

## 2. LITERATURE REVIEW

### 2.1 BASIC INGREDIENTS USED FOR FETA CHEESE MANUFACTURING

#### 2.1.1 Milk

Milk is the most important basic ingredient used for cheesemaking, and its physical, chemical and microbiological properties affect the chemical composition and organoleptic properties of the cheese. Milk of various mammal species and breeds have been used for manufacturing Feta cheese. Goat's milk and cow's milk (Table 2.1) are among the milks that may be used separately or in combination for making Feta cheese, and the properties of the cheese will depend more or less on the composition of the milk (Fox, 1987b; Potter & Hotchkiss, 1995).

**Table 2.1:** Proximate composition of goat's milk and cow's milk (Potter & Hotchkiss, 1995)

Constituent	% in cow's milk	% in goat's milk
Fat	3.80	4.24
Protein	3.35	3.70
Lactose	4.75	4.51
Ash	0.70	0.75
Total Solids	12.60	13.18
Water	87.40	86.82

##### 2.1.1.1 Total solids content

According to Harding (1995), the difference between cow's milk and goat's milk composition is not much, moreover the composition also depends on the breed of the dairy animal. However, Table 2.1 shows that goat's milk is higher in total solids content, especially fat and protein. This is advantageous to the cheese processor in terms of yield since a unit volume of goat's milk produces a significantly higher amount of Feta cheese than cow's milk (Anifantakis, 1990).

### 2.1.1.2 Fat content

Table 2.1 also shows that the average fat content of goat's milk is 4.24% compared with 3.80% for cow's milk. Moreover, goat's milk has a relatively poor creaming ability owing to its small fat globules and the absence of agglutinin, a clustering agent (Macrae *et al*, 1993). Goat milk lipids are higher in short chain fatty acids (C<sub>4</sub> - C<sub>12</sub>), which are more easily attacked by lipase. As a result, Feta cheese made from goat's milk is richer in free fatty acids and hence the cheese has a rancid but pleasant flavour, while that of cow's milk has rather a dull flavour.

The fact that goat's milk lipids are present as small fat globules compared to cow's milk is an advantage in cheesemaking. Small fat globules are easy to incorporate into the cheese curd, while big ones are more easily pressed out of the curd resulting in high losses of fat and low cheese yield (Scott, 1986). Cheese manufacturers reduce this problem by homogenising milk for manufacturing certain cheese types.

### 2.1.1.3 Protein content

The proteins in milk fall into two distinct types, whey proteins which exist in solution and casein in colloidal state. Casein constitute a large proportion of total proteins in both goat's and cow's milk, 95% of which is incorporated into the curd, while most of the whey proteins are lost with the water (Early, 1992).

According to Macrae *et al* (1993), goat's milk has more total protein content, but a slightly lower casein content than cow's milk. As a result, goat's milk form a softer and more friable curd than cow's milk, consequently Feta cheese made from the former is softer in texture.

#### *2.1.1.4 Odour and flavour*

The fact that goat's milk contains about twice as much of capric, caprylic and caproic acids as does cow's milk has been claimed to be a reason for the characteristic odour and flavour associated with goat's milk (Lampert, 1975). Johnson & Peterson (1974) indicated that the goat flavour is a genetic characteristic, but it can be affected by feed, environmental conditions and the presence of the billy goat while milking.

#### *2.1.1.5 Colour*

Goat's milk is almost white in colour as it contains little or no carotene because of the goat's efficiency in converting carotene to vitamin A. As a result, Feta cheese made from goat's milk is typically white in colour, while that of cow's milk has a yellowish tint since it is rich in carotene (Neilsen, Olsen, Lyndon, Sorensen & Skibsted, 1996).

#### *2.1.1.6 Health implications*

Goat's milk forms smaller and more flocculent curds which are more easily digested than cow's milk (Ensminger, Ensminger, Kolande & Robinson, 1986). The former also lacks some of the unique proteins present in the latter, which cause asthma, eczema and other allergic reactions in some people. Therefore goat's milk is often recommended for invalids and infants (Brown, 1981).

### **2.1.2 Starter cultures**

According to Tzanetakis, Vafopoulou-Mastrojinnaki & Litopoulou-Tzanetaki (1995), the natural microflora responsible for fermentation in Feta cheese made from raw goat's milk are lactic acid producing bacteria mostly of the genera *Lactobacillus*, *Lactococcus* (formerly called *Streptococcus*) and *Leuconostocs*. For industrial manufacturing of Feta cheese, a commercial starter culture consisting of one or more of these micro-organisms is commonly used.

The major role of starter cultures in cheesemaking is to convert the milk sugar (lactose) into lactic acid, which in turn causes important and desirable changes necessary in cheesemaking. Such changes include curdling of the milk solids, protein denaturation and development of flavour components. In addition, lactic acid is responsible for the low pH (4.3 to 4.6) which is a characteristic of Feta cheese and it also provides an acidic medium for the activity of proteolytic enzymes. The low pH has a preservation effect by inhibiting and minimising growth of undesired pathogenic and spoilage bacteria (Early, 1992; Pappas, Kondyli, Voutsinas & Mallatou, 1996). For industrial processing of Feta cheese from pasteurised milk, *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* are commonly used as starter culture (Varnam & Sutherland, 1994).

Most of the amino acids of the milk proteins exist in a form that cannot be utilised directly by the starter bacteria, but the presence of a proteinase system is advantageous as it converts the proteins into the absorbable form. Proteinase activity plays an important role during cheese ripening, by hydrolysing casein to soluble and absorbable amino acids and peptides (Cogan, Barbosa, Beuviel, Salvadori, Coccincelli, Fernandes, Gomez Kalantzopoulos, Ledda, Medina, Rea & Rodriguez, 1997).

### 2.1.3 Rennet

The role of rennet in Feta cheesemaking is to convert the milk to a curd which is more stable and firm than that obtained by acid coagulation alone and also to impart desirable changes during ripening (Fox, 1987a). The ability of rennet to perform these functions is due to the presence of the enzyme chymosin, which is secreted in the abomasum (fourth stomach) of the suckling ruminant. Sometimes rennet may contain a combination of chymosin and pepsin in different ratios. In the ancient cheesemaking practice of carrying milk in animal skin sacks, stomachs

and bladders, the milk absorbed the enzymes from the tissues, and these served the renneting functions (Fox, 1987a; Varnam & Sutherland, 1994).

