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# Encyclopedia of Food Chemistry: Milk Proteins

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## Nomenclature

$\alpha$ -LA Alpha-lactalbumin

AA Amino acid

$\beta$ -LG Beta-lactoglobulin

BSA Bovine serum albumin

Ca Calcium

CCP Colloidal calcium phosphate

EtOH-HCl Ethanol-hydrochloric acid

GMP Glycomacropeptide

LF Lactoferrin

MW Molecular weight

pI Isoelectric point

SH Sulfhydryl group

(S-S) Disulfide bond

## Introduction

Milk is a complex biological fluid secreted by the females of all mammalian species. It is produced to meet the nutritional requirements of the neonate such as delivering the energy, essential amino acids (AA) and micro-nutrients required for adequate growth and development. Depending on the mammalian species, the new-born will have very different nutritional requirements leading to large interspecies differences in gross composition and yield of milk (Table 1) (Fox et al., 2015b). Bovine milk (especially from the species *Bos taurus*) is the predominant raw material for dairy products which accounts for ~ 84% of total global milk production and has been subject to extensive characterization (Thompson et al., 2009). Bovine milk proteins and their physical, chemical, functional and nutritional properties will be the focus of this review.

Bovine milk contains ~ 3.4% protein which was initially believed to be in the form of one protein only. Between 1883 and 1885, the Swedish scientist Hammersten (1883) showed that milk proteins could be divided into two groups, casein proteins and serum (whey) proteins, by adjusting the pH of bovine milk to the isoelectric point (pI) of caseins (pH 4.6). At this pH, the casein proteins

**Table 1** Composition of milks of selected mammalian species

Species	Total solids (%)	Protein (%)			Fat (%)	Lactose (%)	Ash (%)
		Total	Casein	Whey			
Cow	12.7	3.4	2.8	0.6	3.7	4.8	0.7
Human	12.2	1.0	0.4	0.6	3.8	7.0	0.2
Sheep	19.3	5.5	4.6	0.9	7.4	4.8	1.0
Goat	12.3	2.9	2.5	0.4	4.5	4.1	0.8
Horse	11.2	2.5	1.3	1.2	1.9	6.2	0.5
Pig	18.8	4.8	2.8	2.0	6.8	5.5	n.a.
Donkey	11.7	2.0	1.0	1.0	1.4	7.4	0.5
Grey Seal	67.7	11.2	n.a.	n.a.	53.1	0.7	n.a.
Polar Bear	47.6	10.9	7.1	3.8	33.1	0.3	1.4

n.a. = Not available.

Adapted from Fox et al. (2015b).

were found to precipitate from milk while the whey protein fraction remained soluble. Later, studies would establish that both casein and whey protein fractions were in turn composed of a number of different proteins.

The concentrations of these two fractions in the milk of any particular mammalian species again differ, and are presumed to be tailored to the nutritional and physiological requirements of the young (Table 1). The whey protein:casein ratio of bovine milk is ~ 20:80 (Jensen, 1995).

## Casein Proteins

Caseins are defined as milk proteins that precipitate from raw skim milk when the pH is adjusted to pH 4.6 (i.e. pI of casein) at temperatures greater than 10 °C. At temperatures less than 10 °C, aggregation of caseins does occur but the aggregates are fine enough to remain in suspension (Fox et al., 2015b).

### Fractionation and Heterogeneity of Caseins

The heterogeneous nature of caseins was first described by Linderstrøm-Lang (1925) who fractionated isoelectric/acid casein using an ethanol-hydrochloric acid (EtOH-HCl) extraction process (O'Mahony and Fox, 2013). The four proteins that were identified from isoelectric casein are:  $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ - and  $\kappa$ -casein, which account for ~38%, ~10%, ~35% and ~15% of total casein, respectively. Variations occur between different casein proteins such as molecular weight (MW) and differences in AA profile (Table 2). More subtle variations (e.g. single AA substitutions) may occur in each of the individual proteins also yielding different forms of the same protein, which is termed micro-heterogeneity. All caseins are phosphorylated, i.e., have phosphate groups attached to side chains of serine, threonine and tyrosine (Klumpp and Krieglstein, 2002), while  $\alpha_{s1}$ - and  $\alpha_{s2}$ -casein are more highly phosphorylated than  $\beta$ - and  $\kappa$ -casein (typically 8, 11, 5 and 1 phosphate groups, respectively (Table 2) (Eigel et al., 1984). The degree of phosphorylation of each casein protein is commonly included in the abbreviated name of the casein protein, e.g.  $\alpha_{s1}$ -8P,  $\alpha_{s2}$ -11P,  $\beta$ -5P and  $\kappa$ -1P.

