Milk Proteins:

Amino acids are the building blocks of proteins. The amino acids differ from each other in their side chain(R).The amino acids are bound together in a protein by peptide bonds which are produced by the reaction of the carboxyl group in an amino acid with the amine group of another amino acid, with loses of water molecule.

Table below shows the amino acids found in whole milk and whey

Amino acid	*Whey*	*Casein*	*Soy*
Alanine	4.6	2.7	3.8
Arginine	2.3	3.7	6.7
Aspartic acid	9.6	6.4	10.2
Cysteine/cystine	2.8	0.3	1.1
Glutamic acid	15.0	20.2	16.8
Glycine	1.5	2.4	3.7
Histidine[a]	1.6	2.8	2.3
Isoleucine[a,b]	4.5	5.5	4.3
Leucine[a,b]	11.6	8.3	7.2
Lysine[a]	9.1	7.4	5.5
Methionine[a]	2.2	2.5	1.1
Phenylalanine[a]	3.1	4.5	4.6
Proline	4.4	10.2	4.5
Serine	3.3	5.7	4.6
Threonine[a]	4.3	4.4	3.3
Tryptophan[a]	2.3	1.1	1.1
Tyrosine	3.3	5.7	3.3
Valine[a,b]	4.5	6.5	4.5

Values are expressed per 100 g of product
[a]Essential amino acid
[b]Branched-chain amino acid

It is clear that caseins contain more amount of imino acid proline , and alcoholic amino acid serine and less amount of some essential amino acids such as

Lucien ,lysine and tryptophan also contain less amount of sulfur containing amino acid cysteine than whey.

Milk proteins: -

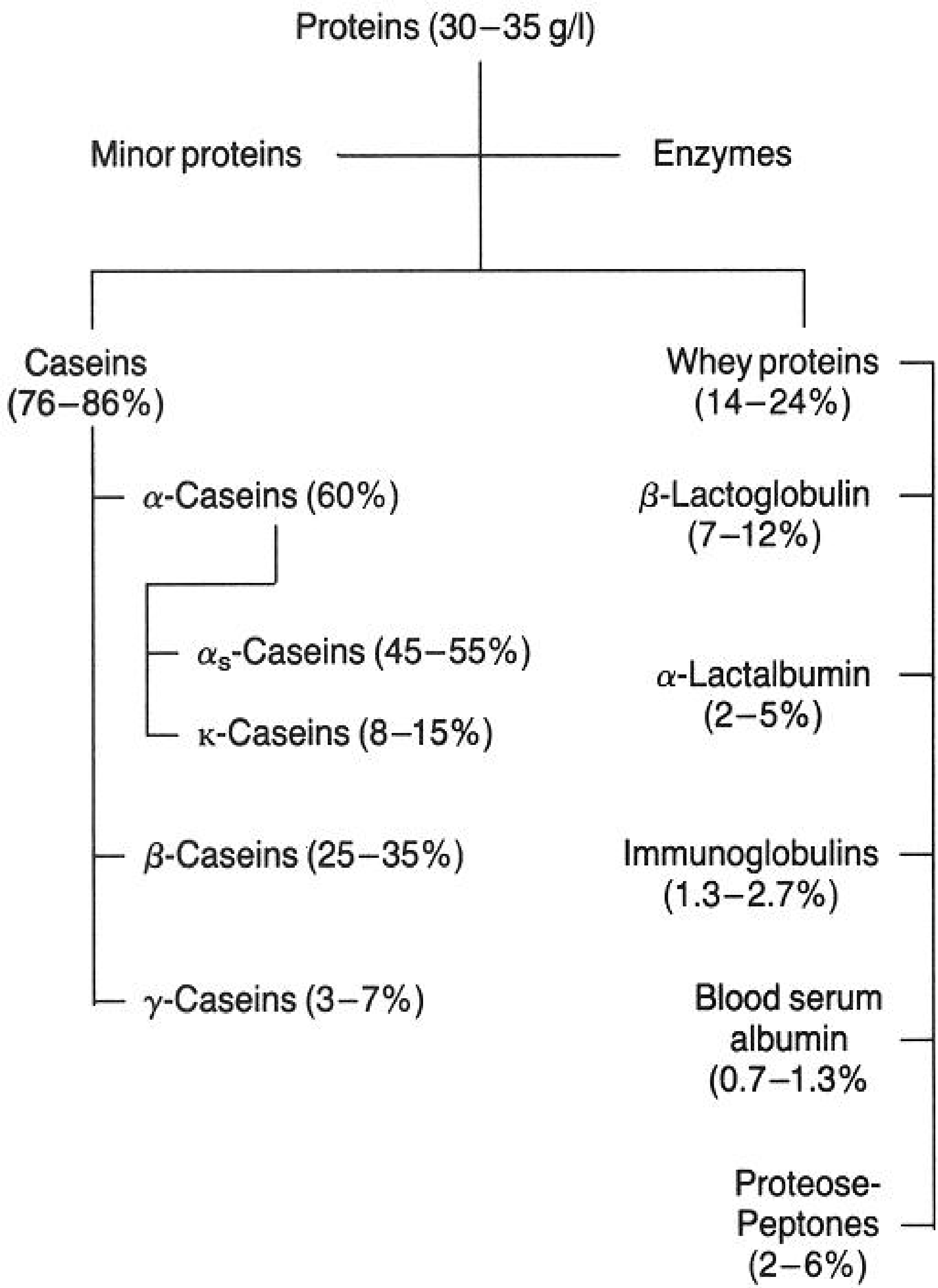
Milk proteins consist of two major groups of proteins called caseins and whey proteins. Of the approximately 3.6% protein in milk, approximately 80% is casein and 20% is whey protein.

