005		48.119	
60	10	C	15.495	2.555	23.232	-6.170	61.273	3.573
		E	18.050		17.062		64.846	
	13	C	17.242	20.826	25.298	-14.030	57.461	-6.796
		E	38.068		11.268		50.665	
120	10	C	15.332	-2.004	26.021	-17.222	58.647	19.328
		E	13.328		8.799		77.975	
	13	C	13.458	-4.272	19.291	-12.163	67.251	16.436
		E	9.186		7.128		83.687	
180	10	C	28.195	4.184	17.399	-3.800	53.916	0.107
		E	32.379		13.599		54.023	
	13	C	27.647	5.388	17.619	-1.248	54.735	-4.142
		E	33.035		16.371		50.593	
360	10	C	11.641	15.515	20.769	-5.981	67.590	-9.535
		E	27.156		14.788		58.055	
	13	C	16.340	20.325	32.841	-16.627	50.818	-3.697
		E	36.665		16.214		47.121	

C = control
E = Neutrase enzyme treated cheese
Neutrase enzyme added at a rate of 0.005% (w/w)
starter culture used (890)
TS = total slow mobility casein fractions
Tβ = total β-casein fractions
$T\alpha_s$ = total α_s-casein fractions

TABLE 4.12

Total casein fractions in each mobility zone (expressed as a percentage of the area of the peaks of an electrogram) in Cheddar cheese made from curd with and without added Neutrase during 360 days of ripening

Ripening period (d)	Ripening treatment (°C)	Treat-ment	TS	E-C	Tβ	E-C	$T\alpha_s$	E-C
1	10	C	12.392	-2.338	43.575	-12.315	44.035	14.654
		E	10.054		21.260		58.689	
	13	C	12.392	-2.338	43.575	-12.315	44.035	14.654
		E	10.054		31.260		58.689	
60	10	C	18.492	1.508	27.153	-13.867	54.355	12.359
		E	20.000		13.286		66.714	
	13	C	24.778	10.600	32.093	-16.850	43.131	6.189
		E	35.378		15.243		49.320	
120	10	C	16.990	-2.301	33.575	-24.577	49.524	26.788
		E	14.689		8.998		76.312	
	13	C	16.355	-2.71	25.705	-21.294	57.940	24.004
		E	13.645		4.411		81.944	
180	10	C	25.413	1.061	16.790	-10.944	57.795	9.888
		E	26.474		5.846		67.683	
	13	C	28.725	9.350	17.559	-13.833	53.717	4.481
		E	38.075		3.726		58.198	
360	10	C	15.952	28.928	25.843	-10.982	58.204	-18.034
		E	44.880		14.861		40.170	
	13	C	18.293	15.782	28.232	-13.759	53.476	-2.024
		E	34.075		14.473		51.452	

C = control
E = Neutrase enzyme treated cheese
Neutrase enzyme added at a rate of 0.01% (w/w)
starter culture used (890)
TS = total slow mobility casein fractions
Tβ = total β-casein fractions
$T\alpha_s$ = total α_s-casein fractions

Legend to

Figure 4.3 Polyacrylamide gel electrograms of Cheddar cheese made from curd with and without added Neutrase during a ripening period of 360 days at 10°C and 13°C

--- Neutrase treated cheese

—— Control Cheese

Starter culture used 850

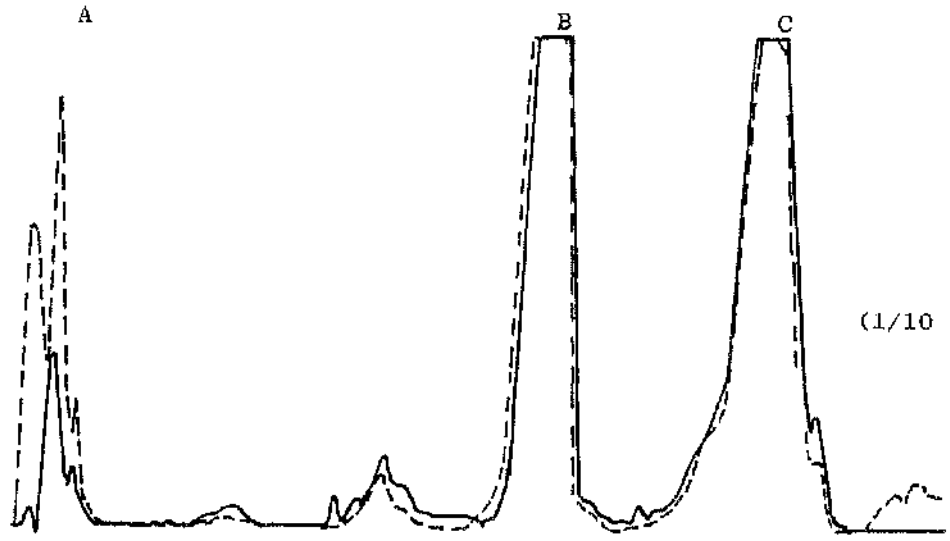
A - Slow mobility casein fractions

B - β-casein fractions

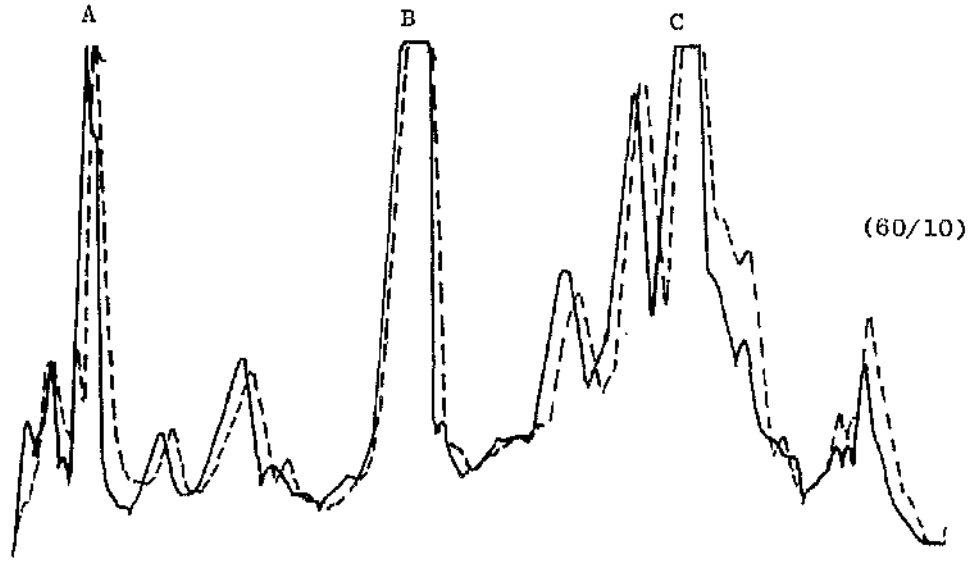
C - α_s- casein fractions

Figures in parenthesis represent (Ripening period (d)/temperature (°C)

Neutrase enzyme was added at a rate of 0.001% (w/w)



(1/10 and 13)



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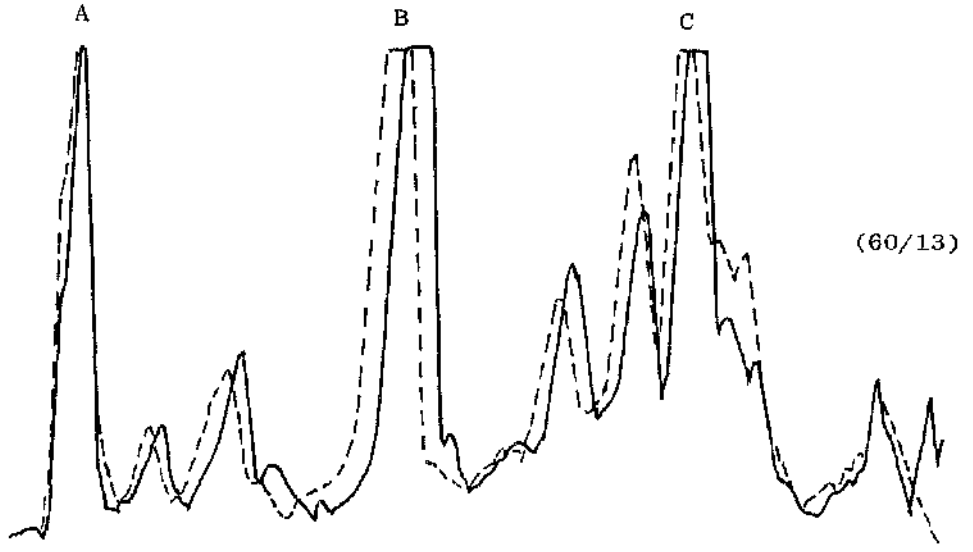
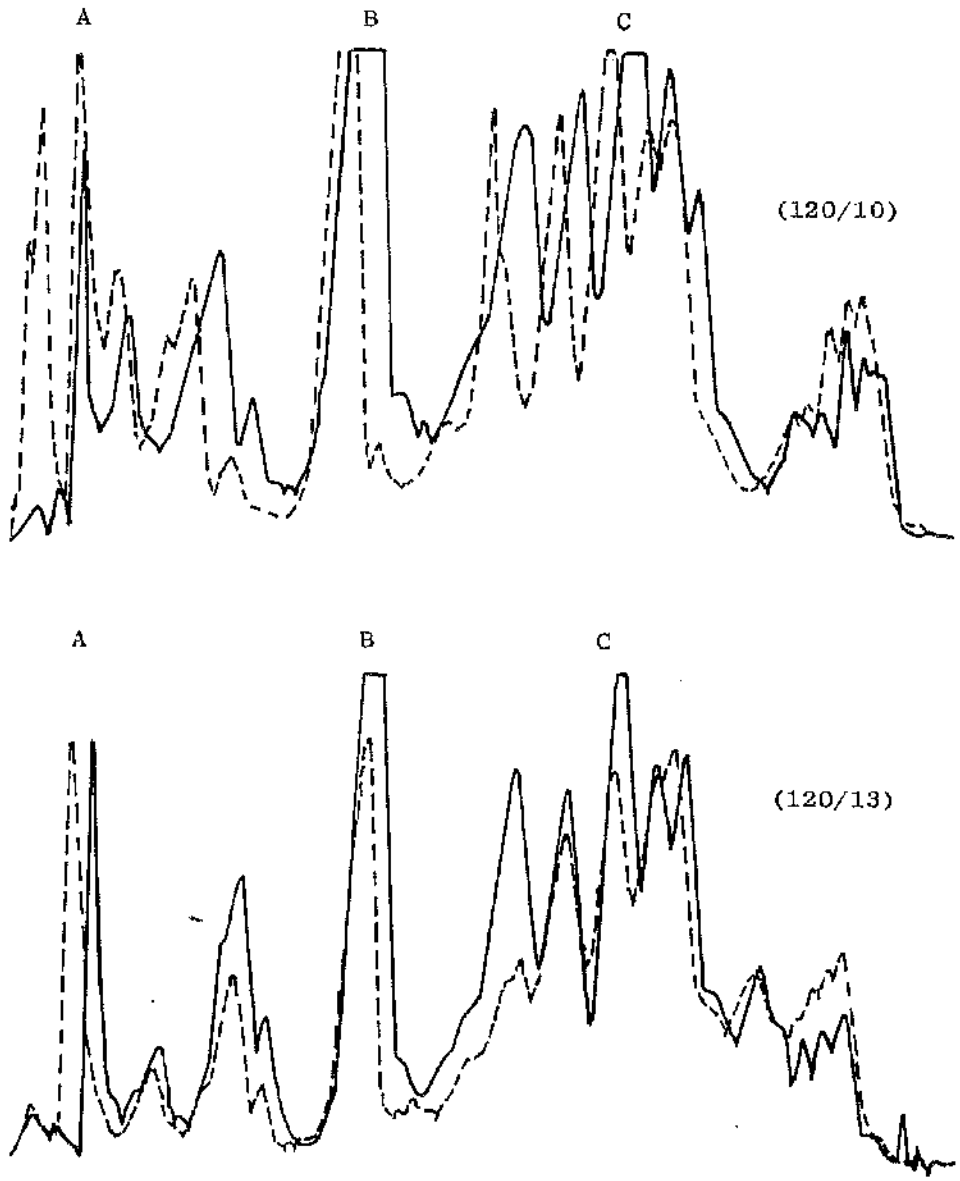
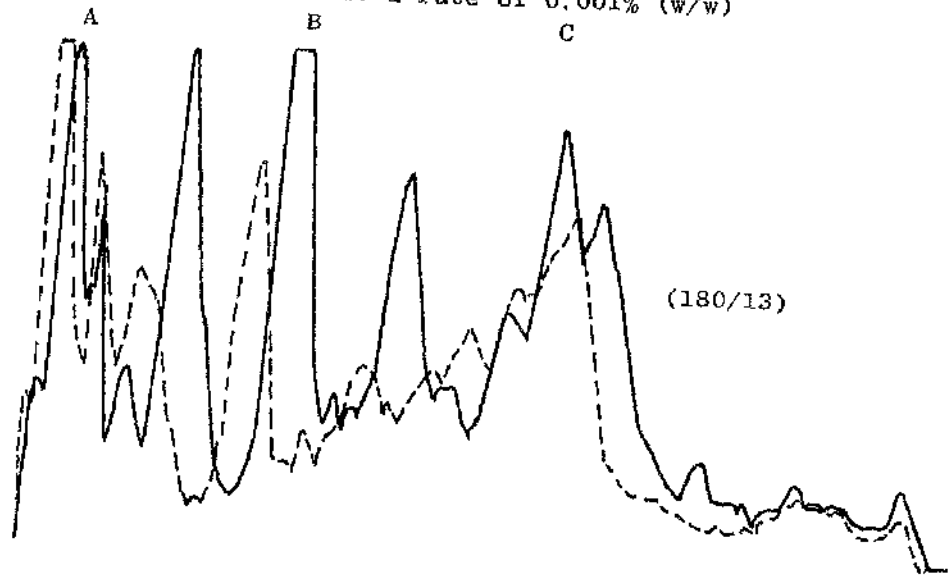


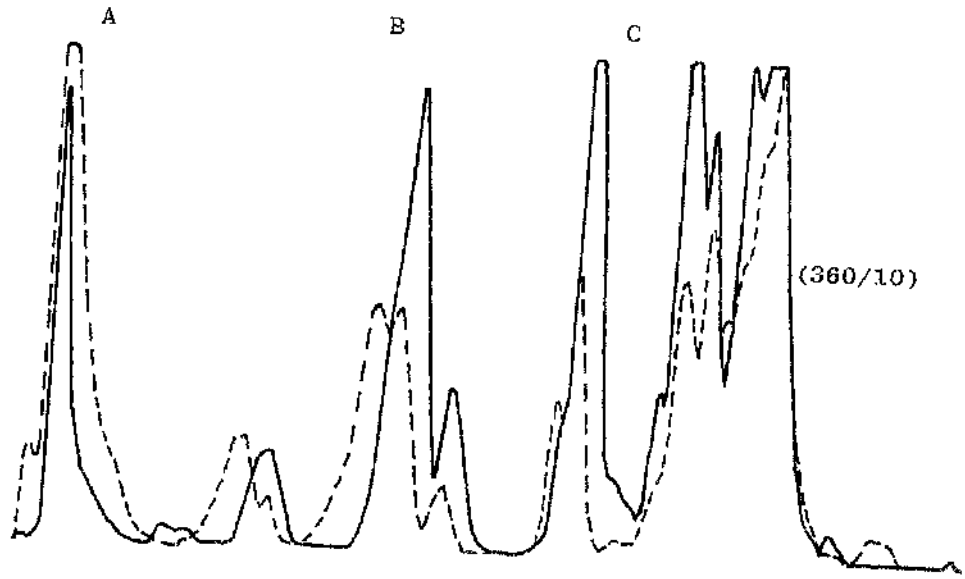
(60/13)

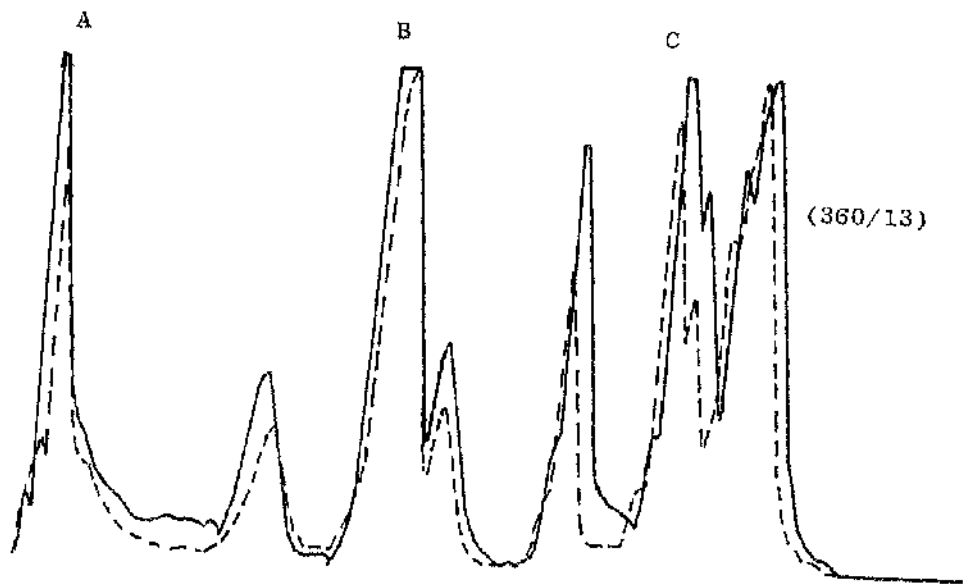
Neutrase enzyme was added at a rate of 0.001%(w/w)



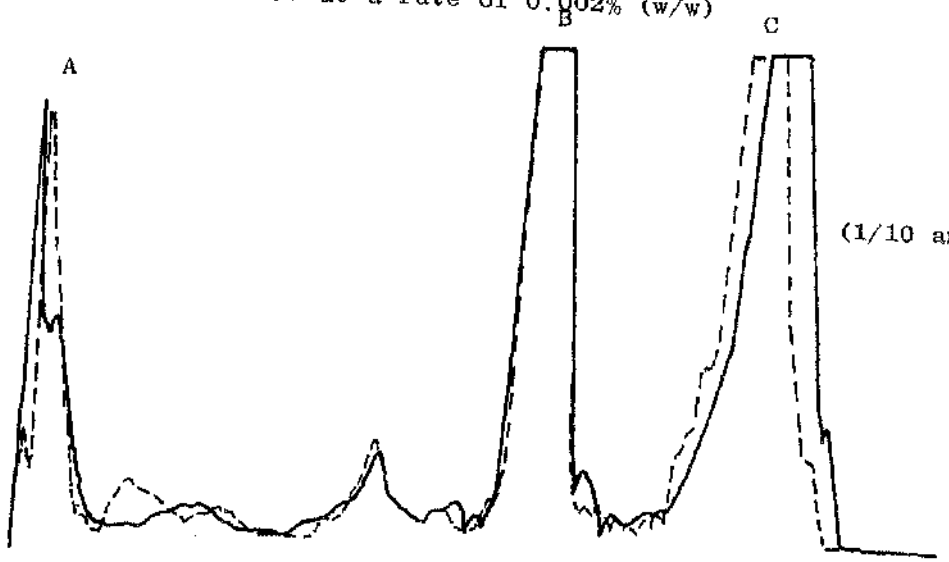
Neutrase enzyme was added at a rate of 0.001% (w/w)

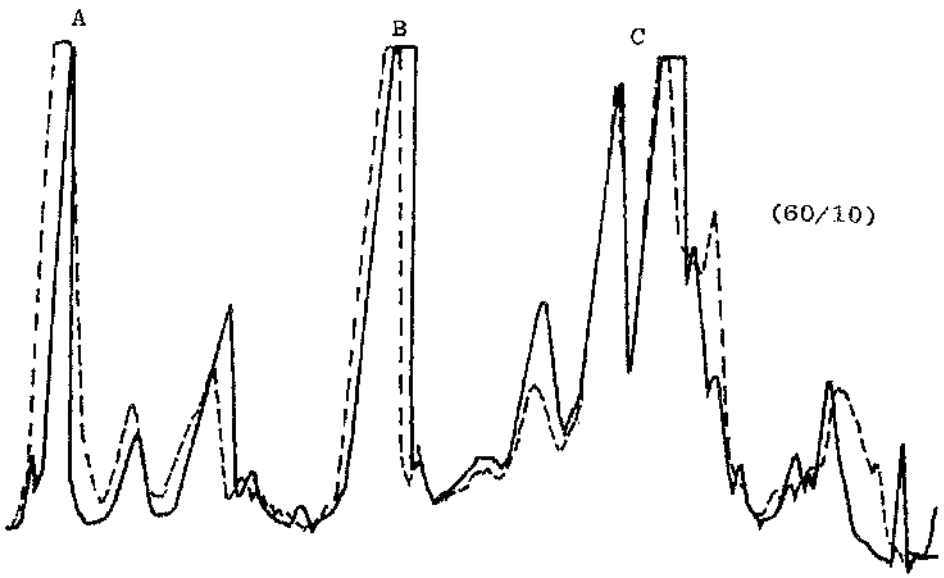


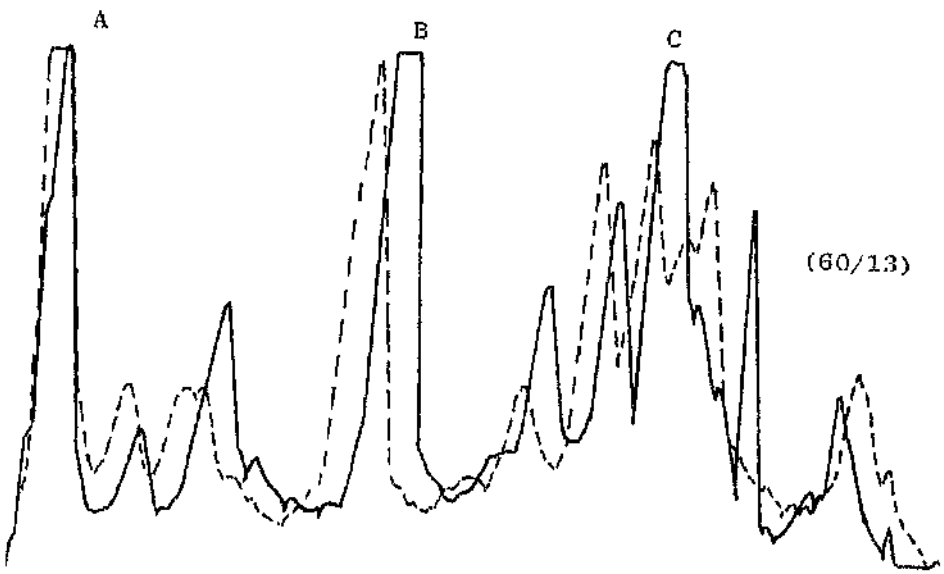




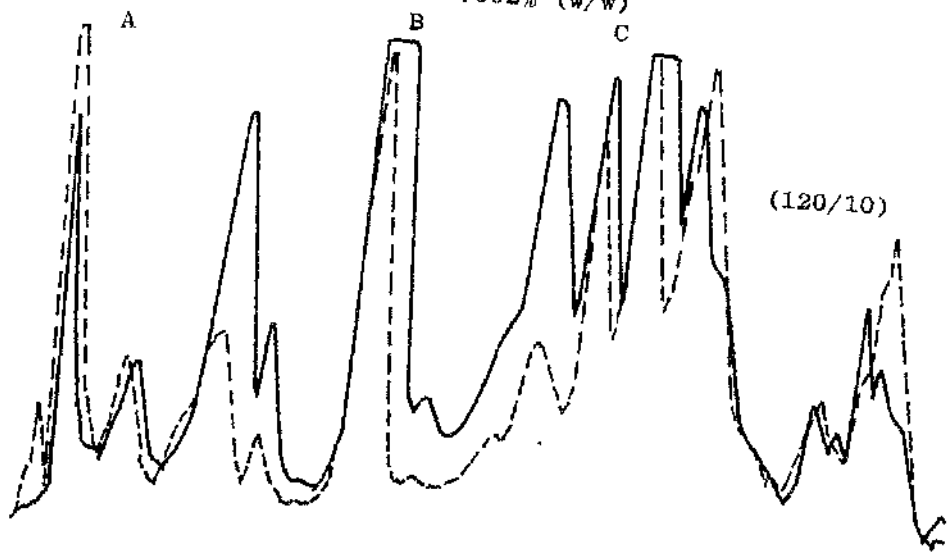
Neutrase enzyme was added at a rate of 0.002% (w/w)

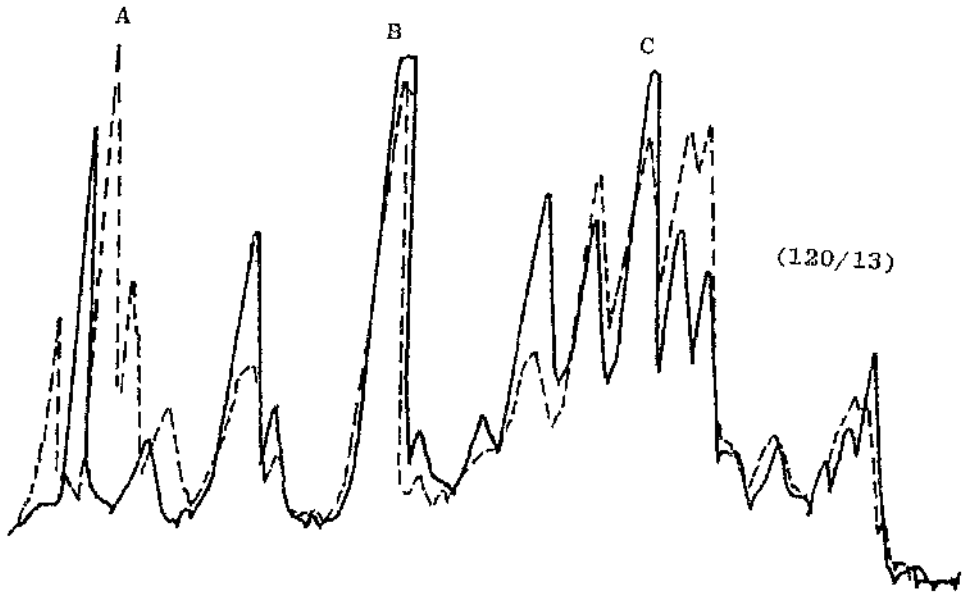


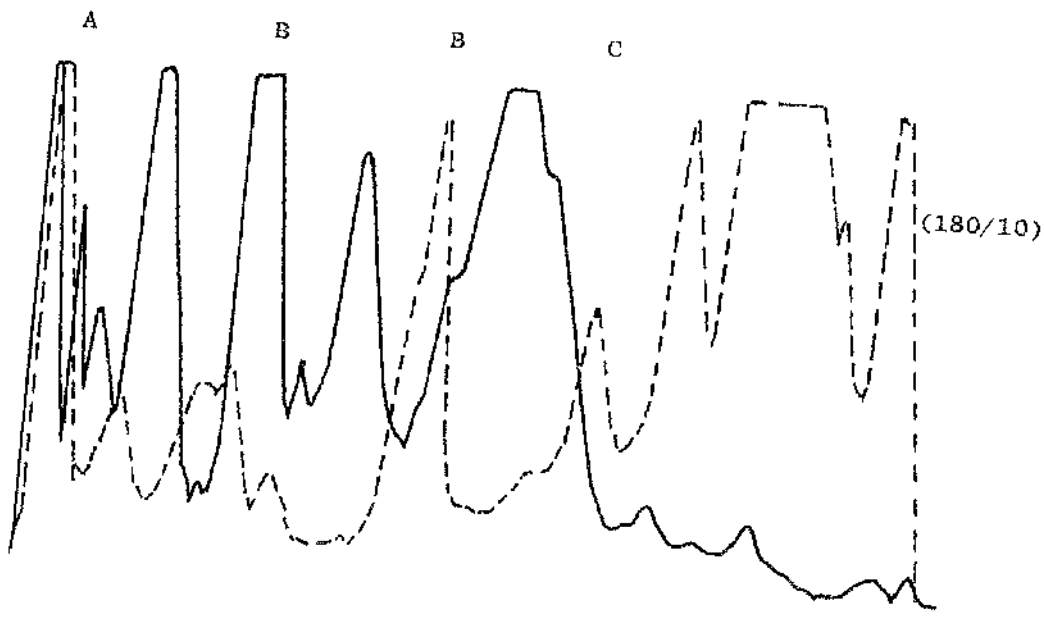




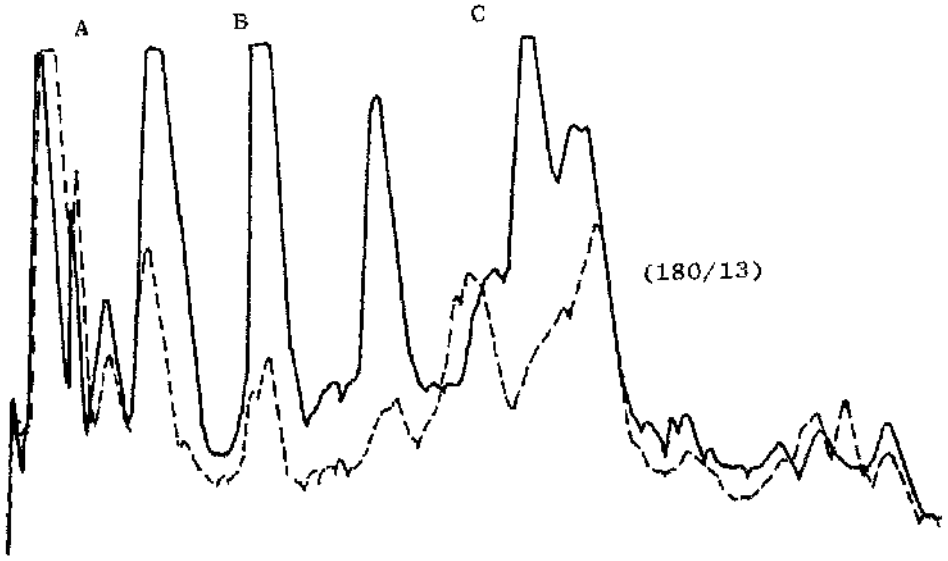
Neutrase enzyme was added at a rate of 0.002% (w/w)

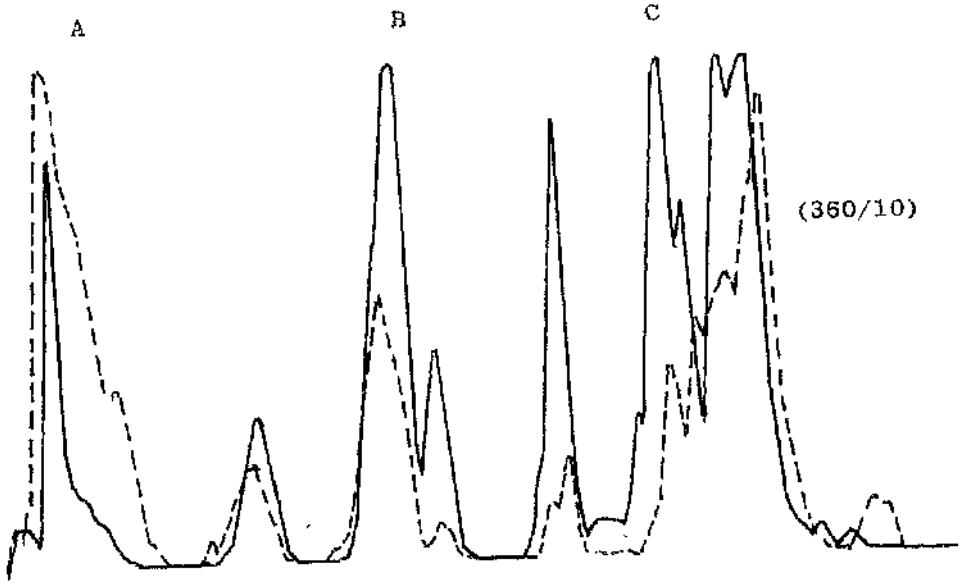


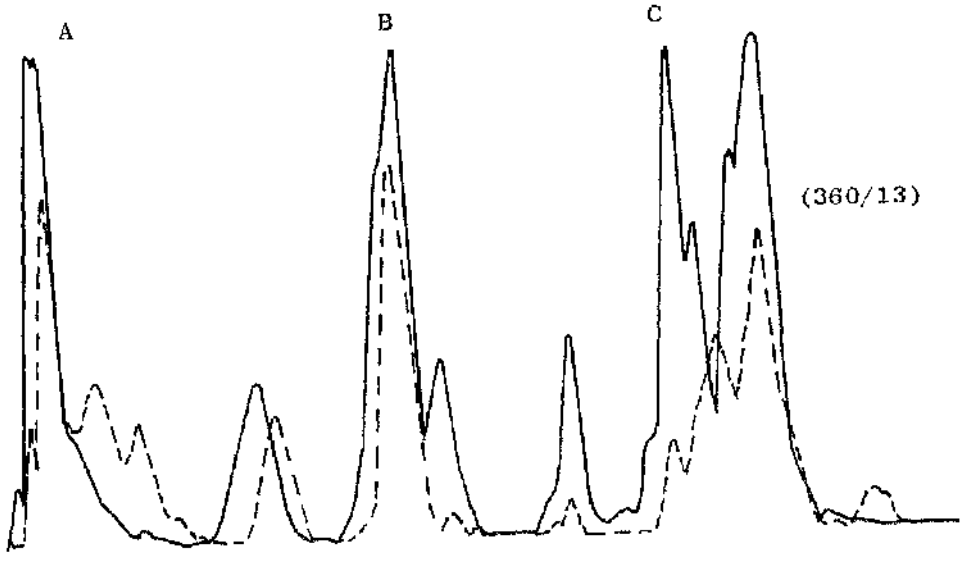




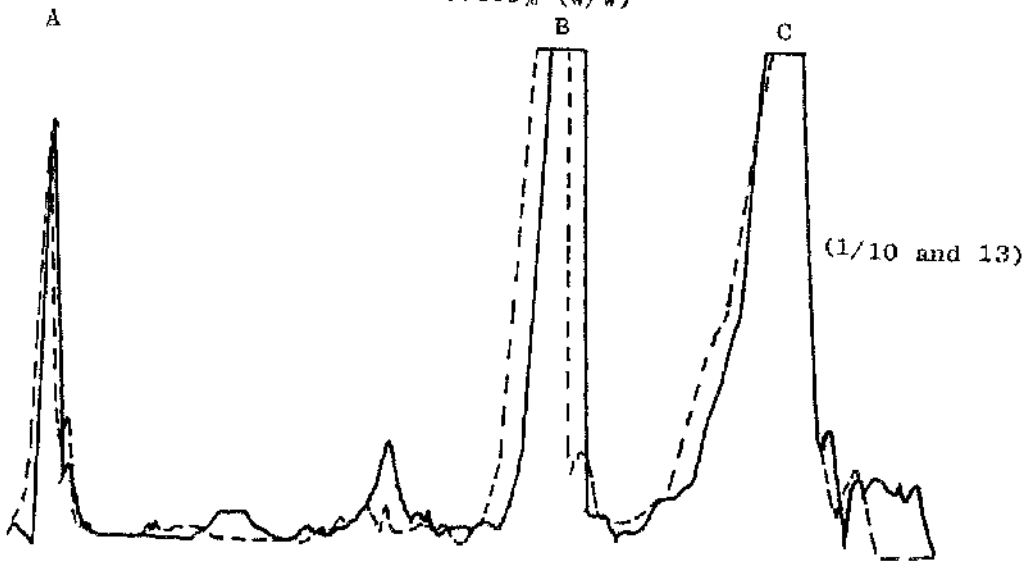
Neutrase enzyme was added at a rate of 0.002% (w/w)

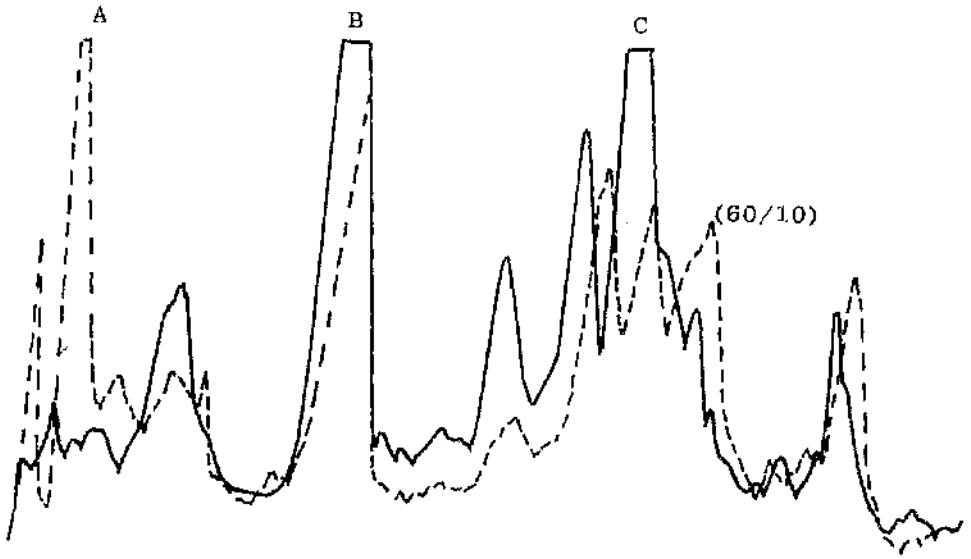


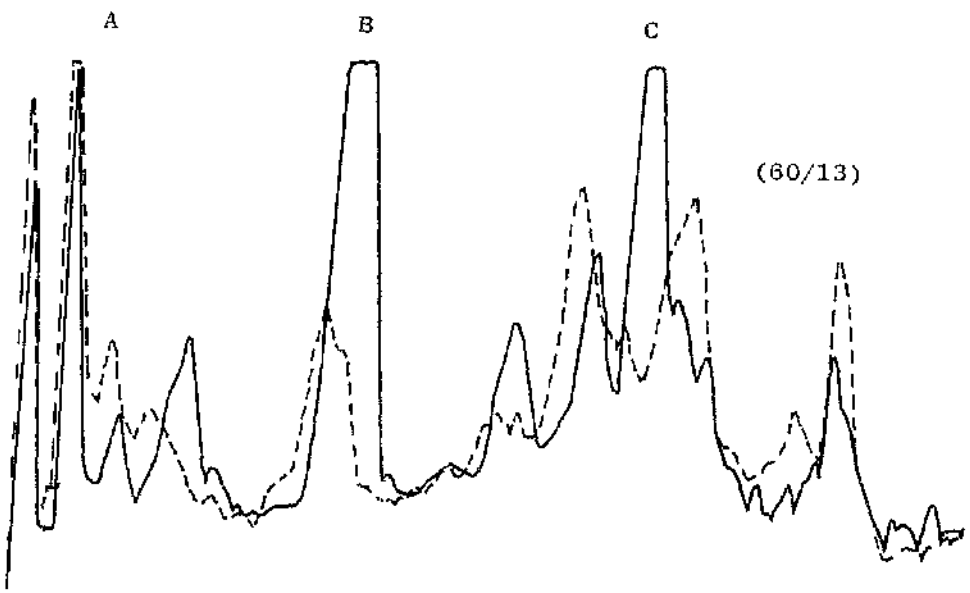




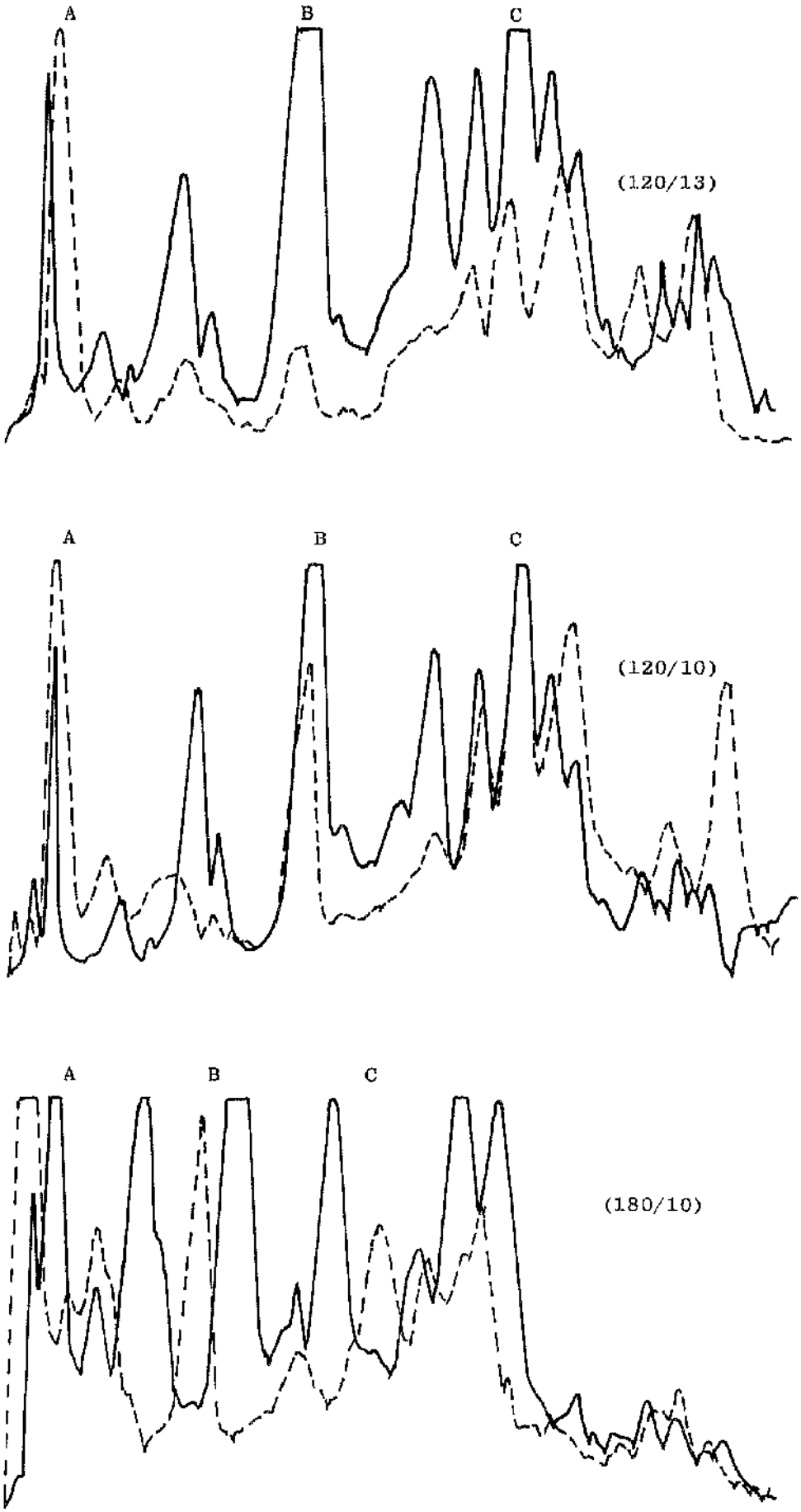
Neutrase enzyme was added at a rate of 0.005% (w/w)



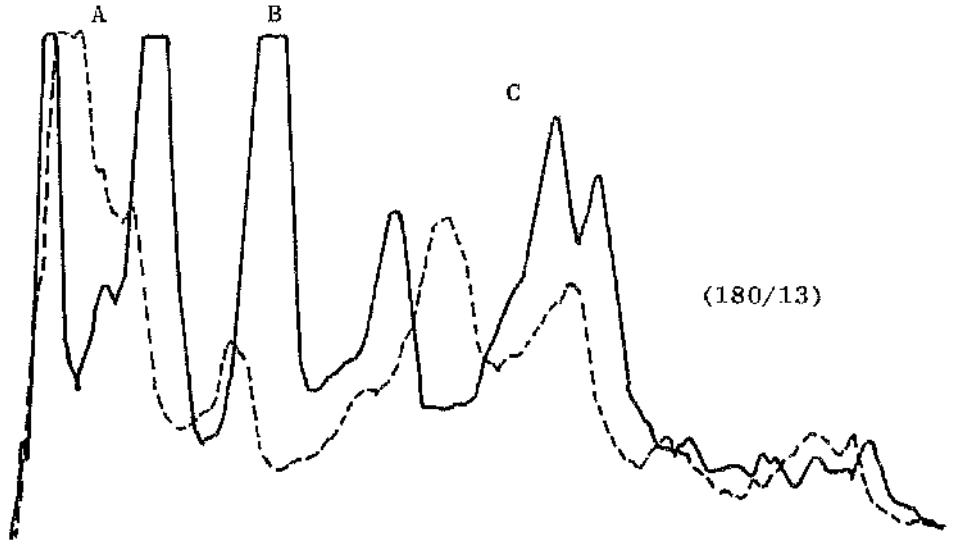


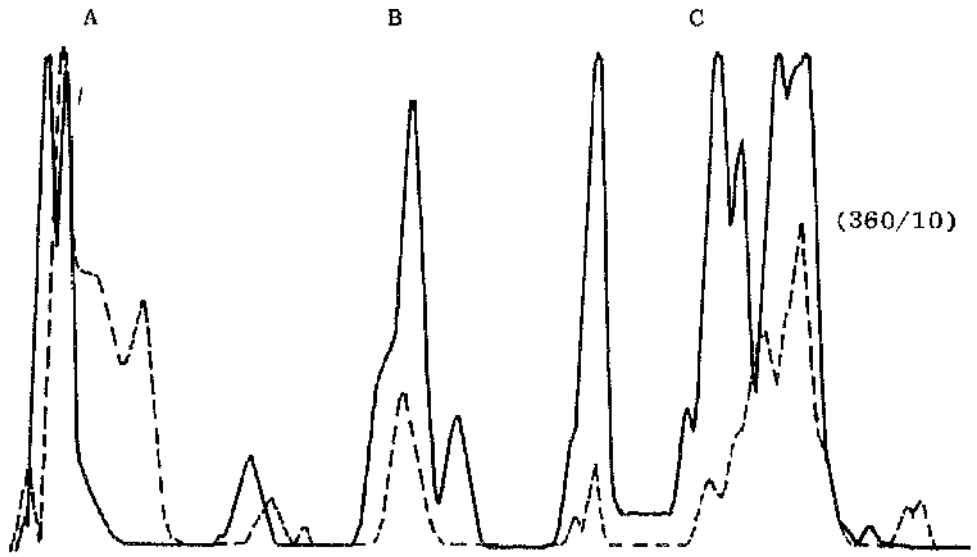


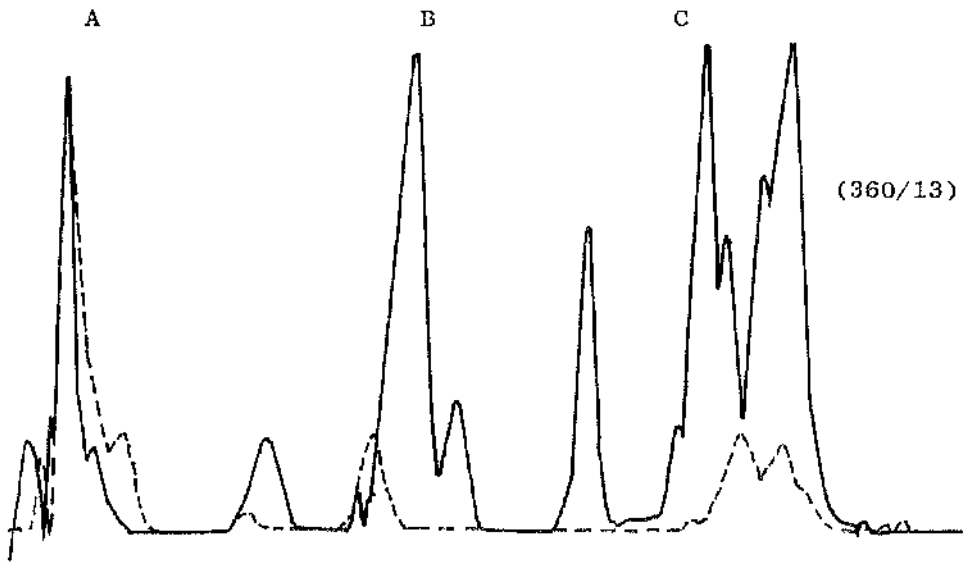
Neutrase enzyme wasadded at a rate of 0.005% w/w)



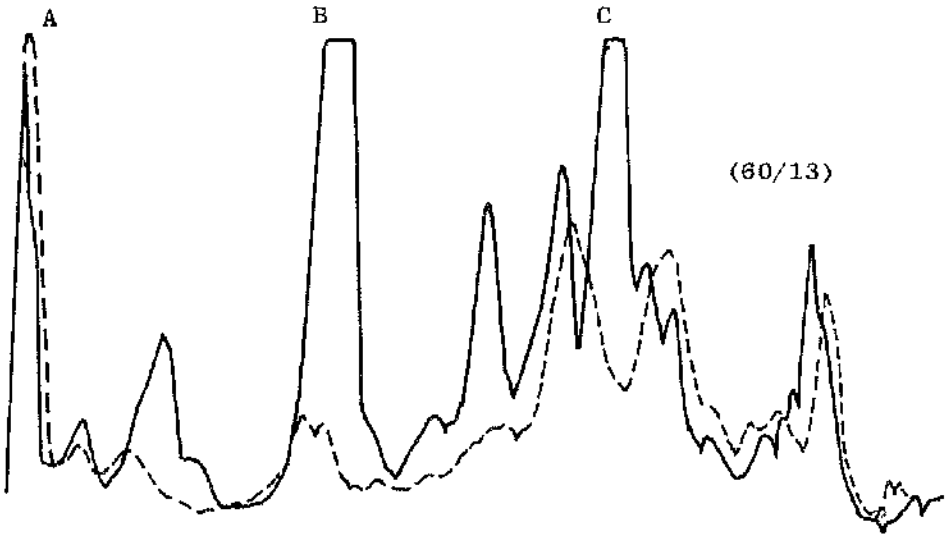
Neutrase enzyme was added at a rate of 0.005% w/w)

