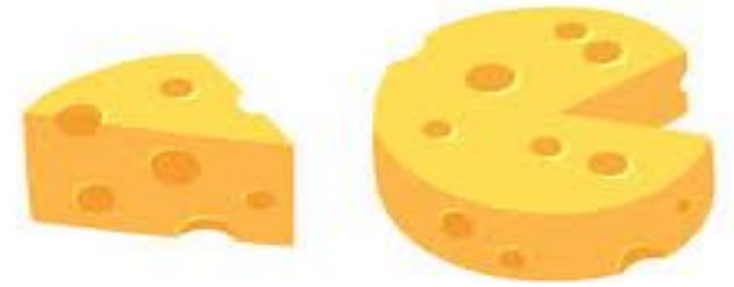


A close-up photograph of various cheeses. In the foreground, there are several small, round mozzarella balls on a wooden cutting board. Behind them are larger wedges of cheddar, a wedge of brie with its characteristic white rind, and a piece of blue cheese with dark mold. The background shows more cheese, including a large wheel of cheddar and a wedge of Swiss cheese with holes.

CHEMISTRY OF CHEESE

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Introduction:



- ❖ Cheese is one of the oldest foods of mankind.
- ❖ In modern times over 400 varieties of cheese are available. They are named on the basis of their characteristics features.
- ❖ All the varieties are grouped into soft, semi hard, hard and very hard type, based on the moisture content of the cheese.
- ❖ Cheddar Cheese is one of the most important variety of cheese. Originated in south-eastern England.
- ❖ Starting from receiving the milk, cheddar cheese manufacturing involves almost twenty stages.

- In the final stage, the fresh cheese is dipped in a liquid paraffin at 104 – 121°C to give on it a coating and allowed to remain in a cold room (ripening room) at 0-4°C for 3 to 6 months.
- When the cheese remains in cold room, the process is known as curing/ripening/souring/maturing.
- During this process the cheese undergoes various physico- chemical and biological changes.
- In the early stages of ripening the cheese is called green cheese.



Biochemical changes in cheese making

- **Flavour Development**

- The mild acidic taste and aroma of green cheese is converted to the characteristic flavour of ripened cheese. During ripening the amino acids of paracasein are liberated as water soluble extract which possess characteristic cheese flavour.
- Alpha amino butyric acid, tyramine are the compounds responsible for flavour generation.
- Diacetyl, a compound produced during citric acid fermentation produces acid flavour.
- Acetic acid, propionic acid, butyric, caproic, capric, caprylic acids, Keto acids and natural carbonyls are also responsible for cheese flavour.

Flavour development

- Hydrolysis of fat by lipolytic enzyme also causes the flavour development in cheese
- Starter bacteria producing lactic acid, propionic acid also contributes towards flavour development
- Bacterial proteinases, lipases and decarboxylases are active at the pH of hard rennet cheese and their end product like amino acids, amines and fatty acids are important as flavour compound.

Body and Texture

- A gradual change happens from rubbery body green cheese to mellow and waxy body in ripened cheese. This is due to loss in moisture content during ripening. Which can be controlled by the temperature of the ripening room and humidity.
- An increase in acidity and decrease in pH takes place. Lowers up to pH 5.10 to 5.05
- Lactose content completely disappears. In some type of cheese, gas production is there.
- During the first few months, organism like starter culture like *S. lactis* predominates, later *Lactobacilli* and other form also dominates.

Chemical Changes

- **Protein:**

1. The main change in protein content in cheese during ripening is to convert the insoluble nitrogen to soluble nitrogen.
2. About one third of the total protein of cheese is converted to soluble form in well ripened hard cheese.
3. The insoluble proteins are converted to products like proteose, peptone, polypeptides, amino acids and ammonia.
4. The proteolysis is induced due to the enzymes derived from micro-organism added as starter, micro-organism present in milk, enzymes present in milk and rennet.
5. The rate of proteolysis increases with increase in temperature of curing, rennet quality, moisture content, less salt, large size of cheese and moderate amount of acid

6. Coating the cheese with paraffin wax reduces the moisture loss in this case.
7. Ripening of cheese is measured in terms of percentage of water soluble nitrogen.
8. Increase in amino acid, amines and volatile acid correlates with gradual increase in cheese flavour.

- **Lactose:**

1. Lactose is converted to lactic acid which is further converted to propionic acid and acetic acid. These acids prevent micro organism from growing.