Milk Protein Fractionation

The nitrogen content of milk is distributed among caseins (75%), whey proteins (18%), miscellaneous proteins (2%) and non-protein nitrogen (5%). This nitrogen distribution can be determined by the Rowland fractionation method:

1. Precipitation at pH 4.6 - separates caseins from whey nitrogen
2. Precipitation with sodium acetate and acetic acid (pH 5.0) - separates total proteins from whey NPN

Ninety-five percent of the nitrogen is associated with protein.
The average concentration of proteins in milk is as follows (although there can be considerable natural variation):


Proteins (30–35 g/l)
Minor proteins
Enzymes
Caseins (76–86%)
Whey proteins (14–24%)
α-Caseins (60%)
α_s-Caseins (45–55%)
κ-Caseins (8–15%)
β-Caseins (25–35%)
γ-Caseins (3–7%)
β-Lactoglobulin (7–12%)
α-Lactalbumin (2–5%)
Immunoglobulins (1.3–2.7%)
Blood serum albumin (0.7–1.3%
Proteose-Peptones (2–6%)

The average concentration of proteins in milk is as follows:

	% of total protein
Total Protein	100
Total Caseins	**78.8**
alpha s1-casein	32.4
alpha s2-casein	8.5
beta-casein	26.1
kappa-casein	9.4
gamma-casein	2.4
Total Whey Proteins	**19.4**
alpha lactalbumin	3.6
beta lactoglobulin	9.8
BSA	1.2
Immunoglobulins	2.4
Proteose peptone	2.4
Miscellaneous	**1.8**

Milk Casein:-

The casein content of milk represents about 80% of milk proteins. The principal casein fractions are α(s1) and alpha(s2)-caseins, ß -casein, and kappa-casein in a percentage ratio of 40:10:40:10. Table below shows some differences between casein fractions:

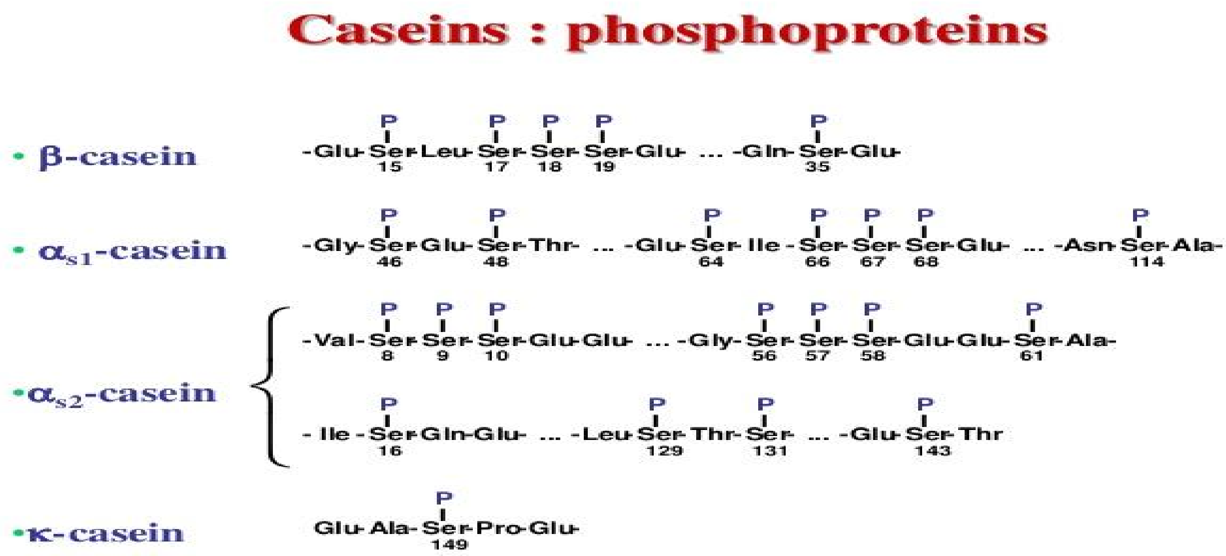
Parameter	Casein α_{s1}	Casein α_{s2}	Casein β	Casein K
Residue of AA (1)	199	207	209	169
Molecular weight (da)	23600	25200	24000	19000
Cysteine residues (1)	-	2	-	2
Group phosphorylating	8 – 9	10- 13	5	1 – 2
Carbohydrates	-	-	-	+
Sensitivity chymosin	+	-	+	++
Calcium sensitivity	++	+++	+	-