For large scale manufacturing of Feta cheese in modern dairies, an extract from lamb and kid vells, in combination or separately, are usually used as a source of chymosin (Scott, 1986).

#### **2.1.4 Salt**

As indicated in Table 1.1 Feta cheese contains about 4 - 5% of sodium chloride (NaCl), which plays an important role in preservation and improving the organoleptic properties of the cheese. It lowers the water activity of the cheese and hence negatively affects growth of undesired micro-organisms and it also influences cheese ripening. NaCl is responsible for the characteristic saltiness of Feta cheese (Pappas *et al.*, 1996).

## **2.2 CHEESEMAKING SCIENCE AND TECHNOLOGY**

### **2.2.1 Heat treatment of milk**

Pasteurisation is the heat treatment usually applied to milk prior to cheesemaking. The conditions for this heat treatment are 63°C for 30 min or 72°C for 15 s.

#### **2.2.1.1 Microbiological effect**

Pasteurisation destroys pathogens and lactic acid bacteria and reduces the load of micro-organisms which would otherwise compete with starter culture bacteria for nutrients (Varnam & Sutherland, 1994). Common milk-borne pathogens like *Campylobacter* and *Salmonella* are among the organisms destroyed. The majority of psychrotrophic spoilage bacteria are also destroyed, but some of the enzymes produced by these bacteria may survive. Their enzymes are partly responsible for the rancid flavour due to lipolytic reactions in Feta cheese produced from

unpasteurised milk and also participate in proteolytic reactions during ripening of the cheese (Fox, 1987a; Early, 1992).

#### *2.2.1.2 Physico-chemical effects*

Pasteurisation results in increased cheese yield by incorporating denatured whey proteins into the curd (Fox, 1987a; Early, 1992). If a more severe heat treatment is applied, the physico-chemical properties of milk are affected. These include formation of large casein micelles and  $\beta$ -lactoglobulin complexes with k-casein through sulphhydryl bonding. The complex inhibits the action of chymosin on casein and hence reduce rennetability of the milk. The latter situation is aggravated by reduced availability of calcium ions in the milk. Poor rennetability of milk can be reversed by addition of calcium chloride at the rate of 0.02% of the milk (Scott, 1986; Fox, 1987a; Early, 1992).

#### *2.2.1.3 Heating technique*

According to Scott (1986), the equipment used for heat treatment of cheesemilk is very variable. In small volume dairy factories, vats or tanks are jacketed to carry hot water or steam which is used as a heating medium. For large scale factory operation, heat exchangers, tubular or multiple-plate type, are commonly used because of their efficiency in heat transfer and economical use.

At any heat treatment temperature, goat's milk fouls the equipment faster than cow's milk. This makes it difficult to ensure uninterrupted continuous processing of large volumes of goat's milk in a cheese plant since the heat exchangers must be shut down and cleaned very often (Kanstanas, Lewis & Grandison, 1995). In traditional technology, milk in large pans or pots was heated directly over a slow fire or indirectly in a water bath (Kosikowski, 1978).

## 2.2.2 Fermentation

Lactic acid bacteria (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* in the case of Feta cheese) obtain energy for their metabolic activities anaerobically by catabolic reactions in which lactose is converted to lactic acid and energy (Keeton, 1983; Bohinski, 1987).

### 2.2.2.1 Advantages of fermentation

Fermentation plays an important role in preservation and providing variety in milk products. Several of the end products enable fermentation to perform these duties, particularly acids and alcohols, which are flavour components, flavour precursors and inhibitory to the growth of pathogenic and spoilage micro-organisms. If the pH of the cheese is maintained below 4.6 through acid production, growth of the toxin producing micro-organism, *Clostridium botulinum*, is also inhibited (Varnam & Sutherland, 1994; Potter & Hotchkiss, 1995).

Starter micro-organisms involved in fermentation are not only catabolic, but they are anabolic as they synthesise many complex vitamins and other growth factors. The net increase in the level of some vitamins may be off-set by the fact that the micro-organisms use some of these for their own metabolic activities. The level of folic acid and choline may increase by up to 100%, while that of pantothenic acid and biotin may increase only slightly (Varnam & Sutherland, 1994; Potter & Hotchkiss, 1995).

Fermentation is also believed to increase bioavailability of some minerals and trace elements especially phosphorus, however there is still some controversy concerning minerals like calcium, magnesium and zinc (Varnam & Sutherland, 1994).

### 2.2.2.2 Lactic acid fermentation

#### a) Homofermentation

Lactose is initially broken down to galactose and glucose by the enzyme  $\beta$  - galactosidase. The process of the biodegradation of this sugar is called glycolysis,

in which pyruvic acid ( $C_3H_4O_3$ ) is an important intermediate. This requires a large number of successive steps, each catalysed by a specific enzyme. When a homofermentative micro-organism like *Lactococcus lactis* ssp. *cremoris* is involved, the sole compound formed is lactic acid, and energy is released for the micro-organism's activities (Bohinski, 1987).

#### b) Heterofermentation

Lactose is converted to glucose and galactose, and subsequently to pyruvic acid in a similar manner as in homofermentation. However, when a heterofermentative micro-organism is responsible for biodegradation of the intermediate (pyruvic acid), other substances apart from lactic acid are also formed. These include diacetyl, acetoin, 2,3 -butanediol, acetic acid, ethyl alcohol, formic acid and carbon dioxide (Keeton, 1983; Bohinski, 1987; Cogan, 1995). According to Cogan (1995), diacetyl and acetic acid are important in flavour perception and carbon dioxide in the texture of fermented dairy products.

Irrespective of the end product, fermentation only releases a very small amount of energy for lactic acid bacteria (LAB) metabolism purposes and hence the end products still contain much of the original energy. Fermentation can be simplified and generalised as indicated in Figure 2.1.

#### 2.2.2.3 Citric acid fermentation

According to Kosikowski (1978), fresh milk contains about 0.2% of naturally occurring citric acid. Some strains of *Leuconostoc* species (*L. cremoris*, *L. dextranicum* and *L. mensenteroides*) and *Lactococcus lactis* subsp. *diacetylactis* can degrade citric acid, with the formation of an important flavour component called diacetyl. Apart from this, aroma compounds like volatile acids, acetic acid, oxalacetate, acetaldehyde, acetone and butylene glycol are also produced. Citric acid fermentation is also known to be responsible for the pin holes seen in Feta

cheese (Figure 2.2) due to production of high amounts of carbon dioxide (Davis, 1976; Tomkins, 1992).

