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WATER-HOLDING PROPERTIES OF MILK PROTEIN PRODUCTS - A REVIEW

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Abstract

Water-holding properties have been well recognized by food technologists among the diversity of functional properties attributed to milk protein products. In general, water-holding is accomplished by a complexity of interactions between water and milk proteins. Besides the term water-holding, synonyms such as water retention, imbibing and hydration have been used to describe this phenomenon. This paper provides a clearer understanding of this parameter by considering some fundamentals of both the molecular structure of milk proteins and the physical interrelationships between water and milk protein powder particles. Differences in water-holding properties of milk protein products are frequently observed and may be due to the nature of the protein and to technological influences. Methods measuring water absorption and methods that measure water retention are applied for the examination of water-holding. By following this distinction, the principles of various methods (e.g., the Baumann method, the absorption capacity test, Farinographic procedures, net tests, filtration tests, modern instrumental techniques) are reviewed.

Key Words: Functional properties, milk proteins, water holding.

Introduction

Milk proteins exhibit a multitude of properties such as digestibility, high nutritive value, 'GRAS' ('generally regarded as safe') conformity and compatibility with other ingredients in food formulations (Kinsella, 1988; Morr, 1989, 1991). In addition, the application of certain powdered dairy products (e.g., some types of casein, caseinates, coprecipitates, and UF proteins and protein hydrolyzates) can improve significantly the texture of many food products. There are typical features attributed to milk proteins which can be characterized as 'functional properties' (Table 1). Among these, water holding of milk protein products has been recognized to be of particular importance in food technology and research. Milk proteins can replace many functional ingredients in a broad variety of foods ranging from dairy products, breads, biscuits, confectionery, meat products, pastries, soups, ice cream, infant foods, margarine, low fat spreads, etc. (for comprehensive surveys see Kirkpatrick and Fenwick, 1987; Modler, 1985; Morr, 1985; De Wit, 1984, 1985; Nienhaus and Reimerdes, 1987). Furthermore, wide-spread use of milk protein products in the non-food area has been promoted (e.g., Harwalkar and Brown, 1989; Southward, 1991; Veerman and Hutten, 1991).

The term 'water holding' covers a variety of properties (Fig. 1) which have been used as synonyms in the literature. Different theories of the principles of water-protein interactions have been reported in the past. According to Kinsella et al. (1989), six basic forms of water associated with proteins can be distinguished: (1) Structural water, which is unavailable for chemical reactions; (2) Hydrophobic hydration water, which surrounds apolar residues in a cage-like structure; (3) Monolayer water, which represents the first absorbed water bonded to protein groups and may be available for certain reactions; (4) Unfreezable water, which includes all water that does not freeze at the sharp transition temperature, depending on the content of polar side chains and on the amino acid composition; (5) Capillary water, which is mechanically held by surface forces in the protein molecule; (6) Hydrodynamic hydration water, which 'loosely'
Figure 1. Parameters used for describing the 'water-holding' properties.

![Diagram showing parameters](image)

Figure 2. Kinds of particle structures of protein powders.

![Kinds of particle structures](image)

Table 1. Functional properties as frequently attributed to milk proteins

<table>
<thead>
<tr>
<th>Property</th>
<th>Types of Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wettability</td>
<td></td>
</tr>
<tr>
<td>Solubility</td>
<td></td>
</tr>
<tr>
<td>Water-holding</td>
<td></td>
</tr>
<tr>
<td>Surface activity</td>
<td></td>
</tr>
<tr>
<td>Emulsifying capacity</td>
<td></td>
</tr>
<tr>
<td>Emulsifying stability</td>
<td></td>
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<tr>
<td>Viscosity</td>
<td></td>
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<tr>
<td>Colloidal stability</td>
<td></td>
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<tr>
<td>Foaming</td>
<td></td>
</tr>
<tr>
<td>Organoleptic properties</td>
<td></td>
</tr>
</tbody>
</table>

In order to simplify the rather complex mechanisms of protein and water interactions in food systems, two categories have been proposed (Kneifel et al., 1991): (1) the part of water that is bound to the molecule and is no longer available as a solvent ('absorbed water'), and (2) the part of water that is entrapped in the protein matrix ('retained water').

