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Denaturation and covalent network formation of wheat gluten, globular proteins and mixtures thereof in aqueous ethanol and water

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ABSTRACT

Food processing often includes heating and/or exposure to solvents as unit operations. Here, the impact of heating at 100 °C in water or aqueous ethanol [10 or 50% (v/v)] on denaturation and covalent network formation of three model proteins [bovine serum albumin (BSA), soy glycinin and wheat gliadin] was examined. Already at room temperature 50% (v/v) ethanol induced disulfide cross-linking between BSA proteins. Increased ethanol concentrations reduced heat-induced polymerization of soy glycinin and wheat gliadin. The use of aqueous ethanol limited the extent of β-elimination, sulfhydryl-disulfide exchange reactions and sulfhydryl oxidation. Gliadin and soy glycinin had higher colloidal stability in 50% (v/v) ethanol than in water. The conformation of BSA and soy glycinin already changed at lower temperatures in 50% (v/v) ethanol than in water. In all media, different proteins influenced each other's denaturation and/or polymerization. During heating in water but not in 50% (v/v) ethanol, gliadin-BSA and gliadin-soy glycinin mixtures polymerized more than expected than the isolated proteins. Thus, phase-separation of proteins did not limit intermolecular disulfide formation. Pretreatment of proteins with aqueous ethanol did not substantially influence their subsequent polymerization during prolonged heating in water. However, ethanol pretreatment of gluten impacted heat-induced polymerization of BSA in gluten-BSA mixtures.

1. Introduction

A key concept in protein science is that form and function are inseparable. Protein aggregation is related to neurodegenerative diseases (Chiti & Dobson, 2006) and sickle cell anemia (De Llano & Manning, 1994), but also to texture and structural properties of food products (Singh, 1991; Totosaus, Montejano, Salazar, & Guerrero, 2002). For instance, polymerization of wheat gluten proteins positively impacts on bread quality (Lagrain, Thewissen, Brijs, & Delcour, 2008). Similarly, the formation of a strong gluten network, mainly through disulfide (SS) bond formation and reshuffling, is crucial for the quality of pasta, some cookie and other cereal-based products (Delcour et al., 2012). Heat-induced denaturation of globular proteins, *i.e.* the transformation of the native to a disordered state, changes protein functionality and often induces gelling. Such aggregation involves both non-covalent interactions and covalent cross-links, the latter mainly SS bonds (Foegeding & Davis, 2011; Mine, 1995). In contrast to albumins and globulins, wheat gluten proteins are soluble neither in water nor in aqueous salt solutions (Osborne, 1907). Also, they do not show endothermic denaturation peaks when analyzed by differential scanning calorimetry (DSC) (Erdogdu, Czuchajowska, & Pomeranz, 1995). In *inter alia* cake and egg noodles, wheat gluten and globular proteins co-exist. Co-protein effects due to interactions and reactions

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between different protein types can impact such food systems (Erickson, Campanella, & Hamaker, 2012; Rombouts, Lagrain, & Delcour, 2012). The influence of different types of proteins on each other's behavior remains to be further investigated.

Protein aggregation in aqueous salt solutions (*e.g.* physiological conditions) has received much attention because of its importance and relevance in life science and food systems. Alcohols are used in products ranging from food additives to preservatives in cosmetics (Nair, 2001) and can impact the stability and folding of proteins (Thomas & Dill, 1993). In food and food system related applications, aqueous ethanol is *e.g.* used for precipitating proteins from cheese whey, purifying protein from soy flakes (Hua, Huang, Qiu, & Liu, 2005; Morr & Lin, 1970) and for marinating meat or fish with beer or wine (Melo, Viegas, Petisca, Pinho, & Ferreira, 2008). Furthermore, some edible films based on soy proteins or wheat gluten are prepared in the presence of aqueous ethanol (Ali, Ghorpade, & Hanna, 1997; Gontard, Guilbert, & Cuq, 1992). Instead of using water to produce vital wheat gluten in a traditional Martin process (Van Der Borght, Goesaert, Veraverbeke, & Delcour, 2005), an energy and water saving method uses (aqueous) ethanol as washout liquid at low temperature (Robertson & Cao, 1998). It results in vital wheat gluten which has better dough mixing properties than the water-equivalent (Robertson & Cao, 2002). Moreover, ethanol pretreatment changes the rheological properties of wheat flour (Robertson et al., 2011). Even at low levels, similar to those in fermenting bread dough, ethanol decreases dough extensibility and makes dough more stiff and tenacious (Jayaram et al., 2014). Notwithstanding the above, the impact of aqueous alcohols on (heat-induced) aggregation of wheat gluten proteins remains to be studied.