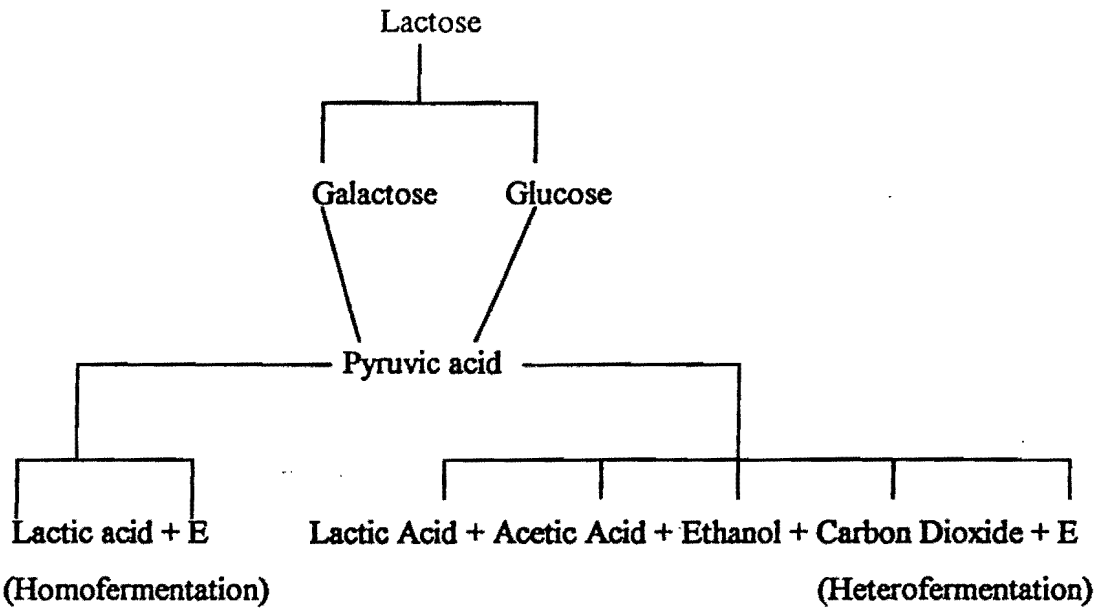


Figure 2.1: Fermentation of Lactose (Modified from Bohinski, 1987).

Depending on the bacterial species involved, citric acid fermentation can lead to formation of various compounds, some as intermediates and others as end products as shown in Figure 2.3 (Fox, 1987a).

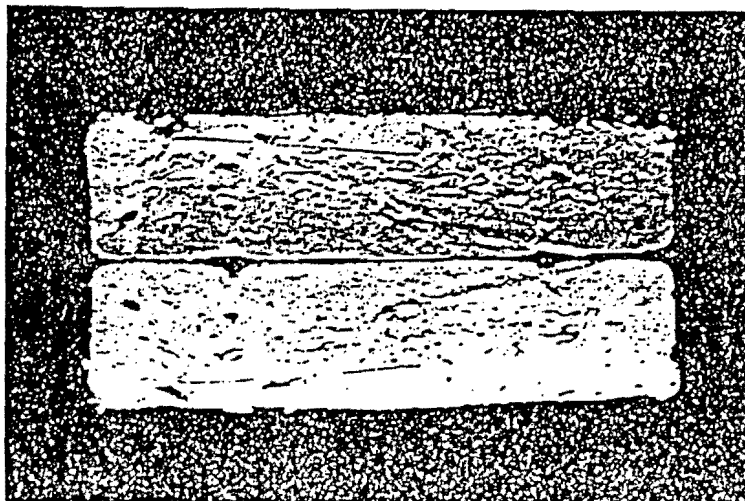


Figure 2.2: Ripe Feta cheese (Davis, 1976).

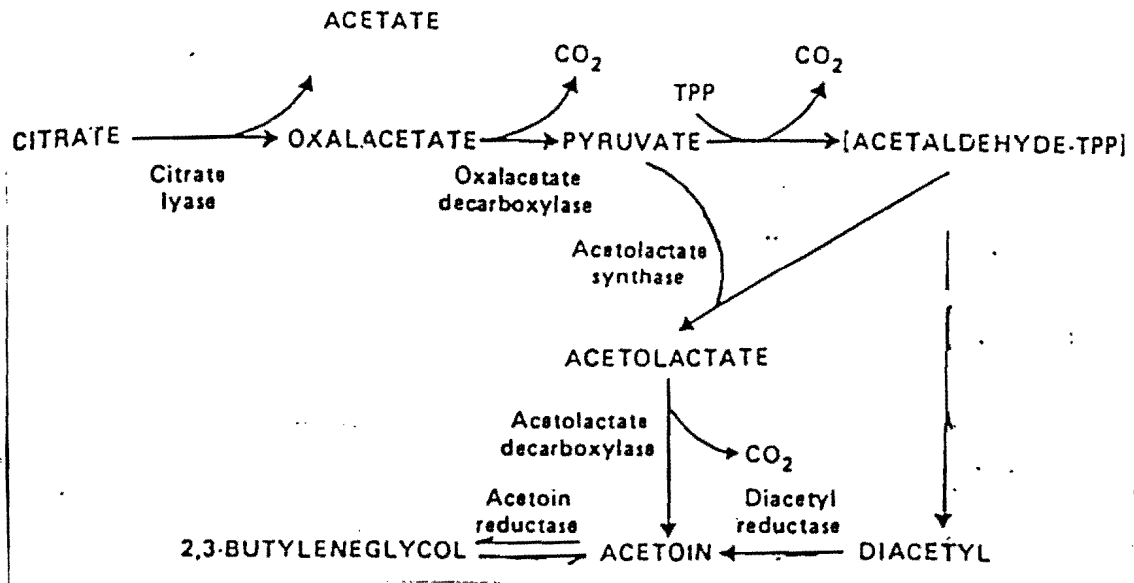
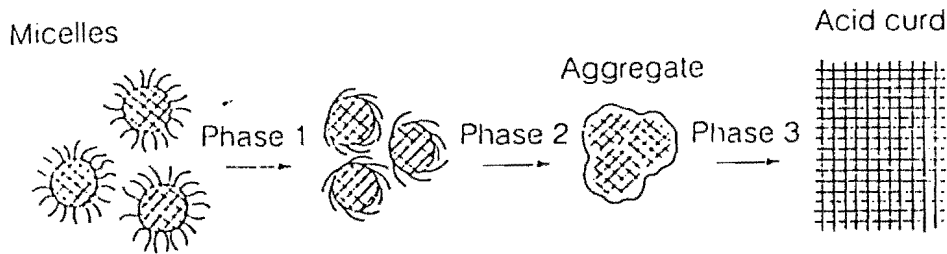


Figure 2.3 Pathway for the metabolism of citrate (Fox, 1987a).

