

Casein – A Unique Milk Protein

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Abstract

Milk is a nutrient-rich food that contains proteins, fats, lactose, vitamins, minerals, enzymes, and other organic compounds. Essential for young mammals, milk proteins provide amino acids, nitrogen, and minerals necessary for muscle and tissue development. In human nutrition, milk is valued for its digestibility and as a source of calcium and phosphorus, crucial for infant growth. Milk proteins are classified into two primary types: caseins, which make up 75–80% of total protein and precipitate under acidic conditions, and serum or whey proteins, which remain soluble. Caseins, existing as micelles, interact with calcium phosphate, stabilizing the protein structure and facilitating controlled destabilization in cheese and yogurt production. Casein-derived peptides exhibit various health-promoting bioactivities, activated through enzymatic proteolysis. These bioactive peptides contribute to cardiovascular health, antioxidative properties, mineral absorption, immune modulation, anti-cariogenic effects, and even support athletic performance. One key function involves promoting remineralization of tooth enamel, enhancing oral health. Casein's multifaceted nature makes it highly valuable in food technology and medical applications, with extensive research focusing on its potential in nutritional supplements. Its slow digestion and mineral-binding abilities support applications in enhancing bone health and preventing conditions like osteoporosis. This diversity of functional benefits and health effects underscores casein's role in nutrition and therapeutic uses.

Keywords: Casein, lactic acid, whey protein, milk proteins, α 1-casein

INTRODUCTION

Milk is a wholesome food containing colloidal dispersion of proteins, emulsion of fat, true solution of lactose, vitamins, minerals, enzymes and minor organic compounds. The basic function of milk proteins is to supply essential amino acids and nitrogen to young mammals and serve as a source of dietary proteins for adults. Milk proteins primarily provide essential amino acids and minerals that are crucial for developing muscles and other tissues in newborn mammals, supporting their growth and bodily functions. It is also important in infant nutrition due to its digestibility and role as a source of calcium and phosphorus [1, 2].

Milk proteins are categorized into two main types and are no longer considered a single, uniform protein group. Caseins make up about 75–80% of the total protein in milk and will precipitate at a pH of 4.6 when the temperature is 30°C. The other portion, known as serum or whey protein, remains soluble under these same conditions. The remaining proteins in milk are small amounts of glycoproteins. Casein proteins in milk form complex clusters, or micelles, along with calcium phosphate. These casein micelles help control calcium phosphate levels in their surroundings using a calcium-binding mechanism. For the dairy industry, maintaining the stability of these micelles or deliberately destabilizing them to make products like cheese and yogurt is essential [3].

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Milk is a source of proteins (casein-derived peptides) that exhibit a range of health benefits due to their bioactivity. These peptides are initially inactive within the parent casein proteins and become active through enzymatic proteolysis. These bioactive peptides exhibit many properties like ACE inhibitory, antioxidant, enhanced mineral absorption, anti-cariogenic activity, immune modulation, opioid, antibacterial activity and improved athletic performance. CPP mainly enhance the localization of ACP (amorphous calcium phosphate) at the tooth surface, inhibiting enamel demineralization and promoting remineralization [4].

Properties of Casein

Casein is a phosphoprotein and exists in the form of micelles in milk. It exists in four subtypes: α 1-casein, α 2-casein, β -casein, and κ -casein, being present in the relative ratio of 3:0.8:3:1, respectively. The casein micelle consists of 94% protein and 6% colloidal calcium phosphate (CCP) on a dry matter basis. Under electron microscopy, casein micelles appear mostly spherical, with sizes ranging from 50 to 500 nanometers in diameter, averaging around 150 nanometers. Their molecular weight spans from 10^6 to over 10^9 daltons, with an average around 10^8 daltons. With a casein concentration of 2.5 grams per 100 milliliters of milk, there are approximately 10^{14} to 10^{16} micelles per milliliter. This suggests that the micelles are packed relatively close together, with the distance between their surfaces being less than the diameter of a single micelle (Table 1) [5].

Table 1. Protein profile (g/L) of milk from different species [2, 5].

