Beneficial and Adverse Effects of Water-Protein Interactions in Selected Dairy Products

ABSTRACT

Water-protein interaction is an important factor in the dehydration, rehydration, solubility, viscosity, gelation, heat stability, browning reaction, and other important properties of dairy products over a wide range of water activities. The ability of the major milk proteins, e.g., casein micelles and whey proteins, to interact with and immobilize water in dehydrated, solution, and gelled milk product systems is largely dependent upon their physicochemical state. This latter phenomenon is largely a function of pH; temperature; protein concentration; total solids concentration; ionic composition; addition of Ca-chelating compounds, stabilizer gums and sugars; and by thermal processing treatments that promote whey protein denaturation, aggregation, and interaction with casein micelles. Examples of the role of water and protein interactions in dairy products are illustrated by casein micelle solvation and voluminosity; whey protein aggregation in heated whey systems; casein micelle aggregation and syneresis in cheese manufacture; viscosity and gelation of sterile milk concentrates; rehydration, solvation, solubility, and functionality of casein, caseinate, whey protein concentrate, and milk powder in formulated food products; viscosity, body, and texture of ice cream and frozen desserts; and viscosity and gelation of cultured dairy products.

INTRODUCTION

Important protein-water interactions in dairy products include endogenous water binding by native milk proteins (e.g., the whey proteins and caseins, casein micelle solvation, and the imbibition of large amounts of water or milk solution by acid or heat-induced casein micelle aggregates or whey protein-casein micelle complex aggregates). Examples of dairy products and processes that depend on milk protein-water interactions for their desirable properties and characteristics include imbibition of water for curd formation and subsequent expulsion of water from the curd by syneresis during the cooking of cheese; imbibition of water to provide viscosity without syneresis in yogurt manufacture; imbibition of water to provide viscosity without excessive coagulation or gelation of sterile milk concentrates; dehydration and rehydration of milk proteins in dried milk and whey products; and imbibition of unfrozen water to control ice and lactose crystallization and provide body and texture in ice cream and other frozen dairy desserts. Protein-water interactions are also peripherally involved in nonenzymatic browning reactions in concentrated and dried dairy products.

Milk proteins, due to their strong ability to interact with and bind water, are highly soluble and functional under appropriate pH, temperature, and ionic conditions. Native whey proteins exist as compact, globular protein molecules with a high degree of secondary and tertiary structure. They are susceptible to heat denaturation that causes them to unfold and become more susceptible to interaction through hydrophobic, calcium bridging, and disulfide interchange mechanisms that result in aggregation in heated whey or interaction with casein micelles in heated milk products (14, 15). Due to their amphiphilic primary structures, the major caseins exist in milk in the form of submicelles that are further assembled into micelles with a colloidal phosphate framework that provides excellent physical stability to them. The caseins are held together by hydrophobic, calcium bridging, and colloidal phosphate bonds in a unique and complex structure that allows...
considerable porosity, thus enabling their micelles to imbibe up to 3.5 g H₂O/g of casein in milk. Heat-induced interaction between casein micelles and denatured whey proteins provides physical stability to the denatured whey proteins and also modifies the ability of the micelles to interact and provide viscosity, gelation, coagulation, syneresis, dispersibility, and solubility to important dairy products.

**MILK PROTEIN-WATER INTERACTIONS**

Water exists in foods in two major forms, e.g., free water and water of lowered or restricted activity (8). Free water exerts a normal vapor pressure, functions as solvent and reactant, and freezes at temperatures below -25°C. Restricted activity water includes tightly bound monolayer water present at water activity (Aₜ) 0 to -.2; structured water that is both hydrogen bonded to protein and polysaccharide polar groups; and clathrate water associated with exposed hydrophobic regions on the protein molecule. Structured water is exchangeable with free water, does not participate readily in chemical and bio-chemical reactions, exhibits a higher than normal heat of vaporization, does not freeze at -25°C, and does not allow normal solute diffusion. Imbibed water is physically immobilized but otherwise similar to free water in physical and chemical properties.