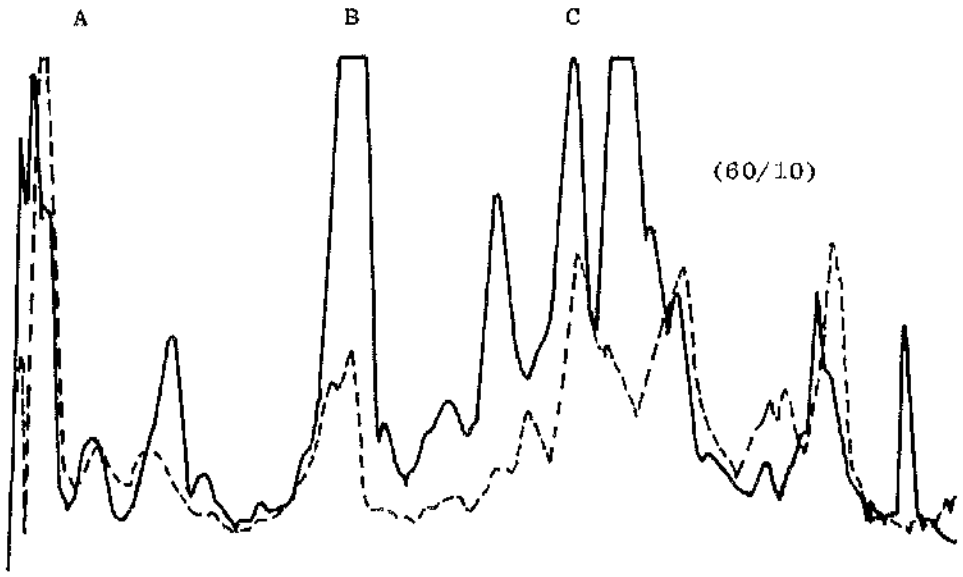


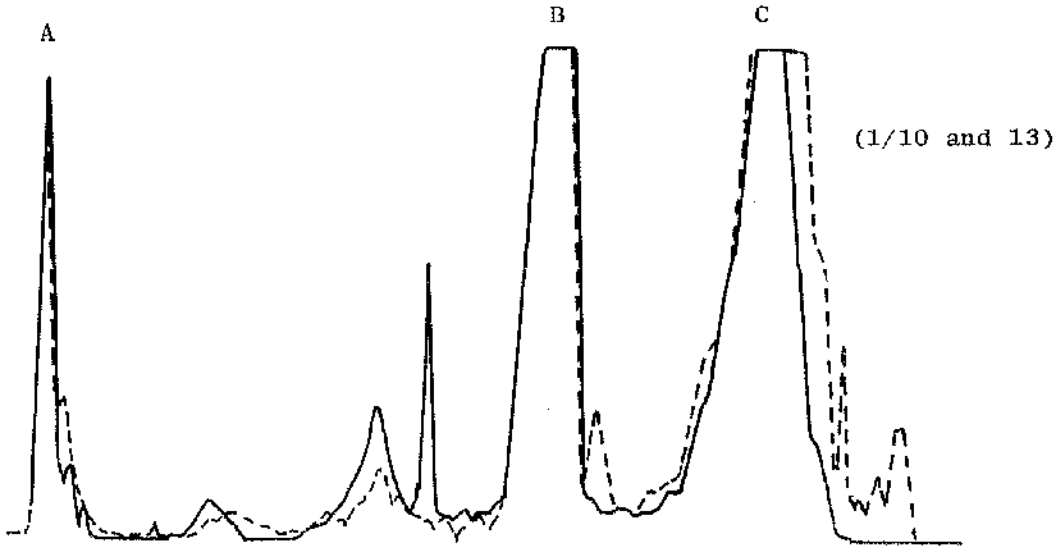




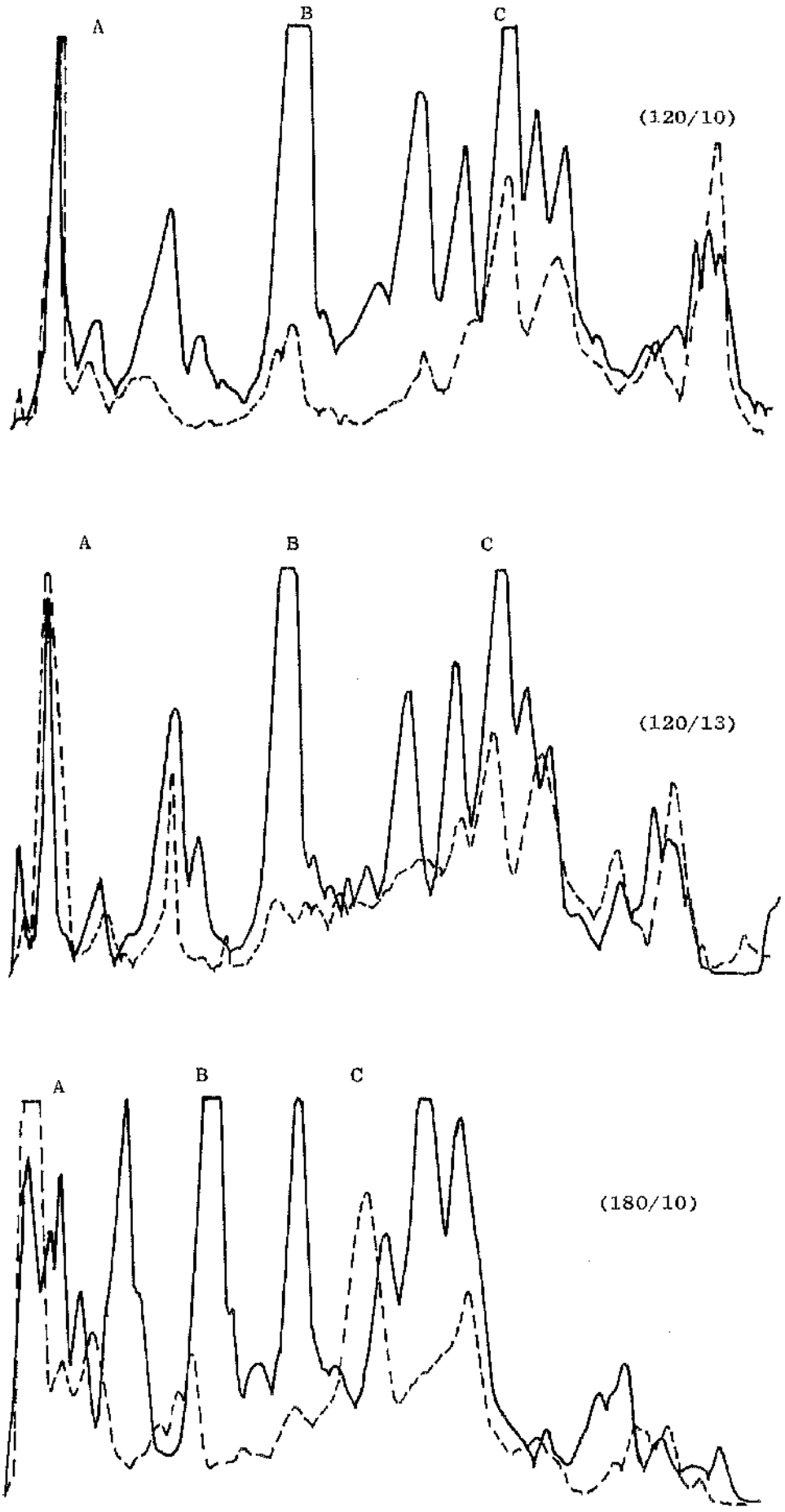
Neutrase enzyme was added at a rate of 0.01% w/w)



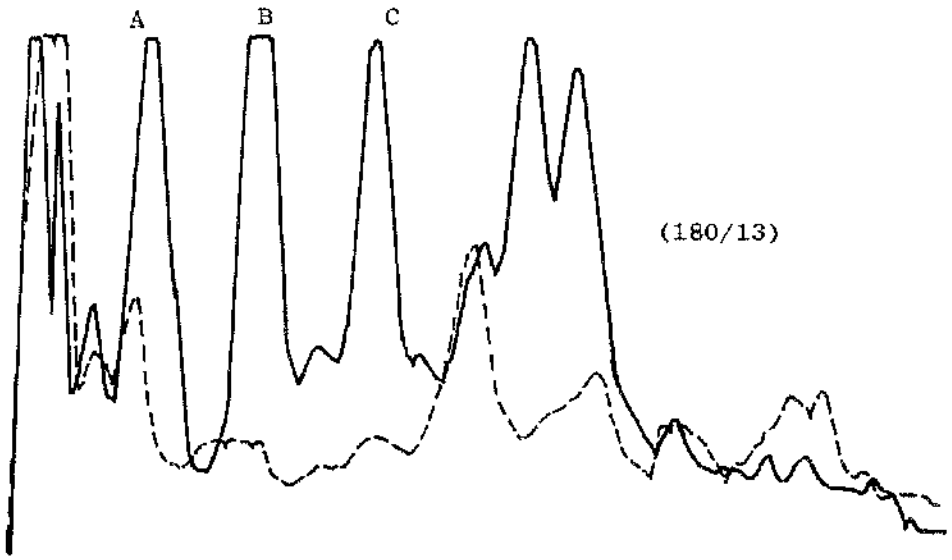


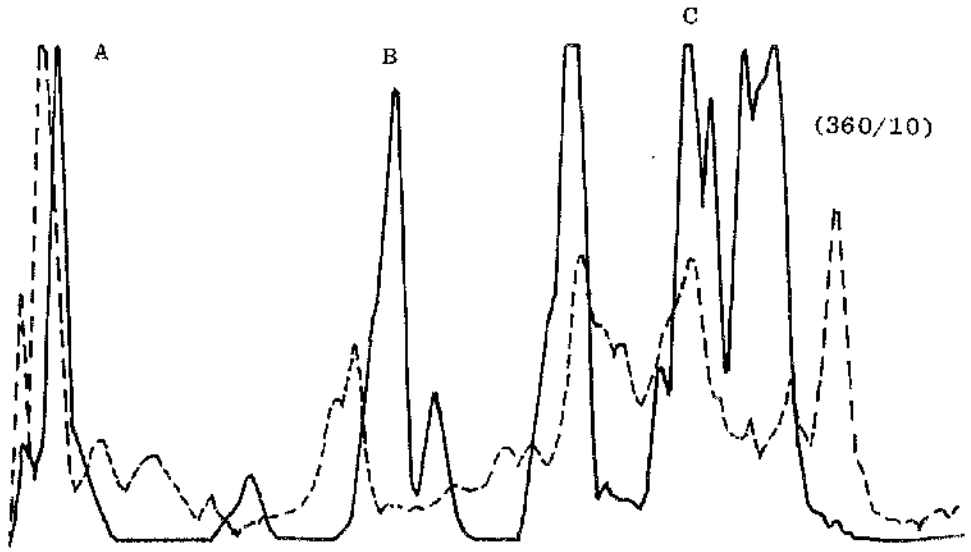


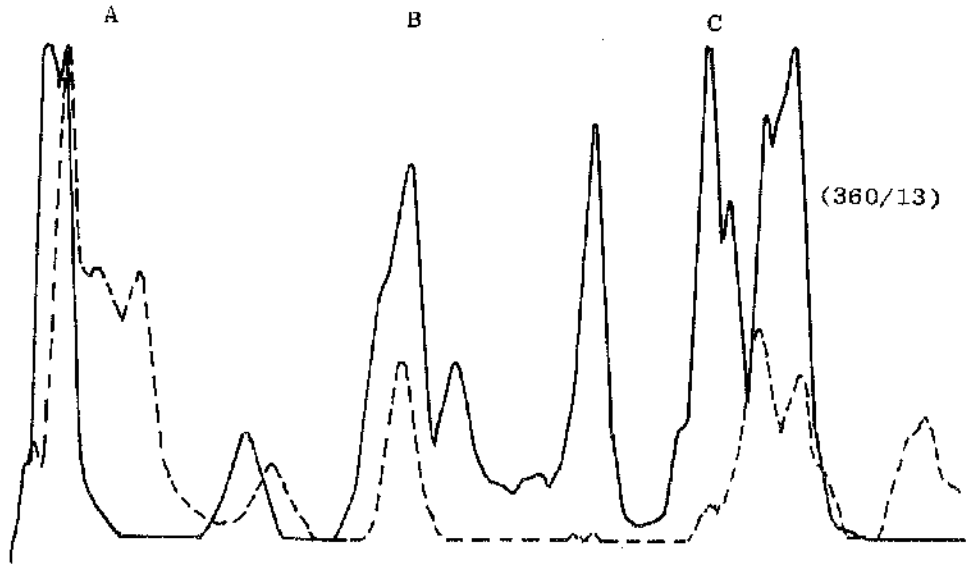
Neutrase enzyme was added at a rate of 0.01% (w/w)



Neutrase enzyme was added at a rate of 0.01% w/w







Legent to

Figure 4.4 : Polyacrylamide gel electrograms of Cheddar cheese made from curd with and without added Neutrase during a ripening period of 360 days at 10 and 13 ^{o}C

Footnote:

--- Neutrase treated cheese

—— Control cheese

Starter culture used 890

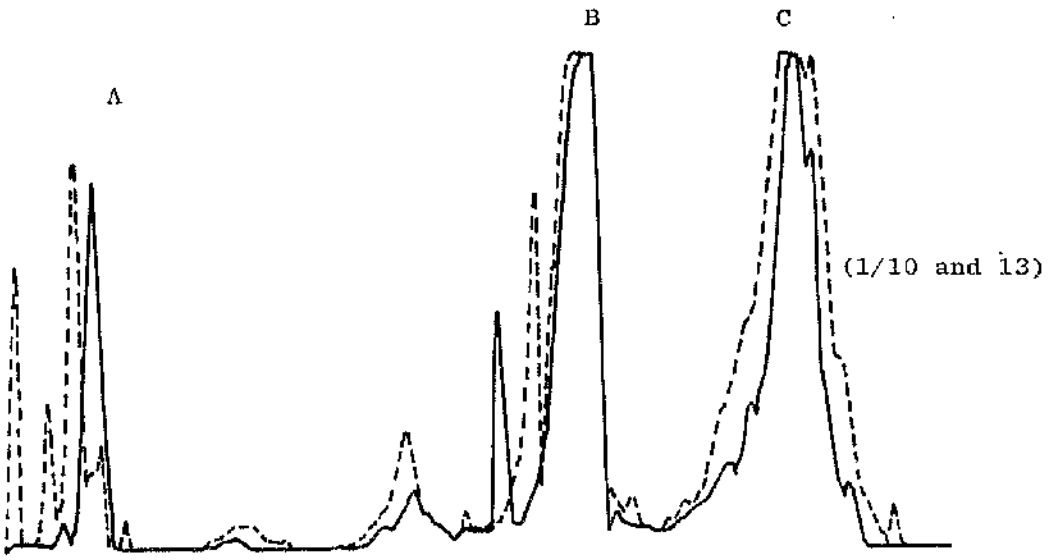
A - Slow mobility casein fractions

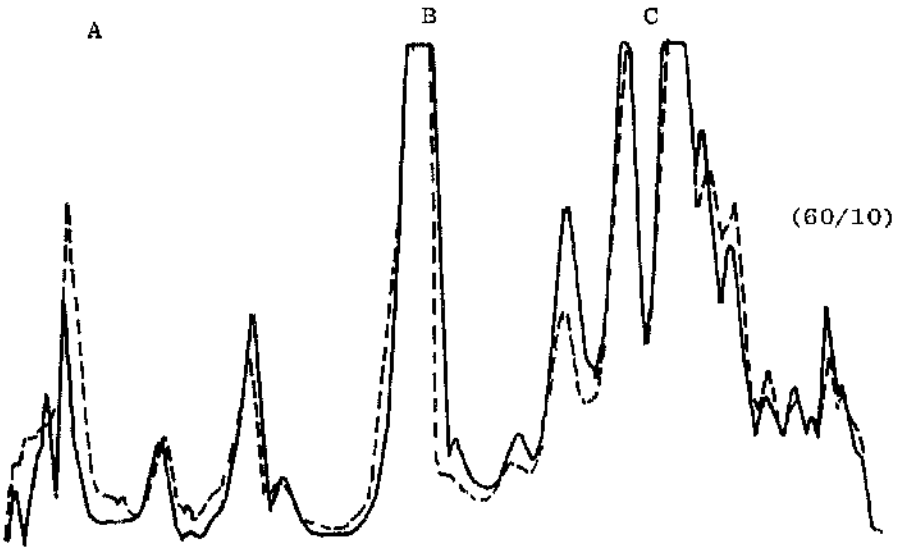
B - β-casein fractions

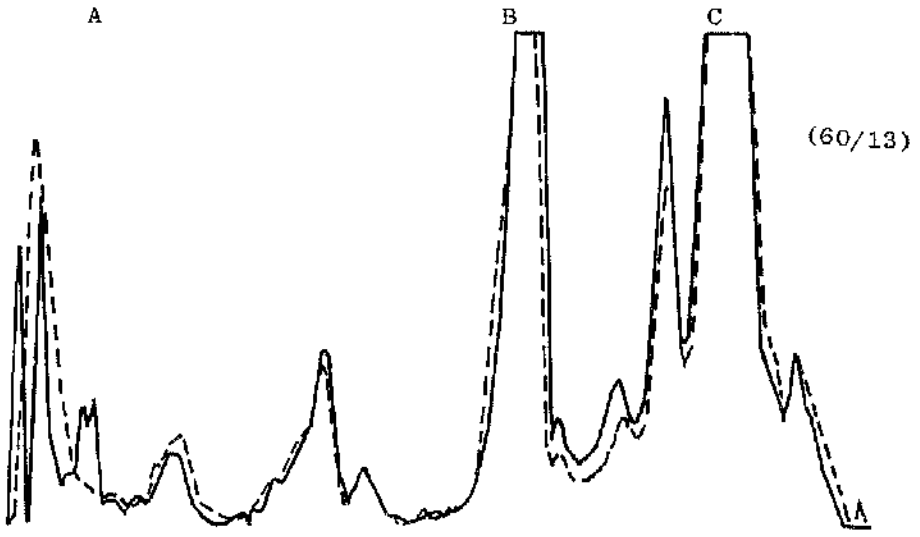
C - α_s-casein fractions

Figures in parenthesis represent (Ripening period (d)/ Ripening temperature (^{o}C))

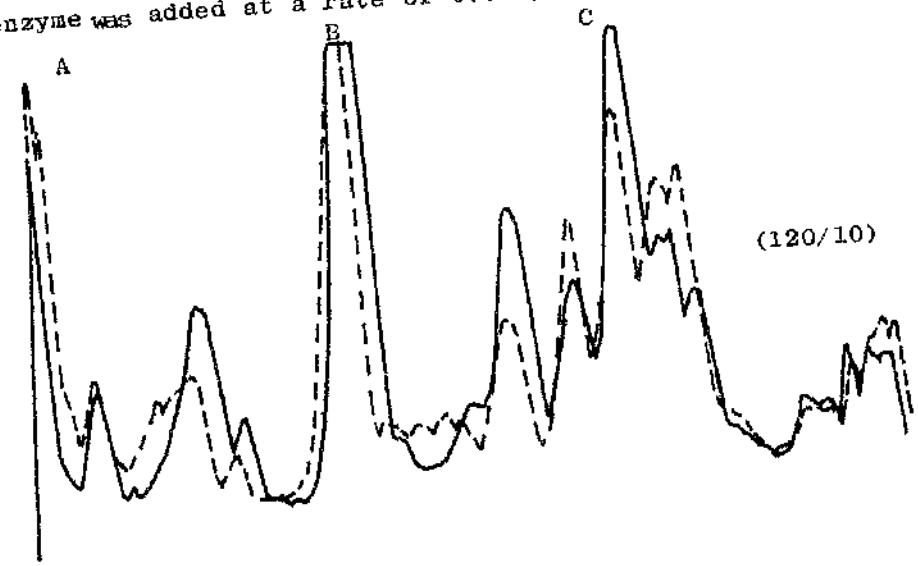
Neutrase enzyme was added at a rate of 0.001% (w/w)

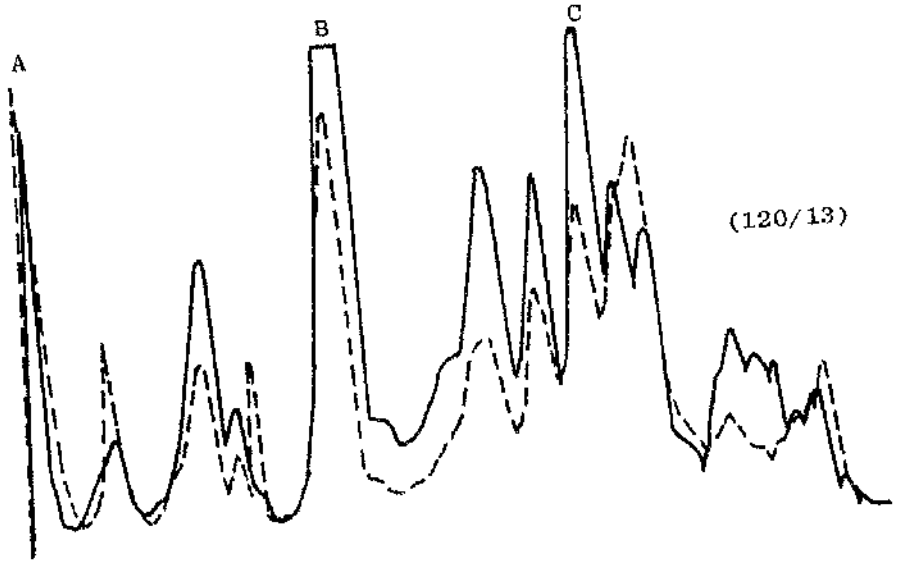


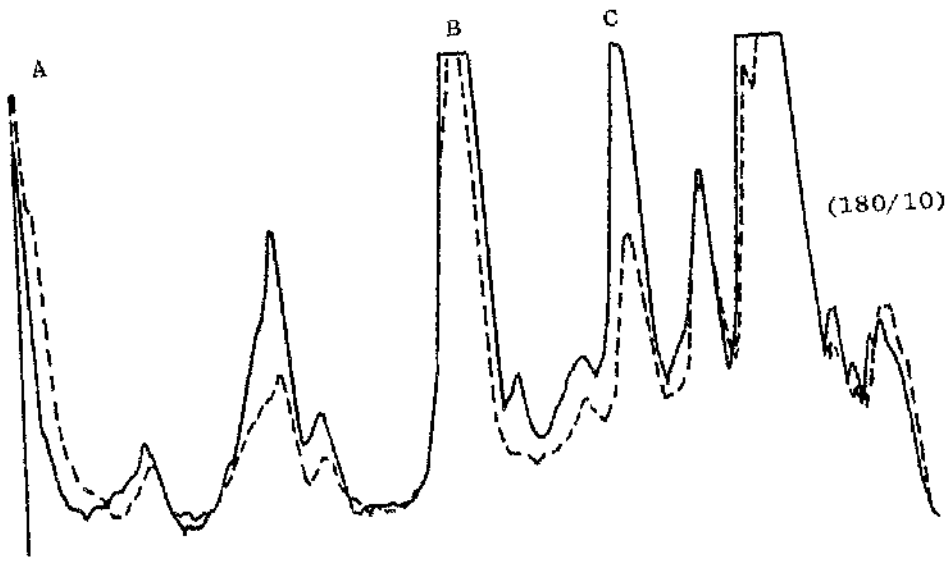




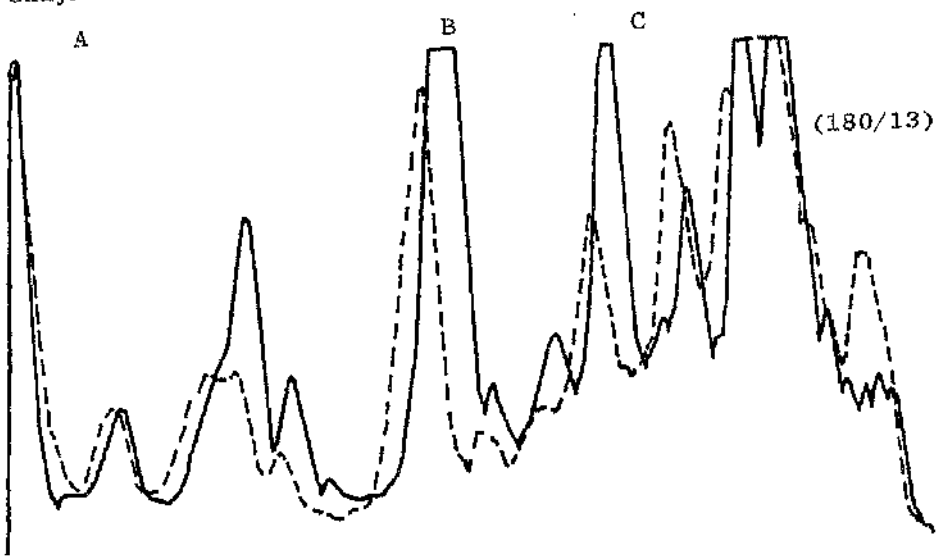
Neutrase enzyme was added at a rate of 0.001% (w/w)

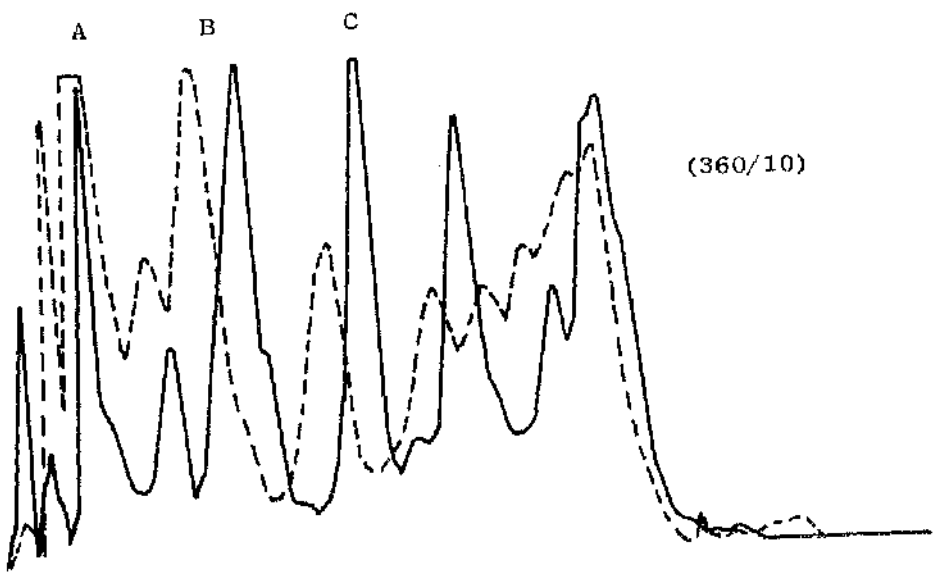


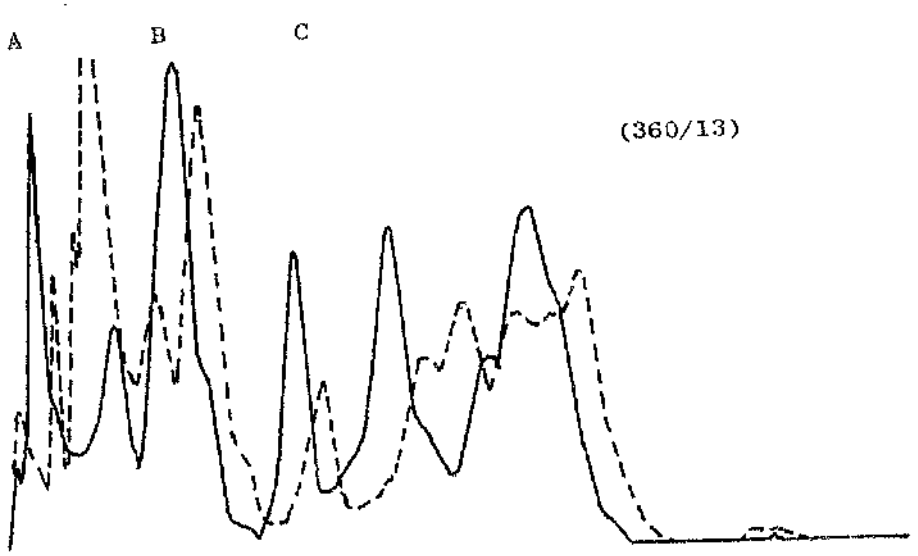




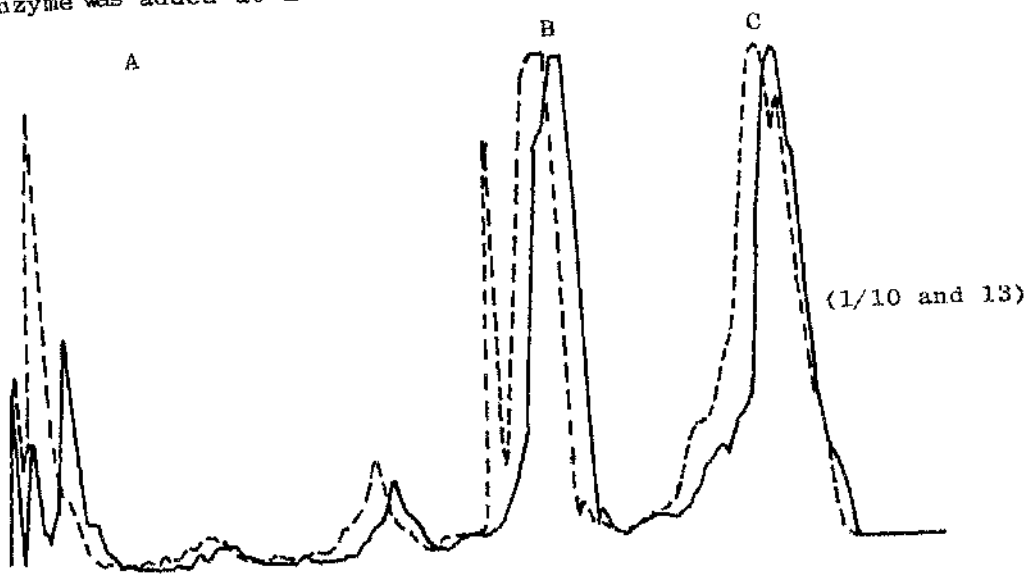
Neutrase enzyme was added at a rate of 0.001% (w/w)

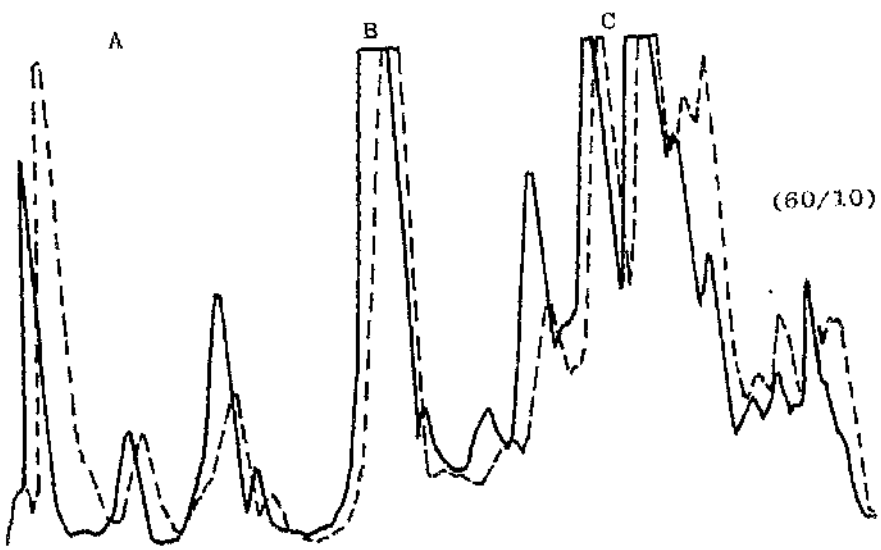


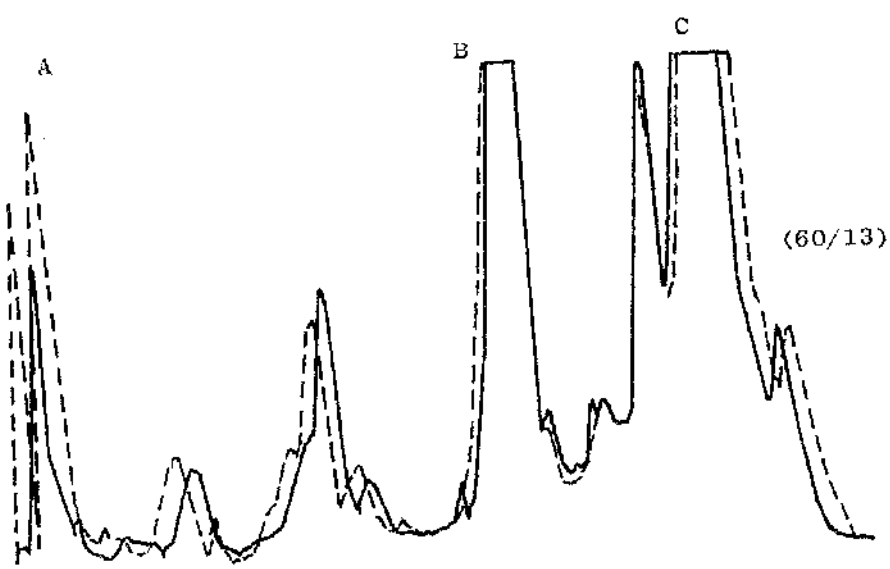




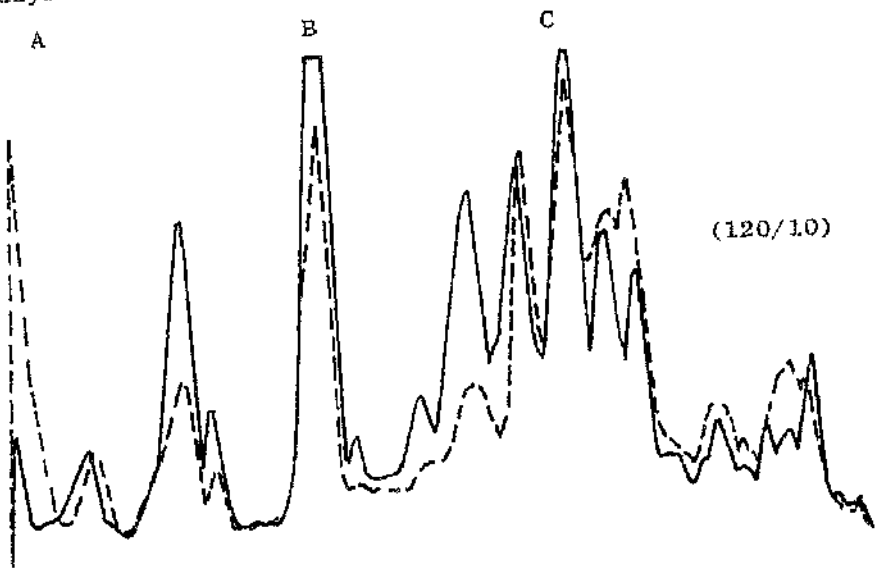
Neutrase enzyme was added at a rate of 0.002% (w/w)

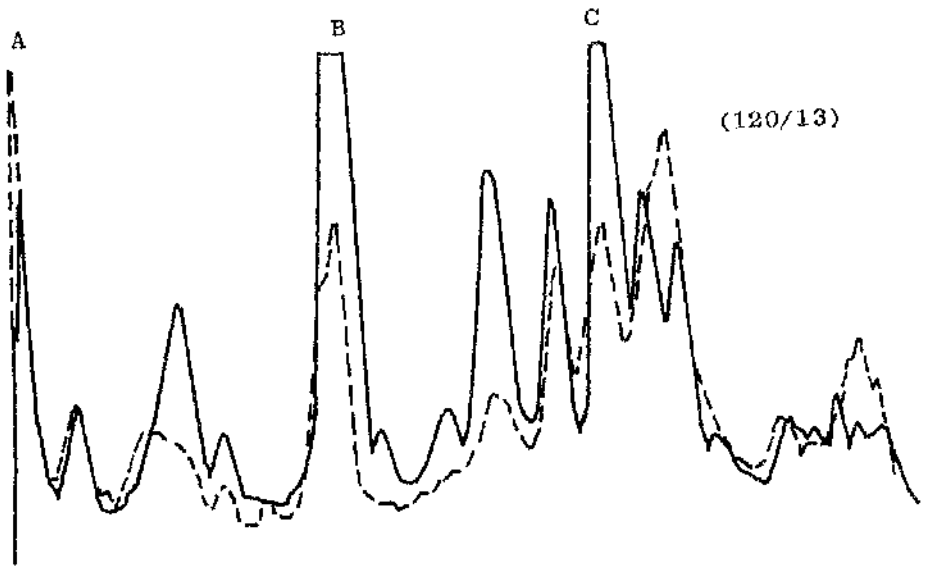


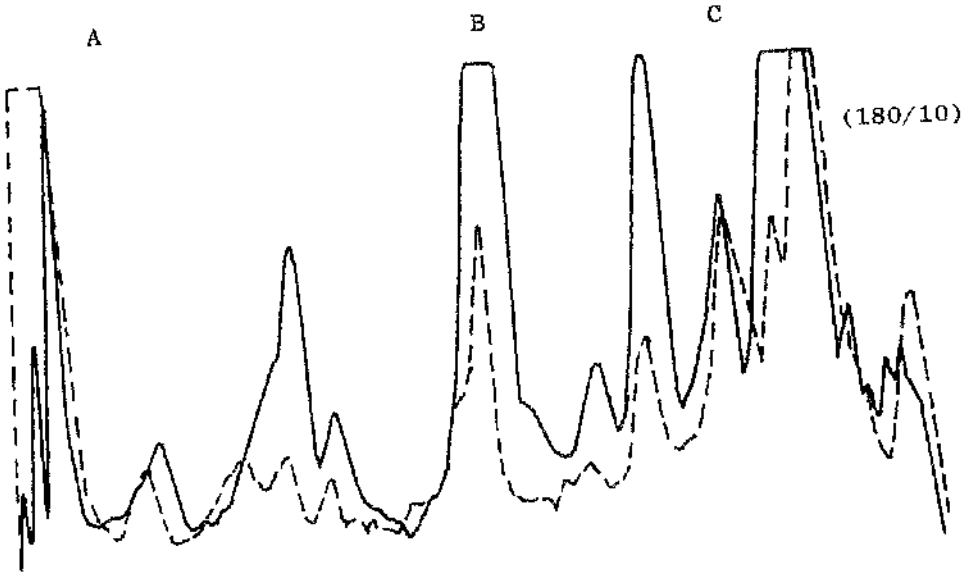




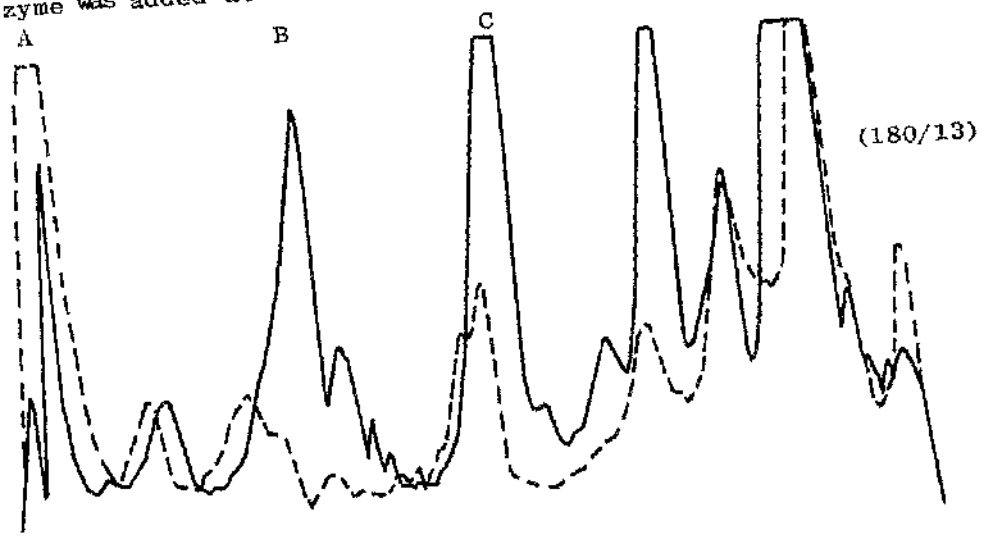
Neutrase enzyme was added at a rate of 0.002% (w/w)

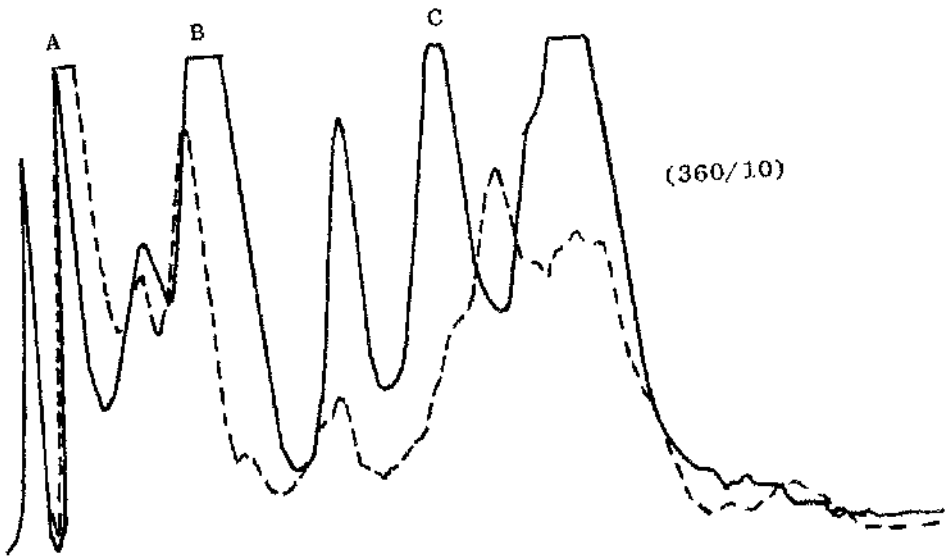


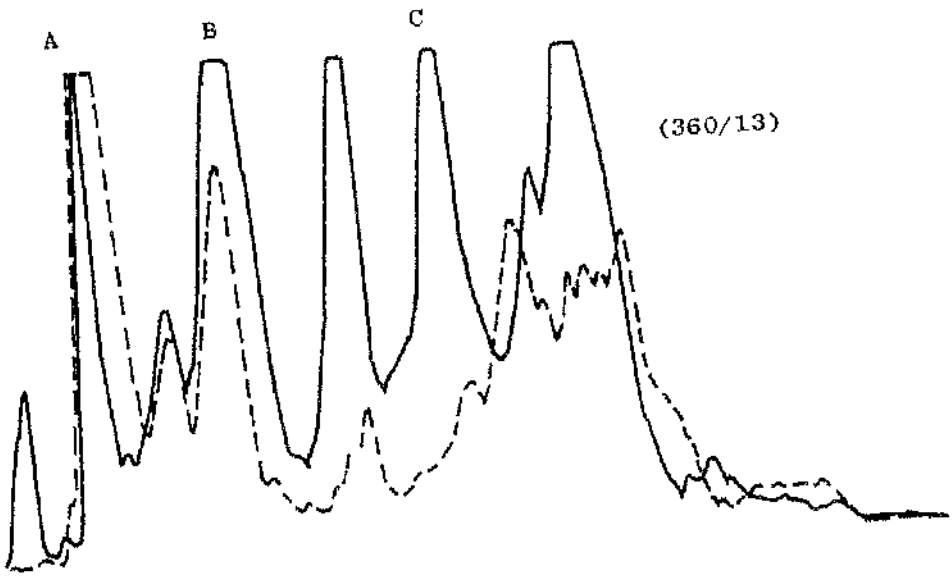




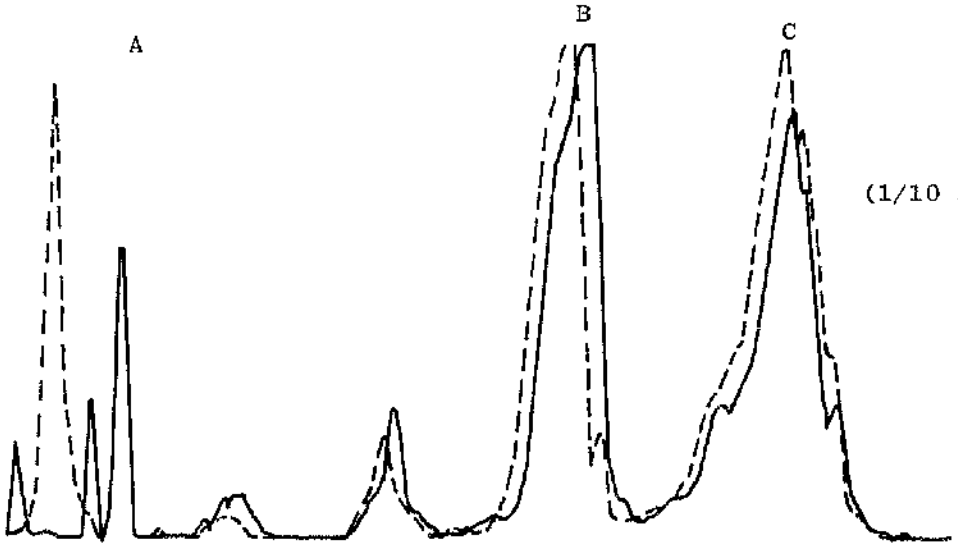
Neutrase enzyme was added at a rate of 0.002% (w/w)



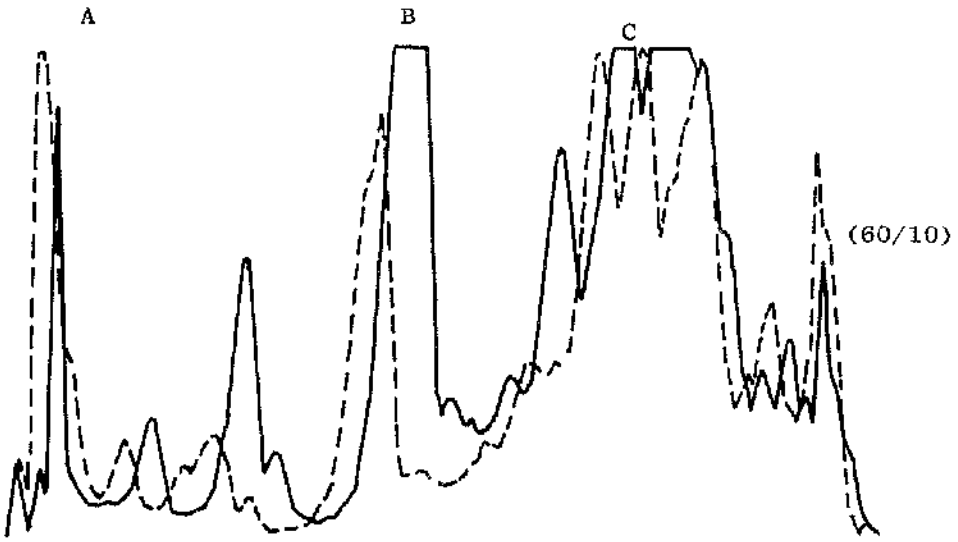




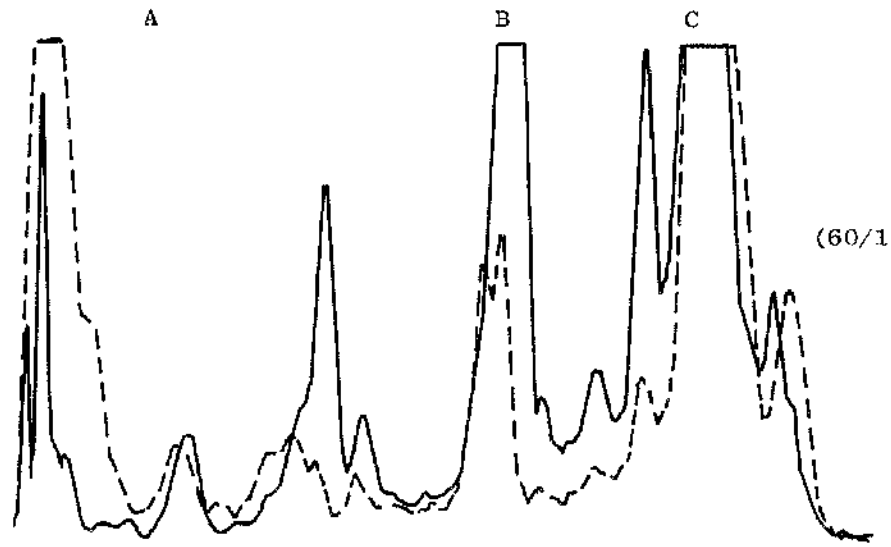
Neutrase enzyme was added at a rate of 0.005% (w/w)



(1/10 and 13)

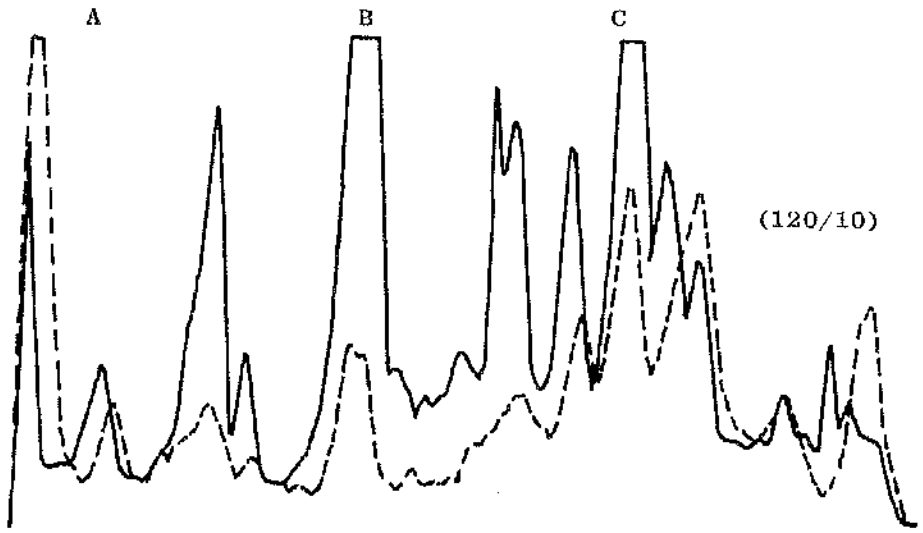


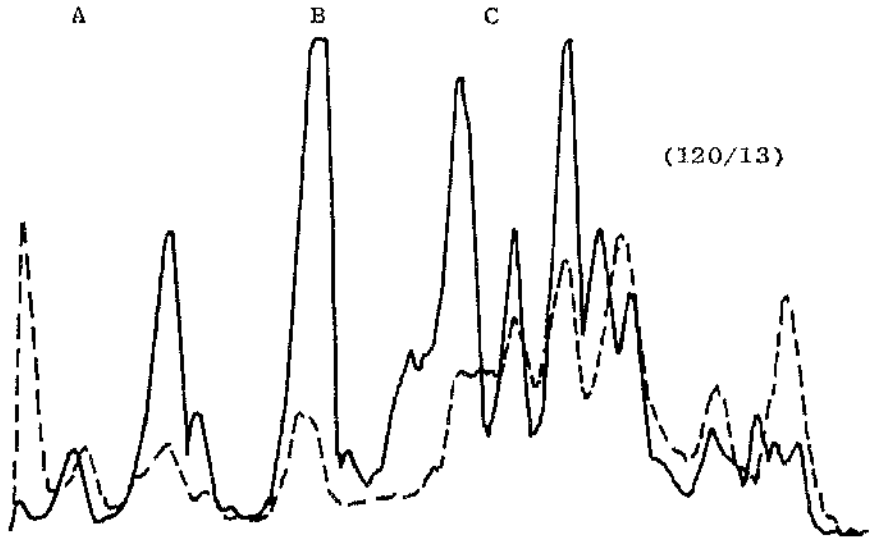
(60/10)

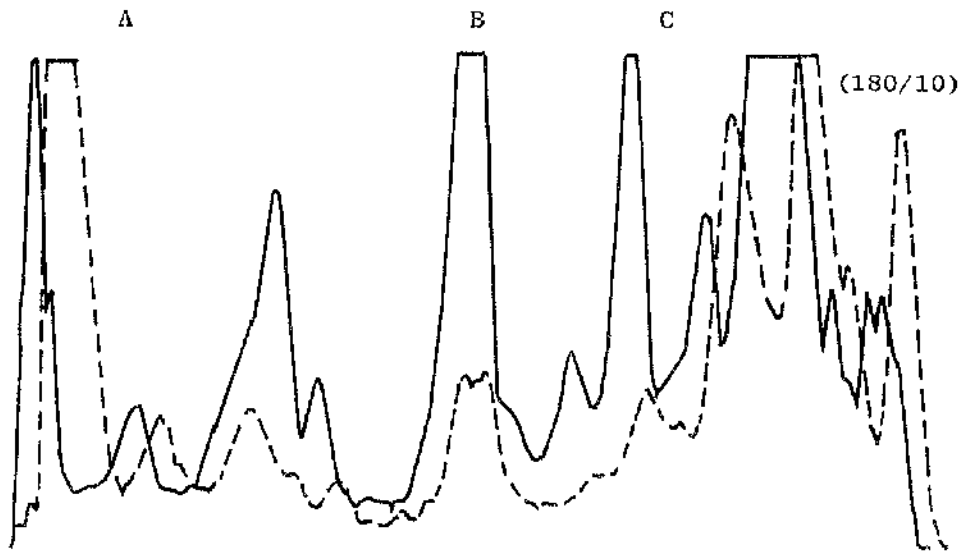


(60/13)

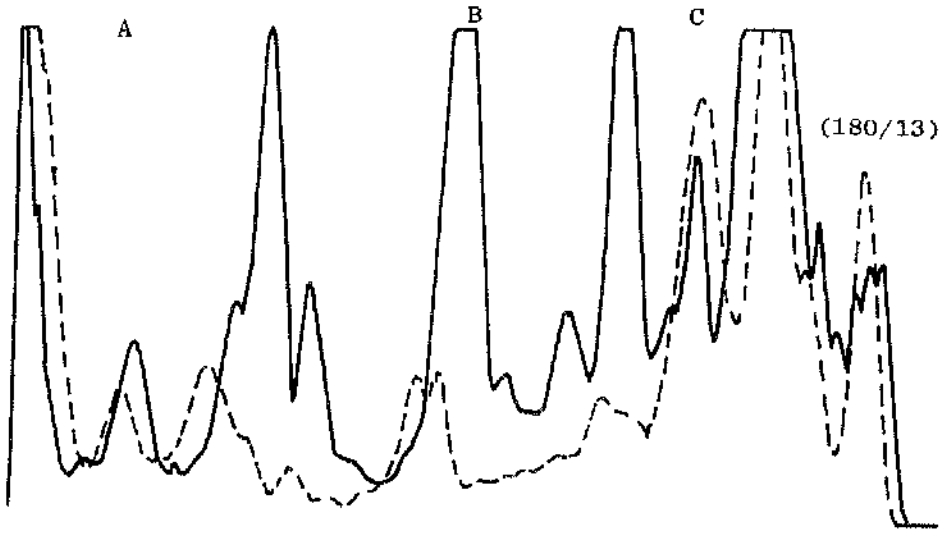
Neutrase enzyme was added at a rate of 0.005% (w/w)