**Table 2** Properties of individual casein proteins

Property	Caseins			
	$\alpha_{s1}$ -	$\alpha_{s2}$ -	$\beta$ -	$\kappa$ -
<b>Molecular weight (kDa)</b>	~23.5	~25.5	~24.0	~19.0
<b>Residues/molecules</b>				
Amino acids	199	207	209	169
Proline	17	10	35	20
Cysteine	0	2	0	2
Phosphate	8–9	10–13	4–5	1–3
Carbohydrate	0	0	0	0–4
<b>Hydrophobicity (kJ/residue)</b>	4.9	4.7	5.6	5.1
<b>Charged residues/molecule</b>	34	36	23	21

Adapted from Fox et al. (2015b).

Additionally,  $\kappa$ -casein is a glycosylated protein, meaning that carbohydrate moieties (e.g., N-acetylneuraminic acid, galactose and N-acetylgalactosamine) are attached to the C-terminal end of the  $\kappa$ -casein molecule. The extent of glycosylation of  $\kappa$ -casein that occurs can vary; 0–4 glycosides may be present on any given  $\kappa$ -casein protein, yielding 9 different possible molecular forms of  $\kappa$ -casein (Dziuba and Minkiewicz, 1996).

## Properties of Caseins

### Structure and Heat Stability

Caseins are relatively small molecules (MW ranging from 19–25 kDa) that lack high levels of secondary ( $\alpha$ -helices and  $\beta$ -turns) or tertiary protein structures. This is somewhat due to high concentrations of the amino acid proline in the proteins: 17, 10, 35 and 20 proline residues are present in  $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ - and  $\kappa$ -casein, respectively (Swaisgood, 1982). Proline inhibits the formation of  $\alpha$ -helices and  $\beta$ -turns due the presence of a cyclic amine in its side chain (Morgan and Rubenstein, 2013). As secondary and tertiary structures are not prevalent in the structural analysis of caseins they are considered to have a non-ordered and open structure (Holt and Sawyer, 1993; Holt et al., 2013).

Protein denaturation occurs when a protein loses its native secondary or tertiary structure, strongly influencing the biological and technological functionality of that protein. As caseins have a low level of secondary or tertiary structure, high temperature treatments have little effect on the caseins, as evidenced by the fact that bovine milk can be heated at 140 °C for ~ 20–30 min without gelation occurring (Singh and Latham, 1993). Such high heat treatments will, however, have other effects on caseins, such as dephosphorylation of AA (Fox et al., 2015a).

### Hydrophobicity and Calcium Sensitivity

All casein proteins have a high concentration (35–45%) of hydrophobic AAs such as valine, leucine, isoleucine, phenylalanine, tyrosine and proline (Swaisgood, 1982). In structured proteins, hydrophobic AAs are generally buried within the protein's tertiary structure; however, as caseins have an open and unordered structure these AAs remain exposed, which leads to caseins being regarded as having a high surface hydrophobicity (Creamer et al., 1982; O'Mahony and Fox, 2013). Hydrophobic bonds readily form between the hydrophobic regions of caseins leading to caseins having a strong tendency to self-associate (Home, 1998).

Hydrophobic amino acids are associated with bitterness, meaning that the hydrolysis of casein molecules has the potential to yield bitter hydrolysates, which is problematic in some applications such as cheese production (Lemieux and Simard, 1992). Caseins can act as amphipatic molecules which adsorb readily at air–water and oil–water interfaces in order to reduce interfacial tension, thereby stabilizing emulsions and foams (Dickinson, 1989).

All caseins (excluding  $\kappa$ -casein) are insoluble in the presence of calcium (Ca) which is naturally present at high concentrations in bovine milk (~30 mM; Gaucheron, 2005), it would therefore be expected that caseins would precipitate out of milk due to their insolubility and Ca-sensitivity.  $\kappa$ -casein, which is Ca-insensitive, and soluble, at all Ca concentrations found in dairy products acts to stabilise the other, Ca-sensitive, caseins from precipitation in milk by combining with both Ca and the Ca-sensitive caseins to form large colloidal structures termed casein micelles (Müller-Buschbaum et al., 2007; O'Mahony and Fox, 2013). Although  $\kappa$ -casein only accounts for ~ 15% of total casein protein in milk, it can stabilise up to 10 times its own weight of the Ca-sensitive caseins *via* the formation of the casein micelle (Fox and Brodtkorb, 2008).