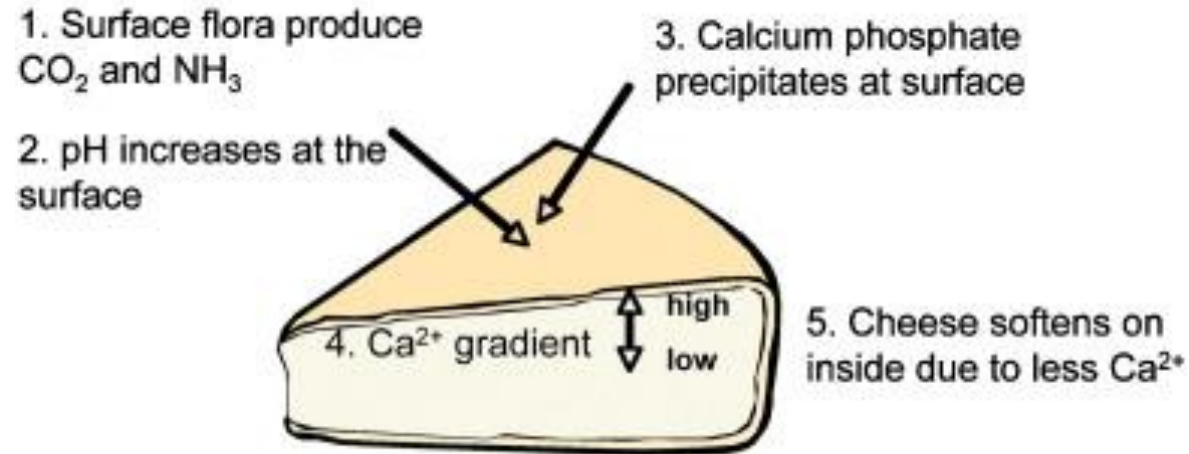
2. Lactose fermenting bacteria, yeast, molds contain an enzyme lactase (β -D-galactosidase) which hydrolyses lactose to glucose and galactose.

3. This hydrolysis can also take place by action of mineral acids such as hydrochloric acid, lactase enzyme present in milk itself and ion exchange resins that is sulfonated polystyrene resins.

4. There are six major types of fermentation reaction in milk like lactic acid, propionic acid, citric acid, alcohol, butyric acid and coliform gassy fermentation.

Bio chemical changes in cheese

After the supply of lactate is exhausted...



Overall increase in pH over time increases plasmin-induced proteolysis

Ripening driven by pH and Ca^{2+} gradients, from the outside in

- **Lipids:**

1. The lipid fraction of cheese contributes more to the development of flavour than any other component. The lipolytic enzymes liberate fatty acids by hydrolysis of the fat during ripening.
2. Amount and type of fatty acids produced determines the flavour and off flavour of cheese.
3. There are three sources of lipases. Those present inherently in normal milk, microorganisms involved in the ripening process and some lipases present in rennet preparations.
4. Lipases are activated by homogenisation and to some extent by agitation, foaming and even changes in temperature.
5. Because of the lipolysis, unclean flavour, bitterness and rancidity is produced.
6. Inoculating lipolytic bacteria in milk (low grade milk) can also produce rancidity.
7. Milk lipases are inactivated at pasteurization temperature, thus pasteurised milk produces a better quality cheese.

Milk-clotting Enzymes

Plant source:

Enzymes from many plant sources may be used as clotting enzymes in cheesemaking but most of the plant proteases are strongly proteolytic and cause extensive digestion of the curd, resulting in reduced yields, bitter flavors and pasty-bodied cheese.

- 1. Papain:** It has powerful milk clotting activity but it is also highly proteolytic. It requires a free sulfhydryl group for its catalytic activity.
- 2. Ficin:** Cheese made with ficin develops bitter flavor which decreases in intensity during curing
- 3. Others:** Bromelain from pineapple has also been considered as a possible substitute for calf rennet. An enzyme extracted from *Withania coagulans* was also used in the manufacture of Surati and Cheddar cheese. Extracts from the flower petals of *Cynara cardunculus* were used for the manufacture of Serra cheese from sheep's milk by Portuguese farmers.

- **Microbial Enzyme:**

The important microbial source of enzymes are

1. *Bacillus*
2. *Micrococcus caseolyticus*
3. *Streptococcus liquifaciens*

- **Fungi Enzyme:**

Example of fungi are

1. *Syncephalastrum racemosum*,
2. *Cladosporium herbarium*,
3. *Aspergillus group*,
4. *Mucor*

- **Animal Rennet:**

- The source of rennet is from the abomasum (4th stomach) of calf of cow, buffalo, goat, sheep and pig.
- Rennet is best among all type of clotting enzyme as it has a high ratio of clotting to proteolytic power.
- Commercially it is called Hansen Rennet. The factory was first established in Denmark in 1874.
- Apart from this, porcine pepsin and bovine pepsin is also used for cheese making.

