Technical Report:

Understanding the Role of Dairy Proteins in Ingredient and Product Performance

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Introduction

Protein is an essential dietary component and sufficient intake is crucial for a healthy and balanced diet. Today, consumers are increasingly aware of and knowledgeable about the benefits of protein in the diet. They recognize the important role it plays in helping manage hunger, sustain energy and maximize performance. Dairy is an important source of high-quality, versatile and multifunctional protein. Many food and beverage manufacturers are trying to incorporate dairy protein in their products. In addition to the excellent source of nutrition, milk protein also provides the clean label desired by consumers as well as a range of functional benefits in finished products: solubility, heat stability, gelling, foaming and emulsification.

Milk is a complex, dynamic nutritional system that provides multiple nutritional and functional benefits. The degree of processing will affect its properties and how it will behave in food systems. The proteins in milk are particularly complex and susceptible to many of the processing conditions used in the dairy and food industry (e.g., shear, heat treatment). Processing results in changes to the milk protein structure, leading to denaturation, aggregation and interactions of proteins. The type and extent of protein interactions can vary depending on many factors such as processing conditions (e.g., time-temperature combination), product composition, pH, protein concentration and ionic strength. These changes in the proteins also can affect the functional properties of dairy ingredients, such as solubility, gelation, heat stability and emulsification, which ultimately affect their performance in the finished products. On the other hand, the heat-induced changes in milk protein functionality contribute to improvement of the sensory properties of dairy and food products, such as yogurt, baked goods and confections. That is why understanding dairy proteins and their functionality can help bring tailored functional properties to dairy ingredients as well as to finished dairy and food products.

This technical report provides food and beverage formulators with an understanding of the milk protein complexity, different protein types and characteristics, as well as research about different dairy proteins. It also examines how processing conditions impact dairy protein performance, and it suggests ways to improve quality and use dairy proteins to create new food products and beverages. See Page 16 for an index of report sections.

Protein structure

Amino acids are the building blocks of proteins. The particular sequence of amino acids in a protein determines its structure, conformation and properties. Depending on the type of amino acids present, different conformations are possible. They are stabilized by various molecular forces. Molecular forces that determine protein conformation are electrostatic interactions, hydrogen bonds, disulfide bonds, dipole-dipole interactions, hydrophobic interactions and van der Waals' forces (**Figure 1**).[1,2,3]

The native structures of proteins are organized at four different levels: primary, secondary, tertiary and quaternary structure (**Figure 2**). The primary structure is the specific amino acid sequence along the covalently linked polypeptide chain (**Figure 2a**). As a result of molecular forces created between the amino acid side chains, the primary structure will fold into an ordered fashion, forming the secondary and tertiary structures that give rise to a uniquely folded native structure that possesses the lowest feasible free energy. The most abundant regular secondary structures found in proteins are the α-helix and the β-pleated sheet. The α-helix emerges through a helical coiling of the amino acid chain and is stabilized by hydrogen bonding between the atoms of the peptide bonds. β-pleated sheets are formed by a linear alignment of some arrays of the amino acid chain (**Figure 2b**). This structure also is stabilized by hydrogen bonding between the strands.

The tertiary structure is the three-dimensional arrangement of the several arrays that are present within the protein. The contributions of inter- and intramolecular interactions are balanced in such a delicate manner that a three-dimensional structure is formed, which is maintained by hydrogen bonding, hydrophobic interactions, van der Waals' forces and electrostatic interactions (**Figure 2c**). Quaternary structure is a superassembly of individual protein molecules. These quaternary structures are generated as a result of interactions between two or more polypeptide chains (**Figure 2d**), formed by the spatial arrangement by noncovalent interactions into a multimeric protein.[2,5]

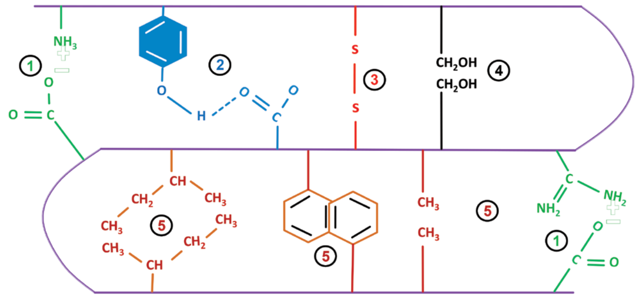

Figure 1. Schematic diagram showing stabilizing forces in proteins: 1. electrostatic interactions; 2. hydrogen bonds; 3. disulfide bonds; 4. dipole-dipole interactions; and 5. hydrophobic interactions.[1]

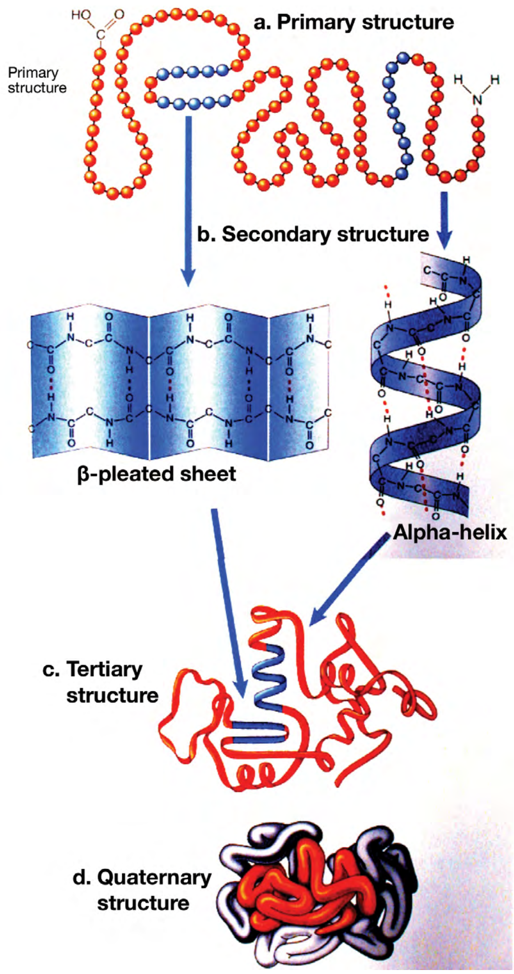

Figure 2. Schematic representation of four different levels of protein structure: a. primary; b. secondary; c. tertiary; and d. quaternary structure.[4]