### Casein Micelle

Holt et al. (2013) summarized the three main biological functions of the casein micelle, based on its structure and stability. It ensures that very high levels of Ca and phosphate can be secreted from the mammary gland and transported to the neonate by ensuring that these minerals are colloidally stable in milk (prevents calcified build-up in mammary glands). The micelle also provides a means of safe excretion of the potentially fibrilligenic caseins (may build up in fibril structures causing blockages) through the mammary gland. Finally, the casein micelle also allows caseins to be retained in the stomach of neonates for a length of time required for sufficient proteolysis of the caseins into smaller peptides which can then be more readily absorbed in the small intestine.

The structure of the casein micelle has been widely debated over recent years (De Kruif and Holt, 2003; Home, 2006, 2008; Farrell et al. 2006; Fox and Brodtkorb, 2008; Dalglish, 2011; Dalglish and Corredig, 2012) and different models for the structure of the casein micelle have been suggested and will be discussed briefly below. It is widely agreed that casein micelles are large, spherical (diameter = 50–500 nm) (de Kruif, 1998; O'Mahony and Fox, 2013) and highly hydrated (3.5 kg H<sub>2</sub>O per kg of protein) (Jeurnink and De Kruif, 1993) structures containing ~ 70,000 individual protein molecules. The dry matter of the casein micelle is 94% protein and 6% low MW species, referred to as colloidal calcium phosphate (CCP). This CCP is composed mainly of Ca (~1,400,00 Ca<sup>2+</sup>) and phosphorus (~1,000,000 PO<sub>4</sub><sup>3-</sup>) with small amounts of magnesium, citrate and other species (O'Mahony and Fox, 2013). The external surface structure of casein micelles is rich in  $\kappa$ -casein where it acts to stabilise the micellar structure and protect against destabilization or aggregation of casein micelles (Dalglish et al., 1989). The glycosylated, hydrophilic C-terminal of the  $\kappa$ -casein molecule (termed macropeptide) can be found protruding ~ 7 nm out of the casein micelle into the aqueous phase of

milk. This creates a hairy  $\kappa$ -casein layer surrounding the casein micelle, responsible for micellar stabilization *via* steric and electrostatic repulsion (zeta potential of  $\sim -20$  mV at pH 6.7) (Müller-Buschbaum et al. 2007; O'Regan et al., 2009).

In comparison, the arrangement of  $\alpha$ - and  $\beta$ -casein and CCP within the internal structure of the casein micelle has yielded greater debate in the various models proposed in recent years. The simplest model was proposed initially by Waugh and Von Hippel (1956) and suggested that the  $\alpha_s$ - and  $\beta$ -caseins are surrounded by a layer of  $\kappa$ -casein. In the intervening period to now, two main models have been developed detailing the internal structure of the casein micelle: the submicelle and the nanocluster model.

