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Rennet coagulation of milk

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Cheese

- Coagulation of casein and trapping fat globules in the formed structure is one of the most important technological ideas of the food fermentations
- Coagulation is achieved by adding specific proteolytic enzymes (rennet) to the milk
- Cheese structure is built up from casein particles

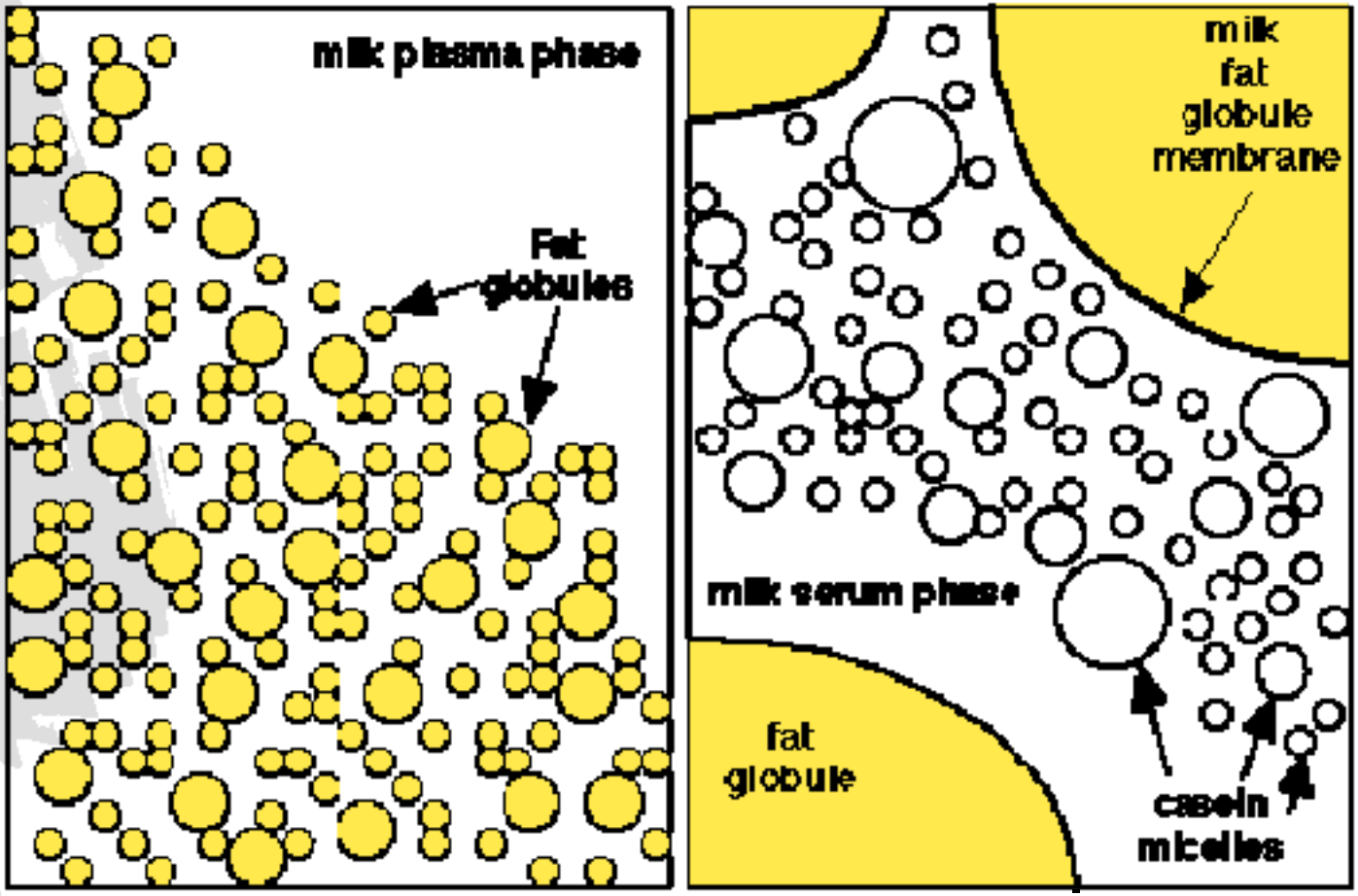
Milk, the main constituent of cheese, is a complex biological fluid

- It contains all nutrients for newborn, about hundred thousand different substances
- Milk composition is variable, depending on the breed, season, climate, health of animal etc.
- Caseins are the most important constituents from cheese making viewpoint

Milk

- Milk is the emulsion of fat globules and suspension of casein micelles in water.
- Milk also contain different bacteria
- Milk proteinases plasmin and cathepsin D are also bound into micelles structure

