Properties of Proteins Contributing to Functionality of Cereal Foods

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The functional properties of proteins in foods, including those in cereal products, are determined by the molecular composition and structure of the individual proteins and their interactions with one another and with other substances. Improving or modifying food characteristics such as viscosity, texture, water absorption, or fat emulsification may involve altering the constituent proteins or adding other proteins. Excellent reviews and symposia have related many aspects of the chemistry of proteins to their contributions to the stability and organoleptic properties of foods (1-3). However, use of new techniques such as amino acid sequence analysis, X-ray crystallography, and NMR in the study of protein structure and physical properties is advancing our concepts of the roles of proteins in food processing, structure, and acceptability.

Table I summarizes some important functional contributions of proteins to foods. The role of proteins, such as those in dough, may be critical, even though protein may be only a small fraction of the food product. Most of the examples in this review are based on studies of proteins in cereal-derived foods, but the basic concepts generally apply to other food systems.

Most plant tissues used for food are storage organs, such as seeds and tubers, where proteins and carbohydrates serve as reserves. In developing endosperm of cereal grains such as wheat, most of the proteins are not dissolved in the cytoplasm but are deposited initially in storage organelles, membranes, and other subcellular structures (4). To facilitate their compact deposition in storage bodies or their insertion into membranes, seed proteins often have unique compositions. Usually such proteins are modified enzymatically after their synthesis and transport, thus accounting for some of their unusual solubility and structural characteristics (5).

One protein may serve both as hydrating agent and fat emulsifier. This versatility reflects the fact that the 21 amino acids have different side chains tied together in varied sequences and amounts. One protein may contain groups that form associations with polar substances and groups that favor a nonpolar environment.

Table I. Functional Properties of Proteins in Foods and Their Applications

<table>
<thead>
<tr>
<th>Property</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsification</td>
<td>Meats, coffee whiteners, salad dressings</td>
</tr>
<tr>
<td>Hydration</td>
<td>Doughs, meats</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Beverages, doughs</td>
</tr>
<tr>
<td>Gelation</td>
<td>Sausages, gel desserts</td>
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<tr>
<td>Foaming</td>
<td>Toppings, meringues, angel food cakes</td>
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<tr>
<td>Cohesion binding</td>
<td>Textured products, doughs</td>
</tr>
<tr>
<td>Textural properties</td>
<td>Textured foods</td>
</tr>
<tr>
<td>Solubility</td>
<td>Beverages</td>
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</tbody>
</table>

Fig. 1. Types of bonds between protein chains.

1 Presented at the AACC 63rd Annual Meeting held in conjunction with the Sixth International Cereal and Bread Congress, Winnipeg, Manitoba, Canada, September 1978.

2 Mention of firm names or trade products does not imply their endorsement or recommendation by the USDA over other firms or similar products not mentioned.
Figure 1 illustrates the types of bonds that develop between sidechain functional groups of amino acids constituting proteins (6). The strongest noncovalent electrostatic bond is between opposite charges of ionized amino acids. Hydrogen bonds between amide or hydroxyl groups also occur, and those between backbone chain peptide amide groups are responsible for α-helical and β conformations. Multiple hydrophobic bonds, although individually weak, can cause associations of considerable tenacity (7). Similar bonds can occur between proteins and substances such as lipids, polysaccharides, and low molecular weight organic molecules. Covalent links due to cystine residues are important intramolecularly in stabilizing chain folding or intermolecularly in forming bonds between chains (8).

**PROTEIN STRUCTURE**

The complete amino acid sequence of some important food proteins has been determined and others are being investigated. Covalent structures explain some aspects of protein functionality. For example, the amino acid sequence in Fig. 2 shows that the first 25 N-terminal amino acids of Ponca wheat α or γ gliadins are mainly hydrophyllic (9,10). Eight glutamine residues, including a sequence of three, can engage in hydrogen bonding. The six proline residues twist the chain at fairly regular intervals. In another portion of this polypeptide chain, near its center, the amino acids are primarily nonpolar; three of the six half-cystine residues are also located in that region (11). The sequences of the various residues probably regulate the folding of the chain through their interactions with one another and with the aqueous environment.

The role of noncovalent bonds in determining protein secondary and tertiary structure has been confirmed by x-ray analysis of protein crystals. Figure 3 shows a model of the chains of the protein α-chymotrypsin in which backbone regions with α-helical, folded β-structure, and random arrangements are further convoluted into globular structures (12). At 2 Å resolution the sites of the individual amino acids can be located, and the proximities of functional groups that contribute to protein tertiary structure can be established. Intramolecular disulfide bonds due to cystine residues connect different regions of the chains (indicated by connecting links in Fig. 3). The conformation of the outer part of the molecule changes with variations of pH or solvent that alters functional group interactions (13).

