

Casein and whey protein of milk: Properties, proteomic insights, and industrial relevance

Abstract

Casein and whey proteins are the two primary protein fractions found in milk, each with distinct properties, structures, and functional roles. Casein proteins, which account for approximately 80% of the protein content in mature bovine milk, are known for their ability to form gel-like structures and their slow digestion rate, making them ideal for nutrition. Whey proteins, comprising the remaining 20%, are rapidly digestible and rich in essential amino acids, making them a popular choice for post-exercise nutrition. Naturally, both protein fractions undergo various post-translational modifications (PTMs) that influence their functional properties, bioactivity, and nutritional value. Casein and whey proteins are integral to the development of functional foods, supplements, and therapeutic agents, with ongoing research exploring their roles in immune modulation, antioxidation, and disease prevention. This review discusses the properties, proteomics, and diverse applications of casein and whey proteins, highlighting their significance in both nutritional science and industrial applications.

Keywords: milk proteins, casein, whey proteins, proteomics, food and pharmaceuticals

Abbreviations: PTMs, post-translational modifications; MS, mass spectrometry; BCAAS, Branched-Chain Amino Acids; BV, Biological Value; SDC, sodium dodecyl sulphate; ESI, electrospray ionization; MALDI, matrix-assisted laser desorption ionization; LD, liquid chromatography; IXC, ion exchange chromatography; CPPs, casein phosphopeptides; TOF, time-of-flight; MS, mass spectrometry; FTICR, Fourier transform ion-cyclotron resonance.

Introduction

Milk proteins have been extensively studied for over 50 years.^{1,2} Despite numerous comprehensive studies investigating the various components of milk proteins, many questions regarding their expression, structure, and modifications remain unresolved.^{3,4} Additionally, Dairy is one of the main sources for high quality protein in the human diet. Processing may, however, cause denaturation, aggregation, and chemical modifications of its amino acids, which may impact protein quality.⁵ Given the significant evolutionary divergence, the presence of multiple genetic variants, and the occurrence of post-translational modifications (PTMs), the milk proteome is highly complex.^{6,7} PTMs such as glycosylation, phosphorylation, disulfide bond formation, and proteolysis can generate a wide array of protein variants from a single gene product.⁸ Proteins derived from milk, specifically casein and whey, are some of the most widely used functional ingredients in the food, pharmaceutical, and cosmetic industries due to their distinct properties, versatile functionality, and beneficial health effects (Figure 1).^{3,7,9,10}



Figure 1 Graphical abstract.

Proteomics is an emerging field that has made significant advances in medicine over the past few decades. However, its vast potential for studying proteomes extends beyond medicine to other scientific disciplines.¹¹ Milk, being a highly heterogeneous and complex fluid, contains numerous genetic variants and isoforms, along with post-translational modifications (PTMs).¹² Given the large number of proteins and peptides present in its matrix, proteomics has proven to be a powerful tool for characterizing milk samples and their derived products.¹³ The technologies developed for separating and characterizing the milk proteome, such as two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and, more notably, mass spectrometry (MS), have enabled exhaustive profiling of the proteins and peptides present in milk and dairy products.^{14,15} These advancements have broad applications in the dairy industry, including the monitoring of key parameters such as microbiological safety, authenticity assurance, and control over transformation processes aimed at enhancing the quality of the final product.⁸

In the present review, we have discussed the structural and functional aspects of casein and whey proteins of milk. The proteomics techniques for the separation and characterization of milk proteins have been discussed in detail with recent advancements like proteomic mass spectrometry: Nano-Electrospray Ionization (nESI). Furthermore, the applications of casein and whey proteins have been extensively discussed in the food, pharmaceutical, and cosmetic industries. This review primarily serves as a source of literature for researchers in academia as well as industry professionals.

Casein and whey protein: Structural aspects and properties

Casein

Caseins are the predominant proteins in bovine milk, accounting for approximately 80% of the total milk protein. Caseins play a crucial role in nutrition, dairy processing, and various industrial applications. Casein is primarily composed of alpha-casein, beta-casein, and kappa-casein.⁷ Casein proteins are amphiphilic, meaning they have both hydrophilic and hydrophobic domains, which contribute to

their unique gel-forming ability in the stomach, resulting in slow digestion and prolonged amino acid release.¹⁶ This property makes casein particularly suitable for muscle recovery and maintenance over extended periods. Figure 2 represents a schematic of micellar casein and milk-derived whey protein manufacture.

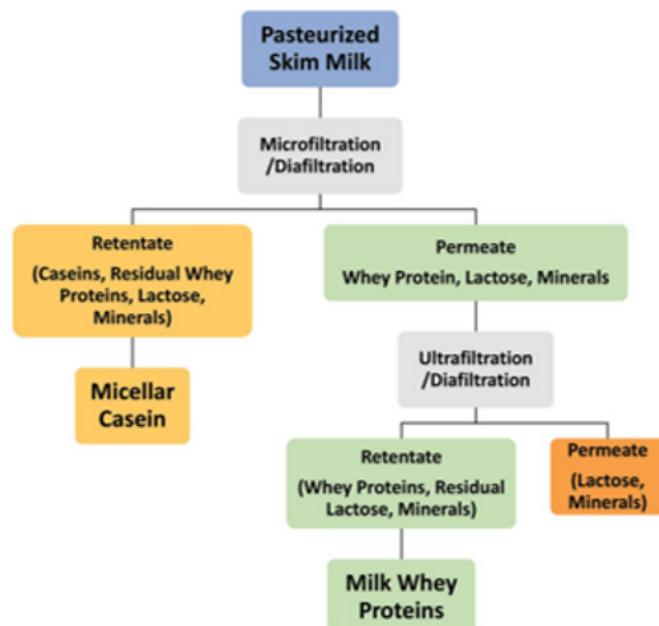


Figure 2 A schematic of micellar casein and milk-derived whey protein manufacture. Reproduced from reference number²⁵ with copyright permission from Elsevier Inc. and Fass Inc. under the terms of the Creative Commons CC by license.