Protein fractions	Ruminants						Non-ruminants		Human
	Cattle	Buffalo	Goat	Sheep (g/100mL)	Red deer	Camel	Horse	Donkey	
Total CN	24.6-28	32-40	23.3-49.3	41.8-52.6	57-84	22.1-26.0	9.4-13.6	6.4-10.3	2.4-4.2
Total WP	5.5-7.0	6	3.7-7.0	10.2-16.1	11-15	5.9-8.1	7.4-9.1	4.9-8.0	6.2-8.3
C:WP ratio	82:18	82:18	78:22	76:24	80:2085:15	73.27:76.24	52:48	56:44	29.71:33.67
Major Caseins									
α S ₁	8-10.47	8.9	0-13.0	2.4-22.1	-	4.9-5.7	2.4	Present	0.77
α S ₂	2.8-3.4	5.1	2.3-11.6	6.0	-	2.1-2.5	0.2	Present	Absent
B-casein	8.6-9.3	12.6-20.9	0-29.6	15.6-39.6	-	14.4-16.9	10.66	Present	3.87
K-casein	2.3-3.3	4.1-5.4	2.8-13.4	3.2-12.23	-	0.8-0.9	0.24	Present	0.14
Major whey proteins									
B-Lg	3.2-3.3	3.9	1.5-5.0	6.5-13.5	-	Absent	2.55	3.3	Absent
A-la	1.2-1.3	1.4	0.7-2.3	1-1.9	-	0.8-3.5	2.37	1.9	1.9-3.4

Casein micelles are highly hydrated, holding around 3–4 grams of water for each gram of protein. Though caseins are hydrophobic, they carry a high charge, contain many proline residues, and have few cysteine residues. There are ~43% hydrophobic amino acids in case of α -S1 casein, ~33% in case of α -S2 casein, ~52% in β -casein, and ~43% in κ -casein. Casein do not form anything more than short lengths of α -helix and have little tertiary structure [6, 7]. The chemical composition of buffalo milk differs greatly from that of bovine milk. Variations in the concentration and organization of casein micelles, as well as mineral content, between these two milk types lead to noticeable differences in cheese-making properties. These include changes in rennet coagulation time (RCT), curd firmness, and moisture retention.

Casein is unique milk protein because it exists in unique micellar form, carries significant amount of calcium, phosphorus and magnesium, lacks definite secondary and tertiary structure and possesses unusually high buffering capacity. Buffalo milk contains higher amounts of calcium, magnesium, and inorganic phosphate as well as higher amounts of calcium and inorganic phosphate associated with the casein micelles than bovine milk [8, 9].

Milk appears white mainly because the tiny casein particles within it scatter a large amount of light. This scattering effect is intensified by the high concentration of these particles, making milk look opaque and white. When shorter wavelengths of radiation, like neutrons and X-rays, are used, they show that the internal structure of the particles is uneven, with variations in density occurring over about 18 nanometers. This behavior suggests that the structure consists of tightly packed spherical sub-units of that size, resembling the raspberry-like look seen in early electron microscope images of casein micelles. Casein itself doesn't react to heat; it only becomes insoluble at temperatures above 120°C. However, it is sensitive to changes in pH and will form a solid when the pH reaches its isoelectric point.

Casein, a protein found in milk, is made up of various proteins that each have different functions, even though they do not have a clear secondary or tertiary structure. The main types of casein proteins are α S1-casein (about 38%), β -casein (36%), k-casein (13%), and α S2-casein (10%). Each of these proteins has a unique amino acid sequence and occupies specific positions within the micelle, allowing them to perform distinct roles. α S1-casein is particularly important for transporting calcium phosphate in milk, and it also act as source of certain antioxidant peptides that can scavenge DPPH radicals. Overall, casein do not exhibit a well-defined structure when they are in their natural state (Table 2).

Table 2. Properties of some milk protein [2, 10].

Property	α -s1 Casein	α -s2 Casein	β -Casein	k-Casein
Molar mass	23,614	25,230	23,983	19,023
Amino acid residue	199	207	209	169
Phosphoserine	8	11	5	1
Cysteine	0	2	0	2
--S—S—linkage/mol.	0	1	0	—
Hexoses	0	0	0	—2.3
Hydrophobicity	25	23	29	22
α -Helix	7?	?	10?	?
Charged Residue	34	36	23	21
Net charge/residue	−0.10	−0.07	−0.06	−0.02
Distribution of charge	Uneven	Uneven	Very uneven	Very uneven
Isoelectric pH	4.5	5.0	4.8	5.6
Association tendency	Strong	Strong	F(T)	Strong
Ca ⁺² Binding	++	++	+	—

Casein is considered a naturally disordered protein, meaning it can function effectively even without a specific structure. Research has shown that α -S1 and β -casein have chaperone-like properties, helping to stabilize the micelle by preventing the clumping of α -S2 and k-casein, respectively. The ability of α S1-casein monomers to come together in water is due to their high hydrophobicity and minimal structural content. α -S2-casein has the most unique primary structure of all the caseins, with a molecular weight of 25,230. This protein contains two cysteine residues and does not have any known carbohydrates. α S2-casein can exist as a dimer and may have some internal disulfide bonds. It is the least likely to aggregate due to its alternating regions of negatively charged and hydrophobic areas. Additionally, it plays a crucial role in transporting calcium phosphate. Its antimicrobial peptide, casocidin-I, can inhibit the growth of *E. coli* and other bacteria.