The various functional groups on the outer region of the protein molecule can interact with appropriate groups in other protein molecules to cause aggregation. X-ray crystallographic analysis of the trypsin-trypsin inhibitor complex indicates that only a small region on the protein surfaces is involved in the association (14). Hydrogen, electrostatic, and hydrophobic bonds all participate to hold the proteins together at the active site.

**POLAR GROUP INTERACTIONS**

The effect of electrostatic forces on the solubility of globular proteins is clearly illustrated by the difference in solubilities of the genetic variants of the milk protein β-lactoglobulin. These proteins have the same shape and size. As shown in Fig. 4, β-lactoglobulins A and B vary by only one charged group, but their solubilities in aqueous salt solutions at neutrality differ considerably (15). The two proteins associate with each other by electrostatic forces, as shown by the solubility curves of A and B mixtures with solubilities intermediate between those of the pure proteins. Wall and Beckwith (16) demonstrated that the solubilities of mixtures of A and B are a function of the amounts of each protein in the solid, the solubility of each pure protein, and the amount of salt in the solutions. Of course, if the pH of protein solutions is made more acidic or basic so that negative or positive charges predominate, the proteins will be highly soluble due to charge repulsion.

In our laboratory we obtained two lines of evidence demonstrating that hydrogen bonding of glutamine amide side chains contribute to wheat gluten insolubility. First, changing the amide groups to methoxyl groups by reaction in alcoholic

![Fig. 3. Conformation of polypeptide chains in α-chymotrypsin A based on x-ray analysis at 2 Å resolution. From Sigler et al (12).](image-url)
HCl alters the protein solubility (17), and second, spectroscopic and solubility data indicate that synthetic polypeptides containing high contents of glutamine are associated by hydrogen bonds (18). Jankiewicz and Pomeranz' study (19) showed that addition of urea markedly decreased the viscosity and cohesiveness of wheat flour dough, as indicated by decreased mixing time and stability as measured in the farinograph. This action of urea is attributed to its disruption of protein-protein hydrogen bonds contributing to gluten cohesion, because urea forms hydrogen bonds with protein polar functional groups according to Kuntz and Brassfield (20).

Proteins are important in maintaining moisture in semimoist foods and baked cereal goods. Kuntz (21) used nuclear magnetic resonance to examine the extent and sites of water-binding on synthetic polypeptides formed from single amino acids. Charged groups on polypeptides or proteins were found to bind five or six water molecules, since the charges enhance the dipole moment of the adjacent water molecules. In contrast, uncharged polar groups attract only one or two water molecules. Nonpolar groups bind very little water but tend to encourage formation of neighboring water crystal lattices due to their hydrophobicity.

**HYDROPHOBIC BONDS**

Many proteins, especially membrane proteins, remain associated even in the presence of strong hydrogen bond-breaking solvents such as 8M urea. Various surfactants such as sodium dodecyl sulfate (SDS) or salts of fatty acids can disrupt the hydrophobic bonds that unite these proteins. Koberhel and Bushuk (22) reported that wheat glutenin disperses well in sodium stearate solution. During gel filtration chromatography on agarose columns, glutenin behaves as if it consists exclusively of high molecular weight proteins in 8M urea. When glutenin is chromatographed on the same column in SDS solution, low molecular weight protein is resolved from the higher molecular weight materials (23, 24). The low molecular weight glutenins, possibly membrane proteins, are associated with the higher molecular components by hydrophobic bonds.

When hydrophobic bonds link lipid to protein, the protein properties may be changed. Charbonnier (25) found differences between the compositions of 70% ethanol extracts of defatted and nondefatted wheat flours. Based on gel filtration analysis, the 70% ethanol extracts of the nondefatted flour contain more high molecular weight protein. The additional extracted protein exists as a lipid complex with certain glutenin proteins that, when free of lipid, are not soluble in 70% ethanol.

The presence of polar lipids in flour improves the functional properties of dough, including the mixing characteristics and dough strength. Addition of polar lipids or surfactants improves the tolerance of doughs to mixing and to the addition of soy proteins for production of breads with acceptable textures (26). The polar lipid seems to enhance aggregation and cohesion of gluten proteins.

**DISULFIDE BONDS**

The role of intermolecular disulfide bonds in functionality of food proteins, especially wheat flour doughs, is being critically reevaluated. Rheological properties of dough are generally explained in terms of either rather extensive intermolecular disulfide crosslinks (27) or fairly linear concatenations of limited disulfide-linked glutenin polypeptide chains (28). Based on the increasing importance attributed to hydrophobic bonds, some workers now minimize the need for extensive intermolecular disulfide bonds. Kasarda et al (29) explain the action of reducing agents on protein properties as the result of conformational changes in the proteins after disulfide cleavage.