## 2.2.3 Curd formation

### 2.2.3.1 Acid curd formation

The normal pH of milk is about 6.6, and as the pH drops due to lactic acid production, casein micelles are destabilised. At the pH of about 4.6, coagulation of the casein occurs. The calcium ions are released and they form a network with casein molecules. This is followed by aggregation of casein, when the positively charged  $\beta$ -casein and the negatively charged  $\alpha$ -casein attract each other. The curd is further strengthened by the collapse of the hair-like  $\kappa$ -casein in a manner shown in Figure 2.4 (Varnam & Sutherland, 1994).



**Figure 2.4:** Acid curd formation (Varnam & Sutherland, 1994).

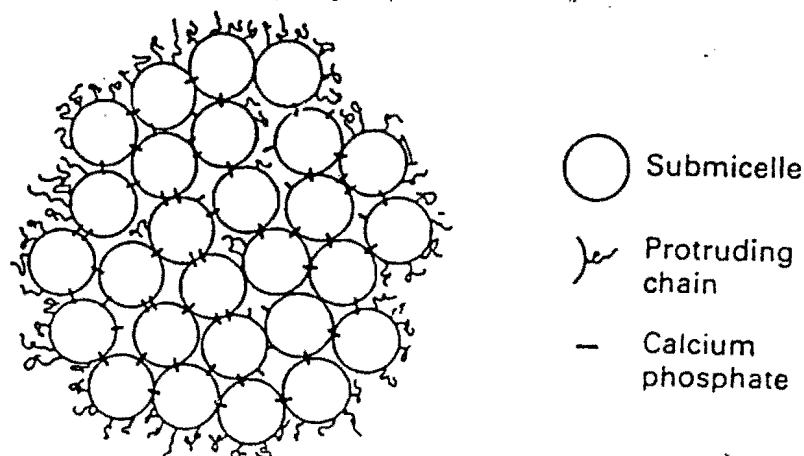
The increased acidity, together with moderate heat of about 35°C and some stirring cause the curd to synerese and expel moisture (whey) from the coagulum, and a product with significantly lower moisture content of about 35 to 60% (compared to 87% moisture in milk) is formed (Fox, 1987a).

### 2.2.3.2 Rennet coagulation

The activity of rennet is caused by:-

- (i) Proteolysis by chymosin (or any other proteolytic enzyme present)
- (ii) Slight acidification
- (iii) Heat application (35°C)
- (iv) Increase in calcium ion concentration

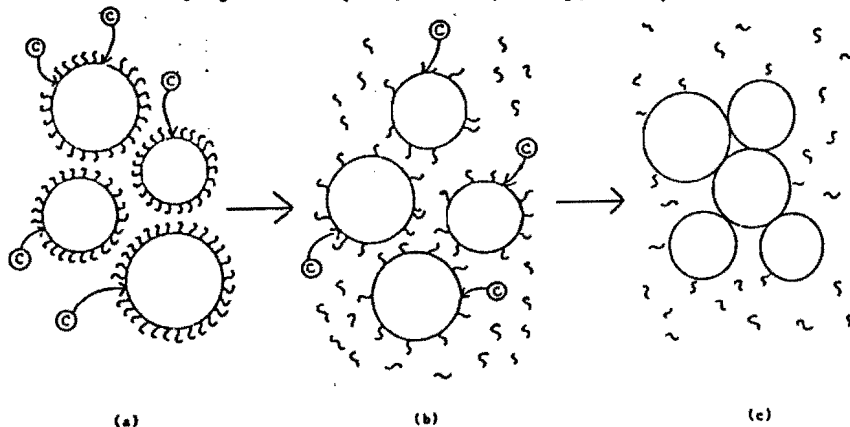
At normal pH of milk, casein molecules (submicelles) exist in clusters which are held together by calcium phosphate and aggregate to form casein micelles illustrated in Figure 2.5.



**Figure 2.5:** Model of casein micelles (Macrae *et al.*, 1993)

On the surface of each micelle chains of the hydrophilic c-terminal ( $\kappa$ -casein) of the submicelles protrude. These make the micelles to be negatively charged on the surface, such that electrostatic repulsion forces keep the micelles apart. Chymosin splits the casein molecules at specific peptide bonds between the 105<sup>th</sup> and 106<sup>th</sup> amino acids, resulting in the removal of the highly acidic and hydrophilic c-terminal chains and hence reducing the repulsion forces which kept the molecules apart (Fox, 1987a; Early, 1992; Macrae *et al.*, 1993)

Once the degree of proteolysis of the  $\kappa$ -casein has reached 80%, the micelles of the original acid curd, which is stabilised by the electrostatic forces and hydrophobic effects, cluster as shown in Figure 2.6. The curd network shrinks further and more water is lost by syneresis (Fox, 1987a; Early, 1992).



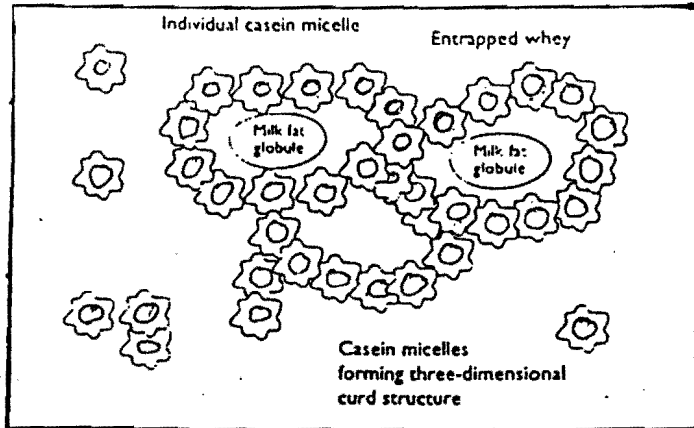
**Figure 2.6:** Schematic presentation of the attack by chymosin (shown as C) on casein micelles (Fox, 1992).

