Caseins

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The milk of mammals is a complex fluid mixture of various proteins, minerals, lipids, and other micronutrients that play a critical role in providing nutrition and immunity to newborns. Casein proteins together with calcium phosphate form large colloidal particles, called casein micelles. Caseins belong to a class of proteins that exhibit open and flexible conformations.

Keywords: milk proteins ; casein ; casein micelles ; cow ; camel ; human ; African elephant ; structures ; functional properties ; nutritional properties

1. Introduction

The term casein was derived from the Latin word *caseus*, meaning cheese. Four casein protein families (κ-, β-, αS1-, and αS2-caseins) have evolved in different mammalian species to maintain specialized roles in milk [1], and their primary function is the provision of nutrients and minerals, especially calcium, to offspring while maintaining fluidity in mammary glands. In addition, caseins are the major milk proteins that provide amino acids as well as immunity to infants, but their functions are significantly affected by their digestibility [2]. In different mammalian species, caseins have been subjected to several evolutionary modifications of their primary sequences and posttranslational modifications by phosphorylation and glycosylation, inducing an overall change in their structural and functional properties. For example, phosphorylation of α- and β-caseins as well as glycosylation of κ-casein are critical modifications, affecting the formation and stability of casein micelles [3].

Caseins are associated with each other and with calcium phosphate in the form of colloidal nanostructures called micelles. Together with the fat globules, whey protein, and minerals in milk, these micelles play important roles in preventing the precipitation of casein proteins and in stabilizing milk as an emulsion [4]. Knowledge about casein micelle formation is limited mainly to bovine milk, which is not even fully understood. According to the available knowledge, αS1-, αS2-, and β-caseins are involved in calcium-binding and are mainly concentrated in the interior of spherical micelles. The calcium-sensitive caseins (αS1-, αS2-, and β-) bind calcium, mainly by electrostatic interactions with colloidal calcium phosphate (CCP), and further aggregate via weaker interactions, including hydrophobic and van der Waals interactions, as well as hydrogen and ionic bonding [5][6]. In addition, κ-casein forms a type of “brush” on the surface of the micelle that interacts with the whey and ensures electrostatic repulsion between micelles [7].

2. Genetics and Biosynthesis of Milk Caseins

2.1. Genetics

Caseins belong to the family of phosphoproteins synthesized in the mammary gland, and they are secreted as roughly spherical, polydisperse (50–600 nm with an average of 200 nm), supramolecular, colloidal aggregates named micelles [8][9]. Caseins are encoded by single autosomal genes, namely *CSN1S1* (αS1-casein), *CSN2* (β-casein), *CSN1S2* (αS2-casein), and *CSN3* (κ-casein), organized as a casein gene locus cluster in a DNA stretch of approximately 250 kb located on chromosome 6 [10]. In all the four selected species, the first two genes, *CSN1S1* and *CSN2*, are close to each other, *CSN3* is the most distant (especially in the elephant), and *CSN1S2* lies in between. The four genes are highly conserved and tightly clustered in camels, whereas they are significantly distant in elephants. Variants (or isoforms) of these genes can result from single nucleotide polymorphisms (SNPs) and nucleotide insertions or deletions. In dromedary camels, the most polymorphic gene is *CSN1S1* (248 SNPs), and the least polymorphic gene is *CSN1S2* (79 SNPs) [10]. Bovine and camel milks contain αS1-, αS2-, β-, and κ-caseins but at the different relative ratios of 38:10:40:12 and 22:9:65.5:3.5, respectively. Human milk lacks αS2-casein, and the relative ratio of αS1-, β-, and κ-caseins in human milk is 3:70:27. African elephant milk lacks both αS1- and αS2-caseins, and it only contains β- and κ-caseins at a ratio of 89:11 [11]. The lack or low level of α-S caseins in the human and African elephant milks supports the suggestion that only κ-casein and an ancient β-casein-like protein are obligatory to form stable casein micelles, and that αS1- and αS2-caseins have developed

in some species later during evolution [12]. The reason(s) behind the genetic variability of caseins and their implications on the technological and nutritional quality of milk is not yet understood and deserves further investigation [8].