Structural aspects of caseins

Caseins are a family of phosphoproteins predominantly found in mammalian milk, comprising four main types: α_{s1} , α_{s2} , β -, and κ -casein.

These proteins are unique due to their flexible, open conformations and lack of a stable tertiary structure, classifying them as intrinsically disordered proteins.¹⁷ The structural organization of caseins allows them to form large, spherical micellar aggregates known as casein micelles, stabilized by calcium phosphate nanoclusters and κ -casein's hydrophilic "hairy" layer, which prevents aggregation through steric repulsion. α - and β -caseins contribute hydrophobic interactions and calcium-binding phosphate regions that facilitate micelle assembly.¹⁸ Unlike globular proteins, casein lacks a well-defined tertiary structure, making it more flexible and capable of forming aggregates under different conditions.¹⁹ The disordered nature of caseins enables them to carry high calcium, and phosphate loads essential for neonatal nutrition while maintaining solubility. These micelles are crucial for delivering nutrients in milk and influence its physical and technological properties.^{20,21}

Functional properties

Casein is known for its excellent emulsification, water-holding, and gelling properties, making it essential in food processing. In cheese production, the coagulation of casein micelles is a critical step, facilitated by enzymatic action or acidification.²² Additionally, casein serves as a slow-digesting protein, contributing to prolonged amino acid release in the human body, which is beneficial for muscle repair and satiety.²³

Digestion and nutritional importance

Casein is digested more slowly than whey protein due to its ability to form a gel-like structure in the stomach, leading to sustained amino acid release.²³ It is a rich source of essential amino acids and bioactive peptides that have been linked to various health benefits, including improved immune function, antimicrobial activity, and potential antihypertensive effects.²⁴

Key characteristics of casein

Slow digestion and amino acid release: Casein forms a gel-like structure when it enters the acidic environment of the stomach. This slow digestion rate of casein allows for a prolonged release of amino acids into the bloodstream, making casein an excellent protein source for sustained muscle repair and growth.²⁶ However, isolated caseins, the digestion rate is actually much faster due to the absence of a three-dimensional structure in the protein.²⁷

Calcium binding: Casein can bind to calcium, which is beneficial for bone health. Its calcium-binding property not only contributes to its nutritional value but also impacts its digestion and bioavailability.²⁸⁻³⁰

Gelation and rheological properties: Casein is unique in its ability to form gels or curds, which is especially important in cheese production. The gelation of casein is essential in creating the desired texture and mouthfeel in dairy products like cheese and yogurt.³¹ The gel-like structure also contributes to casein's slow release of amino acids during digestion, which is beneficial for both satiety and sustained muscle protein synthesis.

Whey protein

Whey protein is the water-soluble fraction of milk protein and represents around 20% of the protein content in bovine milk. The major proteins in whey include beta-lactoglobulin, alpha-lactalbumin, serum albumin, and immunoglobulins. Whey protein is characterized by its rapid digestion and high bioavailability, making it a preferred choice for post-exercise recovery. Table 1 describes the technical data of different milk proteins.⁷

Key characteristics of whey protein:

Fast Absorption: Whey protein is rapidly absorbed, providing an immediate supply of amino acids.^{32,33}

Rich in Branched-Chain Amino Acids (BCAAs): Specifically, leucine, which plays a crucial role in promoting muscle protein synthesis.³⁴

High Biological Value (BV): Whey protein has one of the highest biological values, meaning it provides a complete spectrum of essential amino acids.^{35,36}

Proteomics techniques

Protein separation

The protein separation is the first and important step in proteomics studies. There are different methods and techniques have been developed by researchers for the separation of proteins, but there are two most popular methods for protein separation, *viz.* two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)^{37,38} and Liquid phase separation.^{39,40} Both the methods have been discussed below in detail.