Several factors may affect the interactions between a powdered product and water irrespective if these interactions take place with pure water or water present in a food matrix. The wetting process is influenced mainly by the moisture and the structure of the powder. In this context, the contact angle between the powder particles and water plays an important role. The rate and extent of rehydration depend on mechanical influences (e.g., mixing and stirring). The chemical nature of milk proteins determines the nature of the system (solution, suspension, or dispersion). Subsequently, physical and chemical reactions take place, and a three-dimensional network is formed that takes up and holds the water. This mixture also shows so-called swelling properties and is capable of holding/binding/entrapping water.

Milk protein products act differently in different food product applications. The differences are caused by variations in pH, salt concentration and surface tension as well as by the processing conditions governed by temperature and mechanical effects. For instance, most milk powders show low dispersibility below 40°C, whereas an optimum can be observed at 60°C (Lascelles and Baldwin, 1976). The dispersibility of a milk powder is influenced by its particle dimensions. According to Singh and Newstead (1992), the optimum particle diameter for well-soluble milk powders normally ranges from 150 to 200 μm and is determined by processing conditions. Increasing the particle size gradually improves both the dispersibility and the so-called 'instant' behavior of milk powders. Moreover, the properties of a co-matrix may play a dominant role in the dispersion process. A co-matrix may enable gel formation via trapping of water (Kneifel et al., 1991).

As described above, structure as well as porosity of the powder largely influence the initial phase of wetting. In general, six main forms of structures are common with powder products (Fig. 2). Dried dairy products can be assigned either to the 'globular', 'porous', 'fissured', 'agglomerate' or 'crude' type. Most spray-dried milk protein powders exhibit a typical porous to globular porosity, while roller-dried powders exhibit irregular structures. Micrographs of sodium caseinate powder particles from two different producers are presented in Figures 3a and b. Although both are spray-dried products of comparable particle dimensions, significant differences in their surface structure can be observed. Compared to the powder shown in Fig. 3a, that in Fig.
Water-holding of milk protein products

Figures 3a,b. Scanning electron micrographs of two different sodium caseinates, visualizing the differences in powder structure.

Table 2. Average water-binding capacity of some milk proteins (Kinsella et al., 1989).

<table>
<thead>
<tr>
<th>Component</th>
<th>Bound water (g/100 g product)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caseinate (dry)</td>
<td>5.6</td>
</tr>
<tr>
<td>Caseinate ($a_w = 0.9$)</td>
<td>40</td>
</tr>
<tr>
<td>$\beta$-Lactoglobulin (dry)</td>
<td>6.7</td>
</tr>
<tr>
<td>$\beta$-Lactoglobulin ($a_w = 0.9$)</td>
<td>32</td>
</tr>
<tr>
<td>Native casein micelle</td>
<td>200 - 400</td>
</tr>
</tbody>
</table>

3b exhibits improved water-holding and dispersibility properties due to its pronounced porosity.

Different mechanisms govern the water sorption characteristics of a protein powder particle. A porous powder granule has different micro- and macrocapillary structures which determine its behavior in a fluid medium. Thus, several theories of sorption behavior are based on whether the structure of the sorbent is porous or non-porous (Aguilera and Stanley, 1990). The area and charge of the surface as well as the shape of the capillaries influence directly water absorption and desorption (Jensen and Nielsen, 1982; Sanderson, 1978). The effect of these properties can be described graphically as a hysteresis curve (Aguilera and Stanley, 1990).