Three different points in the reaction are illustrated.

- (a) The  $\kappa$ -casein coat of the micelle is intact, and chymosin has just been added.
- (b) Chymosin hydrolyses  $\kappa$ -casein, but much of the latter still remain and prevents aggregation.
- (c) Almost all of the  $\kappa$ -casein have been hydrolysed and micelles aggregate.

### 2.2.3.3 Structure of the curd

According to Brusgaard (1996), a cheese curd contains three major components - casein, fat and water. Casein form an open casing structure in which fat globules are enclosed as shown in Figure 2.7.

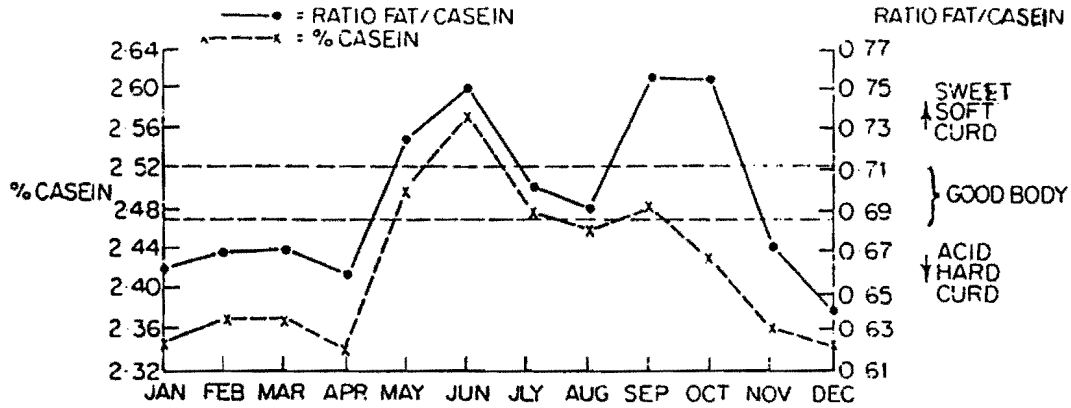


**Figure 2.7:** Structure of a cheese curd (Brusgaard, 1996).

Water is partly bound to casein, and the remainder fills the spaces between the casein matrix and the fat (Scott, 1986). The size and distribution of the fat globules depend on the fat globules' geometry; cheese made from homogenised milk has a more uniform distribution of the fat globules than that made from unhomogenised milk (Fox, 1987a).

The casein framework is composed of chains which are not linear but somehow with an irregular helical spring structure which imparts some elasticity to the curd. The ratio of fat to casein is important in determining the texture of the cheese. When the ratio is out of balance, the body of the cheese is either too soft or too hard (Scott, 1986) as shown in Figure 2.8.

Although it is not specifically stated that the ratios in the figure are for Feta cheese, a similar pattern of change in texture can be expected when the optimum ratio of fat to casein of Feta cheese milk is altered.



**Figure 2.8:** Effect of seasonal variation of casein to fat ratio on the texture of cheese (Scott, 1986).

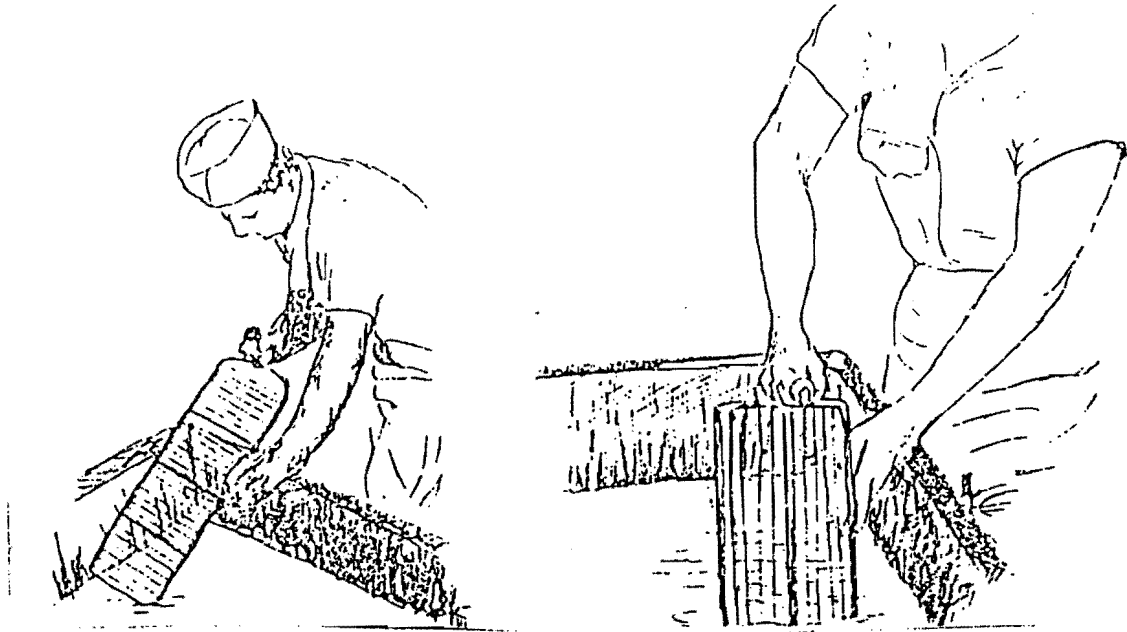
## 2.2.4 Cutting of the curd

### 2.2.4.1 Cutting technique

In traditional cheese processing, primitive forms of cutting methods were employed in which long knives, swords, and tree branches were used. Industrially, a pair of cutting knives shown in Figure 2.9, one with horizontal wires and the second one with vertical wires, are used (Kosikowski, 1978).

A knife is carefully inserted into the curd in an upright position, steadily pushed forward from one side of the vat to the opposite. The horizontal wire knife is used first, only in one direction, lengthwise, whereas the vertical wire knife is applied in two directions, the long and the cross cut (Kosikowski, 1978; Scott, 1986).

In highly mechanized cheese factories, round vats are equipped with rotary wire cutters, and rectangular vats have perpendicular knives which require little manual assistance (Kosikowski, 1978).