2.2. Biosynthesis of Milk and Milk Caseins

Milk is secreted by the mammary glands of animals to provide their offspring with the macronutrients (protein, lipids, and lactose) and micronutrients (minerals and vitamins) required for growth, as well as other components needed to boost immunity, especially during the early stages of life [13]. Different animal species have different nutritional and physiological needs, making milk composition species-specific although sharing some commonalities [14]. During lactation, the mammary epithelial secretory cells secrete huge quantities of the nutrient molecules that make up milk, i.e., proteins, fat globules and soluble components, such as lactose and minerals, while others are transferred from the blood [15]. Proteins are formed in the endoplasmic reticulum by combination of the signal peptides with constituent amino acids and then transported to the Golgi apparatus for posttranslational modification by phosphorylation and glycosylation. The biosynthesis of milk proteins involves complex interactions between insulin, which controls systemic energy status, and intracellular AMP:ATP ratio, which is an indicator of the local energy status, and their regulation of mammalian target of rapamycin (mTOR) mediates the translation initiation and elongation rates of mRNA [16]. Four amino acids, namely, histidine, lysine, methionine, and leucine, are the main limiting essential amino acids involved in regulating milk protein synthesis via mTOR [17]. The gene responsible for the expression and synthesis of casein is signal transducer and activator of transcription 5 (*STAT5*) whose cooperative interaction with glucocorticoid receptor (GR) and CCAAT/enhancer-binding protein-β (C/EBPβ) drives the transcription of β-casein [18]. Differences leading to the various levels of αS1-, αS2-, β-, and κ-caseins in the milks of different animal species are not yet known.

The phosphate groups of the caseins are esterified as monoesters on serine (and to a lesser extent on threonine) with the specific sequence of Ser-X-A (where A is an anionic residue, i.e., Glu, Asp or SerP; and X is any amino acid). Most of the phosphoserine residues in caseins occur in clusters that mainly bind calcium. Glycosylation of threonine residues in proteins may include galactose, galactosamine, and/or N-acetylneuraminic (sialic) acid that occur as tri- or tetrasaccharides [19]. Several of the minor whey proteins are recruited directly from the blood to epithelial secretory cells into milk by passive diffusion or internalization [20]. The essentiality of κ-casein for casein micelle stabilization in the alveolar lumen, as well as for lactation and reproduction, has been confirmed through κ-casein gene null mutations in mice [21][22]. The current κ-casein variants of the *Bos* species may have developed from an ancestral wild type [23].

It has been suggested that casein micelles are highly disordered in non-fixed dynamic structures, in which the different caseins adopt flexible positions while maintaining an overall shape and coherence of the micelle [24]. This feature allows rapid and differential evolution of the various caseins in the different mammalian species. It has recently been shown that caseins interact and form networks with the major whey proteins (β-lactoglobulin, α-lactalbumin, lactoferrin, and serum albumin), and these networks, in turn, interact with the other proteins [25]. The stability of the casein micelles and their destabilization are important for the coagulation of milk in the stomach during digestion as well as in the processing of milk to make yogurt and cheese [25]

3. Molecular Structures of Milk Caseins

3.1. Amino Acid Sequence Identities, and Instability

Caseins are single-chain polypeptides differing in length and amino acid sequence. Milk proteins, in general, and caseins exhibit high heterogeneity due to the existence of different isoforms with variable amino acid sequences. The currently known bovine milk casein variants include 15 variants of β-casein (A1, A2, A3, B, C, D, E, F, G, H1, H2, I, J, K, and L), 11 variants of κ-casein (A, B, C, E, F1, F2, G1, G2, H, I, and J), 10 variants of αS1-casein (A, B, C, D, E, F, G, H, I, and J), and 5 variants of αS2-casein (A, B, C, D, and E) [26], some of which may be involved in posttranslational modification(s) by phosphorylation and/or glycosylation. Some genetic variants have also been identified in other animals, but the available data are not conclusive. Moreover, the genetic factors responsible for these variations are unknown.

3.2. Distribution of Uncharged Polar, Hydrophobic, Aromatic, Isoelectric Points and Polar Amino Acids in Caseins

Protein hydropathy plots are useful fingerprints that can be used to compare the structural content and spatial distribution of the different amino acids in caseins according to their hydrophobic and hydrophilic scores. The hydropathy profile, i.e., the patterns of hydrophilicity and hydrophobicity, as well as the three-dimensional structural plots, supplement the information provided by the amino acid sequence of a protein [27]. The construction of hydropathy plots for a protein sequence is based on the classification of the amino acids into three groups as follows: (i) polar: D, E, H, K, N, Q, and R;

(ii) hydrophobic: A, F, I, L, and M; and (iii) weakly hydrophilic, weakly hydrophobic, or ambiguous amino acids: S, T, W, and Y. Each amino acid is given a hydropathy score between +4.6 (most hydrophobic) and −4.6 (most hydrophilic) [28]. Proline and glycine are excluded because their unique backbone properties are more important than their hydropathies, while cysteine is excluded because its oxidized form lacks the polarizable sulfur [29]. The undulations across the hydropathy profile of these proteins are highly similar, thus uncovering a few key points.