Water-binding Characteristics of Milk Proteins

In contrast to globular proteins, intact casein micelles are capable of binding relative large amounts of water. Water entrapment in the native micellar structure is partly accomplished by the colloidal calcium phosphate, and also by the hydrophilic nature of $\kappa$-casein and its position in the submicelles. As demonstrated by Hardy and Steinberg (1984) with casein, the balance within the 'triangle' water-protein-solutes is of particular importance for its physical stability. Globular proteins such as $\beta$-lactoglobulin display varying degrees of hydration, depending on denaturation, aggregation, and interaction with other proteins (De Wit, 1984, Kinsella, 1984). As a consequence of heat treatment, the protein is unfolded and may exhibit an increased water-binding capacity. While there is still some disagreement, most researchers have reported a slight increase in bound water as the protein denatures. The amount of water bound in denatured milk protein depends on the nature of the protein and the dry matter content (Bech, 1980). Furthermore, the firmness of the heat-induced network largely determines water-holding, because water is entrapped more effectively in a firm structure than in softer gels (Plock and Kessler, 1992). Average water-binding capacities of selected dairy proteins are presented in Table 2.

It has been demonstrated that progressive preheating of milk for the manufacture of sodium caseinate improves the water-holding capacity of the products (Kniefel et al., 1990). When the milk was heated at a rate of 120°C/min, the amount of water held by the caseinate was more than 5-fold the amount held by the non-heated control caseinate (Kniefel et al., 1990). Increased water-holding properties were also observed with polymerized (Korolczuk, 1984) or chemically modified (Canton and Mulvihill, 1983; Kroll et al., 1984) caseins, high-calcium coprecipitates (Thomas et al., 1974; Vattula et al., 1979), and neutralized coprecipitates (Southward, 1985). On an average, skimmed milk powders did not show different water-holding capacities as a function of heat treatment (Knightbridge and Goldman, 1975). These authors tested doughs enriched with several milk protein products. Based on the findings, they made a distinction between 'highly absorptive' (sodium caseinate, soluble coprecipitates), 'medium absorptive' (calcium caseinate, dispersible coprecipitates, insoluble coprecipitates), and 'low absorptive' (skim milk powder,
Figure 4. Influence of enzymatic hydrolysis on the change of water-holding capacity (WHC) of caseins (Abert and Kneifel, unpublished). Casein suspensions were hydrolyzed with Bromelain (E.C. 3.4.22.4) (Biocon, Rosenheim, Germany) and Corolase PN (E.C. 3.4.24.4) (Roehm, Darmstadt, Germany) at pH 6.7 and at 55°C, followed by heat inactivation. Hydrolyzed products were dried using a Buechi 190 Mini laboratory spray drier (Buechi, Flawil, Switzerland), before examination of their WHC.

Table 3. Methods for the assessment of water-holding properties (Kneifel et al., 1991).

<table>
<thead>
<tr>
<th>Measurement of water absorption</th>
<th>Measurement of water retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baumann apparatus</td>
<td>Net test and modifications</td>
</tr>
<tr>
<td>Absorption capacity test</td>
<td>Centrifugation tests</td>
</tr>
<tr>
<td>Spectrophotom. rehydration test</td>
<td>Capillary suction method</td>
</tr>
<tr>
<td>Viscosity strength measurement</td>
<td>Pressure methods</td>
</tr>
<tr>
<td>Farinographic methods</td>
<td>Filtration tests</td>
</tr>
<tr>
<td>Cryoscopic osmometry</td>
<td>Special instrumental techni ques (DSC, NMR, etc.)</td>
</tr>
<tr>
<td>Sorption isotherms</td>
<td></td>
</tr>
</tbody>
</table>

casein, lactalbumin) products. According to Delaney (1976), the water-holding capacity of whey protein concentrate lies within the same order of magnitude as of skim milk powders.

In a recent study (Abert and Kneifel, unpublished) it was shown that some casein hydrolyzates obtained by enzymatic modification exhibited improved water-holding capacity in comparison to non-treated products (Fig. 4). The results depended on the nature of the enzyme used, on whether the milk was preheated or not before casein production, and on the pH conditions during hydrolysis. Negative statistical relationships (P < 0.001) between water-holding capacity and nitrogen solubility as well as emulsion activity index were evident. Other researchers (Mietsch et al., 1989) used Alcalase® or Neutrase® for enzyme treatment of milk proteins and obtained a relatively low water-holding capacity in the treated products.