During gel filtration chromatography, careful fractionation of proteins dissolved in dissociating solvent provides evidence that a range of protein species, differing in type and extent of disulfide bonds, exists in plant seeds, especially endosperms of cereal grains. Most globulins and gliadins and possibly some wheat glutenin proteins have only intramolecular disulfide bonds (Fig. 5). Most acetic acid soluble glutenin molecules have

<table>
<thead>
<tr>
<th>Class</th>
<th>Solubility</th>
<th>Features</th>
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<tr>
<td>Albumins</td>
<td>Salt Solutions</td>
<td><img src="https://example.com/diagram.png" alt="Diagram" /></td>
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<tr>
<td>Globulins</td>
<td></td>
<td></td>
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<tr>
<td>Gliadin</td>
<td>70% Alcohol Solution</td>
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<tr>
<td>Glutenin</td>
<td>1% Acetic Acid</td>
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<tr>
<td>Residue</td>
<td>Reducing Agents or Alkali</td>
<td><img src="https://example.com/diagram.png" alt="Diagram" /></td>
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</table>

Fig. 5. Variation in structure of different wheat gluten proteins depending on their differences in intramolecular and intermolecular disulfide bonds.
limited intermolecular disulfide bonds that result in large asymmetric molecules having ample surface for associative interactions. A remaining protein fraction in wheat flour is insoluble in most protein solvents including acetic acid (30) and SDS (31) solutions. This residue protein must have extensive intermolecular disulfide crosslinks, because it can be solubilized only after reductive cleavage of the disulfide bonds (32).

Huebner and Wall (33) compared the relative amounts of proteins differing in number and extent of disulfide crosslinks for several hard red winter wheats (Fig. 6). The flour varieties with the strongest mixing properties have the largest amount of insoluble protein and glutenin with the highest molecular weight (Glut-I). Apparently the insoluble proteins and the high molecular weight glutenins are the major factors required to produce doughs that can tolerate rigorous mixing for bread production. A suitable mixture of all protein fractions is necessary to achieve good loaf texture and volume, however.

**MODIFICATION OF PROTEIN PROPERTIES**

Protein properties can be modified, within limits, to alter their performance in foods and the changes can be made that retain organoleptic characteristics and nutrition acceptability.

Physical methods of treating proteins can alter protein products. Mixing changes the rheological properties of wheat flour gluten. Heating may result in chemical and physical changes. When corn grain is heated, the albumins, globulins, and zein proteins become less soluble in aqueous or alcohol solutions, as shown in Fig. 7 (34). These insoluble denatured proteins were partially unfolded by heating so that newly exposed groups formed new bonds (physical and disulfide) between neighboring protein molecules. When the residue meals are further extracted with surfactant solutions and solutions containing reducing agents, the insolubilized proteins can be solubilized. Denaturation can be a desirable process in food
applications if it improves protein hydration and results in better textured products and gel and foam production.

Additives also can influence protein performance. Earlier we alluded to the effect of surfactants and lipids on dough protein performance. The effect of reducing or oxidizing agents on disulfide groups of proteins in dough is also well established. The interaction of gluten or other food proteins with gums and pentosans gives interesting effects (35). Addition of gums to dough changes the mixing performance, as shown in Fig. 8. The low protein and weak gluten of the soft wheat Brevor results in poor mixing characteristics of dough prepared from it. Addition of 0.6% alginate to the flour increases the mixing time and dough stability. By forming salt and hydrogen bonds with the protein, the gums improve the protein functionality.

**SUMMARY**

Adaptation of computer and electronic technology to protein research recently is helping to decipher the structures of complex protein molecules in foods. The sequences in which amino acids are arranged, the way in which the molecules are folded, and how they are tied together by disulfide bonds are being determined. This information provides ideas about how the functional groups on the molecules are exposed to the solvent in which they are dispersed and how the proteins interact with other molecules. Solubility of food proteins depends on their net positive or negative charges due to ionizable amino acids. Protein aggregation through hydrogen and hydrophobic bonds contributes viscosity, cohesion, and bonding properties to food ingredients. Lipids bind to proteins mainly through association with hydrophobic groups; the lipid may contribute to cohesion of the protein. Despite current controversy, intermolecular disulfide bonds in gluten best explain many aspects of dough rheology. Additives such as lipids or gums modify protein functionality by combining with the protein molecules.

As more is learned about the basis of cereal and food protein function, we may be able to better develop and select improved plant varieties or chemically and physically modify their proteins to optimize their performance in specific foods.

**LITERATURE CITED**

8. Wall, J. S. Disulfide bonds: Determination, location, and (cont'd on page 313)