Although the scoring value may be altered for isolated amino acids, it estimates the molecular properties and unveils some key similarity features. Firstly, camel and human αS1-caseins show lower and comparatively similar scores compared to bovine milk, which has a higher-than-average score for αS2-casein. Secondly, bovine β- and κ-caseins show substantially lower hydropathy indices compared to the other organisms whose average scoring undulations fall below −0.3. Thirdly, unlike other organisms, bovine caseins show a remarkably similar lower scoring pattern. The bovine, camel, and human αS1-caseins show total hydropathy scores of −110.034, −155.352, and −141.474, respectively, compared to the scores of −163.489 and −129.012 for the bovine and camel αS2-caseins, respectively. Moreover, the bovine, camel, human, and African elephant β-caseins show total scores of −53.08, −59.216, −32.166, and −61.17, respectively, and the bovine, camel, human, and African elephant κ-caseins show total scores of −63.212, −32.135, −52.888, and −38.743, respectively. Lastly, the conserved hydrophobic region in the C-terminal of all these caseins should be functionally responsible for their common characteristic features. The surface hydrophobicities of bovine and camel β-caseins have recently been compared at different pH values (3, 6, and 9) and temperatures (25, 65, and 95 °C) [30]. Because the electrostatic charges are negative and surface hydrophobicity is pH-independent above the isoelectric point, surface hydrophobicity is higher for bovine casein than for camel casein at pH 3 but nearly similar at pH values of 6 and 9. The higher hydrophobicity of bovine β-casein can be explained by the presence of one tryptophan residue and one tyrosine residue in the hydrophobic portion of this protein compared to five tyrosine residues and eight phenylalanine residues but no tryptophan residues in camel β-casein [31][32]. Temperature has a minimal effect on β-casein hydrophobicity, and this effect is less important for camel β-casein than for bovine β-casein [33].

Hydrophobic interactions play major roles in stabilizing the native structure of proteins in an aqueous environment by packing the nonpolar side chains into the compact core of the protein to avoid contact with water. Entropy stabilization of protein structures is achieved via disulfide bonds, hydrogen bonds, hydrophobic interactions, and van der Waals interactions, which make folded proteins more stable than unfolded proteins [34].

3.3. Posttranslational Phosphorylation and Glycosylation of Milk Caseins

In the posttranslational modification of caseins, serine (Ser) residues can be phosphorylated and/or O-glycosylated, while threonine (Thr) residues can only be O-glycosylated. The phosphorylation of caseins affects several of their properties, including calcium binding, micelle stabilization, interactions with proteins, interactions with other molecules, and biological activities [35]. In bovine milk, the phosphorylation of caseins is highly variable with up to 3 phosphate groups on κ-casein, 5 phosphate groups on β-casein, 8 or 9 phosphate groups on αS1-casein, and 10–14 phosphate groups on αS2-casein [36]. The electrophoretic mobility of β-, αS1-, and κ-caseins is slower for camel milk than for bovine milk, suggesting a lower net negative charge [37]. Recently, various isoforms have been reported for camel milk as follows: κ-casein with no phosphate group or a single phosphate group; β-casein with 2, 3, or 4 phosphate groups; αS1-casein with 5, 6, or 7 phosphate groups; and αS2-casein with 7, 8, or 9 phosphate groups [38]. Human β-casein comprises six isoforms having 0–5 phosphate groups per molecule [33].

With preferential phosphorylation at 2–4 sites, the preferential order of phosphorylation for the human β-casein is Ser24 > Ser25 > Ser23 > Ser21 > Thr18 [39]. The African elephant β-casein has been reported to have five isoforms, and one isoform is characterized by a single phosphorylated Ser9 residue and by truncation of the ESVTQVNK peptide sequence, shortening its length to 200 amino acid residues in comparison to the 208 residues of the other four unphosphorylated forms [11]. Human αS1-casein has been described to have three phosphorylation variants—no phosphorylation, one phosphate group at Ser18, and two phosphate groups at Ser18 and Ser26—a pattern that is different from that in ruminants where extensive phosphorylation occurs in the Ser70–Glu78 conserved region [40].

