Milk Proteins

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Abstract
Milk contains many types of proteins, which are classified into two general categories – caseins and whey proteins. Due to their nutritional and functional properties, milk protein products are widely used in the food industry. This entry summarizes the current knowledge on milk protein fractions and their biological properties. The production of the main protein-enriched products is described. In addition, an overview of the current and potential biomedical applications of milk proteins is given.

INTRODUCTION

Milk proteins are usually classified into two major categories: the caseins (CNs), which coagulate or precipitate at pH 4.6, and the serum (whey) proteins that remain in solution at pH 4.6. Because of their high nutritional quality and versatile functional properties, milk proteins are widely used as ingredients in many manufactured foods. More recently, milk proteins have been used for drug delivery and tissue engineering applications.

This entry starts by presenting the bovine milk protein system followed by an overview of the methods for the commercial production of the main milk protein-enriched products. Information on the reported health effects attributed to milk proteins is also given. The entry ends by summarizing the latest research on milk proteins' biomedical applications.

MILK COMPOSITION

The major constituents of milk are water (87.1%), lactose (4.6%), fat (4.0%), protein (3.3%), minerals (0.7%), organic acids (0.17%), and a small number of other constituents present only in trace amounts, e.g., vitamins and enzymes (0.15%). Bovine milk contains about 3.3% milk proteins, discounting non-protein nitrogen, e.g., urea, ammonia, and free amino acids. Around 80% of the total amount of proteins are CNs; the remaining 20% are referred to as whey proteins or milk serum proteins.[1]

Milk Proteins (Lactoproteins)

Proteins present in milk are divided into CNs and whey proteins, which are separated by milk acidification to pH 4.6.

Caseins

Caseins are defined chemically as the milk proteins that precipitate at pH 4.6, which are found in milk and synthesized in mammary glands in response to the hormone prolactin.[2] Caseins express an extraordinary degree of heterogeneity, involving several proteins that diverge substantially in composition and functions.[3] By separating fragments by electrophoresis, it is possible to determine α, β, κ, and γ-CN (α-CN, β-CN, κ-CN, and γ-CN). γ-CN is a proteolytic product obtained after cleavage of β-CN by plasmin. The CN products are discriminated based on their electrophoretic properties and the capacity to precipitate in the presence of calcium, at a concentration of 6 mM and at a temperature of 20°C, into the calcium-sensitive α- and β-CN on one hand and the insensitive κ-CN on the other hand. As milk contains roughly 30 mmol/L Ca, it would be expected that most of the CNs would precipitate in milk. However, κ-CN is soluble at high concentrations of Ca, and it reacts with and stabilizes the Ca-sensitive CNs through the formation of CN micelles.[2–4]

The bovine CN group comprehends four individual gene products known as α_{S1}-CN, α_{S2}-CN, β-CN, and κ-CN in estimated proportions of 4:1:4:1 (w/w). Each of the focal CN components displays additional variability due to different degrees of phosphorylation, glycosylation, disulfide bonding, proteolysis, and genetic polymorphism.[4] Caseins have a relatively high proline content and consequently very little secondary or tertiary structure that can be denatured. Yet, at temperatures higher than 140°C, CNs heat coagulate either by colloidal aggregation or by chemical cross-linking. The colloidal aggregation involves calcium bridges, depending on temperature, calcium concentration, and pH.[1]

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The low content of α-helical structure makes the CNs to be open and flexible, enabling the accessibility for the proteolytic enzymes to the CN molecules and making them easy to degrade. The CNs are capable of associating with each other or self-association, depending on pH, ionic strength, and temperature. Depending on the quantity of phosphoserine residues in the molecule, the different CN components indicate diverse calcium-binding abilities. κ-CN has a very low, if any, calcium-binding capacity with only one phosphoserine, while α_{S1}-CN, α_{S2}-CN, and β-CN, with several phosphoserines, have high binding capacities. About 95% of the CNs in bovine milk are incorporated into large colloidal structures: micelles. These structures are not still entirely understood; however, various models have been proposed. The most denoted and debated models are "the submicelle model," "the hairy model," and the "dual binding model." The proposed CN micelle models have two important aspects in common: the inclusion of calcium phosphate and the κ-CN rich surface. It has been proposed that the CNs are organized into tubular substructures with gaps in between and that κ-CN is grouped at the end of these tubules, on the outer part of the micelle.[3–6]