Table 1 Technical data of different milk proteins

Protein	Solubility	Isoelectric point (pI)	Molecular weight (kDa)	Functional properties
Casein	Low at pH 4.6 (isoelectric point); increases with pH away from pI	~4.6	α -s1: 23–23.6, α -s2: 24.3–25.2, β : 23.6–24, κ : 19	Excellent emulsifying properties, forms gels, used in cheese-making
β -lactoglobulin	Good solubility except near its pI	~5.2	18	Good heat stability, emulsifying properties, gel formation under heat
α -lactalbumin	High solubility over a wide pH range, except near its pI	~4.2	14	High nutritional value, enhances calcium absorption
Immunoglobulins	Varies; generally less soluble at their isoelectric point	Varies; typically, around 7	150–900 (IgA, IgG, IgM)	Immune function, bind pathogens
Lactoferrin	High solubility across a broad range of pH levels	Around 8.0	80	Strong antibacterial and antioxidant activity, iron-binding capacity

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE):

The 2D PAGE is an excellent technique established in the 20th century for the separation of protein from complex systems.⁴¹ This electrophoretic system is a combination of electrophoretic techniques of isoelectric focusing (IEF) and sodium dodecyl sulphate (SDS) PAGE. The technique basically uses two protein properties for its separation, *i.e.*, molecular mass (Mr) and isoelectric point (pI). This technique is a highly effective tool for separating proteins with similar molecular weights (Mr), which are often difficult to distinguish using one-dimensional SDS-PAGE. Similarly, proteins with comparable isoelectric points (pI) are often not well-resolved on IEF gels, especially when analyzing mixtures with high concentrations of specific proteins (*e.g.*, milk). By using a two-dimensional separation approach, resolution is significantly enhanced. Additionally, this method enables researchers to quickly establish a lower limit on the number of proteins present. Readers may follow detailed explanations of 2D-PAGE techniques in various reviews by Gorg *et al.* and Westbrook *et al.*,^{42,43} Despite its powerful capabilities as an analytical technique for resolving proteins, 2D-PAGE has several notable limitations. These include its inability to separate single polypeptide chains weighing more than approximately 150 kDa or less than about 8 kDa. Additionally, 2D-PAGE has a limited dynamic range, meaning it struggles to detect proteins with significantly lower abundance compared to the most abundant ones.^{44,45}

Prefractionation of proteins from a sample is commonly used before 2D-PAGE to improve analysis. This approach can either isolate specific protein subsets or remove highly abundant proteins. By achieving these goals, prefractionation enhances the loading capacity for minor protein components, enabling the detection of low-abundance proteins.¹

Liquid phase separation

Liquid phase separation is one of the common methods of protein separation in recent years for proteomic studies.³⁹ This technique utilizes capillary electrophoresis (CE) and liquid chromatography (LC) together. The liquid phase separation has a distinct advantage as compared to 2D-PAGE; it includes superior sensitivity, little faster, is easy to automate, and with superior dynamic range. In addition, liquid

phase separations can utilize a different separation mechanism (*e.g.* size exclusion, reversed phase (RP), ion exchange), and as such can analyse strongly basic/acidic proteins and proteins of any molecular weight.

A relatively recent advancement in LC-based proteomic analysis is multidimensional LC. First time introduced by Washburn, Wolters, and Yates.⁴⁶ This method involves the sequential use of two or more LC separation techniques (*e.g.*, ion exchange chromatography (IXC) and RP HPLC) coupled with on-line Mass Spectra (MS) and has been successfully applied in various applications. Table 2 represents a comparative summary of 2D-PAGE and LC separation.

Table 2 Comparison of 2D-PAGE and liquid chromatography protein separation techniques for use within proteomic studies

Property	2D-PAGE	Liquid chromatography
Resolution	Very high	Moderate
Separation of		
Acidic/basic proteins	Poor	Good
Low-MW peptides	Poor	Good
High-MW proteins	Poor	Good
Membrane proteins	Poor	Average
	pI	Hydrophobicity
		Reversed phase
Separation based on	Mr	Immunoaffinity
		Ligand affinity
		Ion exchange
Dynamic resolution	Low	Moderate
Automation	Limited	Good
Speed	Hours-days	Minutes-hours

Protein characterization

Protein characterization is essential for a variety of scientific and practical reasons, and it plays a critical role in advancing fields like molecular biology, medicine, and industrial biotechnology. Here are some of the key reasons why protein characterization is important: understanding biological functions, drug development and target identification, disease diagnosis and treatment, and biotechnology

and industrial applications. Below, we have discussed some of the important methods/techniques for protein characterization.

Mass spectrometric analysis

The separated individual proteins need to be identified and, in many cases, need to be comprehensively characterized. The mass spectrometry (MS) primarily covers the proteomics analysis. In recent decades, MS has enabled the rapid, comprehensive characterization of proteins. The soft ionization techniques of electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) are the common methods used in most mass spectrometers designed for biological applications.^{47,48} Major detectors used in such devices range from the simpler quadrupole (Q), time-of-flight (TOF) analysers to more complex devices such as Fourier transform ion-cyclotron resonance (FTICR) analysers.¹

Protein identification in proteomics: process overview

The identification of proteins typically involves a four-step process: protein separation, protein digestion, mass spectrometry analysis of the resulting peptides, and comparison of the observed peptides against a database. Protein digestion can be performed either before or after separation, depending on the technique used.¹¹ In liquid-phase separation, proteins from complex sample mixtures are digested before separation.^{49,50} Conversely, in 2D-PAGE, an *in-gel* or *in situ* digestion is performed after the separation step.⁵¹ The advantage of 2D-PAGE-based methods is their ability to generate a distinct set of peptides from a single protein, making it possible to assign protein identities using peptide mass fingerprinting (PMF) data alone.⁵²

Advancements in proteomic mass spectrometry: Nano-Electrospray Ionization (nESI)