Interactions between amino acid side chains and their immediate environment affect protein aggregation. Small changes in temperature, pH, ionic strength and polarity can impact the conformation of globular proteins. While native and denatured proteins do not aggregate easily in aqueous environments due to buried hydrophobic regions, partially unfolded proteins with notable secondary structure are more prone to aggregate (Chi, Krishnan, Randolph, & Carpenter, 2003). Alcohols are less polar than water and thus weaken hydrophobic interactions and enhance polar interactions thereby facilitating protein denaturation (Thomas & Dill, 1993). Often, proteins denature in aqueous-organic media but not in the corresponding pure organic solvent (Griebenow & Klibanov, 1996). Furthermore, especially the larger alkyl alcohols stabilize α-helical conformations of unfolded proteins (Hirota, Mizuno, & Goto, 1997). Because aqueous alcohols partially unfold proteins, they can induce protein aggregation (Singh, Cabello-Villegas, Hutchings, & Mallela, 2010). With increasing ethanol concentration, bovine serum albumin (BSA), a protein of milk, whey and meat (Belitz, Grosch, & Schieberle, 2009), tends to lose its secondary structure and form aggregates (Liu et al., 2010). Similarly, with increasing alcohol concentrations, partial and progressive dehydration and alcohol binding transforms gel-like sediments of milk and soy proteins into opaque flocks (precipitates) (Boulet, Britten, & Lamarche, 2001). It is clear neither whether alcohol-induced aggregation of albumins and globulins in the above examples is due to non-covalent interactions or covalent cross-links, nor whether and how alcohols would influence heat-induced aggregation.

While food systems often contain more than one protein type, protein denaturation and polymerization have mainly been studied in single protein systems. Due to differences in solubility, proteins in complex food systems can be present in various phases. In this context, Polyakov, Grinberg, and Tolstoguzov (1997) used the term protein thermodynamic incompatibility. They even stated that differences in hydrophilic character between various protein types trigger phase separation and thereby promote interactions between proteins with similar conformation (Polyakov et al., 1997). Given the impact of alcohols on protein conformation and solubility, it is of interest to compare heat-induced polymerization of complex systems in water to that in aqueous ethanol.

Against this background, structural changes during heat treatment of various proteins in aqueous ethanol were compared to those in water. BSA and glycinin, one of the two most abundant soy proteins (Liu et al., 2007) were chosen as model globular proteins. Gliadin, the monomeric protein fraction of wheat gluten consists of α-, γ- and ω-gliadin. It was selected as model prolamin (Wieser, 2007). First, the impact of water and aqueous ethanol on denaturation and covalent network formation of isolated proteins was studied. In addition, sulfhydryl (SH) oxidation, SH-SS interchange reactions and β-elimination reactions were investigated. The second aim was to evaluate the impact of different protein types on each other's denaturation and polymerization during heating in water and aqueous ethanol. Here, the importance of protein incompatibility during heat treatment of complex systems containing different protein types was investigated. Furthermore, the impact of a pretreatment or isolation with aqueous ethanol of proteins was studied on the polymerization behavior of isolated proteins and mixtures thereof.

2. Materials and methods

2.1. Materials

Gluten [83.2% protein, on dry matter (dm) basis] from wheat (cultivar Paragon, RAGT, Ickleton, United Kingdom) and soy glycinin (98.1% dm protein) from soy flour (L.I. Frank, Twello, The Netherlands) were isolated as in Lambrecht, Rombouts, Van Kelst, and Delcour (2015). Gliadin was extracted from gluten (20.0 g) with 70% (v/v) ethanol (250 ml). After centrifugation (10 000 *g*, 10 min), ethanol was evaporated (Rotavapor R3000, Büchi, Flawil, Switzerland) from the supernatant. Gliadin (87.7% dm protein) was freeze dried, ground in a laboratory mill (IKA, Staufen, Germany), and passed through a 250 μm sieve. BSA (fraction V for biochemistry, 98.2% dm protein) was from Acros Organics (Geel, Belgium). All chemicals were at least of analytical grade and from Sigma–Aldrich (Steinheim, Germany) unless specified otherwise. Dithiothreitol (DTT), disodium hydrogen phosphate and sodium dihydrogen phosphate were from VWR International (Leuven, Belgium).

2.2. Protein content

Protein content was determined in triplicate, using an adaptation of AOAC Official Method 990.03 (AOAC, 1995), with an automated Dumas protein analysis system (EAS Variomax N/CN, Elt, Gouda, The Netherlands). Conversion factors (5.7 for gluten and gliadin; 6.25 for soy glycinin and BSA) were used to calculate protein from nitrogen contents.

2.3. Aqueous ethanol pretreatment

BSA, soy glycinin and gluten (500.0 mg dm protein) were shaken for 60 min with 5.0 ml 50% (v/v) ethanol. Gluten (500.0 mg dm protein) was also pretreated with 70% (v/v) ethanol (5.0 ml) in a similar way to simulate conditions during gliadin isolation. Ethanol was evaporated from samples using a Rotational Vacuum Concentrator (Q-lab, Vilvoorde, Belgium, 35 °C, 1.0 mbar). Aqueous ethanol pretreated (EtPT) samples were freeze-dried and ground using a mortar and pestle.