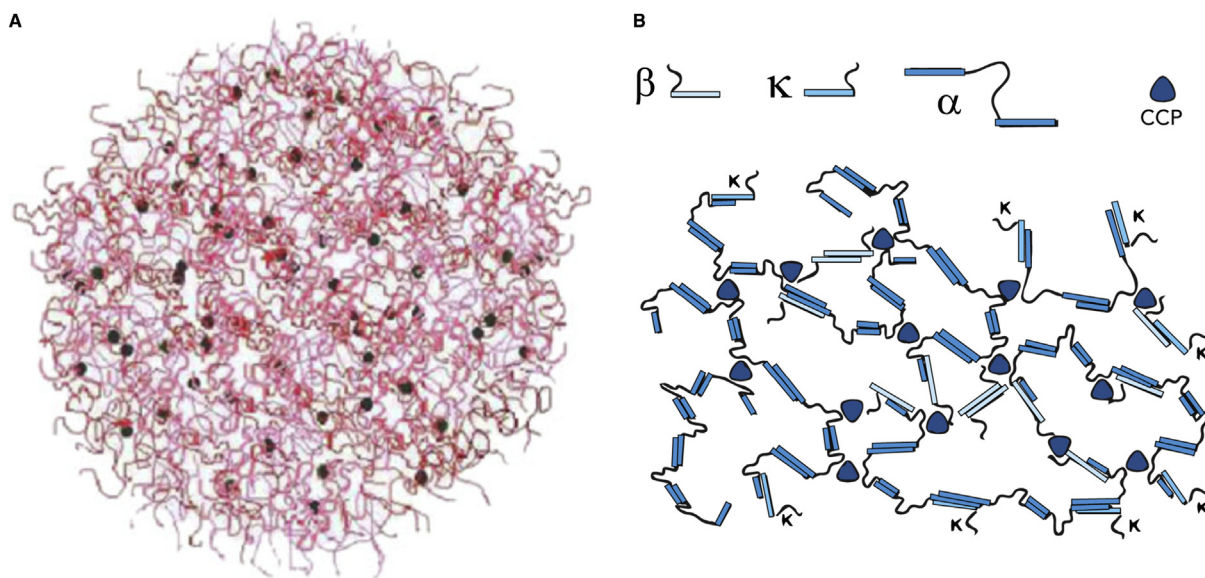
The submicelle model was firstly proposed by Schmidt (1982) and suggests that the casein micelle is composed of smaller submicelles fused together by CCP. This model has not generated widespread support as it relies on the hypothesis that there are two forms of submicelle units present in a casein micelle - a  $\kappa$ -casein-rich and a  $\kappa$ -casein-depleted submicelle. The  $\kappa$ -casein-rich submicelles are located at the surface of the micelle, with the  $\kappa$ -casein-depleted submicelles in the internal structure of the casein micelle. Research has never confirmed that these different submicelles actually exist. Following this, the nanocluster model was introduced by Holt (1992, 1998). This model depicts the internal structure of casein micelles as a tangled web of  $\alpha$ - and  $\beta$ -caseins linked by hydrophobic interactions. This casein web contains nanoclusters of CCP (radius of 2.3 nm) which act to stabilize the casein web structure. Dagleish (1998, 2011) has presented an updated nanocluster model which incorporates large pores within the casein micelle and a more sparse distribution of  $\kappa$ -casein in the  $\kappa$ -casein layer on the surface of the casein micelle.

These pores are large enough to allow (I) easy access to the protruding  $\kappa$ -casein for proteolytic enzymes (important for digestibility and destabilization of caseins) (Diaz et al., 1996) and denatured whey proteins (formation of disulfide linkages between denatured  $\beta$ -lactoglobulin ( $\beta$ -LG) and  $\kappa$ -casein) (Singh and Creamer, 1991; Anema and Li, 2003) and (II) movement of individual proteins in and out of the casein micelle structure (e.g.  $\beta$ -casein leaving and re-entering the micelle on heating and cooling) (Creamer et al., 1977). Both the submicelle and nanocluster models retain the key feature that CCP, which is located within the casein micelle, plays a vital role in stabilizing the Ca-sensitive casein present in the interior of the casein micelle (Fig. 1).

## Whey Proteins

Whey proteins were traditionally separated from caseins *via* isoelectric precipitation of the caseins by adjusting the pH of milk to 4.6. The whey proteins remained in the soluble phase at this pH, producing a whey stream termed acid whey (O'Mahony and Fox, 2013). Another common practice for the separation of the whey from the caseins is through the addition of an enzyme preparation such as rennet (primary enzyme chymosin) to milk, as is done during cheese making, which causes the destabilization (cleaving of  $\kappa$ -casein at the micelle surface), aggregation and precipitation of casein micelles (the curd), leaving the whey proteins and glycomacropeptide (GMP) (C-terminal of  $\kappa$ -casein which is cleaved off by chymosin) in the soluble phase. Whey produced *via* enzymatic coagulation of milk is termed sweet whey and contains GMP.