A significant advancement in proteomic mass spectrometry (MS) techniques is the introduction of nano-electrospray ionization (nESI), as described by Wilm *et al.*⁵³ This technique uses fine capillary tubes to achieve extremely low sample flow rates, as little as 20 nL/min, which substantially extends the period a sample can be scanned. This extended scan time enables detailed analysis of limited or low-abundance samples, enhancing the sensitivity and precision of MS-based proteomic investigations. The increased sensitivity of nESI has proven particularly effective for detecting low-abundance proteins, making it a powerful tool for studying post-translational modifications (PTMs) in trace samples. Several studies have demonstrated the effectiveness of nESI in analyzing PTMs in low-abundance proteins.^{54,55} Furthermore, mass spectrometers incorporating nESI can be coupled with micro- and nano-flow liquid chromatography (LC) systems, providing enhanced separation capacity. This combination allows for greater resolution and accuracy in the detection and quantification of complex proteomic samples.⁵⁶

Proteomics of casein and whey protein

Proteomics involves the large-scale analysis of proteins, with particular emphasis on their structures and functions. In the context of casein and whey proteins, proteomics focuses on an in-depth examination of their structural characteristics, functional roles, and bioactive properties using advanced techniques like mass spectrometry and liquid chromatography. Both casein and whey proteins are derived from milk, but they differ significantly in their amino acid composition, digestion, and biological roles, making them valuable in both nutritional science and food technology.

Casein proteomics

Casein proteins account for about 80% of the protein content in bovine milk. They are characterized by a unique structure with a high degree of phosphorylation, which plays a crucial role in their functional properties. Caseins form a gel or curd when exposed to stomach acid, leading to slow digestion and a gradual release of amino acids, which is beneficial for sustained protein absorption. This slow-digesting property makes casein a popular choice for meal replacements and nighttime protein supplements.

Proteomic studies on casein have utilized high-resolution techniques like mass spectrometry to identify phosphorylation sites, which significantly impact the protein's structure, solubility, and nutritional availability. Additionally, caseins are often analyzed for their interaction with calcium and their role in promoting bone health due to their calcium-binding properties.¹⁵ Other studies have explored how modifications in casein phosphorylation affect its digestibility and functional properties in food products.^{57,58}

Recent studies in casein proteomics have identified multiple bioactive peptides that are released during digestion. These peptides have been shown to exhibit various health benefits, such as antimicrobial, antihypertensive, and immunomodulatory effects.^{59,60} Moreover, casein phosphopeptides (CPPs) have gained attention for their ability to enhance mineral bioavailability, particularly calcium, which is beneficial for bone health.⁶¹

Whey protein proteomics

Whey proteins, comprising about 20% of the protein in milk, are fast-digesting and known for their high biological value due to their rich content of essential amino acids, particularly leucine, which plays a critical role in muscle protein synthesis.^{1,3,59} Whey proteins are highly soluble, which contributes to their rapid absorption and digestion. Whey proteins are primarily composed of beta-lactoglobulin, alpha-lactalbumin, and immunoglobulins, with beta-lactoglobulin being the most abundant protein in whey.

Proteomics of whey proteins have identified a range of bioactive peptides that exhibit antimicrobial, antioxidant, and anti-inflammatory properties. These peptides contribute to the immune-modulating and gut-health-promoting effects of whey protein.^{9,62} Research has also focused on identifying minor whey proteins that have potential health benefits, such as lactoferrin and serum albumin, which possess immunomodulatory and antimicrobial properties.³

Comparative proteomics of casein and whey proteins

Proteomic comparisons between casein and whey proteins provide valuable insights into their respective physiological benefits. While casein is favored for its slow digestion and prolonged amino acid release, whey is preferred for its rapid digestion and superior amino acid profile, making it particularly effective in muscle recovery after exercise. Both proteins have complementary roles in human nutrition, with casein being effective in promoting satiety and muscle preservation over extended periods and whey being ideal for rapid muscle recovery and synthesis.⁶³

Proteomic technologies, such as tandem mass spectrometry (LC-MS/MS), have allowed researchers to characterize the complete proteome of both casein and whey proteins, facilitating the discovery of novel bioactive peptides and providing a deeper understanding of their role in human health. The proteomic analysis of these proteins

also aids in optimizing dairy products for specific health outcomes, such as muscle building, weight management, and immune function enhancement.

Applications of casein and whey protein

Both casein and whey protein are used in diverse applications, particularly in the food, pharmaceutical, and cosmetic industries.

Food industries

Casein and whey proteins play essential roles in the food industry due to their nutritional, functional, and sensory properties. They are widely used in dairy, bakery, meat, beverages, frozen desserts, and nutritional formulas to enhance texture, stability, and nutritional value.

Dairy products: Both casein and whey proteins are widely used in dairy-based foods due to their functional properties, such as emulsification, water-binding, and gelation.