Protein denaturation

The denaturation or unfolding of proteins is the breaking or alteration of stabilizing forces in the native structure. It causes protein to lose or unfold its native structure. Heat, pressure, shear or changes in formulation conditions (e.g., pH or ionic strength) may cause proteins to lose their native structure; the compact native protein molecule begins to unfold into a disordered, random structure (**Figure 3**). Depending on the formulation or processing conditions, protein aggregates linked through intermolecular and intramolecular bonds such as covalent (e.g., disulfide bonds) or noncovalent bonds (e.g., van der Waals' and electrostatic interactions) may be possible. It also is important to note that the general term denaturation can include many denatured forms of a protein, varying from slight changes in the tertiary structure without changes to the secondary structure (e.g., nonnative form) or major changes in secondary and, therefore, tertiary structure.[1]

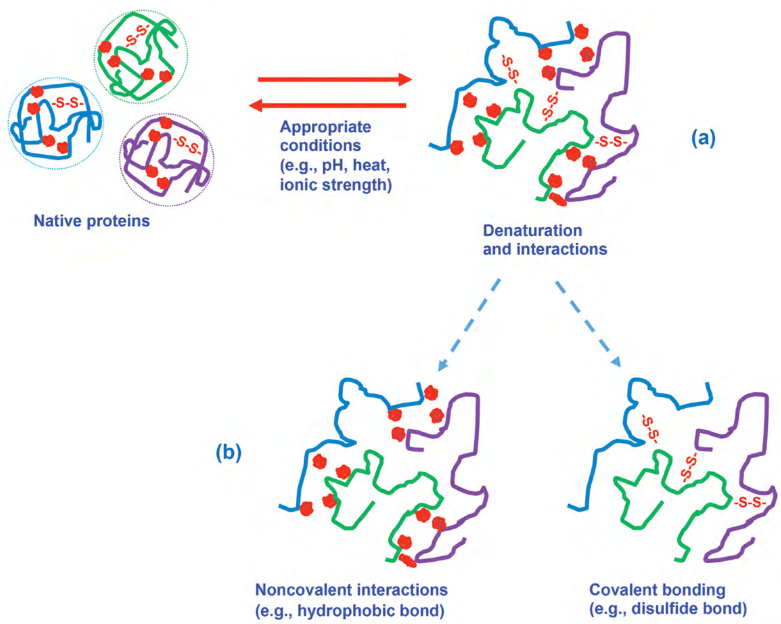

Figure 3. Illustration of heat-induced changes in the structure of native proteins such as: a. denaturation and aggregation of proteins, and b. formation of covalent and noncovalent protein interactions.

Milk proteins: identification, structure and physicochemical properties

Milk is a complex biological fluid containing water, fat, lactose, proteins and minerals (**Table 1**). Water is present as a continuous phase in which other constituents are either dissolved or suspended. Lactose and a portion of the mineral salts are found in the solution; proteins and the remainder of the minerals are found in a colloidal suspension.

TABLE 1: AVERAGE COMPOSITION OF RAW BOVINE MILK[6]

COMPONENT	% (W/W) IN MILK
Water	87.30
Lactose	4.60
Fat	3.90
Proteins	3.30
Casein proteins 2.60	
Whey proteins 0.70	
Minerals	0.70
Organic acids	0.20

Bovine milk contains 30 to 35 grams of protein per kilogram, which is broadly classified into two main categories, namely, casein and whey proteins.[6,7] Casein exists predominantly in the colloidal state; whey proteins exist in soluble form. The casein and whey proteins provide different functional properties and play different roles depending on their state and structure in the aqueous solution.

Caseins and whey proteins have very different structures and, therefore, different physicochemical properties that form the basis for the manufacture of many dairy and food products. The comparison of properties of casein and whey proteins are summarized in **Table 2**. Based on the properties of casein and whey proteins listed in the table, it is apparent the properties of milk proteins influence their behavior in food products. A common example is the precipitation of casein. Lowering the pH of milk via fermentation or direct acidification yields products such as yogurt and cottage cheese. Coagulation of kappa-casein (κ-CN) with rennet leads to production of cheese.

κ-CN is one of four major types of casein molecules, which also include alpha-s1-, alpha-s2- and beta-caseins. The alpha- and beta-caseins are hydrophobic proteins that are readily precipitated by calcium. κ-CN is a distinctly different molecule; it is not calcium-precipitable. As the caseins are secreted, they self-associate into aggregates called micelles in which the alpha- and beta-caseins are kept from precipitating by their interactions with κ-CN. In essence, κ-CN normally keeps the majority of milk protein soluble and prevents it from spontaneously coagulating.