Number per mole (l)

The distinguishing properties of all caseins are

1- their low solubility at pH 4.6.

2-The common compositional factor is that caseins are conjugated proteins, most with phosphate group(s) esterified to serine residues mainly, to a lesser extent, amino acid threonine.The percentage of phosphate in total casein is 0.85 %, αs-casein considered the richest caseinate in phosphates as it contains 0.6 % phosphate, then β-casein contains 0.18%, and K-casein 0.11%.



The importance of phosphate in casein can be explained by:

- These phosphate groups are important to the structure of the casein micelle. Calcium binding by the individual caseins is proportional to the phosphate content.

- From a nutritional point of view, phosphates have the ability to bind

large quantities of calcium and zinc ions.

- Phosphates increase casein solubility.

- It have a relationship with casein thermal stability.

- It plays an important role in casein coagulation with chymosin during the second stage of this process.

Phosphates are covalently bound to protein, and phosphates can be removed from

Casein by harsh heat treatments or pH elevated or by the enzyme phosphatase.

3-The conformation of caseins is much like that of denatured globular proteins. The high number of proline residues in caseins causes particular bending of the protein chain and inhibits the formation of close-packed, ordered secondary structures. When proline is in a peptide bond, **it does not** have **a hydrogen** on the α amino group, it cannot **donate a hydrogen bond to stabilize an α helix**.

- proline led to produce partially folding α helix or naturally un folded protein (naturally denatured proteins) with high resistant against heat treatment this is cause why **casein is very resistant against heat treatment.**

4- Casein is low in sulfur (0.8%) while the whey proteins are relatively rich (1.7%). The sulfur of casein is present mainly in **methionine**, with low concentrations of **cysteine and cystine**; in fact the principal caseins contain only methionine.

5-Caseins contain no disulfide bonds. As well, the lack of tertiary structure accounts for the stability of caseins against heat denaturation because there is very little structure to unfold. Without a tertiary structure there is considerable exposure of hydrophobic residues. This results in strong association reactions of the caseins and renders them insoluble in water.

6-All caseins have high content (35-45%) of apolar amino acids.

Within the group of caseins, there are several distinguishing features based on their charge distribution and sensitivity to calcium precipitation:

Casein Micelle Stability

Colloidal calcium phosphate (CCP) acts as a cement between the hundreds or more of submicelles that form the casein micelle. Binding may be covalent or electrostatic. Submicelles rich in kappa-casein located a surface position, whereas those with less are found in the interior. The resulting hairy layer, at least 7 nm thick, acts to prohibit further aggregation of submicelles by steric repulsion.

The following factors must be considered when assessing the stability of the casein micelle:

1-Role of Ca++:

More than 90% of the calcium content of skim milk is associated with the casein micelle. The removal of Ca++ leads to reversible dissociation of ß -casein . The addition of Ca++ leads to aggregation.

2-Hydrophobic interactions

There is presence of large number of hydrophobic residues clustered together in αS1-, β-, and k-casein as found by amino acid sequence analysis of these proteins. Since these are among the most hydrophobic proteins, role of hydrophobic bonding in the stabilization of casein cannot be ignored. The ability of β-casein to self-associate was reduced after removal of isoleucine and valine at C-terminal end of protein . Additionally, the ability of β-casein to form polymers was destroyed completely after removal of 20 amino acids at C-terminal which are mainly hydrophobic in nature. Various investigators have found that αS1-, β- and k-caseins diffuse out of the micelle at low temperature due to decrease in hydrophobic interactions .

3-Electrostatic interactions

There are many potential sites for strong ion bonding in apolar environment that might play a role in the stabilization of casein micelles. It is not possible to exactly assess the role of various inter- and intramolecular ionic bonds present in αs-, β-, and k-casein in stabilization of casein micelle structure. The ability of k-casein to stabilize the αS1-casein is eliminated when there is carbamylation of lysine residues in k-casein which further demonstrate that ionic interactions play a role in the casein micelle structure . Modification of arginine side chains also affects the casein micelle stability and chymosin-induced coagulation .