Rennet:

- The source of rennet is from the abomasum (4th stomach) of calf of cow, buffalo, goat, sheep and pig.
- Rennet contain two principal enzyme. Renin and Pepsin.

Difference between Pepsin and Renin

Pepsin	Renin
<ul style="list-style-type: none">• pH is 1.8 – 2.7	<ul style="list-style-type: none">• pH is 5.4
<ul style="list-style-type: none">• Pepsin induces proteolysis causes bitterness to cheese	<ul style="list-style-type: none">• Rennin is an extremely powerful clotting enzyme which cause rapid clotting without much proteolysis
<ul style="list-style-type: none">• Pepsin is characteristic digestive enzyme of the weaned animal	<ul style="list-style-type: none">• Rennin is characteristic gastric digestive enzyme of the suckling animals

- The rennet activity can be measured by viscosity change method (Marshall Cup test), Visual precipitation method (Berridge's method), and methods based on proteolysis and NPN release.
- Higher concentration of rennet, the coagulation is faster. Maximum coagulation occurs between 40 – 42°C. There is no coagulation above 65°C or below 10°C.
- Heating of milk delays coagulation because Ca^{++} ions are removed. Other reasons are precipitation of whey protein on casein and rise of pH due to release of CO_2 .
- Acidic milk is more susceptible to coagulation. Higher the concentration of CaCl_2 , faster is the coagulation.

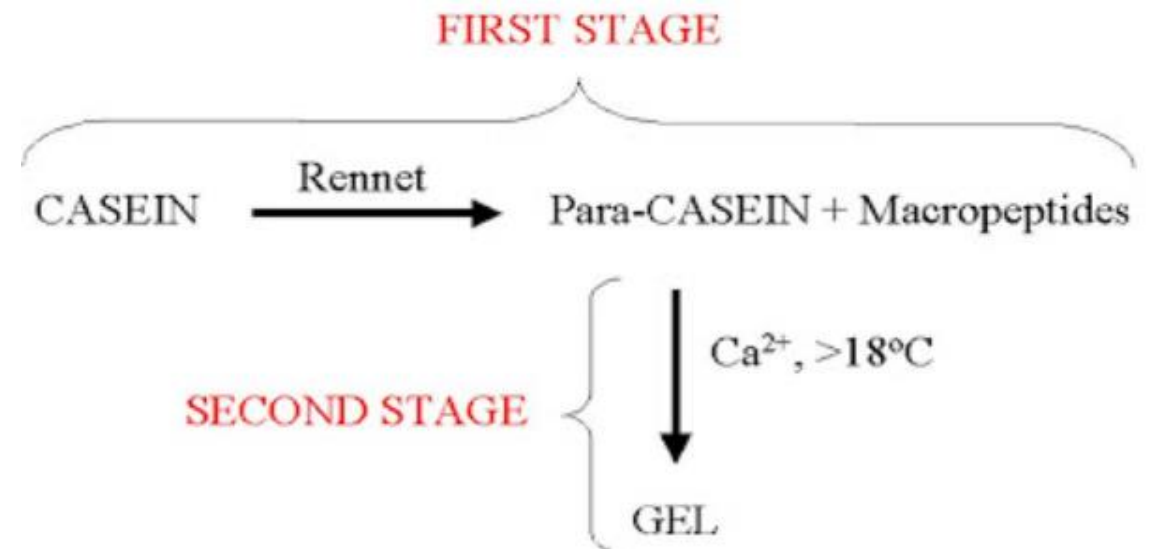
- Homogenization of milk accelerates clotting of milk which may be due to an increase in the degree of aggregation of casein micelles but decrease in curd tension.
- Inhibitors like albumin and globulin retard coagulation while peptone prevents clotting. Various other factors such as composition of milk, aging of milk, use of antiseptic and pH of milk also play a role in coagulation.