In industrial and lab scale applications, the separation and further enrichment of whey proteins from caseins is routinely achieved using unit operations such as chromatography (e.g., ion exchange chromatography) membrane filtration and



**Figure 1** (a) Holt's nanocluster model, depicting an internal protein matrix of  $\alpha$ - and  $\beta$ -casein which contains nanocluster like particles of CCP (●) with steric stabilization provided by the protruding  $\kappa$ -casein at the micelle surface, from De Kruijff et al. (2012). (b) Dual-binding model proposed by Horne (1998), depicting the hydrophobic bonding occurring between  $\alpha$ -,  $\beta$ - and  $\kappa$ -casein, as well as the CCP nanocluster bridging.

centrifugation which exploit the physico-chemical differences (such as molecular weight and charge) between whey proteins and caseins to achieve separation. The form of whey produced from these operations is sometimes termed technical whey.

### Heterogeneity and Fractionation of Whey

Work carried out throughout the 20th century by Palmer (1933), Sorensen and Sorensen (1939), Gordon and Semmet (1953) and Polis et al. (1950) showed that many different whey proteins are present in bovine milk and established that the major proteins of bovine whey are:

- $\beta$ -LG-; (~50% of total whey protein in bovine milk)
- $\alpha$ -Lactalbumin ( $\alpha$ -LA); (~20% of total whey protein in bovine milk)
- Immunoglobulins (Ig); (~10% of total whey protein in bovine milk)
- Bovine serum albumin (BSA); (<10% of total whey protein in bovine milk)
- Lactoferrin (LF); (~1% of total whey protein in bovine milk)

Whey proteins differ in terms of their amino acid composition, size and physicochemical properties (e.g., charge and surface hydrophobicity); however, for the purpose of this review, whey proteins will be discussed as a collective.

### Properties of Whey Proteins

#### Structure and Heat Stability

Whey proteins are highly structured with high levels of secondary and tertiary structure.  $\beta$ -LG, which is the predominant whey protein in bovine milk, is highly structured with more than half of its structure existing in the form of  $\alpha$ -helices (15% of  $\beta$ -LG structure) or  $\beta$ -sheets (50% of  $\beta$ -LG structure) ultimately forming a tightly packed globular structure (Whitney, 1988; Kinsella and Morr, 1984a; Creamer et al., 1983), held together strongly by internal disulfide (S-S) bridges. This highly ordered globular structure is common for most of the major whey proteins, and thus whey proteins are very heat-labile as they are susceptible to loss of structure during processing treatments common in the dairy industry (e.g., high heat treatments, and high-pressure homogenization)

On exposure to temperatures greater than 65 °C, whey proteins tend to unfold from their native structure, exposing AAs such as Cysteine (which contains a free sulfhydryl (-SH) group). The now exposed free-SH group is readily accessible and highly reactive, allowing other denatured whey proteins and proteins containing exposed-SH groups (e.g.,  $\kappa$ -casein) to interact and aggregate *via* the formation of new S-S bonds (Kinsella and Morr, 1984a; Mulvihill and Donovan, 1987; Kinsella and Whitehead, 1989; Singh and Creamer, 1991; Wijayanti et al., 2014).

#### Charge and Hydrophobicity

As seen in Table 3, whey proteins contain high levels of polar and charged AAs. Kinsella and Whitehead (1989) stated that proteins with high levels of polar and/or charged AAs, such as whey proteins, interact strongly with water due to their highly hydrophilic nature. Whey proteins, due to their hydrophilic nature are associated with good solubility characteristics and proteins in whey protein isolate have been shown (Pelegriane and Gasparetto, 2005) to be highly soluble over a wide range of pH (Table 4). Due to their heat sensitivity, the solubility of whey proteins is very temperature dependent, as influenced by denaturation and aggregation.

Due to their charge and hydrophilic nature, whey proteins (unlike the caseins) display a very limited tendency to self-associate and are less sensitive to changes in pH and ionic strength when compared to caseins (O'Regan et al., 2009).

### Functionality of Milk Proteins

The functional properties of proteins are defined as physical and chemical properties that influence the behavior of food systems during processing, storage and consumption. Milk proteins have a wide range of functional properties that lend to their application in numerous food systems (Kinsella and Melachouris, 1976; Kinsella and Morr, 1984a). For the purpose of this article, the functional properties, solubility, gelation and surface activity will be of focus for both casein and whey proteins.