TABLE 2: COMPARISON OF SELECTED PHYSICOCHEMICAL PROPERTIES OF CASEIN AND WHEY PROTEINS[8]

PROPERTIES	CASEINS	WHEY PROTEINS
Structure	Lack well-defined secondary, tertiary and quaternary structure; possess random coil structure	Well-defined tertiary and quaternary structure
Amino acid composition	Low in sulfur-containing amino acids; high in proline	Relatively high in sulfur-containing amino acids; low in proline
Physical state	Exist as large colloidal aggregates called casein micelles	Exist as globular proteins in form of monomer-octamers, depending on pH
Solubility at pH 4.6	Insoluble at pH 4.6	Soluble at pH 4.6
Heat stability	Very heat-stable (can withstand severe heat treatment such as sterilization, ultrahigh temperature (UHT) or retort processing)	Heat-labile (can be completely denatured, particularly when heating at 90°C or higher)
Coagulation by limited proteolysis or ethanol	Can be coagulated by specific, limited proteolysis (e.g., rennet coagulation) or ethanol	Cannot be readily coagulated by enzyme or limited proteolysis or ethanol

CASEINS

Caseins are the major proteins in milk. They represent about 80% of the total nitrogenous material in bovine milk. They exist in the milk as casein micelles. Various models of the casein micelle structure have been proposed since the initial reports in 1969.[9] Because of the amphiphilic nature of casein, it has excellent surface-active and emulsification properties. Caseins have a relatively high charge and contain many proline residues but few cysteine residues.[10] A detailed overview of the structure and properties of casein micelles has been published.[11]

Caseins possess low levels of secondary and tertiary structures, a feature that contributes to their remarkable stability at high temperatures. However, when subjected to severe heat treatment, caseins undergo changes, such as dephosphorylation and proteolysis. Polymerization of caseins can occur as a result of condensation reactions (e.g., Maillard-type reactions) and the formation of lysinoalanine. The changes in casein micelles upon heat treatment include an increase in hydrodynamic diameter, decrease in zeta potential and hydration, and the dissociation of caseins from micelles,[12,13] which have been reviewed in detail.[14,11]

The association and dissociation of casein micelles may occur depending on processing conditions, pH and ionic environment. This is an important property of casein micelles, which forms the basis for various products and functional dairy ingredients such as yogurt, cheese and sodium caseinate (**Figure 4**).

Many technologically important properties of milk, such as heat stability, rennet coagulability and the strength and syneresis properties of rennet gels, are strongly influenced by calcium ions (Ca^{2+}). The binding of Ca^{2+} to caseins is mainly through phosphoseryl residues and also via the carboxylic acid side chains.

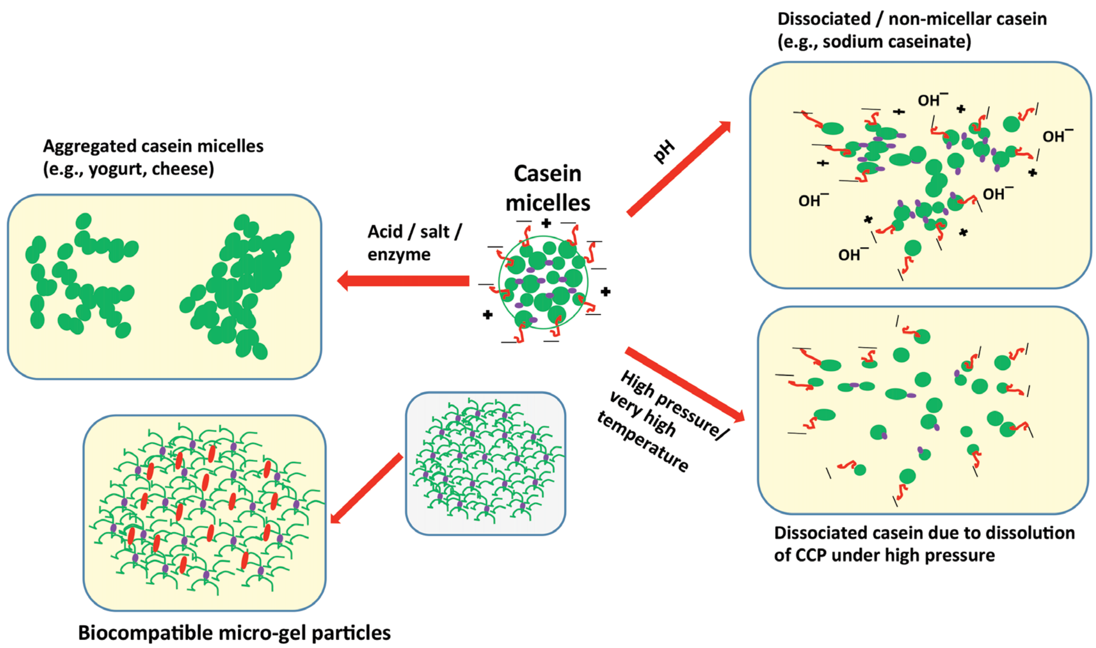

Figure 4. Various approaches to modify the functionality of milk proteins.[15] Schematic diagram showing changes in the casein micelle as affected by change in processing and formulation conditions.

WHEY PROTEINS

Whey proteins or milk serum proteins are the proteins that remain soluble following the isoelectric precipitation of casein at pH 4.6 at 20°C to produce acid whey or following the coagulation of casein by limited proteolysis with rennet to produce sweet whey.[16,17] The average compositions of these two whey types are shown in **Table 3**.

Whey proteins represent about 20% (i.e., 5 to 7 grams per liter) of the total nitrogenous material in bovine milk. The principal whey proteins are β-lactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA) and immunoglobulins (Igs) in decreasing order of whey protein concentration.[3] Whey proteins are predominantly globular with a rather uniform distribution of hydrophobic/hydrophilic amino acids along their polypeptide chains (in contrast to caseins).

TABLE 3: PROTEIN COMPOSITIONS OF SWEET WHEY AND ACID WHEY[18,19,17]

PROTEIN	APPROXIMATE % OF TOTAL WHEY PROTEIN	
	ACID WHEY	SWEET WHEY
β-lactoglobulin	54	45
α-lactalbumin	23	18
Bovine serum albumin	6	5
Immunoglobulins	6	5
Casein-derived peptides	2	20
Enzymes	2	2
Phospholipid-protein complexes	5	5

They lack the amphiphilic nature of casein monomer subunits, a feature that confers on them many unique functional properties.[18] The substantially lower proline content of the whey protein molecules permits a globular conformation with a large amount of helical content, which again explains their strong susceptibility to denaturation by heat.[20]

Whey proteins are sold commercially as food and nutritional ingredients, such as whey powders, whey protein concentrates (WPCs) and whey protein isolates (WPIs). WPCs and WPIs are valuable ingredients in the food industry because of their exceptional nutritional value and important functional properties, such as emulsification, solubility and ability to form heat- or pressure-induced gels.[21,22] The composition of commercial WPCs varies widely,[23,24] affected by various factors such as concentration, seasonal variation, the type of whey (whey source) and the processing methods used to manufacture the WPC (**Table 4**).