4-Hydrogen bonding

The α-helical and β-pleated structures in many globular and fibrous structures are stabilized by hydrogen bonding along the polypeptide chain. Since casein proteins posses very little secondary structure and 72–76% of protein exists in aperiodic form, the degree of stabilization by α-helix and β-structure is very low . Hydrogen bonds between the various components of casein during the formation of highly aggregated casein micelle are possible but in case of ionizable side chains of monomeric proteins which are accessible to water, their contribution to the stability of these monomeric proteins is very less. For the formation of a residue-residue hydrogen bond in case of these monomeric proteins, there must be breakage of water-residue hydrogen bond which has already formed. During the interaction of two subunits of a protein, there are chances of formation of hydrogen bonds between individual monomers as the surface groups are no longer fully hydrated. Hydrogen bond may exist during the formation of aggregated casein micelles and self-association of αS1-casein.

5-Disulfide bonds

Disulfide bonds between cysteine residues during folding of pleated sheet structures, helical segments, and unordered structures leads to the formation of tertiary structure. Both αS2- and k-casein contain cysteine but the degree of disulfide cross-linkages which are normally present in the casein micelle is controversial . It has been reported by many investigators that disulfide cross-linkages contribute to the overall stability of the casein micelle but they are not the driving force for the formation of casein micelle. Slattery in 1978 found that larger micelles have higher molecular weight disulfide-bonded polymers of k-casein. These k-casein molecules are attached with each other and form disulfide-linked aggregates which compose the casein micelle structure .

There are several factors that will affect the stability of the casein micelle system:

Salt content:

affects the calcium activity in the serum and calcium phosphate content of the micelles.

pH:

lowering the pH leads to dissolution of calcium phosphate until, at the isoelectric point (pH 4.6), all phosphate is dissolved and the caseins precipitate.

Temperature:

at 4° C, beta-casein begins to dissociate from the micelle, at 0° C, there is no micellar aggregation; freezing produces a precipitate called cryo-casein.

Heat Treatment:

whey proteins become adsorbed, altering the behavior of the micelle.

Dehydration:

by ethanol, for example, leads to aggregation of the micelles.

When two or more of these factors are applied together, the effect can also be additive.

Methods of caseins precipitation:

1 - **Acid precipitation:**

Addition of acid to milk leads to the transformation of calcium ions associated with caseins and with colloidal calcium phosphate, involved in the binding of caseins with each other to the dissolving and ionizing state, which leads to its separation of colloidal calcium from casein micelles and caseins precipitation due to reaching to their isoelectric point. Acidification causes the casein micelles to destabilize or aggregate by decreasing their electric charge to that of the isoelectric point. At the same time, the acidity of the medium increases the solubility of minerals so that organic calcium and phosphorus contained in the micelle gradually become soluble in the aqueous phase. Casein micelles break and casein precipitates. Aggregation occurs as a result of entropically driven hydrophobic interactions.

Ca-casein micelles + HCl $\longrightarrow$ H-caseinate + $CaCl_2$

Precipitate at pH 4.6

2- Ultra Centrifugal precipitation:

Casein micelles, phosphate, calcium and other salts contents precipitate by centrifugation, and in this method the proportion of dissolved casein is high estimated about 5-20% of total casein.

3- Salting out:

It is a reversible method and the disadvantages of this method is that causes a precipitation of slight percentage of whey proteins with casein.

Casein + $(NH4)_2SO_4$ ⟶ Casein Salts

26.4 g of $(NH4)_2SO_4$ / 100 ml milk

4- Precipitation with the addition of alcohol:

The alcohol pulls out the aqueous layer (Water of Hydration) surrounding casein micelles, which leads to their precipitation.

5-Enzymatic - chymosin (rennet) or other proteolytic enzymes as in Cheese manufacturing. Chymosin, or **rennet**, is most often used for enzyme coagulation. During the **primary stage**, rennet cleaves the Phe(105)-Met(106) linkage of kappa-casein resulting in the formation of the soluble CMP which diffuses away from the micelle and para-kappa-casein, a distinctly hydrophobic peptide that remains on the micelle.

During the **secondary stage**, the micelles aggregate, Calcium assists coagulation by creating iso electric conditions and by acting as a bridge between micelles.

6vbg- Age gelation. Age gelation is an aggregation phenomenon that affects shelf-stable, sterilized dairy products, such as concentrated milk and UHT milk products. After weeks to months storage of these products, there is a sudden sharp increase in viscosity accompanied by visible gelation and irreversible aggregation of the micelles into long chains forming a three-dimensional network.