Milk as Nutraceutical

By

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- **Nutraceutical**, a term combining the words "nutrition" and "pharmaceutical", is a food or food product that provides health and medical benefits, including the prevention and treatment of disease. Such products may range from isolated nutrients, dietary supplements and specific diets to genetically engineered foods, herbal products, and processed foods such as cereals, soups, and beverages.

- The term nutraceutical was originally defined by Dr. Stephen L. DeFelice, founder and chairman of the Foundation of Innovation Medicine (FIM), Crawford, New Jersey.

Gross Comparative Composition

Nutrient	Buffalo milk	Cow milk
Dry matter (%)	16.4	12.8
Fat (%)	6.7	4.0
Solids-not-fat (%)	9.7	8.8
Total protein (%)	4.1	3.3
Casein (%)	3.2	2.7
Whey Protein (%)	0.89	0.67

Buffalo milk contain higher casein and whey protein

Milk Proteins

Protein	Amino Acids
Major Protein	
1) Caseins	
α_{s1}-Casein	199
β-Casein	209
κ-Casein	169
2) Whey Proteins	
α-Lactalbumin	114
β-Lactoglobulin	162
Minor Protein	
Immunoglobulins	-
Lactoferrin	689

Nutraceutical Significance of Whey Proteins

- Good source of sulfur containing amino-acids (cysteine and methionine): 8 times more as compared to casein. Possibly this is mode of anticancer activity.
- Exceptionally rich in BCAAs: leucine, isoleucine & valine
- Contains more leucine than in casein, egg and soy protein
- Humoral response of mice, fed to whey protein diet found to be 5 time more than on diet of either only casein or casein enriched with cysteine
- Antioxidant effects of increased glutathione may be basis for increase lifespan, seen with WP

Whey Proteins: β-Lactoglobulin

- Most abundant whey protein: approximately half of the total protein in bovine whey (not in humans)
- Retinol-binding protein also exists within the β-lactoglobulin.
- *In vivo,* after partial digestion by endopeptidases of pancreas it acts as antimicrobial and also inhibits replication of rotavirus in dose dependent manner
- It binds mutagenic heterocyclic amines and exert anti-carcinogenic effect.

α-Lactalbumin

- It is one of the main proteins found in buffalo milk (approximately 20-25% of whey proteins).
- Recently, a folding variant named "BAMLET" from bovine α-La, which is lethal to tumour cells has been discovered. It selectively enters into tumour cells and induces apoptosis.
- α-La was observed to improve performance in stress-vulnerable individuals by increasing brain tryptophan and serotonin activity.
- Clinical trials suggested that α-La improved sleep in human suffering from nutritional disorders.

