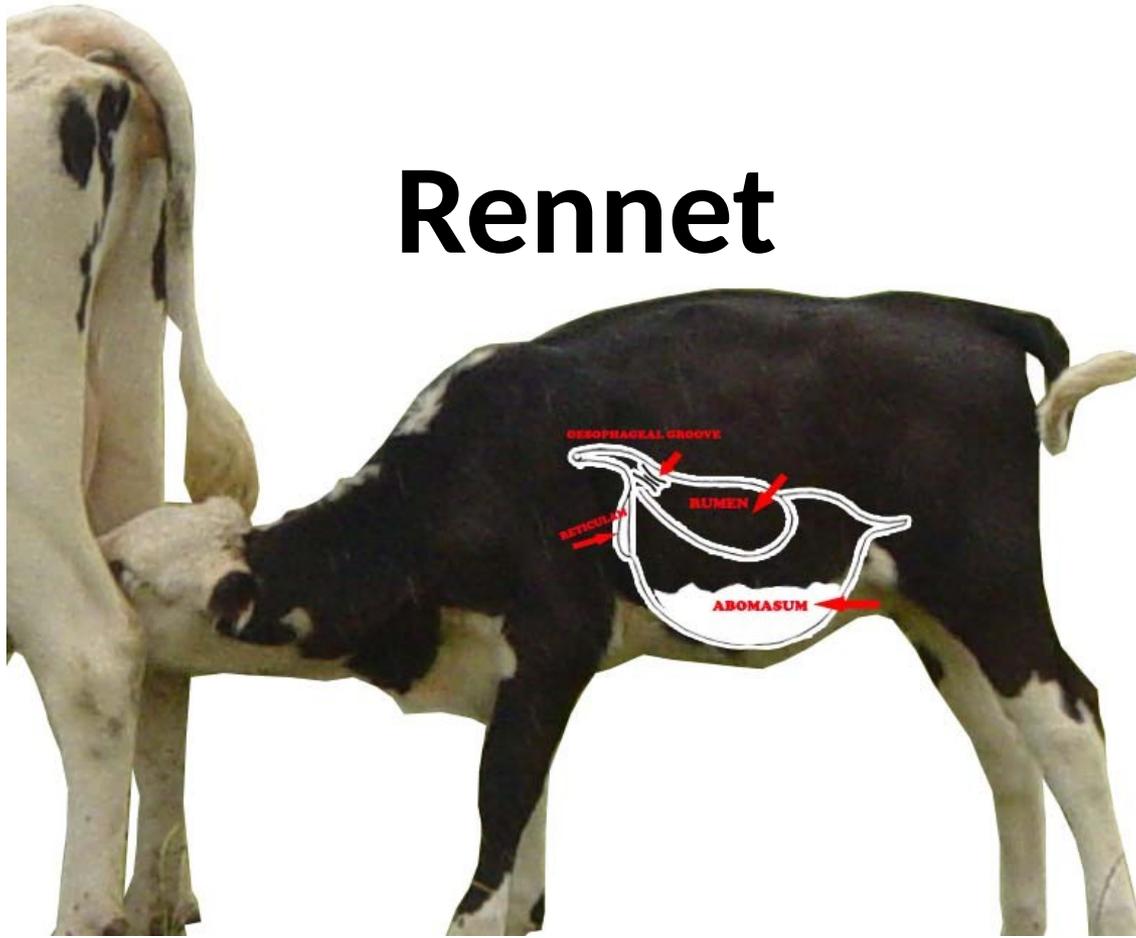


# Rennet



# Rennet

- Milk coagulation is a basic step in cheese manufacturing.
- Calf rennet, the conventional milk clotting enzyme obtained from the fourth stomach of suckling calves was the most widely used coagulant in cheese making all over the world to manufacture most of the cheese varieties.
- The worldwide reduced supply of calf rennet and the increase in cheese production and consumption have stimulated research for milk-clotting enzymes from alternative sources to be used as calf rennet substitutes.
- Further, if calf rennet is used as a coagulant for cheese making, the product has to carry a tag of non-vegetarian which may lead to non-consumption of cheese by vegetarian population of the world.

# Desirable Characteristics of Rennet Substitutes

- ✓ The ratio of milk clotting activity to proteolytic activity should be high. This means that the milk should clot without much proteolysis i.e. breakdown of proteins to peptides. This prevents excessive nonspecific proteolysis during manufacture and hence protects against a weak gel structure, high losses of protein and fat in the whey, and reduced yields of cheese solids. Moreover, it avoids excessive proteolysis during maturation and thus ensures the correct balance of peptides of different molecular weights and hence desirable flavor, body, and functional characteristics in the ripened cheese.
- ✓ It should be thermally stable (comparable to calf rennet) at pH and temperatures used during cheese making. This influences the level of residual rennet in cheese and hence influences the biochemical changes that take place during ripening.
- ✓ It should have low thermal stability at temperatures of whey processing otherwise it may hinder the utilization of cheese whey in various products.
- ✓ It should impart desired flavor, body, and texture characteristics to the finished cheese.