All major milk proteins have dissimilar genetic variants. Genetic polymorphism of milk proteins arises when amino acids are either substituted in or deleted from the amino acid chain. These alterations are the consequence of mutations causing changes in the gene base sequences. The detected genetic variants of CN proteins are: α_{S1}-CN A, B, C, D, E, F, G, H; α_{S2}-CN A, B, C, D; β-CN A1, A2, A3, B, C, D, E, F,G; and κ-CN A, B, C, E, FS, FI, GS, GE, H, I, J.[7]

Milk whey proteins

The amount of whey proteins is approximately 20% of the total milk proteins. These proteins are chemically characterized by being soluble at pH 4.6. The acid whey contains lactalbumins, soluble in 50% saturated $(NH_4)_2SO_4$ or saturated $MgSO_4$, and lactoglobulins. The two major proteins are β-lactoglobulin (β-Lg) and α-lactalbumin (α-La), and also some minor proteins (including blood serum albumin and lactoferrin (LF)).

Compared to CNs, whey proteins have a more ordered secondary and tertiary structure and are usually globular proteins. They are also more heat sensitive, with initiation of denaturation/unfolding at temperatures around 60°C, more hydrophilic, and the majority contains high amounts of α-helical structure.[2]

β-*Lactoglobulin*: β-Lg represents approximately 50% of the whey proteins (~12% total proteins) and contains 162 amino acid residues, with a molecular weight of 18 kDa. It has six cysteine residues, resulting in two disulfide linkages and one very reactive free sulfhydryl group, which in the native state is hidden within the molecule. It is a typical globular protein, and at pH range 5.5–7.5, it exists as a dimer of two monomeric subunits. After decreasing pH to below 5.5, a tetramer is molded, though the protein occurs as a monomer at pH below 3.5. The β-Lg structure has an eight-stranded antiparallel β-barrel establishing the molecular core. Most likely, β-Lg realizes a biological role by transporting vitamin A to the small intestine. By binding many hydrophobic molecules its ability to bind retinol may be incidental.[4,8]

α-*Lactalbumin*: α-La represents approximately 20% of the whey proteins (~3.5% total proteins). It has a molecular weight of nearly 14 kDa and consists of a single polypeptide chain of 123 amino acids. It comprises four tryptophan residues, one larger subdomain with three α-helixes, and one smaller subdomain containing a three-stranded antiparallel β-sheet.[4] It is a component of lactose synthetase, the enzyme involved in the biosynthesis of lactose. Therefore, it has an essential biological function in the synthesis of lactose from UDP-D-galactose and D-glucose. The concentration of lactose in milk is directly linked to the concentration of α-La.[9]

Whey proteins of bovine milk all exhibit genetic polymorphism. Among them, β-Lgs are the most important and highly studied protein genes and, at least, 11 variants are known for β-Lg, of which, A and B variants are the most common: β-Lg A, B, C, D, E, F, H, I, J and α-La A, B, C.[10] There are more whey milk proteins, but are present in very low quantities including serum albumin (also named lactalbumin and milk albuminate), immunoglobulins (IgG, IgA, and IgM), and the proteins of the milk fat globule membrane.[11]

Milk contains roughly 60 indigenous enzymes, which epitomize a minor but vital part of the milk protein system. Some examples are: alanine aminotransferase, catalase, peroxidase, acid and alkaline phosphatases, and protease.[12]

Some specific chemical properties of bovine's milk CNs, α-Lg, and β-Lg are listed in Table 1.

MILK PROTEIN PRODUCTS

There are several milk protein products, e.g., CNs, caseinates, and different whey proteins, which are manufactured from milk by the dairy industry. Milk protein hydrolysates and derived bioactive peptides have arisen as potential ingredients for application in food and mostly in nutraceutical formulations.

The described products are a vast assortment of what is extensively used in biotechnological, biomedical, pharmaceutical, and food industries and research. Both the manufacturing process and applications will be described.