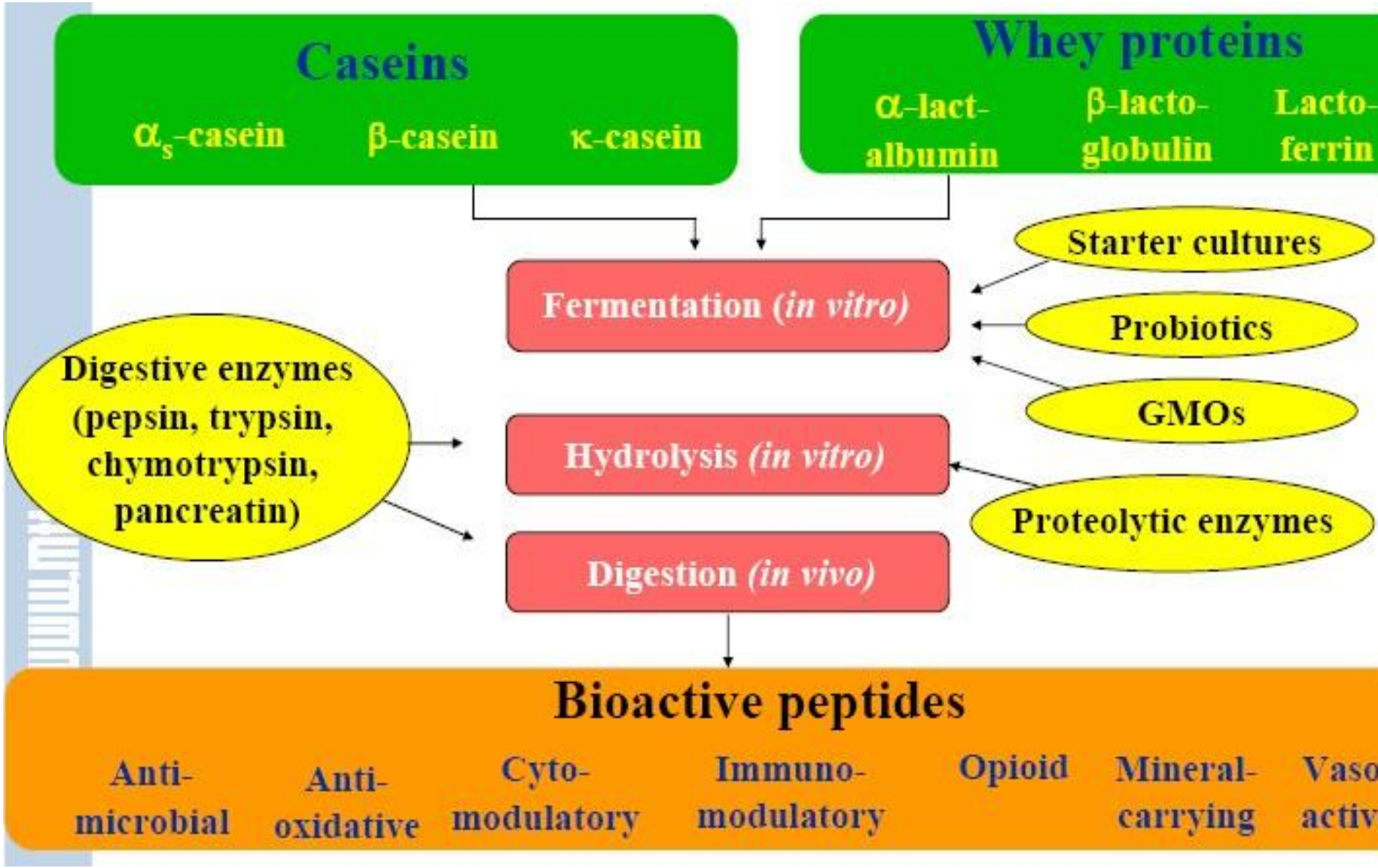
α-Lactalbumin

- Rat trials indicate that α-La protects against ethanol and stress induced gastric injuries in dose dependent manner (optimum dose 200 $mgkg^{-1}$).
- Because of its structurally homology to human protein, purified α-La from buffalo milk is most preferred ingredient in infant formula.
- α-La due to its richness in essential amino acids, ideally suited for fortifying infant formulae.
- Clinical trials with α-La enriched infant formula have shown to exhibit antimicrobial activity also.

Bioactive Peptides

- Bioactive peptides are defined as specific protein fragments from food, that in addition to nutritional value, also exert as nutraceutical/ pharmacological agent.
- Influence multifunctional biological process such as behavior, gastrointestinal, immunological, neurological and nutritional responses

Production of Major Bioactive Peptides from Milk Proteins



Bioactive Peptides from Casein

Casein- Major source of Bioactive peptides

Precursor protein	Bioactive peptides	Regions in the primary structure
β-casein	β-casomorphins (3)	f60-70, f60-66 and f60-64
	β-casokinins (2)	f177-183 and f193-202
	Casein phosphopeptide (4)	f1-28, f2-28, f1-25 and f33-48
α_{s1}-casein	α-casein exorphins (3)	f90-96, f90-95 and f91-96
	α-casokinins (2)	f23-27 and f194-199
	Casein phosphopeptide (4)	f43 -58, f59-79, f1-29 & f46-70
κ-casein	Casoxins (2)	f33-39 and 25-34
	Casoplatelin	f106-116