Bovine κ-casein residues, including Thr154 (41%), Thr163 (29%), Thr152 (14%), Thr142 (7%), and Thr157 (0.1%) [41], can be O-glycosylated with the following molecules: disaccharides (N-acetyl galactosamine and galactose; GalNAcGal); linear and branched trisaccharides (N-acetyl galactosamine, galactose, and N-acetyl neuraminic acid; GalNAcGalNeuAc); and branched tetrasaccharides (N-acetyl galactosamine, galactose, and two N-acetyl neuraminic acids; GalNAc1Gal1NeuAc2) [42]. A detailed study on the glycosylation of human β-casein has indicated that the C-terminal contains the O-glycan peptide (197-LLNQELLLNPTHQYPVTQPLAPVHNPISV-226) [39], which has antimicrobial functionality [43]. Studies have suggested more extensive glycosylation of κ-casein in camel milk [37] and human milk [44] than in bovine milk. The low

percentage of κ-casein and the high level of its glycosylation in camel milk may contribute to its lower pH (6.2–6.7) compared to bovine (6.6–6.7) and human (6.7–6.9) milks [45]. The glycosylation and/or phosphorylation of casein molecules have significant effects on their polarity and functionality.

3.4. Predicted Secondary Structures of Milk Caseins in the Four Animals

The biological functions of proteins are determined by two important characteristics: certain amino acid motifs within the sequence, and the three-dimensional conformation of the protein dictating their surface hydrophilicity/hydrophobicity and structure(s) [46]. The amino acid sequence alone is not able to provide all the information hidden in the sequence because certain amino acid alterations have no effects on the biological function(s), while other alterations may have significant effects. Therefore, the three-dimensional structure of a protein is better conserved than its amino acid sequence during evolution due to its tolerance to changes in the primary structure [47].

4. Casein Micelle Composition and Structure

It has been estimated that a typical bovine milk casein micelle consists of thousands of casein molecules and CCP in a ratio of 94:6 [48]. The phosphoserine residues of the calcium-sensitive caseins associate through their phosphate groups with calcium (or magnesium), which also bind inorganic phosphate, citrate, and water. According to Broyard and Gaucheron [49], different forms of water can be associated with milk caseins as follows: (i) chemically-bound or structural water that is unavailable for chemical reactions; (ii) non-freezable water that is bound to polar amino acids, such as monolayer water; (iii) hydrodynamic hydration water that loosely surrounds proteins; (iv) hydrophobic hydration water that surrounds non-polar amino acids in “cage-like” structures; and (v) capillary water held by surface forces in the proteins. The presence of the different forms of water is responsible for the porous “spongy” structure of casein micelles [50] is formed by cavities (20–30 nm in dimeter) and channels (~5 nm in diameter) [51].

All models agree that the primarily peripheral location of κ-casein is at the surface of the micelle. The “hairy”, negatively charged, glycosylated, hydrophilic glycomacropeptide (GMP, ~7 nm) [5], which protrudes into the aqueous phase, stabilizes micelles and prevents them from aggregating through electrostatic repulsions [5]. The various genetic variants of κ-casein in bovine milk, i.e., A, A^1, B, C, E, F^1, F^2, G^1, G^2, H, I, and J, differ slightly in their primary sequence and functional properties, such as gelation potential [23]. In addition to this genetic polymorphism, variants of κ-casein may differ in their degree of glycosylation. Glycosylation of bovine milk caseins is limited to κ-casein, which can have up to nine glycans per molecule, such as galactose, N-acetylgalactosamine, N-acetylneuraminic acid, and sialic acid [52]. It has been suggested that higher concentrations of κ-casein are associated with smaller micelles in bovine milk [53]. However, higher degrees of glycosylation have been shown to be the main reason for smaller micelle sizes in some cow breeds [54]. A previous study on the Montbéliarde cow breed indicated that the B variant of κ-casein exhibits a relatively higher level of glycosylation and smaller average micelle size (~170 nm) than the A variant, which has a larger average casein micelle size (~207 nm). The amino acids in the GMP, representing the hairy part of κ-casein clipped by renins, are highly conserved and mainly negative with only few positively charged conserved residues near the cleavage site [55]. This part of κ-casein plays an important role in maintaining repulsions between micelles and preventing their coagulation in the mammary gland. The hydrophilic amino acid residues in the central region of the ruminant para-κ-casein (PKC) are strongly conserved, which is more important for its function than the conservation of hydrophobic residues [56]. The GMP of all species lacks cysteines, suggesting that cross-linking of this part of κ-casein is unfavorable for the interaction of micelles with the aqueous environment or with each other via disulfide bonds, which may promote their aggregation.