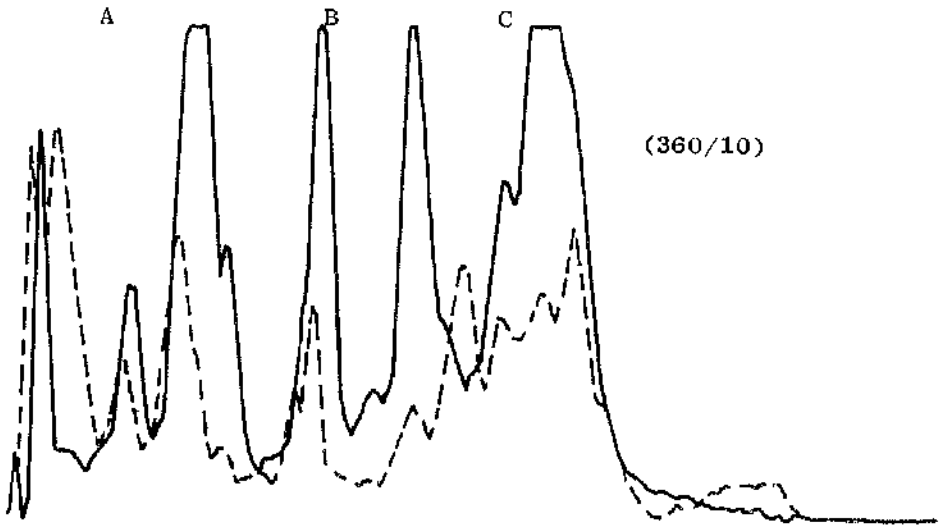


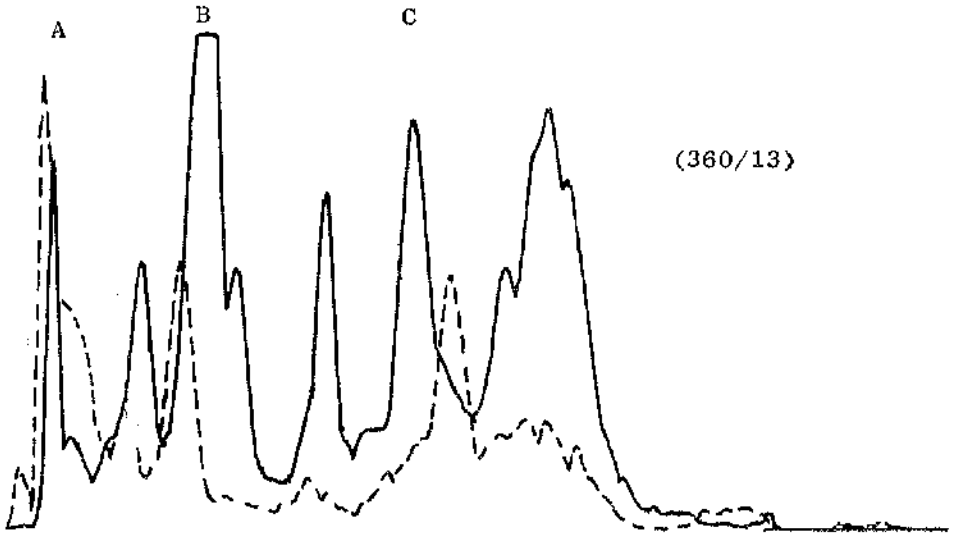




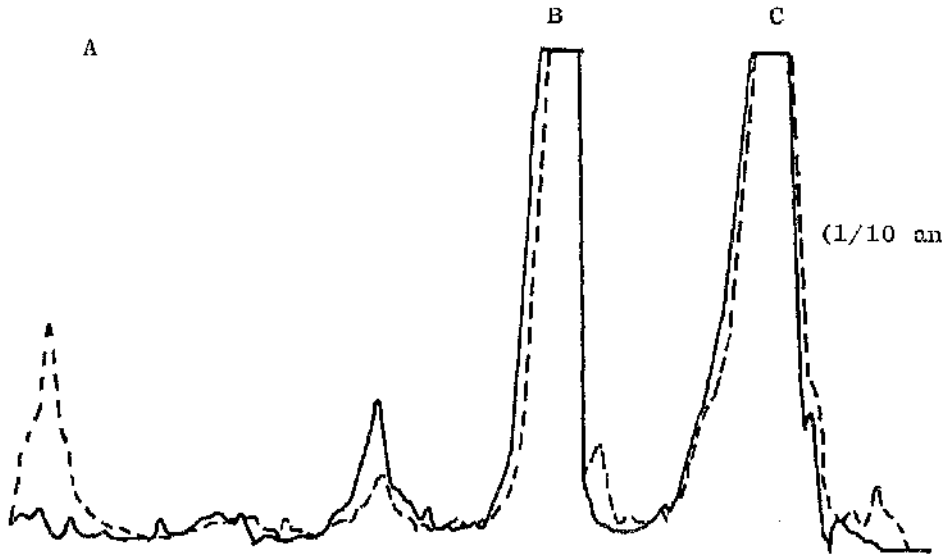
Neutrase enzyme was added at a rate of 0.05% (w/w)



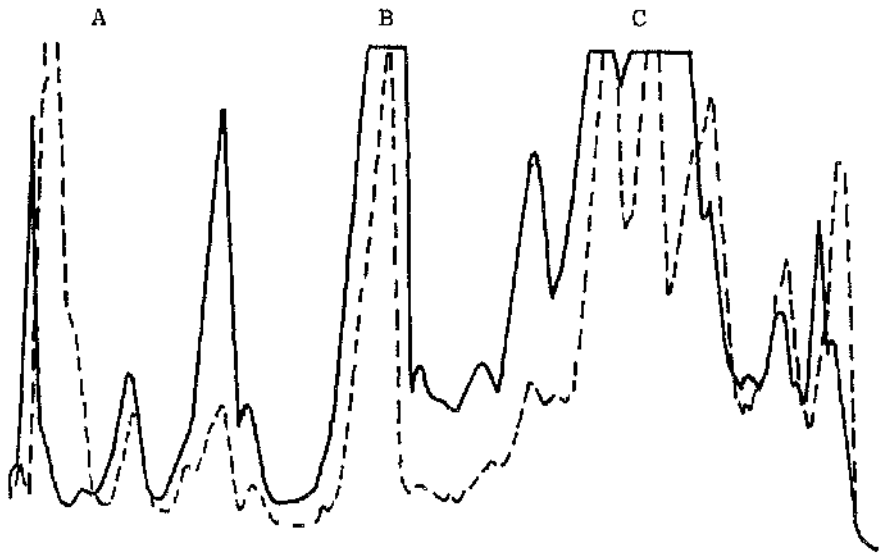




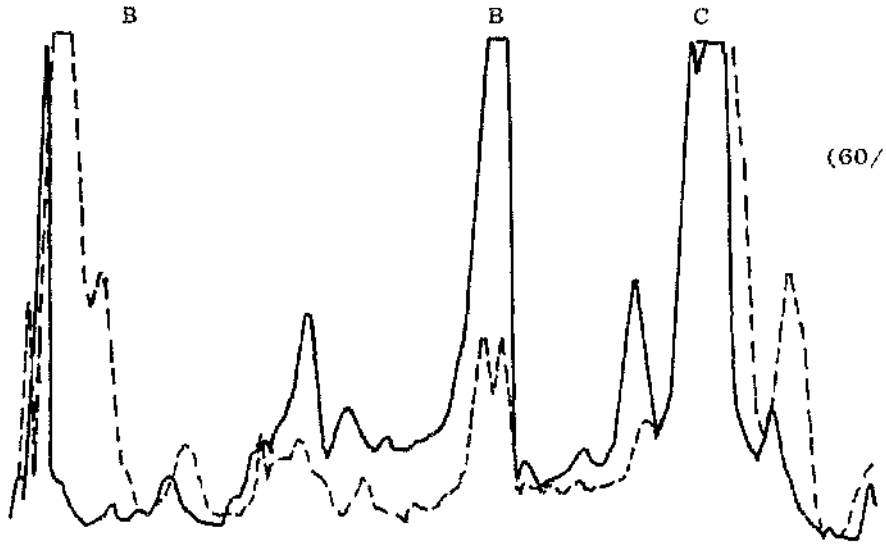
Neutrase enzyme was added at a rate of 0.01% (w/w)



(1/10 and 13)

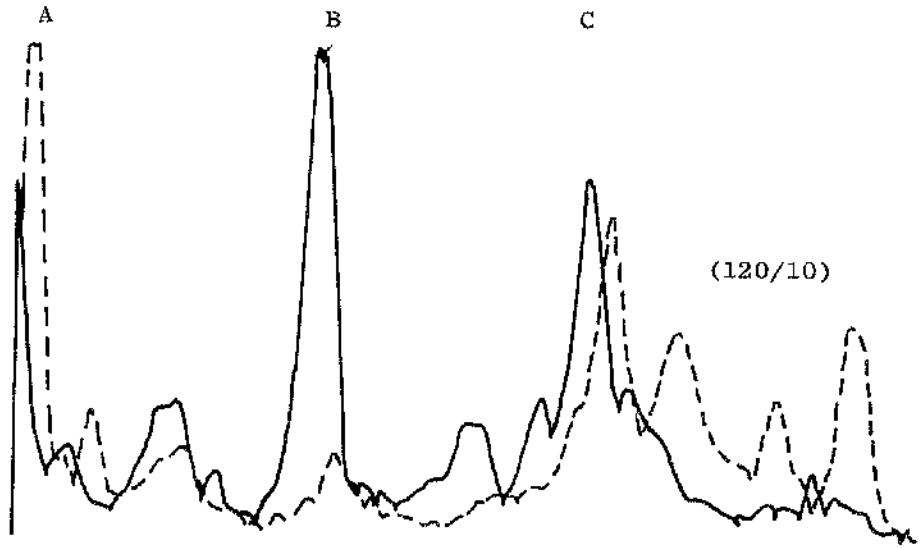


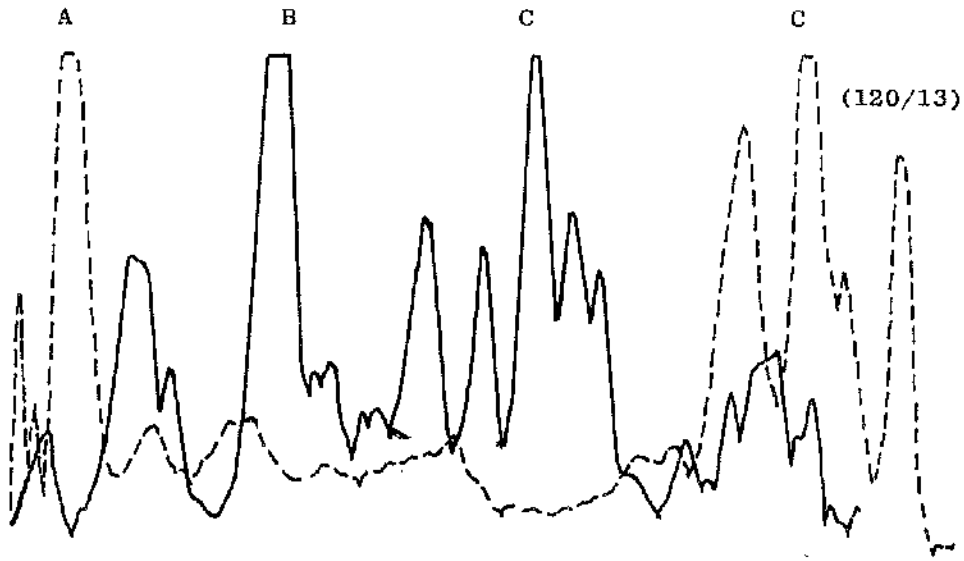
(60/10)

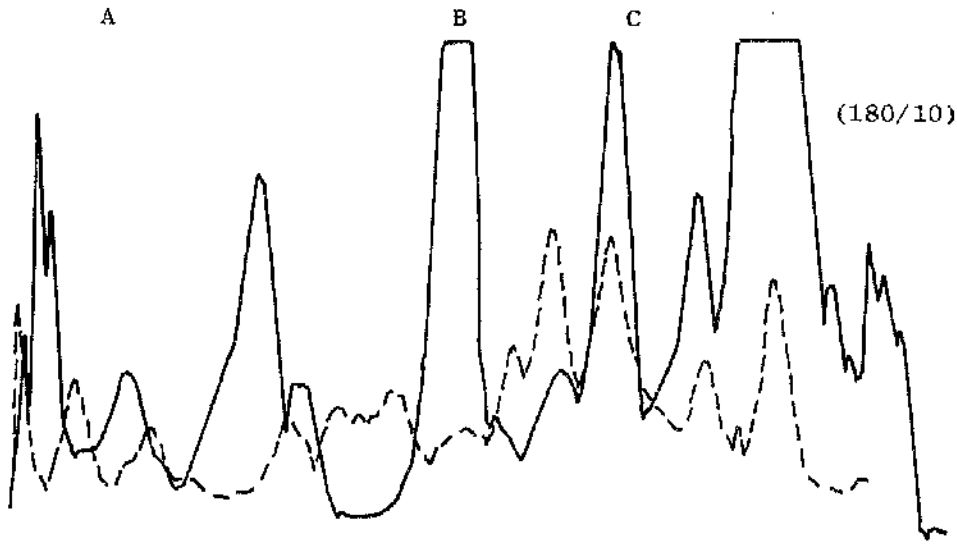


(60/13)

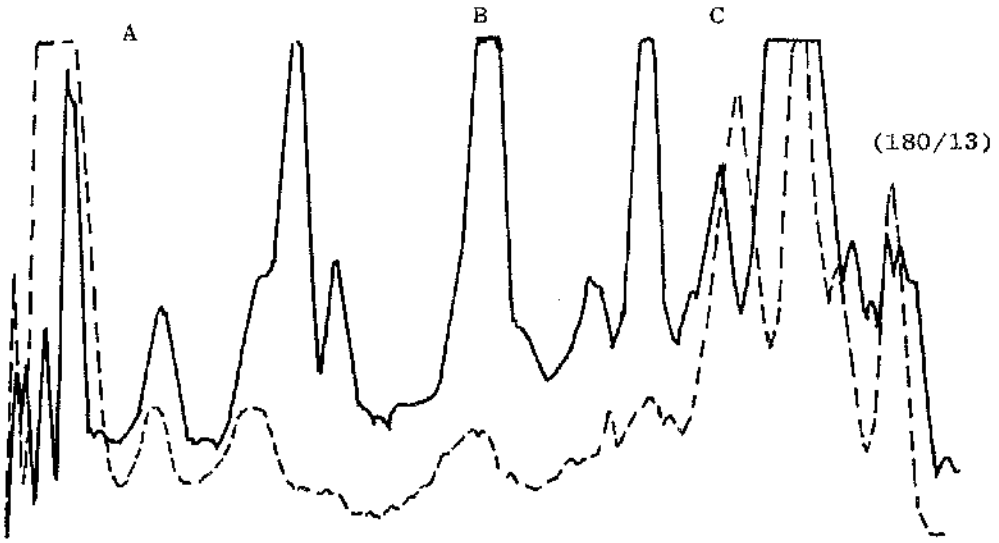
Neutrase enzyme was added at a rate of o.01% (w/w)

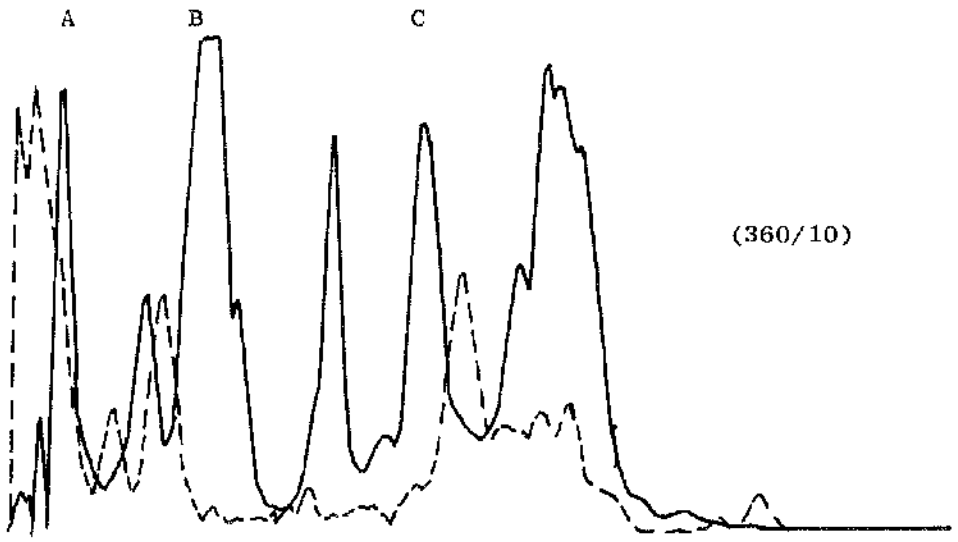






Neutrase enzyme was added at a rate of 0.01% (w/w)





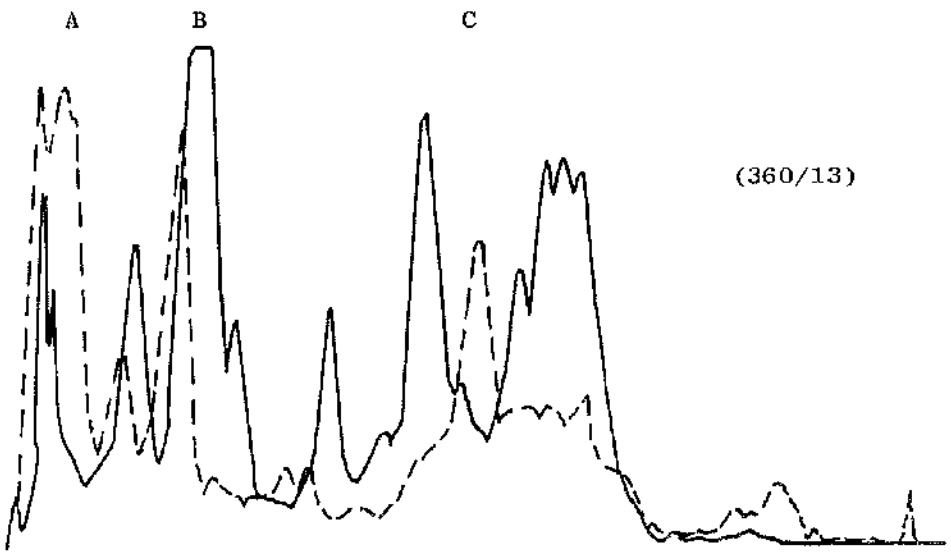
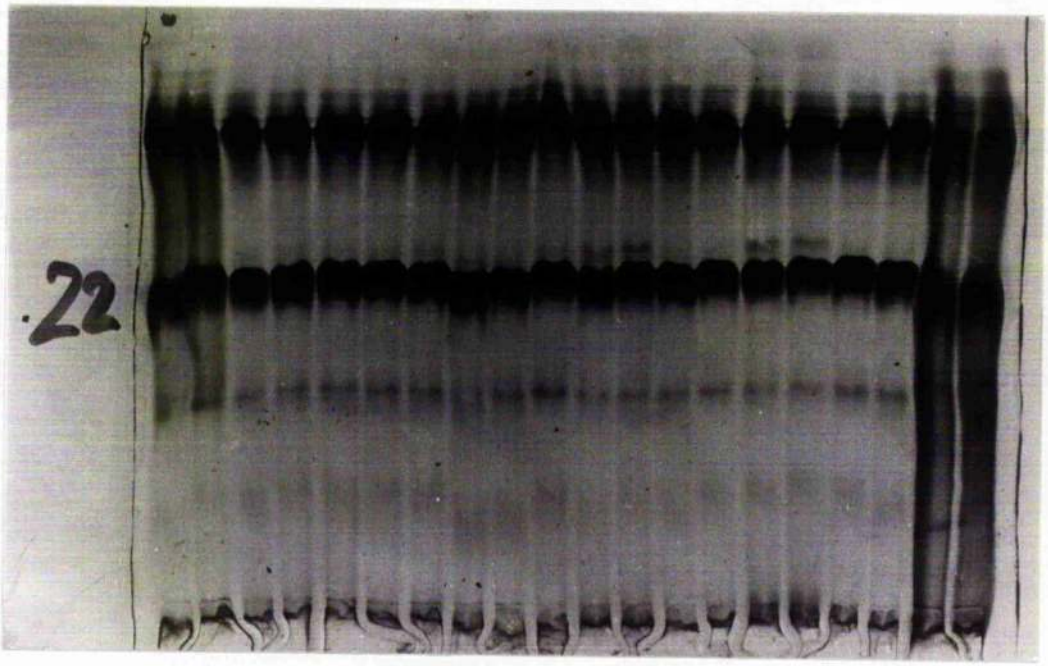


Plate 4.1 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase using starter culture 850 after 1 day of ripening.

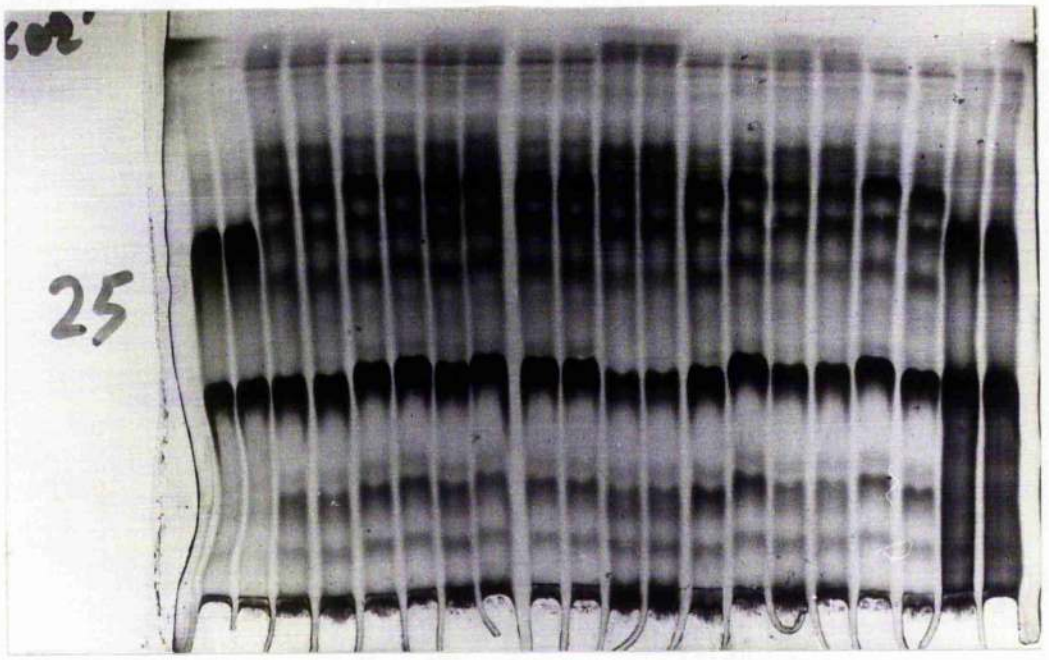
Track No. 1



Track No. 20

Pattern for cheese made from:	Number of tracks (left to right)
Curd with Neutrase (0.001% w/w)	3, 4
Control curd	5, 6
Curd with Neutrase (0.002% w/w)	7, 8
Control curd	9, 10
Curd with Neutrase (0.005% w/w)	11, 12
Control curd	13, 14
Curd with Neutrase (0.01% w/w)	15, 16
Control curd	17, 18
Standard casein	1, 2, 19, 20

Plate 4.2 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase using starter culture 850 and ripened at 10 and 13°C after 2 months of ripening.



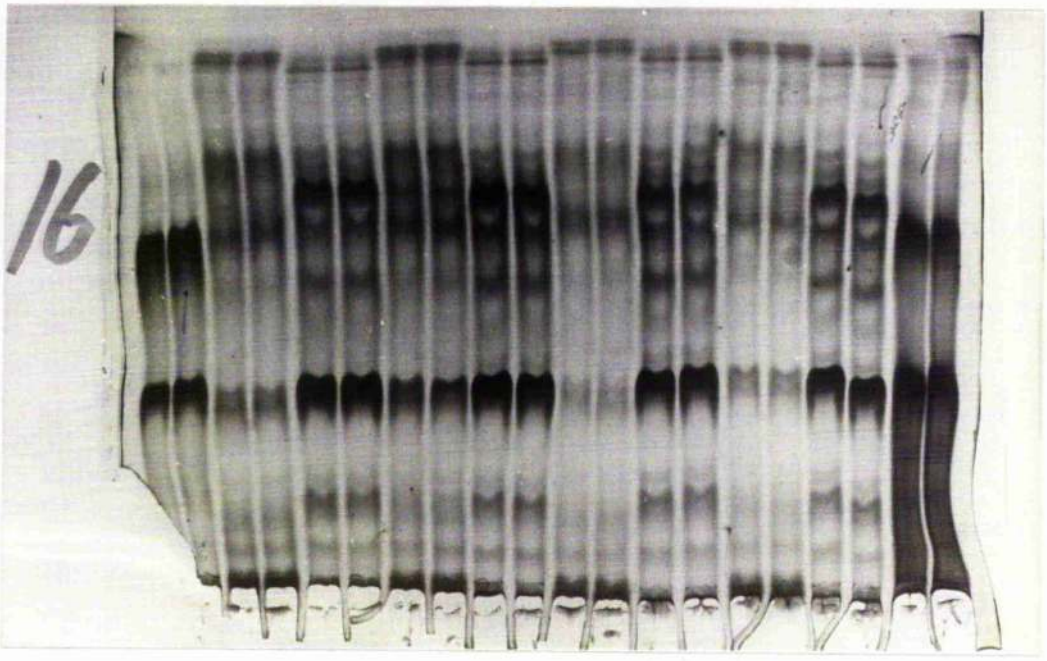
Track No. 1

Track No. 20

Pattern for cheese made from:	Number of tracks (Left to right)
Curd with Neutrase (0.001% w/w) ripened at 13°C	3, 4
Control curd ripened at 13°C	5, 6
Curd with Neutrase (0.001% w/w) ripened at 10°C	7, 8
Control curd ripened at 10°C	9, 10
Curd with Neutrase (0.002% w/w) ripened at 13°C	11, 12
Control curd ripened at 13°C	13, 14
Curd with Neutrase (0.002% w/w) ripened at 10°C	15, 16
Control curd ripened at 10°C	17, 18
Skim milk	1, 2
Standard casein	19, 20

Plate 4.3 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase using starter culture 850 and ripened at 10 and 13°C after 2 months of ripening.

Track No. 1

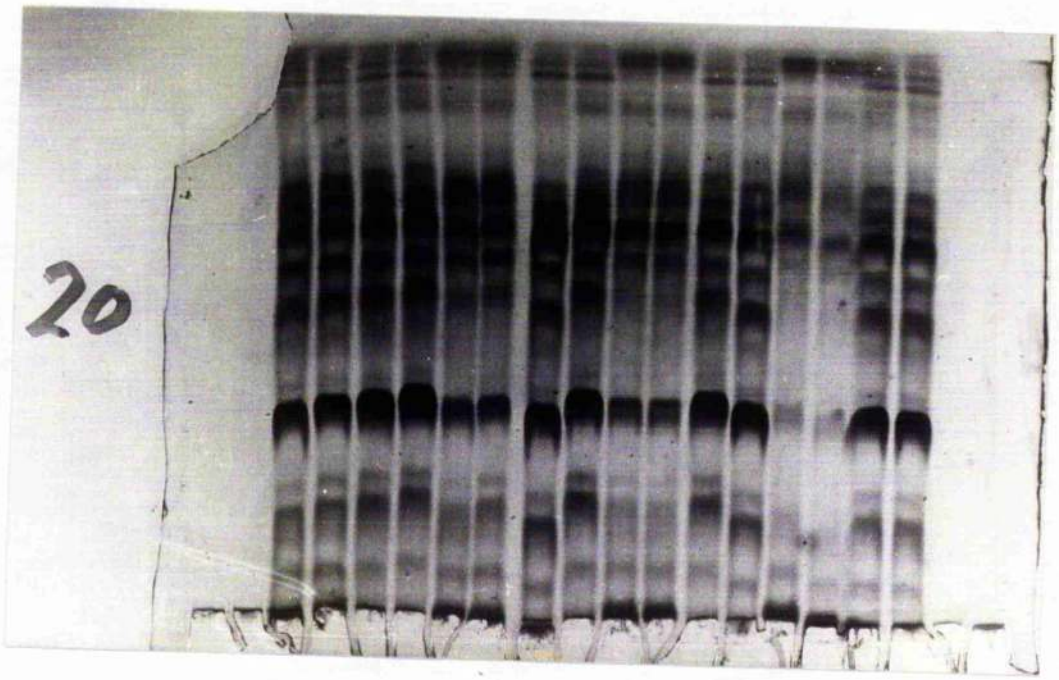


Track No. 20

Pattern for cheese made from:	Number of tracks (left to right)
Curd with Neutrase (0.005% w/w) ripened at 13°C	3, 4
Control curd ripened at 13°C	5, 6
Curd with Neutrase (0.005% w/w) ripened at 10°C	7, 8
Control curd ripened at 10°C	9, 10
Curd with Neutrase (0.01% w/w) ripened at 13°C	11, 12
Control curd ripened at 13°C	13, 14
Curd with Neutrase (0.01% w/w) ripened at 10°C	15, 16
Control curd ripened at 10°C	17, 18
Skim milk	1, 2
Standard casein	19, 20

Plate 4.4 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase using starter culture 850 and ripened at 10 after 4 months of ripening.

Track No. 1

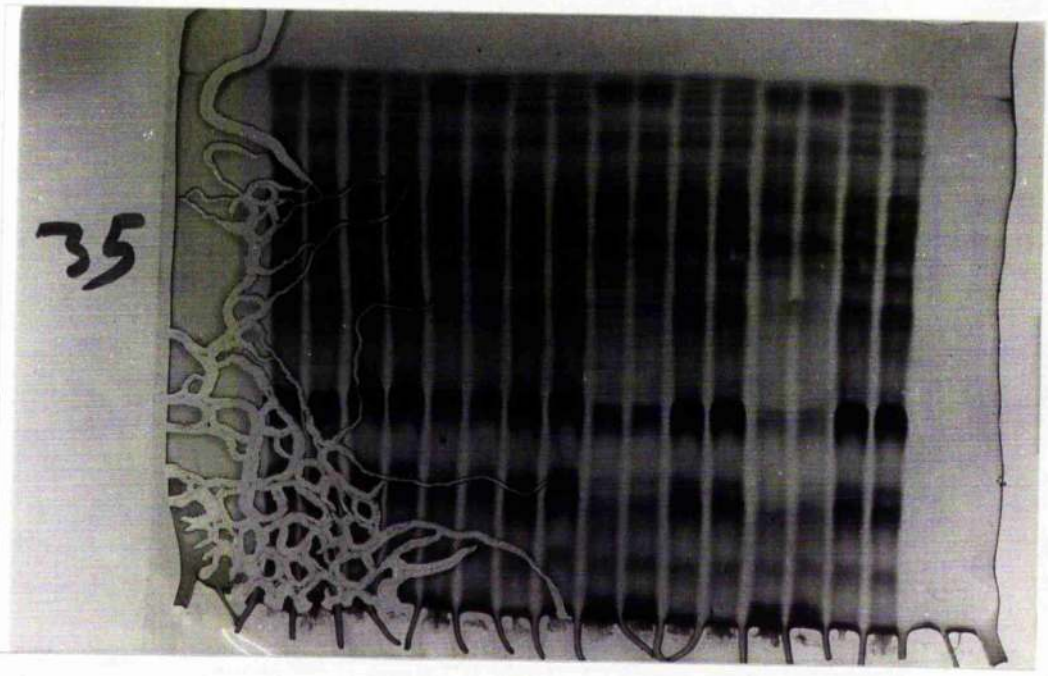


Track No. 16

Pattern for cheese made from:	Number of tracks (left to right)
Curd with Neutrase (0.001% w/w)	1, 2
Control curd	3, 4
Curd with Neutrase (0.002% w/w)	5, 6
Control curd	7, 8
Curd with Neutrase (0.005% w/w)	9, 10
Control curd	11, 12
Curd with Neutrase (0.01% w/w)	13, 14
Control curd	15, 16

Plate 4.5 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase using starter culture 850 and ripened at 13°C after 4 months of ripening

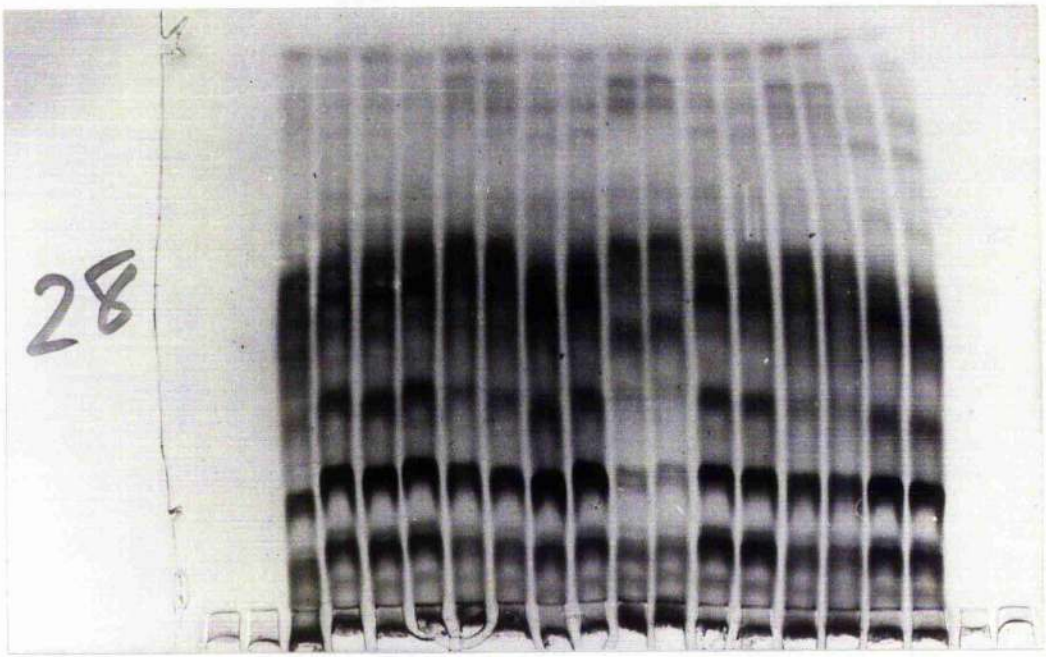
Track No. 1



Track No. 16

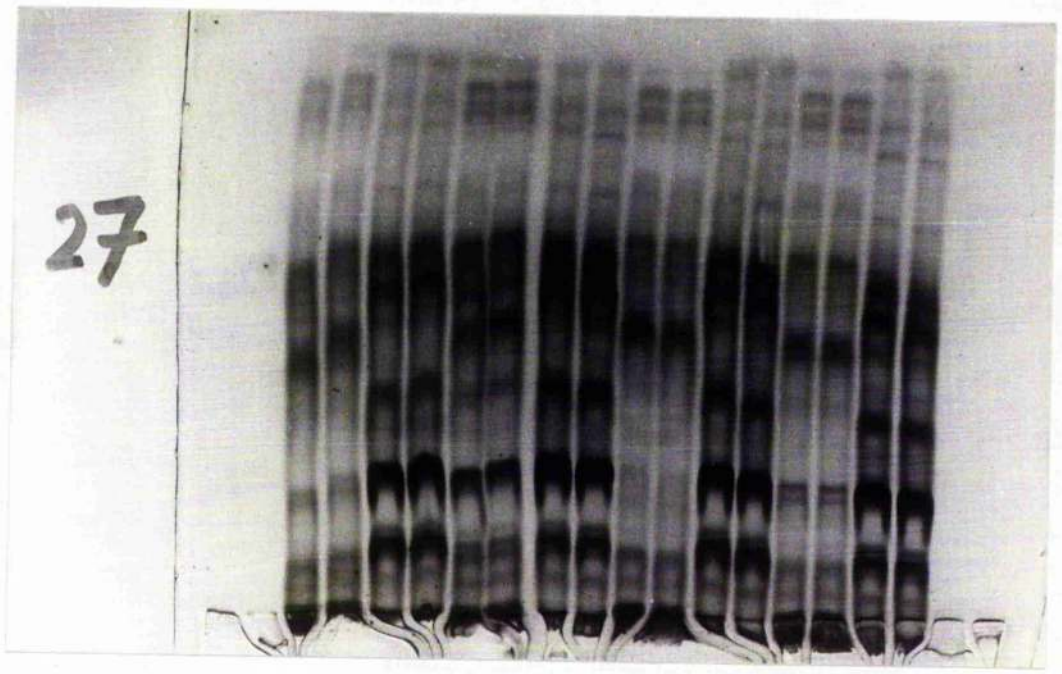
Pattern for cheese made from:	Number of tracks (left to right)
Curd with Neutrase (0.001% w/w)	1, 2
Control curd	3, 4
Curd with Neutrase (0.002% w/w)	5, 6
Control curd	7, 8
Curd with Neutrase (0.005% w/w)	9, 10
Control curd	11, 12
Curd with Neutrase (0.01% w/w)	13, 14
Control curd	15, 16

Plate 4.6 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase using starter culture 850 and ripened at 10 and 13°C after 6 months of ripening.



Pattern for cheese made from:	Number of tracks (left to right)
Curd with Neutrase (0.001% w/w) ripened at 13°C	1, 2
Control curd ripened at 13°C	3, 4
Curd with Neutrase (0.001% w/w) ripened at 10°C	5, 6
Control curd ripened at 10°C	7, 8
Curd with Neutrase (0.002% w/w) ripened at 13°C	9, 10
Control curd ripened at 13°C	11, 12
Curd with Neutrase (0.002% w/w) ripened at 10°C	13, 14
Control curd ripened at 10°C	15, 16

Plate 4.7 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase using starter culture 850 and ripened at 10 and 13°C after 6 months of ripening.



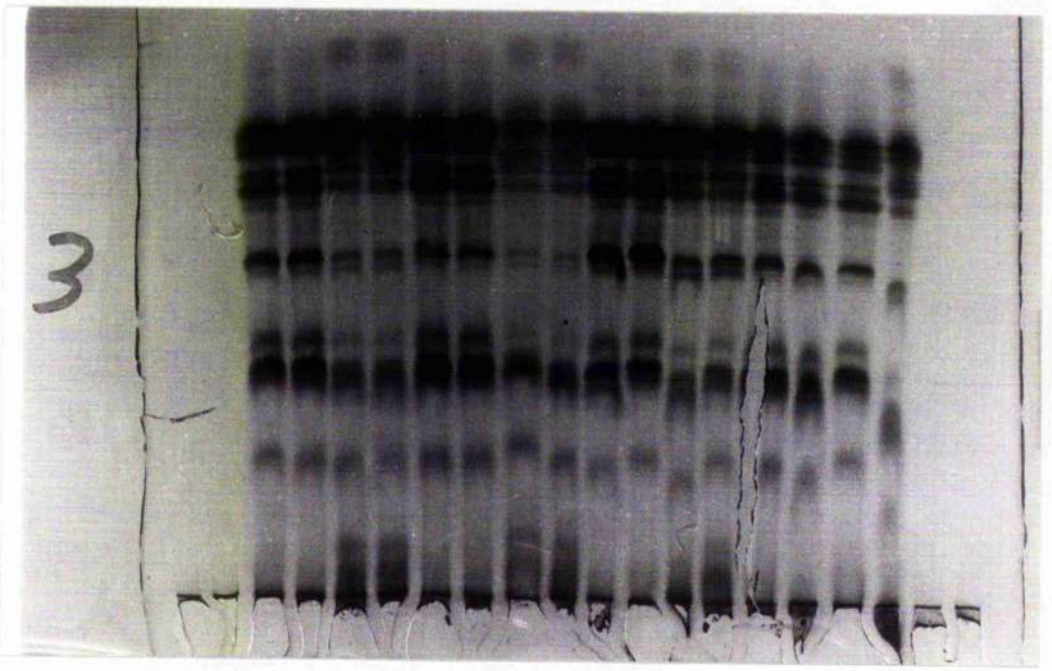
Track No. 1

Track No. 16

Pattern for cheese made from:	Number of tracks (left to right)
Curd with Neutrase (0.005% w/w) ripened at 13°C	1, 2
Control curd ripened at 13°C	3, 4
Curd with Neutrase (0.005% w/w) ripened at 10°C	5, 6
Control curd ripened at 10°C	7, 8
Curd with Neutrase (0.01 w/w) ripened at 13°C	9, 10
Control curd ripened at 13°C	11, 12
Curd with Neutrase (0.01% w/w) ripened at 10°C	13, 16
Control curd ripened at 10°C	15, 16

Plate 4.8 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase using starter culture 850 and ripened at 10 and 13°C after 12 months of ripening.

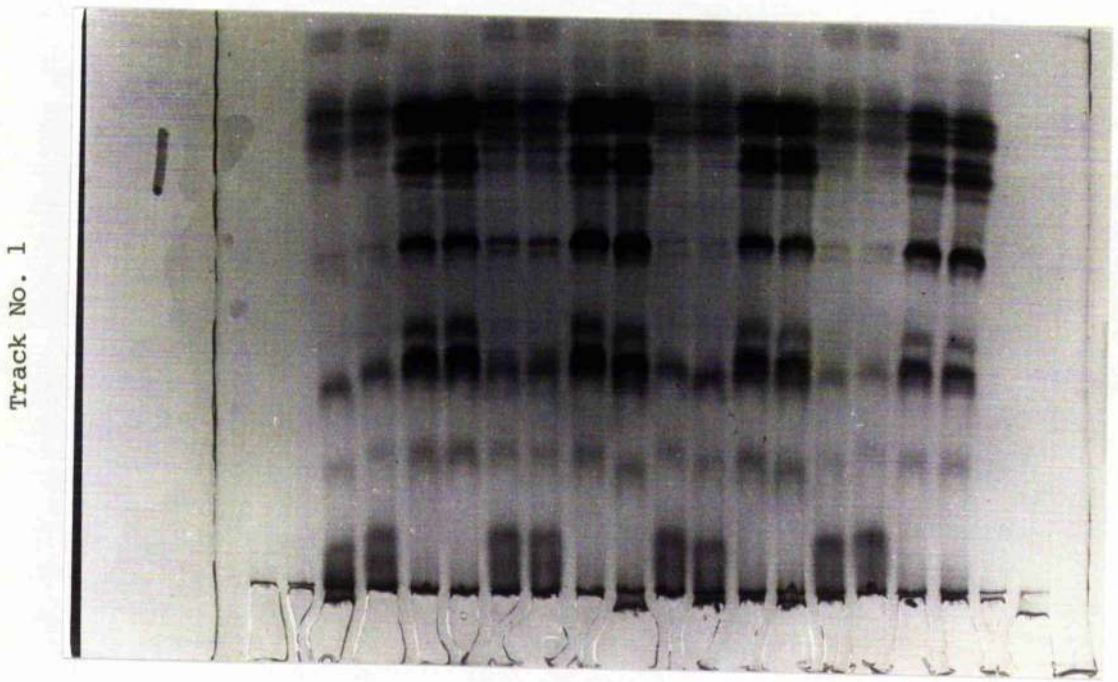
Track No. 1



Track No. 16

Pattern for cheese made from:	Number of tracks (left to right)
Curd with Neutrase (0.001% w/w) ripened at 13°C	1, 2
Control curd ripened at 13°C	3, 4
Curd with Neutrase (0.001% w/w) ripened at 10°C	5, 6
Control curd ripened at 10°C	7, 8
Curd with Neutrase (0.002% w/w) ripened at 13°C	9, 10
Control curd ripened at 13°C	11, 12
Curd with Neutrase (0.002% w/w) ripened at 10°C	13, 14
Control curd ripened at 10°C	15, 16

Plate 4.9 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase using starter culture 850 and ripened at 10 and 13°C after 12 months of ripening.



Pattern for cheese made from:	Number of tracks (left to right)
Curd with Neutrase (0.005% w/w) ripened at 13°C	1, 2
Control curd ripened at 13°C	3, 4
Curd with Neutrase (0.005% w/w) ripened at 10°C	5, 6
Control curd ripened at 10°C	7, 8
Curd with Neutrase (0.01% w/w) ripened at 13°C	9, 10
Control curd ripened at 13°C	11, 12
Curd with Neutrase (0.01% w/w) ripened at 10°C	13, 14
Control curd ripened at 10°C	15, 16

(ii) β-casein Fractions

At the early stages of the ripening period, β-casein appeared as one big band (band number 2) with 1-3 small bands just starting to separate from it. The hydrolysis of the major band of β-casein fraction during the 12 months period of ripening could be summarised as follows:

(a) More extensive breakdown of β-casein could be observed in the experimental cheese compared with the control.

(b) The degree of breakdown of β-casein was dependent on the level of enzyme used (i.e. the greater the amount used the more breakdown took place), and such observation was in agreement to the work reported by Sood & . Kosikowski (1979) and Law & Wigmore (1982).

(c) The hydrolysis of β-casein was slightly influenced by the starter culture (see Tables 4.5 to 4.12).

(d) In general ripening of the cheese at 13°C enhanced the rate of hydrolysis of β-casein; however, the degree of hydrolysis in some cheeses, ripened at 10°C was greater than cheese ripened at 13°C (see Tables 4.5 to 4.12)

(e) The hydrolysis of β-casein in the experimental cheese reached its maximum after 6 months of ripening and apparently increased in the relative proportion of β-casein fractions(s) to the other casein fraction. This could be attributed to the fact that most of the other casein fractions remained degraded and affected the percentage of β-casein as illustrated in Figures 4.3 and 4.4 and Plates 4.1 to 4.9 (Davies, 1984)..

(iii) α_s-casein fractions

α_s-casein fractions appeared in one day old cheese as one major band which is α_{s_1}-casein (band number 3), and 1-4 small bands just separated from it to the top of the gel and/or behind it. The pattern of α_s-casein hydrolysis in Cheddar cheese (control and experimental) could be summarised as follows:

(a) Fraction 3 was hydrolysed more rapidly in the Neutrase treated cheese compared with control cheese, and extensive breakdown was observed after 2 and 4 months of ripening. Such degree of hydrolysis of α_{s_1}-casein was in agreement with the results of Law & Wigmore (1982).

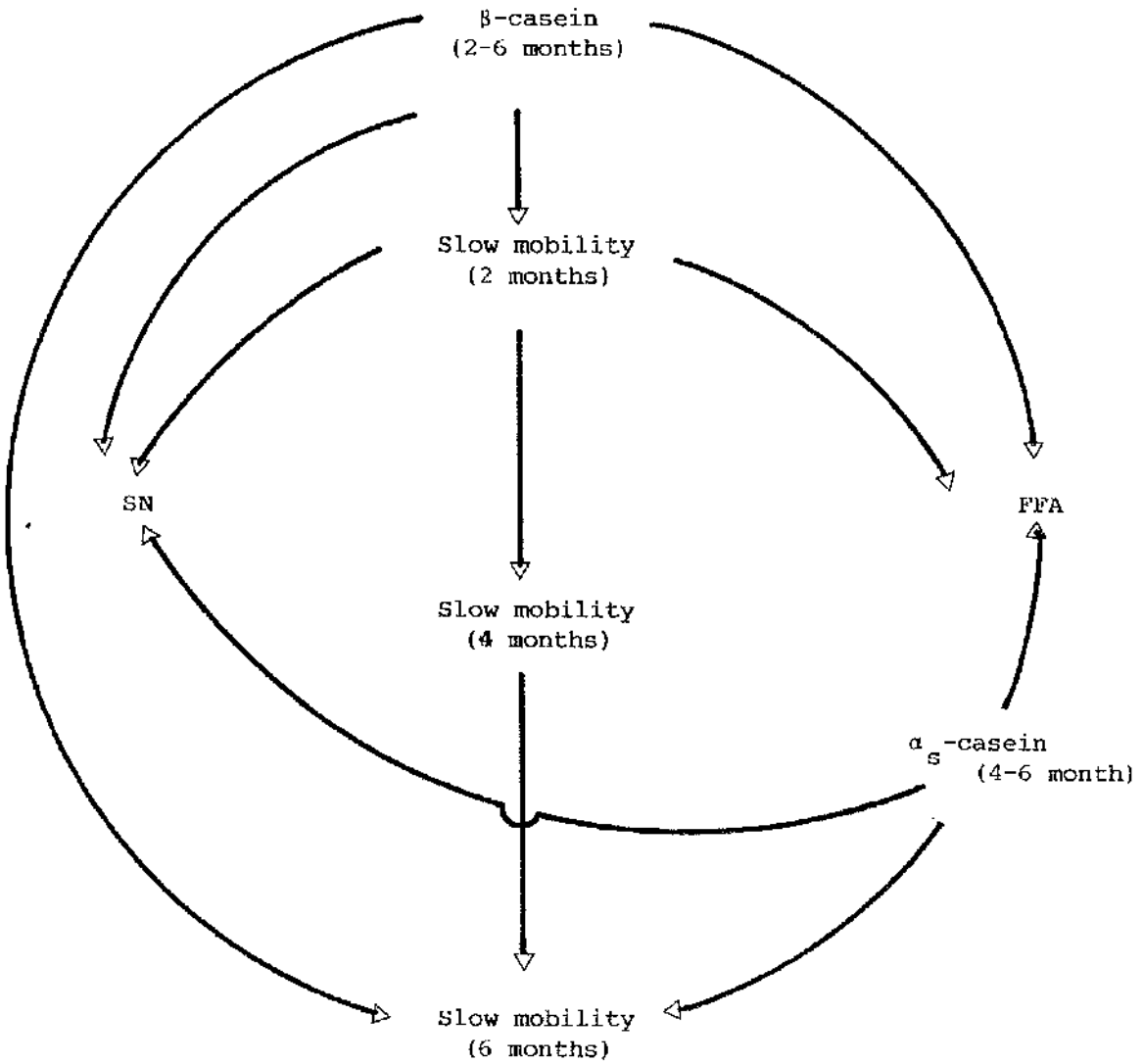
(b) Fractions 1 and 2 increased in size in 6 months old cheeses and decreased after 12 months of ripening.

(c) The gradual increase in the percentage of the total α_s-casein fractions up to 6 months old cheeses could be attributed to the extensive breakdown of β-casein which affect the individual area of the other fractions (see Plates 4.1 to 4.9); however, the decrease after 12 months could be attributed to the hydrolysis of these fractions which results in bands having the same optical density similar to the slow mobility casein fractions, or the production of soluble nitrogen and free amino acids (see Figure 4.5).

(d) The effect of the 2 different temperatures (10 and 13°C) on the hydrolysis of α_s-casein during the ripening period fluctuated dramatically, and it was difficult to draw out any conclusion. Although, the electrophoretic method of analysis provides an excellent resolution of casein hydrolysis in cheese during the ripening period the qualitative and quantitative determination of the progress of hydrolysis could be influenced by factors such as:

- the uneven distribution of the enzyme in the cheese;
- sampling error;
- the effect of reagents (e.g. slight change in the ionic strength of buffer) and/or equipment, i.e. the maintenance of constant electrical current during running the gel, temperature, stain concentration and staining period;
- the presence of some impurities in the chemical reagents which might affect the quality of the gel resulting in some tailing and smearing effects;
- incomplete fat separation from the cheese samples.

Figure 4.5: Schematic illustration showing the degradation of casein fractions in cheese during the maturation period



SN = soluble nitrogen
FFA = free amino acids

After Ridha, Crawford and Tamime (1984)

4.4 Organoleptic Assessment

The cheeses (control and experimental) were assessed and evaluated organoleptically at 2, 4 and 6 months old by 8 panelists as well as the official grader of the Company of Scottish Cheesemakers Ltd.

The cheese qualities evaluated by the 9 panelists were: taste, odour, bitterness, openness, firmness and smoothness, and the scores were awarded on an eleven-point hedonic scale as described in 4.2.6. The overall data wer analysed statistically by using the mean differences between the control and Neutrase-treated cheese where the cheese characteristics were evaluated sparately against each level of added Neutrase (0.001, 0.002, 0.005 and 0.01% w/w); the starter culture (850 and 890), and the ripening period (2, 4 and 6 months).

In this study the cheese samples, i.e. 16 in number, were evaluated in duplicate and at random, and the samples were identified by using numbers in accordance with the statistical tables (Lindley & Miller, 1968). Pieces of apples were provided to the panelists to eat between tasting of the cheese samples in order to clean the palate. The cheese samples were prepared as follows: the blocks of cheese (e.g. 4.75 kg each) were opened from one end, a slice of cheese, (1 cm thick), was discarded and another slice of the same thickness was cut into cubes and provided for evaluation at room temperature, i.e. 20°C.

4.4.1 Taste of the Cheese

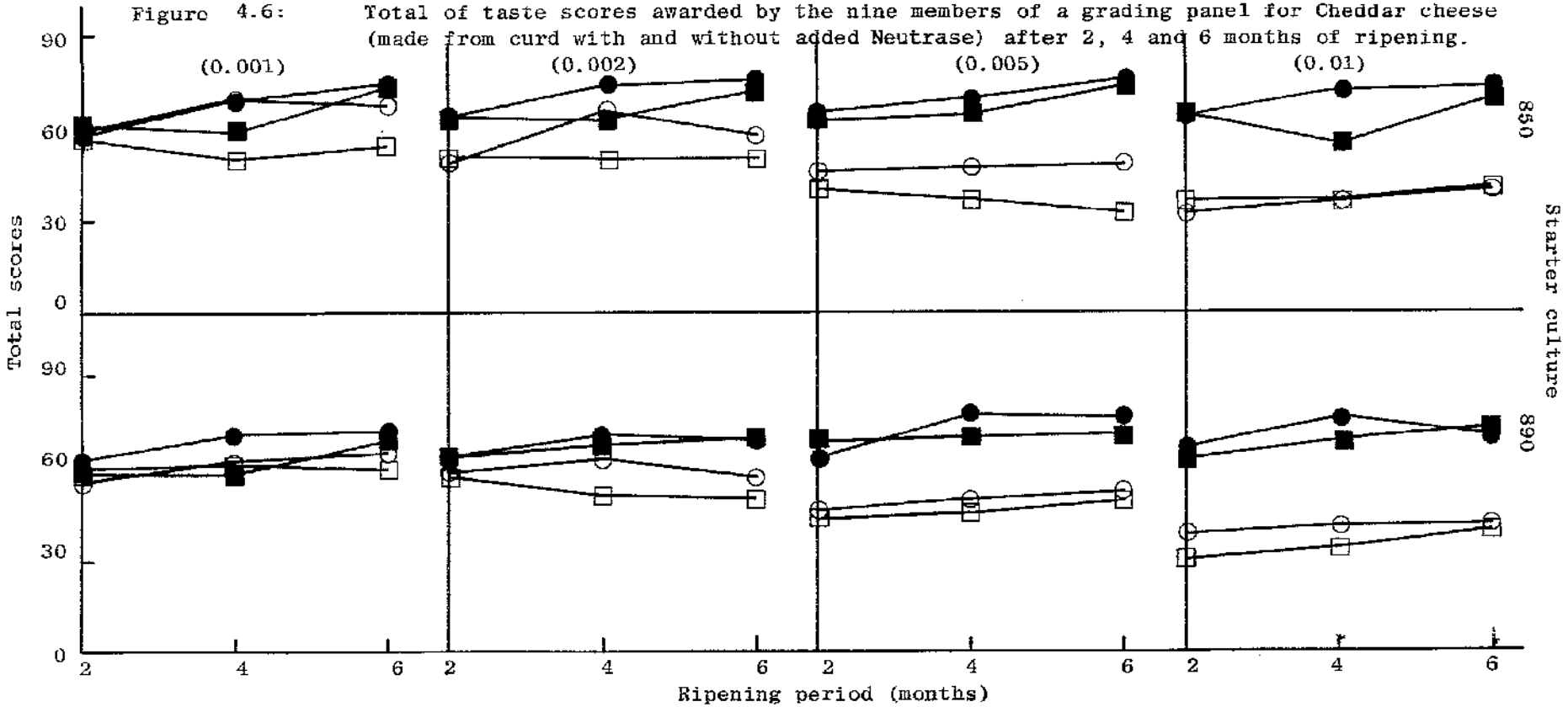
The total scores for the taste of cheese, which were awarded by the panelists is shown in Figures 4.6, and it can be observed that:

(A) the taste of the control cheese ripened at 10 and 13°C for both starter cultures was similar during the ripening period, and the panelists preferred the control cheese.