Caseins and Caseinates

Casein is manufactured from skim milk by one of two methods: precipitation by acid or coagulation by proteolytic enzymes. Fat, whey proteins, lactose, and minerals must be removed from the CN by washing in water to

Table 1 Particular chemical properties of bovine's milk caseins, α-lactalbumin (α-La), and β-lactoglobulin (β-Lg)

Protein	α_{s1}-CN	α_{s2}-CN	β-CN	κ-CN	α-La	β-Lg
Concentration (g/L)	12–15	3–4	9–11	2–4	0.6–1.7	2–4
Phosphorylated	✓	✓	✓	✓	✗	✗
Molecular weight	23,542–23,615	25,226	23,983–24,092	19,006–19,037	14,178	18,277–18,363
Isoelectric point	4.44–4.76	4.44–4.76	4.83–5.07	5.3–5.8	4.2–4.5	5.13
Solubility at pH ~4.6	Insoluble				Soluble	
Heat stability	Very heat stable: tolerate heat at 140°C – 20–25 min				Entirely denatured at 90°C – 10 min	
Physical state in milk	Large colloidal aggregates as micelles				Monomers or small quaternary structures	

Source: Data adapted from Walstra et al.,[1] Gonçalves Ferreira,[12] Fox & McSweeney[13] and Farrell et al.[14]

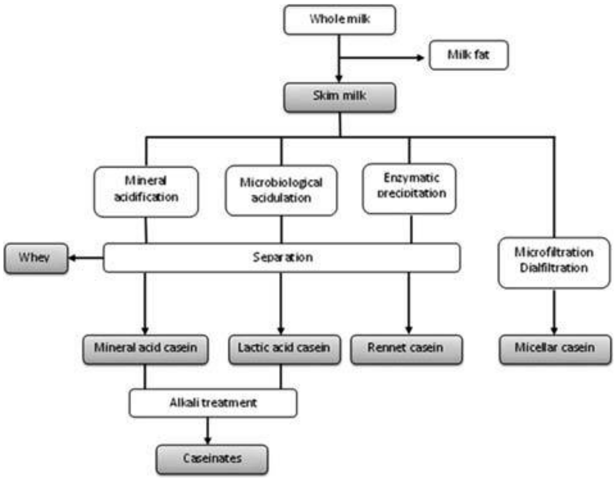

Fig. 1 Production of casein-enriched products.

improve the quality of the final CN product. The product is dried to improve conservation and is used mainly in the food and chemical industries.

In the production of acid CN, the milk pH is decreased to approximately pH 4.6, corresponding to the isoelectric point of CNs. Such acidification can be carried out biologically (by lactic acid bacteria) or by addition of a mineral acid (usually hydrochloric or sulfuric acid) yielding lactic acid CN and mineral CN, respectively (Fig. 1). Rennet CN results from the enzymatic precipitation carried out by several proteolytic enzyme preparations (typically rennet).

Micellar CN, generally referred to as phosphocaseinate, has similar compositional and physical characteristics to the native CN micelles in milk. Micellar CN is manufactured by microfiltration of fresh skim milk using a membrane of suitable pore size, which allows permeation of the serum proteins and soluble constituents whilst retaining the CN micelles. Any soluble permeable constituents are further removed by diafiltration with water and the retained solution is usually spray dried.

Caseinates can be produced from freshly precipitated acid CN curd or from dry acid CN by reaction with a dilute alkali solution. The most commonly used alkali is sodium hydroxide but others like potassium hydroxide, calcium hydroxide, or ammonia can also be used. Caseinates are usually salts of sodium or calcium and have a pH of 6.5–7.0. These products are soluble in water.

Fractionation of caseins

Fractionation of CNs may be needed for a number of reasons, for instance, β-CN is an interesting ingredient for infant formulae, as human milk does not contain it, and κ-CN can be applied as a stabilizer in certain milk products.[15] Most important, the use of individual CN fractions is a condition for the generation and recovery of CN-derived bioactive peptides of high purity. Chromatographic methods can be used to fractionate CNs and have been shown to be better than precipitation procedures[16] but are unsuitable for producing CN for human consumption, due to the utilization of non-food grade reducing agents such as dithiothreitol or β-mercaptoethanol. This issue can be surpassed by using microfiltration of skim milk tailed by anion exchange chromatography using L-cysteine as a food grade reducing agent in the eluting buffer.[17] Table 2 summarizes the nutritional and pharmaceutical use of CNs and their products.