The presence of some levels of β-, αS1-, or αS2-caseins in milk is vital for calcium binding and the micelle internal structure. However, compared to κ-casein, the exact role and the amount of these calcium-binding phosphoproteins necessary for the stabilization of the spatiotemporal organization of the casein micelle remain unknown. This lack of understanding is due to the inter- and intra-differences in the proportions of these proteins in milks from different animal species. The great variation, low sequence identity, and limited conservation of these proteins in different milks place caseins among the most evolutionarily divergent mammalian proteins [57]. Nonetheless, stable and roughly spherical casein micelles are formed by different mammalian species despite this heterogeneity, suggesting that the constitution of casein micelles follows simple and flexible rules. As the presence of αS-caseins is not obligatory in human and African elephant milks, the presence of β-casein is also not vital in milks from knockout mice [58] and in goats that do not express the β-casein gene [58]. Experiments have also shown that approximately 50% of the caseins, predominantly β-casein, dissociate from micelles to the serum phase during cooling of milk [59]. These findings suggest that β- and αS1-caseins may have similar or complementary roles in the constitution and functionality of the casein micelle, a characteristic that may be related to their chaperone activity. The chaperone activity of αS1- and β-caseins, which is critical for the prevention of the aggregation of several proteins (including κ-casein, α-lactalbumin, insulin, lysozyme, alcohol

dehydrogenase, and catalase), is facilitated by a higher frequency of proline residues in β-casein (18%) than in αS1-casein (9.2%) [56]. Studies have suggested that there are two types of β-casein that bind in micelles as follows: (i) a portion that is hydrophobically-bound and that can easily be removed by cooling; and (ii) a portion that is tightly bound to CCP nanoclusters [60].

It has been suggested that αS1-casein is required for efficient transport of β- and κ-caseins from the endoplasmic reticulum to the Golgi apparatus in mammary gland epithelial cells and that β- and κ-caseins are retained in the endoplasmic reticulum in αS1-deficient epithelial cells [61]. The lack of αS1-casein in African elephant milk contradicts the generalization of this proposition and supports the need for its modification. Both αS1- and β-caseins are chaperon proteins that establish a dynamic disorder and inhibit the aggregation and formation of amyloid fibrils by κ-casein [61]. Thus, the previous suggestion by le Parc et al. [55] can be modified to state that a certain minimum proportion of αS1- and/or β-casein is needed to ensure casein functionality and maintain the fluidity of milk. Thus, casein micelles can be viewed as dynamic nanoassemblies composed of submicelles linked together by CCP through their phosphoserine residues. In the core of each submicelle, β- and α-caseins are mainly associated via hydrophobic interactions, while κ-casein mainly resides on the surface of micelles, which stabilizes them through electrostatic and steric repulsions [62]. The level of mineralization has been shown to be higher in camel milk (94 mg/g caseins) than in bovine milk (67 mg/g caseins) [63]. Although the calcium and phosphate partitioning between the micellar and serum fractions is almost similar for camel and bovine milks (60–65%), the partitioning of magnesium and citrate substantially differs in casein micelles with 66% and 33% in camel milk compared to 40% and 10% in bovine milk, respectively.

The casein composition of milk critically affects the structure and properties of casein micelles, especially their size and aggregation behavior. Casein micelles vary in size from very small to very large [6] in bovine, camel, and human milks, and they found that the milks are composed of a large number of small particles (40 nm diameter, 80%) and a small number of large micelles with camel milk having a wider distribution and greater number of large particles. The small particles account for 4–8% of the mass or volume of casein in camel milk with an average size of 260–300 nm compared to 100–140 nm in bovine milk. Despite that the low relative κ-casein percentage correlates with a large average casein micelle size [64], the relationship among casein composition, casein micelle size, and dynamics is poorly understood, warranting further investigation of the effect of phosphorylation and glycosylation considering the variability in casein contents and proportions in different animals.