The denaturation of whey protein occurs when hydrogen, hydrophobic or covalent bonds are affected.[18] This often exposes hydrophobic amino acid side chains that normally are buried within the native three-dimensional structure and, thus, causes an increased reactivity of such groups. Through sulfhydryl-disulfide interchange and hydrophobic interactions, the unfolded protein molecules may associate with each other to form aggregates (**Figure 3**), which will become insoluble as they grow in size. Severe heat treatments can lead to interactions with other protein molecules, which result in intermolecular association and aggregation and, finally, precipitation or gelation, depending on several factors, including the protein concentration, heating and cooling rates, pH and ionic strength.[25,26,18,2,14,3] Descriptions of possible heat-induced changes in the milk proteins has been summarized in **Table 4**.

TABLE 4: DESCRIPTIONS OF POSSIBLE HEAT-INDUCED CHANGES IN PROTEINS[27]

Protein denaturation is any modification in secondary, tertiary or quaternary conformation that is not accompanied by the rupture of peptide bonds involved in the primary structure. The final conformation after denaturation can correspond to a totally (random coil) or partially unfolded polypeptide structure.	**Aggregation or polymerization:** The terms aggregation or polymerization, precipitation, coagulation and flocculation refer to unspecified protein-protein interactions that result in the formation of large complexes with higher molecular weights.	**Gelation** is an orderly aggregation of native and/or (partially) denatured proteins, forming a three-dimensional network structure in which protein-protein and protein-solvent interactions are balanced to produce a well-ordered matrix that is capable of holding significant amounts of water.

Structure–function relationship of milk proteins

The relationship between the structure and function of milk proteins dictates their role in final products. Milk is a colloidal system. Extrinsic and intrinsic factors affect interactions within and between proteins. Extrinsic factors that affect levels of denaturation, aggregation and protein-protein interaction at the molecular level include temperature, protein concentration, pH, ionic strength and type of ion, processing conditions, and external energy such as shear, heat, high pressure or ultrasonication. Intrinsic factors include hydrophobicity, electrostatic interactions, disulphide bonds, molecular weight and amino acid composition (**Figure 5**).[28]

EXTRINSIC FACTORS	INTRINSIC FACTORS
Temperature	Amino acid composition
Pressure	Molecular weight
pH	Hydrophobicity
Protein concentration	Electrostatic interaction
Ionic strength	Number of disulfide bonds and free sulfyral groups
Type of salts (monovalent, divalent)	

Figure 5. Factors affecting protein-protein interactions.[28]

The structure of a food determines the appearance, body, texture, sensory attributes, bioavailability and nutrient delivery in the final product. Therefore, specific formulations and processing conditions can be used to tailor specific protein-protein interactions, which ultimately lead to the development of food products with different structures. Different types of protein interactions depend on the environment in the food system, which are summarized in **Figure 6**. Therefore, knowledge of protein structure and protein-protein interactions is used in the development of dairy ingredients with specific functionalities as well as in the development of final food products and beverages.

The opportunity is to combine the knowledge of structure-function relationships, protein-protein interactions and functional properties to modify processing outcomes such as the denaturation and aggregation pathways of milk proteins. For example, milk proteins possess excellent stabilizing, water-holding and emulsification properties. These properties can be optimized to develop clean-label food products (i.e., minimizing the use of stabilizers and emulsifiers). Achieving tailor-made food structures requires combining the knowledge of physicochemical properties of proteins and the interactions between processing and formulation parameters.

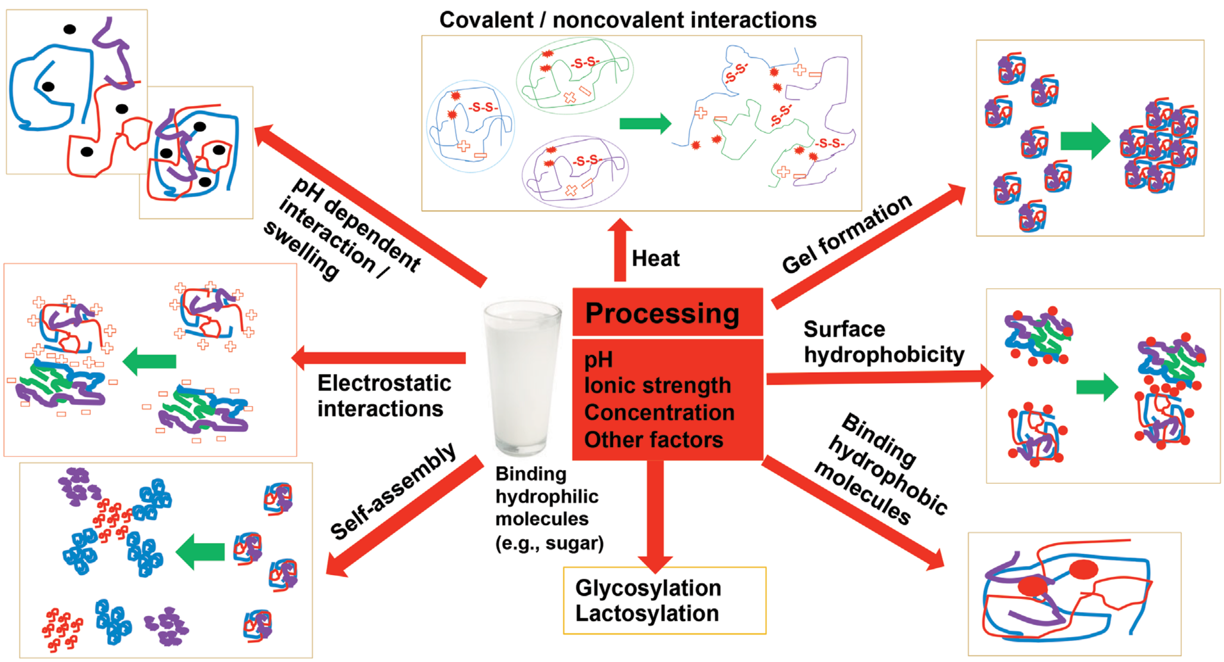

Figure 6. Schematic diagram showing the possibility to manipulate protein interactions as affected by various processing parameters and formulation conditions.[29]