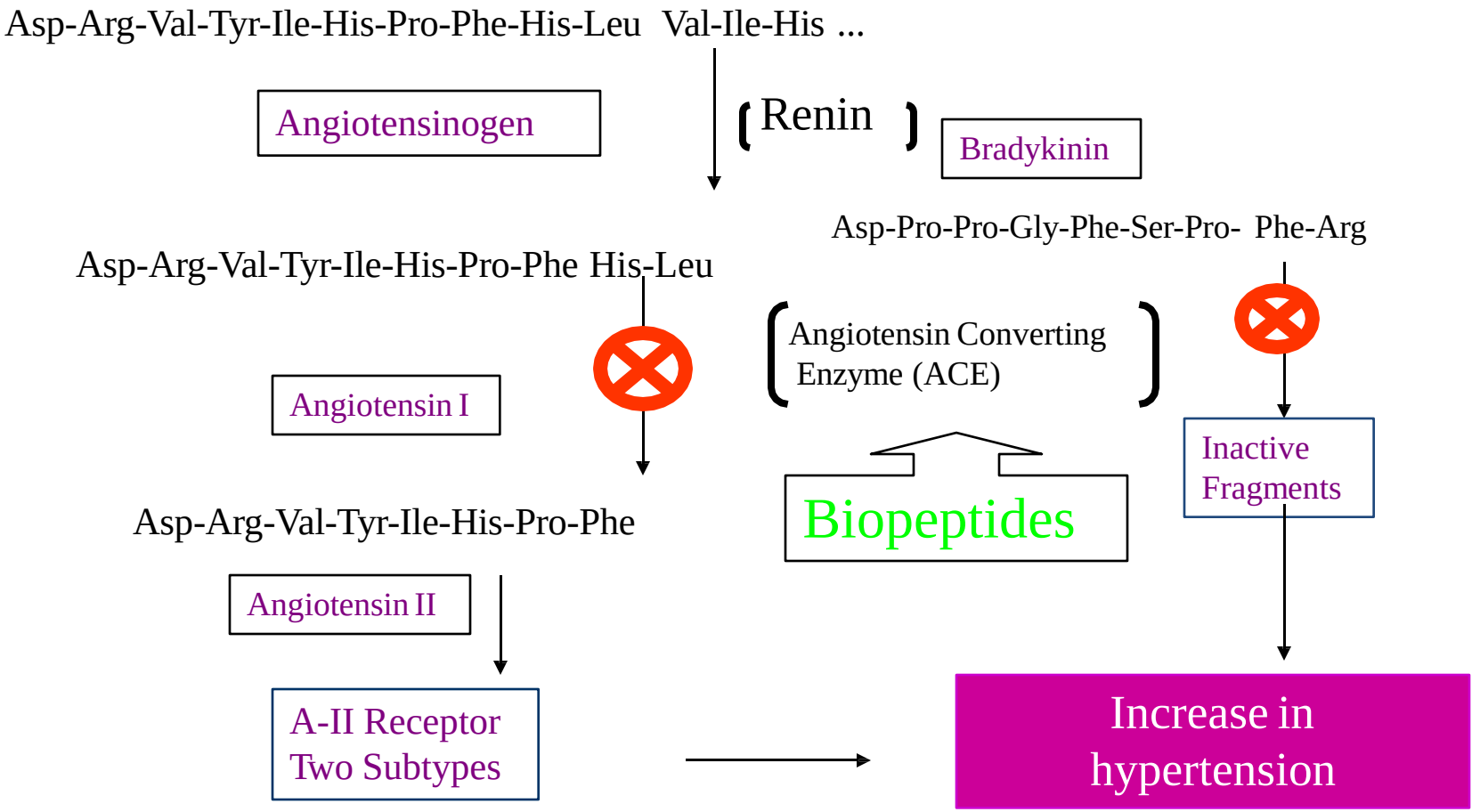
Mineral Binding: Caseinophospho-peptides

- Peptides from α-casein (f43 -58, f59-79, f1-29 and f46-70); β-casein (f1-28, f2-28, f1-25 and f33-48) are major mineral binding peptides
- Produced through *in-vivo* digestion of casein by gastrointestinal proteinases and are relatively resistant to further proteolytic degradation
- Also Produced *in-vitro* by enzymatic digestion with Neutrase, trypsin, chymotrypsin, pepsin and papain
- Caseinphosphopeptides can also be formed during cheese ripening due to plasmin and microbial protease activity

Caseinophospho Peptides for Bone and Calcium Metabolism

- CPPs which contain 2 glutamic acid residues adjacent to serine residue bind calcium
- Form soluble complex with Ca++ and absorb across the intestinal mucosa and prevents formation of insoluble calcium phosphate. Thus, helpful in Vit-D independent absorption
- The excellent bioavailability of calcium from milk and dairy products has, in part, been attributed to the action of CPPs

ACE-INHIBITORY PEPTIDES



ACE Inhibitory Bioactive Peptides

- Peptides derived from milk proteins also have ACE-inhibiting property and thus used as antihypertensive agent.
- ACE inhibitory bioactive peptides are isolated from both fractions of milk protein i.e.,

1. Caseins: α and β caseins
2. whey proteins: α-Lactoalbumin, β-Lactoglobulin.

- Two potent ACE inhibitory tri peptides: val-pro-pro and Ile-pro•pro were isolated from bovine casein, through fermentation with *L. helveticus* and *Saccharomyces cerevisiae*.

Antithromobotic and Opioid Peptides

- Antithrombic peptides inhibit aggregation of platelets.
- Casoplatelin, the casein derived peptides (f106-116, f106-112 and f113-116) are inhibitors of aggregation of platelets
- k-casein fragments f103-111 also prevent blood clotting through inhibition of platelet aggregation
- Opioid properties have been demonstrated for β -casein f60-70
- Typical Opioid peptides of milk, always have Tyrosine at N terminal and Phenylalanine at C terminal