**Figure 2.9:** Cheese curd cutting operation (Kosikowski, 1978).

#### **2.2.4.2 The impact of cutting**

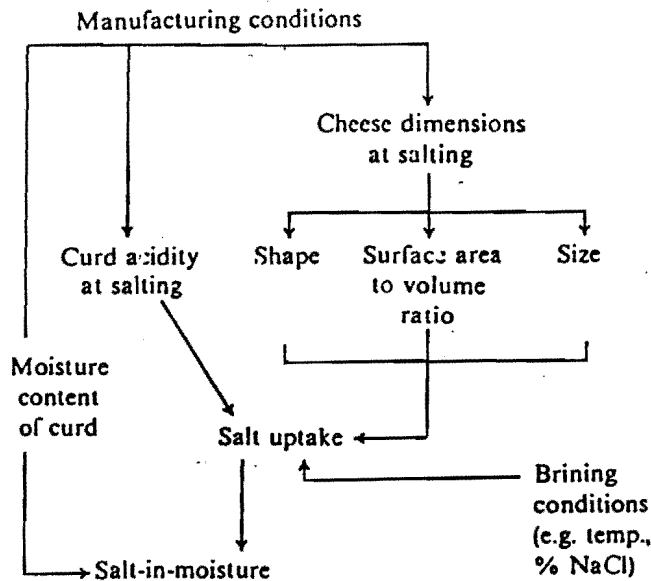
Cutting increases the surface area of the curd and facilitate loss of whey from the curd, allowing the casein matrix to shrink. Gentle uniform cutting keeps the curd from disintegrating into fine particles and thus avoids heavy yield loss (Scott, 1986).

Depending on the distance (spacing) between the wires of the knives, cutting results in breaking the curd into cubes or other uniform shapes. The selection of the specific wire spacing, 1 to 2 cm, determines to an extent, the final moisture level of the cheese. Large cubes give higher moisture cheese than small ones (Scott, 1986).

#### **2.2.5 Salting**

In ancient times, traditionally processed Feta cheese was preserved in sea water (Tsotsanis, 1996), while in commercial manufacturing, the curd is dry salted with coarse salt or brine salted. The importance of salt in cheese has been discussed in Section 2.1.4. The salt sprinkled on the surface of the curd dissolves in the

moisture. Through the diffusion process, salt penetrates into the curd and conversely water (whey) migrates outwards. According to Fox (1987a), the rate of salt absorption and the final salt content depends on factors like geometry of the curd, pH, temperature and moisture content (Figure 2.10).



**Figure 2.10:** - Principal factors that affect salt uptake (Fox, 1987a).

The rate of salt absorption increases with increasing surface area to volume ratio of the cheese. That is, when the curd is cut into smaller pieces, the surface area is relatively larger than in bigger pieces, hence salt absorption occurs fast and time required to attain a desired level is shorter (Fox, 1987a).

When the temperature of the curd is above 32°C, the fat melts and surrounds the surface of the curd and hinders dissolving of the salt in the water, which is a prerequisite to salt absorption (Fox, 1987a).

Curd pH and moisture content are directly related in this regard. At low pH, the moisture content of the cheese is low as the curd tends to synerese due to acidity, and hence salt absorption is slow and the curd quickly becomes saturated with salt at a low level (Fox, 1987a).

## 2.2.6 Ripening

During ripening, biochemical reactions occur which enhance the unique flavour, aroma and textural properties of Feta cheese. The primary reactions that occur involve proteolysis and lipolysis (Early, 1992).

### 2.2.6.1 Lipolysis

#### a) Lipid hydrolysis and rancidity

In traditional cheese made from unpasteurised milk, native milk lipases and the microbial lipases were responsible for breakdown of fats. In pasteurised milk, the former enzymes are inactivated and the latter are more heat resistant but not always present; hence commercial lipases are commonly added (Efthymiou & Mattick, 1964).

As a result of lipolysis, the concentration of volatile and non-volatile free fatty acids increases. According to Varnam & Sutherland (1994), the resultant total free fatty acids and the short chain fatty acids ( $C_4$  to  $C_8$ ) content may be about 50 g/kg. These products, together with the naturally occurring short chain fatty acids (refer to subsection 2.1.1.2) in milk, are responsible for the typical pleasant rancid flavour of Feta cheese. According to Efthymiou & Mattick (1964), the pleasant rancid flavour is associated with free fatty acids from  $C_2$  through to  $C_{10}$ , while  $C_{12}$  and longer fatty acids are responsible for an unpleasant rancidity. The free fatty acids not only function as flavour components in cheese, but are also precursors of other flavour components (Harboe, 1994).

Excessive lipolysis sometimes affect Feta cheese adversely, leading to a rancid flavour defect. This reduces the acceptability of the cheese. It can happen when the cheese milk is spontaneously lipolysed, through processing techniques which induce lipolysis, or addition of too high doses of lipase (Fox, 1983).

b) Lipolytic enzymes specificity

Some of the lipolytic enzymes, specifically pregastric esterases, hydrolyse lipids with preference to the  $S_{n1}$  and  $S_{n3}$  positions (terminal fatty acids) of the triacylglycerols. These positions are predominantly occupied by short chain fatty acids. The 2-monoacylglycerols are only hydrolysed after their conversion to 1- or 3- isomers (Fox, 1983).

Other lipases, including the microbial lipases, are able to sense the length of the fatty acids and prefer to hydrolyse short chains specifically. For these reasons, lipid hydrolysis products are mostly short chain fatty acids (Harboe, 1994). Since goat milk is richer in short chain fatty acids and more susceptible to lipolysis than cow's milk, Feta cheese made from the former is more often associated with rancid flavour than the latter.