(b) Neutrase enzyme added at a rate of 0.001% (w/w) slightly affected the taste of the cheese, and both cheeses (control and experimental) had similar scores (see Figures 4.6). This observation confirms the reported work of Law & Wigmore (1982). However, ripening of such cheese at 13°C resulted in an inferior taste.

(c) the taste of all the cheeses was similar at 2 months old, but

Figure 4.6: Total of taste scores awarded by the nine members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 2, 4 and 6 months of ripening.



Figures in parenthesis represent amount (% w/w) of Neutrase added

●, Control cheese ripened at 10°C

■, Control cheese ripened at 13°C

○, Neutrase treated cheese ripened at 19°C

□, Neutrase treated cheese ripened at 13°C

TABLE 4.13

Statistical analysis of the nine panelists' assessment of the taste of Cheddar cheese made from curd with and without added Neutrase (data is difference between control and experimental)

										SED
Grader	1	2	3	4	5	6	7	8	9	
	1.250	1.187	1.206	2.177	0.865	3.578	2.023	3.219	2.585	0.191
*Enzyme	0.001	0.002	0.005	0.01						
	0.643	1.380	2.651	3.366						0.127
+Starter	850	890								
	2.193	1.827								0.090
Temperature	13°C	10°C								
	2.245	1.775								0.090
Storage	2-months	4-months	6-months							
	1.694	2.191	2.144							0.110
+Starter	850	890								
*Enzyme										
0.001	0.827	0.459								
0.002	1.654	1.106								
0.005	2.947	2.354								
0.01	3.345	3.387								0.180
Temperature	13°C	10°C								
Enzyme										
0.001	0.959	0.327								
0.002	1.772	0.989								
0.005	2.898	2.403								
0.01	3.351	3.381								0.180

cont'd.....

Table 4.13 (cont'd.)

					SED
Temperature	13°C	10°C			
+ Starter					
850	2.652	1.734			
890	1.838	1.815			0.127
Storage	2-months	4-months	6-months		
* Enzyme					
0·001	0.250	0.738	0.941		
0·002	1.083	1.409	1.648		
0·005	2.292	2.915	2.745		
0·01	3.153	3.703	3.243		0.220
Storage	2-months	4-months	6-months		
+ Starter					
850	1.840	2.499	2.240		
890	1.549	1.883	2.048		0.156
Storage	2-months	4-months	6-months		
Temperature					
13°C	1.722	2.506	2.507		
10°C	1.667	1.876	1.782		0.156
+ Starter	850		890		
Temperature	13°C	10°C	13°C	10°C	
* Enzyme					
0·001	1.714	-0.060	0.204	0.714	
0·002	2.081	1.228	1.463	0.748	
0·005	3.500	2.394	2.296	2.413	
0·01	3.314	3.375	3.389	3.386	0.254

cont'd.....

Table 4.13(cont'd.)

							SED
+Starter	850			890			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
*Enzyme							
0.001	0.194	1.321	0.965	0.306	0.154	0.917	
0.002	1.528	1.649	1.786	0.639	1.169	1.509	
0.005	2.389	3.278	3.174	2.194	2.552	2.317	
0.01	3.250	3.749	3.035	3.056	3.656	3.450	0.311
Temperature	13°C			10°C			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
*Enzyme							
0.001	0.167	1.127	1.583	0.333	0.349	0.299	
0.002	1.056	2.122	2.193	1.111	0.696	1.157	
0.005	2.500	3.084	3.111	2.083	2.747	2.380	
0.01	3.167	3.693	3.194	3.139	3.712	3.292	0.311
Temperature	13°C			10°C			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
+Starter							
850	1.889	3.221	2.847	1.792	1.778	1.633	
890	1.556	1.792	2.167	1.542	1.974	1.930	0.220

(+ ve) and (-ve) figures illustrates panelists' preference of the control or the experimental cheese respectively.

* figures indicate percentage (w/w) levels of added Neutrase
+ numbers indicate identity of culture

cont'd.....

Table 4.13(cont'd.)

Source of variation	DF	MS	VR
Grader	8	44.9470	51.492***
Enzyme	3	162.5452	186.213***
Starter	1	14.5235	16.683***
Temperature	1	23.9010	27.381***
Storage	2	10.8318	12.409***
Grader x Enzyme	24	5.5920	6.406***
Grader x Starter	8	4.2280	4.844***
Enzyme x Starter	3	2.2664	2.596
Grader x Temperature	8	7.5040	8.597***
Enzyme x Temperature	3	3.3725	3.864***
Starter x Temperature	1	21.6527	24.805***
Grader x Storage	16	4.4782	5.130***
Enzyme x Storage	6	1.1577	1.326
Starter x Storage	2	1.7766	2.035
Temperature x Storage	2	4.7279	5.416**
Grader x Enzyme x Starter	24	1.1736	1.345
Grader x Enzyme x Temperature	24	2.5411	2.911***
Grader x Starter x Temperature	8	7.6653	8.781***
Enzyme x Starter x Temperature	3	7.9468	9.104***
Grader x Enzyme x Storage	48	2.4200	2.722***
Grader x Starter x Storage	16	2.6686	3.057***
Enzyme x Starter x Storage	6	1.8434	2.112*
Grader x Temperature x Storage	15	8.4173	9.643***
Enzyme x Temperature x Storage	6	1.9261	2.207*
Starter x Temperature x Storage	2	5.3981	6.184**
Residual	170	0.8729	
TOTAL	410	4.6507	

*Significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " "

Temperature = ripening temperature
Storage = ripening period

as the amount of added Neutrase was increased, the taste scores of the cheese progressively decreased.

(d) all the experimental cheeses ripened at 13°C were inferior in taste compared with the other cheeses (control and experimental). This could be due to the enhanced activity of the Neutrase enzyme to produce defects in cheese due to flavour-forming reactions.

The statistical analysis (mean differences) of the 9 panelists assessment of the taste of the cheese and the analysis of variance is shown in Table 4.13, and the following conclusions could be observed:

(i) Differences between the panelists' judgements were highly significant ($p < 0.001$), and varied between 0.865 to 3.219.

(ii) The panelists showed a preference for the taste of the untreated cheese and this was highly significant ($p < 0.001$). In the cheese made with added Neutrase the lowest level of Neutrase addition gave the best taste score. The difference was 0.643 in favour of the control cheese while it was 3.366 with the addition of 0.01% (w/w) Neutrase.

(iii) The panelists showed a preference for the cheese produced from starter culture 890 (significant at $p < 0.001$) where the mean difference was 1.827 compared with 2.193 for the cheese produced using starter culture 850.

(iv) The ripening temperature had a highly significant ($p < 0.001$) effect on the taste of the cheese, and the highest score for taste was obtained when the cheese was ripened at 10°C.

(v) Although the length of ripening period had a highly significant effect ($p < 0.001$) on the taste of the cheese, there were slight differences between 4 and 6 months of ripening (the mean differences were 2.191 and 2.144 respectively).

(vi) The following interactions were found to be significant at different levels and could be grouped as follows:

(a) at $p < 0.001$, panelist x enzyme, panelist x starter culture, panelist x ripening temperature, enzyme x ripening temperature, starter culture x ripening temperature, panelist x ripening period, panelist x enzyme x ripening temperature,

panelist x starter culture x ripening temperature, enzyme x starter culture x ripening temperature, panelist x enzyme x tipening period, panelist x starter culture x ripening period and panelist x ripening temperature x ripening period.

(b) at $p < 0.01$ ripening temperature x ripening period, and starter culture x ripening temperature x ripening period.

(c) at $p < 0.05$ enzyme x ripening temperature x ripening period.

It may be concluded from the above results and/or analysis that the use of Neutrase enzyme did not produce an acceptable taste even with the use of the smallest amount i.e. 0.001% w/w. Such results are not in agreement with what Law & Wigmore (1982) reported. It is possible that the high ripening temperature of 13°C may have adversely affected the taste of the cheese due to the acceleration of off-flavour formation and the growth of undesirable microflora (Law & Wigmore, 1982). Cheese made from starter culture 890 had the best taste compared with starter culture 850 because the latter starter is more proteolytice (see Section 4.3.3).

4.4.2 Odour or smell of the cheese

The total scores awarded for the odour of the cheese by the 9 panelists are shown in Figure 4.7, and it can be observed that the overall results have a similar trend as reported in 4.4.1.

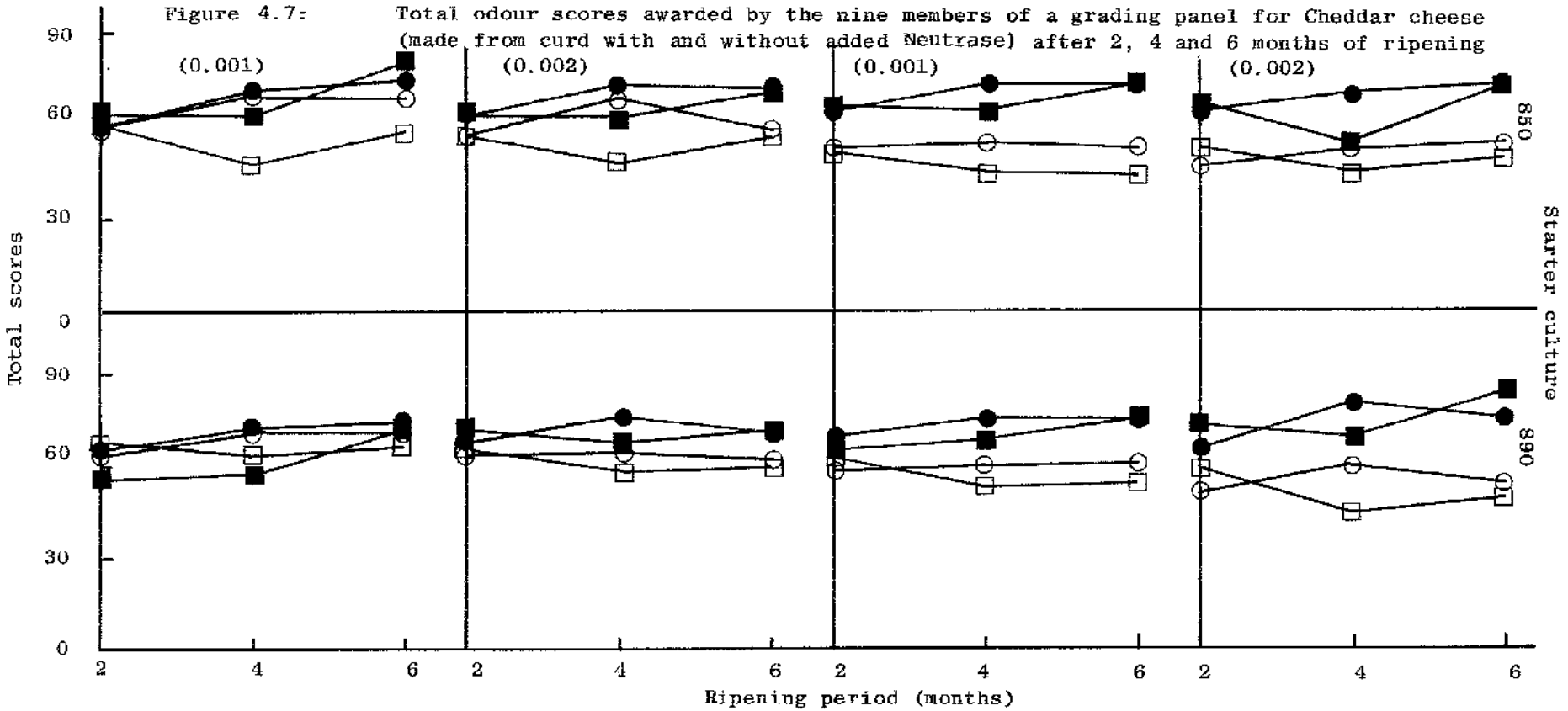
Table 4.14 illustrates the statistical analysis (mean differences) of the data. The following conclusions can be made:

(i) Differences between the panelists' judgements were highly significant ($p < 0.001$) and varied between 0.719 and 2.442.

(ii) Preference was indicated to the odour of the control cheese ($p < 0.001$), and it can be observed that the effect of Neutrase enzyme on the odour of the cheese was minimal at the added rate of 0.001% (w/w).

For example, the mean differences between the control and the experimental cheese (0.001% and 0.01% w/w) were 0.404 and 2.102 respectively.

Figure 4.7: Total odour scores awarded by the nine members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 2, 4 and 6 months of ripening



Figures in parenthesis represent amount (% w/w) of Neutrase added

● , Control cheese ripened at 10°C

■ , Control cheese ripened at 13°C

○ , Neutrase treated cheese ripened at 10°C

□ , Neutrase treated cheese ripened at 13°C

TABLE 4.14

Statistical analysis of the nine panelists' assessment of the odour (smell) of Cheddar cheese made from curd with and without added Neutrase (data is difference between control and experimental)

										SED
Grader	1	2	3	4	5	6	7	8	9	
	0.719	1.219	1.195	0.729	0.458	1.675	0.936	2.375	2.442	0.157
*Enzyme	0.001	0.002	0.005	0.01						
	0.404	1.022	1.694	2.102						0.105
+Starter	850	890								
	1.440	1.171								0.074
Temperature	13°C	10°C								
	1.461	1.150								0.074
Storage	2-months	4-months	6-months							
	0.962	1.360	1.594							0.091
+Starter	850	890								
*Enzyme										
0.001	0.735	0.073								
0.002	1.163	0.881								
0.005	1.991	1.477								
0.01	1.951	2.252								0.148
Temperature	13°C	10°C								
*Enzyme										
0.001	0.624	0.184								
0.002	1.194	0.854								
0.005	1.825	1.563								
0.01	2.206	1.997								

cont'd.....

Table 4.14(con'd.)

					SED
Temperature	13°C	10°C			
+ Starter					
850	1.700	1.180			
890	1.222	1.119			0.105
Storage	2-months	4-months	6-months		
* Enzyme					
0.001	0.056	0.542	0.614		
0.002	0.667	0.968	1.431		
0.005	1.292	1.759	2.032		
0.01	1.833	2.171	2.300		0.181
Storage	2-months	4-months	6-months		
+ Starter					
850	1.062	1.439	1.819		
890	0.861	1.281	1.370		0.128
Storage	2-months	4-months	6-months		
Temperature					
13°C	1.007	1.536	1.840		
10°C	0.917	1.184	1.348		0.128
+ Starter	850		890		
Temperature	13°C	10°C	13°C	10°C	
* Enzyme					
0.001	1.247	0.222	-0.000	0.146	
0.002	1.399	0.928	0.981	0.780	
0.005	2.187	1.634	1.463	1.492	
0.01	1.967	1.936	2.444	2.059	0.209

cont'd.....

Table 4.14 (cont'd.)

							SED
+Starter	850			890			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
*Enzyme							
0.001	0.250	0.982	0.971	-0.139	0.102	0.256	
0.002	0.750	1.015	1.725	0.583	0.921	1.138	
0.005	1.500	1.920	2.313	1.083	1.598	1.750	
0.01	1.750	1.839	2.265	1.917	2.503	2.336	0.256
Temperature	13°C			10°C			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
*Enzyme							
0.001	-0.056	0.955	0.972	0.167	0.130	0.256	
0.002	0.722	1.293	1.556	0.611	0.643	1.307	
0.005	1.333	1.725	2.417	1.250	1.793	1.647	
0.01	2.028	2.173	2.417	1.639	2.169	2.184	0.256
Temperature	13°C			10°C			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
+Starter							
850	1.139	1.795	2.167	0.986	1.083	1.470	
890	0.875	1.278	1.514	0.847	1.284	1.226	0.181

(+ve) and (-ve) figures illustrates panelists' preference of the control or the experimental cheese respectively

* figures indicate percentage (w/w) levels of added Neutrase
+ numbers indicate identity of culture

cont'd.....

Table 4.14 (cont'd.)

Source of variation	DF	MS	VR
Grader	8	24.7369	41.879***
Enzyme	3	60.4043	102.264***
Starter	1	7.8325	13.260***
Temperature	1	10.4846	17.750***
Storage	2	14.7251	24.929***
Grader x Enzyme	24	3.0554	5.173***
Grader x Starter	8	1.4397	2.437*
Enzyme x Starter	3	4.5493	7.702***
Grader x Temperature	8	1.1760	1.991*
Enzyme x Temperature	3	0.2711	0.459
Starter x Temperature	1	4.7018	7.960**
Grader x Storage	16	1.9209	3.252***
Enzyme x Storage	6	0.3582	0.606
Starter x Storage	2	0.8835	1.496
Temperature x Storage	2	1.4973	2.535
Grader x Enzyme x Starter	24	0.9593	1.624*
Grader x Enzyme x Temperature	24	0.8769	1.485
Grader x Starter x Temperature	8	1.2441	2.106*
Enzyme x Starter x Temperature	3	2.7311	4.624**
Grader x Enzyme x Storage	48	1.2931	2.189***
Grader x Starter x Storage	16	1.0973	1.858*
Enzyme x Starter x Storage	6	0.4533	0.767
Grader x Temperature x Storage	15	1.5272	2.585***
Enzyme x Temperature x Storage	6	1.4077	2.439*
Starter x Temperature x Storage	2	0.7924	1.342
Residual	170	0.5907	
TOTAL	410	2.0879	

*Significant at 5 per cent level
** " " 1 " " "
*** " " .0.1 " " "

Temperature = ripening temperature
Storage = ripening period

(iii) The effect of the starter culture (890), the ripening temperature (10°C) and the ripening period (2 months) on the odour of the cheese were highly significant at $p < 0.001$ (i.e. in favour of the control).

(iv) From the statistical analysis the following interactions were significant at different levels:

(a) At $p < 0.001$ grader x enzyme, enzyme x starter culture, grader x ripening period, grader x enzyme x ripening period and grader x ripening temperature x ripening period.

(b) At $p < 0.01$ starter culture x ripening temperature and enzyme x starter culture x ripening temperature.

(c) At $p < 0.05$ grader x starter culture, grader x enzyme x starter culture, grader x starter culture x ripening temperature, grader x starter culture x ripening period and enzyme x ripening temperature x ripening period.

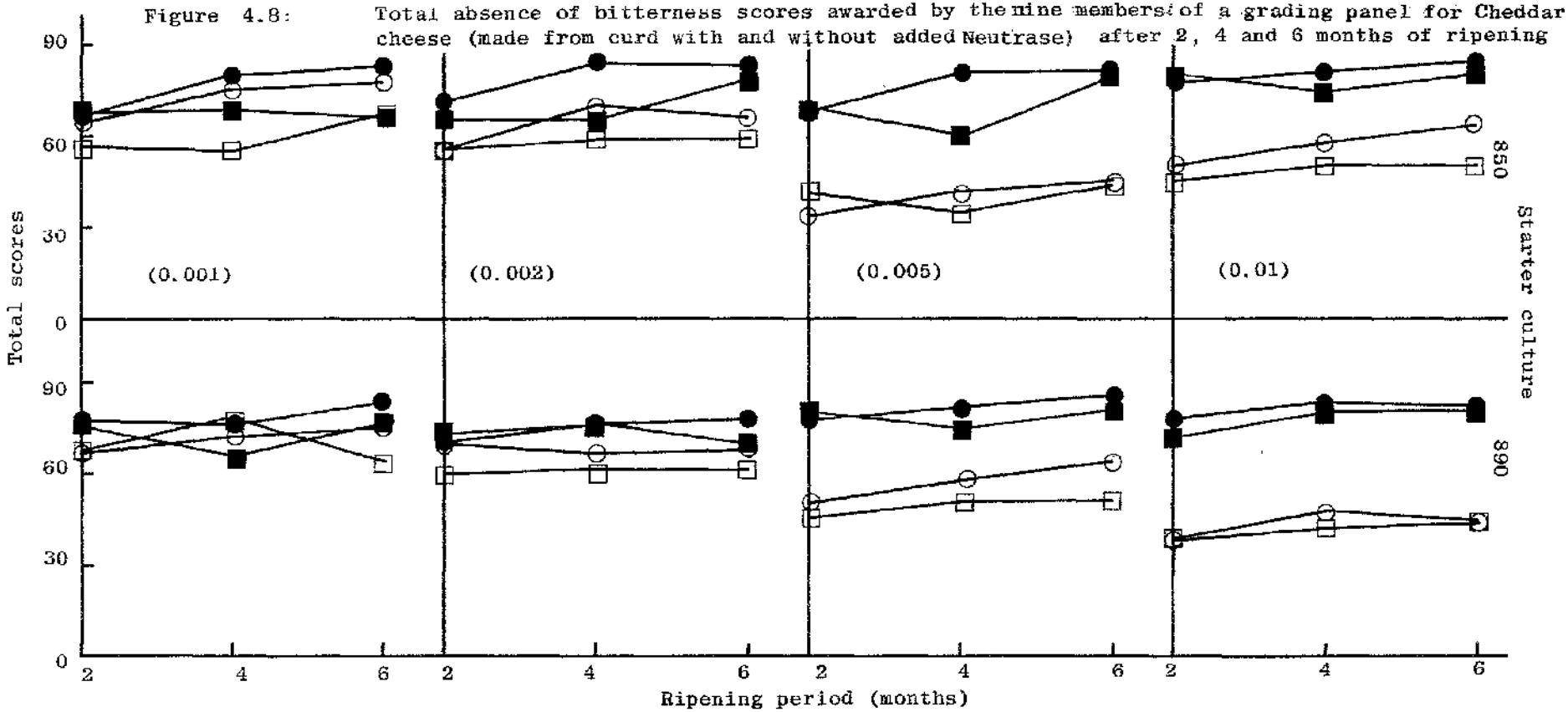
It can be concluded from the above analysis that the Neutrase addition, together with a ripening temperature of 13°C and prolonged ripening produced cheese which were strong in odour, but lacked typical Cheddar cheese odour, and in general were of unacceptable quality ($p < 0.001$). This could be attributed to the production of sulphur compounds (i.e. dimethyl sulphide from cystine and methionine) as a result of enhanced protein hydrolysis in the cheese (Walker, 1959). These sulphur compounds have been found to cause off-flavours in cheese at a level above 35 µg/g (Barnicoat, 1950). Furthermore, Manning & Robinson (1973), Manning (1975) and Manning, Chapman & Hosking (1977) observed that the concentration of methanethiol above 19 µ g/5 ml head space produced flavour defects.

Cheese manufactured with starter culture 890 was highly preferred by the panelists and it was established during the experiments that this particular starter culture had a lower proteolytic activity than starter culture 850 (see Section 4.3.3).

4.4.3 Bitterness in the Cheese

The total scores awarded for the presence of bitterness in cheese (control

Figure 4.8: Total absence of bitterness scores awarded by the nine members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 2, 4 and 6 months of ripening



Figures in parenthesis represent amount (% w/w) of Neutrase added

● , Control cheese ripened at 10°C

■ , Control cheese ripened at 13°C

○ , Neutrase treated cheese ripened at 10°C

□ , Neutrase treated cheese ripened at 13°C

TABLE 4.15

Statistical analysis of the nine panelists' assessment of the bitterness of Cheddar cheese made from curd with and without added Neutrase (data is difference between control and experimental)

										SED
Grader	1	2	3	4	5	6	7	8	9	
	1.948	0.260	2.771	1.667	2.333	4.075	1.776	3.354	3.413	0.233
*Enzyme	0.001	0.002	0.005	0.01						
	0.728	1.473	3.150	4.247						0.156
+Starter	850	890								
	2.612	2.187								0.110
Temperature	13°C	10°C								
	2.606	2.194								0.110
Storage	2-months	4-months	6-months							
	2.285	2.463	2.452							0.135
+Starter	850	890								
*Enzyme										
0.001	1.035	0.422								
0.002	1.732	1.214								
0.005	3.442	2.859								
0.01	4.240	4.253								0.220
Temperature	13°C	10°C								
Enzyme										
0.001	1.052	0.405								
0.002	1.677	1.269								
0.005	3.557	2.744								
0.01	4.137	4.356								0.220

cont'd.....

Table 4.15 (cont'd.)

					SED
Temperature	13°C	10°C			
+Starter					
850	2.989	2.236			
890	2.222	2.152			0.156
Storage	2-months	4-months	6-months		
*Enzyme					
0.001	0.514	0.749	0.922		
0.002	1.347	1.576	1.496		
0.005	3.181	3.099	3.171		
0.01	4.097	4.426	4.218		0.269
Storage	2-months	4-months	6-months		
+Starter					
850	2.333	2.983	2.520		
890	2.236	1.942	2.384		0.190
Storage	2-months	4-months	6-months		
Temperature					
13°C	2.306	2.796	2.715		
10°C	2.264	2.129	2.188		0.190
+Starter	850		890		
Temperature	13°C	10°C	13°C	10°C	
*Enzyme					
0.001	1.825	0.244	0.278	0.567	
0.002	1.872	1.592	1.481	0.946	
0.005	4.076	2.808	3.037	2.681	
0.01	4.182	4.299	4.093	4.414	0.311

cont'd.....

Table 4.15(cont'd.)

							SED
+Starter	850			890			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
*Enzyme							
0.001	0.750	1.599	0.754	0.278	-0.101	1.091	
0.002	1.500	1.919	1.778	1.194	1.233	1.214	
0.005	3.083	3.725	3.517	3.278	2.473	2.826	
0.01	4.000	4.690	4.031	4.194	4.162	4.404	0.381
Temperature	13°C			10°C			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
*Enzyme							
0.001	0.639	1.127	1.389	0.389	0.371	0.456	
0.002	1.222	2.002	1.806	1.472	1.150	1.186	
0.005	3.500	3.503	3.667	2.861	2.695	2.676	
0.01	3.861	4.551	4.000	4.333	4.301	4.435	0.381
Temperature	13°C			10°C			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
+Starter							
850	2.431	3.661	2.875	2.236	2.306	2.165	
890	2.181	1.931	2.556	2.292	1.953	2.212	0.269

(+ve) and (-ve) figures illustrate panelist's preference of the control or the experimental cheese respectively.

* figures indicate percentage (w/w) levels of added Neutrase
+ numbers indicate identity of culture

cont'd.....

Table 4.15(cont'd.)

Source of variation	DF	MS	VR
Grader	8	63.559	48.683***
Enzyme	3	274.576	210.312***
Starter	1	19.516	14.948***
Temperature	1	18.308	14.023***
Storage	2	1.432	1.097
Grader x Enzyme	24	11.309	8.662***
Grader x Starter	8	4.770	3.653***
Enzyme x Starter	3	2.344	1.796***
Grader x Temperature	8	10.101	7.737***
Enzyme x Temperature	3	5.523	4.230**
Starter x Temperature	1	12.600	9.651**
Grader x Storage	16	6.967	5.336***
Enzyme x Storage	6	0.545	0.417
Starter x Storage	2	10.275	7.870***
Temperature x Storage	2	3.873	2.966*
Grader x Enzyme x Starter	24	1.750	1.340
Grader x Enzyme x Temperature	24	2.707	2.074**
Grader x Starter x Temperature	8	7.491	5.738***
Enzyme x Starter x temperature	3	5.792	4.437**
Grader x Enzyme x Storage	48	2.639	2.022***
Grader x Starter x Storage	16	3.784	2.898***
Enzyme x Starter x Storage	6	2.121	1.625
Grader x Temperature x Storage	15	6.831	5.233***
Enzyme x Temperature x Storage	6	0.688	0.527
Starter x Temperature x Storage	2	3.266	2.502
Residual	170	1.306	
TOTAL	410	6.492	

*Significant at 5 per cent level
** " " 1 " " "
*** " "0.1 " " "

Temperature = ripening temperature
Storage = ripening period

and experimental) are illustrated in Figure 4.8 and the trend of bitterness in the cheeses is similar to that reported in 4.4.1 and 4.4.2. However, the degree of bitterness in the cheese was influenced by the amount of added Neutrase and the ripening temperature at 13°C.

The statistical analysis (mean differences) of the bitterness in the cheeses is shown in Table 4.15, and the analysis of variance could be summarised as follows:

(i) The panelists' showed great differences in their detection of bitterness in the cheese which varied between 0.260 and 4.075 and was significant at $p < 0.001$.

(ii) The use of Neutrase at 0.01% (w/w) had a pronounced effect on the bitterness scores for the cheese. For example, the mean differences between the control and the experimental cheeses (0.001 and 0.01% w/w) were 0.728 and 4.247 respectively, and this was significant at $p < 0.001$ (confirming the work of Law & Wigmore, 1982).

(iii) The mean differences between the effect of starter cultures 890 and 850 were 2.187 and 2.612 respectively, and were possibly due to the low proteolytic activity of the former starter culture (see Section 4.3.3).

(iv) The ripening temperature had a highly significant effect ($p < 0.001$) on the level of bitterness in the cheese and the mean differences between the control and the experimental cheeses were 2.606 and 2.194 when the cheeses were ripened at 13 and 10°C respectively.

(v) Although the length of the ripening period had a significant effect ($p < 0.001$) on the degree of bitterness in the cheese, this effect did not increase in cheese beyond 4 months. The self-regulatory mechanism of the Neutrase (Law & Wigmore (1982) may have had some influence.

(vi) Different interactions were found to be significant at different levels as follows:

(a) At $p < 0.001$; grader x enzyme, grader x starter culture, enzyme x starter culture, grader x ripening temperature, grader x ripening period, starter culture x ripening

period, grader x starter culture x ripening temperature, grader x enzyme x ripening period, grader x starter culture x ripening period and grader x ripening temperature x ripening period.

(b) At $p < 0.01$; enzyme x ripening temperature, starter culture x ripening temperature, grader x enzyme x ripening temperature and grader x starter culture x ripening temperature.

(c) At $p < 0.05$; ripening temperature x ripening period.

From the above analysis, it may be observed that the degree of bitterness in the cheese was highly influenced by the rate of added enzyme, the ripening temperature and the starter culture.

4.4.4 Openness of the Cheese

The total scores awarded for the openness of the cheese (control and experimental) are shown in Figure 4.9. The degree of openness in the cheese was primarily influenced by the level of enzyme and temperature of ripening of the cheese. It can be observed from Figure 4.9 that the trend of openness in the cheese was similar to trends for other cheese characteristics (Figures 4.6-4.8).

Table 4.16 summarises the statistical analysis (mean differences) of the 9 panelists, and the following observations may be made:

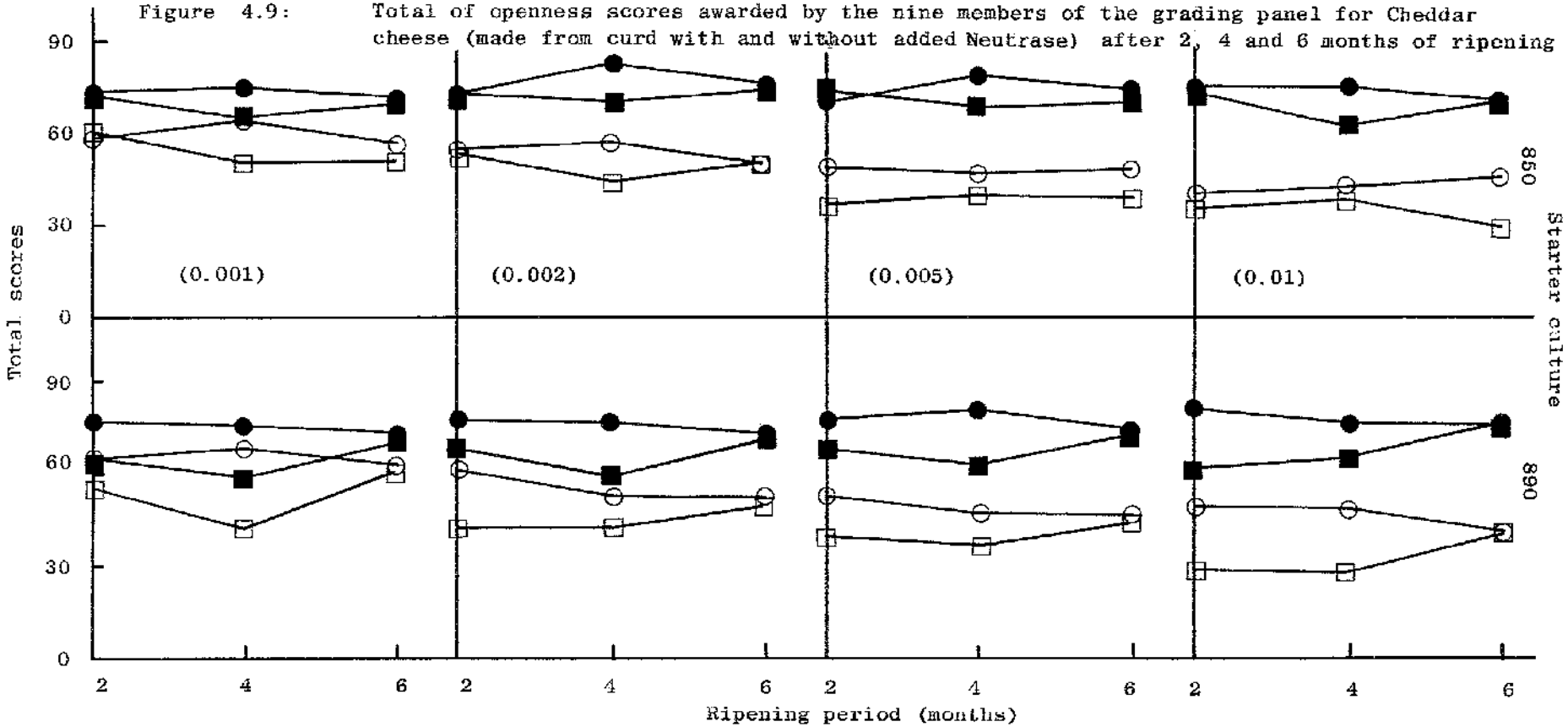
(i) Differences between the panelists judgement was highly significant ($p < 0.001$) and varied between 1.320 and 3.750.

(ii) Addition of Neutrase had a highly significant effect ($p < 0.001$) on the openness of the cheese and the mean differences were 1.319 (0.001% w/w) and 3.735 (0.01% w/w).

(iii) The mean differences between starter cultures 850 in the control and the experimental cheeses were 2.778 and 2.507 respectively, and were significant at $p < 0.05$.

(iv) The ripening temperture had a significant effect ($p < 0.001$) on the openness of the cheese, and the mean differences between the control and the experimental cheeses were 2.913 and 2.372 which were ripened at 13 and 10°C respectively.

Figure 4.9: Total of openness scores awarded by the nine members of the grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 2, 4 and 6 months of ripening



Footnote: Figures in parenthesis represent amount (% w/w) of Neutrase added

● , Control cheese ripened at 10°C

■ , Control cheese ripened at 13°C

○ , Neutrase treated cheese ripened at 10°C

□ , Neutrase treated cheese ripened at 13°C

TABLE 4.16

Statistical analysis of the nine panelists' assessment of the openness of Cheddar cheese made from curd with and without added Neutrase (data is difference between control and experimental)

										SED
Grader	1	2	3	4	5	6	7	8	9	
	2.021	1.320	2.881	3.750	2.403	2.769	3.264	3.146	2.228	0.180
*Enzyme	0.001	0.002	0.005	0.01						
	1.319	2.389	3.126	3.735						0.120
+Starter	850	890								
	2.778	2.507								0.085
Temperature	13°C	10°C								
	2.913	2.372								0.085
Storage	2-month	4-month	6-month							
	2.507	2.796	2.624							0.104
+Starter	850	890								
*Enzyme										
0.001	1.597	1.042								
0.002	2.489	2.290								
0.005	3.214	3.037								
0.01	3.812	3.659								0.170
Temperature	13°C	10°C								
*Enzyme										
0.001	1.505	1.133								
0.002	2.595	2.184								
0.005	3.400	2.851								
0.01	4.151	3.320								0.170

cont'd.....

Table 4.16 (cont'd)

					SED
Temperature	13°C	10°C			
+Starter					
850	3.097	2.459			0.120
890	2.729	2.286			
Storage	2-months	4-months	6-months		
*Enzyme					
0.001	1.264	1.273	1.421		
0.002	2.153	2.691	2.324		
0.005	2.986	3.393	2.998		
0.01	3.625	3.827	3.754		0.208
Storage	2 months	4-months	6-months		
+Starter					
850	2.674	3.000	2.660		
890	2.340	2.592	2.589		0.147
Storage	2-months	4-months	6-months		
Temperature					
13°C	2.771	3.086	2.882		
10°C	2.243	2.506	2.367		0.147
+Starter	850		890		
Temperature	13°C	10°C	13°C	10°C	
*Enzyme					
0.001	1.835	1.358	1.176	0.908	
0.002	2.671	2.306	2.518	2.062	
0.005	3.620	2.808	3.181	2.894	
0.01	4.262	3.361	4.040	3.278	0.240

cont'd.....

Table 4.16 (cont'd.)

							SED
+Starter	850			890			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
*Enzyme							
0.001	1.417	1.585	1.788	1.111	0.961	1.054	
0.002	2.139	2.979	2.349	2.167	2.403	2.300	
0.005	3.194	3.486	2.962	2.788	3.330	3.034	
0.01	3.944	3.949	3.542	3.306	3.705	3.966	0.295
Temperature	13°C			10°C			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
*Enzyme							
0.001	1.167	1.766	1.583	1.361	0.781	1.258	
0.002	2.500	2.812	2.472	1.806	2.570	2.176	
0.005	3.556	3.396	3.250	2.417	3.390	2.746	
0.01	3.861	4.370	4.222	3.389	3.284	3.286	0.295
Temperature	13°C			10°C			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
+Starter							
850	2.889	3.250	3.153	2.458	2.750	2.167	
890	2.653	2.922	2.611	2.028	2.263	2.566	0.208

(+ve) and -ve) figures illustrates panelist's preference of the control or the experimental cheese respectively.

* figures indicate percentage (w/w) levels of added Neutrase
\+ numbers indicate identity of culture

cont'd.....

Table 4.16 (cont'd.)

Source of variation	DF	MS	VR
Grader	8	25.8228	33.053***
Enzyme	3	116.7476	149.438***
Starter	1	7.9198	10.137**
Temperature	1	31.5878	40.433***
Storage	2	3.0475	3.901*
Grader x Enzyme	24	1.6917	2.165***
Grader x Starter	8	2.0357	2.606**
Enzyme x Starter	3	0.9757	1.249
Grader x Temperature	8	3.2875	4.208***
Enzyme x Temperature	3	1.1713	1.499
Starter x Temperature	1	1.0336	1.323
Grader x Storage	16	3.8861	4.974***
Enzyme x Storage	6	0.7538	0.965
Starter x Storage	2	1.1225	1.437
Temperature x Storage	2	0.0418	0.054
Grader x Enzyme x Starter	24	0.6914	0.885
Grader x Enzyme x Temperature	24	0.8904	1.036
Grader x Starter x Temperature	8	1.1221	1.436
Enzyme x Starter x Temperature	3	0.4384	0.561
Grader x Enzyme x Storage	48	0.8087	1.035***
Grader x Starter x Storage	16	3.8122	4.880***
Enzyme x Starter x Storage	6	1.1450	1.466
Grader x Temperature x Storage	15	1.8701	2.394*
Enzyme x Temperature x Storage	6	2.4922	3.190**
Starter x Temperature x Storage	2	3.7466	4.796**
Residual	162	0.7812	
TOTAL	402	2.7168	

* Significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Temperature = ripening temperature
Storage = ripening period

(v) Although the ripening period had a significant effect ($p < 0.01$) on the openness of the cheese up to 4 months old, a slight decrease was observed at 6 months old.

(vi) The following interactions were significant at different levels:

(a) At $p < 0.001$; grader x ripening temperature, grader x ripening period, grader x enzyme x ripening period and grader x starter culture x ripening period.

(b) At $p < 0.05$; grader x starter culture, enzyme x ripening temperature x ripening period and starter culture x ripening temperature x ripening period.

(c) At $p < 0.01$; grader x ripening temperature x ripening period.

It can be concluded, that the openness observed in the experimental cheeses could be primarily influenced by the proteolytic activity in the cheese which affect the matrix formation due to casein hydrolysis and/or syneresis.

4.4.5 Firmness of the Cheese

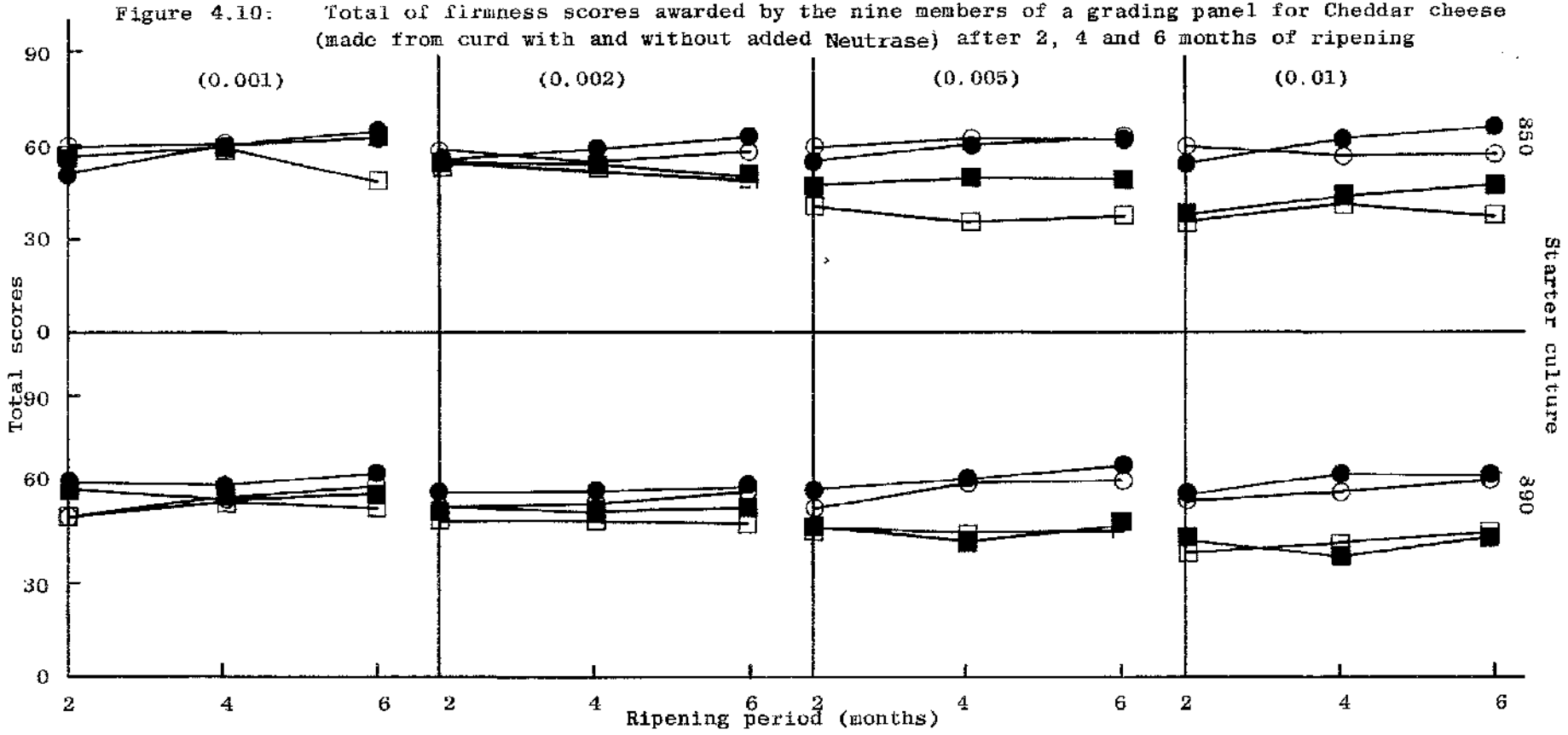
The total scores awarded by the 9 panelists for the firmness of the cheese are shown in Figure 4.10, and it can be observed that the enzyme-treated cheese were characterised by having a very soft body compared with the control cheese. The addition of a small amount of Neutrase i.e. 0.001% w/w, slightly affected the firmness of the cheese. Ripening of the cheese (control and experimental) at 13°C resulted in a cheese which was soft in body compared with cheeses ripened at 10°C.

The reason(s) for differences in body scores may be attributed to enhanced proteolytic and/or enzymatic activities of the starter culture and the added Neutrase (Law & Wigmore, 1982 and see Section 4.3.3.2).

The statistical analysis (mean differences) of the panelists is summarised in Table 4.17 and the following conclusions may be drawn:

(i) Differences between the panelists' judgement were highly significant ($p < 0.001$), and the mean differences for the individual panelists varied between 0.644 and 2.990.

Figure 4.10: Total of firmness scores awarded by the nine members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 2, 4 and 6 months of ripening



Figures in parenthesis represent amount (% w/w) of Neutrase added

●, Control cheese ripened at 10°C

■, Control cheese ripened at 13°C

○, Neutrase treated cheese ripened at 10°C

□, Neutrase treated cheese ripened at 13°C

TABLE 4.17

Statistical analysis of the nine panelists' assessment of the firmness of Cheddar cheese made from curd with and without added Neutrase (data is difference between control and experimental)

										SED
Grader	1	2	3	4	5	6	7	8	9	
	1.042	2.990	0.991	2.323	0.240	0.774	1.620	1.281	-0.644	0.179
*Enzyme	0.001	0.002	0.005	0.01						
	0.344	0.645	1.612	2.117						0.119
+Starter	850	890								
	1.239	1.120								0.084
Temperature	13°C	10°C								
	1.343	1.016								0.084
Storage	2-months	4-months	6-months							
	0.885	1.276	1.377							0.103
+Starter	850	890								
*Enzyme										
0.001	0.359	0.330								
0.002	0.659	0.631								
0.005	1.657	1.566								
0.01	2.280	1.953								0.168
Temperature	13°C	10°C								
*Enzyme										
0.001	0.584	0.105								
0.002	0.735	0.555								
0.005	1.799	1.424								
0.01	2.255	1.979								0.168

cont'd.....

Table 4.17(cont'd.)

					SED
Temperature	13°C	10°C			
+Starter					
850	1.617	0.861			
890	1.069	1.171			0.119
Storage	2-months	4-months	6-months		
*Enzyme					
0.001	0.014	0.402	0.618		
0.002	0.375	0.579	0.981		
0.005	1.236	1.764	1.835		
0.01	1.917	2.358	2.076		0.205
Storage	2-months	4-months	6-months		
+Starter					
850	1.000	1.294	1.423		
890	0.771	1.258	1.332		0.145
Storage	2-months	4-months	6-months		
Temperature					
13°C	1.069	1.315	1.646		
10°C	0.701	1.237	1.109		0.145
+Starter	850				
Temperature	13°C	10°C	13°C	10°C	
*Enzyme					
0.001	0.908	-0.190	0.250	0.400	
0.002	0.674	0.644	0.796	0.466	
0.005	2.228	1.087	1.370	1.762	
0.01	2.658	1.903	1.852	2.055	0.237

cont'd.....

Table 4.17(cont'd.)

							SED
+Starter	850			890			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
*Enzyme							
0.001	-0.083	0.501	0.659	0.111	0.302	0.576	
0.002	0.306	0.594	1.077	0.444	0.565	0.884	
0.005	1.528	1.591	1.852	0.944	1.937	1.818	
0.01	2.250	2.488	2.104	1.583	2.228	2.048	0.290
Temperature	13°C			10°C			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
*Enzyme							
0.001	0.139	0.501	1.111	-0.111	0.302	0.124	
0.002	0.472	0.594	1.139	0.278	0.565	0.823	
0.005	1.417	1.814	2.167	1.056	1.715	1.503	
0.01	2.250	2.349	2.167	1.583	2.367	1.985	0.290
Temperature	13°C			10°C			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
+Starter							
850	1.417	1.560	1.875	0.583	1.028	0.971	
890	0.722	1.069	0.819	1.446	1.246		0.205

(+ve) and (-ve) figures illustrate panelist's preference of the control or the experimental cheese respectively

* figures indicate percentage (w/w) levels of added Neutrase

+ numbers indicate identity of culture

cont'd.....

Table 4.17 (cont'd.)

Source of variation	DF	MS	VR
Grader	8	55.3063	72.885***
Enzyme	3	73.2450	97.185***
Starter	1	1.5260	2.011
Temperature	1	11.5862	15.269***
Storage	2	9.7112	12.798***
Grader x Enzyme	24	2.5426	3.351***
Grader x Starter	8	1.5618	2.058*
Enzyme x Starter	3	0.5444	0.717
Grader x Temperature	8	5.3123	7.001***
Enzyme x Temperature	3	0.4451	0.587
Starter x Temperature	1	19.8546	26.165***
Grader x Storage	16	4.3223	5.696***
Enzyme x Storage	6	0.9083	1.197
Starter x Storage	2	0.3565	0.470
Temperature x Storage	2	1.9449	2.563
Grader x Enzyme x Starter	24	0.9385	1.237
Grader x Enzyme x Temperature	24	0.4446	0.586
Grader x Starter x Temperature	8	1.3697	1.805
Enzyme x Starter x Temperature	3	4.3929	5.789***
Grader x Enzyme x Storage	48	1.1943	1.574***
Grader x Starter x Storage	16	2.0282	2.673***
Enzyme x Starter x Storage	6	1.0312	1.359
Grader x Temperature x Storage	15	0.9504	1.252
Enzyme x Temperature x Storage	6	0.6084	0.802
Starter x Temperature x Storage	2	0.1050	0.138
Residual	170	0.7588	
TOTAL	410	2.9626	

*Significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Temperature = ripening temperature
Storage = ripening period

(ii) The effect of Neutrase (as the rate was increased), ripening temperature at 13°C and the ripening period were highly significant ($p < 0.001$), and in favour (as indicated by firmer body scores) of the control cheese, ripening temperature at 10°C and ripening period of 2 months.

(iii) Although mean differences between the starter cultures (850 and 890) were 1.239 and 1.120 respectively, these were not statistically significant.

(iv) The following interactions were observed:

(a) At $p < 0.001$ between grader x ripening temperature, grader x ripening period, enzyme x starter culture x ripening temperature, grader x enzyme x ripening period and grader x starter culture x ripening period.

(b) At $p < 0.01$ between grader x starter culture.

It can be observed from the above analysis that the Neutrase enzyme had reduced the firmness of the cheese (as the rate of addition is increased) due to the greater proteolysis that took place in the cheese. These results confirm those of Law & Wigmore (1982). Ripening the cheese at 13°C (control and experimental cheese) also reduced the firmness of the cheese, an effect which may have been due to enhanced activity of the enzyme(s) in the cheese, resulting in change of the physical characteristics of the cheese.