Antimicrobial Peptides from Milk

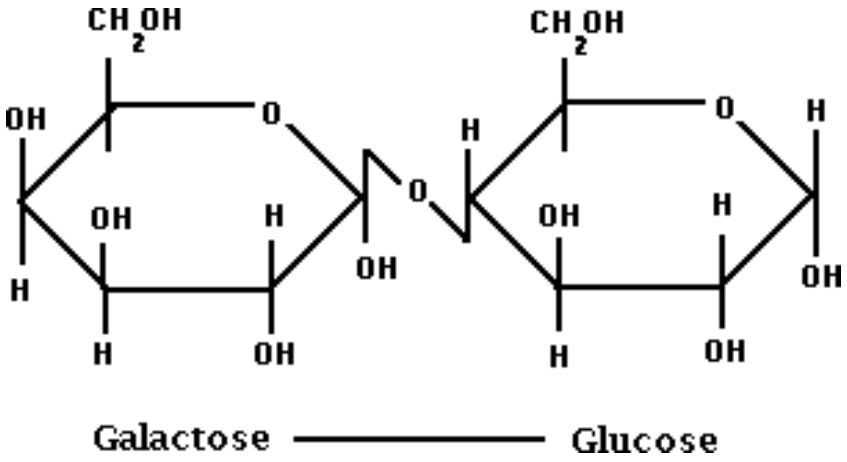
- **Antimicrobial peptides isolated from lactoferrin, αs1 casein have wide range of bactericidal, yeasticidal and fungicidal activities**
- **α s1 Casein derived peptide "Isracidin" and "Casocidin" isolated from α s2 casein inhibit *in-vitro* growth and in vivo multiplication of *S. aureus* and *Candida albicans* and also have protective effect against staph *aureus, Strepto pyogenes, monocytogenes and E.coli.***

Glycomacropeptide: Nutraceutical Values

- Glycomacropeptide (GMP) is formed from α-casein during cheese production (action of chymosin)
- Binds to carbohydrate moiety of enterotoxins of *vibrio cholerae* and *Escherichia coli, responsible for* mimicking the enterotoxin receptor sites.
- GMP helps to reduce dental caries, by preventing cariogenic bacterial adhesion. Also effective against four important human influenza virus strains.
- GMP also inhibits platellets aggression
- Rich source of sialic acid - Stimulation of brain development

Lactose

- Major carbohydrate in milk
- Disaccharide
  - Glucose and galactose molecule
- Only present in the mammary gland



Lactose

- Lact =milk, Ose= sugar so lactose means milk sugar
- Scientific name is β-O-D-galactopyranosyl-(1-4)-α-D- glucopyranosyl
- lactose is a reducing sugar

Concentration in milk

- Cow milk 4.1-4.5 (%)
- Buffalo milk 5.0-5.2 (%)
- Human milk 6.98 (%)

- Comprises 52% of milk SNF, and 70% of whey solids
- Not as sweet as sucrose
- Maillard reactions
- Lactose is water soluble, occurring as a molecular solution in milk, in cheese making, most of the lactose remains dissolved in the whey

•LAB - **ß -D-galactosidase (lactase)**, an enzyme that splits these monosaccharides : result is increased sweetness, and depressed freezing point

•Other enzymes from LAB – Lactic Acid (Fermented Products)