Methods for Water-holding Capacity Measurement

According to Chou and Morr (1979), the term 'water-holding capacity' can be defined as a quantitative indication of the amount of water retained within a protein matrix under certain defined conditions. It usually also includes entrapped water. In this context, 'hydration' must also be considered. It is expressed in grams of water associated with or occluded by 1 gram (dry weight) of protein (Mulvihill and Fox, 1989) and is closely related with the so-called voluminosity (Swaisgood, 1982; Walstra, 1979) according to the following equation:

$$ V = v_2 + d_1 v_1^0 $$ (1)

where

- $V$ = Voluminosity (cm³/g protein)
- $v_1^0$ = Specific volume of pure water
- $v_2$ = Specific volume of the dry protein
- $d_1$ = Hydration (g water/g protein)

Different methods that are usually applied for estimating the water-holding properties of food proteins have been described by Kneifel et al. (1991). In general, the testing procedures are either tests under model conditions or tests in actual food systems (Harper, 1984). Many of the methods used in laboratories are arbitrary and empirical and are applied as so-called internal methods. Because of the lacking comparability of these tests due to different measuring principles, standardized procedures that enable a specific and reliable characterization of the milk protein products should be developed. Another problem arises from the fact that many testing procedures were originally developed for substances other than milk proteins and had, therefore, to be modified and adapted.

Methods for the assessment of the water-holding capacity are based mainly on the application of either an external force such as pressure, centrifugation, and capillary suction of a porous material being in contact with the sample, or on the evaluation of swelling when measuring the fluid uptake or the amount of water released during filtration. In these methods, the amount of water released or held by the sample is examined. Despite the difficulty of evaluating the actual mechanism associated with a given method, an attempt has been made to distinguish between the procedures that measure water absorption and those that register water retention (Table 3). In the following section, the different testing principles will be described according to this distinction.
Water-holding of milk protein products

Figure 5. Principles of some methods for the examination of water-holding properties of milk protein products:
a: the Baumann apparatus; b: the Absorption Capacity Test; c: the Spectrophotometric Rehydration Test; d: the Modified Net Test; e: the Capillary Suction Test; and f: the Filtration Test.

Water Absorption Measurement

Baumann apparatus

This is a classical device for testing the water uptake of a powdered product (Baumann, 1967). It consists of a thermostatted funnel connected to a horizontal graduated capillary attached to the top of the funnel (Fig. 5a).

The powder is dusted onto a wetted filter paper that is placed on a fritted glass filter set on the top of the funnel. The water uptake by the sample at equilibrium is read off from the graduated capillary in milliliters and is expressed on a dry basis. Although a glass lid is used to minimize losses due to evaporation, a blank value should be determined and considered in the calculation.
One drawback of this method arises from the fact that only small amounts (usually 20-500 mg) of powder are tested. Due to the principle of this procedure, the results reflect the wettability properties rather than the water-holding characteristics. Thus, the procedure is useful primarily for studying the very first steps of water uptake of a prevalingly non-soluble powder when brought into contact with water. Hermansson (1972) monitored the water-uptake of different food proteins by means of the Bauman apparatus and found that soybean protein was distinctly superior to sodium caseinate and to a whey protein concentrate in terms of water-holding. Different times ranging from about 20 minutes (whey protein concentrate) to about 150 minutes (soybean protein) were needed by the samples to reach an equilibrium. The Baumann method is of advantage for predicting the water-binding capacity of hydrocolloids (Wallingford and Labuza, 1983).

