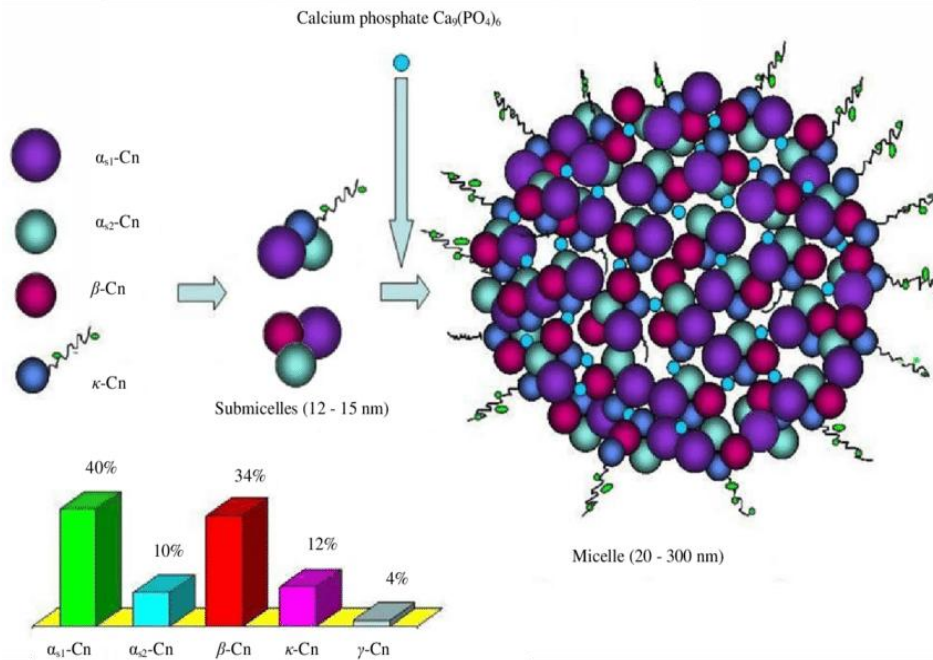


## Milk Coagulation: -

The main group of milk proteins, the caseins, are not soluble in water (colloids or suspension) and exist in milk as small particles (<300 nm) called micelles.



In normal milk, aggregation is prevented by two factors. If one of these factors is removed the micelles will aggregate and form a gel like jells.