Changes in protein structure and protein-protein interaction as well as interactions of proteins with other components in the food system contribute to the textural and functional properties (e.g., gelling, viscosity) of the final products. Proteins can interact with other proteins or with other components present in the food systems, such as carbohydrates, fats and minerals. This makes the system more complex but provides opportunities to develop products with novel texture. For example, protein gels, in the presence of lipid-containing small fat globules with narrow particle size distribution, have been reported to have smooth texture and higher gel strength.[30] This suggests that it also may be possible to manipulate the texture and smoothness of the final product by manipulating interactions between proteins and lipids in the food systems.

A protein molecule's charge at a given pH is important because it affects the electrostatic repulsion and interaction of proteins (**Figure 7**).[31,32] The repulsive forces can be altered by changing the pH of the protein solution or by the addition of ions or salt to the protein solution, which allows for tailored protein-protein interactions.

This is one of the reasons for reduction of electrostatic repulsive forces upon an increase in ionic strength.[33] The type of salt (e.g., monovalent salts vs. divalent salts) also affects the protein-protein interactions and the type of gel formed. The salt concentration required to change gel microstructure depends on the salt's position in the Hofmeister series.[34]

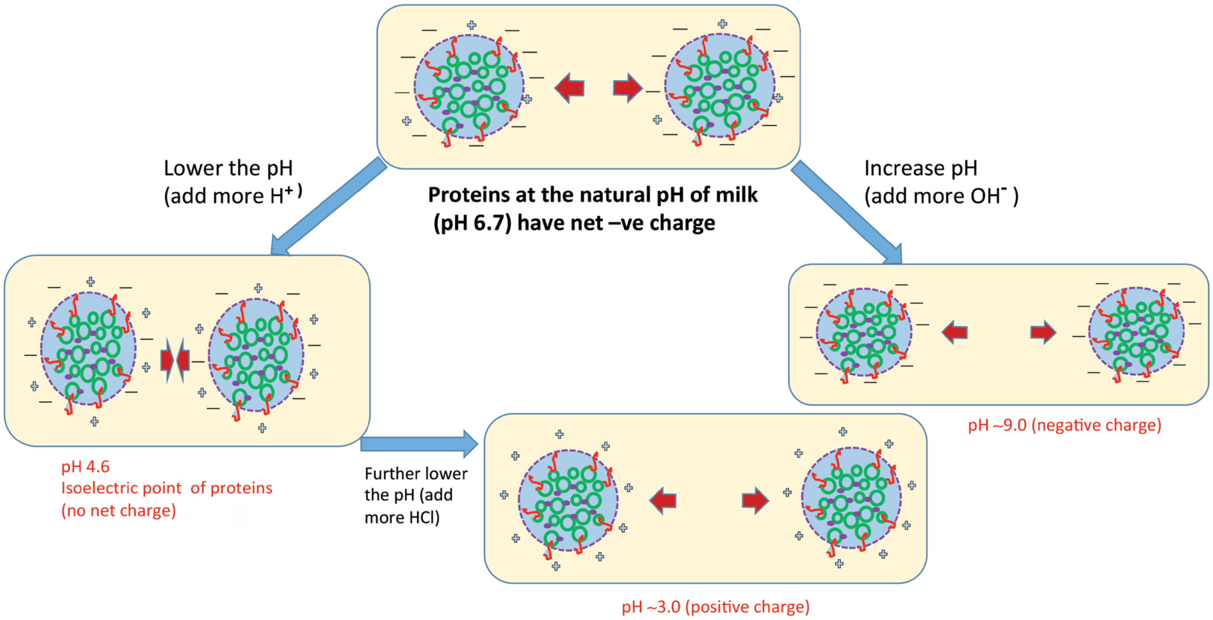

Figure 7. Net negative charge: Schematic diagram showing effect of pH on the charges of protein molecules and protein interactions.[35]

MILK PROTEIN FUNCTIONALITY

In addition to being an excellent source of nutrition, milk proteins play an important role in providing desirable functional properties in final products. Dairy ingredients are used as functional ingredients for a range of applications in the food industry (**Table 5**). Ingredients such as whole milk powder (WMP), skim milk powder (SMP), milk protein concentrates (MPC), WPCs and WPIs are used in the formulations of nutritional and meal replacement beverages or recombined milk products.

In many instances, these foods and beverages are subjected to severe heat treatments such as ultra-high temperature (UHT) treatment or retort sterilization to extend shelf life and ensure food safety for human consumption. Therefore, dairy ingredients intended for food and beverage applications that require the protein system to maintain its solubility, such as in ready-to-drink (RTD) beverages, must be heat-stable and able to withstand commonly used severe heat treatments.

Heat stability is the ability of proteins to survive severe heat processing without detrimental changes, such as excessive turbidity, increased viscosity, phase separation, precipitation or gelation, during or shortly after processing.[41] Heat stability of milk is a function of its protein stability.[42] The application of heat leads to levels of denaturation and aggregation, resulting potentially in thickening or gelation of the mixture.[43] In some cases where heat stability is desired, denaturation and aggregation of whey proteins can be detrimental. Examples of poor heat stability consequences during processing are:

- Restriction on the concentration of solids (total solids) that can be processed
- Reduced process efficiency
- Reduced ability to tolerate time and temperature during processing

Therefore, heat stability is one of the most important processing considerations when selecting ingredients for use in food and beverages.