Absorption capacity test

This method was developed by Seiler (unpublished) and is derived from the Baumann apparatus testing principle. It was applied for the first time by Kneifel et al. (1992). A plastic tube to which a paper membrane is attached at the lower end is filled with the powder sample and put on a frit (porosity 0) for a chosen time (Fig. 5b). The frit itself is in contact with water, at ambient temperature, which passes through the frit and the membrane into the powder cylinder. Weighing is carried out before and after water absorption and also after the wet and dry phases have been separated. These procedures allow determination of the following parameters: (1) the vertical propagation height of water into the powder (mm); (2) the amount of wetted powder (g) per area (cm²); (3) the amount of water (g) absorbed by 1 cm³ of dry powder; (4) the amount of water (g) absorbed by 1 g of dry powder.

With this method, the coefficient of variation of parameter (4) lies within the range of 2.4 and 12.6% (Kneifel et al., 1992). In analogy to the Baumann apparatus method, the absorption capacity test characterizes mainly the initial phase of water uptake, depending on factors such as wettability, capillarity, particle size, and dissolution effects. The rehydration initiation phenomenon of a powdered sample can, therefore, be studied by using this procedure. However, this method may be affected by the solubility of the test material in water, because highly soluble powders may diffuse back into the water via the membrane and the frit.

Spectrophotometric rehydration test

This method is based on continuous spectrophotometric measurements of the change in transmission density of the dispersed powder as a function of time (De Wit and Klarenbeek, 1986). The device consists of a cylindrical tube with a fritted glass bottom onto which a known amount of the sample is placed (Fig. 5c). The glass tube is connected to a spectrophotometer (set at 600 nm) equipped with a flow-through cell and an X-Y recorder. A defined volume of water is circulated by means of a peristaltic pump. An optical index that defines a kinetic relationship between the reconstitution properties and the absorption characteristics of the powder is then calculated.

More recently, a modification of this method has been introduced. Samples of protein-water mixtures are removed from the tube at two different times and are measured in a separate spectrophotometer (De Wit, 1989). The rehydration behavior is estimated based on the differences between the two readings. As reported by those authors using this rehydration method, the procedure has shown an acceptable reproducibility when a defined set-up of the testing assembly is used.

Viscosity strength measurement

Intrinsic viscosity of a protein-water mixture increases by the same factor by which the volume fraction is increased during hydration. Based on this criterion, rotation viscometers have been used for calculating the hydration capacities of casein micelles (Dewan et al., 1973), whey protein concentrates (McDonough et al., 1974), casein solutions (Korolczuk, 1982a), acidic milk protein concentrates and caseins (Korolczuk, 1982b), as well as polymerized casein derivatives (Korolczuk, 1984). Cross-linked caseins of high viscosity exhibited higher hydration levels than untreated proteins. Korolczuk (1982a) used a defined formula for calculating the water-holding capacity from the data obtained by viscosity measurements. The equation has experimentally been proven valid for 68 casein samples. In contrast to models described earlier, this equation was found to be valid for a relatively broad range of protein concentrations (Korolczuk, 1982a).

Farinographic methods

The Brabender farinograph technique has been mainly used for measuring the water absorption by flours, doughs and soybean products. Only a few modified methods have been reported regarding its use in measuring the water absorption characteristics of flour and protein blends. Knightbridge and Goldman (1975) studied factors affecting the water-absorption capacity of dried milk products in doughs. Based on this technique, milk protein products have been classified by their suitability as dough ingredients. Heat-precipitated whey proteins, e.g., take up between 70 and 147 g of water per 100 g of powder (Short, 1980). A relationship between the water-holding capacity and the protein content of the whey protein powders was observed. Other reports on farinographic testing (Delaney, 1976; Guy et al., 1974)
describe the water-holding behavior of different whey protein concentrates used in doughs. In analogy to the procedures used for routine examination of dough, the constant-flour-weight (300 g) or constant-dough-weight (480 g) methods have been applied by most users of this method.

Cryoscopic osmometry

This technique has been chosen to test thickening agents such as hydrocolloids and only in one case to test dairy products (Tarodo de la Fuente and Alais, 1975). These authors used this method to monitor the solvation behavior of casein in milk. In principle, cryoscopic osmometry measures a colligative property, related to the ability of a substance to depress the freezing point. The freezing point is then converted into an effective osmotic concentration expressed as milliosmoles per kilogram of water (Rey and Labuza, 1981).