Mechanism of rennet action in cheese making

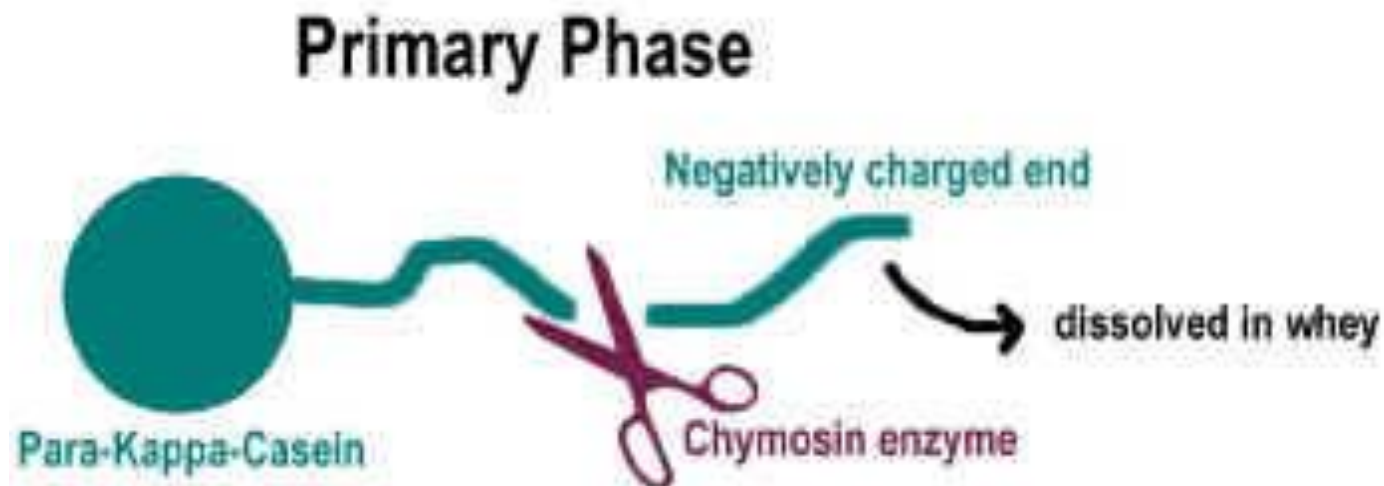
- In cheese making, enzymatic coagulation of milk involves modification of the casein micelles via limited proteolysis of kappa-casein by proteinase preparations followed by Ca^{2+} -induced aggregation of the rennet-altered micelles.

In cheese preparation, milk clotting occurs in two or three separate phases.

- **Primary Phase:** The enzyme rennet attach K-casein to destroy its stabilizing capacity.
- **Secondary Phase:** non enzymatic phase in which the destabilized system clots in the presence of calcium ions and the slow general hydrolysis components followed by clotting.



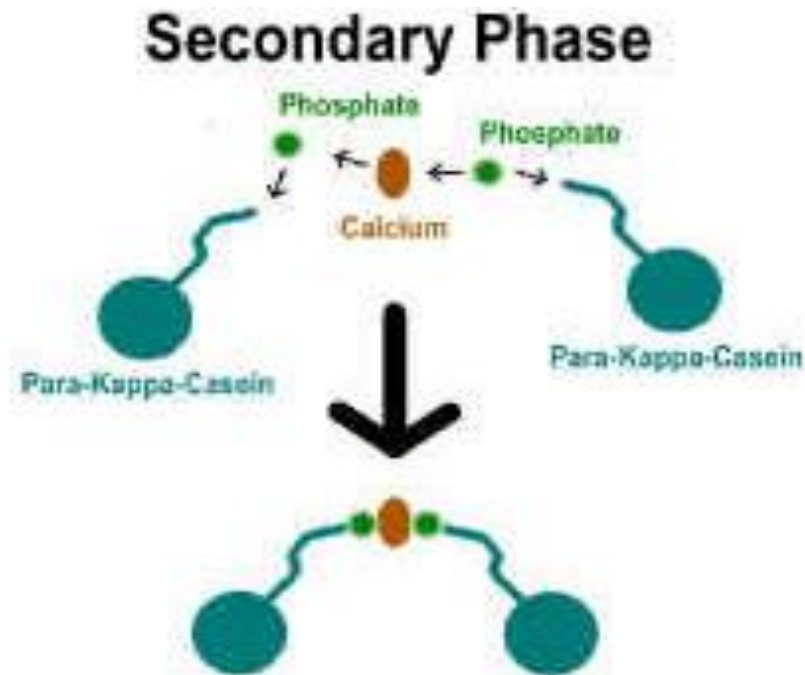
- **Primary Phase:**
- Kappa-Casein is the only casein hydrolyzed during rennet coagulation. kappa-Casein is hydrolyzed at its Phe105-Met106 bond to produce para-kappa-casein (kappa-casein fragment 1-105) and glycomacropeptides (kappa-CN f106-169).
- Glycomacropeptides diffuse into the aqueous phase; para-kappa-casein remains attached to the micelle core.



Secondary Phase:

Removal of the macropeptides from micelles reduces zeta (surface) potential of the micelles from -20 to about -10 mV and also removes the steric stabilizing layer.

When about 85% of total kappa-casein is hydrolyzed, colloidal stability of the micelles is reduced so much that they coagulate at temperatures above about 20°C in the presence of Ca^{2+} .



Thank you