5. Functional Properties of Milk Caseins

5.1. Milk Types

For health reasons, it is important to understand the different types of milk. A1 β-casein milk is the most abundant milk, it is obtained from cows varieties, such as like Holstein Friesian and Jersey, while A2 β-casein milk is mostly obtained from cows, such as Gir and Sahiwal, and milk from camels and goats [65][66] Compared to A2, A1 milk is relatively cheaper and easier to find. Recently, there has been a difference of opinion about the health effects of the A1 milk type. A1 milk contains BCM-7 (Beta-casomorphine-7) derived from the A1 β-casein during the digestion, which is not present in A2 milk and has been linked to several undesirable health effects [67].

5.2. Coagulation Properties

Coagulation and clotting of milk caseins are initiated by either the neutralization of the negative charges of the “hairy” hydrophilic parts of κ-casein by acids or by the cleavage of renin enzymes, leading to destabilization of the milk emulsion and precipitation of caseins [68]. Renin enzymes cleave the Phe^{105}-Met^{106} bond of the mature bovine milk κ-casein, producing the hydrophilic GMP (C-terminal amino acid residues 106–169 of the mature bovine κ-casein corresponding to amino acids 127–190 of the whole protein with an approximate molecular weight of 6800 Da) and the hydrophobic PKC (N-terminal amino acid residues 1–105 of the mature bovine κ-casein corresponding to amino acids 22–126 of the whole protein with an approximate molecular weight of 12,000 Da) [19]. In κ-casein, serine occurs at higher levels than threonine in PKC and at lower levels in GMP, suggesting a functional preference for O-glycosylation over phosphorylation in GMP [24]. Furthermore, GMP is unique as it lacks aromatic amino acids and is rich in branched amino acids.

The casein composition and micelle structure of milk affect its coagulation and digestibility in the stomach [2], with β-casein having greater effects on digestibility than κ- and α-casein [69][70]. Recently, Zou et al. [71] compared the digestibility of camel, human, and bovine milks by in vitro infant digestion. They observed that human milk does not form a clot in the stomach, however bovine milk forms hard curd, which is in agreement with others [72]. Gastric emptying time in infants, which is shorter for human milk than for bovine milk, correlates with the formation of firm clots of αS1-casein compared to the formation of soft aggregates from β-casein [2]. The nature of the clot formed in the stomach affects the further

digestibility of the proteins in the intestine [73]. However, camel milk behaves differently as it instantaneously forms floccules that assemble into a single soft clot of proteins entrapping fat globules [74]. The hardness/softness of the stomach coagulum influences gastric emptying, protein digestion kinetics, and passage through the stomach, and it also affects the digestion in the lower gut and the release of amino acids, especially in individuals with digestive disorders or discomfort [72].

Stomach renins, e.g., chymosin (EC 3.4.23.4) and pepsin (EC 3.4.23.1), hydrolyze κ-casein at low pH values (1.5–2.5), causing the release of GMP into the whey and coagulation of the hydrophobic PKC in the curd [52]. Both chymosin and pepsin clot milk under specific conditions, and they also lead to proteolysis, which ripens the cheese. Chymosin is a principal proteinase that hydrolyzes the Phe-Met bond, and pepsin has a higher and an unspecific proteolytic activity towards peptide bonds, involving aromatic amino acids (Phe, Tyr, and Trp) [52]. The coagulation properties of milk are affected by the κ-casein variants as well as their interaction with the variants of β-casein and β-lactoglobulin [23]. Chymosin-induced coagulation properties of milk are also enhanced by glycosylation [75]. After the initial formation of aggregates by acid, digestion of bovine milk in the stomach proceeds by pepsin and chymosin, leading to the hydrolysis of κ-casein, which occurs at much lower levels in camel and human milks. Bioactive antioxidant, antimicrobial, ACE inhibitory, and dipeptidyl peptidase IV (DPP IV) inhibitory peptides have recently been identified in bovine, camel, and human milks after intestinal digestion [73]. Most of these peptides are derived from β-casein, which is predominant in camel milk. A BLAST search against the UniProt database has indicated that camel β-casein protein shares 69.3% and 61.3% sequence identity with bovine and human β-casein, respectively. It has also recently been shown that camel milk proteins are as equally digestible as their bovine and human counterparts under infant gastrointestinal digestion conditions, and they have been suggested as prospective substitutes to the use in infant formula [74].