Monitoring of sorption isotherms

Basically, water absorption characteristics of powders in an atmosphere of defined relative humidity can be described by means of typical sorption isotherms (Aguilera and Stanley, 1990; Chou and Morr, 1979; Mulvihill, 1992; Ozimek et al., 1992). The weight uptake after exposing the powder sample to an atmosphere of given water activities (e.g., over saturated salt solutions) can serve as a measure for water absorption. Corresponding values are expressed as g of water/100 g of protein product (Grufferty and Mulvihill, 1990). In many proteins, a moisture equilibrium will be obtained within 24 hours (Hagenmaier, 1972). However, 4 days were needed to equilibrate a para-casein preparation (Geurts et al., 1974). Whey powders usually exhibit differences in adsorption of moisture due to lactose recrystallization. Lactose-free skim milk powders often give isotherms of sigmoid shape (Kinsella, 1984).

Methods for Water Retention Measurement

Net test and modifications

The net test combines filtration and centrifugation procedures and is carried out using a special plexiglass equipment (Hermansson and Lucisano, 1982; Wierbicki et al., 1957). The assembly introduced by the former authors consists of a tube in which the gel is formed, a filter paper to be placed on a net (200-μm mesh) fixed between the upper and the bottom tube (inner diameter of 11 mm). Following gel formation in the upper tube (closed with a rubber stopper at the lower end), the gel is cooled and the stopper is removed. Then the upper tube is connected with the lower part and the whole assembly is centrifuged at 790 g. Moisture loss of the gel is assessed by weighing the gel before and after centrifugation. The result must be corrected for the water uptake by the filter paper. One advantage of this method is that the low speed centrifugation used usually limits structural breakdown of the gel. Thus, only weakly retained or non-retained water will be separated.

A modification of this testing principle was described by Kneiefel et al. (1992) and was applied for the examination of several milk protein powders. In this study, the Amicon Micropartition System MPS-1 (Amicon Corp., Danvers, MA), available commercially, was used to centrifuge the mixture. Previously, this device was also applied for the assessment of the amount of water-soluble nitrogen substances in caseinates (Kneiefel and Beurel, 1990). The system consists of two plastic tube units with a filter in between and connected by clips. The filter has to be punched out from a Schleicher & Schuell filter paper sheet no. 287 (this type was found to be optimal based on the results of preliminary trials). A 12% powder suspension in distilled water is stirred with 500 rpm for 1 hour at 80°C (Fig. 5d). The mixture is then made up to the initial weight with water to compensate for the amount of evaporated water. The sample reservoir is filled up to the mark with the mixture. Four devices are placed into a conventional Gerber centrifuge and centrifuged at ambient temperature for 10 minutes at 1,100 rpm. The mean weight of the four filtrates is used as a measure for the water-holding capacity. In a comparison with two other methods for the characterization of water-holding properties of a variety of milk protein powders, relatively low variation coefficients ranging from 0.5 to 5.7% were calculated (Kneiefel et al., 1992). In general, net tests can be assigned more or less to the category of so-called applied tests, since they enable a simulation of actual food systems.

Other centrifugation tests

Many centrifugation methods have been described; the tests are based on high-speed or low-speed centrifugation of protein-water mixtures that are prepared under defined conditions (e.g., Hermansson and Lucisano, 1982; Luther et al., 1983; Sollars, 1973; Sternberg et al., 1976; Thompson et al., 1969). In principle, the protein-water mixture is centrifuged in a tube and either the amount of the liquid released or the protein with the remaining water is weighed and the supernatant is discarded. One important drawback of high-speed techniques is the possible damage of the network structure. Thus, methods which apply relatively lower centrifugation g-values are preferably used because they prevent structural changes.