Milk structure



Main components of milk, changes in content

- Protein
- Fat
- Lactose
- Minerals
- Water

- Breed, genetic selection
- Feeding
- Climate
- Technological process

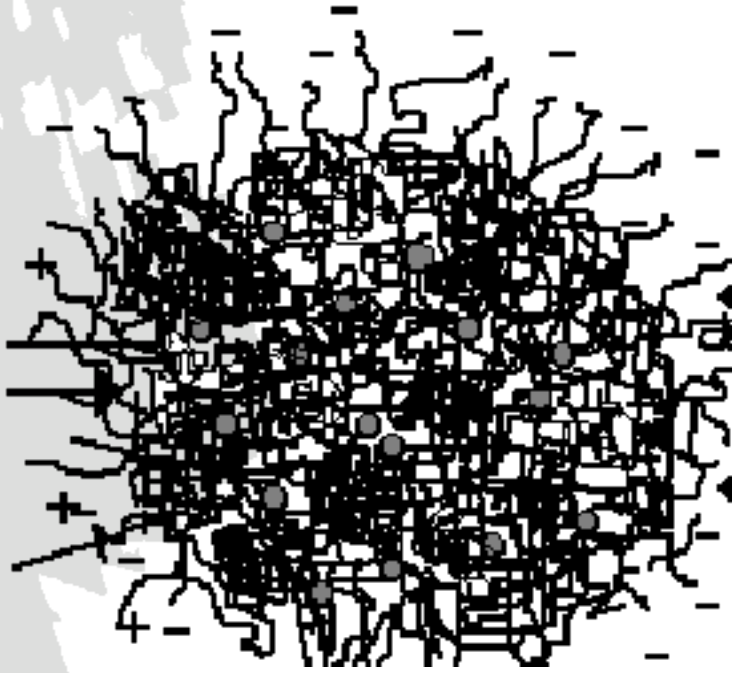
Milk protein composition

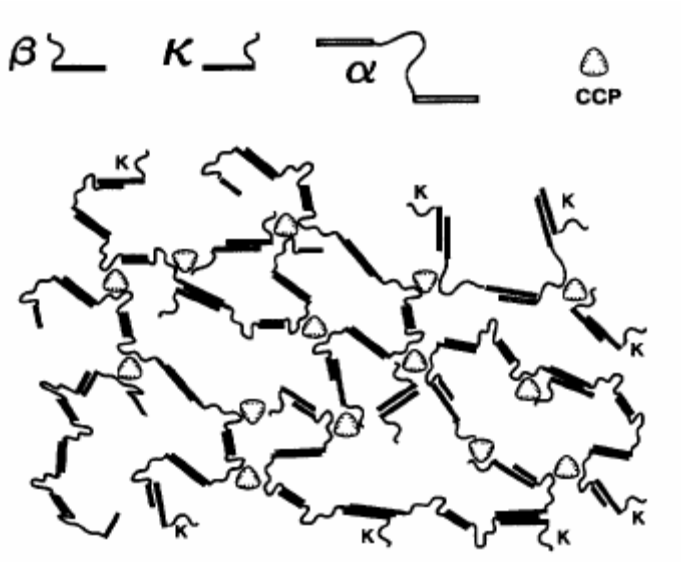
Fraction	%.
αs1 -casein	34-40
αs2 -casein	11-15
β-casein	25-35
κ-casein	8-15
β-laktoglobuline	7-12
α-laktalbumine	2-4
Serum albumine	0.5-2
Immunoglobulines	
Enzymes	

Structure of caseins determines the peculiarities of proteolysis

- Caseins are phosphoproteins, M~20kD, synthesized in mammary gland
- Bovine casein particles are built up from four proteins
- Caseins have low levels of secondary and tertiary structures. In bovine milk about 90% of casein exists as macromolecular aggregates termed micelles, with molecular weight about 10^8 kD and mean diameter of 200nm.

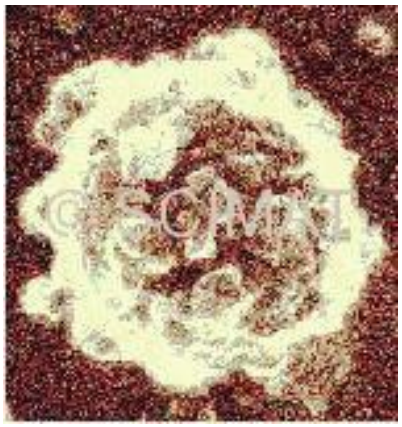
Models of caseins proposed by Holt(1992) and Horne(1998)





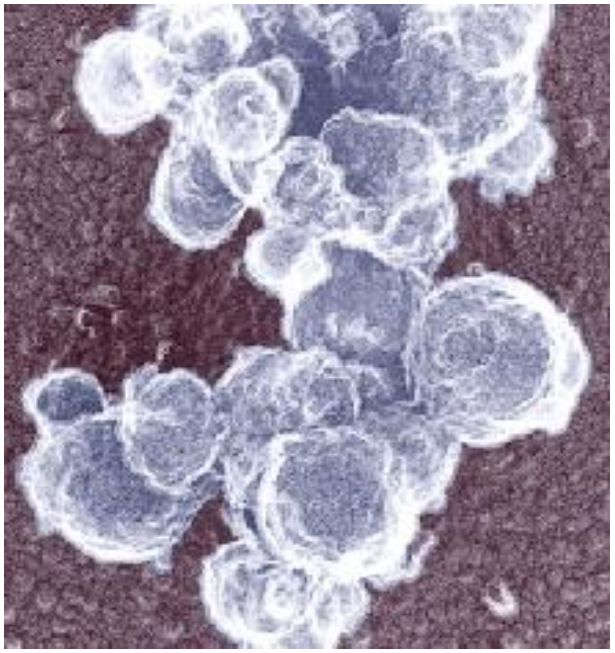
Casein particles

- Contain on dry weight basis 94% of protein and 6% of minerals
- Micelles are hydrated containing up to 3.3 g water per gram of protein
- Micelles are porous occupying about 4 ml/ g
- Native casein micelles are quite stable during technological treatment