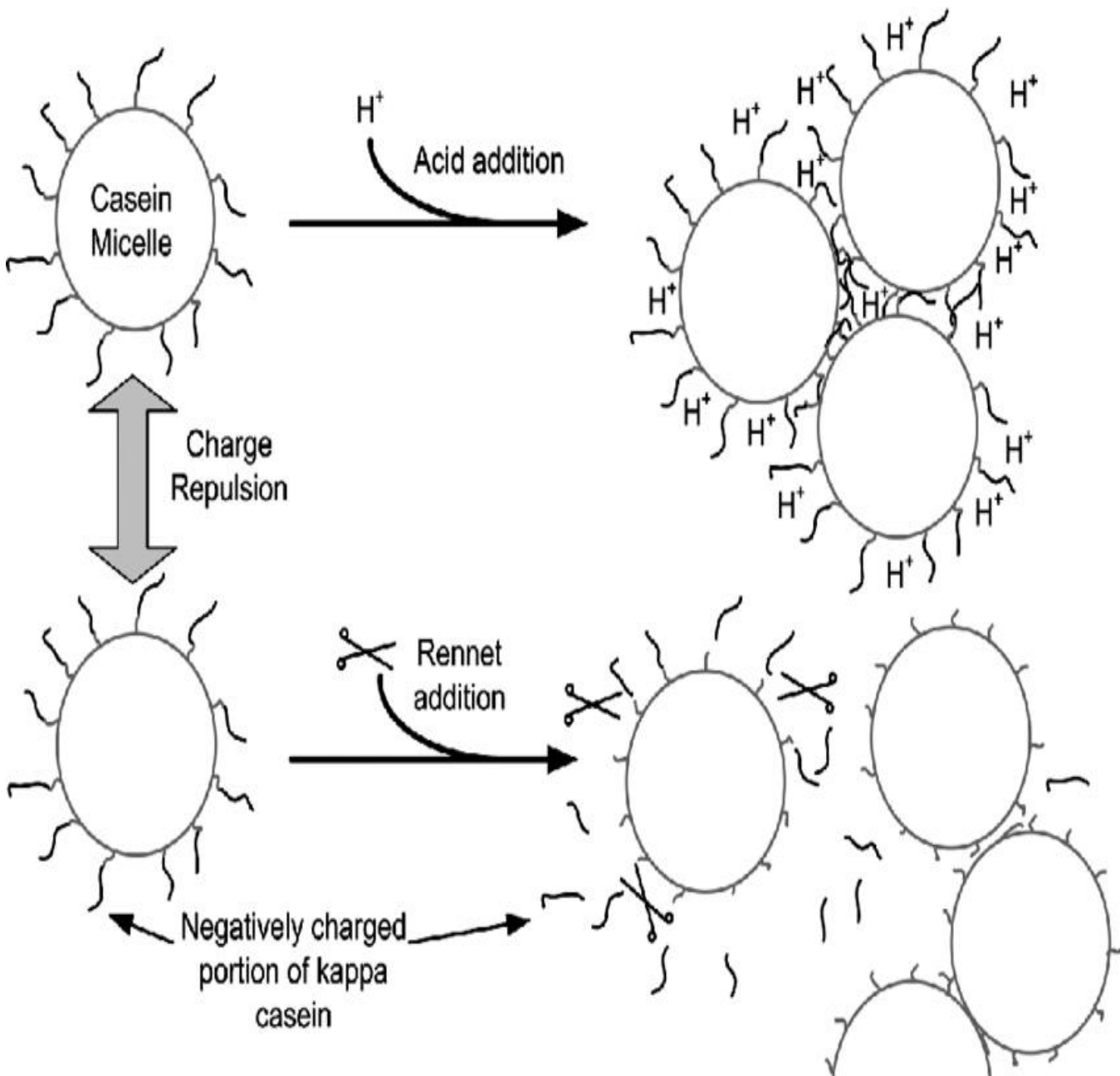
1-The first factor is a negative charge on the micelles. At the pH of milk, the micelles are negatively charged they helps prevent the micelles from sticking together.

2-The second stabilizing factor is a 'hairy' layer of surface-active protein, called kappa-casein (K-casein), on the surface of the micelle. This layer helps prevent the micelles from sticking together

there are two ways to coagulate milk;

1- one is to neutralize the negative charge on the micelle. That can be able by acidification or a combination of high temperature and acidification(acid coagulation).

2-The other is to remove the hairy layer from the micelles. That's called enzymic coagulation.



## Acid coagulation

-Acid milk gels can be formed by lactic bacteria or the use of acidifying agents such as gluconic-delta-lactone (GDL is slowly hydrolyzed to gluconic acid in the presence of water).

- use pure vinegar acid, lactic acid, citric acid or any other food grade organic acid. sometimes a natural acid such as lemon juice is added.

The acid can be added drop by drop to warm milk (about 80 - 90°C, just after boiling).

-Acid coagulation is used in the production of cottage cheese, and quark as well as other fermented milk products such as yoghurt, commercial butter milk, kefir etc.

## Enzymic Coagulation of Milk: -

The three stages of enzymic coagulation are: -

### (1) Primary Stage (First Stage) Enzymatic Stage: -

Coagulants are aspartate-proteinase, endopeptidase, which can split proteins into relatively large fragments.

In the first stage, the enzyme (rennet) cuts off a specific fragment  $\kappa$ -casein. At the natural pH of milk, about **80% of  $\kappa$ -casein** must be cleaved to permit the enzymatic coagulation of milk involves modification of the casein micelles by limited proteolysis by selected proteinases, called rennet or coagulating enzymes specifically split off a specific part of these  $\kappa$ -casein hairs, hydrolyzing the Phe-Met peptide bond (at amino acid 105–106), producing para- $\kappa$ -casein (f1- 105A.A.)

and macro peptides (f106- 169 A.A.); and thus destabilization of the whole micelles , macro peptides also called glycol macro peptide since they contain most or all of the sugar groups attached to k-casein).