More information about the coagulation of milk caseins is available from experimental results on cheese and yogurt quality. These studies are largely available for bovine milk and to some extent for camel milk, but not for human and African elephant milks. Compared to bovine milk, the low κ-casein in camel milk is associated with larger casein micelles, poor milk coagulation properties, and soft/fragile cheese [76]. Similarly, bovine milk with large casein micelles is associated with slow rennet coagulation and reduced cheese firmness [75]. The softness of camel milk cheese is affected by other factors, including high percentage of β-casein and higher proteolytic activity [76]. Further studies on the coagulation properties of milks from different mammalian species may allow identification of the different factors that affect casein coagulation after treatment with renin enzymes and/or with acids.

5.3. Ethanol Stability of Milk

In a study by de la Vara et al. [77], ethanol stability of sheep, goat, and cow milk was significantly influenced by pH; a pH increase from 5.7 to 7.1 increased stability significantly. Alhaj et al. [78] reported that camel ethanol stability is affected by sodium chloride (NaCl) concentration and the variation of other minerals that influences the ionic strength. The addition of NaCl to camel milk enhances its sodium and potassium balance, thereby stabilizing the casein micelles.

5.4. Heat Sensitivity

Compared to bovine milk caseins, camel milk caseins are less stable at higher temperatures (100–130 °C), and their heat coagulation rate is influenced by pH levels [79][80]. For instance, the coagulation time of camel milk at 130 °C and pH 6.7 is 2–3 min, whereas the coagulation time of bovine milk is 40 min [81]. The reduced level of κ-casein (5% of total casein in camel milk compared with 13.6% in bovine milk) and the absence of β-lactoglobulin may be responsible for the poor stability of camel milk at high temperatures. Camel milk has poor heat stability and cannot be sterilized at natural pH, but the addition of casein and calcium has been found to play a significant role in camel milk heat stability [82].

5.5. Proteolysis Sites and Products

Milk caseins are subject to hydrolysis by a wide range of proteolytic enzymes, including endogenous, gastric, and bacterial enzymes, such as pepsin, chymosin, trypsin, chymotrypsin, elastase, carboxypeptidase A, carboxypeptidase B, and leucine amino peptidase (LAP). The open conformation of the caseins explains the ability of proteolytic enzymes to access target bonds and to cause rapid and extensive degradation of casein to smaller peptides [83]. Quadrupole time-of-flight (Q-TOF) mass spectrometry analysis of hydrolytic peptides has discovered that human milk contains several endogenous proteolytic enzymes: plasmin and trypsin (cleave after lysine K and arginine); cathepsin D and elastase (cleave after alanine, isoleucine, leucine, proline, and valine); pepsin and chymotrypsin (cleave after phenylalanine, leucine, tryptophan, and tyrosine); and proline endoperoxidase (cleaves after proline) [84]. Similar enzymes are also present in the gastrointestinal tract of humans and in the milks of other animal species [83].

β-casein is more prone to enzymatic hydrolysis than the other caseins, leading to the release of a large number of peptides. The lack of disulfide bonds and the abundance of proline residues impart an open structure for β-casein that makes it readily available for enzymatic hydrolysis [1]. Plasminogen and its activators, associated with the casein micelle, are mainly responsible for casein degradation by plasmin (EC 3.4.21.7) [84]. The conversion of plasminogen to plasmin is activated by heat treatment during milk processing, and plasmin is heat tolerant and can withstand temperatures as high as those used in ultraheat treatment [85]. Plasmin has a preference to hydrolyze N-terminal Lys-X bonds but is also capable of slowly hydrolyzing Arg-X bonds. Moreover, plasmin readily hydrolyses β- and αS2-caseins as well as slowly hydrolyzes αS1-casein, but k-casein is highly resistant to plasmin [86]. The main proteolytic products produced from β-casein by plasmin include C-terminal peptides [γ1-casein (29–209), γ2-casein (106–209), and γ3-casein (108–209)] and their complementary N-terminal peptides [proteose peptone 8 fast (PP8 fast, 1–28), proteose peptone 8 slow (PP8 slow, 29–105/107), and proteose peptone 5 (PP5, 1–105/107)] [87]. Plasmin has been reported to hydrolyze αS2–casein, resulting in the following 14 fragments: 1–24, 71–80, 115–149, 115–150, 150–165, 151–165, 153–170, 166–173, 167–173, 174–181, 182–188, 182–197, 153–207, and 198–207 [88]. Although plasmin is less active towards αS1-casein, this casein has several cleavage sites where its hydrolysis starts with the cleavage of six fragments (1–22, 91–100, 91–103 103–124, 106–124, and 194–199) from the N-terminal and central parts of the protein, followed by nine other fragments (1–34, 35–90, 80–90, 80–103, 104–124, 104–199, 106–199, 125–199, and 152–199) [89]. Plasmin has also been demonstrated to hydrolyze κ-casein to release the following five fragments: 1–16, 1–24 [90], 1–3, 17–21, and 22–24 [89]. The plasmin hydrolytic peptides of β-casein [91] and κ-casein [92] have been demonstrated to have strong antibacterial properties.