Capillary suction methods

In addition to the so-called capillary volumeter (Hofmann, 1975), which has been developed primarily...
for the examination of meat, a special device measuring the capillary suction potential has been described by Labuza and Lewicki (1978) and used for testing gelatin, starch and carrageenan gels. A diagram of this assembly is shown in Figure S5e. The gel to be measured is placed in a polypropylene cup, layered with filter paper with a predetermined moisture content, sealed with a rubber stopper pierced by a glass capillary with a bore of 0.3 mm. This capillary avoids pressure formation during the time when the cup is closed. After storing the cup with the sample at 6°C for 72 hours, the equilibrium water content of the filter paper is determined. A large contact surface and a thin gel layer should be ensured to achieve fast movement of water from the gel into the filter paper. The measurement is affected by the initial gel concentration, the moisture of the filter paper, and the temperature of the equilibration experiment. A variation coefficient as low as 2.5% is reported for this method (Labuza and Lewicki, 1978).

Pressure methods

In analogy to the capillary suction test, pressure procedures are used mainly for the assessment of meat products (e.g., Lee and Patel, 1984). A specimen is placed in a Universal Testing Machine for compression along the vertical axis and the released fluid is collected on preweighed dry filter paper sheets. The amount of the expressed fluid is calculated from the weight gain.

Another pressure method was described for testing milk proteins (Kabus, 1972). The sample is weighed on a filter paper and pressed between two solid plates under defined conditions. The whole assembly is covered with aluminum foil to prevent water evaporation during the procedure.

Filtration tests

As schematically shown in Figure S5f, a dispersion or a solution of a powdered sample in water is prepared according to a standardized stirring and mixing procedure (Kneifel et al., 1990; Rustad and Nesse, 1983). After a given equilibration time, the volume of water released from an aliquot of the mixture is measured. This method has been used mostly to screen caseinates to be incorporated in processed cheese as additives. When using this method, the water-uptake of the filter as well as the ability of the protein gel to clog the pores of the filter paper have to be considered. The coefficient of variation of this method is relatively high (Kneifel et al., 1992).

Another filtration test was described by De Wit (1988) who estimated water binding in yogurt samples containing whey proteins as gelling additives. In that study, the amount of fluid drained from 150 ml yogurt during 1 hour was measured with graduated tubes, after storing the samples for 25 hours at 4°C.

Special instrumental techniques

During the last decade, differential thermal analysis systems have become a powerful tool for an in-depth study of physical changes in food systems. The general purpose of these measurements is to monitor the difference between enthalpy changes that occur in a sample and in some inert reference material during heating. Generally, the methods used to accomplish this objective may be divided into three types: (1) Differential thermal analysis (DTA), (2) the Boersma DTA, and (3) Differential scanning calorimetry (DSC). Details of these techniques have been extensively reviewed by Lund (1983).

DSC has been used mostly to study protein denaturation (e.g., De Wit, 1988) and starch gelatinization (e.g., Wootton et al., 1974). However, it has also been demonstrated by some authors (Rüegg et al., 1974; Rüegg and Blanc, 1976) that this technique is suitable for observing the hydration phenomenon in milk proteins. Berlin et al. (1973) used DSC for the estimation of the amount of freezable water in whey protein concentrates.

Nuclear magnetic resonance (NMR) has also been shown to be a valuable technique for investigating kinetic properties of water as well as protein-water interactions of milk proteins (Di Nola and Brosio, 1983; Farrell et al., 1989; Kuntz, 1971). Lelièvre and Creamer (1978) used NMR in a study of protein-water interactions during the setting of curd, and Callaghan et al. (1983) used it to characterize the state of water in cheese. Methods such as infrared and Raman spectroscopy are mainly of academic interest but may also contribute to the understanding of the behavior of nonfreezable water (Schnepf, 1989).

X-ray and neutron scattering techniques have made it possible to identify water molecules within protein structures. One limitation of this procedure is that the protein must be in a crystalline state and only the position of the oxygen atom can be determined (Schnepf, 1989).