β -casein is the second most abundant protein in milk, with a molecular weight of 23,983 and five phosphoserine residues. The protein's net charge, phosphoserine content, and α -helical structures are mainly concentrated in the first 40 amino acids at the N-terminal end, while the C-terminal end contains many polar residues that contribute to its high hydrophobicity. β -casein is important for influencing the surface properties of the casein micelle and contains an antioxidant peptide that exhibits antioxidant activity. K-casein is the fourth major component of milk proteins and is soluble over a wide range of

calcium ion concentrations, unlike other types of casein, such as α S1, α S2, and β -casein. This ability to remain soluble in calcium has led researchers to recognize its role in stabilizing casein micelles, which helps prevent the precipitation of casein in milk. K-casein stabilizes micelle formation and contains peptides like casoxins, which act as opioid antagonists, and casoplatelin, which inhibits platelet aggregation. The molecular weight of reduced k-casein is approximately 19,023, and it exists as a mixture of high molecular weight polymers. Notably, k-casein is the only major casein component that has carbohydrates attached to the highly soluble macromolecule formed after chymosin hydrolysis. Each k-casein monomer contains only one or two phosphate residues, contributing to its solubility in calcium.

There are several genetic variants of casein proteins, each with different numbers of phosphoserine residues, particularly in α S2-casein, which shows significant variability in its phosphorylation. Another notable characteristic of caseins is the high number of proline residues, especially in β -casein, which significantly influences their secondary and tertiary structures. Additionally, all casein proteins have different regions along their chains that are either hydrophilic or hydrophobic. α S-caseins are the main type of casein, containing 8 to 12 seryl phosphate groups, while β -casein has about 5 phosphoserine residues and is more hydrophobic compared to both α S-caseins and k-casein.

α S-caseins and β -caseins, which are highly phosphorylated, are very sensitive to calcium salt concentrations and tend to precipitate when there are high levels of Ca^{2+} ions. In contrast, k-caseins are glycoproteins that contain only one phosphoserine group, which allows them to remain stable in the presence of Ca^{2+} ions. This stability helps protect the other calcium-sensitive caseins from precipitating and contributes to the overall stability of the casein micelle.

Casein is not affected by heat, becoming insoluble only at temperatures above 120°C. However, it is sensitive to pH and will precipitate at its isoelectric point. The caseins' inability to form stable structures is largely due to their high content of proline, an amino acid that disrupts structure. As a result, casein lacks stable secondary and tertiary structures.

Each of the four types of casein can have different numbers of phosphate groups linked to their serine or threonine residues. α S2-casein typically exists as a dimer, while k-casein can form structures ranging from dimers to decamers, depending on how the intermolecular disulfide bonds are arranged. There are 10 distinct molecular forms of k-casein, which vary based on the level of glycosylation, and it is the only type of casein that is glycosylated.

Manufacture of Casein

Milk proteins divided into two main categories in 20°C, 4.6 pH which are separable and non-separable. The separable protein is known as casein, while the other part is known as whey [11]. Based upon functional properties of casein, there are two basic types named according to clotting agents. These are called acid casein and rennet caseins. Acid caseins are divided into mineral acidified and biological acidified. Casein is commercially produced from skim milk. The casein begins to precipitate with diluted acid (1 M) added gradually reduce to pH 4.6 with sulphuric acid (H_2SO_4), hydrochloric acid (HCl) or lactic acid ($\text{C}_3\text{H}_6\text{O}_3$) is used as precipitated acid. Precipitation can happen at any temperature, but below around 6°C, the resulting aggregates are very small and stay suspended in the liquid, although they can be separated by low-speed centrifugation. At higher temperatures (30–40°C), the aggregates become larger and easily settle out of the solution. When temperatures exceed about 50°C, the precipitate tends to become stringy and harder to manage.