Coagulation

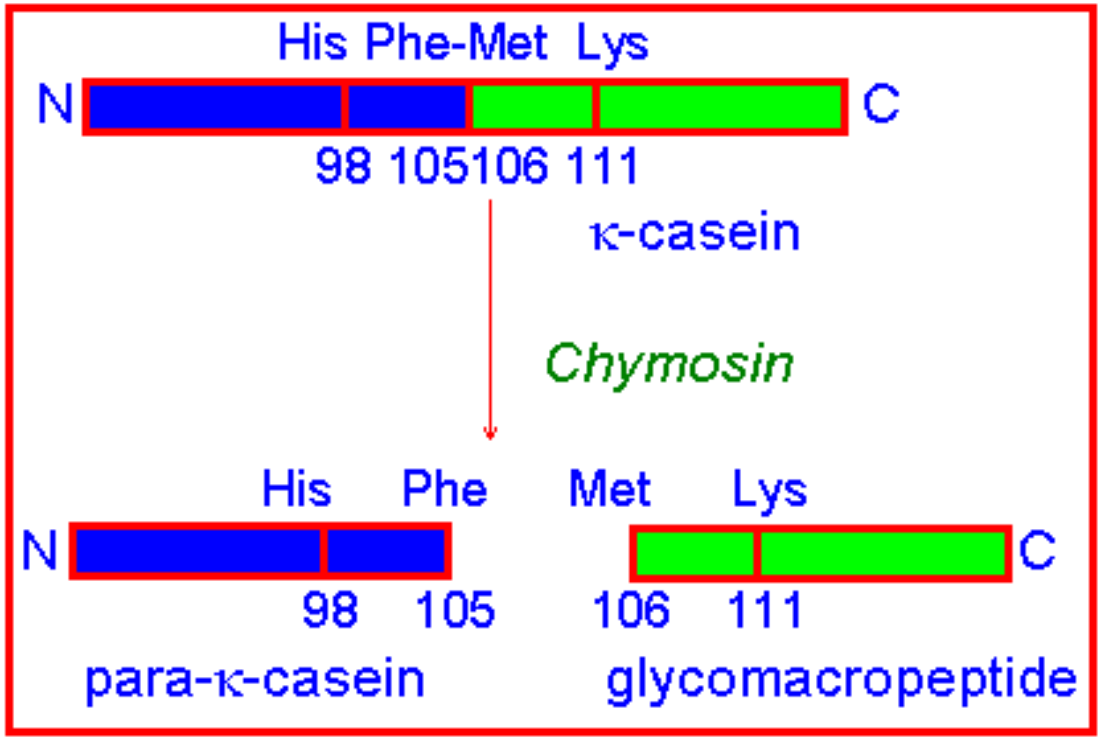
- It happens when the casein micelles stick together
- Casein micelles are hydrophobic and their natural tendency is to aggregate
- In normal milk this process is prevented by glucomacropeptide and negative charge on the micelles



Enzymatic coagulation

- The primary phase of rennet coagulation involves the specific enzymatic modification of casein micelles
- Aggregation of the rennet- altered micelles is the secondary phase of coagulation