#### Solubility

The solubility of a protein is defined as the amount of protein that goes into solution or colloidal dispersion under specified conditions (temperature and ionic strength) and does not sediment out by defined centrifugal force (Morr et al., 1985; IDF, 1995). The solubility of a protein is regarded as the most important functional property as it is a prerequisite for many other functional properties and acts as a useful index of overall protein functionality. If a protein has good solubility, its potential applications are naturally expanded (Kinsella and Morr, 1984a; Zayas, 1997c). The solubility of a protein is ultimately decided by a combination of

**Table 3** Amino acid composition of the major whey proteins in bovine milk

AA	$\beta$ -LG	$\alpha$ -LA	BSA	LF
Aspartate	11	9	41	53
Asparagine	5	12	13	44
Threonine	8	7	34	52
Serine	7	7	28	50
Glutamate	16	8	59	30
Glutamine	9	5	20	68
Proline	8	2	28	44
Glycine	3	6	15	74
Alanine	14	3	46	98
Cysteine	5	8	35	38
Valine	10	6	36	66
Methionine	4	1	4	4
Isoleucine	10	8	14	26
Leucine	22	13	61	106
Tyrosine	4	4	19	34
Phenylalanine	4	4	27	43
Tryptophan	2	4	2	17
Lysine	15	12	59	78
Histidine	2	3	17	14
Arginine	3	1	23	58
Total	162	123	581	987

Adapted from Kinsella and Whitehead (1989).

**Table 4** Protein solubility values of whey protein isolate solutions<sup>a</sup> over a pH and temperature range (Pelegri and Gasparetto, 2005)

Temperature ( $^{\circ}$ C)	pH	Protein solubility (g/100 g)
40	3.50	87.1 $\pm$ 0.03
	4.50	81.8 $\pm$ 0.12
	5.65	86.3 $\pm$ 0.02
	6.80	87.7 $\pm$ 0.02
	7.80	92.8 $\pm$ 0.49
43	3.50	87.7 $\pm$ 0.28
	4.50	78.8 $\pm$ 0.41
	5.65	89.0 $\pm$ 0.88
	6.80	83.9 $\pm$ 0.26
	7.80	88.9 $\pm$ 0.35
50	3.50	87.1 $\pm$ 0.06
	4.50	72.2 $\pm$ 0.26
	5.65	89.6 $\pm$ 0.05
	6.80	74.6 $\pm$ 0.01
	7.80	88.6 $\pm$ 0.32
57	3.50	82.0 $\pm$ 0.20
	4.50	64.9 $\pm$ 0.16
	5.65	87.6 $\pm$ 0.50
	6.80	75.6 $\pm$ 0.08
	7.80	85.2 $\pm$ 0.32
60	3.50	80.7 $\pm$ 0.18
	4.50	62.4 $\pm$ 0.06
	5.65	92.4 $\pm$ 0.10
	6.80	68.2 $\pm$ 0.15
	7.80	87.8 $\pm$ 0.05

<sup>a</sup>Whey protein solutions were made by adding  $\sim$  0.5 g of whey protein isolate (94.3% protein) to a volumetric flask and making to 50 mL volume.



(I) the balance of intermolecular hydrophobic and electrostatic interactions, which themselves, are controlled by surface hydrophobicity and (II) the state (native or denatured) of the protein under specific environmental conditions of temperature, pH and ionic strength (O'Regan et al., 2009).

### Gelation

Gelation refers to the ability of proteins to form a gel under practical conditions during processing or storage of a food product (Zayas, 1997b) and is a very important functional attribute in many food systems, e.g., yoghurt and cheese production. A gel is defined as a system containing a relatively small proportion of solid in a relatively large proportion of liquid, yet the system has mechanical rigidity, thus a protein gel is composed of a three dimensional network of proteins entrapping a large amount of water (Kinsella and Whitehead, 1989). A protein gel is formed when protein molecules are altered in a way which allows unfolding (denaturation) to occur, yielding polypeptide regions which are capable of forming various interactions (protein–protein and protein–water), resulting in a three-dimensional, cross-linked network (O'Regan et al., 2009). The alteration of milk proteins to form gels is most commonly achieved *via* thermal denaturation or enzymatic coagulation.

### Surface Active Properties

Molecules with surface active properties are used routinely in food processing to stabilize emulsions (oil dispersed in water, O/W, or water dispersed in oil, W/O) and foams (air dispersed in a liquid phase) in formulated foods (e.g., mayonnaise, cake batter). In order for proteins to act as surface active agents they must be amphipathic, meaning they contain both hydrophilic and hydrophobic regions (Kinsella and Morr, 1984a). Milk proteins adsorb readily at the interface and surface of emulsions and foams, respectively, and act to reduce interfacial and surface tension between the hydrophobic and hydrophilic phases, in turn stabilizing emulsions and foams (Table 5).