Whey Protein-Enriched Products

Whey is the serum or liquid remaining after the removal of fat and CN from milk during the manufacture of cheese or acid and rennet CN. It contains whey proteins, lactose, minerals, and a small amount of fat. Whey as a by-product of the manufacturing process of cheese and rennet CN is known as sweet whey and has a pH of 5.9–6.6. Manufacture of mineral-acid-precipitated CN yields acid whey with a pH of 4.3–4.6. Table 3 shows the main differences between sweet and acid whey.

Whey and whey protein-enriched solutions are usually pasteurized using minimum temperature and holding times and maintained at low temperature to minimize microbial spoilage and physico-chemical deterioration of the proteins and other constituents that would affect the functional and organoleptic properties of the end products.

Table 2 Casein-enriched products and their nutritional and pharmaceutical use

Type of casein	Caseins in general	α-Casein/rennet casein	β-Casein	κ-Casein	Sodium caseinate	Phosphocaseinate (micellar casein)
Applications	Water dispersible protein Special dietary foodstuffs Toothpaste	Special dietary foodstuffs	Infant formulae	Stabilizer in certain milk products	Candy for space feeding Enriched dairy drink for infants Meat replacement	Dental filling material Mineral binding activity
Reference(s)	[16–24]	[14–18,23,24]	[16–21,23,24]	[16–18,23,24]	[21–26]	[27]

Table 3 Characteristics of sweet and acid whey

Type of whey	Sweet whey	Acid whey
Total solids	6.4	6.5
Water	93.6	93.5
Fat	0.05	0.04
True protein	0.55	0.55
Non-protein nitrogen	0.18	0.18
Lactose	4.8	4.9
Mineral/ash content	0.5	0.8
pH	Minimum: pH 5.6	Maximum: pH 5.1

Source: Data adapted from Nielsen.[28]

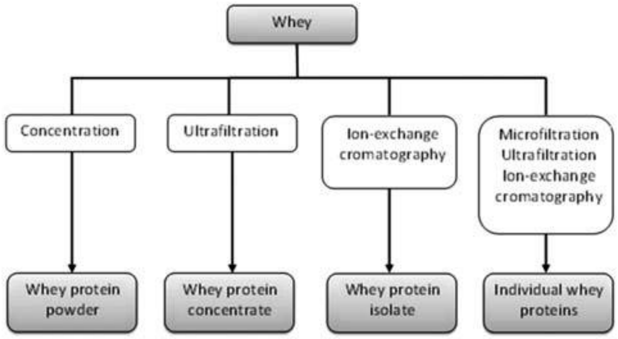

Fig. 2 Production of whey protein-enriched products.

Table 4 Individual whey proteins and their nutritional and pharmaceutical applications

Fractionated whey	α-La	β-Lg	Lactoperoxidase (LPO)	Lactoferrin (LF)	Immunoglobulins (Igs)	Glycomacropeptide (GMP)
Applications	Infant formula		Cold sterilization of milk (antibacterial activity)	Milk-based infant formulae (iron absorption and protection against enteric infection in neonates)	Formulae for preterm infants	Nutrition of patients with phenylketonuria (contains no Phe, Tyr, Trp, Lys, or Cys residues)
Reference(s)	[32–41]	[35,42–44]	[46,47]	[48]	[48–50]	[50,51]

Whey-derived products include whey protein powder, whey protein concentrate (WPC), whey protein isolate (WPI), and individual whey proteins like β-Lg or α-Lg (Fig. 2).

Whole whey powders containing less than 15% protein are produced by concentrating whey by evaporation alone or in combination with reverse osmosis followed by spray drying. Demineralized and demineralized–delactosed whey powders containing 15–35% protein are produced by reverse osmosis, electrodialysis or ion-exchange and/or lactose crystallization to reduce the mineral and/or lactose whey contents.

WPCs are produced under mild conditions of pH and temperature by ultrafiltration of whey. They can be in liquid or dry form and have a protein content that typically ranges from 34% to 85%. When the protein concentration exceeds 90%, the product is known as WPI. WPI is produced from whey by ion-exchange chromatographic methods and usually contains 90–95% protein.