Chymosin attack, 1-st step of milk coagulation

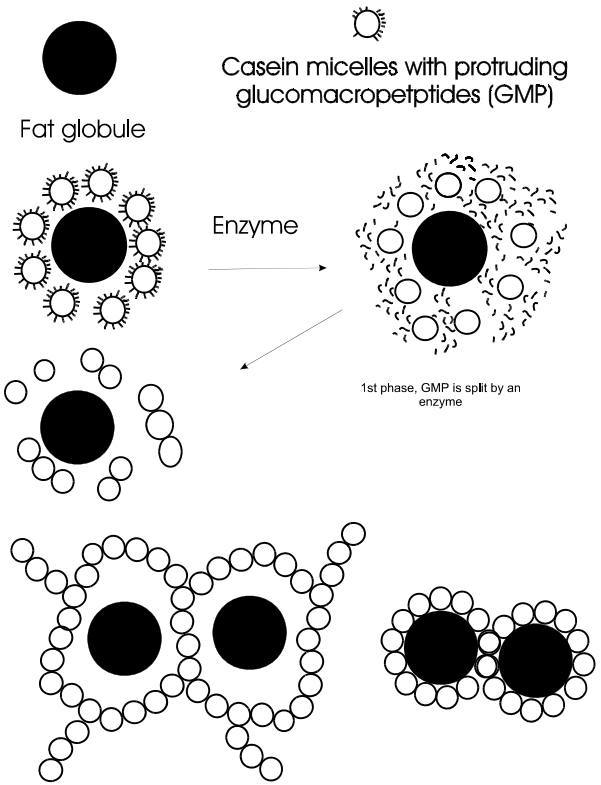


Rennet

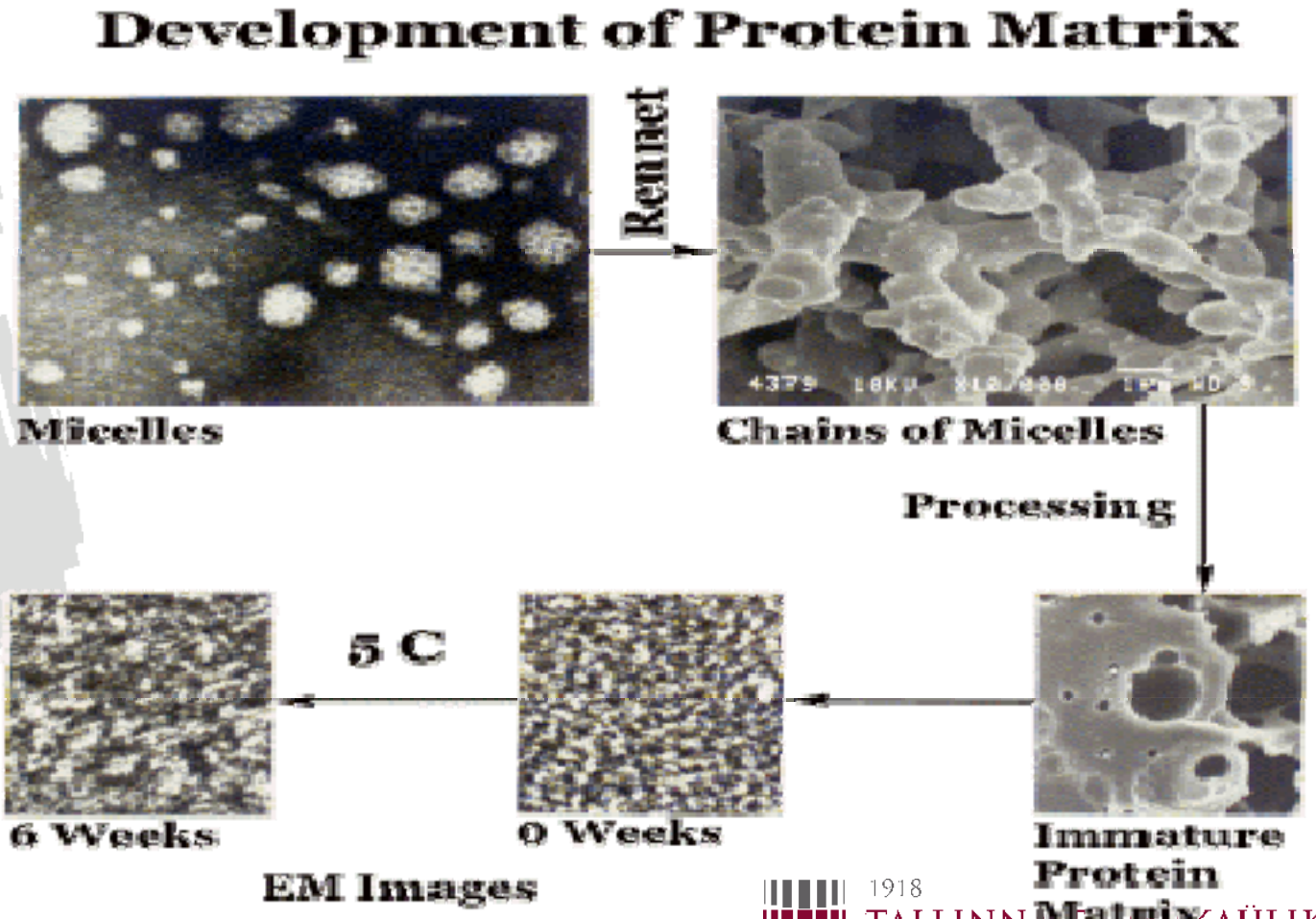
- Several proteinases will coagulate milk under suitable conditions, but most of them are too proteolytic
- Chymosin is the best, most of it is produced by microorganisms today, for example ChyMax (Chr. Hansen)
- Bovine pepsin mixed with chymosin is also used (Stabo)

Start of aggregation

- Rennet coagulation follows the specific hydrolyses of micelle stabilizing surface layer during this step glucomacropeptide is lost
- At the natural pH of milk (6.7), about 80% of κ-casein must be cleaved to permit aggregation of the micelles
- After loosing its water-soluble tail κ-casein can no longer keep the casein particles separated, the diameter of casein micelles is reduced 7-10 nm



Changes in protein structure, 1-st day



Formation of three dimensional structure

- The casein clusters continue to grow until they form a continuous three dimensional network which traps water, fat and bacteria
- The strength of the gel must be high enough when mechanical cutting is started

Factors that affect the hydrolysis of κ-casein

- **Acidity.** The pH optimum for first stage of rennet action in milk is about 6.0
- **Temperature**. The optimum temperature for milk by chymosin at pH 6.6 is around 45°C.
- **Ionic strength**
- Protein variants (**Genotype**)