Whey proteins, in particular, can undergo heat-induced denaturation, aggregation and gelation (**Figure 8**). The ability to form heat-induced gels to achieve desired sensory and textural properties in foods is an important functional property of whey proteins. These gels can be classified as “fine-stranded” or “particulate” (by appearance, microstructure or rheological properties). The type of the structure will contribute to different textural properties of the final products. Development of noncovalent (mostly hydrophobic) and disulfide-linked protein aggregates are possible during whey protein aggregation and gelation.[44,45,46]

TABLE 5: SELECTED FUNCTIONAL PROPERTIES OF DAIRY INGREDIENTS AND THEIR APPLICATIONS IN THE FINAL PRODUCTS[36,37,38,39,40]

NO.	PROPERTY	DESCRIPTION	EXAMPLES OF SELECTED APPLICATION
1	Water-binding	Interacts with product components to provide high water-holding capacity	Meat products Bakery products Confectionary Imitation cheese Frozen desserts Prepared foods
2	Viscosity	Interactions with other product components, concentration, protein structure and heat processes all contribute	Soups and sauces Yogurts Puddings Beverages
3	Emulsification	Ability to maintain two immiscible liquids (e.g., water and oil) in a stable emulsion	Coffee whitener Ice cream Salad dressings Sausages (meat emulsions) Soups, sauces and dips Mayonnaise Processed cheese
4	Foaming	Ability to form stable foams at air-water interfaces, providing excellent whipping ability (i.e., ability to incorporate and hold air in the product)	Ice cream Frozen desserts Whipped cream and toppings Aerated confections (e.g., nougat and marshmallows) Cakes and mousses Meringues
5	Gelation	Provides physical structure to food products by cross-linking proteins; improves mouthfeel in certain applications	Yogurts Bakery Custards Confectionary Meat products Prepared foods
6	Solubility/ heat stability	Ability to remain in solution under different processing conditions and concentrations, in addition to varying conditions such as changes in pH, mineral levels and heat process	Recombined, UHT and sterilized milk Soups and sauces Infant and clinical nutrition Coffee whitener Sports beverages Protein-fortified juice beverages
7	Opacity/clarity	Visual attributes from opacity in beverages to clarity in high-acid sports beverages	Fortified milk beverages Protein sports beverages Chocolate Confections/caramels Sauces Dressings
8	Flavor/color development	Usually associated with the Maillard reaction, providing desirable characteristics such as uniform brown color and caramelized flavors	Confectionary, caramels Soft sweets and confection coatings Baked goods (dough, cake, muffins, crackers) Sauces/soups

Protein gelation traditionally has been achieved by heating, but other physical and chemical processes also can be used.[47] Pressure is another physical means. Chemical means include acidification, enzymatic cross-linking and the use of salts. These cause modifications in the protein structure and interactions of proteins with proteins and with other components in the solutions. The characteristics of each gel will vary depending on factors such as protein concentration, degree of denaturation caused by pH, temperature, ionic strength and/or pressure.[48] The protein-protein and protein-solvent interactions also are reported to be influenced by factors that affect protein gelation as well as affecting the type and properties of gels.[49,31]

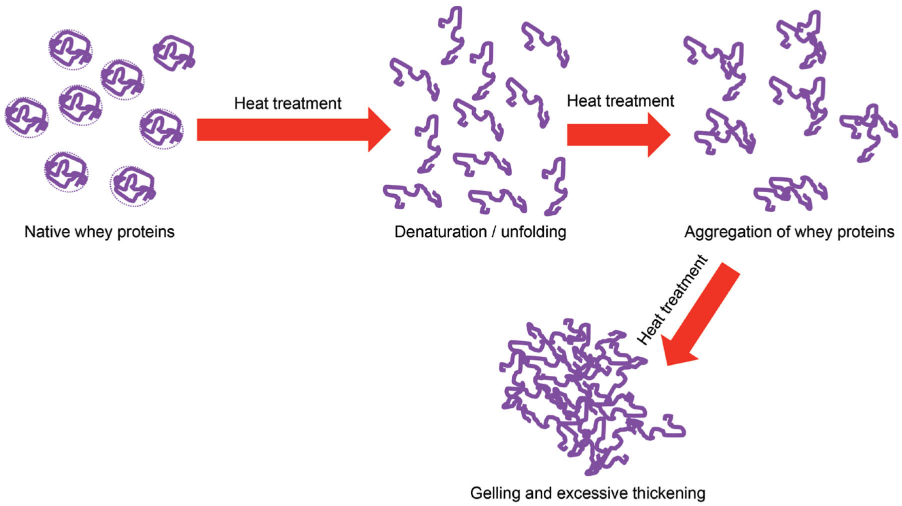

Figure 8. Illustration showing effects of severe heat treatments, such as UHT or retort sterilization, of whey protein solutions. Severe heat treatment of whey protein solutions can lead to denaturation, aggregation and gelation or excessive thickening of whey proteins.