In Table 4 are summarized whey protein products' applications in the food and pharmaceutical industries.

Fractionation of whey proteins

For some applications selected isolated proteins may be desirable. For instance, for preparation of infant formulae α-La appears to be more appropriate since β-Lg is the most allergenic of the bovine milk proteins to the human infant (in fact human milk does not contain β-Lg), justifying in this way the commercial interest for fractionating α-La and β-Lg.

Table 5 Whey protein-enriched products and their nutritional and pharmaceutical uses

Whey product	Whey protein concentrates (WPC)	Whey protein isolates (WPI)	Whey protein hydrolyzate (WPH)	Delactosed whey powder	Demineralized whey powder	Demineralized–delactosed whey powder
Protein concentration	35–85%	90–95%	94%	~30%	15–35%	15–35%
Applications	Geriatric/hospital/ liquid diets Infant formulae Pharmaceutical formulae	Nutritional proteinbar/ beverages Pharmaceutical formulae	Hypoallergenic infant formulae Pharmaceutical formulae	Dietary supplementation		
Reference(s)	[33,52]	[53]	[53]	[33]	[33]	[33]

Whey protein fractionation processes are mainly based on membrane separation and chromatographic techniques. Table 5 summarizes the current food and pharmaceutical applications of some individual whey proteins.

Casein–whey protein coprecipitates

Whey proteins can be coprecipitated with CN by first heating milk, at its natural pH, to temperatures that denature the whey proteins and induce their complexation with CN, followed by precipitation of the milk protein complex by acidification to pH 4.6 or by addition of $CaCl_2$ combined with variable levels of acidification. Such products, referred to as high-, medium-, or low-calcium CN–whey protein coprecipitates based on the calcium content, generally exhibit poor solubility. Insoluble coprecipitates can be dissolved in sodium tripolyphosphate and sodium hydroxide.[54]

Coprecipitates are used as carbohydrate-free and low-lactose infant food. High-calcium coprecipitate is used as a cake mix for diabetics and soluble low-calcium coprecipitate in nutrition, for imitation rice.

BIOLOGICAL EFFECTS OF MILK PROTEINS

General Health Benefits

Proteins have biological activities associated with the intact proteins themselves or with amino acid sequences within the proteins. These activities can be triggered by the intact protein on hydrolysis by proteolytic enzymes, microbial proteolytic activity, or by common food processing treatments (heating and acid/alkaline conditions).

The first food-derived bioactive peptide was identified in 1950 when Mellander reported that milk protein CN and its phosphorylated peptides enhanced vitamin D-independent bone calcification in rachitic infants.[55] Biologically active peptides can be generated in vivo during gastrointestinal digestion of milk protein or released in vitro on hydrolysis of milk proteins; these biologically active peptides include angiotensin-converting enzymes (ACE) inhibitors, antithrombotic, opioid agonists, opioid antagonists, antimicrobial, immunomodulatory and anxiolytic peptides, and CN phosphopeptides. Some CN derivatives have antimicrobial effect (Table 6), ameliorate intestinal calcium uptake, and also show proinflammatory and immunomodulatory properties. A study exposed that α_{S1}-CN may be, as well, a leukocyte-derived peptide and possesses immunomodulatory properties.[58] Caseins have shown a protective effect in experimental bacteremia by stimulating myelopoiesis. Their hydrolyzates are protective in diabetic animals, decrease the tumor growth, and weaken colicky symptoms in infants. Casein-derived peptides confirm some antihypertensive effects. Glycomacropeptide, derived from κ-CN, exhibits antibacterial and antithrombotic activities. Starter and non-starter bacteria, predominantly lactic acid bacteria, are generally highly proteolytic and are capable of generating bioactive peptides from milk proteins during the

Table 6 Antimicrobial activity of milk casein derivatives

Antimicrobial compound	Microorganism	Reference(s)
Lactenin (generated following the treatment of milk with rennin)	Streptococci	[56,57]
Caseicin A and B peptides	*Cronobacter sakazakii* *Escherichia coli*	
Casocidin I	*Escherichia coli* *Staphylococcus carnosus* *Cronobacter sakazakii* *Listeria innocua* *Streptococcus mutans*	
Isracidin	*Staphylococcus aureus* *Listeria monocytogenes* *Candida albicans*	

fermentation of milk-based products. ACE-inhibitory, immunomodulatory, anti-oxidative, antimutagenic, and opioid activities have all been identified in fermented milk and/or cheeses.[59]