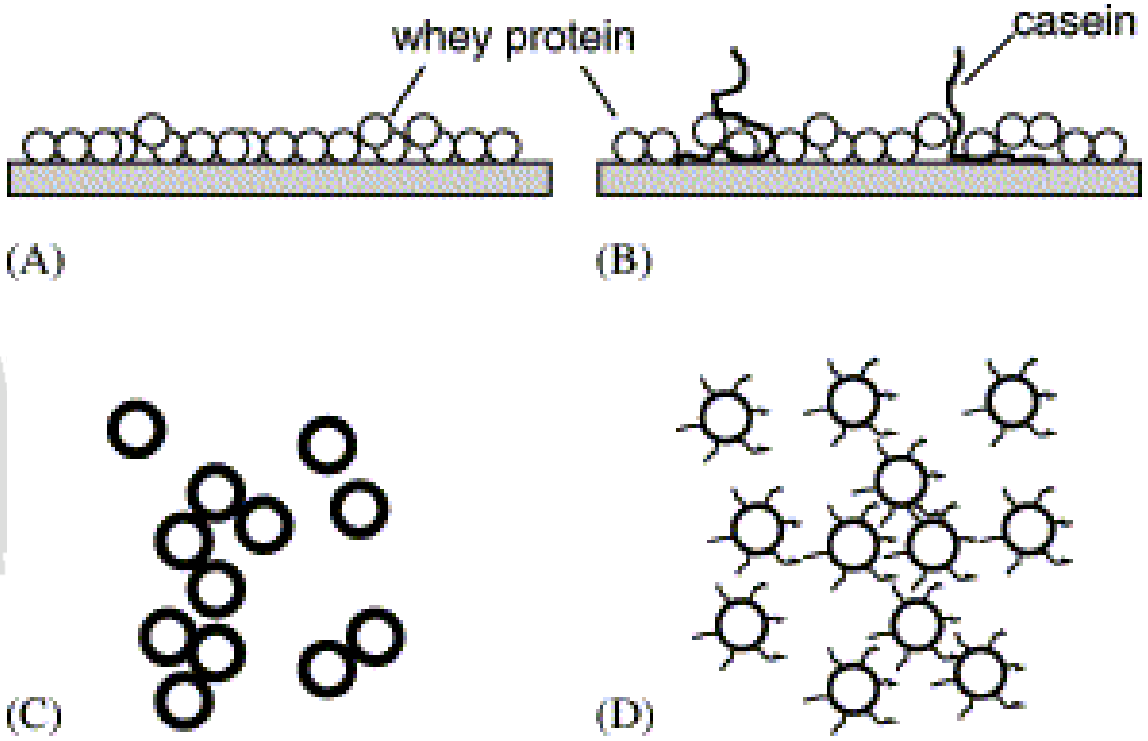
Effect of technological parameters: effect of temperature

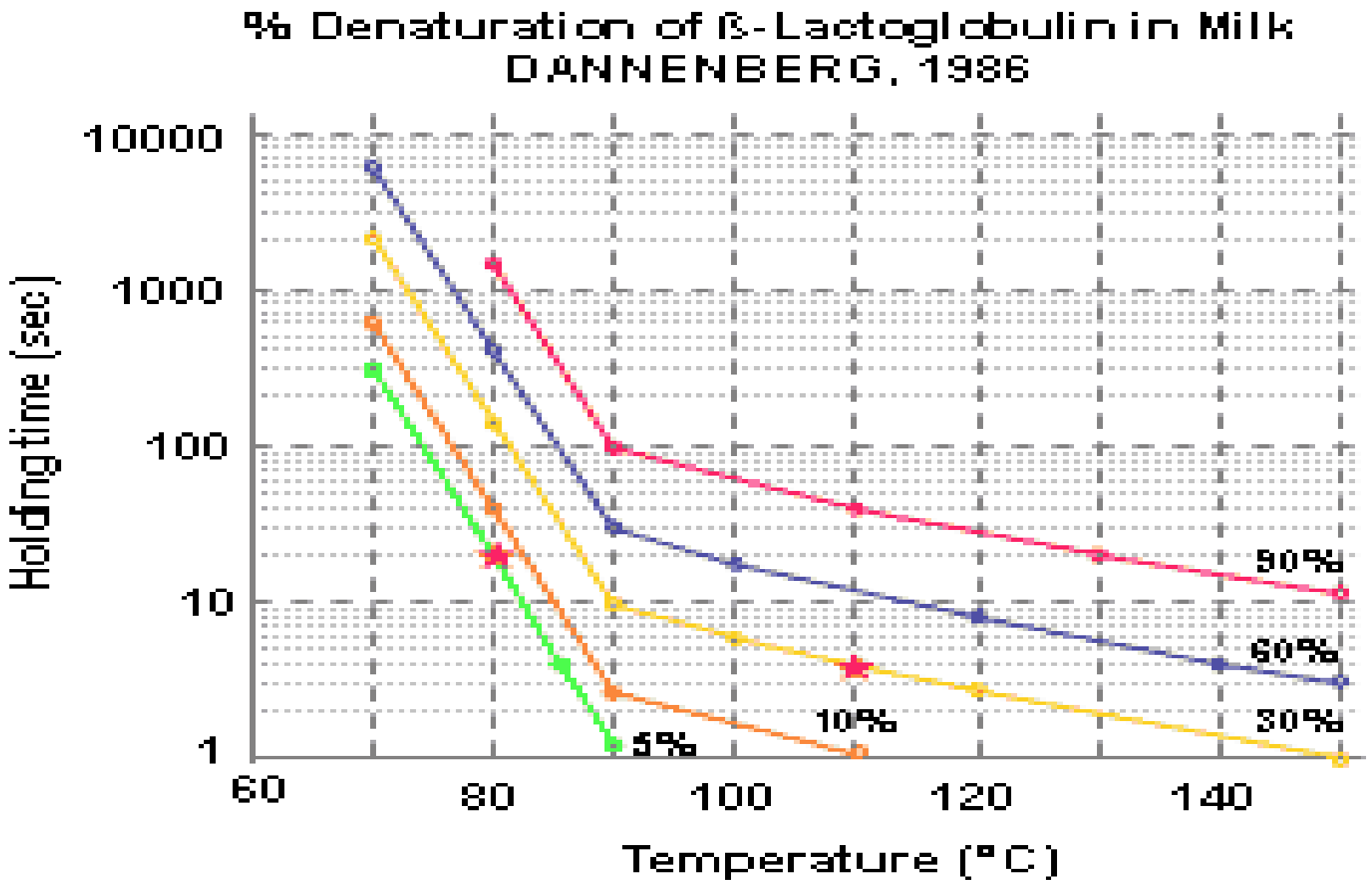
- Optimum coagulation temperature of milk for most cheese varieties is 30-32°C. At the temperature less than 30°C the gel is weak and difficult to cut without excessive yield loss. At temperatures less than 20°C the second stage of renneting, coagulation, do not occur, but the primary stage goes to completion