HEAT TREATMENT AND MILK PROTEINS: EFFECTS ON FUNCTIONALITY

Heat treatment is an essential unit operation employed in the dairy industry to make products microbiologically safe, extend shelf life and modify the functional properties of milk products.[17,14,44,50,51] **Table 6** lists examples of the most common heat treatments used in dairy processing.[17,14]

TABLE 6: COMMON HEAT TREATMENTS EMPLOYED IN THE COMMERCIAL PROCESSING OF MILK AND MILK PRODUCTS[17,14]

HEAT TREATMENT	TEMPERATURE/TIME CONDITIONS
Thermization	65°C/30 seconds
Pasteurization	72°C/15 seconds
Preheat treatment for milk powder	80 to 120°C/2 to 10 minutes
Preheat treatment for yogurt manufacture	90°C/5 to 10 minutes
UHT sterilization	140°C/3 to 20 seconds
Sterilization/retorting (batch/in-container)	110 to 120°C/5 to 20 minutes

These heat treatments all lead to various degrees of denaturation, aggregation and interactions of proteins.[50,52,53] Immunoglobulins, lactoferrin and BSA are sensitive to thermal processing. Partial denaturation of these proteins occurs during commercial pasteurization. β-LG and α-LA are extensively denatured during preheat treatments used for milk powder manufacture and during UHT treatment. Depending on the severity of heat treatment, they form varying proportions of high-molecular-weight aggregates linked via disulfide bonds or hydrophobically linked aggregates. Also, in addition to formation of soluble aggregates such as dimers and trimers of β-LG, α-LA and BSA, disulfide-linked complexes between caseins (κ-CN and α_{s2}-CN) and whey proteins are formed.[50,54,52] Such process-induced interactions also can be linked to specific functional properties.

Upon the heating of milk, where both casein and whey protein are present, whey proteins interact with the casein micelles to form casein-whey protein complexes (**Figure 9**).[55,56,52,53] Most studies conclude that thiol/disulfide interchange reactions, which lead to the formation of intermolecular disulfide bonds, play an important role in the heat-induced aggregation of β-LG and its interaction with other proteins, including the caseins. Studies also report that, as well as the aggregation by intermolecular covalent (disulfide) bonds, noncovalent interactions (such as hydrophobic or ionic interactions) also are involved in the heat-induced interactions of milk proteins. The interactions between β-LG and κ-CN have been considered very important for functionality of many dairy products. Two disulfide bridges and a free sulfhydryl group present in the native structure of β-LG play an important role in its heat-induced interactions with κ-CN (**Figure 9**).[57,14,58,59,52]

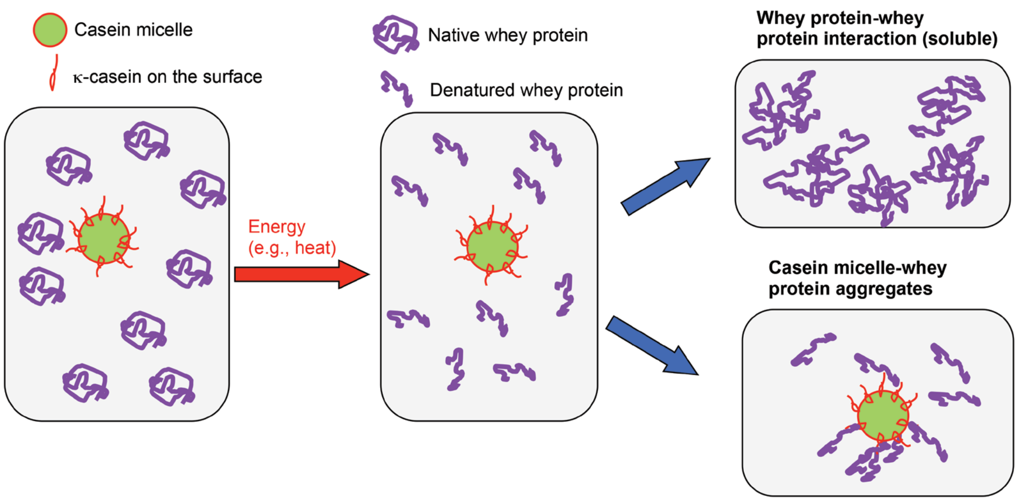

Figure 9. Schematic diagram showing possible protein interactions in the heat-treated milk system.

Some well-known examples of the beneficial effects of the heat-induced functionality of milk proteins include heat-induced gelation of whey proteins,[3] preheat treatments for improving the heat stability of milk powder, evaporated and sterilized milk products;[60,61] improved functional properties of milk powder;[62] and improved yogurt texture.[63] This topic has been reviewed in detail.[52]

HEAT TREATMENT AND WHEY PROTEINS: EFFECTS ON FUNCTIONALITY

Upon heating, whey proteins can denature and form soluble aggregates or insoluble aggregates (gels) by interacting with other whey proteins (see **Table 5** for description of terminology) in the system.[64,65,66,67,68,69,70,46,71,72,73] Notably, different whey proteins have different responses to heat treatment in terms of conformational changes, altered bonding patterns, formation of interprotein aggregates via disulfide bond interchange and altered hydrophobic associations. There are distinct differences in the denaturation behaviors of individual whey proteins because different whey proteins have different thermal transition temperatures (**Table 7**). Because of the heterogeneity of the whey protein system, and because the individual proteins exhibit different responses to heat, the thermal denaturation and aggregation of the total whey proteins reflect the collective response of the component proteins.[74]

TABLE 7: THERMAL DENATURATION TEMPERATURES AND ENTHALPIES OF WHEY PROTEINS[25,75,17]

WHEY PROTEIN	TD (°C)	TTR (°C)	ΔH (kJ/mol)
β-LG	78	83	311
α-LA	62	68	253
BSA	64	70	803
Ig	72	89	500

TD = the initial denaturation temperature; TTR = the temperature at the differential scanning calorimetry peak maximum; ΔH = the enthalpy of denaturation.

SOME COMMERCIAL APPLICATIONS OF HEAT TREATMENTS FOR PROTEIN FUNCTIONALITY

1. Yogurt

Preheat treatments (e.g., 90°C for 10 minutes) are commonly applied to milk used in yogurt manufacture and are reported to improve the textural, microstructural and rheological properties of yogurt.[76,77] The heat-induced interactions between β-LG and κ-CN, via sulfhydryl-disulfide interchange, are believed to be central to the improvement in the yogurt texture.[14,52] Also, yogurts made from preheated milks have higher pHs at gelation and produce considerably firmer gels than yogurts made from unheated milks.[76,63,56,78]

2. Milk powders

During the manufacture of milk powders, a range of preheat treatments are applied to milk with the aim to produce milk powders with specific functional properties. Skim milk powders are broadly classified as low-, medium- and high-heat powders. This classification is usually based on the whey protein nitrogen index (WPNI), which is the amount of undenatured whey protein present in the powder. It is related to the denaturation of whey proteins as a result of specific heat treatment that is used during the manufacture of milk powders, particularly during preheat treatment, evaporation and drying.[51] The interactions between denatured whey proteins and casein micelles ultimately influence the functionality of milk powders. These protein interactions also affect how the casein micelles behave during further processing such as evaporation and drying.[79] During evaporation, the casein micelle size increases primarily due to the aggregation of the micelles or association of whey proteins with the micelles.