α-Lactalbumin revealed antiviral, antitumor, and antistress properties. Diets rich in α-La were anxiolytic, lowered blood pressure in rats, led to a superior weight gain in malnourished youngsters and prevented diarrhea.[60] Lactoferrin (LF) was the first iron-binding protein described in milk by Johansson in 1960.[61] It exhibits antibacterial (specifically microbiostatic properties against *Escherichia coli*),[61,62] antifungal, antiviral, antiparasitic, and antitumor activities. It is protective with respect to intestinal epithelium, stimulates bone growth, and accelerates recovery of the immune system function in immunocompromised animals. LF exhibits a variety of effects on the host immune system, ranging from inhibition of inflammation to promotion of both innate and adaptive immune responses.[63] Although the mechanisms underlying these LF immunomodulatory properties have not been fully elucidated yet, evidence indicates that the capacity of this molecule to directly interact with antigen presenting cells (APC), i.e. monocytes/macrophages and dendritic cells, may play a critical role. At the cellular level, LF modulates important aspects of APC biology, including migration and cell activation, whereas at the molecular level it affects expression of soluble immune mediators, such as cytokines, chemokines, and other effector molecules, thus contributing to the regulation of inflammation and immunity.[64,65] Additionally, LF-derived peptides inhibit ACE activity in vitro and reduce systolic blood pressure in hypertensive rat and displayed potential as orally effective antihypertensive compounds.[66] Other milk proteins also have potential therapeutic properties: proline-rich polypeptide exhibits a variety of immunotropic functions, as well as promotion of T-cell maturation and inhibition of autoimmune disorders. It was shown to increase or stabilize the instrumental activity of daily living status in patients with Alzheimer's disease; lysozyme is effective in treatment of periodontitis and prevention of tooth decay; and, finally, lactoperoxidase shows antibacterial properties.[67]

Cancer Protection

Animal and cell culture studies suggest that milk proteins, especially whey, have anticarcinogenic properties. This activity seems to be linked to components rich in sulfur amino acids, cysteine and methionine, and holding the γ-glutamylcysteine residue, which makes cysteine (and methionine, in times of cysteine deficiency) readily bioavailable for the synthesis of glutathione, a strong xenobiotic deactivating and antineoplastic agent.[68] Binding of iron by LF may make this potential pro-carcinogen unavailable for intestinal damage, whereas the vitamin B-binding proteins make their vitamins, potential anticancer agents, more bioavailable and keep them from use by intestinal microorganisms.[68]

BIOMEDICAL APPLICATIONS

Milk proteins are widely used in the food industry for their nutritional and functional properties. The fact that milk proteins are inexpensive, readily available, natural, and non-toxic makes them interesting for potential biomedical applications. Temporary replacement implants, tissue-engineering scaffolding, membranes for promoting wound healing, and drug delivery carriers are the most promising target applications.

Drug Delivery

Milk proteins are natural vehicles for bioactives. Several of their structural and physico-chemical properties facilitate their functionality in delivery systems: excellent surface and self-assembly properties, binding of ions and small molecules, pH-responsive gel, brilliant gelation properties, suitable for programmable release, conjugates with synergistic combinations of properties, interactions with other macromolecules to form complexes, crucial for protecting sensitive payload, swelling performance, biocompatibility and biodegradability, many shielding abilities, assisting to control the bioaccessibility of the bioactive, and stimulate its bioavailability.[69]

The versatility and potential of CNs and whey proteins in drug delivery applications are well patented in the research work summarized in Tables 7 and 8.

Tissue Regeneration and Wound Healing

Recent advances in tissue engineering have focused on finding more biocompatible, non-toxic materials to imitate natural tissue components.[100]

Besides biocompatibility and biodegradability, milk proteins may also present some bioactivity. For example, the uniqueness of LF as a skeletal regenerative molecule lies in its ability to favorably modulate the responses of the various cell types involved in musculoskeletal regeneration. Human LF is also known to promote neovascularization. This molecule reveals pleiotropic functions, encourages the proliferation and differentiation of osteoblast cells, and bars osteoclast-mediated bone resorption.[101]

In Table 9 are summarized the main reported studies using both CN and whey proteins in tissue engineering and wound healing.