Based on the “leaky gut” theory [93] and the “autism model”, which propose exorphins and serotonin as the important mediators in the development of autism, the effects of β-CM-7 on autism have been studied [94]. With respect to type 1 diabetes mellitus (T1DM), it has been proposed that β-CM-7 may contribute to the impairment of gut-associated immune tolerance and enhance the autoimmune reactions leading to destruction of β cells [90]. Some studies have found an association between an early ingestion of bovine milk by infants and the increased risk for the development of T1DM [95] [96]. The opioid activity of human β-CM-7 (Tyr-Pro-Phe-Val-Glu-Pro-Ile) has been found to be 3–30 times lower than that of bovine β-CM-7 (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) according to the guinea pig ileum longitudinal muscle/myenteric plexus preparation assay [97].

Apart from the possible harmful effects of milk opioid peptides, milk hydrolysates and peptides are considered beneficial for health [98].

5.6. Chaperone Activities

Molecular chaperons stabilize other proteins against unfolding, aggregation, and precipitation under conditions of thermal and other environmental stress situations. The mechanism of chaperone action is not fully understood, but researchers have suggested that it involves hydrophobic interactions and complex formations with partially folded proteins to enable their solubilization through hydrophilic regions. The less ordered and non-compact secondary and tertiary structures of caseins enable them to possess chaperone activity through their hydrophobic and hydrophilic regions. The chaperone activity is responsible for the solubilization of target proteins and the prevention of their aggregation and fibrillation. For example, it has been observed that αS1-casein, but not β- and κ-caseins, prevent the aggregation and precipitation of reduced insulin and α-lactalbumin [99]. αS2- and κ-caseins have minimal chaperone activity and can form amyloid fibrils, which are inhibited by αS1- and β-caseins [100]. Dissociation of κ-casein from casein micelles is a prerequisite for amyloid fibril formation, and oxidation of methionine residues enhances their formation [100]. The large number of proline residues, the extent of exposed hydrophobic surfaces, the polar phosphorylated residues, and the N-terminal hydrophilic domain may explain the chaperone activity of β-casein [101]. Phosphorylation of αS1- and β-casein plays an important role in the chaperone activity of these caseins [102]. Bovine β-casein exhibits higher chaperone activity against alcohol dehydrogenase aggregation than camel β-casein, which may be due to a higher net charge and a greater surface hydrophobicity of bovine β-casein, leading to stronger amphiphilicity and “detergent” properties [103]. The chaperone-like activities of milk caseins are important not only for their biological functions but are also expected to play a role during food processing, e.g., the stability of ultrahigh temperature (UHT) milk [4].

5.7. Nanoencapsulation Properties

Casein micelles, sized 50 to 500 nm, are natural carriers of calcium and phosphate, and they have great potential to serve as nanoencapsulation carriers for a variety of hydrophilic and hydrophobic bioactive components [104]. Examples of bioactive compounds that have been encapsulated in reassembled casein micelles or sodium caseinates include ω-3 polyunsaturated fatty acids [105], vitamin D2, vitamin D3 [106], vitamin E, β-carotene [107], catechins [107], quercetin [104],

folic acid [108], and anticancer drugs [109]. Casein micelles are stable during food processing, safe, protective against oxidation, and highly bioavailable. Thus, the diversity of casein structures present in the milk of different animal species will allow for the effective and tailored design for encapsulation of different bioactive nutraceuticals [110]

6. Nutritional Properties and Applications

Casein polymorphism is influenced the composition, texture, and functional properties of milk products [111]. Studies have shown that casein polymorphism has been associated with ischemic heart disease, cardiovascular disease, type 1 diabetes, sudden infant death syndrome, neurological disorders, such as autism and schizophrenia, lactose intolerance, and various allergies [112][113]. Therefore, careful attention should be paid to casein polymorphism, and deeper research is needed to verify the range and nature of its interactions with the human body, especially in the gastrointestinal tract. In this research the focus would be limited on diabetes, allergies, and autism.

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