In the manufacture of high-protein powders, concentration of milk by ultrafiltration, in particular diafiltration prior to drying, can cause dissolution of colloidal calcium phosphate, resulting in loosening of the casein micelle structure and possible swelling of the casein micelles. Increasing the degree of concentration causes progressive breakdown of micellar structure from an intact micelle to a swollen diffuse micelle and, finally, to a smaller fragmented micellar structure. Such changes in the casein micelle predispose the milk system to further protein-protein interactions during spray drying and, consequently, impact the functionality of the ingredients.[79,80,81]

NONHEAT MODIFICATIONS TO FUNCTIONAL PROPERTIES OF MILK PROTEINS

Stability and functional properties of milk proteins such as viscosity, gelling and emulsification can be modified through physicochemical modifications of milk proteins (i.e., using heat, shear, high-pressure processing, ultrasonication, pH, ionic strength and charge manipulations); through enzymatic modifications (e.g., transglutaminase (TGase) cross-linking of proteins); or through chemical modifications (e.g., succinylation, lactosylation and ligand binding). Such approaches can be used to tailor the functional properties of milk proteins to meet customer expectations.

Enzymatic cross-linking and modifications of milk proteins through TGase have been reported to enhance the functional properties of milk proteins,[82] including increasing the gel strength, water-holding capacity[83] and viscosity of acid gels.[84,85,86] This allows for the potential to manufacture low-fat yogurt without the addition of gums[86] and can help prevent serum separation or syneresis caused by changes in temperature or physical impacts. TGase treatment of milk protein also can enhance heat stability of milk proteins.[87,88,89] The enzymatic modifications also can help improve the surface activity and emulsification properties of milk proteins.[90]

Chemical modifications such as succinylation of milk proteins[91] make it possible to develop new products through formation of electrostatically stable complexes between anionic native proteins and protein derivatives with a net positive charge. The microlayering of sodium caseinate submicelles to stratify the thin film layer via step transition could play an important role in stability of food emulsions.[92,90]

Recently, carbon dioxide treatment of milk proteins has been used to optimize the ratio of micellar to nonmicellar casein in milk intended for the manufacture of yogurts with customized body, texture and flavor. It also has been used to improve functional properties (solubility, emulsification and heat stability) of MPC and micellar casein concentrate (MCC).[93]

Another approach is the application of high-pressure processing (HPP) for the preservation and modification of various aspects of foods, including alteration of their functional properties.[94,95,96,97,98] It has been reported as a physical tool for protein modification and, thus, has the potential to produce new dairy products with modified texture and functional properties. HPP is gaining acceptance for food processing and preservation because of increased consumer demand for microbiologically safe, nutritious, clean-label and "freshlike" food products with an acceptable shelf life.[99,94,95,96] HPP leads to protein denaturation, aggregation and gelation by altering the delicate equilibrium between the bonds that stabilize the native structure of proteins.[100,101,102,103,104] The impact is different from heat and is dependent on the type of protein, pH, ionic strength, the applied pressure and pressurizing temperature, and the duration of the pressure treatment.[105,102,106,107] The destabilization can affect the aggregation behavior, pressure-induced functionality such as gel formation, and physical, rheological and microstructural properties of whey proteins.[108,109,110,111] Comprehensive chapters on the effects of HPP on milk proteins can be reviewed.[53,112,51,113]

Ultrasonic processing is an emerging technology in food and dairy applications. Ultrasound refers to sound waves above the frequency of human hearing (~ >18 kHz). When ultrasound passes through a liquid, bubble nuclei present in the liquid grow by bubble coalescence and rectified diffusion. When these bubbles reach a critical size, they collapse under near-adiabatic conditions (without gain or loss of heat), generating extreme conditions that in the surrounding liquid include intense shear forces, turbulence and microstreaming. This is known as acoustic cavitation,[114] and these physical effects are used in the processing of food. Ultrasound-induced physical effects are finding increased use in dairy processing, in applications such as the enhancement of whey ultrafiltration,[115] reduction of product viscosity,[116] homogenization of milk fat globules and altering fermentation characteristics,[117] sonocrystallization of lactose[118] and the cutting of cheese blocks.[119]

The increased availability of efficient, large-scale continuous flow through ultrasonic systems has enabled these processes to move from the laboratory to commercial operations throughout Europe and the United States.[120] Ultrasonic processing is establishing itself as a significant food-processing technology and has the significant potential to modify the functional application of dairy proteins, both casein and whey. It has the capability for large commercial scale-up and good payback on capital investment.[120]

Conclusion

Milk and, in particular, milk proteins are fascinating and complex systems designed by nature. They can be processed into a huge array of nutritious and great-tasting food products and beverages. With the evolution of food science and technology, we are continually learning to harness the potential of milk proteins to provide increasing nutritional and functional benefits for the development and marketing of food products. Today's food consumers are becoming ever more discerning, demanding new products as well as improved flavors and texture, all with ingredient labels that are simple and clear. Milk and, specifically, milk ingredients are uniquely positioned to provide an ever-widening opportunity for food scientists to meet and exceed consumer expectations.

For more information about dairy ingredient research, visit ThinkUSAdairy.org or USDairy.com. For assistance with new or improved products using dairy ingredients, contact Dairy Technical Support at techsupport@ThinkUSAdairy.org.

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