Conclusions

Water-holding properties of a protein are the result of a broad array of factors governing protein-water interactions in food systems. Although there are several theories describing water-holding characteristics of proteins, it has not been possible to define exactly the term 'water-holding'. Thus, it is useful to use terms such as 'bound', 'free', or 'structural' water specifically in context with the measuring technique and the environmental conditions employed. There are many methods available that provide information on the mechanisms of hydration and
Water-holding of milk protein products

water binding. However, many of them measure the complexity of chemical, physical, and mechanical parameters of the powder solution, dispersion, or suspension. This implies that each method should be viewed as one piece of a complex puzzle and a sufficient characterization of the water-holding property can only be achieved when based on a set of different tests. The modified net test seems to offer some advantages over other measuring principles such as filtration and absorption capacity tests in terms of precision.

Many of the important functional properties of proteins in foods are related to the interaction of water with food proteins. Milk proteins offer a considerable potential in mediating and promoting functional effects. It has, therefore, become an important challenge for the food scientists to develop, improve, and sufficiently characterize the functionality of these substances.

Acknowledgments

Thanks are due to Mr. V. Fryder from Nestlé Research for taking micrographs of the caseinate samples.

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Water-holding of milk protein products


Discussion with Reviewers

J. N. De Wit: What is meant by 'water not available as a solvent'? Is that water absorbed because of steric exclusion of other solutes, or is this highly structured water around polar or apolar groups? Which techniques are used to characterize that type of water?

Authors: As mentioned in the text, the division into two categories of 'bound water' was primarily chosen in order to simplify the complexity of protein-water interactions. Depending on the protein product to be exam-
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ined, 'water not available as a solvent' may cover both the steric exclusion and the highly structured water. To elucidate the nature of that types of water in a gel, it would therefore be necessary first to consider the nature of the protein (e.g., whether the protein may bind water ionically, through hydrogen bond reinforcement or rather excludes it because of containing hydrophobic side chains) (see Labuza and Busk, 1979) and its physical properties (e.g., solubility, dispersibility, etc.), and then to choose the methodology (several methods will be necessary in most cases) to be applied.

P. S. Kindstedt: The 'water-holding' of natural cheese can vary considerably depending on the type and age of cheese. I believe that the states of water in cheese plays an important role in determining textural and rheological characteristics, therefore a better understanding of how water exists in cheese is needed. Which analytical strategies would you suggest to use in order to characterize the water phase and its state of 'boundness' in cheese?

Authors: Water-holding properties of cheese are strongly affected by pH and salt content as well as by technological conditions (e.g., pressure, temperature). Geurts et al. (1974) (text ref.) reported that several methods (water non-solvent for various solutes, sorption isotherms, isopestic methods) would be necessary for a sufficient characterization of water binding properties in cheese. Additionally, NMR can serve as a useful tool. Based on NMR, it has been concluded (Callaghan et al., 1983, text ref.) that there is strong evidence that water diffusion in cheese is confined to surfaces within the protein matrix. In general, if cheese is considered, a suggestive methodological strategy should not only focus the final product, but also the curd and the 'young' cheese. In this context, the survey on physical properties of curd syneresis given by Walstra et al. (1987) is recommended for reading.

P. S. Kindstedt: Dried grated Italian cheeses such as Parmesan and Romano have been popular in the U.S. for many years. Recently, a growing market has developed for dried grated cheeses that traditionally have not been available in grated form, such as Cheddar. The hygroscopic properties of such cheeses can be quite different than those of Parmesan and Romano, leading to problems with functionality. Which analytical strategies would you suggest to use to evaluate the water-binding characteristics of dried grated cheese?

Authors: In order to evaluate such products, a rather simple method used for assessing the hygroscopicity properties of whey powders (comment: with this category of products, hygroscopicity problems are also frequently observed) might be adopted. This method has been described in a booklet by NIRO Atomizer (1978) and is based on equilibrating a certain amount of product on a frit, under an atmosphere of defined humidity accomplished by a saturated NH₄Cl solution. A special glassware equipment is necessary.

Additional References