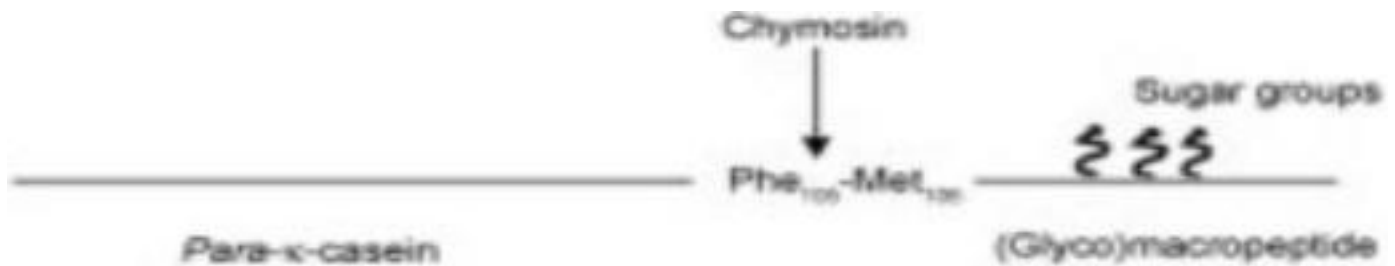
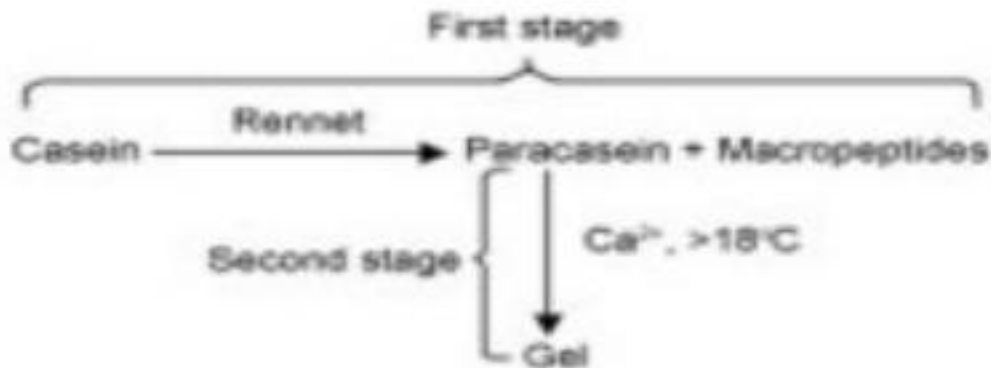


Fig. 1  $\kappa$ -Casein.

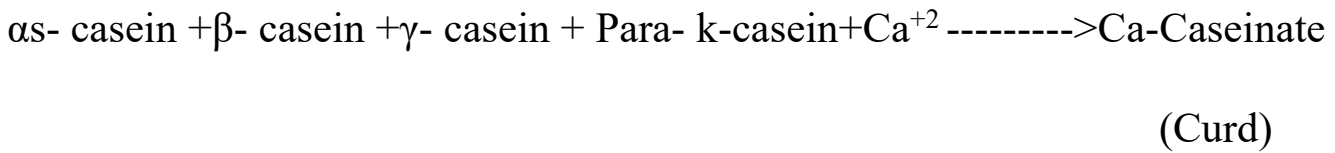


-The hydrophilic macro peptides diffuse into the surrounding medium(water soluble) while the para-k-casein remains attached to the cured(water insoluble and Ca-sensitive peptide)

K-Casein-----> Para- k-casein + Casino Macro peptide (CMP)(Glyco- Macro peptides )

## (2) Secondary Stage (Secondary or Second Stage) Non-Enzymatic Stage: -

The next stage is the physical process of calcium-induced aggregation of the rennet-altered micelles to form a gel. After losing its water-soluble tail, k-casein can no longer keep the casein particles separated and they begin to form chains and clusters. The clusters continue to grow until they form a continuous, three dimensional network which traps water, fat and some salts inside, and forms a gel, like Jell-o.



### **(3)The Third Stage (Tertiary Stage):-**

includes a slow proteolysis of curd and producing long chain peptide, this phase occur during cheese storage or cheese ripening, during ripening, rennet causes milk protein to break down, giving the typical cheese taste.

### **Factors Effecting Milk Enzymatic Coagulation:-**

#### **1-Effect of pH.**

Lower pH increases enzyme activity and neutralizes charge repulsion between micelles. Therefore, both primary and secondary stages of coagulation proceed more quickly at lower pH.