During the enzymatic coagulation of casein, the pH of the milk remains unchanged. This process occurs in two stages. In the first stage, the enzyme breaks the bond between phenylalanine and methionine (residue 105–106) in the k-casein molecule, resulting in the formation of para k-casein and glycomacropeptide (GMP). The hydrophilic GMP is then released into the whey. This process

destabilizes the casein micelles. In the second stage of the reaction, the remaining para k-casein forms a three-dimensional clot when calcium ions are present. As a result of the action of rennet on casein micelles, curd is formed. The curd is then cooked to 50°C, and the temperature of the whey is gradually increased by 1°C per minute (Figure 1).

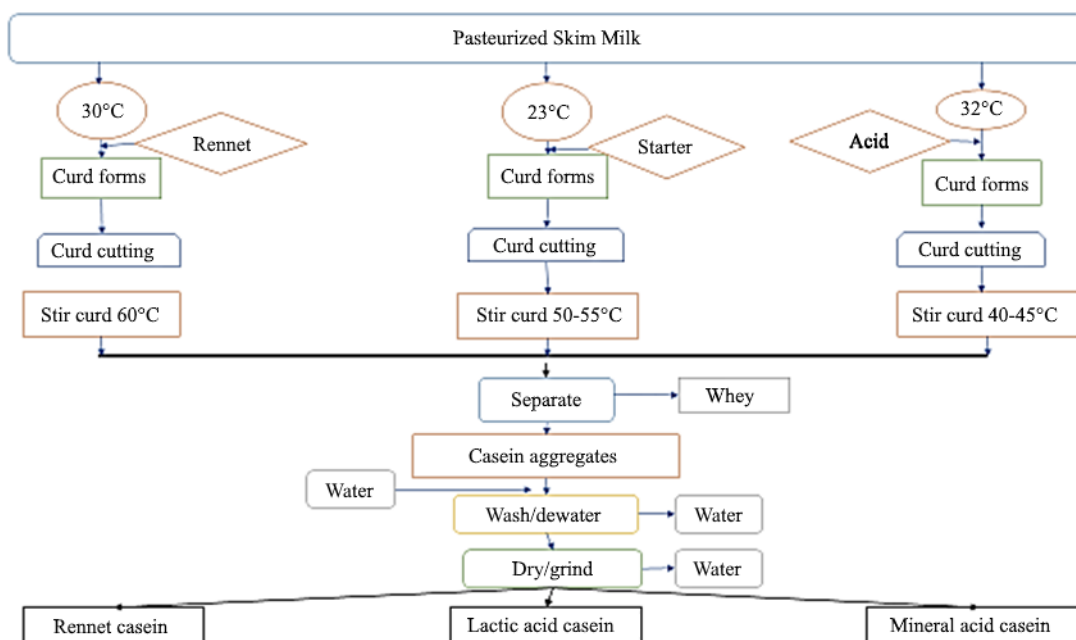


Figure 1. Process flowchart for the production of rennet casein, lactic acid casein, and mineral acid casein from pasteurized skim milk.

This heating helps increase the firmness of the curd and inactivates the coagulant. Washing the casein curd is a crucial step in making casein, as effective washing leads to significant quality improvements. During this process, large amounts of lactose, minerals, and acids become trapped within the curd, making it difficult to remove them easily during the washing. Thoroughly removing lactose is crucial because even small amounts can react with casein when heated, leading to undesirable effects due to the Maillard browning reaction. The rate at which lactose diffuses out depends on the size and permeability of the curd particles, as well as the purity, quantity, and flow rate of the wash water. Smaller and more permeable curd particles are essential for effective washing. The casein curd needs to be washed three times, with each wash lasting 15 to 20 minutes. After the whey is drained, wash water should be added in the same amount as the whey that was removed. Once the wash water is drained, the curd is pressed, crushed, and dried with hot air until it reaches a moisture content of 12%. The dried casein is then milled and packaged into granules measuring 0.5 to 1.0 mm in size. High-quality rennet casein should be white or slightly yellowish; a darker color indicates poor quality, often due to a higher lactose content.

Health Benefits of Caseins

Casein is a protein that offers both nutritional and functional advantages. It has a strong ability to bind minerals like calcium, magnesium, and phosphorus. Additionally, casein is a rich source of bioactive peptides, which are specific protein fragments that positively affect bodily functions and overall health [12]. Most bioactive peptides in fermented dairy products are derived from the caseins. These bioactive peptides exhibit many properties like ACE inhibitory, enhanced mineral absorption, anti-cariogenic activity. It is commonly employed in various food formulations due to its functional characteristics. Casein is used to make a wide range of milk and dairy products. Many of the recognized bioactive peptides are created using digestive enzymes, primarily pepsin and trypsin. For instance, angiotensin-converting enzyme (ACE)-inhibitory peptides and calcium-binding phosphopeptides (CPPs) are typically produced using trypsin.