4.4.6 Smoothness of the Cheese

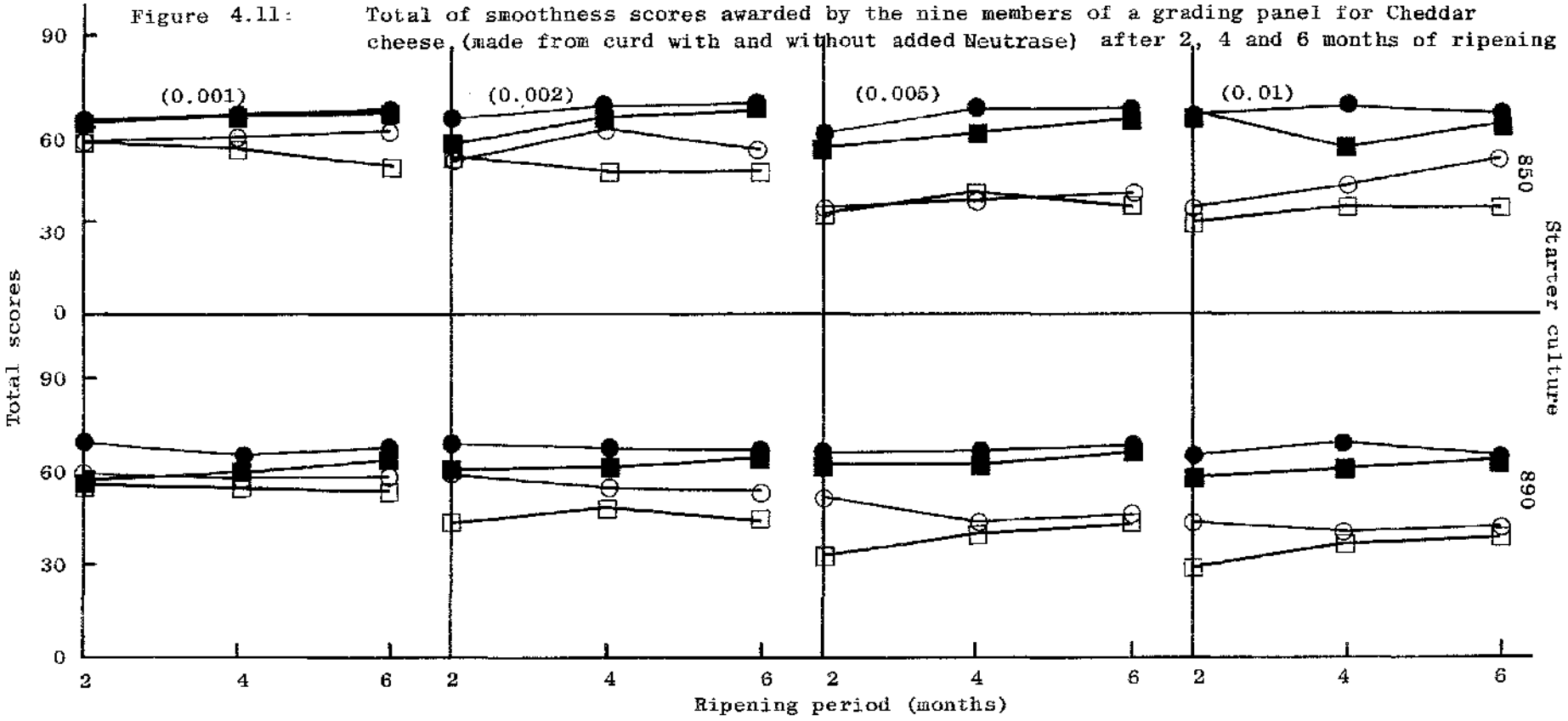
The total scores awarded for the smoothness of the cheese by the 9 panelists during the ripening period are illustrated in Figure 4.11, summarised as follows:

(i) The enzyme treated cheese was more crumbly than the control cheese even at the level of 0.001% w/w added Neutrase.

(ii) Cheeses ripened at 13°C (control and experimental) were more crumbly than the cheeses ripened at 10°C.

(iii) Slight differences of smoothness were observed in the cheeses (control and experimental) prepared with starter cultures 850 and 890.

Figure 4.11: Total of smoothness scores awarded by the nine members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 2, 4 and 6 months of ripening



Figures in parenthesis represent amount (% w/w) of Neutrase added

●, Control cheese ripened at 10°C

■, Control cheese ripened at 13°C

○, Neutrase treated cheese ripened at 10°C

□, Neutrase treated cheese ripened at 13°C

TABLE 4.18

Statistical analysis of the nine panelists' assessment of the smoothness of Cheddar cheese made from curd with and without added Neutrase (data is difference between control and experimental)

										SED
Grader	1	2	3	4	5	6	7	8	9	
	1.021	1.663	0.995	2.667	0.885	1.400	3.162	2.708	2.156	0.195
* Enzyme	0.001	0.002	0.005	0.01						
	0.882	1.550	2.239	2.736						0.130
+ Starter	850	890								
	1.964	1.740								0.092
Temperature	13°C	10°C								
	2.189	1.515								0.092
Storage	2-months	4-months	6-months							
	1.778	1.890	1.888							0.113
+ Starter	850	890								
* Enzyme										
0.001	1.101	0.663								
0.002	1.644	1.456								
0.005	2.273	2.205								
0.01	2.837	2.635								0.184
Temperature	13°C	10°C								
* Enzyme										
0.001	1.021	0.743								
0.002	1.917	1.183								
0.005	2.692	1.786								
0.01	3.127	2.346								0.184

cont'd.....

Table 4.18(cont'd)

					SED
Temperature	13°C	10°C			
+Starter					
850	2.392	1.536			
890	1.986	1.493			0.130
Storage	2-months	4-months	6-months		
*Enzyme					
0.001	0.639	0.884	1.122		
0.002	1.500	1.378	1.772		
0.005	2.070	2.343	2.304		
0.01	2.901	2.955	2.354		0.225
Storage	2-months	4-months	6-months		
+Starter					
850	1.902	2.136	1.854		
890	1.653	1.643	1.923		0.159
Storage	2-months	4-months	6-months		
Temperature					
13°C	2.070	2.171	2.326		
10°C	1.485	1.609	1.450		0.159
+Starter	850		890		
Temperature	13°C	10°C	13°C	10°C	
*Enzyme					
0.001	1.430	0.772	0.661	0.714	
0.002	2.00	1.289	1.833	1.078	
0.005	2.772	1.774	2.612	1.799	
0.01	3.365	2.310	2.889	2.382	0.260

Cont'd.....

Table 4.18(cont'd)

							SED
+ Starter	850						
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
* Enzyme							
0.001	0.750	1.312	1.241	0.518	0.457	1.004	
0.002	1.583	1.500	1.850	1.417	1.256	1.694	
0.005	1.944	2.630	2.244	2.196	2.055	2.365	
0.01	3.329	3.103	2.080	2.472	2.806	2.628	0.318
Temperature	13°C			10°C			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
* Enzyme							
0.001	0.417	1.090	1.556	0.861	0.679	0.689	
0.002	1.750	1.778	2.222	1.250	0.978	1.322	
0.005	2.696	2.713	2.667	1.444	1.972	1.942	
0.01	3.417	3.103	2.861	2.385	2.806	1.846	0.318
Temperature	13°C			10°C			
Storage	2-months	4-months	6-months	2-months	4-months	6-months	
+ Starter							
850	2.125	2.592	0.458	1.679	1.681	1.249	
890	2.015	1.750	2.194	1.292	1.537	1.651	0.225

(+ve) and (-ve) figures illustrates panelist's preference of the control or the experimental cheese respectively.

* figures indicate percentage (w/w) levels of added Neutrase
+ numbers indicate identity of culture

cont'd.....

Table 4.18 (cont'd.)

Source of variation	DF	MS	VR
Grader	8	34.9294	38.302***
Enzyme	3	70.7198	77.542***
Starter	1	5.4229	5.946*
Temperature	1	49.1352	53.879***
Storage	2	0.5963	0.654
Grader x Enzyme	24	4.2489	4.659***
Grader x Starter	8	5.4012	5.923***
Enzyme x Starter	3	0.6491	0.712
Grader x Temperature	8	7.3632	8.074***
Enzyme x Temperature	3	2.0329	2.229
Starter x Temperature	1	3.5472	3.890*
Grader x Storage	16	3.9811	4.365***
Enzyme x Storage	6	2.5787	2.828**
Starter x Storage	2	2.8567	3.133**
Temperature x Storage	2	1.1065	1.213
Grader x Enzyme x Starter	24	1.1043	1.211
Grader x Enzyme x Temperature	24	0.4180	0.458
Grader x Starter x Temperature	8	2.4877	2.728**
Enzyme x Starter x Temperature	3	0.8809	0.966
Grader x Enzyme x Storage	48	0.8731	0.957
Grader x Starter x Storage	16	1.6585	1.819*
Enzyme x Starter x Storage	6	1.5397	1.688
Grader x temperature x Storage	15	2.6643	2.922***
Enzyme x Temperature x Storage	6	1.8871	2.069
Starter x Temperature x Storage	2	2.7614	3.028*
Residual	167	0.9120	
Total	407	2.9386	

* Significant at 5 per cent level
** " " 1 " " "
*** " " 0.1 " " "

Temperature = ripening temperature
Storage = ripening period

(iv) The smoothness of the cheese treated with Neutrase did not improve after 2 months ripening period compared with the control. This may be attributed to the protein hydrolysis.

The statistical analysis (mean differences) of the scores awarded by the 9 panelists for the smoothness of the cheese is given in Table 4.18, and the following conclusions may be made:

(i) Differences between the individual panelists' judgement varied between 0.885 to 3.162, and this was highly significant ($p < 0.001$).

(ii) Although the Neutrase-treated cheese was crumbly compared with the control ($p < 0.001$), the mean difference between them was 0.882 to 2.736 when the enzyme was added at 0.001 and 0.01% w/w respectively. Such results confirm the work of Law & Wigmore (1982).

(iii) The starter culture had little effect on the smoothness of the cheese (significant at $p < 0.001$), and the panelists' preference was in favour of starter culture 890.

(iv) Cheeses (control and experimental) ripened at 13°C were very crumbly compared with the cheeses ripened at 10°C (significant at $p < 0.001$), and the mean differences between the control and the experimental cheese ripened at 13 and 10°C were 2.189 and 1.515 respectively.

(v) The mean differences between the control and the experimental cheese was as high as 1.778 after 2 months of ripening, but this difference did not increase after 4 and 6 months and it was not significant at any level.

(iv) The following interactions were found to be significant at the following levels:

(a) At $p < 0.001$; grader x enzyme, grader x starter culture, grader x ripening temperature, grader x ripening period and grader x ripening temperature x ripening period.

(b) At $p < 0.005$; enzyme x ripening period, starter culture x ripening period and grader x starter culture x ripening temperature.

(c) At $p < 0.01$; starter culture x ripening temperature, grader x starter culture x ripening period and starter culture x ripening temperature x ripening period.

It can be concluded from the above analysis that the experimental cheeses were very crumbly and the degree of crumbliness was dependent on the amount of added Neutrase, which could be responsible for the extensive proteolysis. Ripening of the cheese (control and experimental) at 13°C produced a crumbly product which could be due to the enhanced activity of the enzymes in cheese.

4.5 Conclusion

The failur of Neutrase enzyme to produce a clean typical flavour and acceptable texture characteristics in Cheddar cheese could be mainly attributed to the high protein breakdown which resulted in the liberation of some bitter peptides and other degradation products. This conclusion on the inferior characteristics of Cheddar cheese made with added Neutrase is not in agreement with other reported work on the use of Neutrase in Cheddar cheese (Law, 1981 and Law & Wigmore, 1982). The effect of the Neutrase enzyme on the texture characteristics of the cheese (i.e. loss of the cheese matrix formation) was also influenced by the extent of proteolysis that took place during the ripening period. Other defects, which had been detected in the cheese made with added Neutrase were brittleness and mottled colour (see Plate 4.10). The degree of mottling and brittleness which occurred in some parts of the cheese could be attributed to the uneven distribution of the enzyme at the salting stage although it must be stressed that the salt and enzyme were effectively mixed with the milled curd and allowed to dissolve before hopping.

The cheeses (control and experimental) were kept up to 12 months and assessed by 3 professional graders whose comments were that all the experimental cheeses did not meet consumer acceptability standards, especially for taste and odour characteristics. Similar comments were given to the control cheeses ripened at 13°C; however, the cheeses (i.e. control) which were ripened at 10°C were still of satisfactory quality after 12 months storage and the scores awarded were between 3-6 points out of 8.

The addition of neutral proteinase enzyme to cheese curd resulted in

Legend to

Plate 4.10 : The discolouration or mottling in Cheddar cheese made from curd with and without added Neutrase after 2 months of ripening. In all cases the control cheese are on the right of the plates.

Key:

4088	Neutrase treated cheese (0.001% w/w) ripened at 10°C
5107	Control cheese ripened at 10°C
0210	Neutrase treated cheese (0.001% w/w) ripened at 13°C
2081	Control cheese ripened at 13°C
2784	Neutrase treated cheese (0.002% w/w) ripened at 10°C
4765	Control cheese ripened at 10°C
0677	Neutrase treated cheese (0.002% w/w) ripened at 13°C
2022	Control cheese ripened at 13°C
2344	Neutrase treated cheese (0.005% w/w) ripened at 10°C
7585	Control cheese ripened at 10°C
4622	Neutrase treated cheese (0.005% w/w) ripened at 13°C
8846	Control cheese ripened at 13°C
5825	Neutrase treated cheese (0.01% w/w) ripened at 10°C
6988	Control cheese ripened at 10°C
4924	Neutrase treated cheese (0.01% w/w) ripened at 13°C
4558	Control cheese ripened at 13°C

PLATE 4.10


4088
5107
0210
2081
2784
4765
0677
2022
2344
7585
4622
8846
8869
9884
4924
4558

increased proteolysis in cheese during a 2 month ripening period. The effect of this increased proeolysis was to produce cheese with a softer body. The cheese produced with the addition of neutral proteinase had a more intense odour and taste than the control cheese, but these characteristics were not typical of high quality Cheddar cheese. Defects such as bitterness, mottled colour and weak body were associated with some of the cheese produced from enzyme-treated curd. The changes brought about by the addition of the enzyme did not increase to any large extent after 4-6 and 12 months.

Based on the results of these experiments and in order to overcome the problem of the method of enzyme addition, as well as day-to-day variation in compositional quality of milk and to confirm the effect of this enzyme on accelerated Cheddar cheese ripening in more detail it was decided to use Neutrase coated on salt and to produce Cheddar cheese in a semi-large sale, e.g. 2255 litre quantities.

SECTION II

Cheddar cheese (3 trials) was produced from 2255 litres of milk as illustrated in Figure 2.2, using starter culture 850. After the milling stage, the curd was divided into four equal portions, i.e. control and the remaining three portions which were treated with different levels of Neutrase enzyme coated on salt. The rates of enzyme added to the curd were 0.002, 0.003 and 0.005% (w/w). The primary objective of these trials was to produce commercial size rindless cheese from the same batch of milk so that to minimise the effect of seasonal variation on the quality of cheese in relation to evaluating Neutrase addition, and furthermore, to confirm if possible, the observations reported in Section I.

4.6 Materials and Methods

4.6.1 Chemical analysis of cheese

The methods for the determination of fat, moisture, salt, pH, total nitrogen, soluble nitrogen, free amino acids and gel electropheresis of the cheese were described in 2.3.1, 2.3.2, 2.3.3, 2.3.4, 2.3.5, 2.3.6, 2.3.7 and 2.3.8 respectively.

4.6.2 Rheological properties of the cheese

The brittleness and hardness of the cheese were measured as described in 2.3.9.

4.6.3 Organoleptic assessment

The cheese was assessed organoleptically by the official grader of the Company of Scottish Cheesemakers Ltd., Glasgow, U.K. and two professional panelists from Dairy Technology Department (WSAC) after 1, 2, 3 and 4 months of ripening. The scores for flavour intensity, off-flavour and bitter flavour were awarded as described by Law & Wigmore (1982), and an overall description of these characteristics is illustrated in Table 4.19.

4.7 Results and Discussion

4.7.1 The chemical composition of one-day old cheese

The chemical composition of one-day old cheese (control and Neutrase-treated cheese) is summarised in Table 4.20, and it can be observed that all the cheeses comply with the existing legal standard in the United

TABLE 4.19

Organoleptic scheme used to evaluate Cheddar cheese made from curd with and without added Neutrase

Cheese characteristics	Rating scale	Category of assessment
Flavour intensity	0-8	mild (0-2), medium (2-4), strong (4-6), very strong (6-8)
Off-flavour intensity	0-4	slight (0-1), definite (2), strong (3), very strong (4)
Bitter flavour intensity	0-4	slight (0-1), definite (2), strong (3), very strong (4)
Flavour quality	0-8	under quality (0-2), quality (2-4), good quality (4-6), very good (6-8)
Colour defect	0-4	slight mottled (0-1), definite (2), mottled (3), severly mottled (4)
Elasticity	0-8	very elastic (0-2), elastic (4), inelastic (6), very inelastic (8)
Openness	0-8	very open (0-2), open (4), close (6), very close (8)
Firmness	0-8	very soft (0-2), soft (4), hard (6), very hard (8)
General acceptability	0-20	unacceptable (0-10), acceptable (10-15) very acceptable (15-20)

TABLE 4.20

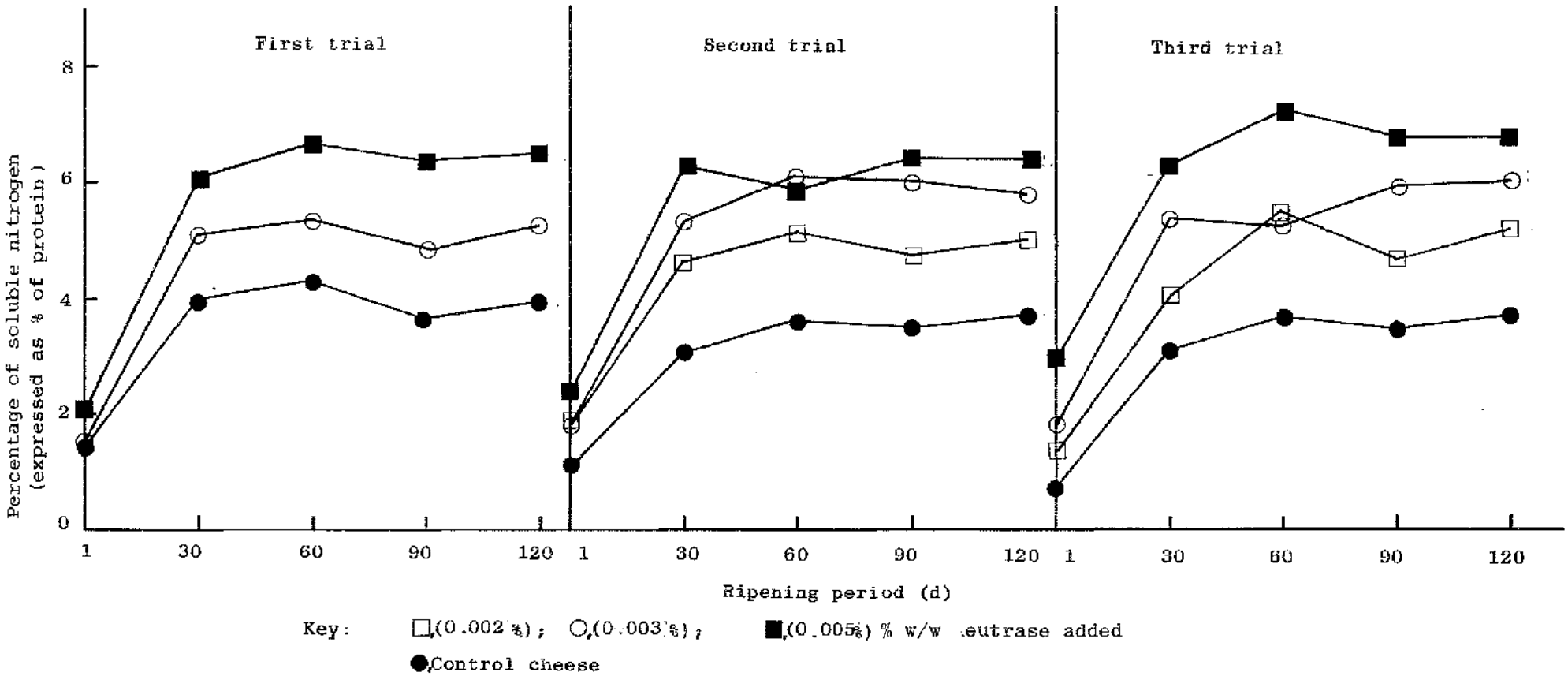
Chemical composition of one day old Cheddar cheese made from curd with and without added Neutrase

Trial No.	Treatment	Moisture %	Fat %	FDM %	MFFC %	TN %	SN %	Salt %	SM %	pH %
1	C	36.52	33.00	51.18	54.51	23.22	1.37	1.73	4.74	5.12
	(0.003)	34.31	34.00	51.76	51.98	24.54	1.45	1.67	4.87	5.32
	(0.005)	35.73	35.00	54.97	54.97	22.54	1.90	1.62	4.53	5.17
2	C	35.92	33.00	51.50	53.61	23.67	1.01	1.59	4.43	5.18
	(0.002)	34.36	35.00	53.32	52.86	23.58	1.81	1.63	4.71	5.27
	(0.003)	35.24	34.00	52.50	53.39	23.65	1.75	1.70	4.82	5.17
	(0.005)	34.63	34.00	52.01	52.47	23.46	2.26	1.69	4.88	5.26
3	C	37.65	34.00	54.53	57.05	23.49	0.68	1.64	4.36	5.10
	(0.002)	37.21	34.00	54.15	56.38	24.15	1.27	1.75	4.70	5.17
	(0.003)	37.04	35.00	55.59	56.98	24.69	1.75	1.67	4.51	5.17
	(0.005)	38.33	33.00	53.51	57.21	24.05	2.83	1.68	4.38	5.20

Figures in parentheses represent rate (% w/w) of Neutrase enzyme added

C = control (untreated cheese)

Figure 4.12: The extent of protein hydrolysis (% soluble nitrogen) during the ripening of Cheddar cheese (made from curd with and without added Neutrase).



Kingdom (e.g. maximum 39% moisture and minimum 48% fat in dry matter). In the present study the moisture content in the cheese varied between 34.31 and 38.33. It is possible to suggest that the latter level of moisture in the cheese could influence the rate of ripening of Cheddar cheese.

The pH values of one-day old cheeses were rather high, but from preliminary experiments it was observed that the starter culture used continued to produce acid during the early stages of the ripening period and if the cheddared curd was milled above 0.6% lactic acid the cheese tended to be acidic. For these reasons the cheddared curd was milled at higher than normal pH levels.

The soluble nitrogen liberated in the control cheese varied between 0.68 and 1.37% while it varied between 1.27 and 2.83% in the enzyme-treated cheese.

Total nitrogen and salt levels in the cheese were similar in the control and experimental cheeses.

4.7.2 Nitrogenous substances

4.7.2.1 The level of soluble nitrogen in the cheese

Figure 4.12 illustrates the level of soluble nitrogen present in Cheddar cheese made from curd with and without added Neutrase enzyme during the 4 months ripening period and the following may be observed:-

(a) The enzyme treated cheese contained higher level of soluble nitrogen compared with the control and this finding agreed with the results of Law & Wigmore (1982).

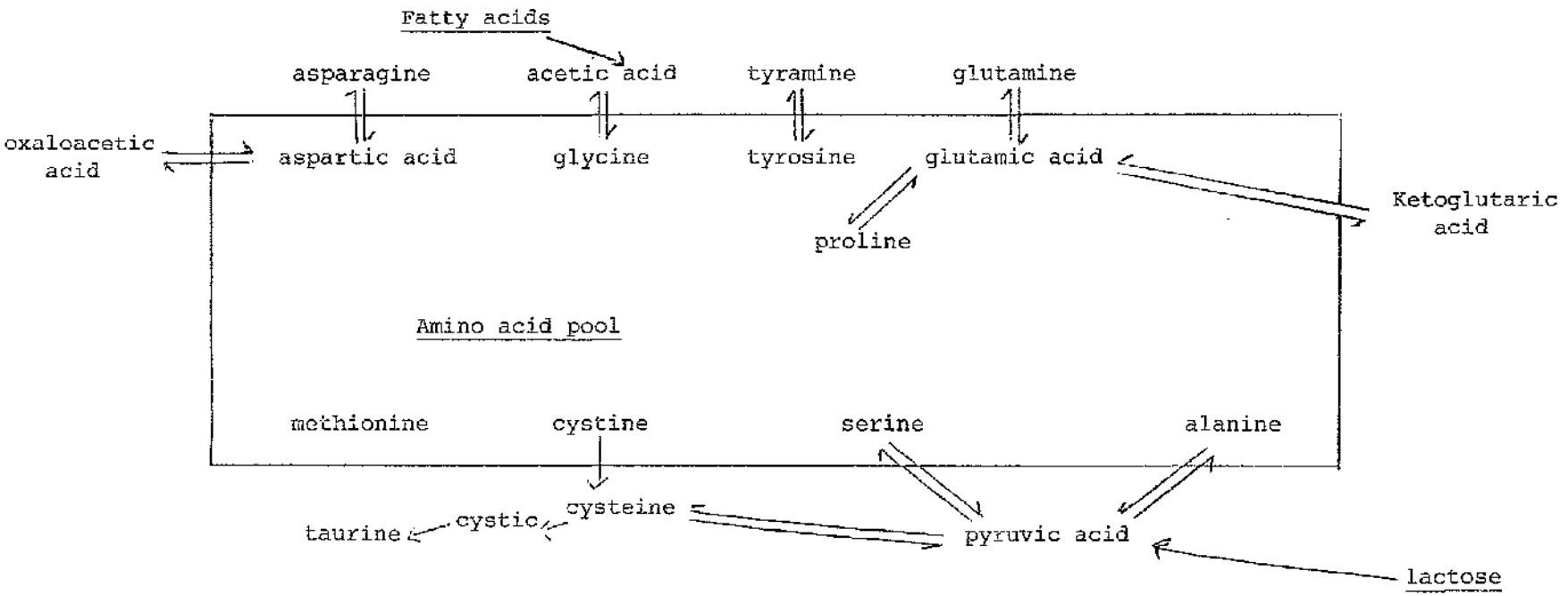
(b) The amount of soluble nitrogen in the experimental cheeses progressively increased in relation to the amount of Neutrase enzyme added to the curd.

(c) The amount of soluble nitrogen in the cheese treated with 0.005% (w/w) Neutrase (i.e. the third trial) was higher than in those cheeses treated with the same level of enzyme, in other trials. The cheese in the third trial had the highest moisture content (Table 4.20).

4.7.2.2 The level of free amino acids in the cheese

The level of the individual free amino acids in the three trials (control

Figure 4.13: Diagrammatic representation of a possible amino acid 'pool' developed during cheese ripening



After Scott (1981)

and experimental) are shown in Appendix 9, and it was difficult to observe any pattern of the liberation of amino acids in these cheeses during the ripening period. Different levels of free amino acids could be observed between the three trials and the reasons for such difference(s) could be attributed to:

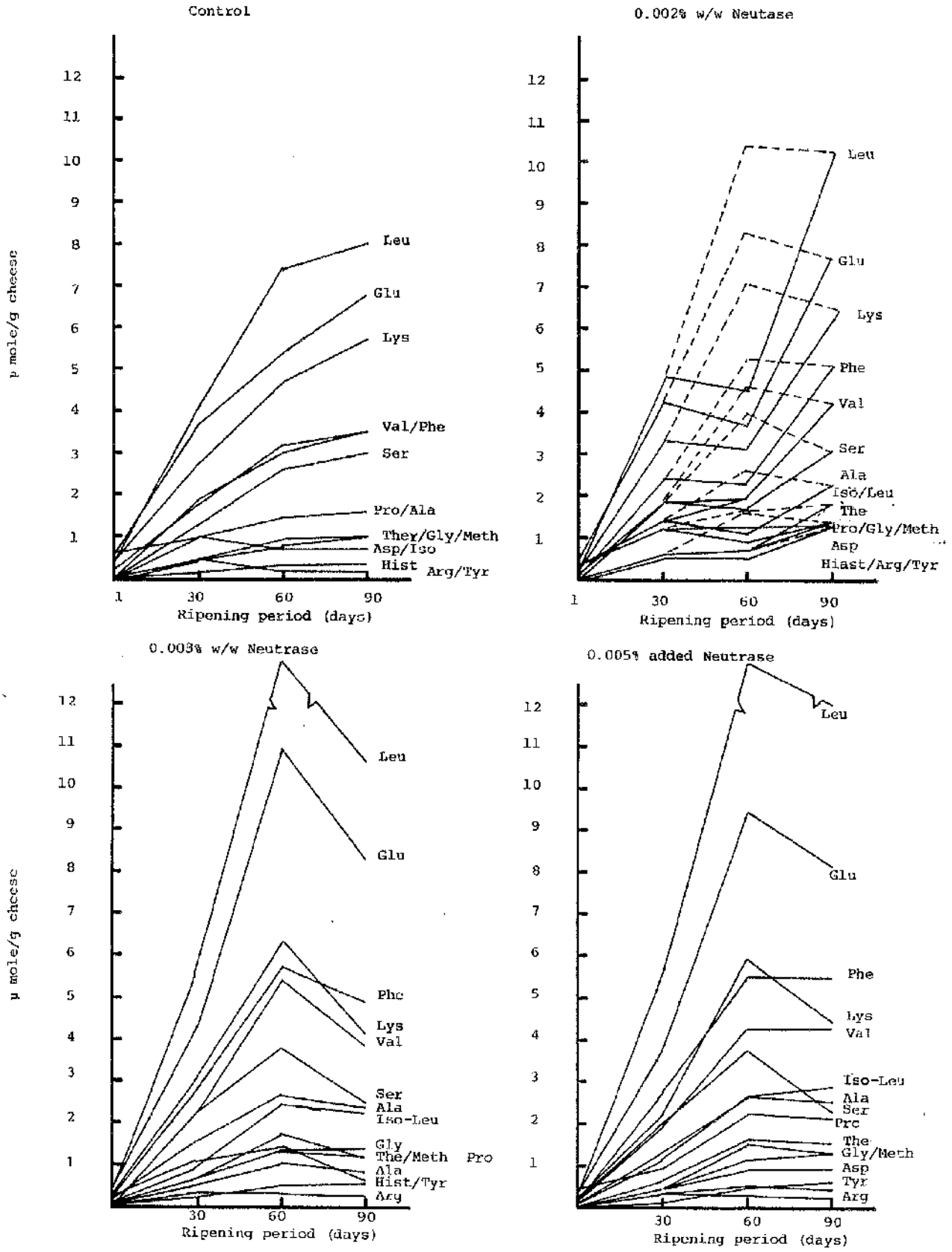
- the variation of the protein level in the cheese;
- the uneven distribution of the enzyme in the cheese;
- the amount of moisture present in the cheese;
- in some of the experimental cheeses, syneresis had occurred resulting in the loss of fluid from the cheese. This fluid probably contained protein breakdown products including amino acids.
- the enzymes that survived the heat treatment of the cheese milk;
- the utilisation of some of these amino acids by the cheese microflora (Manson, 1984);
- the conversion of some free amino acids into fatty acids either by chemical or microbial reactions (Scott, 1981), and Figure 4.13 illustrates some possible reactions.

In order to minimise the effect of some of the factors mentioned above which may alter the level of free amino acids in the cheese, it was decided to take the average of these trials and determine whether or not a certain pattern of free amino acids in the cheese would appear.

Table 4.21 summarises the average level of free amino acids in the cheese (control and experimental) up to 3 months of ripening, and due to reason(s) beyond my control, the analysis of the cheese at 4 months old could not be carried out.

Sixteen different amino acids have been detected in the cheeses (control and experimental), and Figure 4.14 illustrates the changes in the level of amino acids in these cheeses up to 3 months old. It could be observed, however, that the level of amino acids progressively increased in the cheese during the ripening period and the lowest level was observed in the control cheese. Furthermore, the liberation of certain acids was greater than others, and some of these amino acids did not

Figure 4.14: The spectram of the individual free amino acids in Cheddar cheese made from curd with and without added Neutrase during 3 months of ripening



---, theoretical based on proportion of the amino acids to soluble nitrogen

TABLE 4.21

The average level of individual free amino acids (μ-mole/g) in Cheddar cheese made from curd with and without added Neutrase

	One day old				One month old				Two month old				Three month old			
	C	0.002*	0.003*	0.005*	C	0.002*	0.003*	0.005*	C	0.002*	0.003*	0.005*	C	0.002*	0.003*	0.005*
Lysine	0.43	0.34	0.32	0.28	2.76	3.34	3.00	2.27	4.75	3.10	6.36	5.97	5.75	6.36	4.18	4.50
Histidine	0.08	0.08	0.07	0.06	0.20	0.20	0.21	0.17	0.46	0.20	0.56	0.54	0.44	0.4	0.55	0.64
Arginine	Trace	Trace	Trace	Trace	0.57	0.32	0.29	0.33	0.30	0.20	0.37	0.39	0.19	0.36	0.27	0.23
Asparatic acid	0.07	0.04	0.04	0.04	1.06	0.50	0.48	0.43	0.70	0.45	1.00	0.93	0.85	0.99	0.88	0.92
Thereonine	0.02	0.03	0.02	0.03	0.53	0.69	0.68	0.62	0.97	0.73	1.73	1.63	1.09	1.40	1.16	1.57
Serine	0.08	0.08	0.08	0.16	1.32	1.95	2.23	2.01	2.63	1.77	3.87	3.81	3.01	3.19	2.57	2.38
Glutamic acid	0.51	0.42	0.45	0.35	3.74	4.20	4.25	3.75	5.46	3.61	10.97	9.53	6.85	7.73	8.31	8.15
Proline	0.64	0.51	0.45	0.41	1.06	1.28	1.01	0.98	1.55	0.94	2.48	2.26	1.62	1.30	1.68	2.11
Glycine	0.03	0.03	0.03	0.03	0.47	0.45	0.69	0.43	0.85	0.58	1.31	1.19	1.14	1.31	1.32	1.31
Alanine	0.13	0.14	0.11	0.11	1.16	1.42	1.54	1.31	1.58	1.13	2.69	2.64	1.64	2.35	2.46	2.58
Valine	0.07	0.08	0.07	0.08	1.93	1.94	2.25	1.99	3.08	1.99	5.48	4.32	3.54	4.34	3.87	4.38
Methionine	0.24	0.02	0.03	0.03	0.44	0.53	0.65	0.62	0.79	0.57	1.39	1.54	0.96	1.23	1.25	1.30
Isoleucine	0.02	0.03	0.05	0.05	0.38	0.59	0.90	1.11	0.67	0.76	2.43	2.68	0.77	1.97	2.30	2.92
Leucine	0.29	0.32	0.29	0.35	4.60	4.85	5.60	5.75	7.44	4.52	16.07	15.30	8.05	10.31	10.68	12.06
Tyrosine	0.09	0.08	0.06	0.06	0.47	0.42	0.43	0.44	0.46	0.19	0.58	0.64	0.33	0.38	0.42	0.46
Phenylalanine	0.17	0.20	0.25	0.23	1.84	2.47	2.82	2.81	3.25	2.30	5.70	5.57	3.56	5.14	4.98	5.59

C = Control

*The amount of Neutrase (%w/w) added

increase beyond a certain level. Therefore, it was decided to divide/ classify these amino acids into three groups in order to simplify the discussion.

(a) The amino acids which were found in small concentration (< 1 μ mole/g cheese), i.e. histidine, arginine, aspartic acid and tyrosine. The level of these amino acids was found to increase steadily up to 2 months and then start to decline after 3 months (see Figure 4.14). A summary of the level of these amino acids in the cheeses (control and experimental) was:

	μ mole/g cheese							
	Initially				at 3 months Neutrase			
	% (w/w) added Neutrase				% (w/w) added Neutrase			
Amino acids	Control	0.002	0.003	0.005	Control	0.002	0.003	0.005
Histidine	0.08	0.08	0.07	0.06	0.44	0.43	0.55	0.64
Arginine		t r a c e			0.19	0.36	0.27	0.23
Aspartic acid	0.07	0.04	0.04	0.04	0.85	0.92	0.88	0.92
Tyrosine	0.09	0.08	0.06	0.06	0.33	0.38	0.42	0.46

The low concentration of these amino acids in the cheeses could be attributed to their low amount in the protein (Walstra & Jenness, 1984) and see Table 4.22. The increased level of these amino acids in the experimental cheeses compared with the control could be attributed to the high protein breakdown in these cheeses, and this agreed with the results of Law & Wigmore (1982). However, the decreased level of these amino acids at 3 months old could be attributed to the self regulatory mechanism of the enzyme (Law & Wigmore, 1982) the syneresis took place in the cheese, the utilisation by the microflora, and to the conversion of some amino acids to fatty acids, amines and other amino acids (see Figure 4.13 and Harper & Kristoffersen, 1959).

Scott (1981) reported that histidine and arginine are bitter in taste, aspartic acid to be broth-like in taste and tyrosine has little or no taste.

Schörmuller (1968) and Weaver & Kroger (1978), reported that arginine was responsible for the repulsive unpleasant, bitter-sweet taste in the cheese. However, in this present study, these amino acids were found in small amounts in both the control

and the experimental and and are unlikely to have had any marked influence on the undesirable taste(s).

(b) The amino acids, which were found < 3 μ mole/g cheese, were therionine, proline, glycine, methionine, alanine and isoleucine. The level of these amino acids in the control cheeses were found to increase steadily during the ripening while it is either decreased or levelled-off in the experimental cheese after 2 months (see Table 4.21 and Figure 4.14). The level of these amino acids in the cheeses (control and experimental) could be summarised as follows:-

μ mole/g cheese

	Initially % (w/w) added Neutrase				at 3 months old % (w/w) added Neutrase			
Amino acid	Control	0.002	0.003	0.005	Control	0.002	0.003	0.005
Therionine	0.02	0.03	0.02	0.03	1.09	1.4	1.16	1.57
Proline	0.64	0.51	0.45	0.41	1.62	1.3	1.68	2.11
Glycine	0.03	0.03	0.03	0.03	1.14	1.31	1.32	1.31
Methionine	0.24	0.02	0.03	0.03	0.96	1.23	1.25	1.30
Isoleucine	0.02	0.03	0.05	0.05	0.77	1.97	2.30	2.92
Alanine	0.13	0.14	0.11	0.11	1.64	2.35	2.46	2.58

The increase in the level of these amino acids in the experimental cheeses compared with the control could be attributed to the high protein breakdown in these cheeses (Law & Wigmore, 1982) while the decreased level after 2 months ripening could be attributed to the same factors mentioned above.

However, Scott (1981) reported that methionine and isoleucine were bitter in taste, and glycine, proline and therionine were sweet in taste. Green & Manning (1982) reported that methanethiol, which was an essential flavour compound in Cheddar cheese could have been derived frm methionine. However, the level of methionine and isoleucine in the experimental cheese compared with the control, and the presence of bitterness in neutrase cheese could be associated with these amino acids. Proline, which was thought to be bitter in taste (Manson,1984), did not increase more in the experimental cheese than in the control except in the cheese treated with 0.005% w/w Neutrase. It is doubtful therefore if proline contributed to the bitterness in the cheese.

(c) The amino acids, which were found in levels between 3 and 16 μ mole/g cheese, were:- lysine, serine, glutamic acid, valine, leucine and phenylalanine. These amino acids form the major amino acids consistuents of the casein fractions (see Table 4.22), and this level increased steadily in the control cheese (up to 3 months ripening), confirmed by Weaver & Kroger 1978).
In the experimental cheeses the highest level of these amino acids were found in 2 months old cheese, and progressively decline at 3 months (see Figure 4.14), which could be attributed to the same factors mentioned in Section (a) above. A summary of the level of these amino acids in the cheese (control and experimental) were found to be as follows:-

μ mole/g cheese

	Initially % (w/w) added Neutrase				at 3 months old % (w/w) added Neutrase			
Amino acid	Control	0.002	0.003	0.005	Control	0.002	0.003	0.005
Lysine	0.43	0.34	0.32	0.28	5.75	6.36	4.18	4.50
Serine	0.08	0.08	0.08	0.16	3.01	3.19	2.57	2.38
Glutamic acid	0.51	0.42	0.45	0.35	6.85	7.73	8.31	8.15
Valine	0.07	0.08	0.07	0.08	3.54	4.34	3.87	4.38
Leucine	0.29	0.32	0.29	0.35	8.05	10.31	10.68	12.06
Phenylalanine	0.17	0.20	0.25	0.23	3.56	5.14	4.98	5.59

It may be observed from the above summary that the amino acids content of the experimental cheeses was greater than the control. Scott (1981) reported that the taste of lysine, leucine and phenylalanine were bitter, and valine and serine were sweet while glutamic acid was broth-like - at least one of the panelists identified the flavour and odour as being meaty and reminiscent of meat broth. Such a report and the high level of lysine, leucine and phenylalanine found in the experimental cheeses may help to explain the presence of bitterness detected by panelists.

4.7.2.3 Casein hydrolysis

PAGE was used to study the extent of hydrolysis of casein fractions in cheese (control and experimental) during the four months ripening period and the gels were scanned at A 0.5 OD. Many peaks were observed in each electrogram, and these peaks could be divided into the following mobility bands:

TABLE 4.22

Amino acids composition of some milk proteins
(mol per kg protein)

Component	α_{s_1}-casein (B)	α_{s_2}-casein	β-casein (A^2)	κ-casein (B)
Glycine	0.38	0.08	0.21	0.11
Alanine	0.38	0.32	0.21	0.79
Valine	0.47	0.55	0.79	0.21
Leucine	0.72	0.52	0.92	0.42
Isoleucine	0.47	0.44	0.42	0.68
Proline	0.72	0.40	1.46	1.05
Phenylalanine	0.34	0.24	0.38	0.21
Tyrosine	0.42	0.48	0.17	0.47
Tryptophan	0.08	0.08	0.04	0.05
Serine	0.68	0.67	0.67	0.68
Thereonine	0.21	0.59	0.38	0.74
Cysteine	0.00	0.08	0.00	0.11
Methionine	0.21	0.16	0.25	0.11
Arginine	0.25	0.24	0.17	0.26
Histidine	0.21	0.12	0.21	0.16
Lysine	0.59	0.95	0.46	0.47
Asparagine	0.34	0.55	0.21	0.37
Aspartic acid	0.30	0.16	0.17	0.21
Glutamine	0.64	0.59	0.88	0.74
Glutamic acid	1.02	0.99	0.75	0.68

After Walstra & Jenness (1984)

(i) slow mobility

(ii) α_s-casein

(iii) β_s-casein

The extent of hydrolysis of casein during the ripening period is illustrated in Appendix 10. Typical gel electrograms are illustrated in Figure 4.15 and Plates 4.11 to 4.17, and the overall pattern of casein hydrolysis could be summarised as follows:

(i) Slow mobility bands

The number of casein fractions in this mobility zone ranged between 3 and 7, and more fractions were observed in the Neutrase treated cheese. As mentioned elsewhere (see Section I), the experimental cheese contained a higher percentage of these fractions than the control which could be attributed to the Neutrase enzyme activity on β-casein (Law & Wigmore, 1982 and Marcos et al., 1978). During the ripening period, the percentage of these fractions has increased in the control and experimental cheeses, and the level was greater in the Neutrase treated cheese. Furthermore, the experimental cheese showed fluctuations in the percentage and the number of bands during the ripening period which could be attributed to the uneven distribution of the enzyme in the cheese and it is most likely that the casein fraction(s) that fall within such mobility was readily hydrolysed (Ridha, Crawford & Tamime, 1984). The products of hydrolysis could be amino acids, peptides and/or soluble nitrogen (see Figure 4.5).

(ii) β-casein

The number of bands of β-casein in the control and the experimental cheeses ranged from 1 to 2, and it can be observed that β-casein was progressively reduced as the cheese became older. The degree of β-casein hydrolysis was greater in the experimental cheese than in the control cheese (see Appendix 10). The major band of the β-casein which was readily hydrolysed was number 2, and the degradation of the band contributed to the increase of the bands in the slow mobility zones (Marcos, et al., 1978). β-casein in the control cheese showed a progressive and steady decrease during the ripening period. In the experimental cheese, the β-casein fraction was more extensively hydrolysed than other fractions in relation to the level of enzyme used and the duration of ripening; however, some fluctuation in the extent of

Legend to

Figure 4.15: Typical polyacrylamide gel electrograms of Cheddar cheese made from curd with and without added Neutrase during 4 months of ripening

Footnote: Figures in parentheses represent rate (% w/w) of added Neutrase

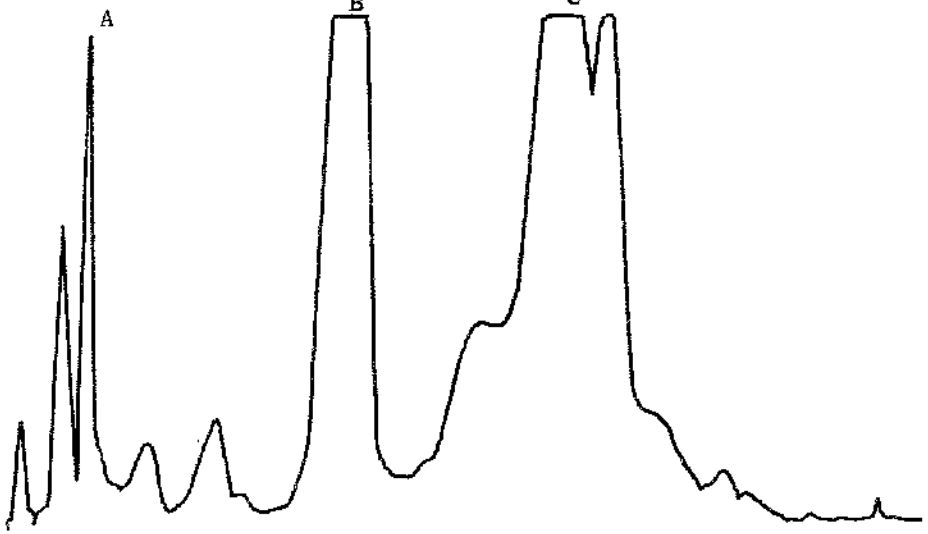
A - Slow mobility casein fractions

B - β-casein fractions

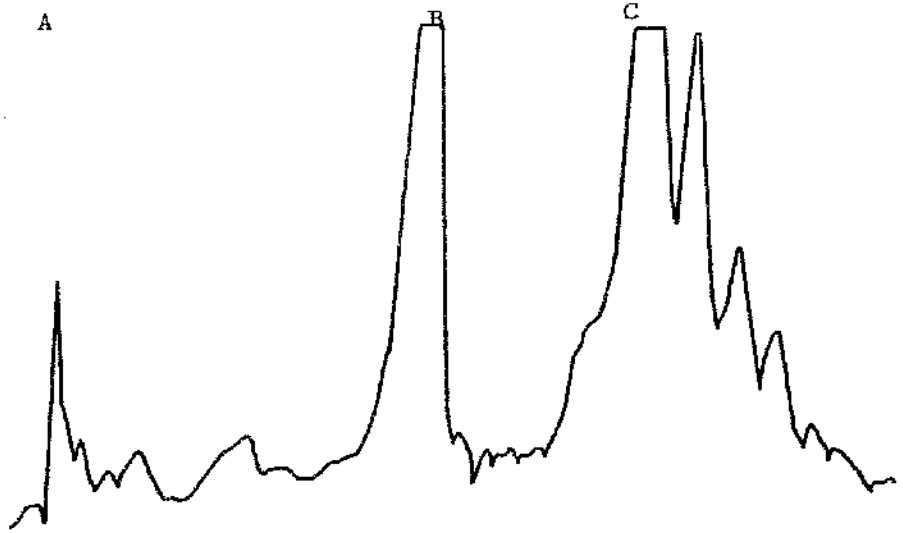
C - α_s-casein fractions

Control cheese

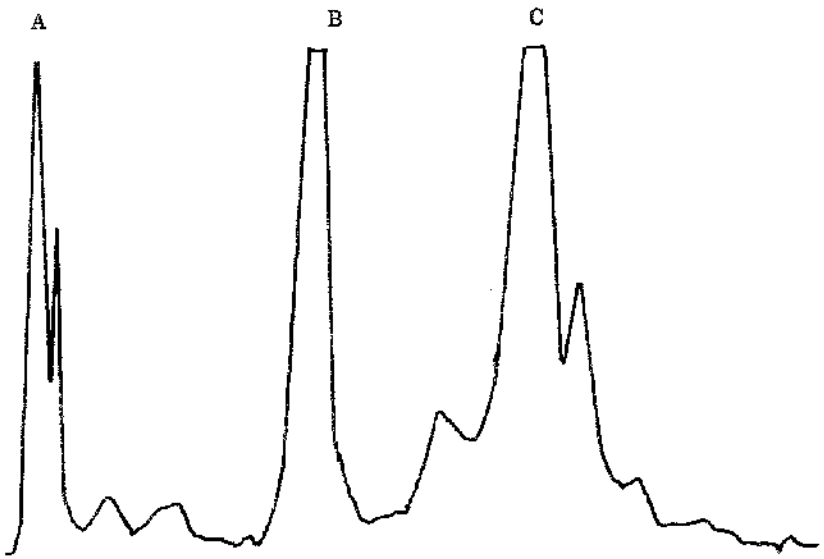
One day old



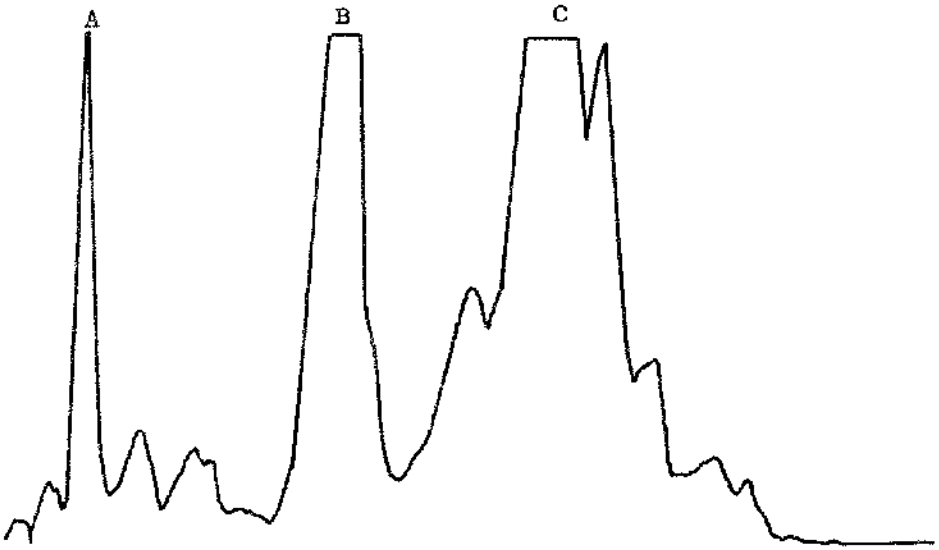
(0.003)



(0.002)

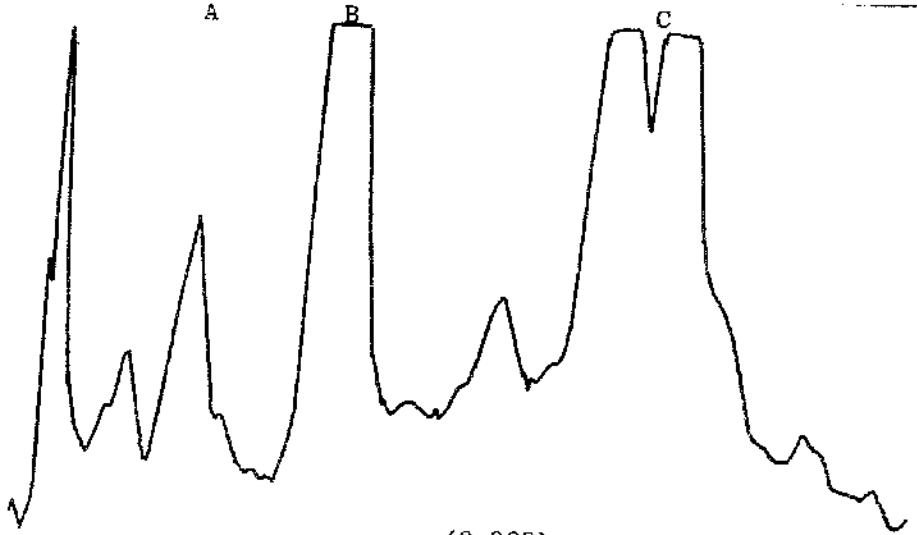


(0.005)

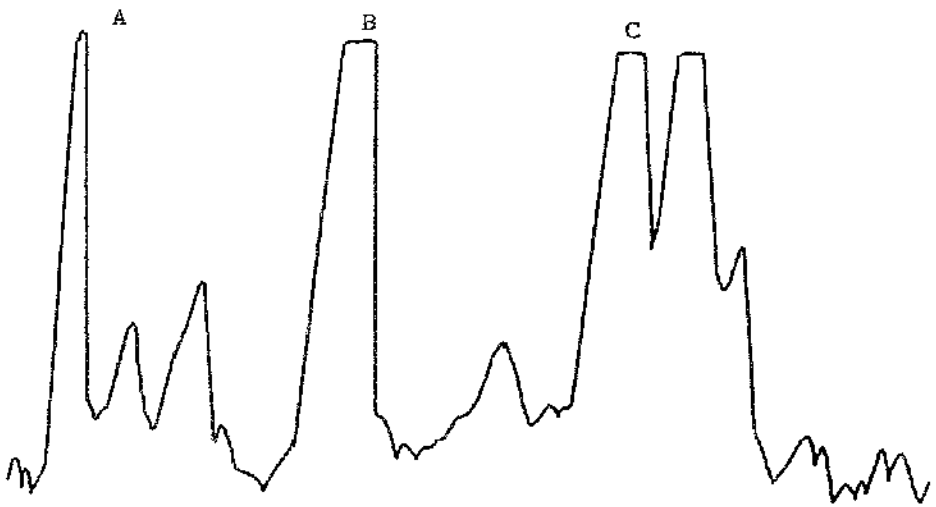


cont'd.....