## Nutritional Properties of Milk Proteins

### Delivery of Amino Acids

The main nutritional function of bovine milk proteins is to act as the principal source of nitrogen and amino acids which are required for growth and maintenance of protein synthesis (Tipton and Wolfe, 2001). Amino acids are classified as essential or non-essential, with Rose et al. (1948) defining essential amino acids as those which cannot be synthesized in sufficient amounts by the body to maintain growth or nitrogen balance. Milk proteins have a high biological value as all essential amino acids (Histidine, lysine, phenylalanine, leucine, threonine, valine, tryptophan, methionine and isoleucine) are present at relatively high levels in the major milk proteins ( $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ -,  $\kappa$ -CN,  $\beta$ -LG and  $\alpha$ -LA) (Table 3). Whey proteins have higher levels of essential AAs (Hambræus and Lönnerdal, 2003) while caseins, in addition to delivering AAs, play a vital role in delivery of calcium *via* the CCP in casein micelles.

### Biologically-Active Proteins and Peptides

Biologically-active compounds are those that affect biological processes, beyond the nutritional value, in a way which has an impact on body function. For milk proteins, the proteins themselves, as well as the peptides formed from their proteolytic digestion, may be biologically active. For the purpose of this review the effect that milk proteins and peptides have on the immune, cardiovascular and nervous systems will be discussed.

Milk proteins and peptides that have a beneficial effect on the immune system are termed as either immunomodulatory or antimicrobial. Immunoglobulins (whey proteins) are an example of immunomodulatory proteins, and are in fact a family of proteins with varied structure and functions; however, their structures and functions critically revolve around their ability to identify and bind specific antigens presented by bacteria and viruses which aids host protection (Schroeder et al., 2010). During proteolytic breakdown of LF in the stomach by pepsin, an antimicrobial peptide, lactoferricin, is formed which targets Gram-negative bacteria and binds to their cell wall. Once attached to the cell wall, lactoferricin causes the release of lipopolysaccharides, which irreversibly damages the cell wall, leading to further morphological changes to the structure and function of the Gram-negative bacteria (Bellamy et al., 1992; Appelmelk et al., 1994; Tomita et al., 2002).

Antithrombotic milk peptides have a positive effect on the human cardiovascular system. These peptides are mainly formed during the enzymatic break down of  $\kappa$ -casein and have been shown to interrupt the formation of thrombi which, when formed, can block veins, arteries or the chambers in the heart (Clare and Swaisgood, 2000; Mills et al., 2011).

The nervous system of newborn infants has been shown to be affected by opioid peptides from milk, which obtain their name due to pharmacological similarities to opium. The major opioid peptides of milk are formed during the proteolytic digestion of  $\beta$ -casein and are called  $\beta$ -casomorphins, which are thought to be biologically potent (Teschemacher et al., 1997; Clare and Swaisgood, 2000).  $\beta$ -casomorphins have only been found in the intestinal tract and blood plasma of newborn infants and have yet to be identified in children or adults. The specific activity of  $\beta$ -casomorphins is somewhat unclear, while it has been shown to be associated with stimulating food intake and increasing the output of insulin in infants (Xu, 1998; Mills et al., 2011).