The subject of protein-water interaction (e.g., protein hydration) has been reviewed by Chou and Morr (1) and Kinsella and Fox (6, 7). The extent of protein-water interaction is dependent on a number of factors including water content and key fundamental properties of the protein system that depend on pH, ionic composition and activity, and temperature. Processing treatments that alter the protein content, water content and activity, pH, ionic composition and activity, and physicochemical state of the protein molecule strongly influence the extent of protein-water interaction in dairy products. For example, heat treatments that promote whey protein denaturation result in only a slight increase in protein-water interaction, which is of minor importance in most dairy products. However, treatments that cause whey protein aggregation in whey systems and interaction of whey proteins with casein micelles in milk systems generally reduce the availability of their polar groups to interact with water. Such protein aggregations under suitable conditions provide substantial viscosity or result in formation of a gel structure capable of imbibing large amounts of free water. Additionally, the loosely structured, porous casein micelles imbibe considerable amounts of water as milk solution in fluid milk products. Processing treatments that promote interaction and aggregation of casein micelles are responsible for the increase in viscosity and gelation of cultured milk, cheese, and sterile milk concentrates, primarily by increasing the amount of imbibed water retained within the heat-induced micelle microstructure. Reversible and irreversible changes in the milk proteins caused by heating greatly influence the dispersibility, solubility, and functionality of spray dried milk, whey, and milk protein concentrate products.

Table 1 lists the hydration values for two major milk proteins (1). The BET monolayer values have special significance for dried milk products at Aₜ < .2, whereas the hydration data for Aₜ .92 applies for fluid dairy products. These values demonstrate that milk protein-water interactions involving hydrogen bonding and clathrate water structures do not totally account for the observed changes in viscosity, gelation, and other physical properties of dairy products.

Casein micelles are porous colloidal particles formed in the bovine mammary system from spherically shaped submicelles, and they possess a unique calcium phosphate framework that provides a degree of rigidity sufficient to resist deformation when pelleted by preparative ultracentrifugation (11, 14). Their physicochemical properties are summarized in Table 2. Of prime importance to this topic is the high value for the voluminosity, e.g., 3.5 to 6 cc/g dry micelle. This large voluminosity value accounts for their high degree of solvation (Table 3). Micelle solvation due to imbibed water is less at pH 6.6, the pH of fresh milk, than at pH 5 or 7.5. The increased solvation value at pH 5 is probably due to partial micelle aggregation at pH 5, which is approaching the isoelectric point of the caseins. The explanation for the increased micelle solvation at pH 7.5 is not known but may be due to deposition of additional colloidal phosphate from the milk solution onto the micelle to increase its ability to imbibe water.
TABLE 1. Milk protein hydration values by different methods.1

<table>
<thead>
<tr>
<th>Water hydration</th>
<th>H2O in Dry micelle</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g/g)</td>
<td>(g/g)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method</th>
<th>Casein</th>
<th>β-Lactoglobulin</th>
<th>Casein</th>
<th>β-Lactoglobulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>BET Monolayer method</td>
<td>.05</td>
<td>.067</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equilibrium moisture contact method, Aw .92</td>
<td>.40</td>
<td>.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfreezable water by nuclear magnetic resonance</td>
<td>.39</td>
<td>. . .</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Chou and Morr (1).

Warming milk to 35 to 40°C greatly reduces the solvation of casein micelles. Electron microscopic and ultracentrifugal data confirm that warming results in the formation of 2 to 3-μm micelle aggregates, which presumably are responsible for the viscosity and gelation in certain manufactured milk products (12, 13). Although the data are not available, it is postulated that heating milk products to temperatures of ≥ 90°C causes aggregation of casein micelles that results in increased viscosity, gelation, and coagulation due to imbibition of additional amounts of free water (9, 10).

PROTEIN-WATER INTERACTIONS IN DAIRY PRODUCTS

Table 4 gives compositional data for selected dairy products (2). Because milk fat does not interact with water it should not affect appreciably the Aw values for these products. For this reason their compositions on a fat-free basis are listed. Because Aw of these products containing ≥ 50% water generally ranges between .92 and 1.0 (15), all products other than nonfat dry milk and other dried milk products are assumed to have Aw in this range. This assumption is consistent with data indicating that Aw values for most cheese products range from .95 to .988 (7).

For the remainder of this paper it will be assumed that those compositional and processing factors that influence viscosity, gelation, and related physiochemical properties of dairy products do so mainly through their ability to modify the state of dispersion of casein micelles and casein micelle-denatured whey protein complexes. Much of this hypothesis is corroborated by the excellent review on the microstructure of milk and dairy products (3).