• **Lactose intolerance**

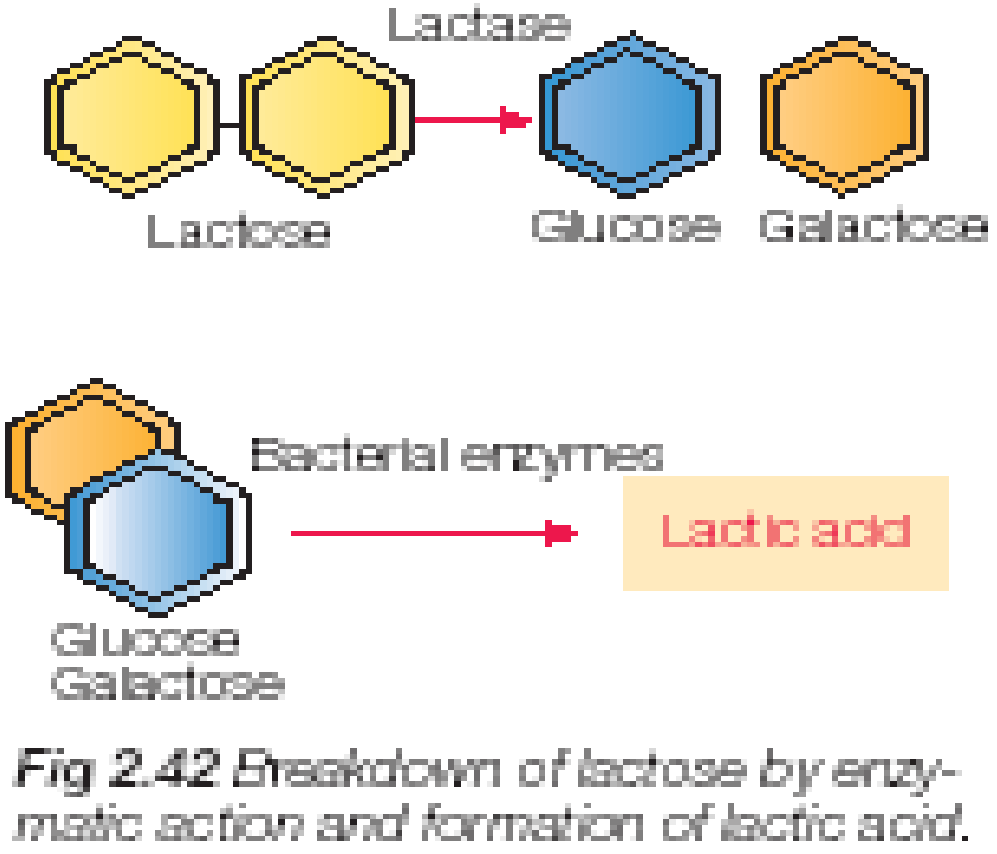


Fig 2.42 Breakdown of lactose by enzymatic action and formation of lactic acid.

Lactose: Nutraceutical Action

- Its metabolite, Galactose prevents mental retardness
- It influence iron absorption.
  - Reduces ferric form of food iron to ferrous form.
  - Body utilizes only ferrous form.
- Slow rate of absorption: So blood glucose level does not increase rapidly.
- Milk is suitable for diabetic persons with highly valuable milk proteins.

Lactulose as Bifidus Factor

- Formed during heating of milk
- Alkali isomerisation process is used for commercial production
- Utilized selectively by bifidobacteria
- 0.5% lactulose in infant formula: stimulate the bifidobacteria without laxative effect
- 1.2–1.5% lactulose in diet increase bifidobacteria, lower pH and inhibite pathogenic gram ⁻ve bacteria

Other Nutraceutical Values of Lactulose

- Control chronic constipation : Laxative
- Control salmonella colonization
- Inhibit dehydroxylation of primary bile acids resulting in less cholesterol absorption
- Exert anti- toxin and Anti-carcinogenic effects
- Protect against DNA damage
- Showed promising result in treatment of Shigella carrier

- **Malliard and pre Malliard reactions**
  - **Loss of nutritional value**
  - **Development of brown colour**
  - **Development of characteristic ‘sterilized milk’ flavour**
  - **alteration of heat stability on re-heating**

- **At temperatures above 100 °C - reaction between lactose and protein-resulting in a brownish colour.**

- **The series of reactions, occurring between amino groups of amino acid residues and aldehyde groups from milk carbohydrates, is called the Maillard reaction or browning reaction.**

Browning Reactions

✓ Maillard browning

reducing sugar + amine ⟶ brown pigments + flavors

✓ Caramelization

sugar —high temps→ brown pigments + flavors

✓ Enzymatic browning

phenolics —polyphenoloxidase→ brown pigments + flavors

Effects of Maillard Reaction

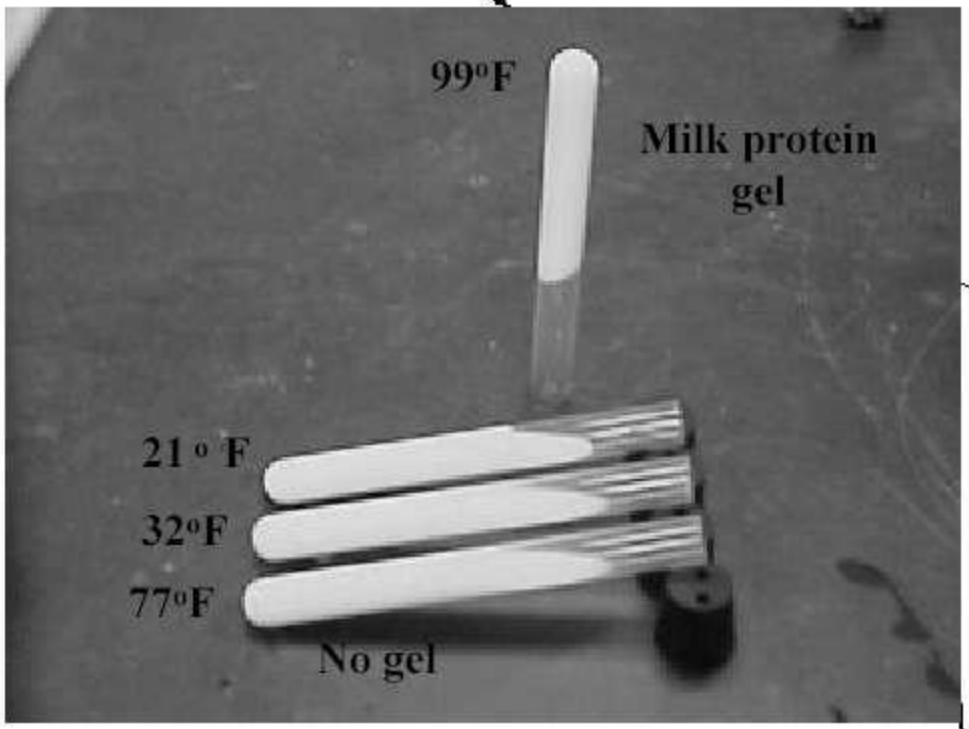
- **Desirable:**
  - Color - bread crust, syrup, meat
    Flavor - coffee, cocoa, meats
    Antioxidants
- **Undesirable**
  - Color - changes in color during storage
    Flavor - changes during processing and storage
    Nutritional loss - essential amino acids,
    Vitamins (vit c), palatability and digestibility

