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Macromolecular changes and nano-structural arrangements in gliadin and glutenin films upon chemical modification Relation to functionality☆

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ABSTRACT

Protein macromolecules adopted for biological and bio-based material functions are known to develop a structured protein network upon chemical modification. In this study, we aimed to evaluate the impact of chemical additives such as, NaOH, NH_4OH and salicylic acid (SA), on the secondary and nano-structural transitions of wheat proteins. Further, the effect of chemically induced modifications in protein macromolecular structure was anticipated in relation to functional properties. The gliadin-NH_4OH-SA film showed a supramolecular protein organization into hexagonal structures with 65 Å lattice parameter, and other not previously observed structural entities having a characteristic distance of 50 Å. Proteins in gliadin-NH_4OH-SA films were highly polymerized, with increased amount of disulfide crosslinks and β-sheets, causing improved strength and stiffness. Glutenin and WG proteins with NH_4OH-SA showed extensive aggregation and an increase in β-sheet content together with irreversible crosslinks. Irreversible crosslinks hindered a high order structure formation in glutenins, and this resulted in films with only moderately improved stiffness. Thus, formation of nano-hierarchical structures based on β-sheets and disulfide crosslinks are the major reasons of high strength and stiffness in wheat protein based films.

1. Introduction

The biological macromolecules, e.g. proteins, starch, natural rubber, etc., are characterized for their structural and film forming properties to be utilized for bio-medical or industrial purposes [1,2]. Wheat gluten (WG) protein is a complex blend of monomeric and polymeric macromolecules, commercially available from the bioethanol and starch industries, and widely tested for the production of bio-based materials [3–5]. The mixture of proteins in the WG have a molecular weight ranging from 30,000 to 10 million Da, with the ability to form the largest polymers in nature [6,7]. Due to the complex polymeric nature (intra- and inter-disulfide crosslinks, etc.), the structural elucidation of WG proteins at molecular level is rather difficult. However, structural understanding of WG is important to utilize its polymeric properties for state of the art applications, i.e. bio-based materials, functional medical health-care devices, etc. WG-based materials in the form of films, coatings, adhesives and foams have been produced and characterized for their structural and functional properties, e.g. mechanical, gas barrier and water resistance [8–11]. In addition, the component proteins of WG, the gliadin and glutenin, have been studied for their properties related to materials production [12–14]. The structural conformation and functional properties of fractionated gliadin and glutenin proteins differ greatly from pristine or commercial WG. Gliadin proteins are monomeric in their native state held together with low energy van der Waals forces and electrostatic interactions and are primarily intra-molecular disulfide bonded [15]. Glutenin proteins in their native state form large polymeric structures in which polypeptide chains are connected with intra- and inter-chain disulfide linkages [6,16]. Both the gliadin and glutenin interact and form polymers of varying size and shape through various molecular interactions when hydrated or during processing [17]. The molecular interactions among protein films

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can be studied quantitatively by swelling or protein solubility, and also qualitatively in terms of protein structure and morphology by X-ray scattering, infra-red spectroscopy and chromatographic techniques [18]. A number of factors, e.g. amount and type of plasticizer, processing temperature and the use of additives have an effect on the structural and functional properties of the final bioproducts [12]. The use of additives such as NaOH, NH_4OH and salicylic acid (SA), contributes positively to functional properties of WG based films, by inducing changes in protein macromolecular structure [9,19,20]. Salicylic acid through its radical scavenging mechanism reduces/delays the disulfide crosslinking and increases the processing window of gluten proteins [9]. Alkaline additives such as, NaOH and NH_4OH, denature the initial aggregated protein structure and provide opportunities for new protein–protein interactions during film formation [19,21].

The aim of the present study was to investigate the protein macromolecular changes and nano-structural arrangements in gliadin and glutenin films upon chemical modifications (NaOH, NH_4OH and SA). The main focus was addressed on the protein macromolecular morphology, polymerization behavior and protein–protein interactions, as well as relation of protein structural changes to the mechanical properties of films.

2. Materials and methods

2.1. Materials

Wheat gluten was supplied by Lantmännen Reppe, Lidköping, Sweden. The protein content of dry gluten powder was 77.7% (modified NMKL Nr 6, Kjeltec, Nx5.7, www.NMKL.org), the starch content was 5.8% (Ewers, polarimetric method) and the moisture content was 6.9% of the dry powder (NMKL 23, 1991), as described by the supplier. Glycerol (99.5% purity) was supplied by Karlshams Tefac AB, Sweden. Urea, NaOH and sodium dodecyl sulfate (SDS) were purchased from Duchefa, Netherlands. NaH_2PO_4 was purchased from J.T. Baker, Netherlands. NH_4OH (25% solution), trifluoro acetic acid (TFA) and isocratic grade acetonitrile were purchased from Merck, Germany. Dithiothreitol (DTT) and salicylic acid (SA) were sourced from Saveen Werner AB, Sweden and VWR international, Sweden, respectively. Gradient grade acetonitrile used in RP-HPLC was purchased from Honeywell, Germany.