- a) Cheese production: Casein plays a vital role in cheese making as it forms curds when coagulated with rennet or acid, while whey protein remains in the liquid whey.³¹
- b) Yogurt and fermented products: Whey proteins enhance creaminess and texture due to their water-holding capacity.⁶⁴

Bakery and confectionery: Casein and whey proteins improve the texture, shelf life, and nutritional quality of baked goods. Casein improves dough strength and elasticity, leading to better volume and texture in bread, and helps reduce staling and extends shelf life.¹² Whereas whey proteins in confections act as emulsifiers in chocolate and caramel and enhance foam stability in marshmallows.³²

Meat and seafood products: Casein and whey proteins are used to improve the texture, water retention, and protein content of processed meat and seafood products. Casein in meat products enhances water retention and reduces cooking loss. It also improves binding properties in sausages and meatballs.⁶⁵ Whey proteins in seafood products improve gelation and texture in surimi-based products.⁶⁶

Ice cream and frozen desserts: Casein and whey proteins are used to improve the texture, creaminess, and stability of frozen desserts. Casein in ice cream enhances emulsification and prevents ice crystal formation. It has been found that it also improves melting resistance in these products. Whey proteins in frozen desserts increase aeration, overrun, and enhance creaminess and mouthfeel.^{67,68}

Nutritional and infant formulas: Both casein and whey proteins are used in nutritional formulas for infants, athletes, and individuals with special dietary needs. Casein in infant formula provides slow-digesting proteins for prolonged satiety and enhances calcium absorption.³³

Sports nutrition: Whey protein is commonly found in protein powders, bars, and supplements designed for post-workout recovery. Casein is often incorporated into nighttime protein supplements to support muscle repair during sleep.^{23,69}

Pharmaceutical industries

Casein and whey proteins play a significant role in the pharmaceutical industry, offering diverse applications in drug delivery, wound healing, tissue engineering, and clinical nutrition. Their bioactive, biodegradable, and biocompatible properties make them valuable for creating innovative pharmaceutical formulations.

Drug delivery systems: Casein and whey proteins are extensively used as carriers for drug delivery due to their biodegradability,

biocompatibility, and controlled-release properties. Casein-based drug carriers like micelles and nanoparticles made from casein are used for the targeted delivery of hydrophobic drugs. They offer a slow and sustained release, improving drug bioavailability.^{70,71} Whey protein-based nanocarriers are used for the controlled release of anticancer drugs and bioactive compounds and especially protect the drug from degradation and enhance stability. Most of the whey proteins are used for the encapsulation of drugs of bioactive molecules.¹⁴

Immunotherapy and vaccines: Casein and whey proteins are being explored as adjuvants and carriers in vaccines and immunotherapy. Casein acts as an immunomodulator and is used as a carrier for antigens in oral vaccines. It enhances mucosal immunity due to its biocompatibility and ability to protect antigens from gastric degradation.⁷² Whey protein fractions, such as lactoferrin, show antiviral and antibacterial activity. Generally used in vaccine formulations to enhance immune responses.⁷³

Wound healing and tissue engineering: Casein and whey proteins are used in wound healing formulations and tissue engineering due to their bioactive properties. Casein hydrolysates contain bioactive peptides that promote cell proliferation and tissue repair. It is reported in hydrogel forms for biofilm formation and faster healing.⁴ These whey proteins are rich in essential amino acids, which promote the collagen production and cell growth during tissue regeneration and are considered as suitable materials for wound dressing.¹⁰

Nutraceutical and clinical nutrition: Both casein and whey proteins are used in pharmaceutical grade nutraceuticals and clinical nutrition products for patients with malnutrition, sarcopenia, and chronic diseases. Casein in clinical nutrition provides slow-release amino acids, making it suitable for chronic disease management and muscle wasting conditions and used in enteral nutrition formulas for cancer and elderly patients.²³ Whey is used in cancer cachexia and sarcopenia therapy due to its high biological value and ability to promote muscle synthesis. Included in oral nutritional supplements for patients with chronic conditions.⁷⁴

Antimicrobial and antioxidant applications: Casein and whey proteins contain bioactive peptides with antimicrobial and antioxidant properties, making them suitable for pharmaceutical preservation and therapeutics. Casein-derived peptides exhibit antimicrobial properties against pathogens like *E. coli* and *S. aureus*. Also, it has been used in antimicrobial coatings and formulations.¹³ Whey proteins have cysteine-rich peptides that promote glutathione production, enhancing cellular antioxidant capacity.⁷⁵

Protein-based therapeutics: Casein and whey proteins are used as protein carriers and in protein therapeutics due to their stability and biocompatibility. Casein is used as a carrier for enzymes and therapeutic proteins. It protects bioactive proteins from degradation in the gastrointestinal tract.^{71,76}

Functional foods: Bioactive peptides derived from casein and whey have been used in the development of functional foods that promote health benefits beyond basic nutrition, such as lowering blood pressure or improving immune function.⁷⁷

Cosmetic industries

Casein and whey proteins have versatile applications in the cosmetic industry due to their hydrating, anti-aging, repairing, and brightening properties. They are widely used in skincare, haircare, body care, and anti-aging products, making them essential ingredients in modern cosmetic formulations.

Skin moisturizers and hydrating products: Casein and whey proteins are widely used in moisturizing creams, lotions, and serums due to their water-binding properties and ability to form a protective film on the skin. Casein improves moisture retention by forming a thin film on the skin's surface and provides a soft and smooth texture, enhancing skin feel.^{5,78} Whey protein is rich in amino acids, which enhance the skin's natural moisture barrier. Hence used in anti-aging and hydrating serums for its firming properties.