    Toxicity/mutagenicity

Heat treatments

- Thermization-65°C/15S
- Pasteurization-
  - LTLT 63°C/30min.
  - HTST 72°C/15S
- Forwarming-80-90°C/2-10min.
- Sterilization
  - In Container-110-115°C/15min
  - UHT-130-140°C/3-5S

Effect of temperature



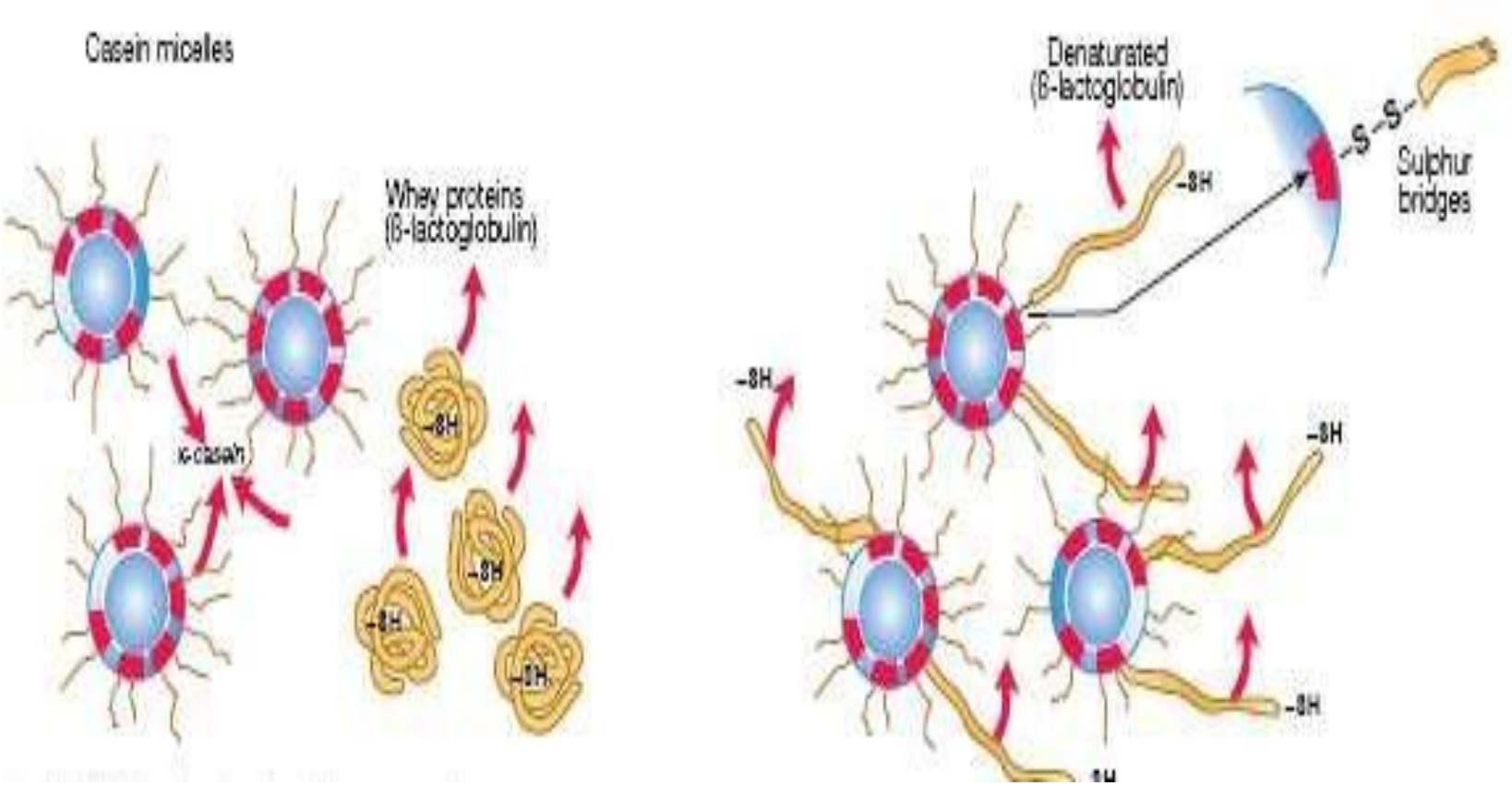
Heat treatment of milk

- 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

- Denaturation of whey proteins and their association with casein micelles

- Denaturation begins at 65°C and is almost completed when whey proteins are heated to 90°C for 5 minutes
  - Change in texture and viscosity
  - alteration of heat stability on re-heating