#### 2.2.6.2 *Proteolysis*

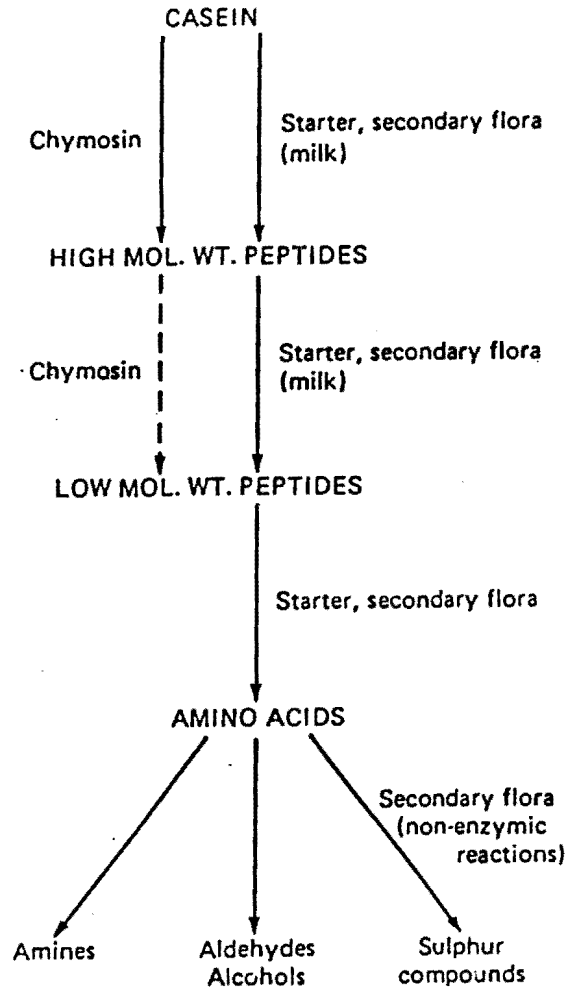
a) Mechanism of proteolysis

In many cheeses a certain portion (about 2 to 6%) of the milk coagulant's proteolytic activity is retained in the cheese curd and the rest of the proteolytic activity is lost with the whey (Brusgaard, 1996). The activity of the enzyme chymosin on the milk proteins continues through the ripening process. Chymosin further breaks down para-casein molecules into peptides and amino acids, but its activity on beta-casein is only limited (Varnam & Sutherland, 1994).

At a certain stage during ripening, the activity of chymosin ceases due to unfavourable conditions like increase in pH and decrease in temperature, and secondary micro-flora originating from the starter culture may take over proteolysis and break down amino acids further, as shown in Figure 2.11 (Fox, 1987a).

In many cheeses, especially those made from goat's milk, the most predominant amino acid at the end of ripening is usually proline, followed by glutamic acid,

valine, leucine, isoleucine, histidine and lysine, which in all make up as much as 60% of the total free amino acids content (Fresno, Tornadijo, Carballo, Bernado & Gonzalez-Preito, 1997)



**Figure 2.11** - Breakdown of casein during cheese ripening (Fox, 1987a).

This amino acid profile is typical of cheeses in which the starter microflora consists of *Lactococcus* and *Lactobacillus*. These micro-organisms have special peptidases for hydrophobic peptides rich in proline (Fresno *et al.*, 1997). During storage of the cheese, peptides and amino acids dissolve in the whey and decrease with decrease in moisture content of the cheese (Macrae *et al.*, 1993).

b) Textural changes

Proteolysis also result in softening of the cheese due to calcium phosphate precipitation which weakens the casein network during ripening. The effect is highly dependent on the pH value, in most cases high pH induce activity of proteinase and further soften the cheese (Varnam & Sutherland, 1994).

According to Fox (1987a), chymosin is to a large extent responsible for the texture development in cheese. Its action is more favoured near the centre of the curd where the salt content is low.

#### 2.2.6.3 Flavour components

Bitter peptides in cheese originate mostly from  $\alpha$  - casein and  $\beta$ -casein fractions with high average hydrophobicity (Q). Peptides with a Q- value of  $< 5.44$  kJ/residue tend to be non-bitter, and those with  $Q > 5.86$  are bitter, whereas no predictions can be made about peptides with Q-values ranging between 5.44 and 5.86 kJ/residue (Gomez, Garde, Gaya, Medina & Munez, 1997). According to Scott (1986), phenylalanine is one of the amino acids which cause bitterness when occupying the terminal position of casein fragments.

Rennet produces bitter peptides from both  $\alpha$ - and  $\beta$ - casein, whereas starter culture proteinases release bitter peptides mostly from  $\beta$  - casein. Peptidases from some strains of lactic acid bacteria are very effective in degrading bitter peptides and subsequently reducing the bitter flavour in cheese (Gomez *et al.*, 1997).

### 2.3 DEVELOPMENTS IN FETA CHEESEMAKING

In recent years, several attempts have been made to improve the quality of Feta cheese manufactured from cow's milk, with the intention of making it resemble the traditional version as much as possible. Many developments have been made in the

production of the cheese such as developments in mechanization and manipulation of ingredients.

### **2.3.1 Ultra filtration and reverse osmosis**

Due to higher content in total solids, especially fat and protein, the cheese yield is significantly higher when goat's or sheep's milk is used for Feta cheesemaking than when cow's milk is used (Anifantakis, 1990). This has led to the concentration of milk solids using techniques like ultra-filtration (UF) and reverse osmosis in Feta cheese manufacturing. UF was found to be advantageous in improving the cheese yield by 25 to 30%, the concentrate had better rennetability properties (80% less rennet is used) and fat losses were also reported to be minimal. These improvements occur when the milk was concentrated to the ratio 1 : 4.5 (El-Gazzar & Marth, 1991).

UF Feta cheese had almost the same overall composition as the traditional product except that the former had 3 to 5% more moisture than the latter due to the high water holding capacity of whey proteins retained in UF cheese (El-Gazzar & Marth, 1991). The UF cheese also had a different texture, no mechanical holes and hence a different mouth feel which usually made the cheese less acceptable (Renner & El-Salam, 1977). When UF milk concentrate is used, it is always necessary to make changes in the basic Feta cheesemaking procedure and hence it causes some inconvenience and unusual complications.

### **2.3.2 Skim Milk Retentate powder**

De Block, De Ville & Petit (1996) made a contribution in Feta cheese manufactured by adding skim milk retentate powder to the cheese milk. The cheese produced this way had a higher yield and the production was economical. However, the composition of this cheese did not meet the specifications as it was

found to have a much lower fat content (13%) than the traditional version (reference cheese) which had about 22% (m/m) fat content.

### **2.3.3 Low heat skim milk powder and anhydrous milk fat**

Jana & Thakar (1996) produced Feta cheese using high solid content (37 to 38%) recombined milk prepared by mixing low heat skim milk powder (LHSMP) and anhydrous milk fat (AMF). This was meant to help in regions with low or fluctuating supplies of fresh milk and where there are long distances between the milk production areas and the cheese processing centres. Feta cheese of moderate taste, good colour and good consistency was produced from the high solids recombined milk. However, this was reported to have certain limitations, which include:-

- Fast acid development
- Poor rennetability
- Insufficient firmness of the curd
- Syneresis and drainage problems
- High residual lactose content

Corrective measures were necessary but found to be time consuming as well as uneconomical.