Control cheese

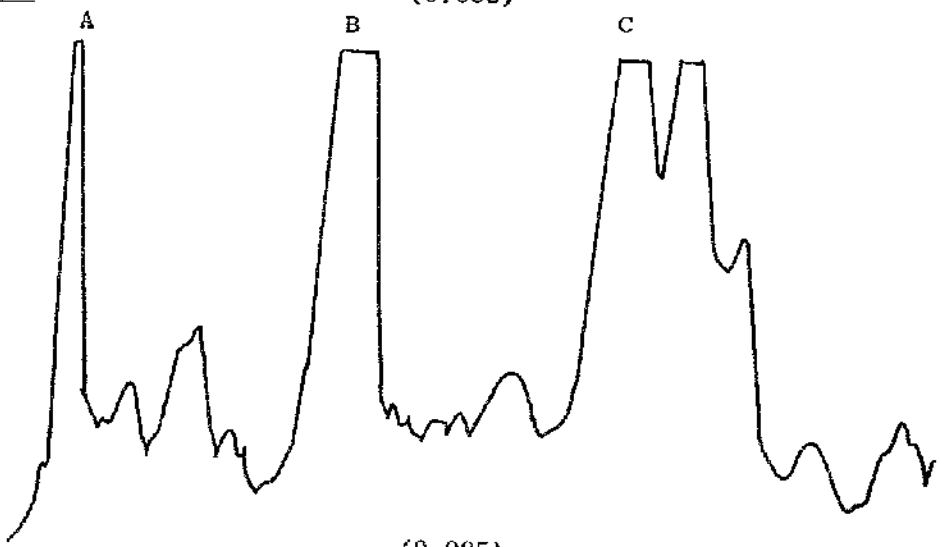


(0.002)

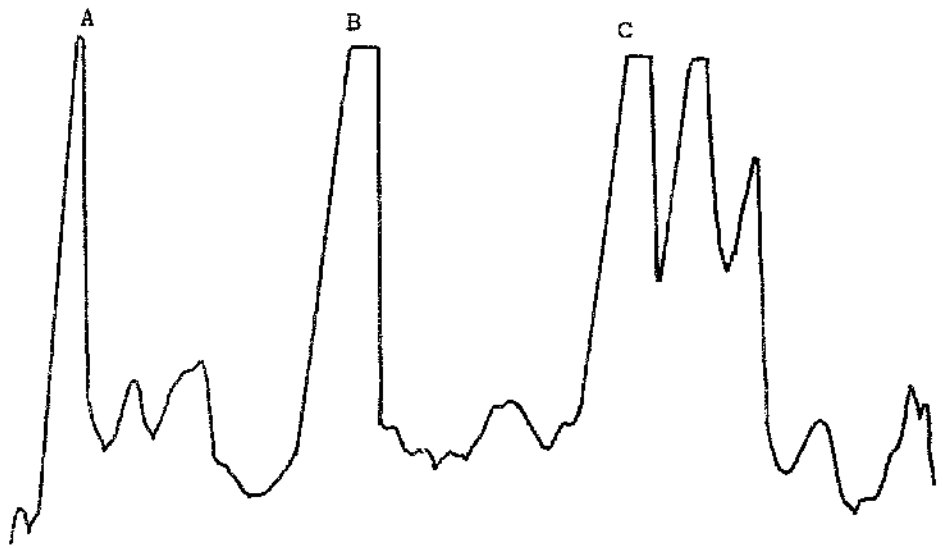


One month old

(0.003)



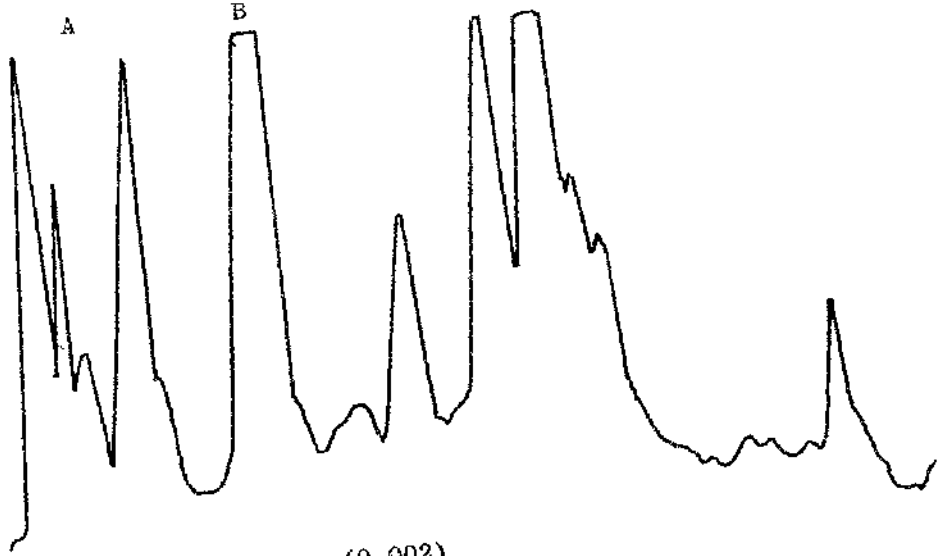
(0.005)



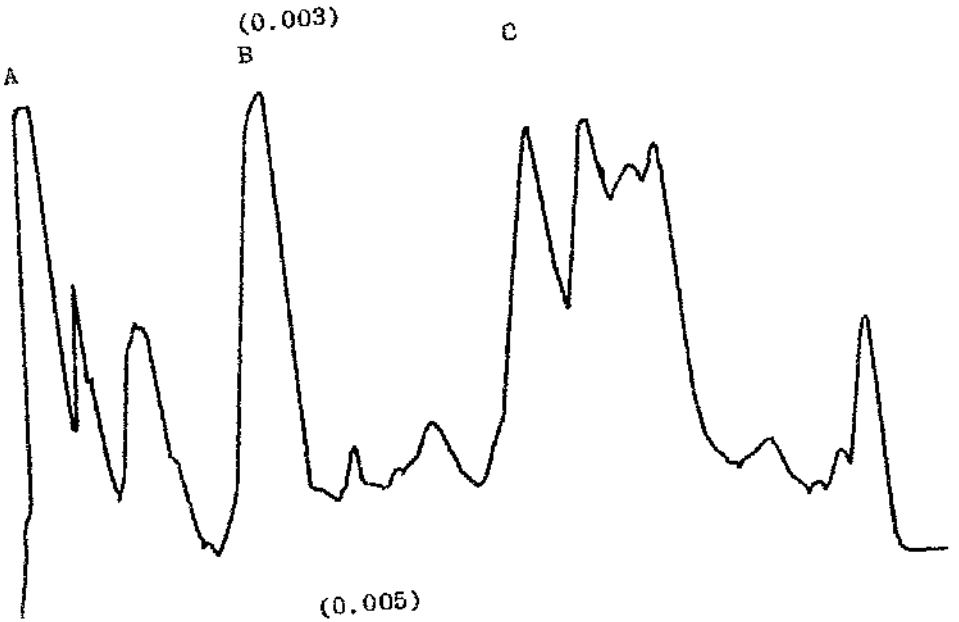
cont'd .....

Two months old

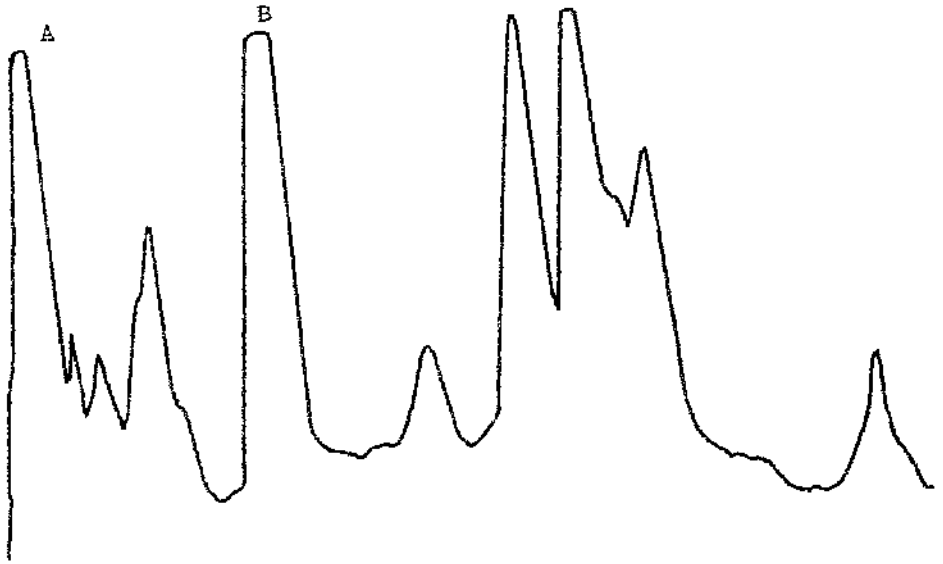
Control cheese



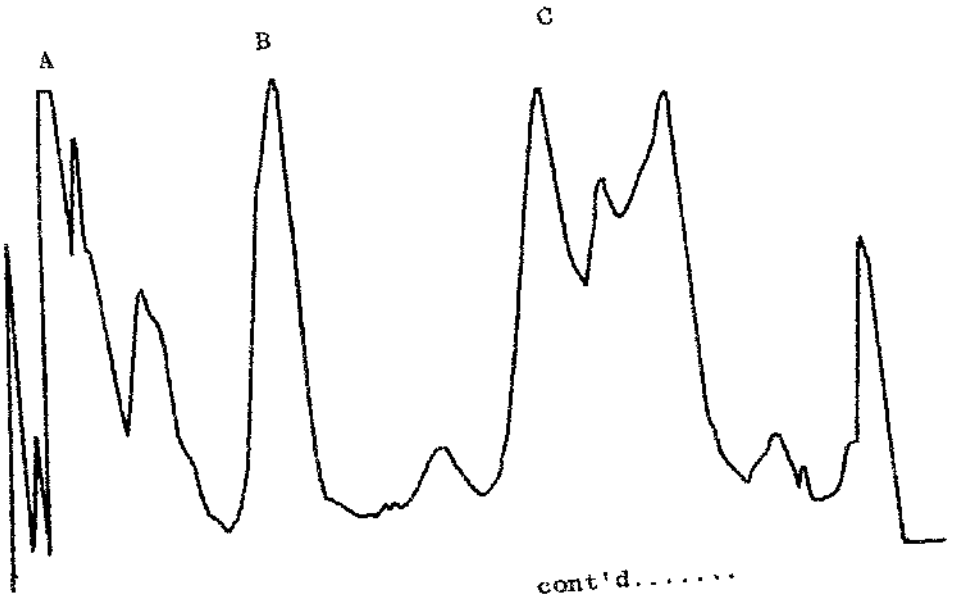
(0.003)



(0.002)



(0.005)



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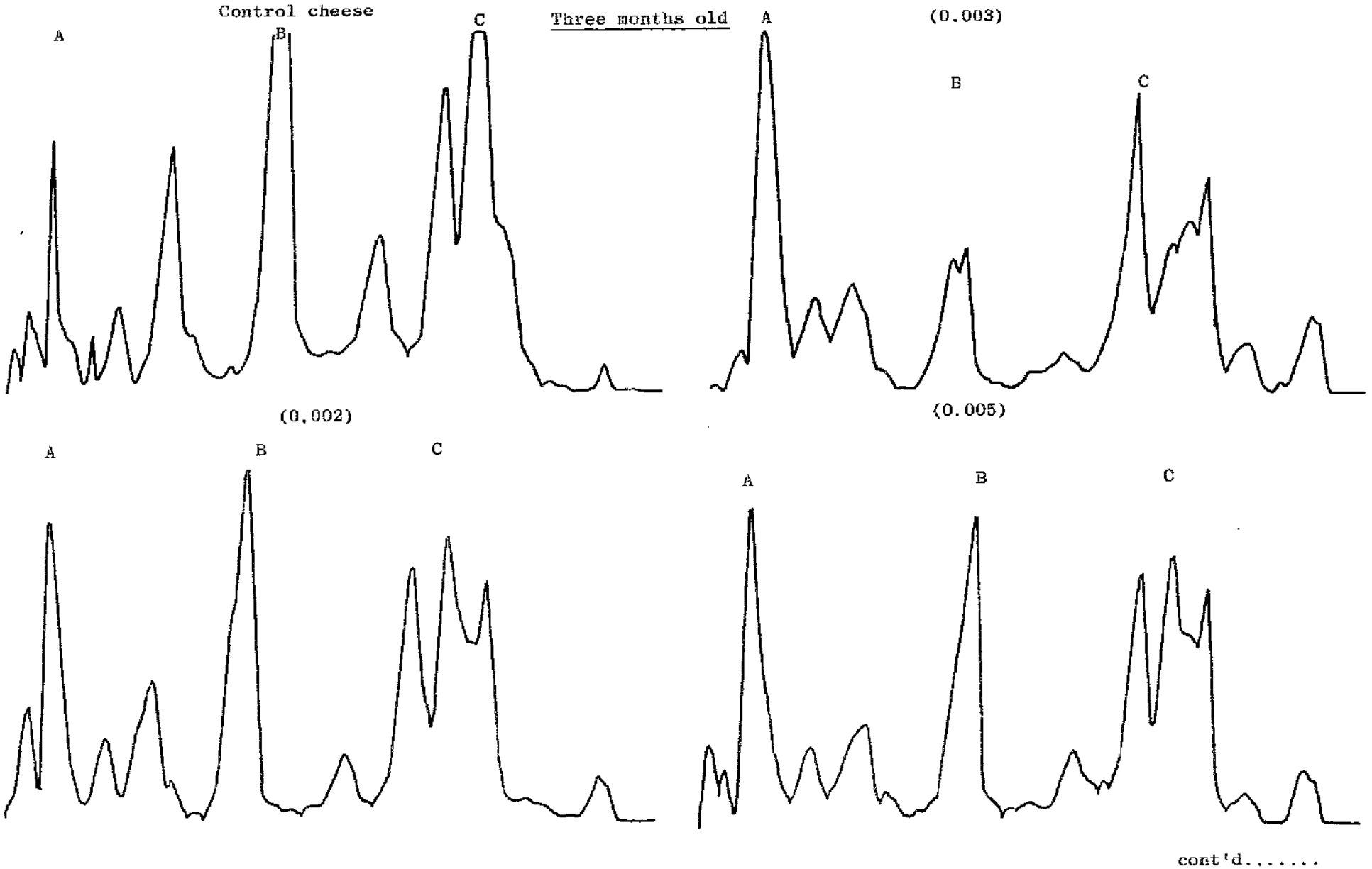
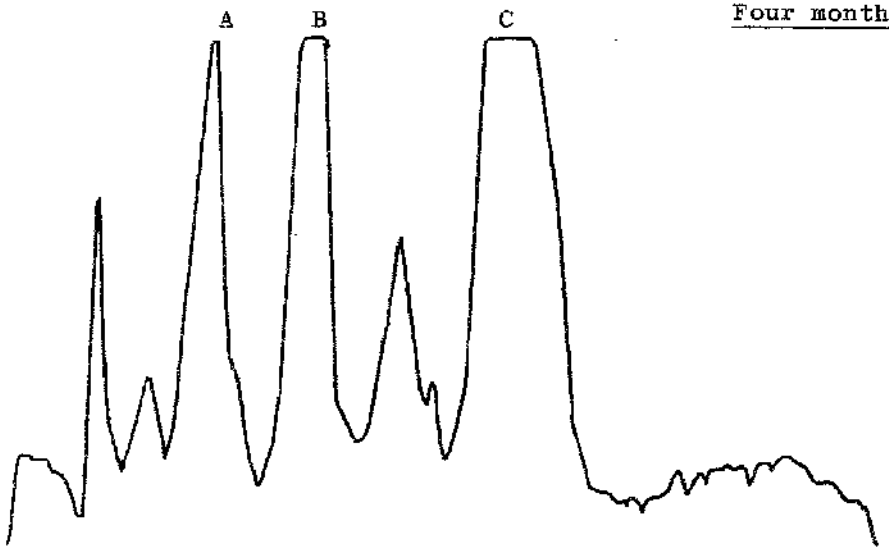
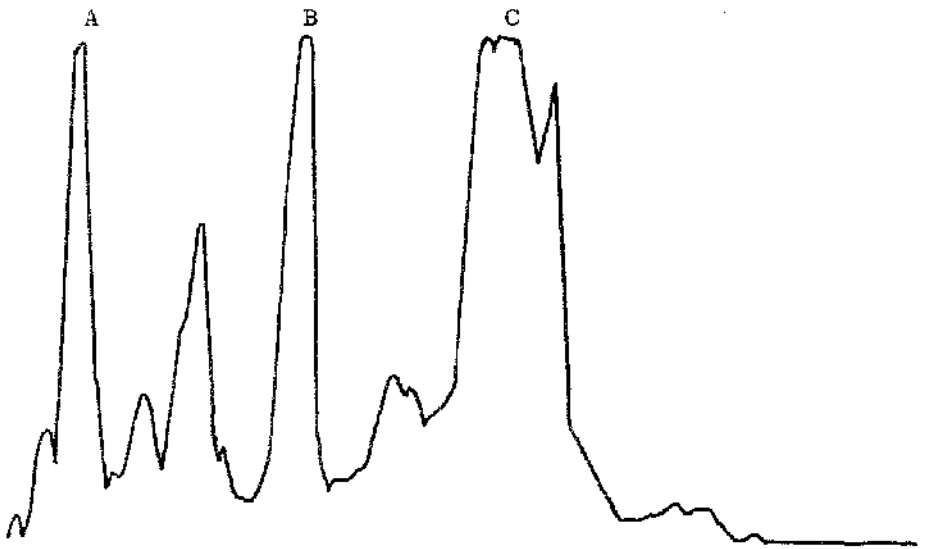

Control cheese
A
B
C
(0.002)
Three months old
A
(0.003)
B
C
(0.005)
A
B
C
A
B
C
cont'd.....

Four months old

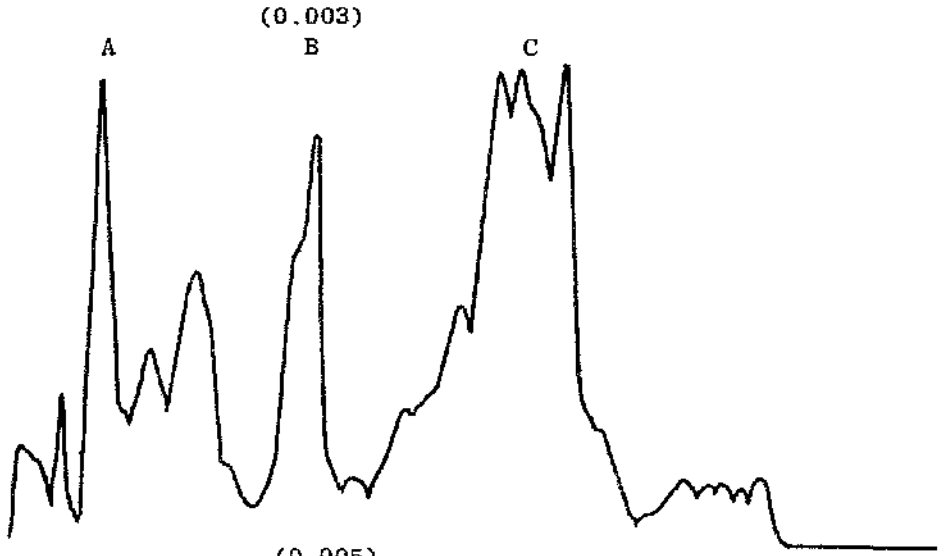
Control cheese



(0.002)



(0.003)



(0.005)

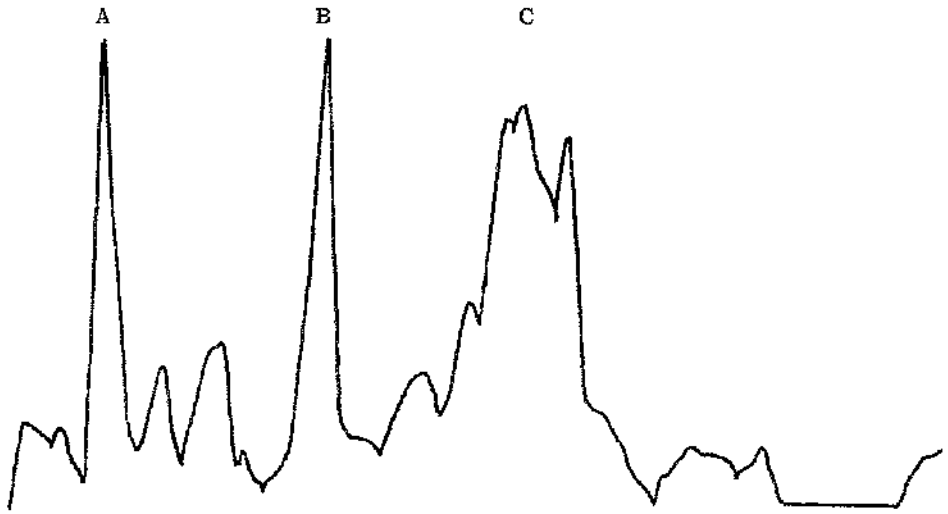
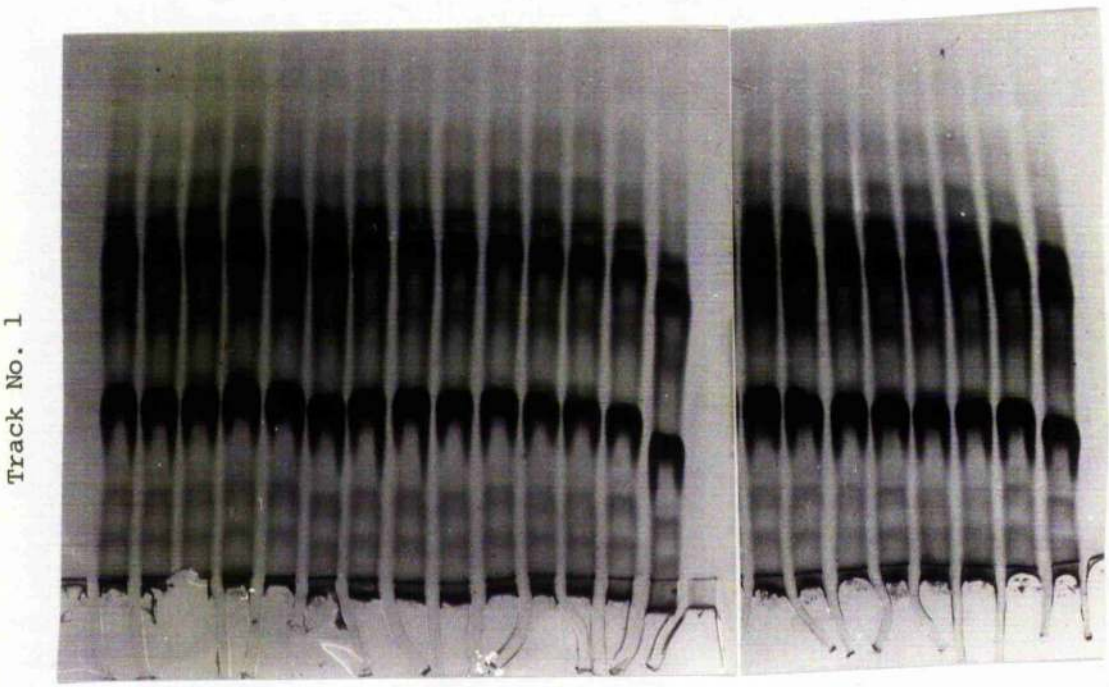


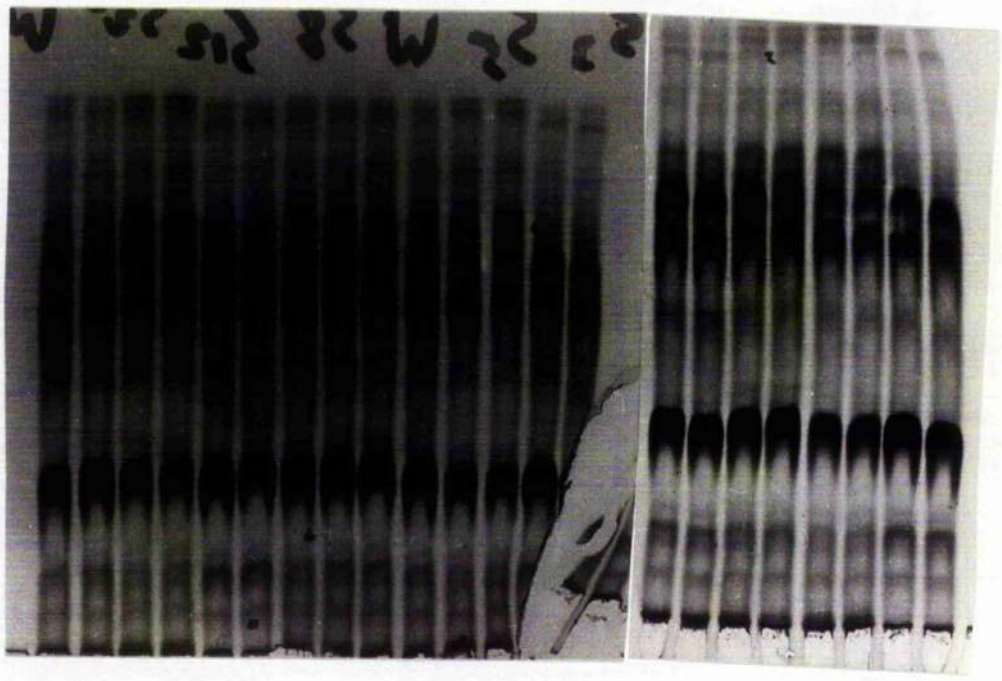
Plate 4.11 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase after 1 day of ripening.



Trial No.	Pattern for cheese made from:	Number of tracks (left to right)
1	Curd with Neutrase (0.003% w/w)	1, 2
	Curd with Neutrase (0.005% w/w)	3, 4
	Control curd	5, 6
2	Curd with Neutrase (0.002% w/w)	7, 8
	Curd with Neutrase (0.003% w/w)	9, 10
	Curd with Neutrase (0.005% w/w)	11, 12
	Control curd	13, 4
3	Curd with Neutrase (0.002% w/w)	15, 16
	Curd with Neutrase (0.003% w/w)	17, 18
	Curd with Neutrase (0.005% w/w)	19, 20
	Control curd	21, 22

Plate 4.12 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase after 1 month of ripening.

Track No. 1

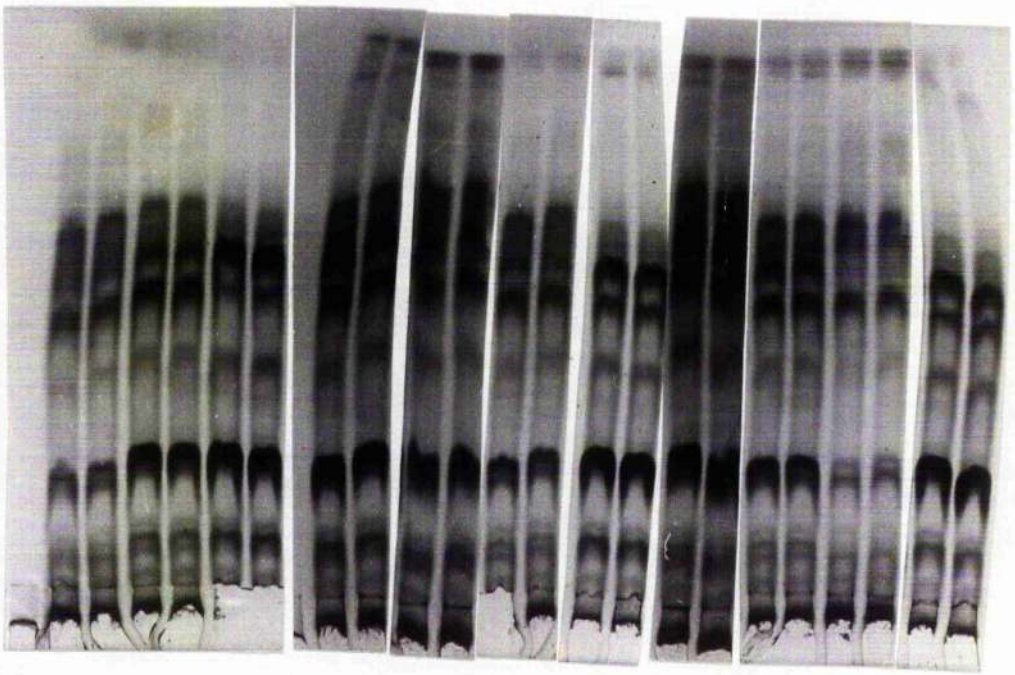


Track No. 22

Trial No.	Pattern for cheese made from:	Number of tracks (left to right)
1	Curd with Neutrase (0.003% w/w)	1, 2
	Curd with Neutrase (0.005% w/w)	3, 4
	Control curd	5, 6
2	Curd with Neutrase (0.002% w/w)	7, 8
	Curd with Neutrase (0.003% w/w)	9, 10
	Curd with Neutrase (0.005% w/w)	11, 12
	Control curd	13, 14
3	Curd with Neutrase (0.002% w/w)	15, 16
	Curd with Neutrase (0.003% w/w)	17, 18
	Curd with Neutrase (0.005% w/w)	19, 20
	Control curd	21, 22

Plate 4.13 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase after 2 months of ripening.

Track No. 1

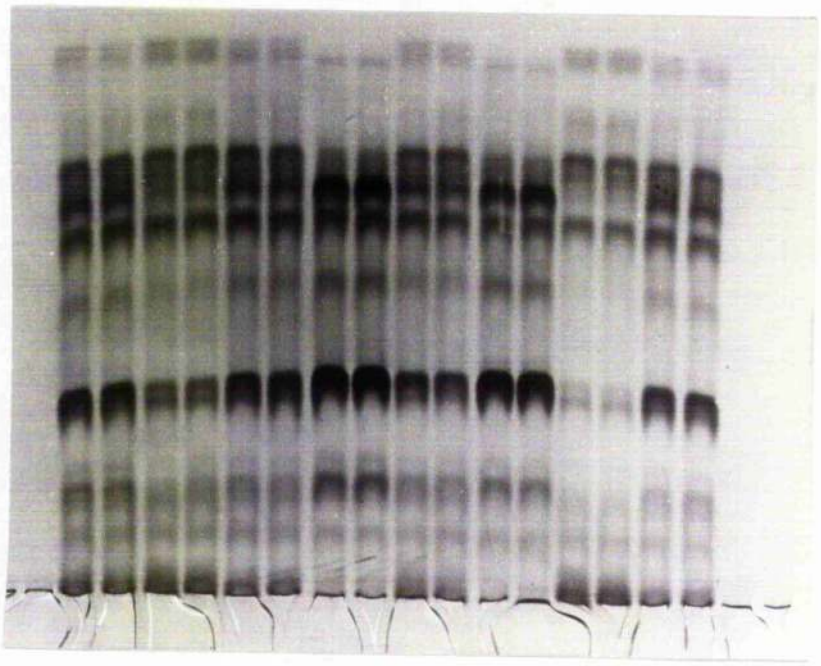


Track No. 22

Trial No.	Pattern for cheese made from:	Number of tracks (left to right)
1	Curd with Neutrase (0.005% w/w)	1, 2
	Curd with Neutrase (0.003% w/w)	3, 4
	Control curd	5, 6
2	Curd with Neutrase (0.002% w/w)	7, 8
	Curd with Neutrase (0.003% w/w)	9, 10
	Curd with Neutrase (0.005% w/w)	11, 12
	Control curd	13, 14
3	Curd with Neutrase (0.002% w/w)	15, 16
	Curd with Neutrase (0.003% w/w)	17, 18
	Curd with Neutrase (0.005% w/w)	19, 20
	Control curd	21, 22

Plate 4.14 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase after 3 months of ripening.

Track No. 1

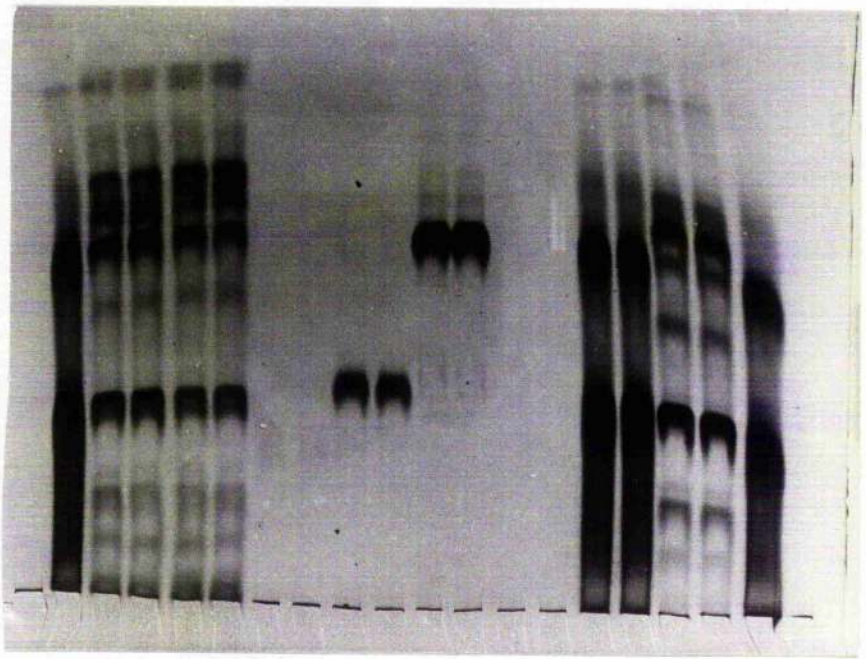


Track No. 16

Trial No.	Pattern of cheese made from:	Number of tracks (left to right)
2	Curd with Neutrase (0.002% w/w)	1, 2
	Curd with Neutrase (0.005% w/w)	3, 4
	Curd with Neutrase (0.003% w/w)	5, 6
	Control curd	7, 8
3	Curd with Neutrase (0.003% w/w)	9, 10
	Control curd	11, 12
	Curd with Neutrase (0.005% w/w)	13, 14
	Curd with Neutrase (0.002% w/w)	15, 16

Plate 4.15 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase after 3 months of ripening.

Track No. 1

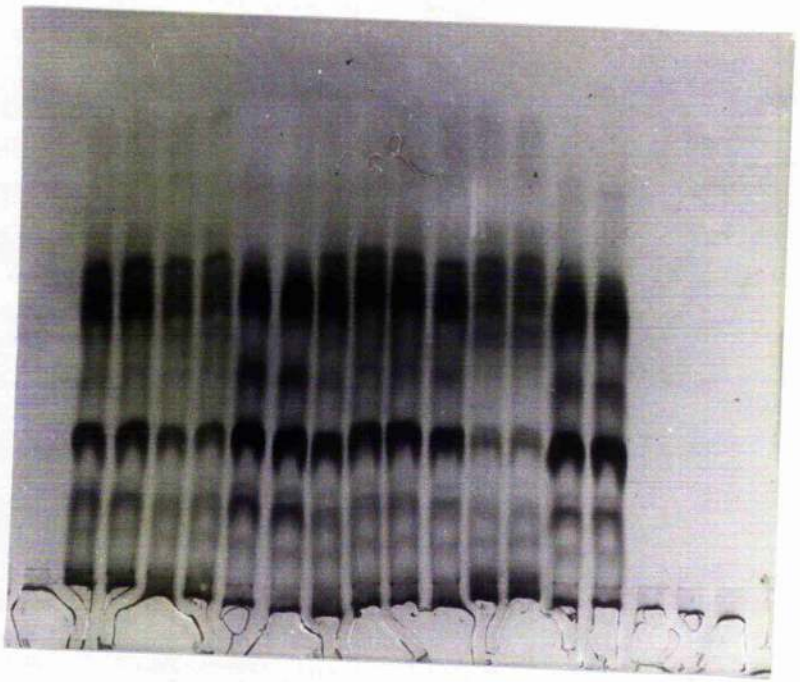


Track No. 15

Trial No.	Pattern for cheese made from:	Number of tracks (left to right)
1	Standard casein	1
	Curd with Neutrase (0.003% w/w)	2, 3
	Curd with Neutrase (0.005% w/w)	4, 5
	Pure β- casein	6, 7
	Pure α_{s1}-case	8, 9
	Standard casein	11, 12
	Control curd	13, 14
	Standard casein	15

Plate 4.16 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase after 4 months of ripening.

Track No. 1

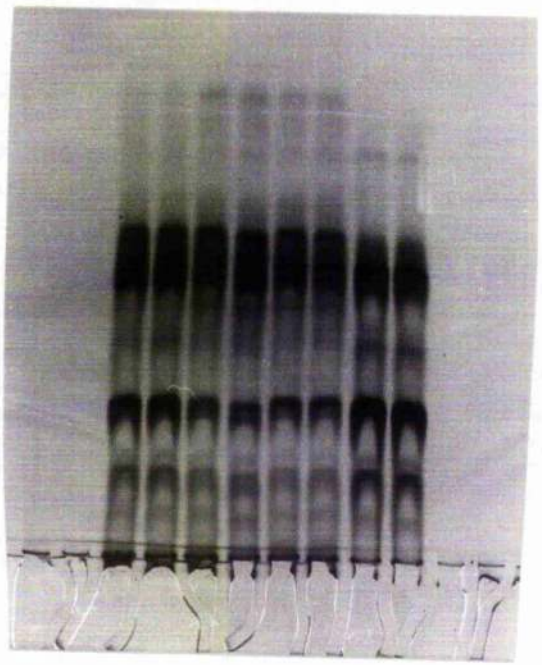


Track No. 14

Trial No.	Pattern for cheese made from:	Number of tracks (left to right)
1	Curd with Neutrase (0.003% w/w)	1, 2
	Curd with Neutrase (0.005% w/w)	3, 4
	Control curd	5, 6
3	Curd with Neutrase (0.002% w/w)	7, 8
	Curd with Neutrase (0.003% w/w)	9, 10
	Curd with Neutrase (0.005% w/w)	11, 12
	Control curd	13, 14

Plate 4.17 : The electrophoretic pattern of Cheddar cheese made from curd with and without added Neutrase after 4 months of ripening.

Track No. 1



Track No. 8

Trial No.	Pattern for cheese made from:	Number of tracks (left to right)
2	Curd with Neutrase (0.002% w/w)	1, 2
	Curd with Neutrase (0.003% w/w)	3, 4
	Curd with Neutrase (0.005% w/w)	5, 6
	Control curd	7, 8

hydrolysis of β-casein was also observed and could be attributed to the uneven distribution of the enzyme.

(iii) α_s-casein

The number of α_s-casein fractions varied between 2 and 12 bands in the control cheese while it varied between 4 and 11 bands in the experimental cheese (see Appendix 1C). The hydrolysis of the individual casein fractions did not follow a similar trend in the cheese (control and experimental), which is due to the appearance of very minor peaks at position 2 or 3 of α_s-casein fraction. The formation of such minor peaks could be attributed to the following:-

- interference from the integrator,
- the possible formation of new peaks,
- some tailing effect of the peaks in the gel.

However, the overall hydrolysis of total α_s-casein was similar in all the treatments which showed slow hydrolysis during the ripening period and the possible product(s) of hydrolysis is illustrated previously in Figure 4.5.

Conclusion

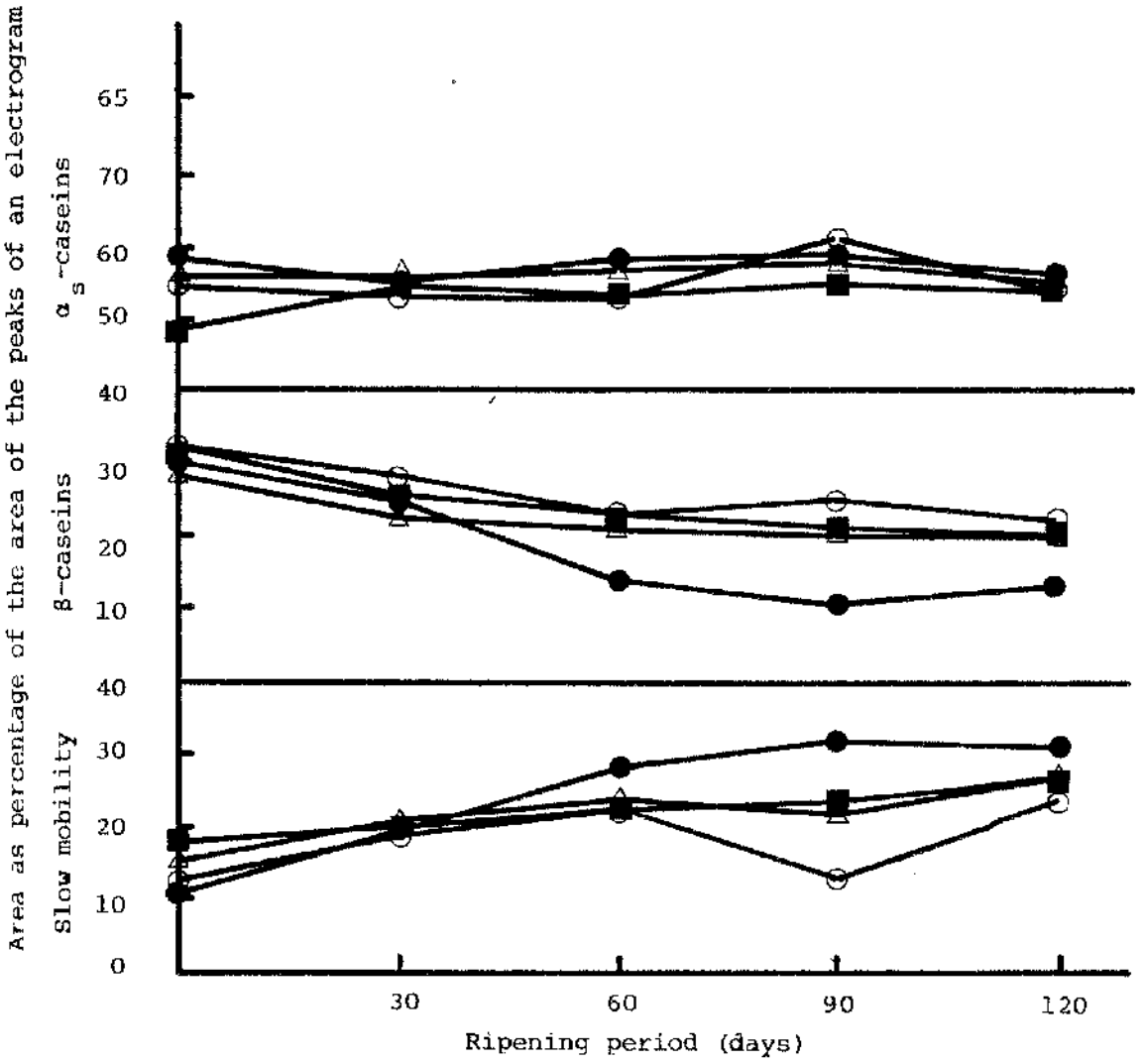
Different patterns of casein hydrolysis were observed in the present 3 trials due to factors mentioned above. However, the average of 3 trials of the total peaks in each mobility zone is calculated and the overall pattern is illustrated in Figure 4.16, from which may be observed the following:-

- slow mobility bands increase and level off after 60 days; however, the decrease in the control cheese could be related to the fact that α_s-casein was less hydrolysed than in the experimental cheeses.
- β-casein progressively decreased during the ripening period and maximum hydrolysis was observed in Neutrase treated cheese at a rate of addition of 0.005% (w/w).
- α_s-casein hydrolysis in all the cheeses remained linear.

4.8 Organoleptic assessment

The cheeses (control and experimental) were assessed and evaluated by

Figure: 4.16: Variation in the area of casein fractions (calculated as percentage of the area of the peaks of an electrogram) in Cheddar cheese made from curd with and without added Neutrase after different periods of ripening



○ , control cheese

■ , cheese prepared with 0.002% (w/w) Neutrase

△ , cheese prepared with 0.003% (w/w) Neutrase

● , cheese prepared with 0.005% (w/w) Neutrase

three professional graders after 1, 2, 3 and 4 months for flavour intensity, off-flavour, bitter or taste flavour, flavour quality, elasticity, openness, firmness, general acceptability and colour defects. The results of this experiment confirms the pattern of results reported in Section I, and the following may be observed:-

(A) The score of the flavour intensity of the cheese (control and experimental) is illustrated in Figure 4.17, and the mean scores awarded by the graders could be summarised as follows:-

- the control cheese produced less flavour than Neutrase treated cheese;
- the flavour intensity in the Neutrase-treated cheese at 2 months was equivalent to 'natural' cheese of 4 months old;
- greater flavour intensity was influenced with the level of enzyme added.

(B) The graders' scores for off-flavour and bitter taste intensities in the cheese (control and experimental) were similar, and the mean scores awarded are shown in Figures 4.18 and 4.19. The following observations may be made:-

- Neutrase-treated cheese was inferior to the control due to the development of off-flavour and bitter taste;
- the degree of off-flavour and bitter taste in the Neutrase-treated cheese was affected by the level of enzyme added, and the highest intensities of these defects were observed in 3 months old cheese;
- a slight reduction in off-flavour and bitter taste was observed in the experimental cheeses at 4 months old, and the reason(s) for such phenomena is not well established.

(C) The scores awarded by the 3 graders for the flavour quality of the cheese (control and experimental) is illustrated in Figures 4.20 to 4.23, and it may be observed that the Neutrase-treated cheese had slightly better quality after 1 and 2 months ripening. However, the control cheese at 3 and 4 months was superior in quality compared with the Neutrase-treated cheese. The quality of the experimental cheese deteriorated after 3 months old.

Figure 4.17: Means of flavour intensity scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) during 4 months of ripening

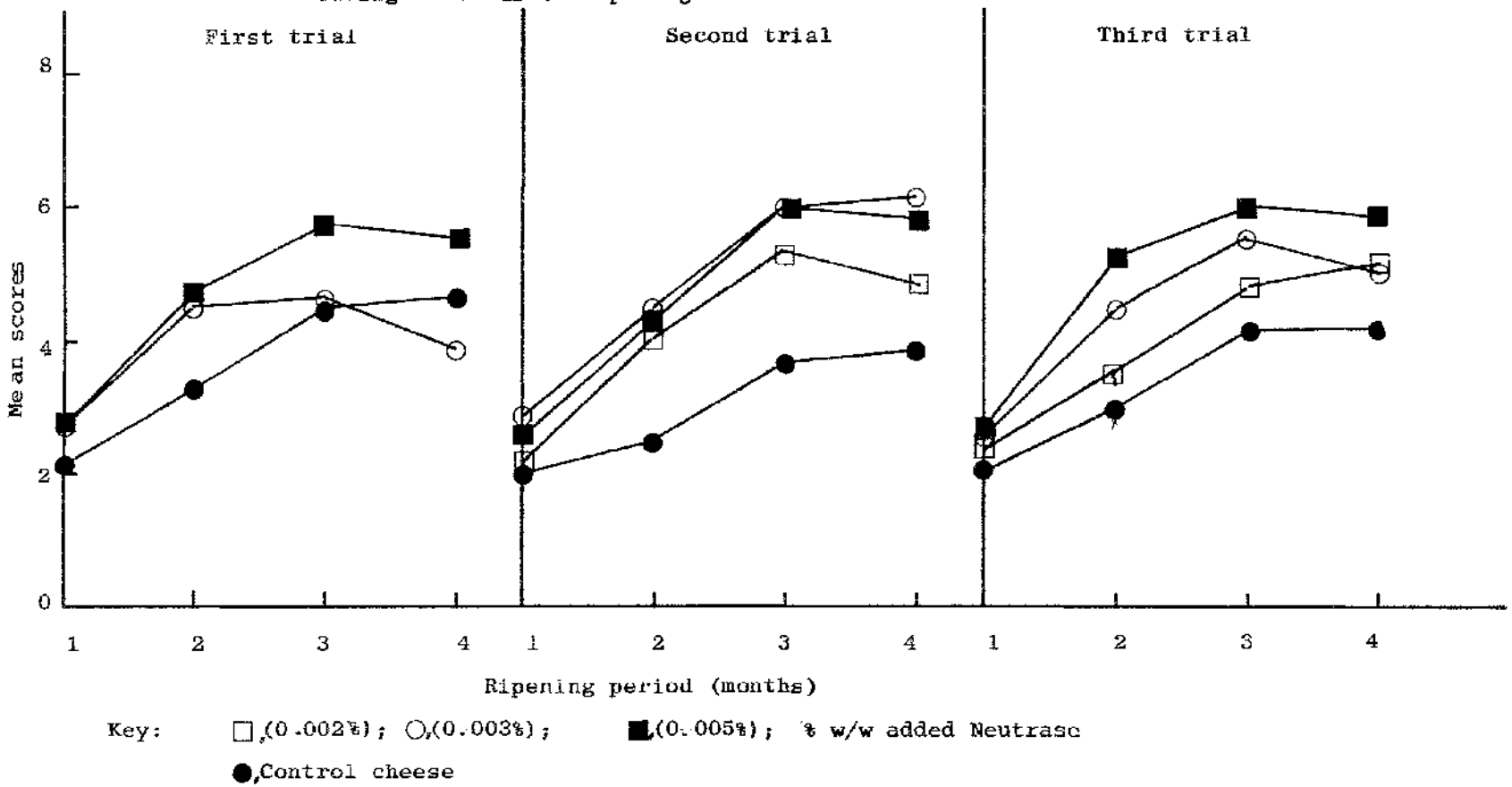
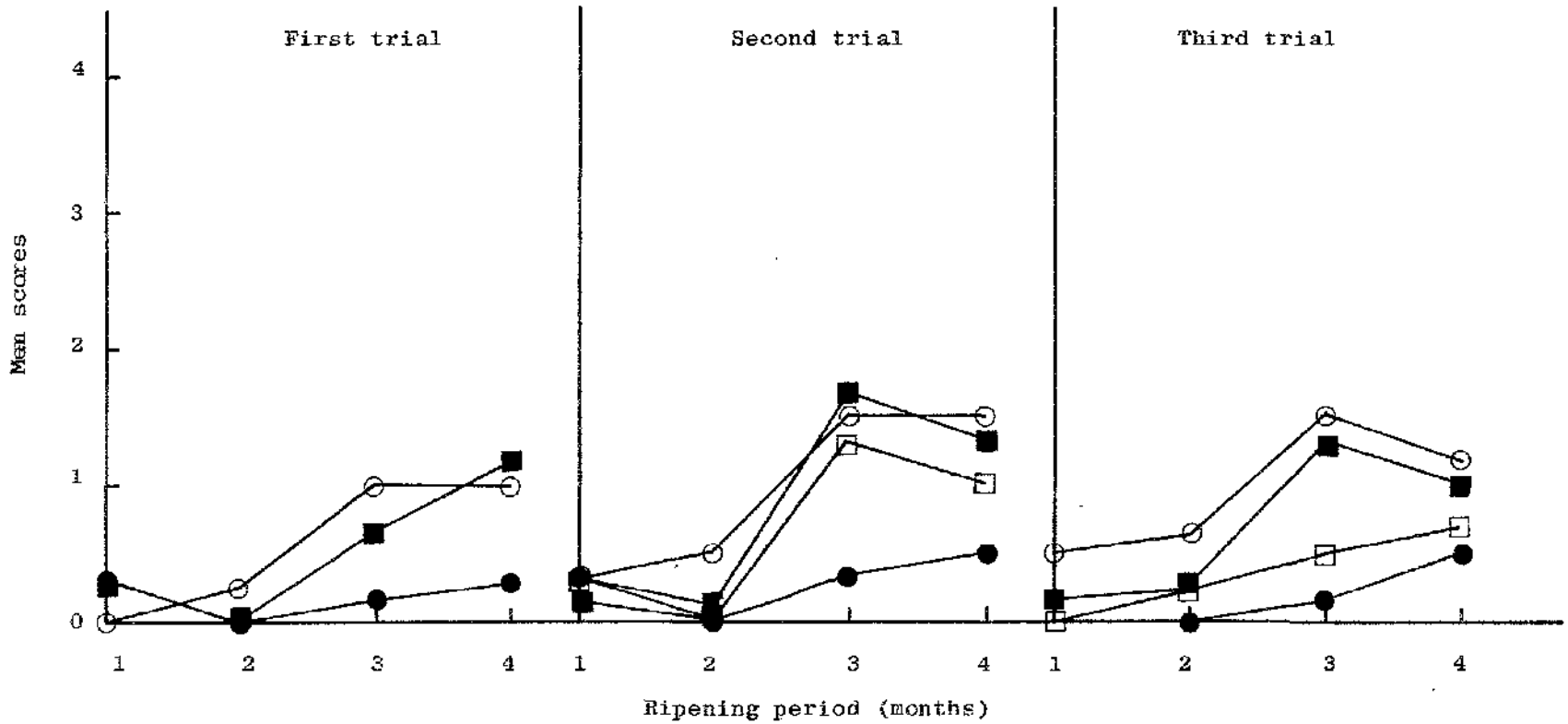


Figure 4.18: Means of off-flavour intensity scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) during 4 months of ripening.