β-lactoglobulin bound to the κ-casein by sulphur bridges



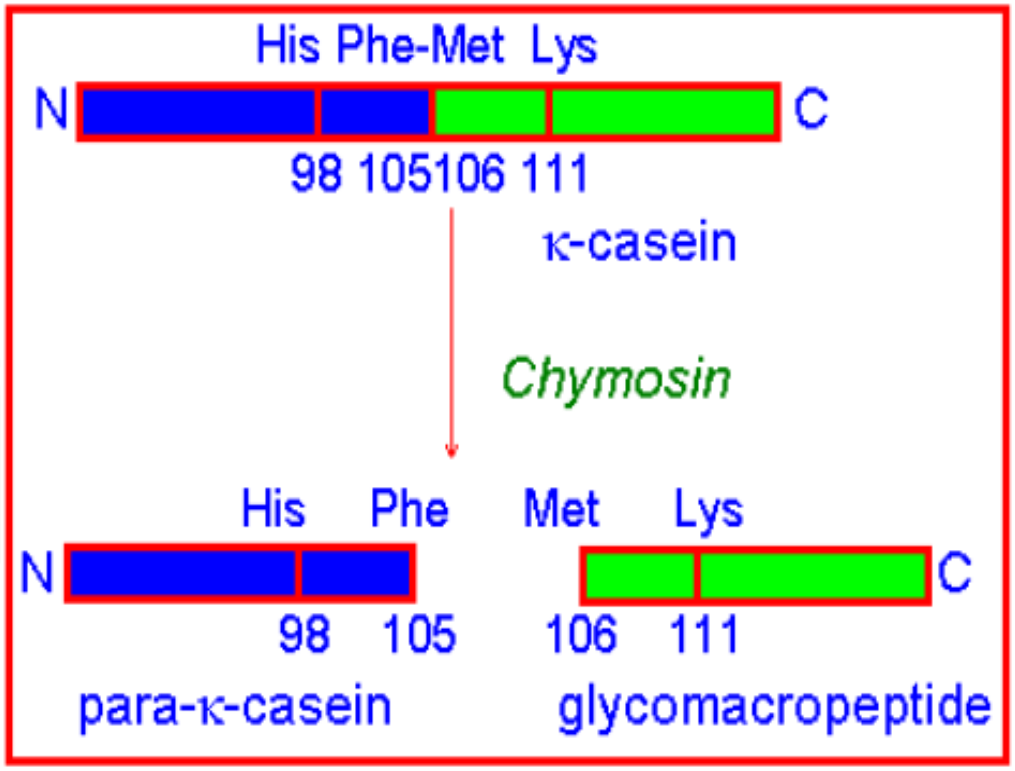
- **The higher the pasteurisation temperature at constant holding time, the softer the coagulum**
- **Milk intended for cheese making should therefore not be pasteurised, or at any rate not at higher temperatures than 72°C for 15 – 20 seconds.**

- β-lactoglobulin in particular is bound to the κ-casein fraction by sulphur bridges
- undesirable phenomenon in production of semi-hard and hard types of cheese

Enzymatic coagulation

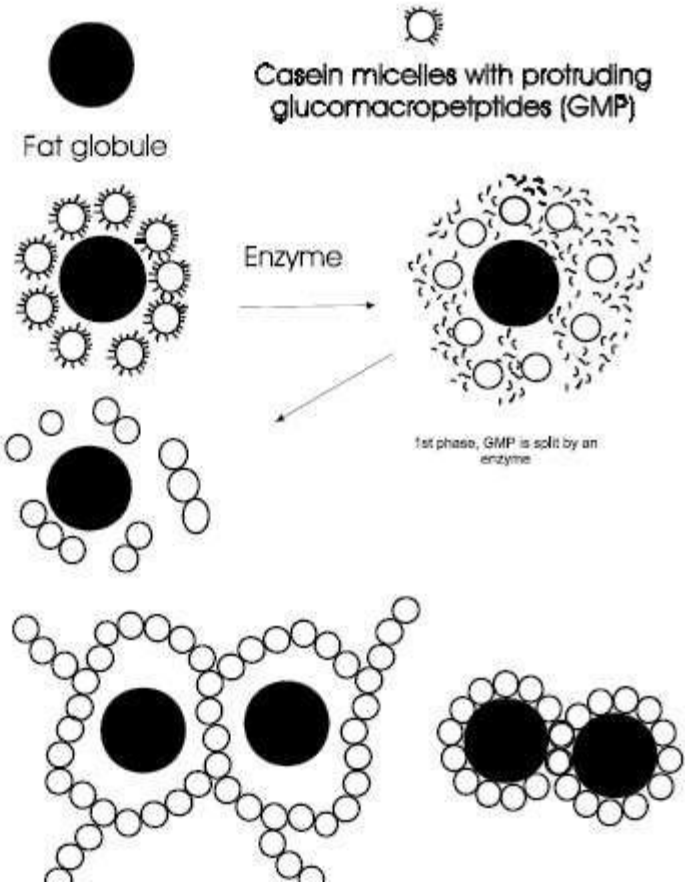
- The primary phase of rennet coagulation involves the specific enzymatic modification of casein micelles
- Aggregation of the rennet- altered micelles is the secondary phase of coagulation