#### **2-Effect of Calcium .**

Calcium is not required for the primary stage (i.e., enzyme hydrolysis of k -casein) but is essential to aggregation of the casein micelles(secondary stage). At low levels of calcium the primary phase goes to completion. Then, direct coagulation can be induced by adding sufficient calcium chloride.

#### **3-Effect of temperature.**

The optimum coagulation temperature for most cheese is 32-37C°, the. At temperature less than 30C the gel is weak and difficult to cut with excessive yield loss due to fines loss. At temperatures less than 20C° coagulation does not occur, due mainly to the very high temperature coefficient of the secondary phase but the primary stage goes to completion and the milk will then coagulate quickly when

warmed. . At higher temperatures (above 55-60°C), depending on pH and enzyme) the rennet is denatured.

#### **4-Effects of heat treatments.**

Mild heat treatment such as pasteurization decreases the rate of the secondary stage. coagulation is prolonged or prevented by preheating milk at temperatures above about 70°C (depending on the length of exposure). The effect is due to the interaction of  $\beta$ -lactoglobulin with K-casein via sulphhydryl-disulphide interchange reactions; both the primary and, especially, the secondary phase of coagulation are adversely affected and during heat treatment calcium and phosphate move from ionic soluble to non-ionic colloidal (insoluble) form which decreases the rate of the secondary stage (caseins aggregation) .

#### **Rennet substitutes: -**

Due to

1-Increasing world production of cheese and the decreasing supply of young calf stomachs , the supply of calf rennet has been insufficient.

2-Some countries such as India the religion restrictions prevent the slaughtering of calves

3- The production of coagulant from calves is not economic.

Above cause led to a search for suitable and cheap calf rennet substitutes. There are many sources of enzymes, ranging from animal, plants, fungi, and microbial sources, that will substitute for calf rennet.

### **1-Animal: -**

Such as pig, cow, sheep, Goat and chicken Pepsin. It acts much like bovine chymosin, but the pH of the milk has to be slightly decreased to achieve rapid clotting. This type of coagulants coagulate sweet milk at pH 6.6 difficulty, this is one reason why it is used in cheese making at a 1:1 ratio with rennet.

### **2-Plant:-**

Several plants proteases exist, including ficin from fig juice , papain from papaya tree and bromelin from pineapple. It was reported that most plant proteases are strongly proteolytic (have low MCA to PA ratio) and cause extensive digestion of the curd. This has resulted in reduced yields, bitter flavors and pasty-bodied cheese. However coagulant from the flower of thistle (*Cynara cardunaculus*) it has been used successfully for many years in Portugal to make native ripened Serra cheese. In India, enzyme derived from (*Withania coagulans*) has been used successfully for cheese making .In Sudan, enzyme derived from (*Solanum dohium* ) is used for making of a soft cheese (Jibna beida).

### **3-bacterial coagulants:-**

Some bacteria including *Bacillus subtilis* ,*B. cereus*, *B. coagulans*,, *B.polymyxa* are produced rennet like enzyme .The bacilli enzyme preparations are not suitable for cheese making because of extensive proteolytic activity .This has resulted in bitter flavors cheese.

### **4-Molds:-**

Nowadays microbial coagulants (from molds) cover a considerable part of the demand for of coagulating enzymes worldwide. Microbial coagulants are produced

by fermentation. Most widely applied are enzymes from *Rhizomucor miehei*, *Rhizomucor pussilus*, and *Cryphonectria parasitica* ), these coagulants are commercially available in the cheese making industry and the best calf rennet substitutes.

### **Requirements of suitable coagulating enzymes**

- 1-High ratio of clotting to proteolytic activity (C/P).
- 2-Proteolytic specificity. Structure and flavour of ripened cheese depends on the type of proteolysis caused by the coagulant during cheese curing.
- 3-Stable and able to coagulate milk at the normal pH of milk .
- 4-The coagulant must be eliminated by pasteurization to prevent proteolysis in products made from whey.
- 5-Distribution between curd and whey. Only 0-15% of rennet remains in the curd, but small amounts of residual rennet are significant to ripening of aged cheese.
- 6-Low price.
- 7-High storage stability.