Heat treatment of milk

- causes denaturation of whey proteins and complex interactions among denaturated whey proteins, casein micelles, minerals and fat globules.
- The interactions of whey proteins with casein micelles interfere with the rennet coagulation process, resulting in long coagulation times and weak curd structure

Depending on pH and milk composition different protein aggregates are present




% Denaturation of ß-Lactoglobulin in Milk
DANNENBERG, 1986
Holding time (sec)
10000
1000
100
10
1
60
80
100
120
140
Temperature (°C)
90%
60%
30%
10%
5%

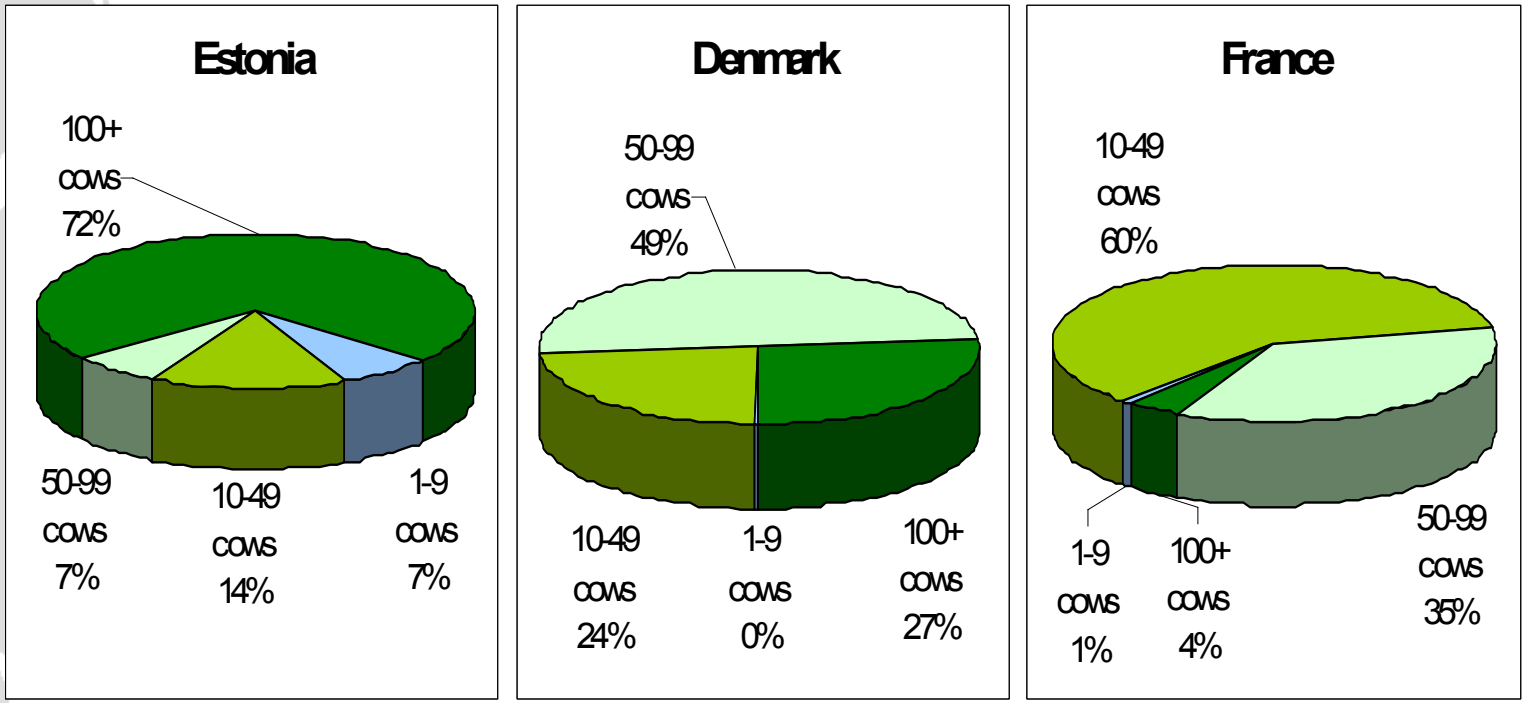
Methods for improving renneting properties of heated milk