Jana & Thakar (1996) further pointed out that the LHSMP and AMF flavour usually associated with recombined milk was also present in the Feta cheese. Moreover, an alternative technology was required to enable the homogenisation step to be eliminated as it led to fat globule participation in the protein matrix, thereby affecting cheese structure and texture.

## **2.3.4 Bleaching of the milk fat**

### *2.3.4.1 High temperature bleaching*

In an attempt to improve the colour of cow's milk Feta cheese, milk fat from cow's milk was bleached in a bath of silicon oil at temperatures between 180 and 280°C (Neilsen, Olsen, Lyndon, Sorensen & Skibsted, 1996). The bleaching mechanism involved removal of carotenoids, which also act as antioxidants. As a result, increased cholesterol oxidation in the Feta cheese was reported. This was aggravated by increased oxidation of heat-treated milk fat by thermal hydrolysis of lipid hydroperoxides. This oxidation led to development of undesired rancid flavour and accumulation of toxic oxysterol in the cheese.

### *2.3.4.2 Chemical Bleaching*

Treatment of the cream from cow's milk with chlorophyll or 0.00048% of benzoil peroxide resulted in production of Feta cheese with a white colour without adversely affecting the flavour of the cheese (Zerfiridis & Kristoffersen, 1968).

## **2.3.5 Other non-dairy additives**

### *2.3.5.1 Coagulants and coagulation catalyst*

Recombined and reconstituted milks exhibit poor rennetability as a result of irreversible precipitation of the minerals calcium and phosphorus. Even when pasteurised milk is used for cheesemaking, the rate of coagulation is slower and the resultant coagulum is weaker than when unpasteurised (raw) milk is used. Addition of calcium chloride at the rate of 0.02% of the weight of the cheese milk or addition of aqueous extracts of flowers of *Cynara* sp were effective in counteracting rennetability defects (Jana & Thakar, 1996). Ionic calcium plays an important role in forming intermolecular bridges during curd formation (Varnam & Sutherland, 1994).

Extracts from some plants have been tried as milk coagulating agents, although there is no evidence that these can be applied in Feta cheese processing. Examples of such plants are given in Table 2.2.

**Table 2.2** Plants giving extracts that will coagulate milk (Tomkins, 1992).

<b>Common name</b>	<b>Scientific name</b>
Burdock	<i>Arctium minus</i>
Bittersweet	<i>Solanum dulcamara</i>
Mallow	<i>Malva sylvestris</i>
Thistle	<i>Cirsium and Carlina spp</i>
Fig tree	<i>Ficus carica</i>
Hogweed	<i>Heracleum sphondylium</i>
Knapweeds	<i>Centaurea spp</i>
Lady's bedstraw	<i>Galium verum</i>
Ragwort	<i>Senecio jacobaea</i>
Spearworts	<i>Ranunculuc spp</i>
Nettle	<i>Urtica dioica</i>
Teasel	<i>Dipsacus fullonum</i>
Yarrow	<i>Achillea millefolium</i>
Spurge	<i>Euphorbia lathyris</i>

#### 2.3.5.2 Lipolytic agents

Feta cheese manufactured from pasteurised cow's usually lacks the characteristic flavour normally associated with goat's milk cheese. For fullest flavour, a commercial lipase (kid and lamb extract in the ratio 2: 1) was added to the milk at the rate of 7.5 g/500 kg milk (Zerfiridis & Kristoffersen, 1968). Cheese produced with other classes of commercial lipases produced atypical rancid, bitter and unclean flavours, and also developed peppery and soapy flavour defects (Efthymiou & Mattick, 1964).

Despite the positive aspects of using non-dairy additives, there are strict regulations concerning the use of additives in dairy products. In many countries, the regulations are based on the FAO/WHO Food Standards Committee recommendations, but such regulations still vary from country to country (Scott, 1986). This threatens the international market of Feta cheese containing additives.

### **2.3.6 Low fat version of Feta cheese**

World wide, there is an overwhelming demand for reduced fat food products and the food scientists and manufacturers are challenged to satisfy this trend. According to Mann (1996), a Greek research team manufactured Feta cheese from low fat milk using a traditional method. Milk containing 1.5%, 3.0% and 4.5% fat were used and compared with milk with 6.0% fat as control.

Significant increases in protein and moisture content of the cheese with decrease in milk fat content was reported, while the solid-non-fat contents and cheese yield decreased. Apart from these, lipolysis, proteolysis, body, texture and flavour scores of the cheese were adversely affected by reduced fat content. Modifications either in technology or formulations are hence necessary to improve the quality and acceptability of the cheese (Mann, 1996).

Zwaginga (1990) indicated that if the current trend of low fat products is not controlled in cheese formulations, the ultimate product will be a cheese with a rubber-like texture and a bitter taste. To mask these defects, the cheese formulations will have to include mixtures of ingredients that will result in losing the natural image of cheese and the traditional cheesemaking process. Zwaginga (1990) also suggested that if critical limits are approached, manufacturers should stop the adaptation of the process and concentrate on the development of new products based on the availability of raw material and the know-how.

### 2.3.7 Rennet substitutes

According to Varnam & Sutherland (1994), a shortage and corresponding expense of mammalian rennet has led to the use of proteolytic enzymes from microorganisms like *Mucor miehei*, *M. pusillus* and *Endothia parasitica* as substitutes. However, these enzymes are much less specific in hydrolysis (less specific to Phe<sub>105</sub> - Met<sub>106</sub> bond) and hence affect the quality of the resultant cheese adversely. This is due to the fact that, unlike the mammalian proteinase which contains a small fraction of pepsin in addition to chymosin, microbial enzyme is purely chymosin (Kandarakis, Anifantakis & Moschopoulou, 1995).

According to Kandarakis *et al.*, (1995), microbial proteinase was successfully used in place of rennet for manufacturing many kinds of cheeses including Feta. Although the Feta cheese had almost the same composition and organoleptic properties as the mammalian rennet cheese, its rate of whey drainage was slightly slower. After slight alterations in the Feta cheesemaking technique were made, the cheese had a higher moisture content.