Yogurt

Yogurt premix is formulated to contain milk, nonfat dry milk, sugar, and stabilizer gum. It is heated to ≥ 75°C to solubilize dry ingredients and to form denatured whey protein-casein micelle complexes. The heated premix is homogenized to disperse the milk fat, cooled and fermented to pH 4.5 with thermophilic, lactic acid cultures to provide a smooth, viscous, and partially gelled product with minimal syneresis. The added sugar, lactose, and stabilizer gum provide for proper casein micelle aggregation during acid development by the culture bacteria. Without these limitations and the added restriction due to interaction with denatured whey proteins the casein micelles would continue to aggregate and would eventually lose their abil-
TABLE 4. Composition of selected dairy products.¹

<table>
<thead>
<tr>
<th></th>
<th>Milk fat (%)</th>
<th>Protein (%)</th>
<th>Solids-not fat (%)</th>
<th>Water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>3.5</td>
<td>3.5</td>
<td>9.0</td>
<td>87.5</td>
</tr>
<tr>
<td>Concentrated skim milk</td>
<td>0.3</td>
<td>10.0</td>
<td>27.0</td>
<td>73.0</td>
</tr>
<tr>
<td>Evaporated milk</td>
<td>7.9</td>
<td>7.0</td>
<td>18.3</td>
<td>73.8</td>
</tr>
<tr>
<td>Whipping cream</td>
<td>36.0</td>
<td>2.2</td>
<td>5.9</td>
<td>57.3</td>
</tr>
<tr>
<td>Ice cream</td>
<td>12.5</td>
<td>4.0</td>
<td>25.1</td>
<td>62.1</td>
</tr>
<tr>
<td>Yogurt</td>
<td>4.8</td>
<td>5.0</td>
<td>11.8</td>
<td>83.1</td>
</tr>
<tr>
<td>Cottage cheese</td>
<td>0.4</td>
<td>16.9</td>
<td>20.4</td>
<td>79.0</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>32.0</td>
<td>22.0</td>
<td>27.8</td>
<td>37.0</td>
</tr>
<tr>
<td>Butter</td>
<td>80.5</td>
<td>2.5</td>
<td>3.1</td>
<td>16.5</td>
</tr>
<tr>
<td>Nonfat dry milk</td>
<td>0.8</td>
<td>35.9</td>
<td>96.2</td>
<td>3.0</td>
</tr>
</tbody>
</table>

¹Fundamentals of Dairy Chemistry (2).

ity to retain free water of imbibition as is the case in cottage cheese manufacture. Electron microscopic evidence of the importance of casein micelle-denatured whey protein complex aggregation and added stabilizer gums for manufacturing high quality yogurt is given by Kalab et al. (4, 5). This consideration is also consistent with recent results demonstrating the influence of heat treatment intensity on the viscosity and gelation properties of yogurt (16).

Cottage Cheese

Skim milk to be used for making cottage cheese is usually subjected to minimal heat processing (e.g., HTST pasteurization at 72 to 74°C for 15 s or its equivalent) prior to fermentation with lactic acid bacteria to produce a gel at pH 4.6. Some manufacturers use slightly higher and more drastic temperature conditions to promote whey protein denaturation and interaction with casein micelles to increase cottage cheese curd yields. However, it is necessary not to overdo this reaction, because if too much whey protein is interacted with casein micelles, curd formation will likely be inhibited, and problems related to incomplete moisture removal by syneresis will result. The cottage cheese curd is cut at pH 4.5 and cooked to 55 to 60°C to promote casein micelle coagulation and expulsion of whey by syneresis. This latter process must be completed in order to produce a cottage cheese product with proper moisture content. Electron microscopic evidence confirms that the casein micelles in cottage cheese curd are much more closely associated than in yogurt and it is this closeness of the micelles that is responsible for syneresis for moisture removal (3).

Cheddar Cheese

Raw or pasteurized milk is fermented to pH 6.0 to 6.2 with lactic acid bacteria culture and treated with rennet enzyme coagulant to hydrolyze the 105-106 peptide bond of the κ-casein component of the casein micelles. This enzymatic cleavage releases a 7000-dalton macroglycopeptide fragment from the micelle, thus increasing its hydrophobicity, decreasing its zeta potential, and rendering it susceptible to aggregation and curd formation (14). The resulting curd is cut and cooked to 45 to 55°C to promote casein micelle aggregation and syneresis to expel whey and provide a product with the required moisture content. It is essential that milk for Cheddar cheese manufacture not be subjected to excessive heating, because this would complex denatured whey proteins with the casein micelles, thus inhibiting the availability of κ-casein for hydrolysis by rennet and interfering with curd formation. Excessive heating of milk for Cheddar cheese manufacture would also inhibit the cheddaring process needed to mat casein micelles together to provide proper body and texture in the cheese product.