Chymosin attack, 1-st step of milk coagulation

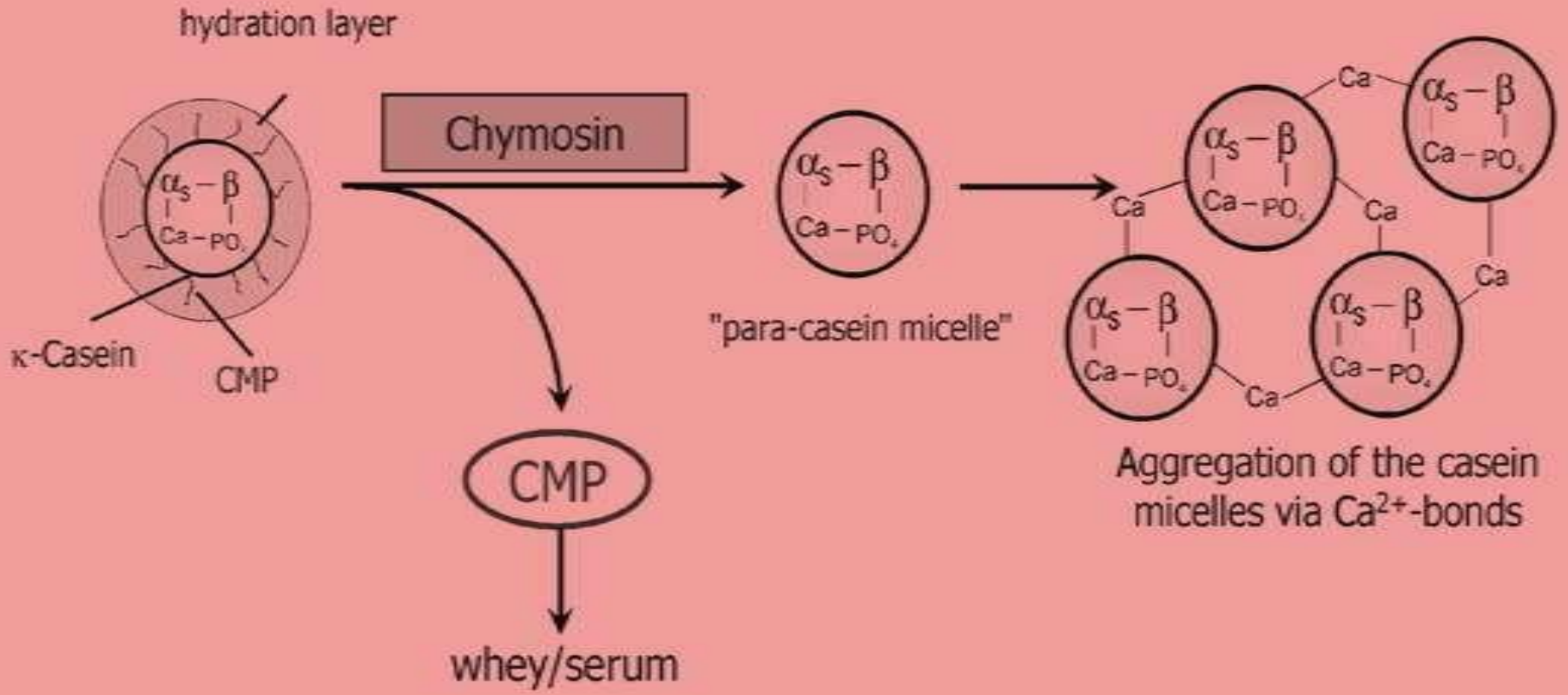


Start of aggregation

- 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



Release of CMP during renneting



Milk:	3,3 – 4,5 g/l	κ-Casein	(10 – 15 % of total protein)
	1,2 – 2,5 g/l	CMP	(4 – 6 % of total protein)
Sweet whey:	1,0 – 2,5 g/l	CMP	(15 - 20 % of total protein)