- Acidification to pH values below 6.2, acidification reduces charge repulsion of casein micelles
- Rising the temperature slowly and holding milk in the vat with or without starter (preripening)
- Calcium chloride addition. Ca-ions reduce electrostatic resistance of micelles

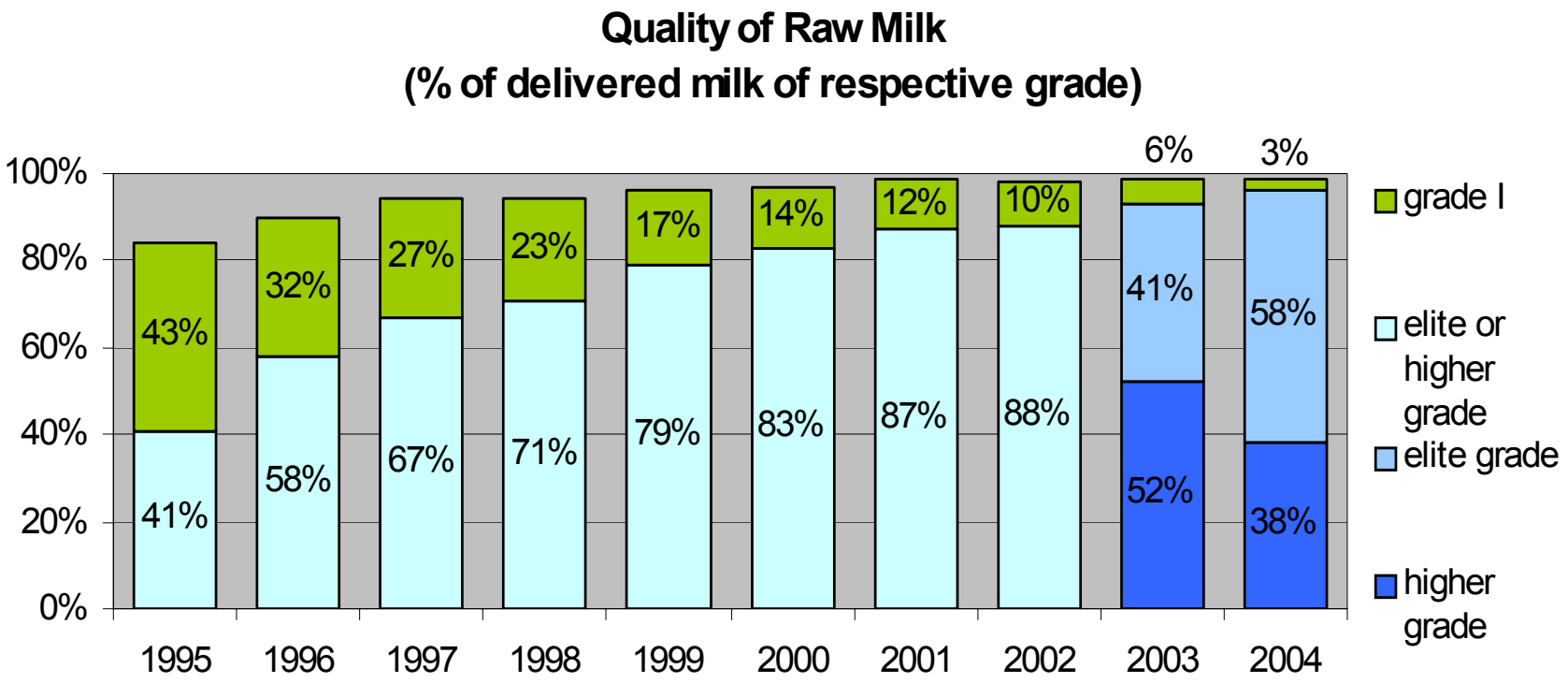
Renneting properties of Estonian farm milk

- The milk production of Estonia is concentrated into big farms
- The number of dairy cows in last ten years has diminished about two times, milk production per cow has raised
- Some farms are already using automatic milking systems