Anti-aging and firming products: Both casein and whey proteins are used in anti-aging creams and serums due to their collagen-boosting properties and ability to improve skin elasticity. Casein contains bioactive peptides that stimulate collagen production, reducing wrinkles and fine lines and further enhancing skin elasticity and firmness.⁷⁹ Whey protein promotes skin regeneration by supporting fibroblast growth. And therefore used in firming and lifting creams for its skin-tightening effect.⁸⁰

Hair care and conditioning products: Casein and whey proteins are popular ingredients in shampoos, conditioners, and hair masks due to their repairing and strengthening properties. Casein forms a protective film over hair strands, enhancing shine and smoothness and improving hair elasticity, and reducing breakage. Whey protein is rich in cysteine and amino acids, which strengthen hair fibres and are used in volumizing shampoos and conditioners for improved hair texture.⁸¹

Summary and future prospective

Casein and whey protein are both highly valued for their distinct properties and biological activities. While casein's slow-digesting nature makes it suitable for prolonged amino acid release, whey protein's rapid digestion promotes fast muscle recovery. Recent advances in proteomics have provided a deeper understanding of the bioactive peptides in these proteins, highlighting their potential in various nutrition, biomedical, and health applications. Both proteins continue to play a critical role in food science, nutrition, pharmaceuticals, and cosmetics, with ongoing research to explore their full potential in improving human nutrition and health. In a nutshell, the casein and whey proteins have been studied well in the literature. However, more research is needed to understand the complete milk proteome for its further application in different domains.

Acknowledgments

None.

Funding

None.

Conflicts of interest

The authors declare no conflict of interest related to this publication.

References

- O'Donnell R, Holland JW, Deeth HC, et al. Milk proteomics. *Int Dairy J*. 2004;14(12):1013–1023.
- Farrell HM Jr, Bleck GT, Brown EM, et al. Nomenclature of the proteins of cows' milk—sixth revision. *J Dairy Sci*. 2004;87(6):1641–1674.
- Kazimierska K, Kalinowska-Lis U. Milk proteins—their biological activities and use in cosmetics and dermatology. *Molecules*. 2021;26(11):3253.
- Madureira AR, Tavares T, Gomes AMP, et al. Physiological properties of bioactive peptides obtained from whey proteins. *J Dairy Sci*. 2010;93(2):437–455.
- Van Lieshout GAA, Lambers TT, Bragt MCE, et al. How processing may affect milk protein digestion and overall physiological outcomes: a systematic review. *Crit Rev Food Sci Nutr*. 2020;60(14):2422–2445.
- Gai N, Uniacke-Lowe T, O'Regan J, et al. Effect of protein genotypes on physicochemical properties and protein functionality of bovine milk. *Foods*. 2021;10(10):2409.
- Nayik GA, Gull A, Masoodi L, et al. Milk proteins: chemistry, functionality and diverse industrial applications. *Cogent Food Agric*. 2024;10(1):2377686.
- Holland JW. Post-translational modifications of caseins. *Milk Proteins*. 2008;107–132.
- Olvera-Rosales LB, Cruz-Guerrero AE, García-Garibay JM, et al. Bioactive peptides of whey: obtaining, activity, mechanism of action, and applications. *Crit Rev Food Sci Nutr*. 2023;63(30):10351–10381.
- Davari N, Bakhtiary N, Khajehmohammadi M, et al. Protein-based hydrogels as promising materials for tissue engineering. *Polymers (Basel)*. 2022;14(5):986.
- Aebersold R, Mann M. Mass spectrometry-based proteomics. *Nature*. 2003;422:198–207.
- De la Fuente MA, Singh H, Hemar Y. Recent advances in the characterisation of heat-induced aggregates of whey proteins. *Trends Food Sci Technol*. 2002;13(8):262–274.
- Hayes M, Ross RP, Fitzgerald GF, et al. Casein-derived antimicrobial peptides generated by *Lactobacillus acidophilus*. *Appl Environ Microbiol*. 2006;72(3):2260–2264.
- Farooq MA, Aquib M, Ghayas S, et al. *Polym Adv Technol*. 2019;30(9):2183–2191.
- Ji Z, Yu Z, Du Q, et al. Comparative proteomic study of casein micelles in human and animal milks. *LWT*. 2025;220:117560.
- Kim J. Pre-sleep casein protein ingestion in post-exercise recovery nutrition. *Phys Act Nutr*. 2020;24(2):6–10.
- Holt C, Timmins PA, Errington N, et al. A core-shell model of calcium phosphate nanoclusters stabilized by beta-casein phosphopeptides, derived from sedimentation equilibrium and small-angle X-ray and neutron-scattering measurements. *Eur J Biochem*. 1998;252(1):73–78.
- Dalgleish DG, Corredig M. The structure of the casein micelle and its changes during processing. *Annu Rev Food Sci Technol*. 2012;3:449–467.
- de Kruif CG, Huppertz T. Casein micelles: size distribution in milks from individual cows. *J Agric Food Chem*. 2012;60(18):4649–4655.
- Pastrana EF, D'Oria G, Mota-Santiago P, et al. Emulsification of milk fat and skimmed milk curds. *Int Dairy J*. 2025;162:106153.
- Lucey JA. Milk protein gels. In: *Milk Proteins*. 2008:449–481.
- Li Q, Zhao Z. Acid- and rennet-induced coagulation behavior of modified casein micelles. *Food Chem*. 2019;291:231–238.
- Boirie Y, Dangin M, Gachon P, et al. Slow and fast dietary proteins modulate postprandial protein accretion. *Proc Natl Acad Sci U S A*. 1997;94(26):14930–14935.
- Meisel H, Fitzgerald RJ. Biofunctional peptides from milk proteins. *Curr Pharm Des*. 2003;9(16):1289–1295.
- Carter BG, Cheng N, Kapoor R, et al. Microfiltration-derived casein and whey proteins from milk. *J Dairy Sci*. 2021;104(3):2465–2479.
- Boulier A, Denis S, Henry G, et al. Casein structures affect postprandial amino acid delivery. *Food Chem*. 2023;415:135779.
- Mandalari G, Adel-Patient K, Barkholt V, et al. In vitro digestibility of β -casein and β -lactoglobulin. *Regul Toxicol Pharmacol*. 2009;55(3):372–381.