Sterile Milk Concentrates

Retort and UHT sterilized milk concentrates are subjected to sufficient heat treatment during forewarming and sterilization to drastically alter their proteins and to result in several water-related defects (3, 14). Conventionally sterilized evaporated milk has heat stability problems that arise from coagulation of denatured whey protein-casein micelle complexes. This latter problem is controlled by proper forewarming of the milk to alter the proteins prior to concentration under vacuum and also by adding phosphate or citrate salts to stabilize the complexed micelles. The object of these processing treatments is to provide the proper degree of coagulation of the whey protein-casein micelle complexes to develop sufficient viscosity to prevent fat separation and settling of mineral deposits from the product during extended storage.

Control of storage-induced whey protein-casein micelle complex aggregation in UHT sterile milk concentrate is accomplished by adding hexamethaphosphate prior to sterilization. Although the mechanism for this improvement of storage stability, which is necessary to prevent gelation, is not known, the polyphosphate ions probably bind calcium ions and complex with micelles to increase their zeta potential sufficiently to retard their aggregation and imbibition of free water.

Ice Cream and Frozen Desserts

Ice cream mix is formulated from milk, concentrated milk, cream, sugar, corn sweetener, stabilizer gums, and emulsifiers. The mix is pasteurized at sufficiently high temperatures of > 75°C for 30 min to disperse and solubilize dry components. This heat treatment also denatures and promotes interaction of denatured whey proteins and casein micelles. The mix is aged sufficiently to allow the proteins, stabilizer gums, and sugars to become fully hydrated before freezing. This latter process is necessary to develop adequate viscosity in the mix to facilitate air incorporation during freezing and to control ice and lactose crystallization in the product during hardening and storage.

Dried Milk and Whey Products

Dried nonfat and whole milk powders, whey powder, and milk protein concentrates are manufactured to meet compositional and functional standards. The severity of the heat treatments used for pasteurization, forewarming, concentration, and drying must be carefully controlled to produce products that meet standards for dispersibility, solubility, and functionality. These latter factors are largely controlled by regulating the severity of the heat treatments used for manufacturing the dried products. High heat milk powder is manufactured from milk that has been given a severe forewarming treatment prior to vacuum concentration and spray drying. This product contains its milk proteins in the form of denatured whey protein-casein micelle complexes, which exhibit limited ability to rehydrate and resolubilize but which provide optimal functionality for bakery and other related uses. Low heat milk powder is produced from minimally heated milk, which has its proteins in undenatured whey protein and casein micelles in the uncomplexed form. This product exhibits excellent dispersibility, solubility, flavor, color, and functionality due to its ability to rehydrate and solubilize its proteins.

It is necessary to protect dried milk products from atmospheric moisture. Resorption of moisture by these products during storage can result in increased nonenzymatic browning and protein conformation changes that cause loss of solubility and functionality (1). Dried milk products with \( A_w \) of about .1 are readily susceptible to lipid oxidation and associated flavor development.

**CONCLUSIONS**

Milk proteins interact with water by several different mechanisms and at several different \( A_w \) levels (1, 6, 7). Their hydrophilic amino acid residues and peptide bonds hydrogen bond .05 g H\(_2\)O/g protein at \( A_w \).1 to .2 and .4 g H\(_2\)O/g protein at \( A_w \).92. Milk proteins in solution associate with additional water as structured, clathrate, multilayer, capillary, and loosely associated water (1, 6, 7). This associated water affects the physicochemical properties of the milk proteins and alters their ability to interact and complex to form additional quaternary structure in the processing of certain milk products. As a result, the complexed milk protein structures are able to immobilize large amounts of free water in certain
dairy products (e.g., yogurt, acid-type and rennet-type cheese curd, and in UHT sterile milk concentrates). This phenomenon is also important in providing viscosity and crystallization control in ice cream and frozen desserts and is important for providing physical stability to evaporated milk products. The processor of dairy foods must understand and properly manipulate these protein-water interactions in order to manufacture successfully most of the dairy products commercially available today.

REFERENCES