Key: □,(0.002%); ○,(0.003%) and ■,(0.005%) % w/w added Neutrase
●,Control cheese

Figure 4.19: Means of bitter flavour intensity scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) during 4 months of ripening

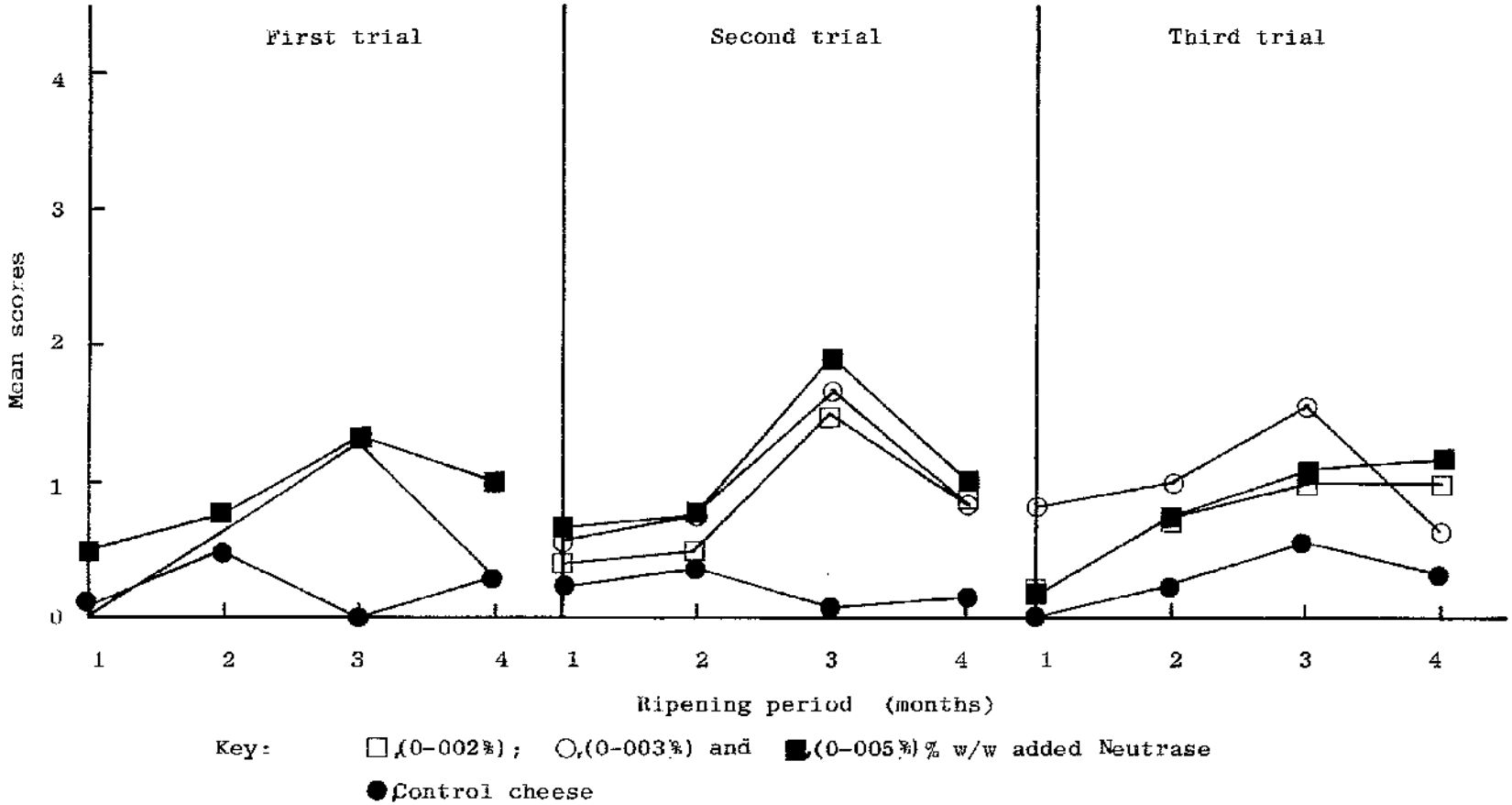
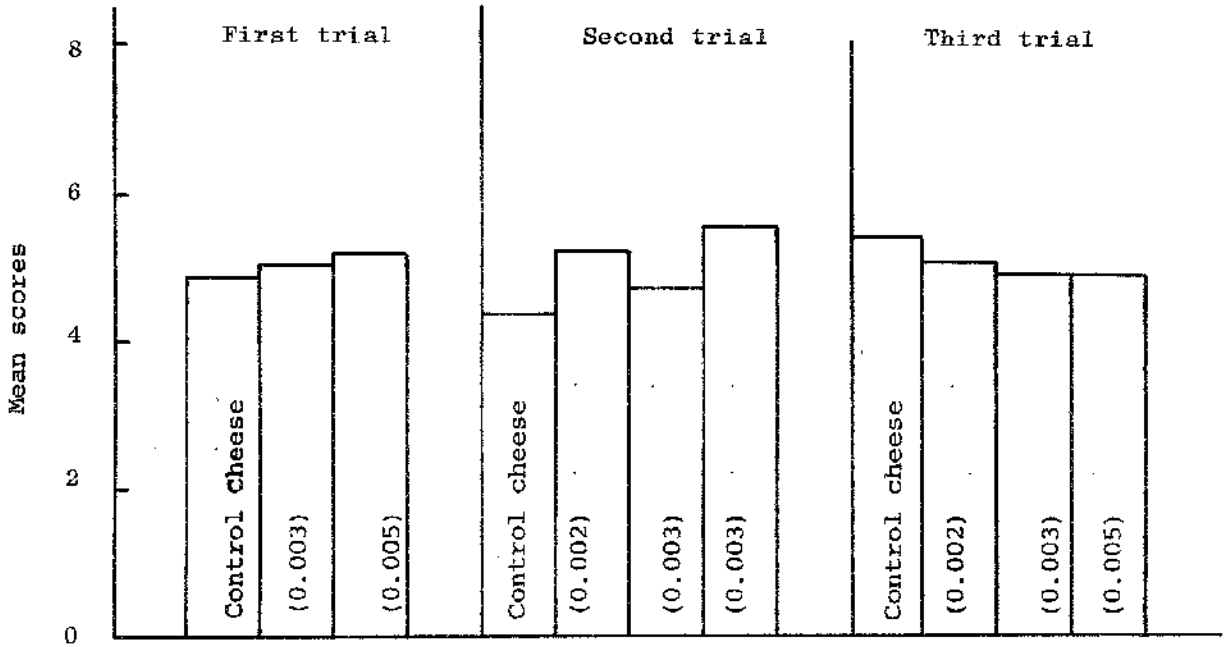
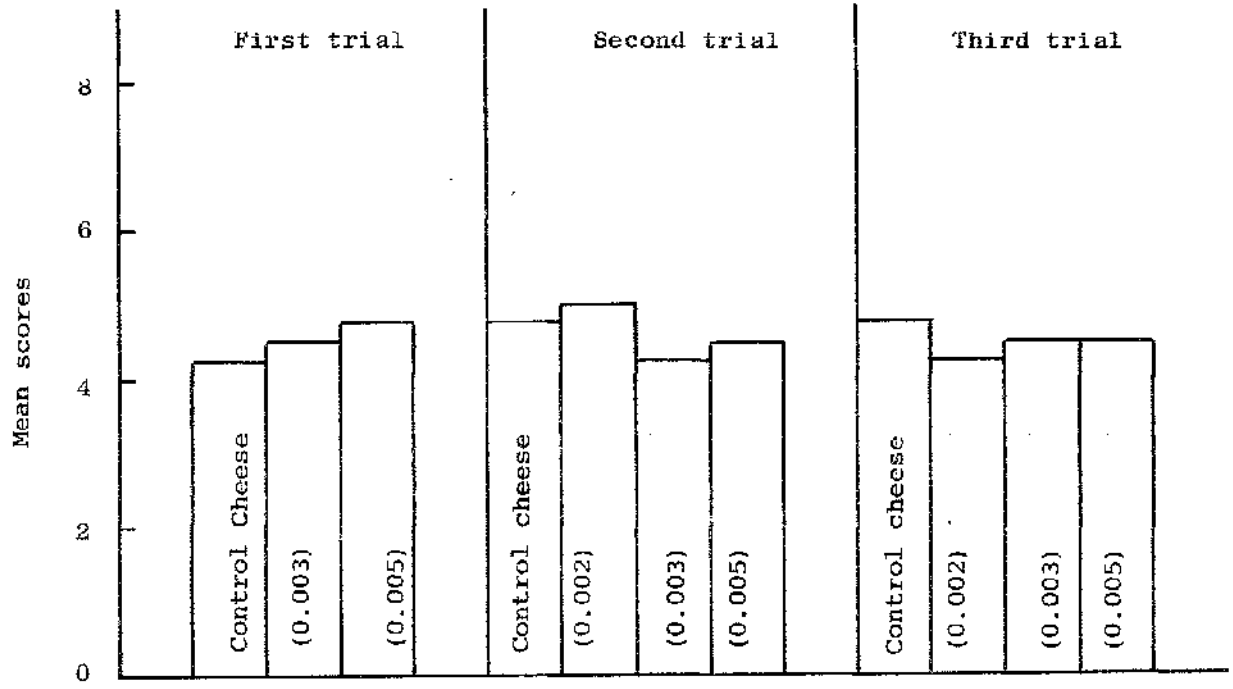


Figure 4.20: Means of flavour quality scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 1 month of ripening



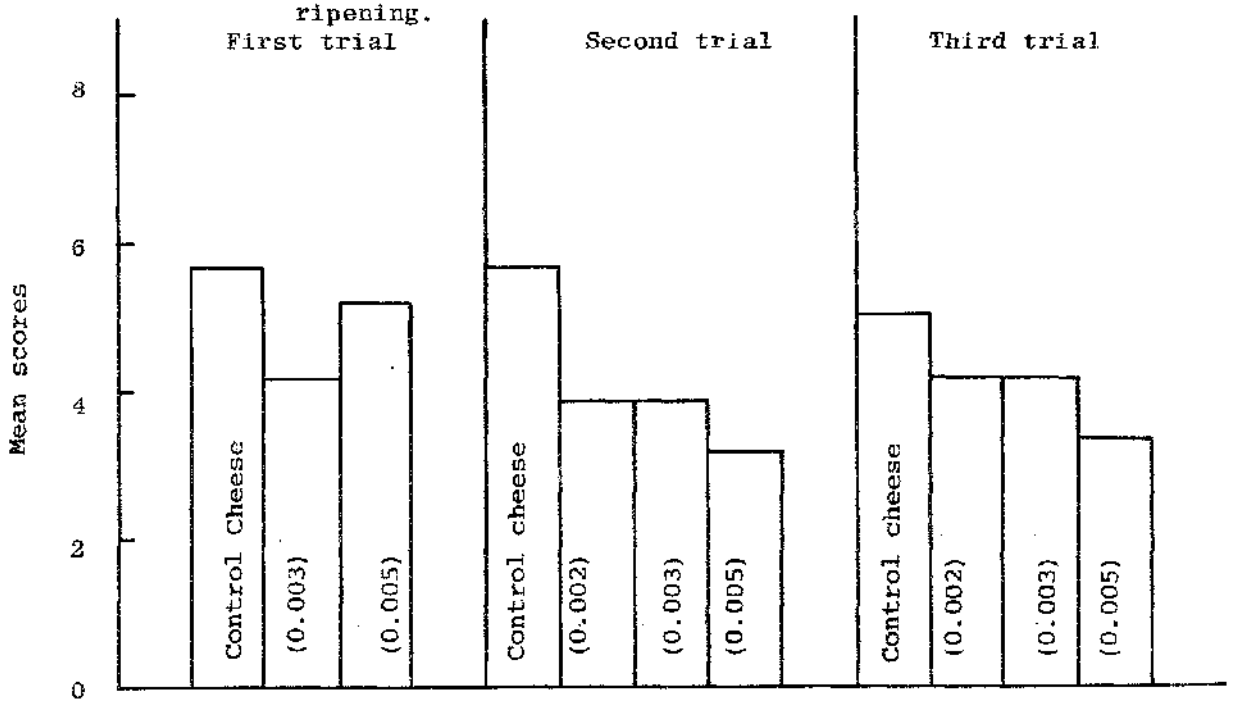
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.21: Means of flavour quality scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 2 months of ripening.



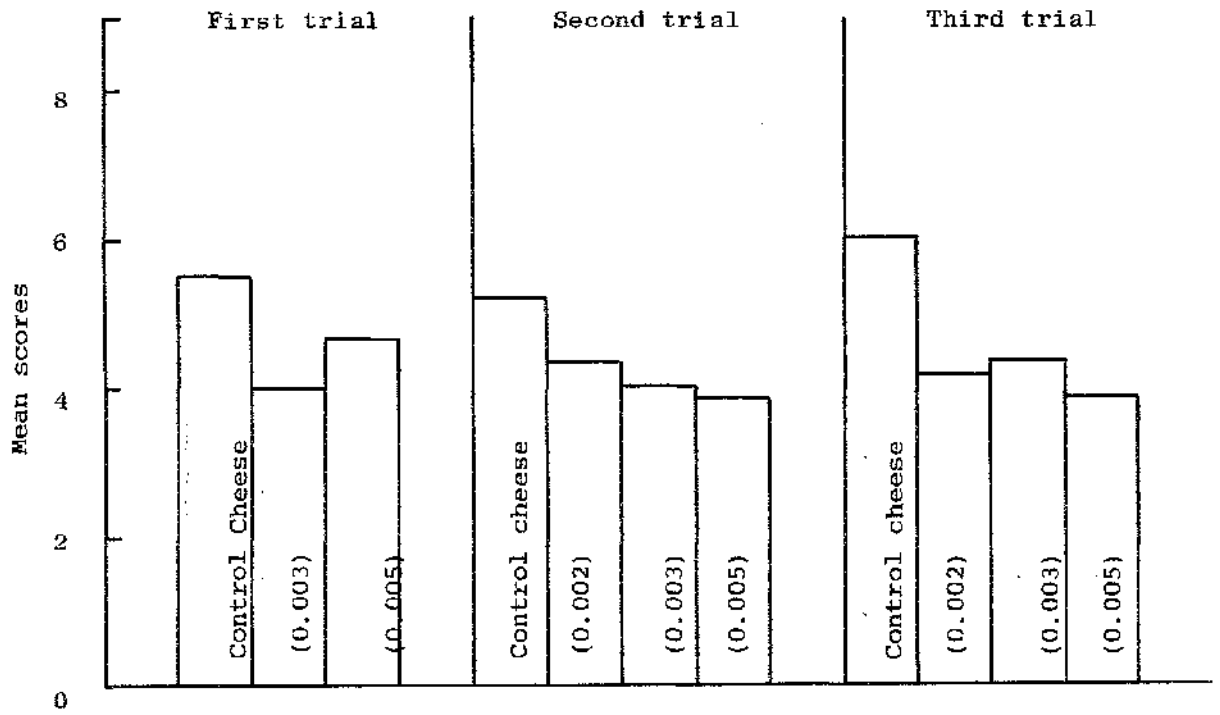
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.22: Means of flavour quality scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 3 months of ripening.



Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.23: Means of flavour quality scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 4 months of ripening.



Figures in parentheses represent amount (% w/w) of Neutrase added

(D) The means of the elasticity scores awarded by the graders' is illustrated in Figures 4.24 to 4.27, and it may be observed that the Neutrase-treated cheese is inelastic compared with the control during the ripening period. The young cheese was categorised as inelastic (see Figures 4.24 to 4.27), possibly due to the casein matrix, however, as the cheese became older, it became more elastic.

(E) The means scores of the openness awarded by the graders' is illustrated in Figures 4.28 to 4.31, and the following aspects could be observed:-

- close texture characteristic was only observed in the control cheese;
- open texture characteristic was predominant in the Neutrase-treated cheese and became more pronounced as the cheese became older (i.e. after 3 and 4 months old);
- the degree of openness in the Neutrase-treated cheese was greater as the level of added enzyme was increased.

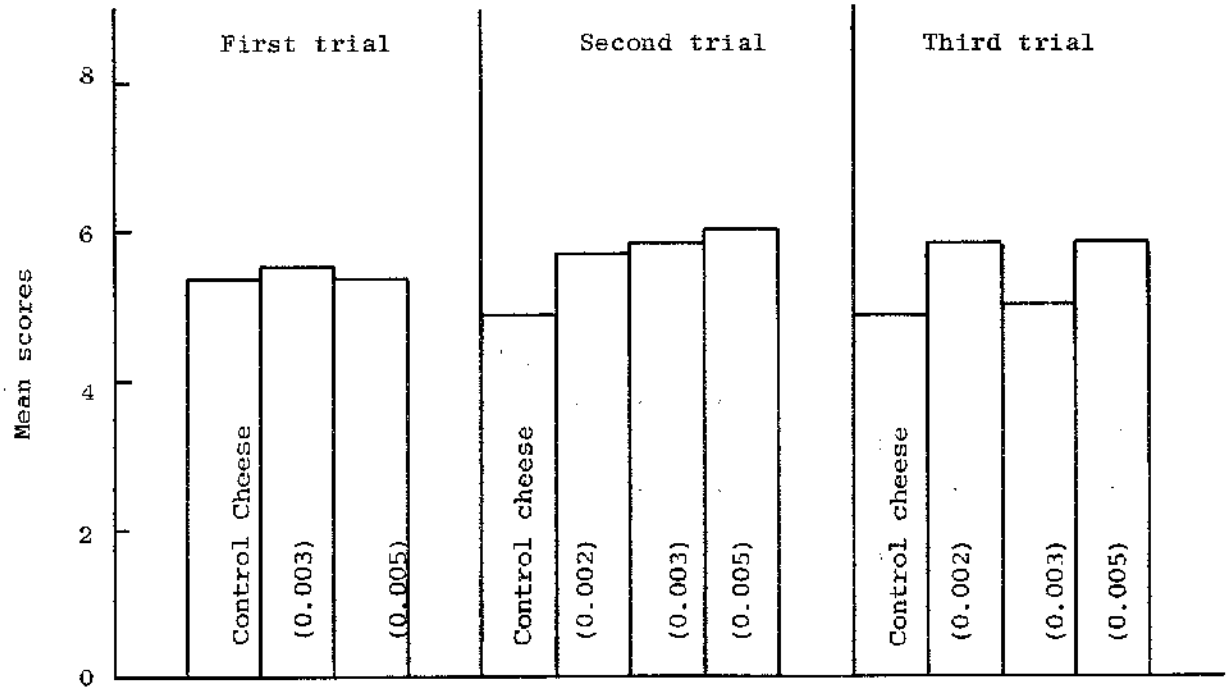
(F) The graders' results for the firmness of the cheese (control and experimental) are illustrated in Figures 4.32 to 4.35, and from the means scores of the firmness awarded by the graders' the following observations may be made:-

- the Neutrase-treated cheese was softer compared with the control cheese;
- the cheeses (control and experimental) became softer as the cheese became older;
- the Neutrase-treated cheese became softer with increasing amounts of added enzyme.

(G) The control cheese was more acceptable than the Neutrase-treated cheese, and the scores awarded by the graders' are shown in Figures 4.36 to 4.39. It can be observed, however, that despite the faults mentioned above, the Neutrase-treated cheese was still acceptable.

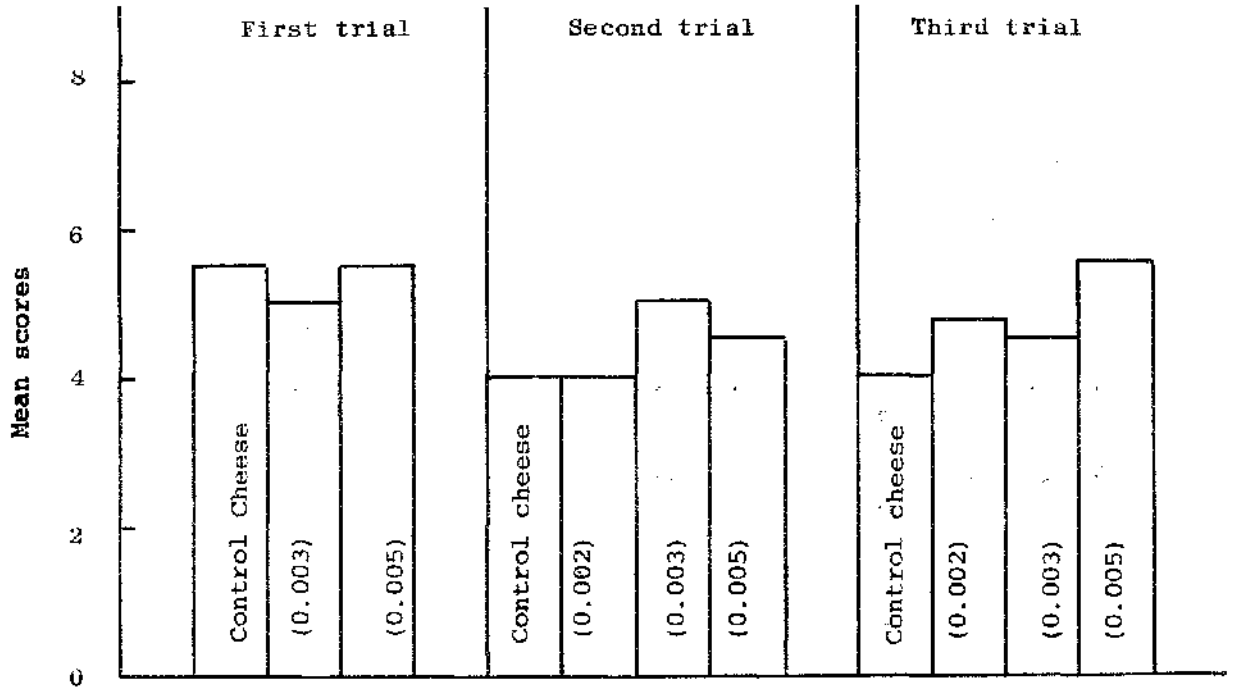
(H) Discolouration and mottledness of the experimental cheeses was evident (see Plate 4.18), and the graders' comments regarding the colour of the cheese is shown in Figure 4.40, however, such defect could be a contributory factor of down grading the cheese.

Figure 4.24: Means of elasticity scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 1 month of ripening



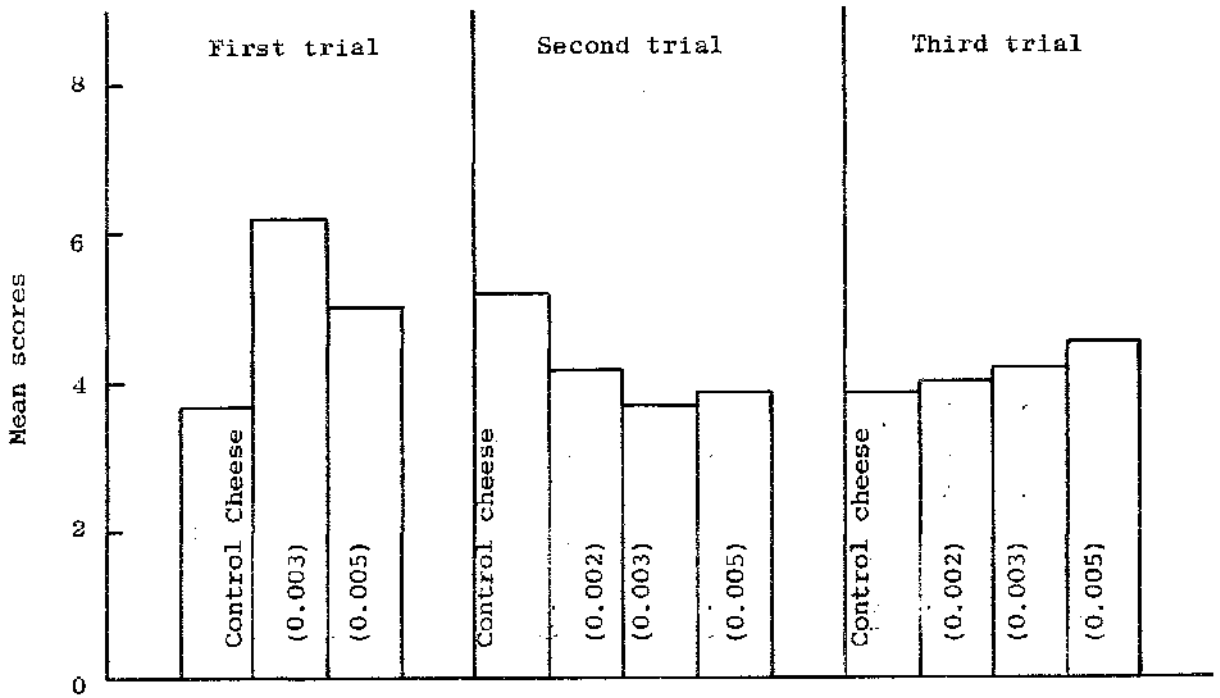
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.25 Means of elasticity scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 2 months of ripening



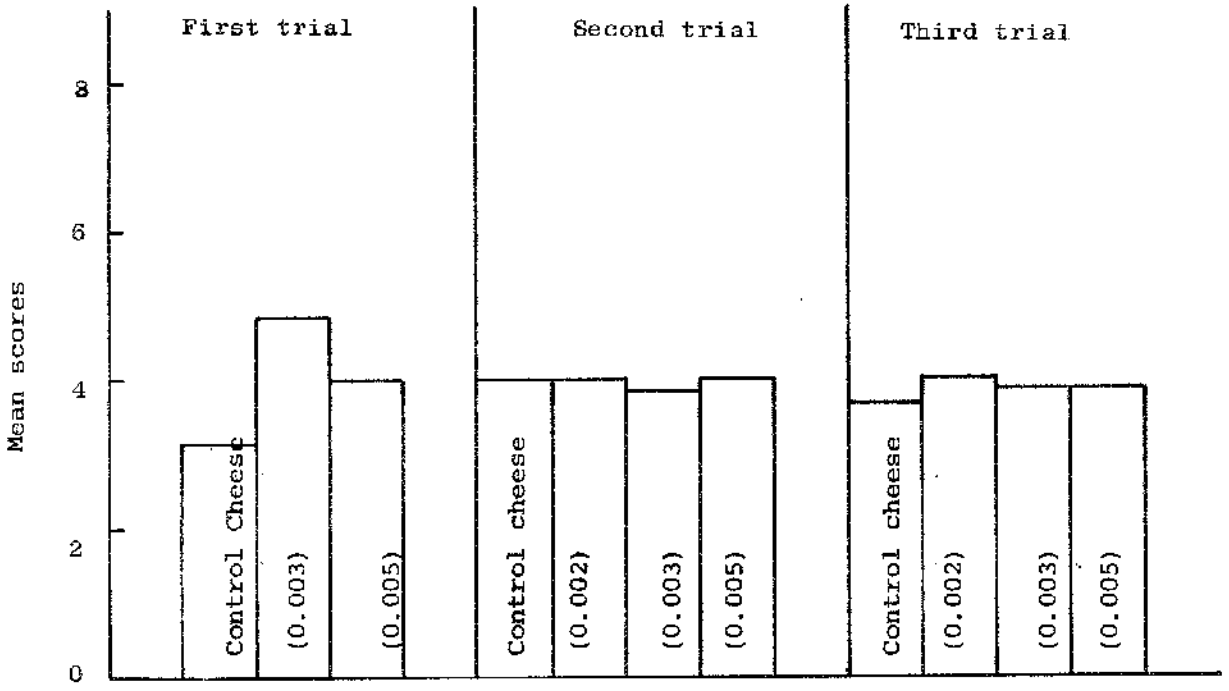
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.25: Means of elasticity scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 3 months of ripening



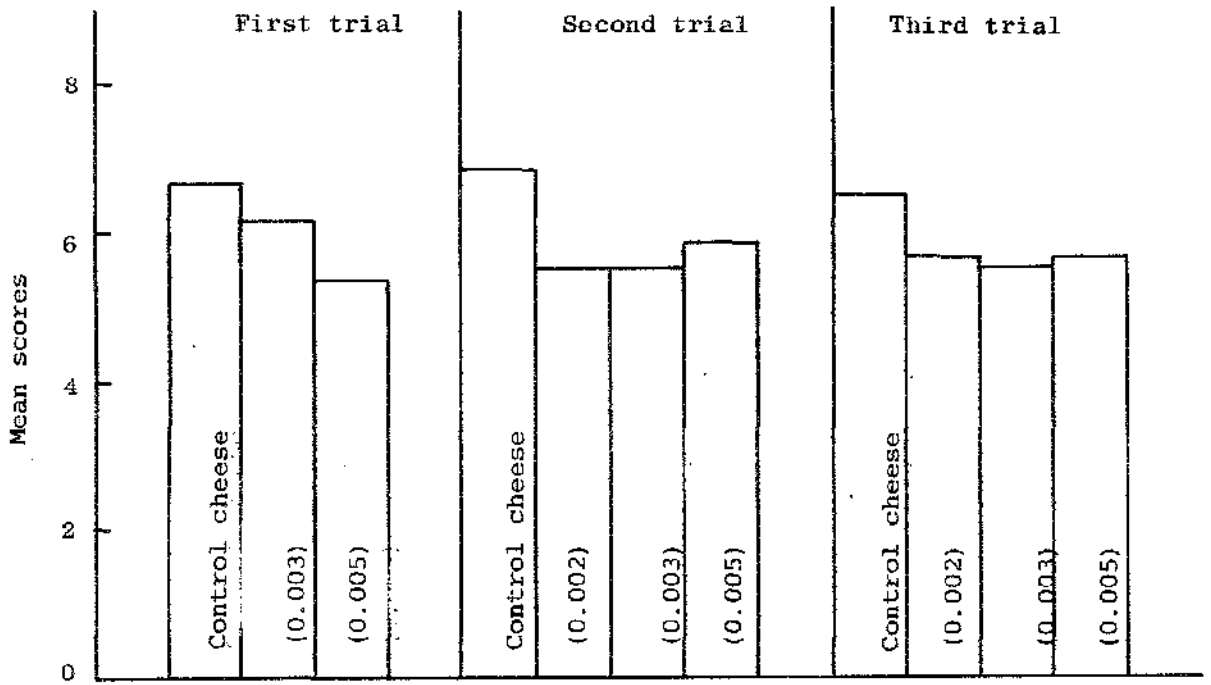
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.27: Means of elasticity scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 4 months of ripening



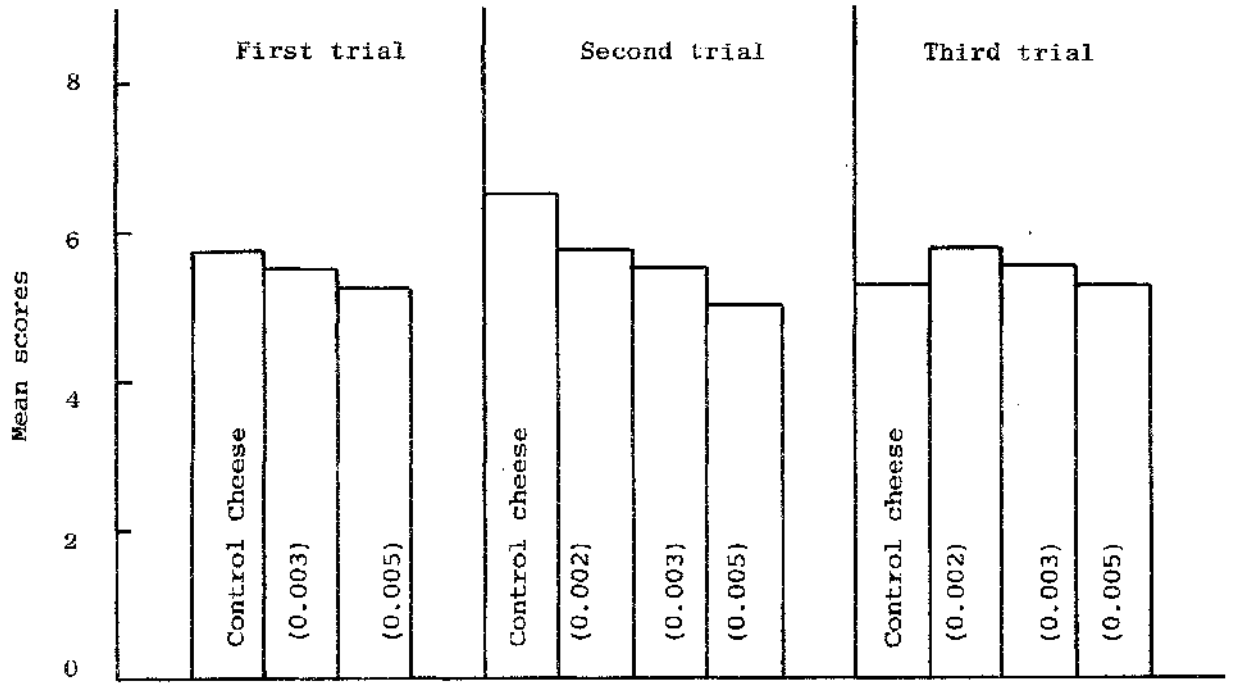
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.28: Means of openness scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 1 month of ripening.



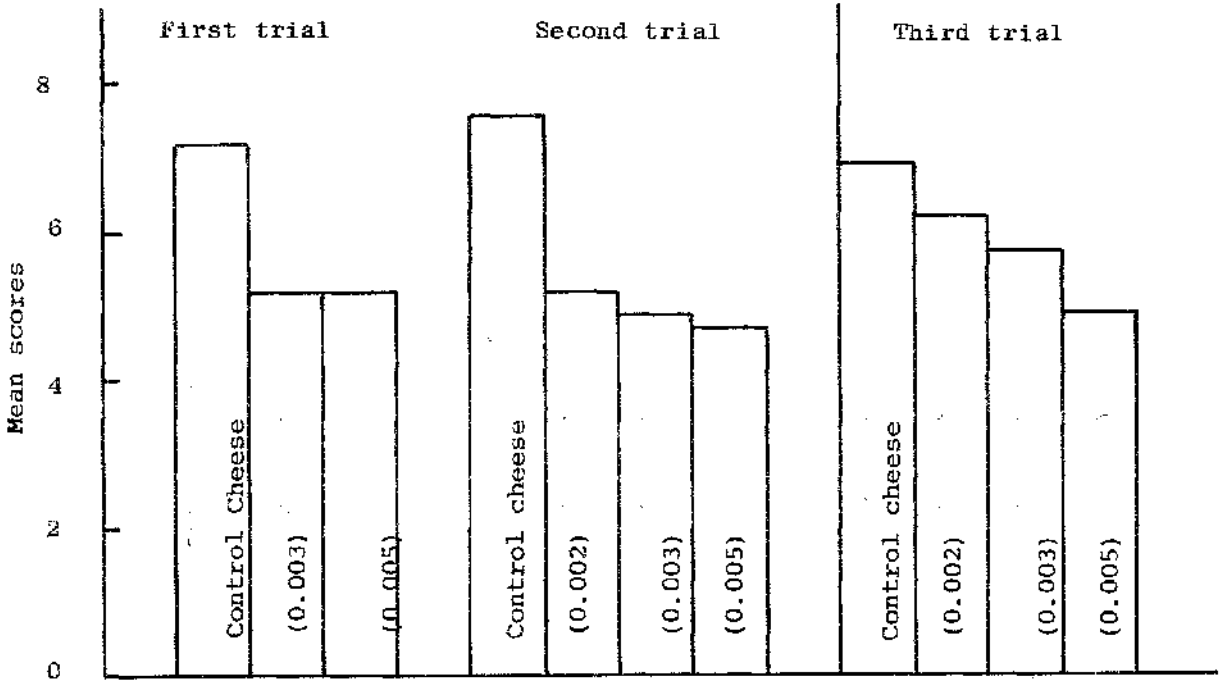
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.29: Means of openness scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 2 months of ripening.



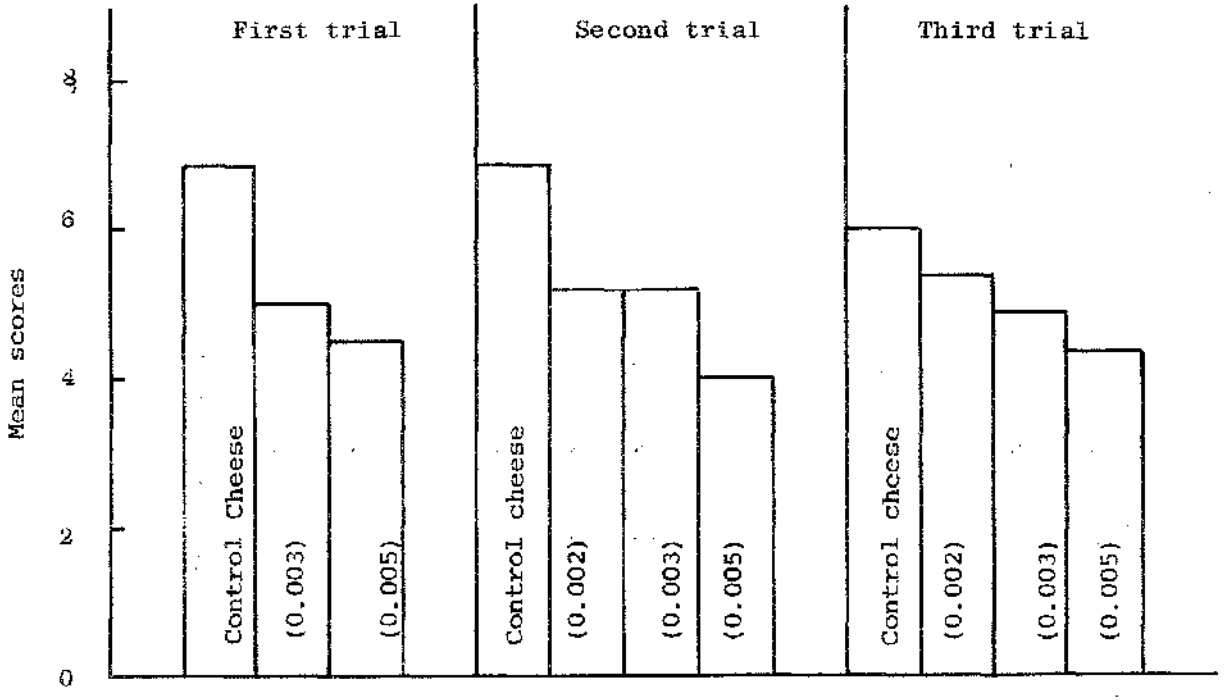
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.30: Means of openness scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 3 months of ripening



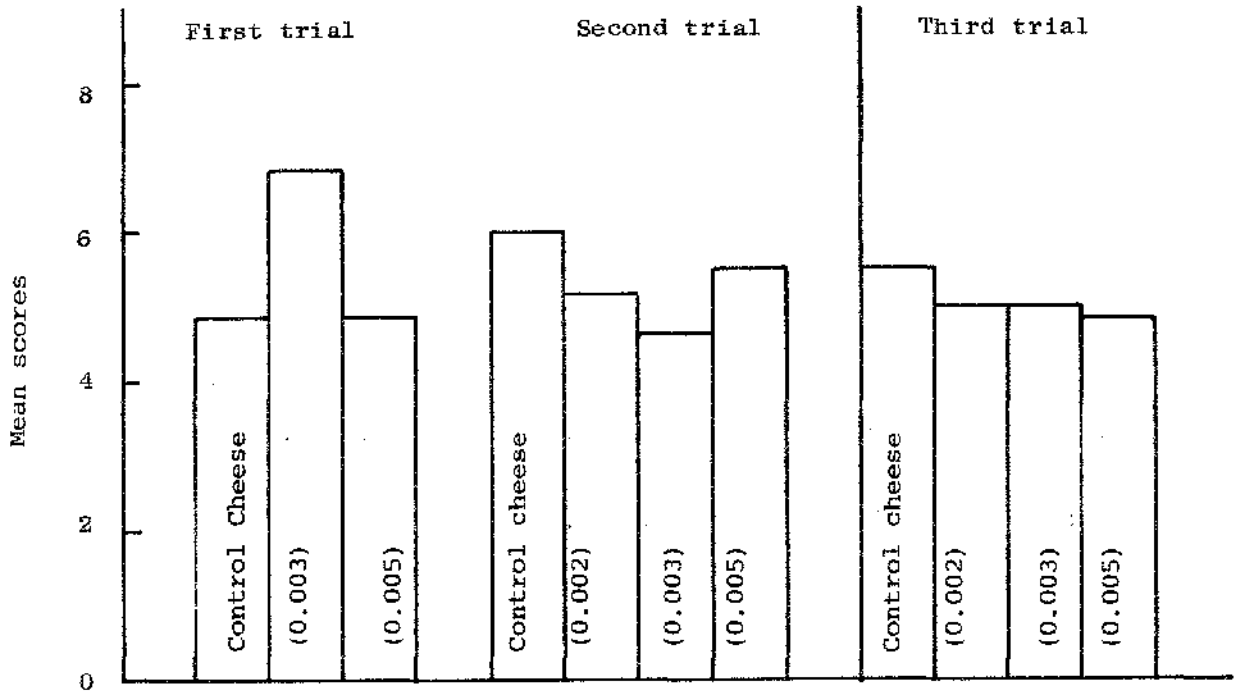
Figures in parentheses represent amounts (% w/w) of Neutrase added

Figure 4.31: Means of openness scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 4 months of ripening



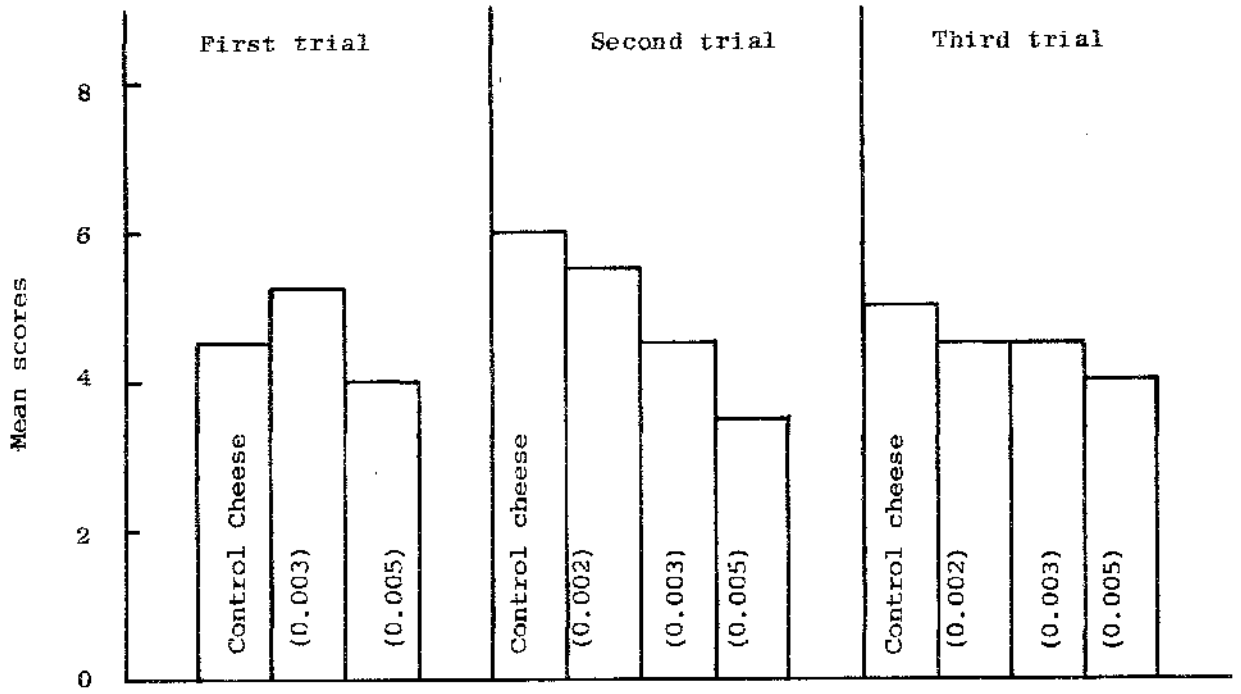
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.32: Means of firmness scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 1 month of ripening



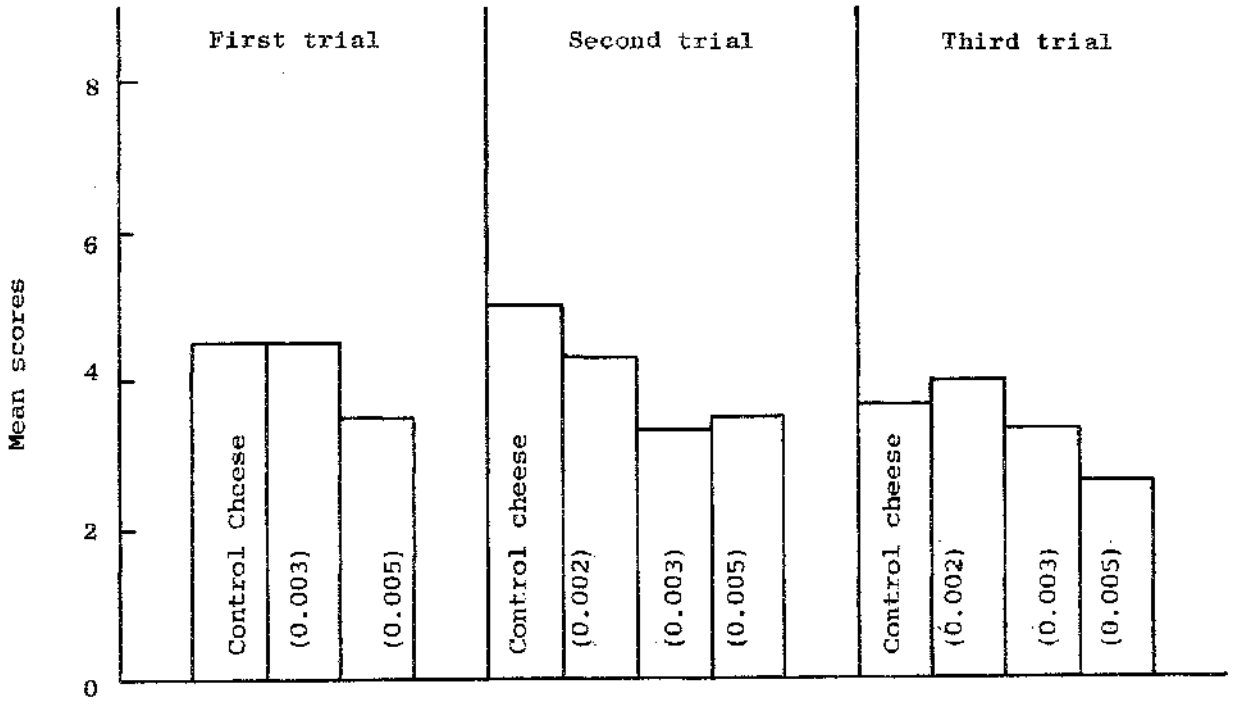
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.33: Means of firmness scores awarded by the three members of a grading panel for Cheddar cheese (made form curd with and without added Neutrase) after 2 months of ripening



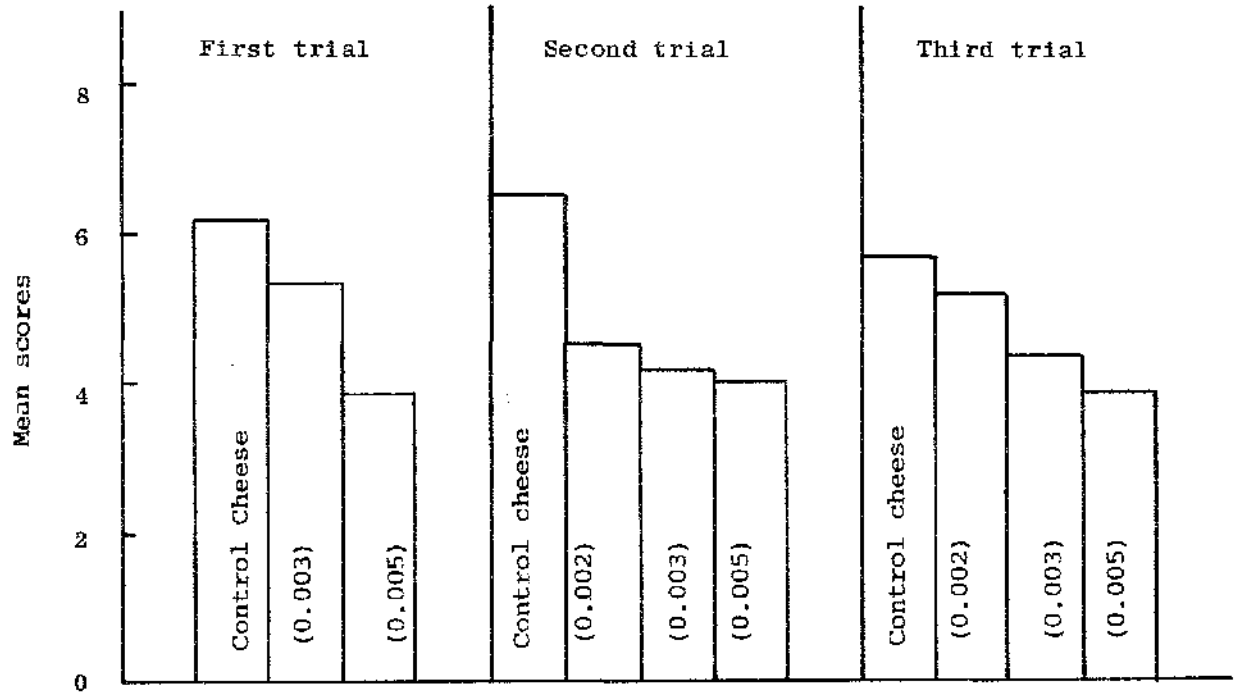
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.34: Means of firmness scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 4 months of ripening



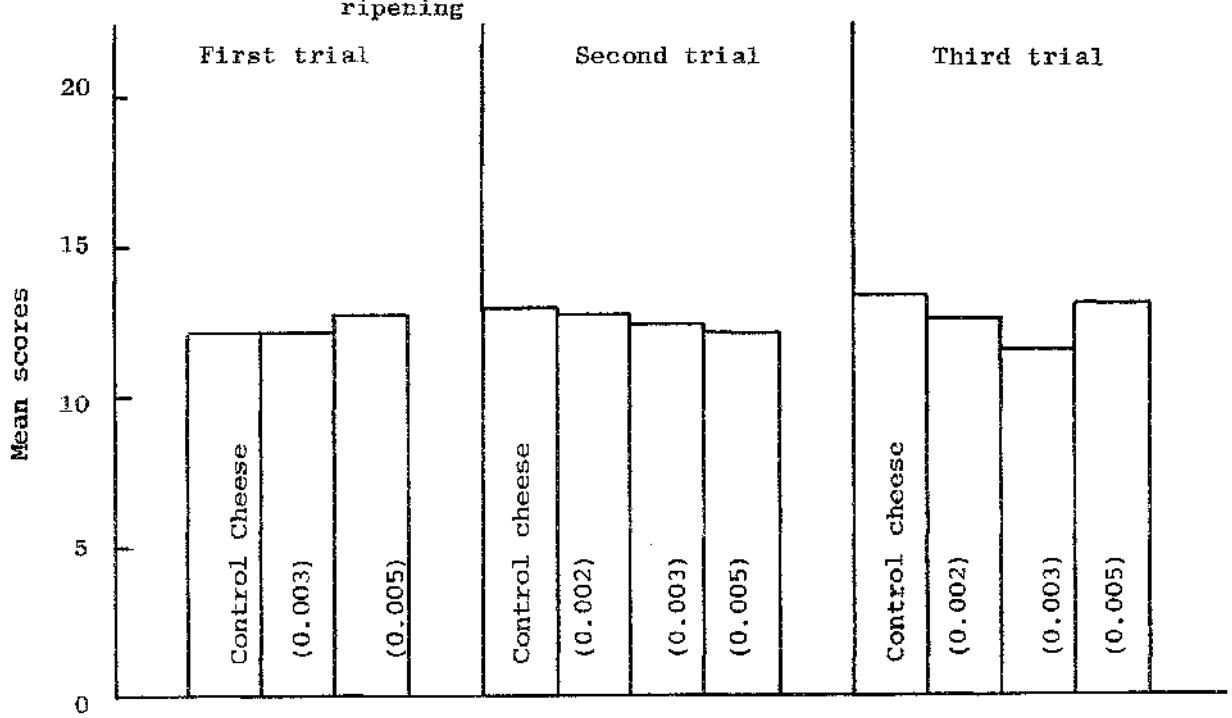
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.35: Means of firmness scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 3 months of ripening



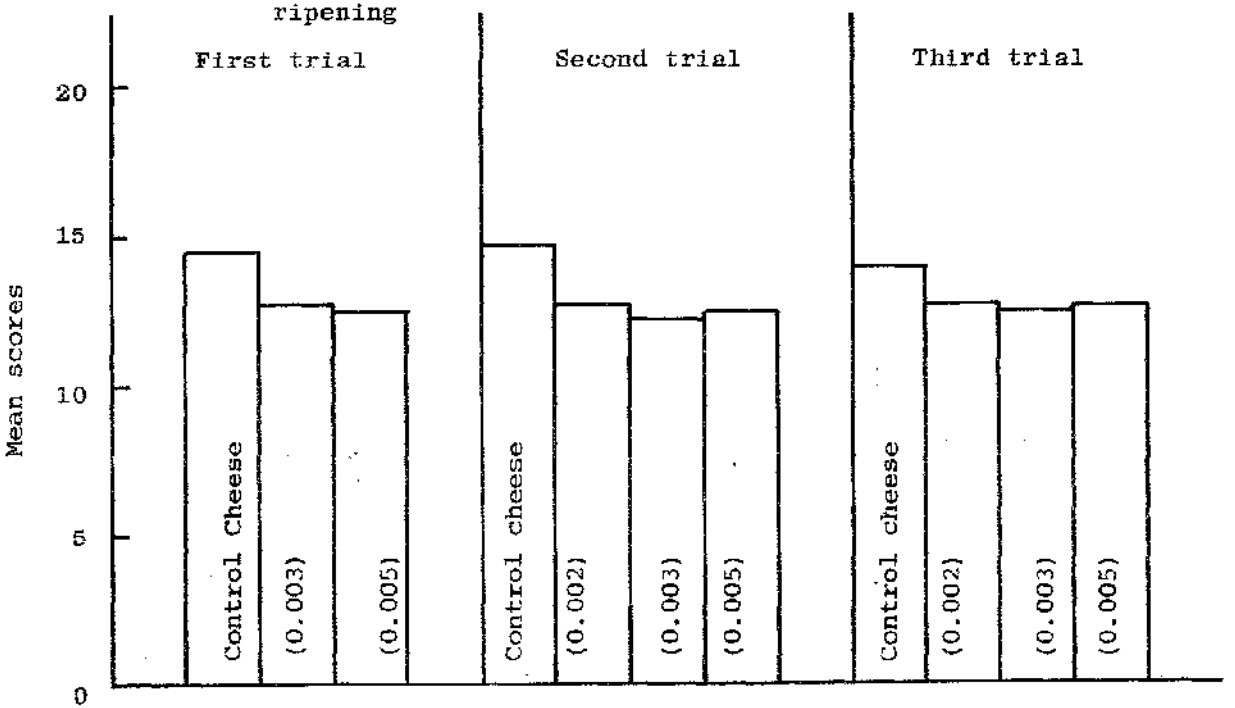
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.36: Means of general acceptability scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 1 month of ripening



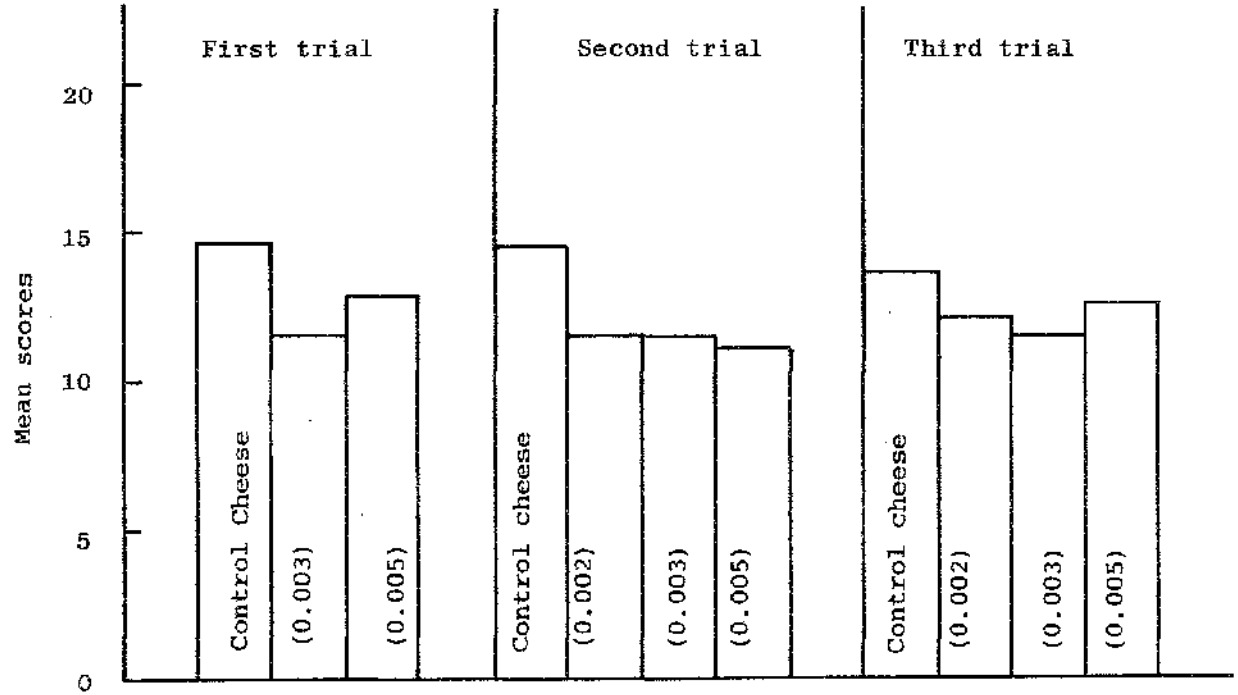
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.37: Means of general acceptability scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 2 months of ripening



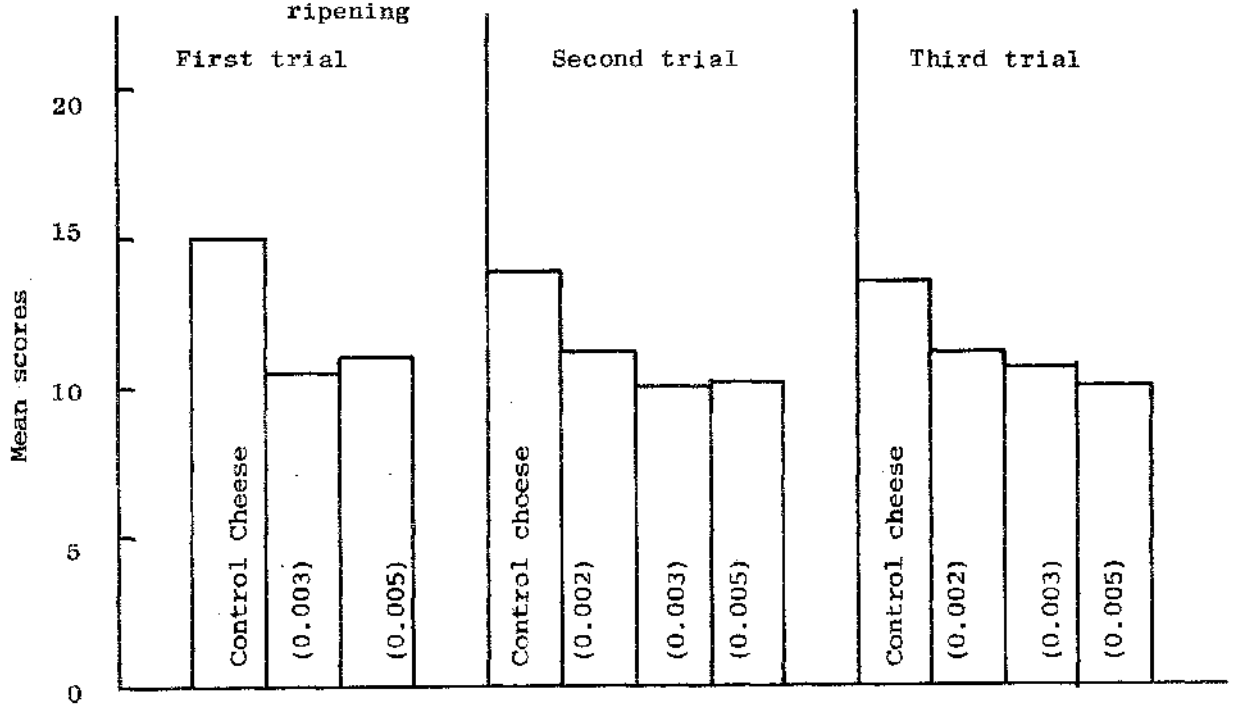
Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.38: Means of general acceptability scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 3 months of ripening



Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.39: Means of general acceptability scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) after 4 months of ripening



Figures in parentheses represent amount (% w/w) of Neutrase added

Figure 4.40: Means of mottling scores awarded by the three members of a grading panel for Cheddar cheese (made from curd with and without added Neutrase) during 4 months of ripening.