28. Nwachukwu ID, Aluko RE. Food protein structures and functionality. In: *Food Protein Structures*. 2021;1–33.

29. Liao W, Liu S, Liu X, et al. Novel calcium-binding peptide from casein hydrolysate. *Food Funct.* 2019;10(12):7724–7732.

30. Cross KJ, Huq NL, Palamara JEA, et al. Physicochemical characterization of casein phosphopeptide–amorphous calcium phosphate nanocomplexes. *J Biol Chem.* 2005;280(15):15362–15369.

31. Fox PF, Uniacke-Lowe T, McSweeney PLH, et al. *Dairy Chemistry and Biochemistry*. Springer; 2015.

32. Mulcahy EM, Fargier-Lagrange M, Mulvihill DM, et al. Characterisation of heat-induced protein aggregation in whey protein isolate. *Food Chem.* 2017;229:66–74.

33. Lönnerdal B. Nutritional and physiologic significance of human milk proteins. *Am J Clin Nutr.* 2003;77(6 suppl):1537S–1543S.

34. Bo T, Fujii J. Primary roles of branched-chain amino acids and their metabolism in physiology and metabolic disorders. *Molecules.* 2024;30(1):56.

35. González-Weller D, Paz-Montelongo S, Bethencourt-Barbuzano E, et al. Proteins and minerals in whey protein supplements. *foods.* 2023;12(11):2238.

36. Ha E, Zemel MB. Functional properties of whey, whey components, and essential amino acids. *J Nutr Biochem.* 2003;14(5):251–258.

37. Rabilloud T. Two-dimensional gel electrophoresis in proteomics. *Proteomics.* 2002;2(1):3–10.

38. Rabilloud T, Chevallat M, Luche S, et al. Two-dimensional gel electrophoresis in proteomics: past, present and future. *J Proteomics.* 2010;73(11):2064–2077.

39. Issaq HJ, Conrads TP, Janini GM, et al. Methods for fractionation, separation and profiling of proteins and peptides. *Electrophoresis.* 2002;23(17):304–3061.

40. Issaq HJ. The role of separation science in proteomics research. *Electrophoresis.* 2001;22(17):362–3638.

41. O'Farrell PH. High resolution two-dimensional electrophoresis of proteins. *J Biol Chem.* 1975;250(10):4007–4021.

42. Westbrook JA, Yan JX, Wait R, et al. Zooming-in on the proteome: very narrow-range immobilized pH gradients reveal more protein species. *Electrophoresis.* 2001;22(14):2865–2871.

43. Görg A, Obermaier C, Boguth G, et al. The current state of two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis.* 2000;21(6):1037–1053.

44. Vuong GL, Weiss SM, Kammer W, et al. Improved sensitivity proteomics by postharvest alkylation and radioactive labeling. *Electrophoresis.* 2000;21(13):2594–2605.

45. Gygi SP, Corthals GL, Zhang Y, et al. Evaluation of two-dimensional gel electrophoresis-based proteome analysis. *Proc Natl Acad Sci U S A.* 2000;97(17):9390–9395.

46. Washburn MP, Wolters D, Yates JR. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat Biotechnol.* 2001;19(3):242–247.

47. Fenn JB, Mann M, Meng CK, et al. Electrospray ionization for mass spectrometry of large biomolecules. *Science.* 1989;246(4926):64–71.

48. Tanaka K, Waki H, Ido Y, et al. Protein and polymer analyses up to m/z 100,000 by laser ionization TOF MS. *Rapid Commun Mass Spectrom.* 1988;2(8):151–153.

49. Graves PR, Haystead TAJ. Molecular biologist's guide to proteomics. *Microbiol Mol Biol Rev.* 2002;66(1):39–63.

50. Gingras AC, Gstaiger M, Raught B, Aebersold R. Analysis of protein complexes using mass spectrometry. *Nat Rev Mol Cell Biol.* 2007;8(8):645–654.

51. Shevchenko A, Tomas H, Havliš J, et al. In-gel digestion for mass spectrometric characterization. *Nat Protoc.* 2006;1(6):2856–2860.

52. James P. Protein identification in the post-genome era. *Q Rev Biophys.* 1997;30(4):279–331.

53. Wilm M, Mann M. Analytical properties of the nanoelectrospray ion source. *Anal Chem.* 1996;68(1):1–8.

54. Chen S, Zeng J, Zhang Z, Xu B, Zhang B. Recent advancements in nano-electrospray ionization interfaces. *J Chromatogr Open.* 2022;2:100064.