**Milk-clotting Enzymes from Plants:** Enzymes from many plant sources may be used as clotting enzymes in cheese making 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.

**Papain:** The latex of the plant *Carica papaya* yields papain and several other proteases. It has powerful milk clotting activity but it is also highly proteolytic.

**Ficin:** Ficin is present in the latex of several species of the genus *Ficus* (fig) such as *Ficus glommarata*, *Ficus religiosa* and *Ficus carica* but the best source is *Ficus carica*. Cheese made with ficin develops bitter flavor which decreases in intensity during curing.

**Others:** Bromelain from pineapple has also been considered as a possible substitute for calf rennet. An enzyme extracted from *Withania coagulans* also used in the manufacture of Surti and Cheddar cheese. Extracts from the flower petals of *Cynara cardunculus* used for the manufacture of Serra cheese from sheep's milk by Portuguese farmers.

# Microbial Rennet

- ✓ A large number of microorganisms are known to produce milk clotting enzymes. The possibility of a few of the microorganisms proving successful as rennet substitutes may appear from the fact that they play an important part during the manufacture of cheese.
- ✓ Microbial enzymes are known to exhibit considerable variations in the range of activity, substrate specificity and mode of action. Even more important is the fact that they can be produced economically on any desired scale.
- ✓ Many hundreds of bacterial and fungal cultures have been investigated for milk clotting enzymes and their proteolytic abilities.
- ✓ Milk clotting enzymes from bacteria like *Streptococcus liquefaciens*, *Micrococcus caseolyticus*, *Bacillus cereus*, *B. polymyxa*, *B. mesentericus*, *B. coagulans* and *B. subtilis* have been used as coagulating enzymes. Milk clotting enzymes from fungi are also used during cheese making. Some of the fungi producing such enzymes are *Aspergillus nidulans* and *Cladosporium herbarum*.

## Micoorganisms used in commercial production of microbial rennet

<b>Name</b>	<b>Source of Enzyme</b>
Hannilase	<i>Mucor miehei</i>
Rennilase	<i>Mucor miehei</i>
Fromase	<i>Mucor miehei</i>
Miki	<i>Mucor miehei</i>
Marzyme	<i>Mucor miehei</i>
Novadel	<i>Mucor pusillus</i>
Noury	<i>Mucor pusillus</i>
Meito	<i>Mucor pusillus</i>
Emporase	<i>Mucor pusillus</i>
Suparen	<i>Endothia parasitica</i>
Mikrozyme	<i>Bacillus subtilis</i>
Sure curd	<i>Endothia parasitica</i>

- The protease of the *Mucor miehei* coagulants degrade casein fairly rapidly in the pH range 5.5 -7.0; but in spite of this, the incidence of bitter cheese using this enzyme is low. The enzyme is very sensitive to temperatures in the region 37-45°C and is destroyed at 70°C. This enzyme is used successfully for many types of cheese.
- *Mucor pusillus* extract is also used as microbial rennet. It is highly proteolytic than calf rennet or the *Mucor miehei* extract. It tends to give hard curds because of its high proteolytic activity and the curd tends to lose fat into the whey, thereby giving lower yield as compared to others. Thus, this enzyme is usually used in combination with other enzymes.

## Recombinant chymosin

- ✓ Many rennet substitutes are used for cheese making but many of these proteolytic enzymes from microbial or plant origin cause flavor, texture and yield changes in certain types of cheese that are different than those produced by calf rennet. Further, they are not suitable for long ripening cheeses as they have a different range of non-specific activities than chymosin and do not produce the correct flavors on prolonged ripening.
- ✓ Recombinant rennet/chymosin can be prepared by gene transfer technology in which the bovine chymosin is cloned in a suitable production strain and the enzyme is produced by fermentation.
- ✓ This enzyme can be isolated and used in cheese making as a coagulant having properties similar to that of calf rennet.
- ✓ *E. coli*, *Kluyveromyces lactis* and *A. niger* var. *awamori* have been successfully used for production of calf rennet.
- ✓ In addition to the benefit that such chymosin can be produced in large-scale fermentors at low cost, recombinant and highly pure chymosin also has some other advantages such as specific, low proteolytic activity, predictable coagulation behavior and vegetarian approval.