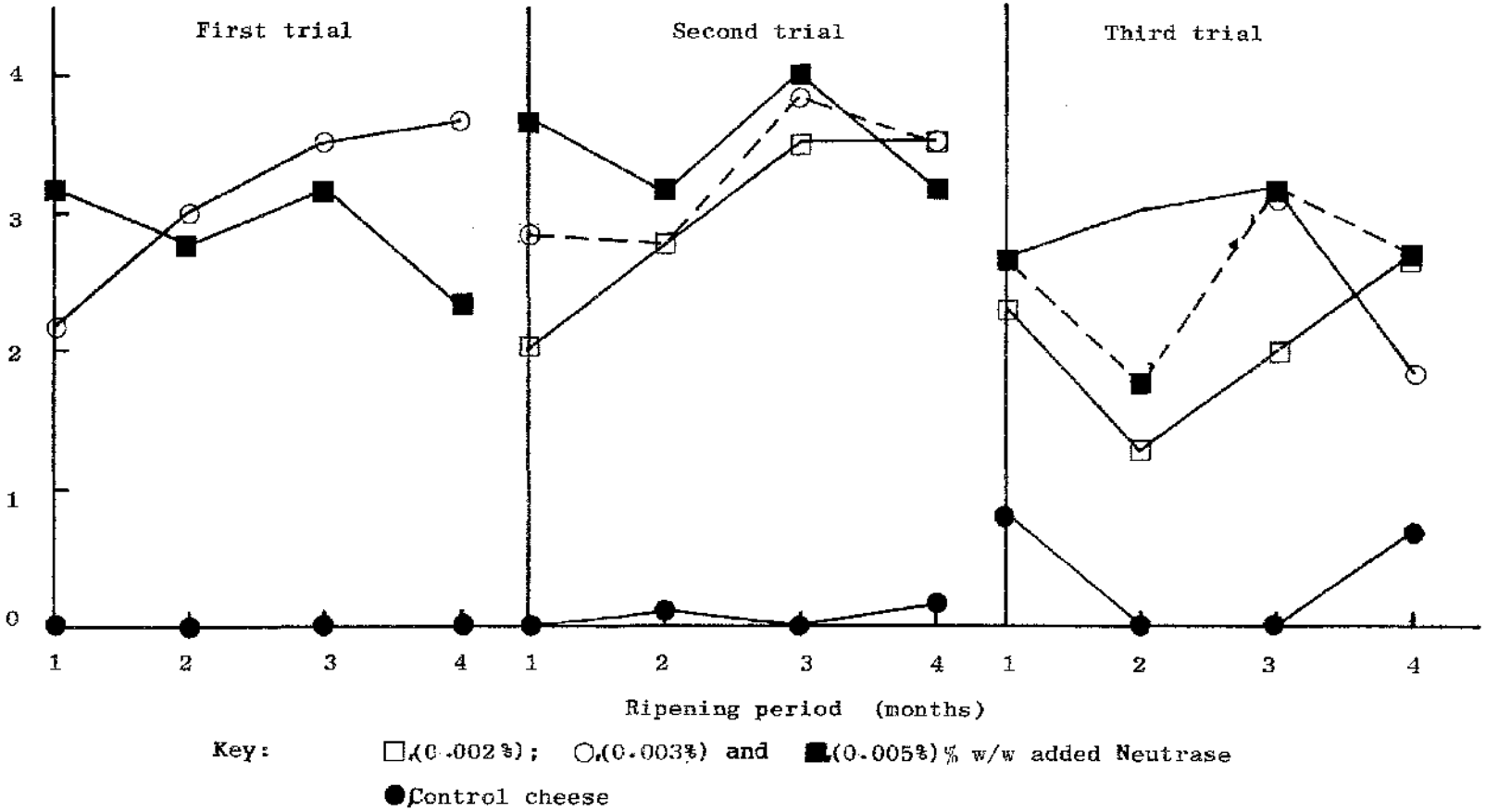


Plate 4.18 : The discolouration or mottling in Cheddar cheese made from curd with and without added Neutrase after 2 months of ripening.

Key:

0873	Control cheese
5058	Neutrase treated cheese (0.003% w/w)
3576	Neutrase treated cheese (0.005% w/w)
7335	Neutrase treated cheese (0.002% w/w)

4.9 The rheological properties of the cheese

The measurement of brittleness and hardness characteristics of the cheese (control and experimental) using the Instron Food Testing Instrument during the ripening showed similar trends in crumblyness and firmness as assessed by the panel in Neutrase treated cheese (see Table 4.24 and Figures 4.41 to 4.48). As the level of added enzyme increased the cheese became softer in body and more brittle. Such results could be attributed to the high proteolysis taking place in the cheese (see Figure 4.12), and this agreed with the early report of Law & Wigmore (1982). Neutrase-treated cheese became softer in body and more brittle as the cheese became older, and this also could be due to the increased proteolysis during the ripening. The differences between the cheeses treated with the same level of enzyme could be attributed to firstly, the uneven distribution of the enzyme which agreed with the result of Law & Wigmore (1982), and secondly, the consistency of the cheese as this could be affected by the salt diffusion, moisture content and chemical and biological reaction in the cheese (Al-Obaidi, 1980).

4.10 Conclusion

The biochemical changes which occurred in the experimental cheeses in this trial were similar to those for the preliminary trial reported in Section I. The addition of Neutrase enzyme to the cheese curd increased the proteolytic activity in the cheese and resulted in the liberation of more soluble nitrogen and increased casein hydrolysis compared to the control. These effects increased the flavour intensity of the cheese. However, the enzyme-treated cheese had the following defects:-

- bitter and unacceptable flavour(s);
- open and crumbly texture;
- brittle and softer body cheese;
- discoloured or mottled appearance.

The extent of these defects depended on the amount of enzyme added, and these faults are primarily attributed to the proteolytic activity of Neutrase. For example, the bitter taste is associated with the liberation of peptides, peptones, poly-peptides and free amino acids, and these nitrogenous compounds can also result in off-flavour development in the cheese. The defects of body and texture characteristics of the

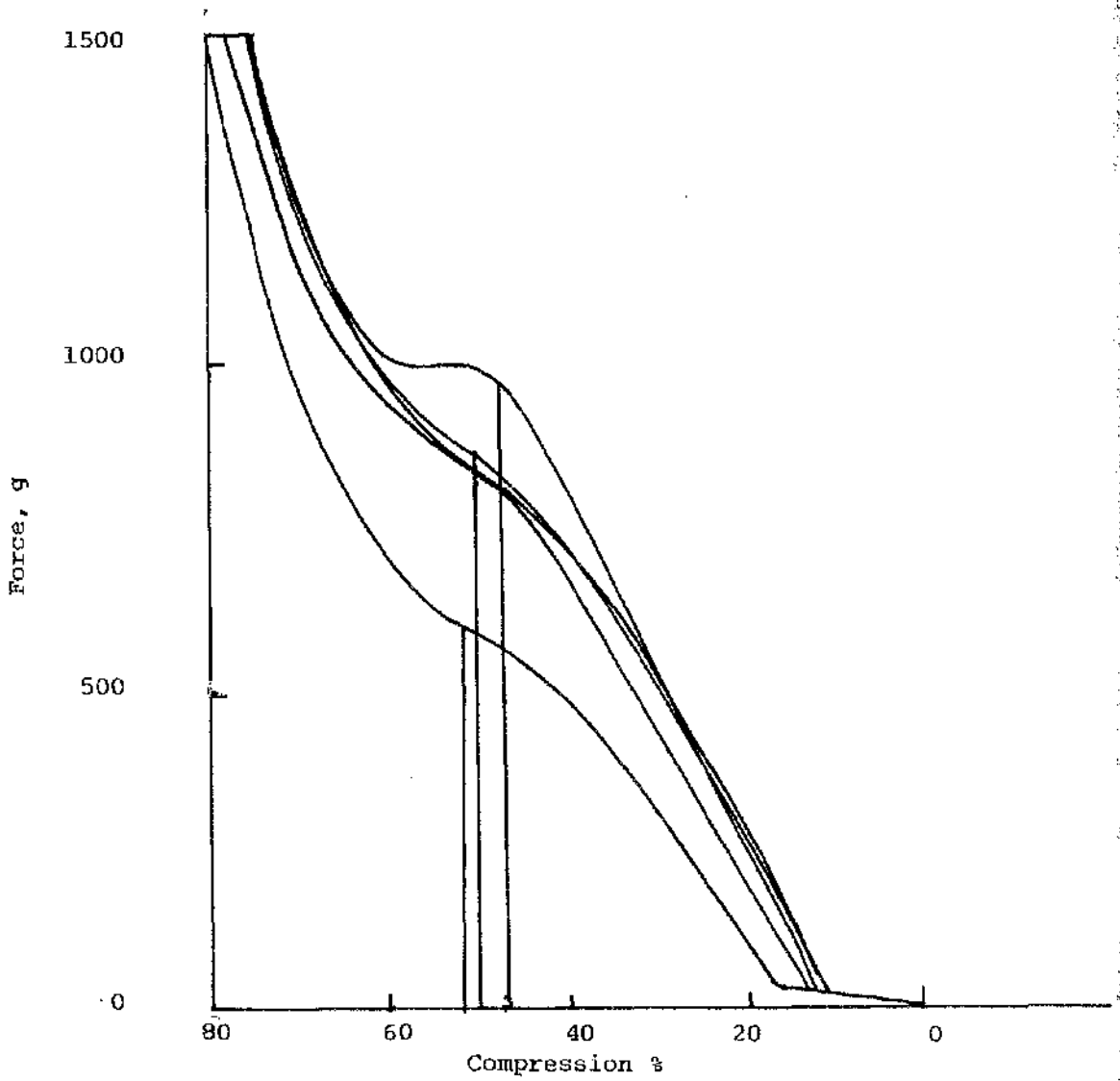
TABLE 2.24

Rheological properties of Neutrase-treated cheese during 4 months of ripening (average of 5 readings from each sample)

Ripening period (month)	Level of enzyme (% w/w)	Brittleness (as % of control)	Hardness (as % of control)
1	0.002	*	94
	0.002	*	110
	0.003	*	95
	0.003	*	159
	0.003	*	98
	0.005	*	81
	0.003	*	61
	0.005	*	62
2	0.002	46	79
	0.002	105	108
	0.003	117	115
	0.003	37	77
	0.003	86	106
	0.005	48	79
	0.005	47	93
	0.005	36	79
3	0.002	23	49
	0.002	95	142
	0.003	106	112
	0.003	30	78
	0.003	35	76
	0.005	26	75
	0.005	23	64
	0.005	25	86
4	0.002	66	85
	0.002	58	70
	0.003	167	132
	0.003	65	104
	0.003	60	67
	0.005	34	56
	0.005	31	82
	0.005	10	38

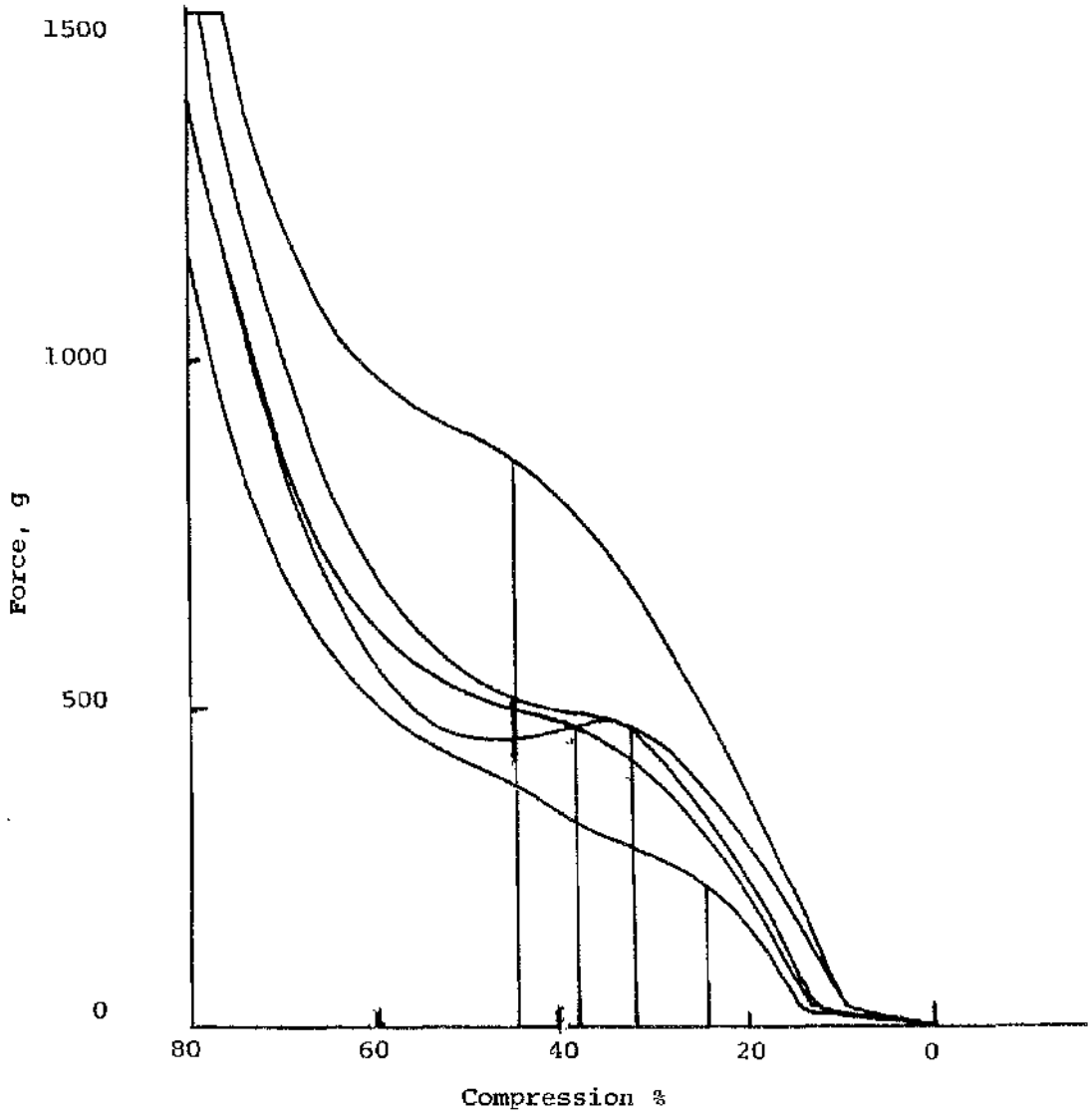
*Brittleness was not measured

Figure 4.41 Compression curves (up to 80%) from five individual readings of the same sample of a 2-month-old control Cheddar cheese



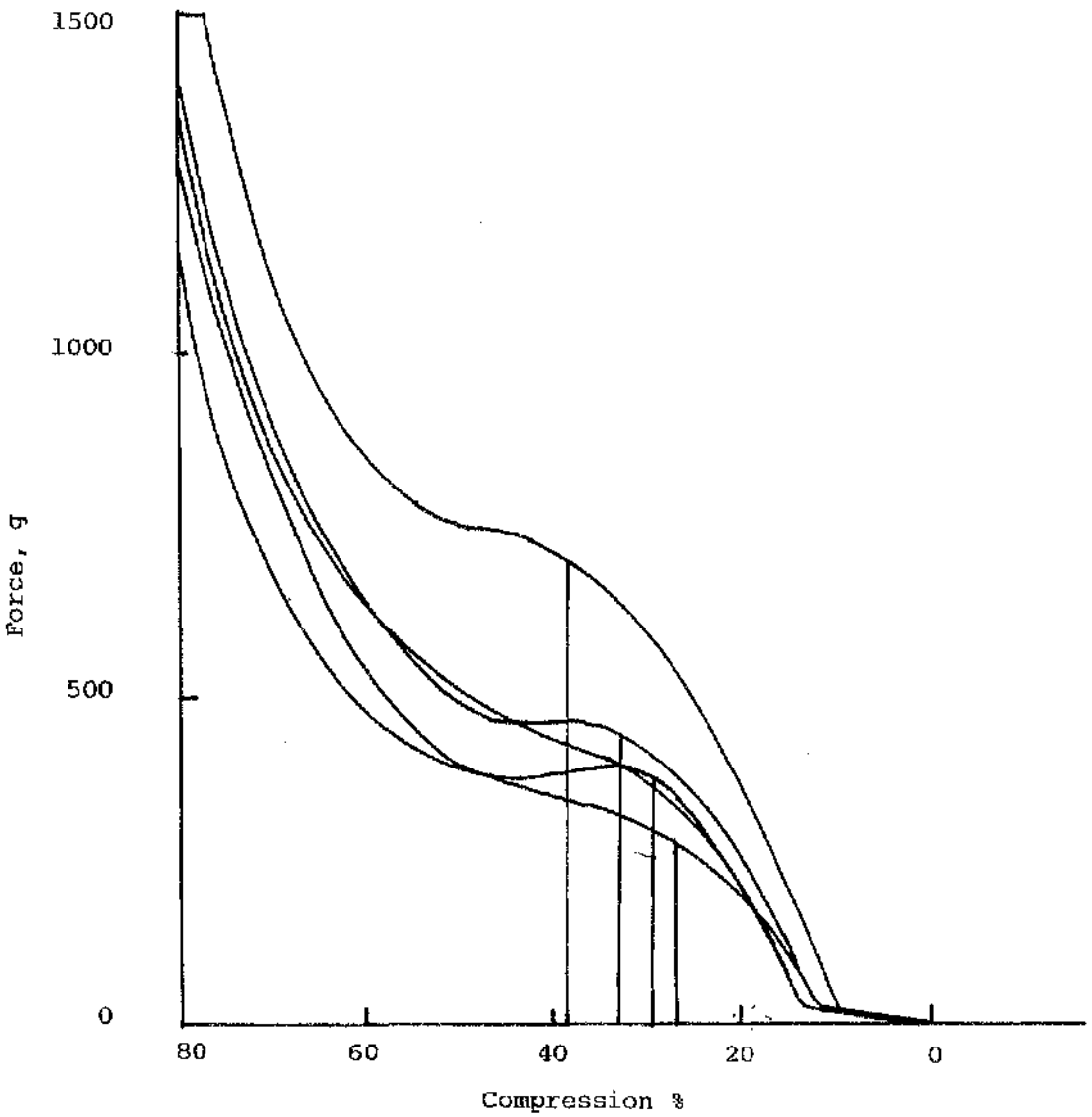
Vertical lines from the compression scale to the inflexion point on the curves mark the area to the right of the lines used to calculate the 'work' required to break the cheese samples (brittleness). The total area under the complete curves was taken as a measure of their hardness.

Figure 4.42 Compression curves (up to 80%) from five individual readings of the same sample of a 2-month-old Cheddar cheese prepared with 0.002% added Neutrase



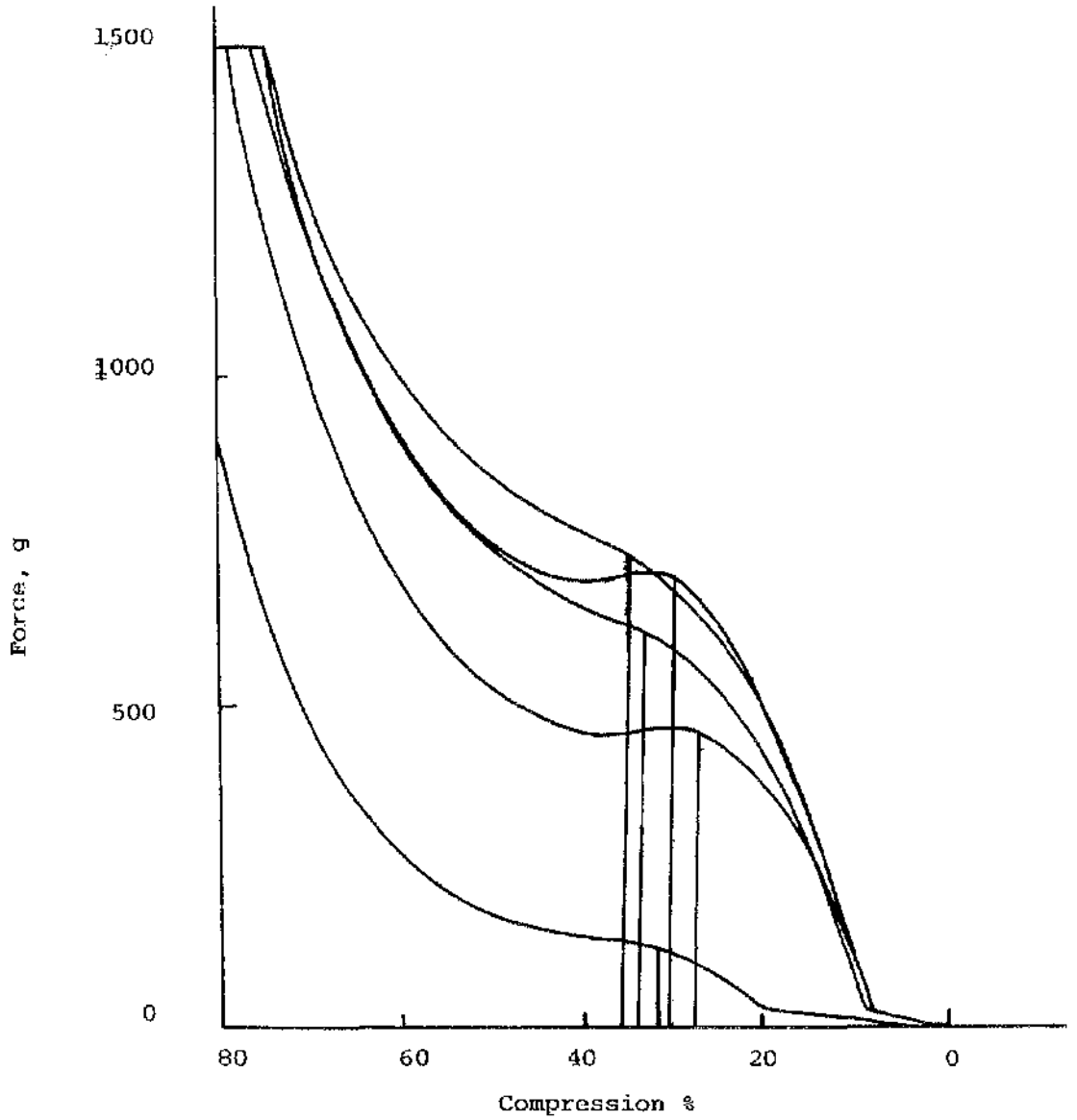
Vertical lines from the compression scale to the inflexion point on the curves mark the area to the right of the lines used to calculate the 'work' required to break the cheese samples (brittleness). The total area under the complete curves was taken as a measure of their hardness.

Figure 4.43 Compression curves (up to 80%) from five individual readings of the same sample of a 2 month-old Cheddar cheese prepared with 0.003% w/w added Neutrase



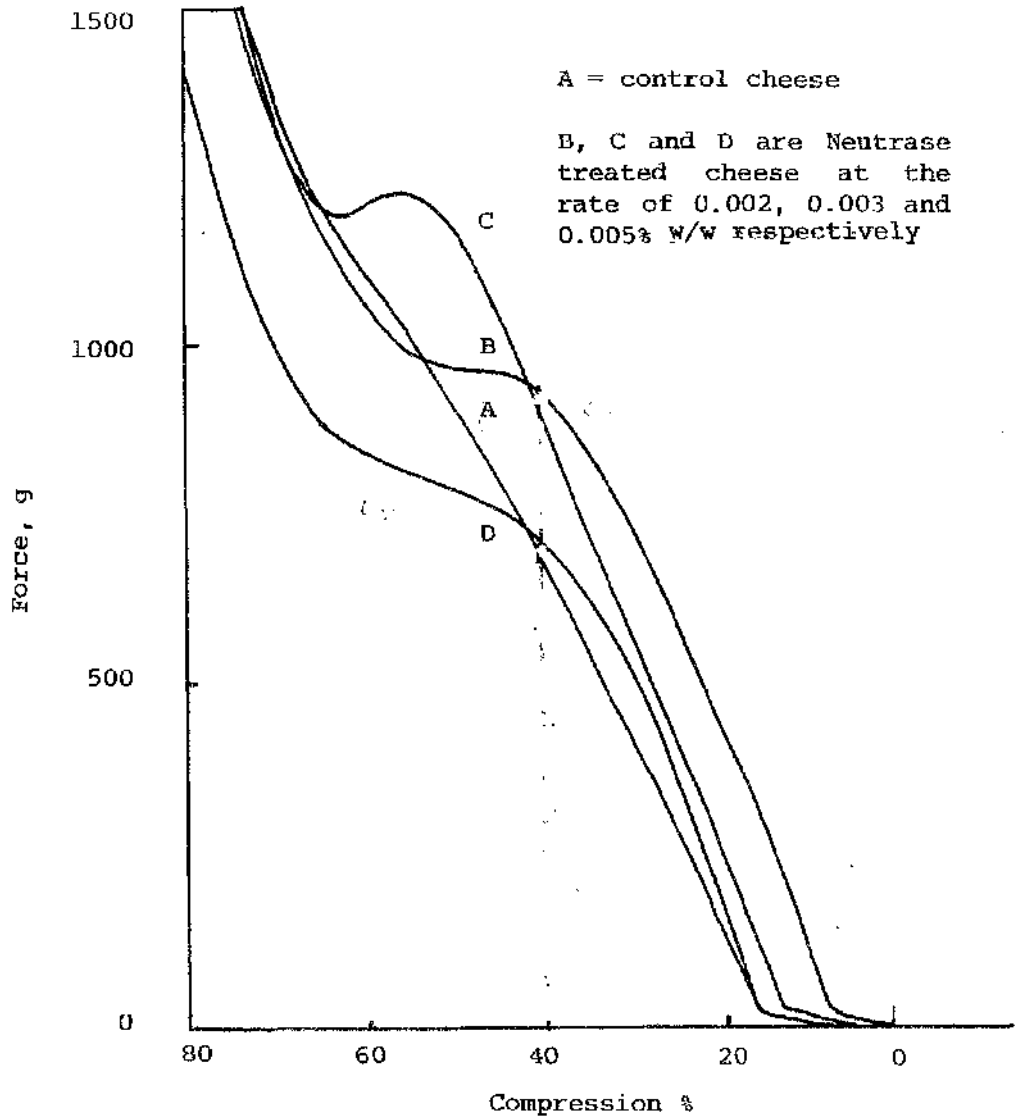
Vertical lines from the compression scale to the inflexion point on the curves mark the area to the right of the lines used to calculate the 'work' required to break the cheese samples (brittleness). The total area under the complete curves was taken as a measure of their hardness.

Figure 4.44 Compression curves (up to 80%) from five individual readings of the same sample of a 2 month-old Cheddar cheese prepared with 0.005% w/w added Neutrase



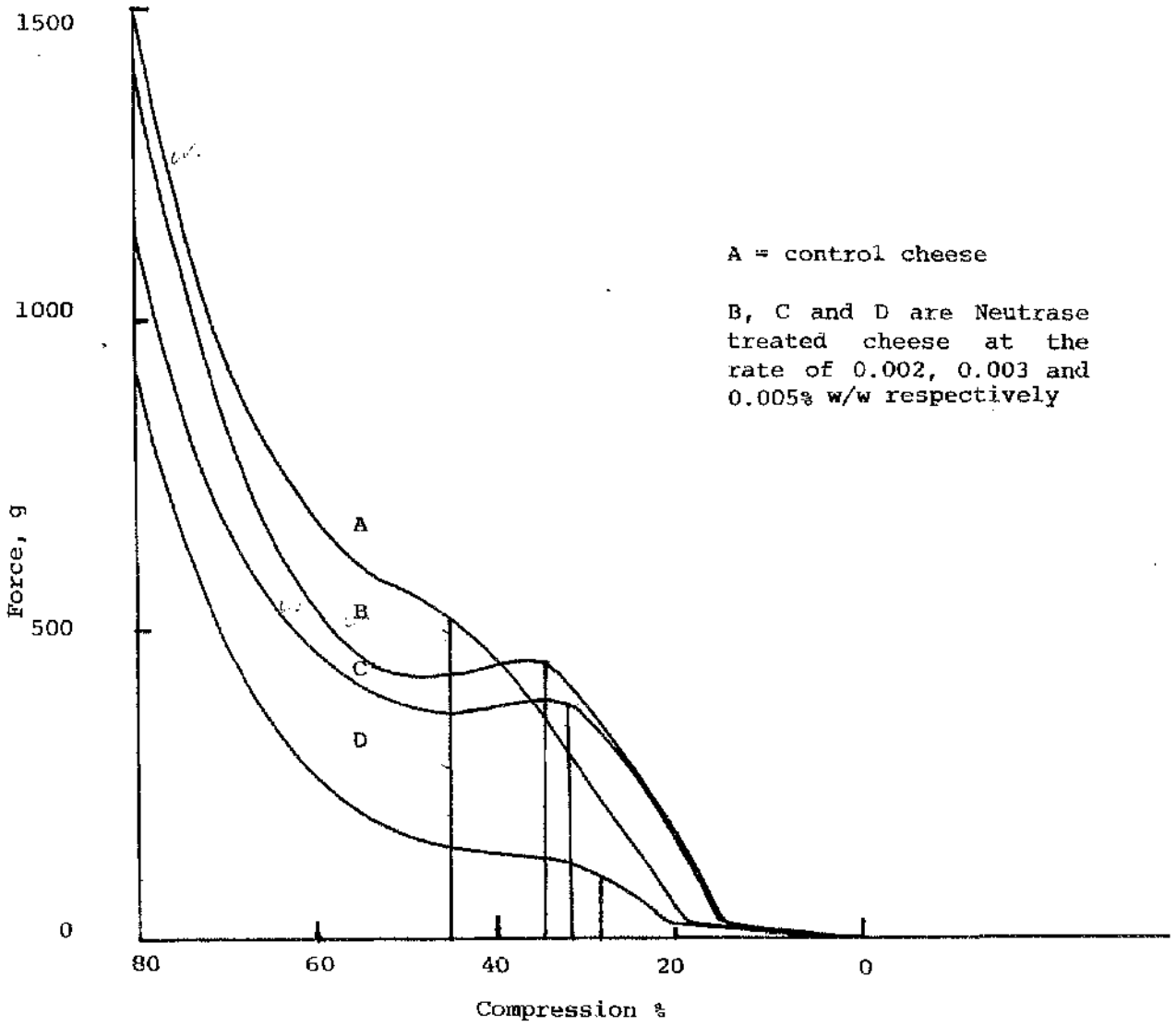
Vertical lines from the compression scale to the inflexion point on the curves mark the area to the right of the lines used to calculate the 'work' required to break the cheese samples (brittleness). The total area under the complete curves was taken as a measure of their hardness.

Figure 4.45 Compression curves (up to 80%) from measurements of one-month-old Cheddar cheese made from curd with and without added Neutrase, obtained in an Instron Food Testing Instrument.



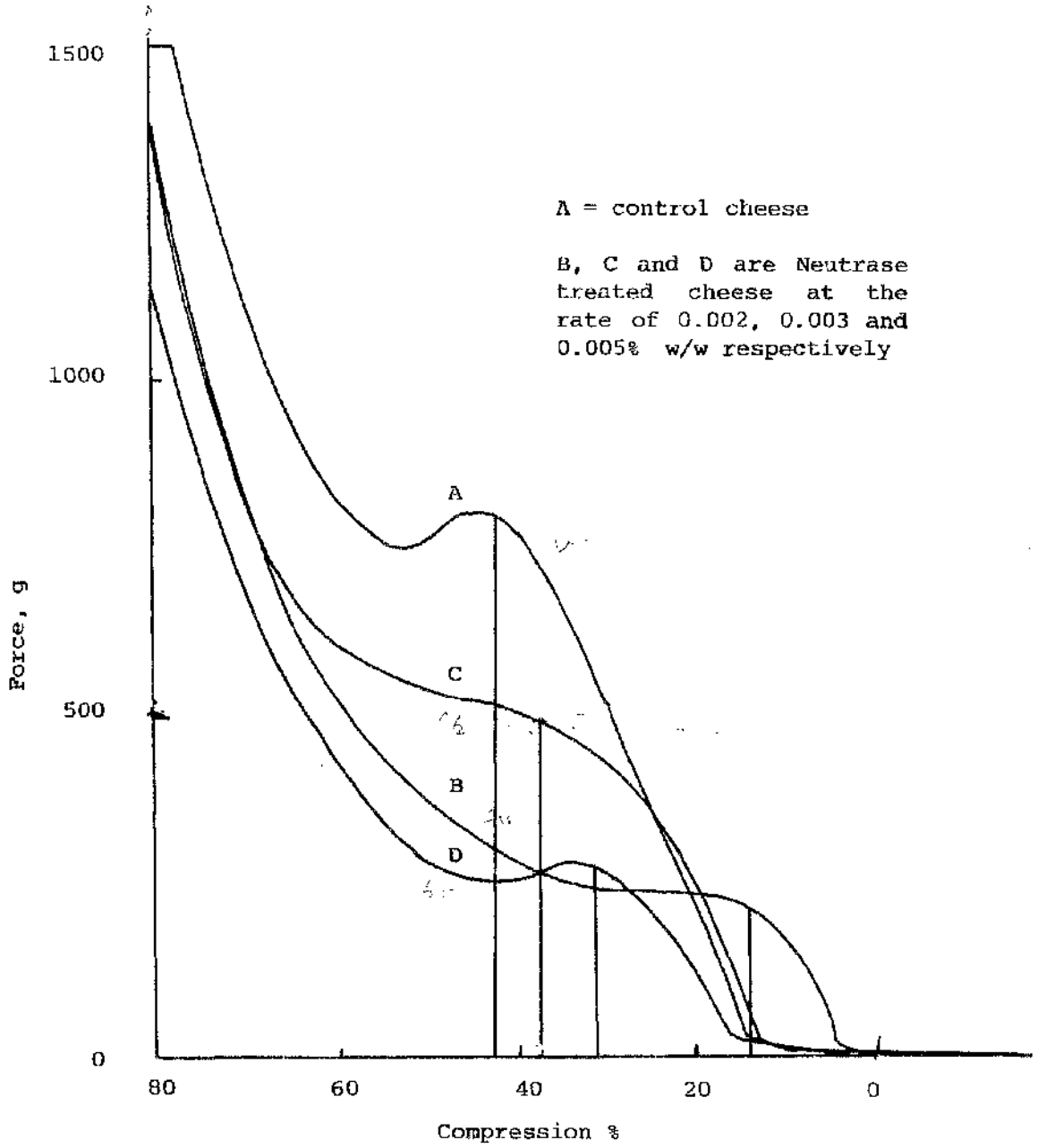
Vertical lines from the compression scale to the inflexion point on the curves mark the area to the right of the lines used to calculate the 'work' required to break the cheese samples (brittleness). The total area under the complete curves was taken as a measure of their hardness.

Figure 4.46 Compression curves (up to 80%) from measurements of 2-month-old Cheddar cheese made from curd with and without added Neutrase, obtained in an Instron Food Testing Instrument.



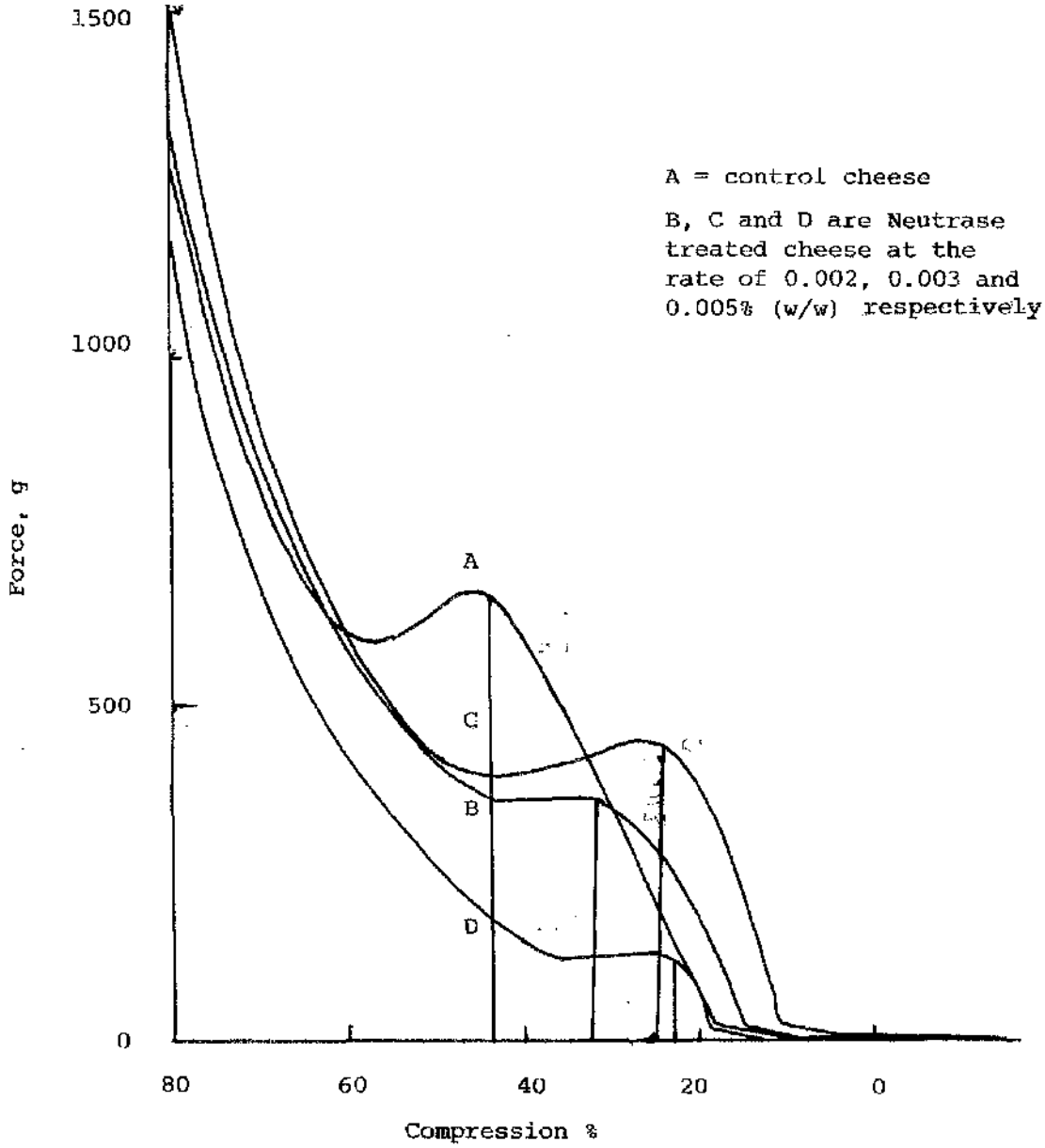
Vertical lines from the compression scale to the inflexion point on the curves mark the area to the right of the lines used to calculate the 'work' required to break the cheese samples (brittleness). The total area under the complete curves was taken as a measure of their hardness.

Figure 4.47 Compression curves (up to 80%) from measurements of 3-month old Cheddar cheese made from curd with and without added Neutrase, obtained in an Instron Food Testing Instrument



Vertical lines from the compression scale to the inflexion point on the curves mark the area to the right of the lines used to calculate the 'work' required to break the cheese samples (brittleness). The total area under the complete curves was taken as a measure of their hardness.

Figure 4.48 Compression curves (up to 80%) from measurements of 4-month-old Cheddar cheese made from curd with and without added Neutrase, obtained in an Instron Food Testing Instrument



Vertical lines from the compression scale to the inflexion point on the curves mark the area to the right of the lines used to calculate the 'work' required to break the cheese samples (brittleness). The total area under the complete curves was taken as a measure of their hardness

Neutrase-treated cheese may also be attributed to proteolysis where the casein matrix is broken down.

In order to overcome the above-mentioned defects in the experimental cheeses, the enzymatic activity has to be stopped when the desired flavour intensity in the cheese has been achieved, i.e. after 3 months ripening or longer, depending on the amount of added Neutrase. One effective method of inactivating the enzyme is the application of heat and certain trials have been carried out to manufacture processed Cheddar cheese (see Chapter 5).

CHAPTER FIVE

Accelerated ripening of Cheddar cheese used for the preparation of processed cheese

5.1 Introduction

Processed cheese was first produced at the beginning of this century in the United States of America by the Kraft Company. The primary objective is to extend the shelf life of the product which could be achieved by applying heat to inactivate the microflora and the enzymes in the cheese. Various forms of processed cheese may be prepared and cheese of difference composition and qualities may be blended. According to Meyer (1973), the advantage of the processed cheese industry to the community could be summarised as follows:

(a) Economic, particularly political/economic advantages;

(b) Factory technical advantages;

(c) The varied and valuable properties of processed cheese products;

(d) The unlimited variation and combination possibilities in the creation of new processed cheese types.

The production of processed cheese in some western European countries is illustrated in Table 5.1, and it could be observed that the most important countries for theproduction of cheese are the Federal Republic of Germany, France, United Kingdom and Italy.

Processed cheese is mainly produced by blending 30% of matured cheese (age 8-10 months) and 70% of young cheese (age 1-4 months). The latter cheese imparts to the processed cheese the smooth texture, firm body and water-holding capacity, while the older cheese enhances the flavour and good melting properties (Sutherlands, 1975).

The state of knowledge of this product in terms of processing, manufacture, 'acceleration' and organoleptic qualities, have been discussed in recent books (Meyer, 1973, and Thomas, 1977).

Information on the manufacture of processed cheese has been reviewed by several authors (Kristoffersen, Mikolajcik & Gould, 1967; Mann, 1969 and 1970; Galesloot & Langeveld, 1973; Mann, 1975; Sutherlands,

TABLE 5:1

Factory processed cheese
production in some European countries
1,000 tonnes

Country	1980	1981	1982
Belgium	20.2	19.5	19.6
Denmark	9.1	10.0	8.0
France	79.4	84.0	84.3
Germany	119.5	124.6	123.5
Ireland	10.0	8.0	8.0
Italy	25.0	19.7	19.7
Netherlands	19.7	22.2	21.4
U.K.	21.3	21.6	22.1

Data compiled from EEC (1981, 1982, 1983)

1975; Mann, 1978; Thomas et al., 1980; Marshall & Doperalski, 1981; Mann, 1981 and 1983).

The objective of this present study was the utilisation of the Neutrase-treated cheese for the production of processed cheese. As mentioned elsewhere, this enzyme could be used to accelerate the flavour development in 'natural' Cheddar cheese (incidentally, this enzyme is permitted to be used in the manufacture of processed cheese, Statutory Instrument, 1984, No. 847), but to overcome certain defects obtained from the enzyme-treated cheese it was decided toevaluate the quality of processed cheese made from:

(a) 'Natural' Cheddar cheese (young)

(b) Neutrase-treated cheese (young)

(c) 'Natural' Cheddar cheese (young and old)

5.2 Materials and Methods

5.2.1 Manufacture of processed cheese

Fourteen batches of processed cheese were produced from different cheeses and the overall recipes are illustrated in Table 5.2. The emulsifying salts and stabilisers used were Joha S.E. and Joha T obtained from Fibrisol Services Ltd., London, UK.

The Joha S.E. (E 450a, E 450b and E 450d) consisted of the following compounds:

tetrapotassium pyrophosphate, potassium polyphosphate and potassium tripolyphosphate,

and Joha T (E 339) consisted only of trisodium orthophosphate.

The cheese was mixed with the ingredients in a Stephan Universal machine, Western Germany (type or Model UMM/SK40F, made in West Germany, and heated to 90°C for 5 minutes. The product was packed in Pukkafilm laminated hot weld pouches and stored at 5°C.

5.2.2 Analysis of the cheese

5.2.2.1 Determination of the moisture in the cheese

The moisture content in the cheese was determined as described in 2.3.2.

TABLE 5.2

The type of cheese base and the ingredients used to produce processed Cheddar cheese

No. of trials	Cheese base		Wt. of cheese approximately (kg)	Ingredients added to all cheese base material
3	Control (3 months old)	A1, A2 A3	9	3% Joha SE 0.5% Joha T 0.5% Sald water was adjusted to 45% before the processing
3	Cheddar cheese curd with 0.003% (w/w) added Neutrase (3 months old)	B1, B2 B3	9	
3	Cheddar cheese curd with 0.005% (w/w) added Neutrase (3 months old)	C1, C2 C3	9	
2	Control (3 months old) Control (18 months old) Total	D1, D2	6 3 9	
3	Control (3 months old) Control (18 months old) total	E1, E2 E3	7 2 9	

5.2.2.2 Organoleptic assessment

The cheeses were assessed and evaluated by five panelists from the Dairy Technology Department (West of Scotland Agricultural College) for the general acceptability of the flavour, body and texture on a scale of 0-20 as:

0-5	unacceptable
6-10	acceptable
11-15	very good
16-20	excellent

5.3 Results and Discussion

The moisture content of the processed Cheddar cheese varied between 42.64 and 44.55% (see Table 5.3). Such variation could be attributed to the variation in the moisture lost during processing and packaging. From preliminary trials, it was observed by adjusting the moisture content in the cheese to a maximum of 45%, the moisture content in the processed cheese falls within the existing legal standard in the UK (e.g. 43% moisture).

The average scores for the general acceptability are given in Table 5.3, and the following may be made:

(i) Processed cheese produced from cheese curd containing Neutrase were awarded grade scores between 8.2 and 15.4 - acceptable to very good. The highest acceptable scores were awarded to processed cheese made from Cheddar cheese curd to which 0.005% Neutrase had been added. Such results confirm that the Neutrase-treated cheese may provide the flavour and the body and texture characteristics required in processed cheese.

(ii) Processed cheese produced from young cheese (i.e. control) scored higher than the cheese produced from different mixtures of young and very matured Cheddar cheese (e.g. the acceptability varied between 7.8 and 9.4 for the young control cheese and 5.8 to 7.4 for the mixture of cheeses). All these cheeses were least acceptable compared with Neutrase-treated cheese, and this could be

TABLE 5.3

The moisture content and the average scores awarded by the 5 panelists for the general acceptability of the processed cheese

Treatement Code*	Moisture (%)	General acceptability	Amount of Neutrase added to the cheese curd % (w/w)
A1	43.51	9.4	none
A2	43.29	7.8	none
A3	44.55	8.4	none
B1	43.01	14.4	0.003
B2	42.82	8.2	0.003
B3	43.75	9.4	0.003
C1	43.48	15.4	0.005
C2	44.04	10.8	0.005
C3	42.79	13.4	0.005
D1	42.70	6.6	none
D2	43.65	6.4	none
E1	43.37	5.8	none
E2	42.64	5.8	none
E3	42.77	7.4	none

*Coding as described in Table 5.2

attributed to:

- lack of flavour in the young cheese;
- texture was not smooth;
- development of undesirable flavour due to the age of the controlled cheese, e.g. 18 months.

5.3 Conclusion

The efficacy of using the enzyme-treated cheese vis-a-vis control cheese for the production of processed cheese could be achieved at a young age (i.e. Neutrase-treated cheese at 3 months), and hence blending of cheeses at different ages may not be required.

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