**Table 5** Functionality and applications of milk protein ingredients

	Solubility		Gelation		Surface Activity	
	Casein	Whey	Casein	Whey	Casein	Whey
<b>Description</b> (Kinsella and Melachouris, 1976; Kinsella and Morr, 1984b; Zayas, 1997a; O'Regan et al., 2009)	<p>-Soluble to high conc. at pH values outside of pH 4–5 (casein pI = 4.6).</p> <p>-Caseins' heat stability allows them to remain soluble after heat treatment.</p> <p>-Solubility improved by use of calcium chelators (e.g., citrates).</p>	<p>-Soluble over entire pH range encountered in food applications (at low ionic strength).</p> <p>-Solubility decreases at high ionic strength due to salting out effect (de Wit and van Kessel, 1996).</p> <p>-Thermal denaturation and aggregation causes a reduction/loss in solubility.</p>	<p>-Casein gels are formed from milk by acid and enzymatic coagulation</p> <p>-Rennet gelation occurs via proteolysis of the <math>\kappa</math>-casein layer of the casein micelle leading to micelle destabilization and aggregation.</p> <p>-Acid gelation of casein occurs on adjustment of pH to 4.6 (pI of caseins) and produces gels that are prone to syneresis (whey expulsion).</p>	<p>-Due to their heat labile nature, whey proteins have excellent thermal gelation properties.</p> <p>-The characteristics (hardness, elasticity and turbidity) of the gels formed is dependent on the solution environmental conditions: mainly pH and ionic strength.</p>	<p>Both types of milk proteins are known to have high surface-activity properties leading to their use as emulsion and foam stabilizers in food applications.</p> <p>Caseins, due to their open structure, allow for a large surface coverage at the interface/surface of an emulsion or foam.</p> <p>The emulsion properties of both casein and whey proteins are improved via conjunction with polysaccharides as forming a polysaccharide portion on the interface protruding outwards into the emulsion continuous phase, increasing steric stabilization of the emulsified droplets (Drapala et al., 2016).</p>	<p>Whey proteins, in their native globular state, adsorb in thicker films at the interface/surface of emulsions or foams. Denatured whey protein aggregates are associated with enhanced surface activity.</p>

	<i>Solubility</i>		<i>Gelation</i>		<i>Surface Activity</i>	
	<i>Casein</i>	<i>Whey</i>	<i>Casein</i>	<i>Whey</i>	<i>Casein</i>	<i>Whey</i>
<b>Applications &amp; Ingredients</b> (O'Regan et al., 2009)	<u>Beverages</u> Caseins used in nutritional beverages which require high heat treatments for protein fortification -Sodium caseinate	<u>Beverages</u> Whey proteins are used in the production of protein beverages due to their solubility over a wide pH range. –WPC, WPI	<u>Dairy Products</u> The formation of a casein gel is a core step in the production of cheese. Different cheese varieties are produced depending on the gelation mechanism (enzymatic vs acid)	<u>Dairy Products</u> Some cheese varieties such as ricotta are produced by forming a whey protein coagulum/curd using heat and acid gelation.	<u>Beverages</u> Caseins, due to their stability, are used as emulsifiers and stabilizers in cream liqueurs which is a challenging environment. -Sodium caseinate	<u>Beverages</u> Whey protein solubility at low pH lends to their use as emulsifiers in protein fortified fruit juices or soft drinks which contain volatile oil flavor compounds. –WPC, WPC
	<u>Dairy Products</u> Caseins and caseinates are commonly used in the production of analog cheese where one of their main functions is to bind water and emulsify oil. -Rennet casein, acid casein	<u>Bakery Products</u> Due to their solubility, whey proteins are incorporated into baked goods to retain water and to provide additional functionality. –WPC	Both caseins and whey proteins are utilized in the production of yoghurts. This not only improves the nutritional value of the yogurt but also allows increased yield, viscosity and gel strength (reduced syneresis)		<u>Dairy Products</u> In the production of table spreads, a water in oil emulsion is created. Emulsifiers are used to stabilise this emulsion but due to the very high level of oil in the dispersed phase, casein ingredients are used to further provide stabilization to the emulsion. -Sodium caseinate	<u>Bakery Products</u> Whey protein ingredients are used as a replacement for egg proteins in many bakery applications as both very effectively stabilise foams (e.g. meringues). –WPC, WPI
	<u>Meat Products</u> Caseins act to bind large amounts of water, assisting in fat emulsification and improving the final texture in the production of processed meat. -Sodium caseinate	<u>Confectionary Products</u> Protein-rich bars are produced with whey protein ingredients to increase their level of protein and to control texture via their water binding capabilities (Hogan et al., 2012) -Hydrolyzed WPC	Casein gelation occurs and is vital in the production of kefir which is a fermented dairy beverage. As fermentation occurs the pH of the kefir drops which causes acid induced gelation leading to an increase in the beverage viscosity.	<u>Textured Products</u> Whey proteins are used in the production of surimi due to their gelation properties, as a replacement for beef plasma protein or potato starch (Hsu and Kolbe, 1996). –WPC		<u>Meat Products</u> Fat used in processed meat product production is pre-emulsified with whey proteins to allow for the formation a strong fat containing gel network during cooking. –WPC

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