Angiotensin-converting enzyme (ACE) is a type of dipeptidyl carboxypeptidase and zinc metalloprotease that acts as a dipeptide hydrolase. It is commonly linked to the renin-angiotensin system, where it converts angiotensin I into angiotensin II, a powerful vasoconstrictor. ACE exists in a soluble form in blood and various body fluids. Casokinins (from casein) and lactokinins (from whey protein) have been identified in fermented milks or in hydrolysates of milk proteins, produced using specific enzymes like pepsin, trypsin, and chymotrypsin [11].

The stomach and intestinal contents of adult humans fed milk or yoghurt have been found to contain CPPs [2, 3]. Calcium-binding phosphopeptides (CPPs) can bind to and dissolve minerals, making them potentially beneficial for preventing conditions like osteoporosis, dental cavities, hypertension, and anemia. However, animal studies examining the ability of CPPs to improve the bioavailability of minerals, particularly calcium, have produced mixed results.

In general, animal studies using tracers have revealed a positive effect of CPPs on calcium absorption. Dental caries (tooth decay) is a pathological disease characterized by the loss of dental hard tissue due to its chemical removal from the surface by an acid or chelation without bacterial involvement. Some of dietary acids (carbonic acids, citric acids and phosphoric acids) mainly weaken our enamel, leading to dental erosion. CPP mainly enhances the localization of ACP (amorphous calcium phosphate) at the tooth surface, which inhibits the enamel demineralization and promotes remineralization.

CCP also have antioxidant properties. it acts to potentially scavenge superoxide, hydroxyl radicals and stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) [13] and 2,20-azinobis-3-ethylbenzothiazoline-6-sulfonic acid; (ABTS) [14] radicals, as well as lower oxidation in iron-induced peroxidation of liposomes containing arachidonic acid [15]. Calcium-binding phosphopeptides (CPPs) derived from bovine casein have both primary and secondary antioxidant properties. They work by directly scavenging free radicals and sequestering metal pro-oxidants that can promote oxidation. Additionally, casoplatelins, which are peptides derived from casein, can inhibit the aggregation of platelets activated by ADP and prevent the binding of the human fibrinogen γ -chain to specific receptors on the platelet surface [16–18]. Furthermore, the κ -casein fragment f103–111 can prevent blood clotting through inhibition of platelet aggregation but is not able to affect fibrinogen binding to ADP-treated platelets [2, 4]. κ -caseinoglycopeptides from several animal species are a source of antithrombotic peptides. The sequence of amino acids in f106–171 of sheep's κ -casein, known as κ -caseinoglycopeptide, was shown to decrease thrombin-induced platelet aggregation and collagen-induced platelet aggregation in a dose-dependent manner [19].

Casein-derived peptides possess antimicrobial properties as well. For example, caseicidin, which is produced by digesting casein with chymosin at neutral pH, was one of the first defense peptides to be purified. It has shown effectiveness against various bacteria, including *Staphylococcus spp.*, *Sarcina spp.*, *Bacillus subtilis*, *Diplococcus pneumoniae*, and *Streptococcus pyogenes* [20]. Two other peptides were isolated from a peptic hydrolysate of α 2-casein, namely f (183–207) and f (164–179); the former exhibited higher antimicrobial activity than the latter, although both possessed comparable hemolytic effects [21]. Another fragment of α 1-casein, called isracidin f (1–23)), has shown antibiotic-like activity in vivo against *Staphylococcus aureus* and *Candida albicans*. It has the ability to protect sheep and cows from mastitis [22–25].

CONCLUSION

Casein is a multifaceted protein that provides a variety of nutritional and functional benefits. The diversity of structures and nutrients makes them essential for various biological functions. It provides important amino acids, calcium and phosphorus for tissue development and bone health. Bioactive peptides of casein that work through an enzymatic process. Provides many health benefits Including antioxidants antimicrobial peptides. Its ACE inhibitory properties are beneficial to cardiovascular health. Immune support and oral health. The unique characteristics of casein have led it to focus on food

technology and medical applications. This is because the potential of casein in food and nutritional supplements has been the subject of extensive research. Slow digestion characteristics of casein coupled with a high level of mineral binding ability and possible applications in beneficial mineral absorption. This is especially beneficial in preventing osteoporosis and other conditions.

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