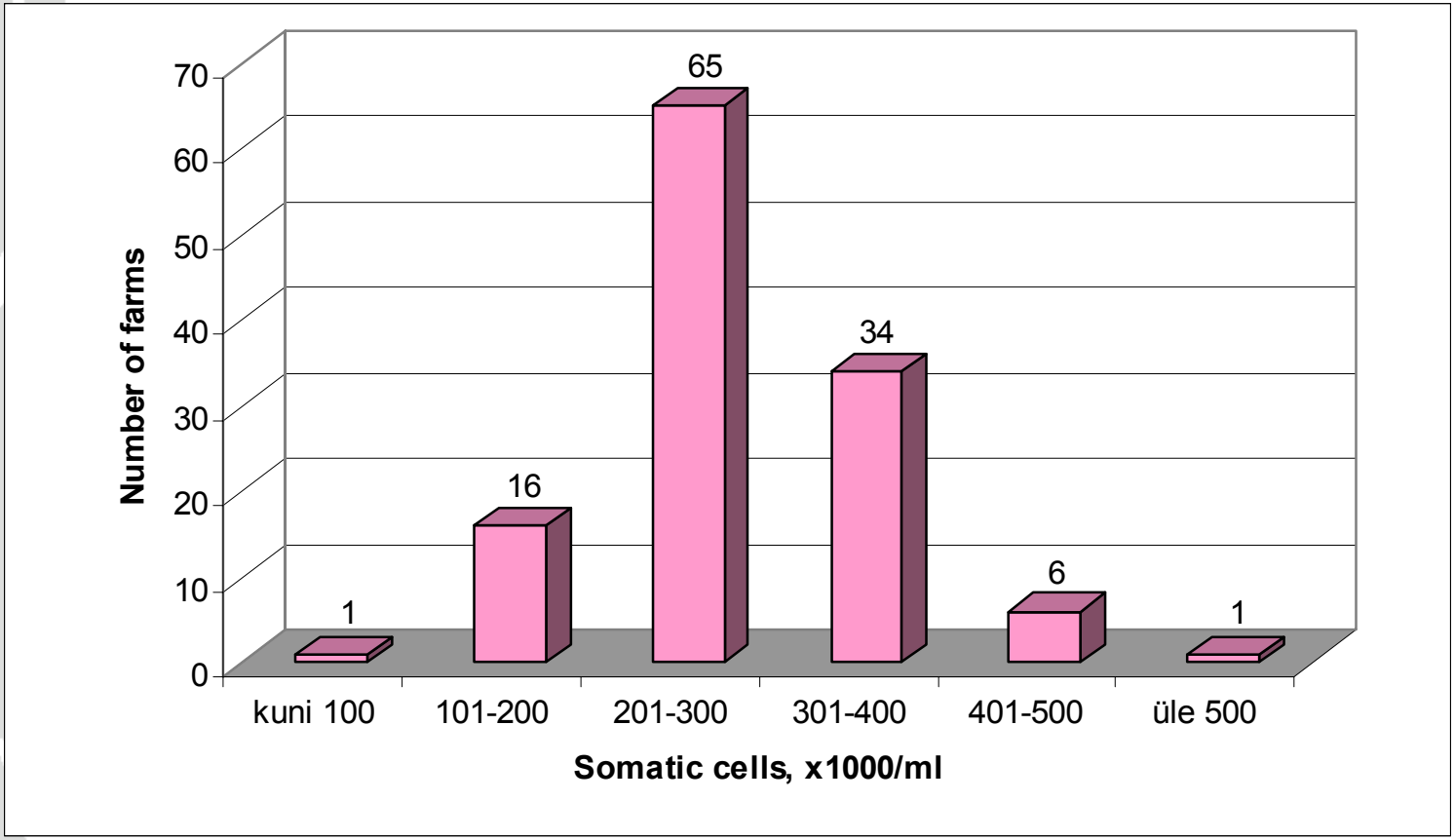
Per Cent of Dairy Cows Living in Herds with the Respective Size



Quality of Raw Milk 2004



Somatic cells in farms milk



Influence of cold storage and mechanical factors (transportation, pumping, etc..)

- Milk is collected by big trucks and distances for transportation are quite long.
- Temperature and duration of cold storage of milk before processing, truck transportation to dairy plant and pumping there through long pipelines also affects technological quality of milk

Influence of thermal treatment and cold storage on renneting

	Rt	Strength,g	Rt, day 5	Strength, Day 5
Raw milk	5.24	18.67	7.15	15.60
65°C	5.51	10.09	6.21	5.75
78°C	8.37	8.15	8.53	6.57
65°C*			6.32	16.74

Cold storage and heating

- Our results showed that storage of raw and pasteurized milk at 0°C for 24 h prolonged rennet coagulation and resulted in weaker coagulum comparing with samples stored at 6°C.
- Raw milk is the best for cheese making, it has the shortest rennet clotting time and highest gel strength, UHT milk did not coagulate during hours.

Conclusions

- Milk for cheese making should be collected daily
- Pasteurization temperature should not exceed 72°C.
- Mechanical treatment (pumping, stirring) has strong effect on clotting time, but the gel strength was almost not influenced
- Residues of disinfectants in tubes and tanks should be avoided, as these could brake down the gel structure and inhibited starters growth
- Milk produced in Estonia is suitable for cheese making