CONCLUSION

Milk contains a mixture of proteins, each having unique attributes for nutritional, biological, and food applications. The major proteins present in milk include the CNs, β-Lg, and α-La. Minor but also important proteins are LF and lactoperoxidase. This entry presented an overview of manufacturing processes, biological activities, and applications in the food and nutraceutical fields.

Table 7 Drug delivery systems based on caseins

Vehicle	Drug(s)	Application	Reference(s)
Film coating	Diltiazem	Oral controlled drug release	[70]
Composite	Phenytoin	Oral improvement of release	[71]
	Ibuprofen	Oral, N.A.	[72]
	Diclofenac	Oral controlled drug release	[73]
Hydrogel	Vitamin B12	Oral controlled drug release	[74]
(micro)beads	Loratadine	Oral controlled drug release	[75]
	Diethylstilboestrol	Parenteral controlled drug release	[76]
Microparticles	Methotrexate	N.A., controlled drug release	[77]
	Acetaminophen	N.A., controlled drug release	[78]
	β-Lactoglobulin	Oral controlled drug release	[79]
	5-Fluorouracil	Implantation, site-specific (tumor) and controlled drug release	[80]
Microspheres	Levonorgestrel	Parenteral controlled drug release	[81]
Microcapsules	Acetaminophen	Oral controlled drug release	[82]
Nanocarriers	Vitamin D2	Oral controlled drug release	[83]
	Curcumin	Oral site-specific (tumor) drug release	[84]
	Mitoxantrone	Oral site-specific (tumor) drug release	[85]
	Celecoxib + Budesonide	Oral site-specific (intestine) controlled drug release	[86]
	Mitoxantrone	Oral drug release	[85]
	Paclitaxel Vinblastine	Oral drug release	[87]

Table 8 Drug delivery systems based on whey proteins

Protein type	Vehicle	Drug/bioactive	Application	Reference(s)
WPC	Hydrogel	Caffeine	Controlled delivery	[88]
WPI	Alginate/whey microparticles	Insulin	Improvement of oral bioavailability	[89]
	Alginate/whey protein beads	Theophylline	Controlled drug delivery	[90]
	Alginate/whey protein microspheres	Riboflavin	Controlled drug delivery	[91]
	Beads	Retinol	Protection against oxidation and sustained delivery	[92]
	Aerogel	Ketoprofen	Controlled diffusive drug delivery	[93]
	Hydrogel	Anthocyanin-rich bilberry extract	Improved stability and controlled delivery of anthocyanins	[94]
	O/W/O double emulsions	Flumethrin®	Sustained release of hydrophobic drugs for topical uses	[95]
β-La	Nanoparticles	Caffeine	Controlled delivery	[96]
	Nanocomplexes	EGCG	Protection against oxidative degradation and sustained release	[97]
Succinylated β-La	Tablet	Riboflavin	Controlled drug delivery	[98]
	Tablet	Probiotic microorganism	Intestinal delivery	[99]

Table 9 Applications of milk proteins in tissue engineering and wound healing

Vehicle	Applications	Delivery	Reference(s)
Hydrogel of lactoferrin	Bone tissue regeneration	Injection	[100]
Bovine osteopontin-coated hydroxyapatite and gold model surfaces	Bone tissue regeneration	Implant	[101]
Genipin-cross-linked casein	Regeneration after peripheral nerve injury	Injection	[102]
Lactoferrin	Bone tissue regeneration	Oral	[103]
Hydrogel of κ-casein	Neuronal tissue regeneration	Injection	[104]
Bovine lactoferrin C-Lobe	Corneal epithelial regeneration after wound	Topical	[105]
Whey protein isolate/diethylene glycol	Culture of human keratinocytes and fibroblasts cells	Film	[106]
Whey protein isolate or lactalbumin blended with polycaprolactone	Culture mouse fibroblast	Film	[107]

Recently, milk proteins have received considerable interest for drug delivery and tissue engineering applications. However, compared to other biopolymers, their use is still limited, especially in the field of tissue engineering, and thus, further investigation is required.

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