55. Pan P, Gunawardena HP, Xia Y, et al. Nanoelectrospray ionization of protein mixtures. *Anal Chem.* 2004;76(4):1165–1174.

56. Smith RD, Shen Y, Tang K. Ultrasensitive and quantitative proteome analyses. *Acc Chem Res.* 2004;37(4):269–278.

57. Zhang Y, Guo Y, Jiang C, et al. A review of casein phosphopeptides: enrichment, identification, and biological properties. *Food Biosci.* 2024;59:104217.

58. Hewa Nadugala B, Pagel CN, Raynes JK, et al. The effect of casein genetic variants, glycosylation and phosphorylation on bovine milk protein structure, technological properties, nutrition and product manufacture. *Int Dairy J.* 2022;133:105440.

59. Fernández-Tomé S, Hernández-Ledesma B. Gastrointestinal digestion of food proteins and bioactive peptides. *Mol Nutr Food Res.* 2020;64(21):2000401.

60. Akbarian M, Khani A, Eghbalpour S, et al. Bioactive peptides: synthesis, sources, and mechanisms. *Int J Mol Sci.* 2022;23(3):1445.

61. Chakrabarti S, Guha S, Majumder K. Food-derived bioactive peptides in human health: Challenges and opportunities. *Nutrients.* 2018;10(11):1738.

62. Zhao C, Ashaolu TJ. Bioactivity and safety of whey peptides. *LWT.* 2020;134:109935.

63. Jensen HB, Poulsen NA, Møller HS, et al. Comparative proteomic analysis of casein and whey. *J Dairy Res.* 2012;79(4):451–458.

64. Tamime AY, Robinson RK. Microbiology of yoghurt and starter cultures. In: *Tamime and Robinson's Yoghurt*. 3rd ed. Woodhead; 2014.

65. Jin SK, Lee SH, Kim SH, et al. Effect of non-meat proteins on pork emulsified sausages. *Food Sci Anim Resour.* 2024;44(5):1069–1079.

66. Guo G, Li Q, Du L, et al. Whey protein effects on surimi gel properties. *Food Sci Technol Int.* 2025;31:1–10.

67. Sofjan RP, Hartel RW. Effects of overrun on structural and physical characteristics of ice cream. *Int Dairy J.* 2004;14(3):255–262.

68. Eisner MD, Wildmoser H, Windhab EJ. Air cell micro structuring in a high viscous ice cream matrix. *Colloids Surf A Physicochem Eng Asp.* 2005;263(1-3):390–399.

69. Tang C. Chemical properties of whey protein and muscle growth. *Sports Health.* 2025.

70. Shaker MA, Elbadawy HM, Al Thagfan SS. Enhancement of atorvastatin oral bioavailability via encapsulation in polymeric nanoparticles. *Int J Pharm.* 2021;592:120077.

71. Wang L, Jia W, Yang Q, et al. Casein nanoparticles as oral delivery carriers for improved bioavailability and hypoglycemic activity of apigenin. *Food Hydrocoll.* 2024;146:109194.

72. Tavares GM, Croguennec T, Carvalho AF, et al. Milk proteins as encapsulation devices and delivery vehicles: Applications and trends. *Trends Food Sci Technol.* 2014;37(1):5–20.

73. Wakabayashi H, Yamauchi K, Takase M. Lactoferrin research and applications. *Int Dairy J*. 2006;16(11):1241–1251.
74. Wirunsawanya K, Upala S, Jaruvongvanich V, et al. Whey protein supplementation improves body composition and cardiovascular risk factors in overweight and obese patients: a systematic review and meta-analysis. *J Am Coll Nutr*. 2018;37(1):60–70.
75. Moro T, Brightwell CR, Velarde B, et al. Whey protein hydrolysate increases amino acid uptake, mTORC1 signaling, and protein synthesis in skeletal muscle of healthy young men in a randomized crossover trial. *J Nutr*. 2019;149(7):1149–1158.
76. Zahariev N, Draganova M, Zagorchev P, et al. Casein-based nanoparticles: a potential tool for the delivery of daunorubicin in acute lymphocytic leukemia. *Pharmaceutics*. 2023;15(2):471.
77. Yigit A, Bielska P, Cais-Sokolinska D, et al. Whey proteins as a functional food: Health effects, functional properties, and applications in food. *J Am Nutr Assoc*. 2023;42(8):758–768.
78. Secchi G. Role of protein in cosmetics. *Clin Dermatol*. 2008;26(4):321–325.
79. Al-Rawhani AH, Adznam SN, Zaid ZA, et al. Effectiveness of whey protein supplementation on muscle strength and physical performance of older adults: A systematic review and meta-analysis of randomized clinical trials. *Clin Nutr*. 2024;43(10):2412–2426.
80. Gorouhi F, Maibach HI. Role of topical peptides in preventing or treating aged skin. *Int J Cosmet Sci*. 2009;31(5):327–345.
81. Sardana K, Sachdeva S. Role of nutritional supplements in selected dermatological disorders: A review. *J Cosmet Dermatol*. 2022;21(1):85–98.