2.2. Methods

2.2.1. Extraction of gliadin and glutenin fractions

Gliadin and glutenin were extracted from commercial WG powder based on their solubility in 70% ethanol as described by Blomfeldt et al. [14]. Briefly, WG powder (16 g) was mixed in 70% ethanol (200 mL). The mixture was shaken for 30 min at 300 rpm (Hunkel Ika, Werk KS 500) and centrifuged afterwards for 10 min at 12,000 rpm (Beckman J2.21, US). Gliadin proteins were collected in the supernatant which was concentrated by rotary evaporation. Glutenin was collected as a rubbery pellet and chopped into small pieces. Both the gliadin and glutenin were lyophilized and ground into powder (Yellow Line A10, IKA) and stored at −20 °C for further use. The protein content of gliadin and glutenin was measured by the Dumas method [22] (nitrogen volatilization by Flash 2000 NC Analyzer, Thermo Scientific, USA) and was found to be 91% for gliadin and for glutenin 75%.

2.2.2. Compression molding

The WG, gliadin and glutenin fractions (7 g) were mixed with glycerol (3 g) by hand in a mortar and pestle followed by a laboratory grinder (Yellow Line A10, IKA) using 3 pulses of 10 s, scraping of the mixing chamber between pulses. The films with additives contained NH_4OH (5%), SA (1%) and NaOH (3%) either separately (5% NH_4OH) or in combinations (5% NH_4OH-1% SA) and (3% NaOH-1% SA). In the cases where salicylic acid was added it was mixed with the dry protein in a laboratory grinder using 2 pulses of 10 s before glycerol addition. NH_4OH (25% aqueous solution) was added to the glycerol before mixing with protein. NaOH was first dissolved in water and then added to glycerol before mixing with protein, maintaining the same total level of water as in the NH_4OH samples.

The sample names were abbreviated as gliadin (Glia), glutenin (Glut), wheat gluten (WG), glycerol (Gly) followed by the additive(s) present. The protein–glycerol/protein–glycerol-additive mixtures were placed in the center surrounded by a 0.5 mm thick aluminum frame with a 100 mm × 100 mm central opening to control the size and thickness of the film. The protein powders or mixtures were then molded at 130 °C in a pre-heated hydraulic press (Polystat 400s, Schwabenthan, Germany) between aluminum plates (also pre-heated) with polyethylene terephthalate release film on both sides. A molding force of 100 kN was applied for 10 min to all samples. The sheets were removed from the press and cooled in the frame between two aluminum plates at room temperature. The sheets were removed from the frame when cooled using a scalpel. Samples were stored at −20 °C until further analysis.

2.2.3. Tensile testing

Using a sample cutter (ISO 37, type 3, Elastocon AB, Sweden), dumbbell-shaped tensile specimens (minimum of 9 from each film) were punched from the hot-pressed films. These specimens had a narrow section with a width of 4 mm and length of 16 mm in the test direction. Before testing tensile specimens were conditioned at 50% relative humidity (RH) and 23 °C for 48 h, which was found to be long enough to achieve the equilibrium moisture content (data not shown). The thickness of the test section was measured on each specimen at 5 locations using a Mitutoyo IDC 112B indicator with dial gage stand and averaged. Tensile testing was performed on an Instron 5566 universal test machine at 23 °C and 50% RH using a crosshead speed of 100 mm/min, 30 mm initial clamp separation, and a 100 N load cell using Bluehill software (Instron, Sweden). The stress was calculated from the applied force divided by the cross sectional area of the reduced width section and strain was calculated from the crosshead displacement divided by the narrow section length (16 mm). The initial slope of the stress–strain curve was used for the calculation of the Young's modulus and for toe compensation (ASTM D638-08).

2.2.4. Size exclusion high performance liquid chromatography

Amount and size distribution of proteins were measured according to method and definition by Gupta et al. [23]. Due to aggregated state of the proteins in the films leading to low solubility, the original method was modified according to Gällstedt et al. [24]. All the extractions were done in triplicate using 16.5 mg of protein for each sample (samples were chopped to approx. 0.2 mm × 0.2 mm). Serial extraction using 1.4 mL buffer for each extraction step was performed as described by Gällstedt et al. [24]. Phosphate buffer (pH 6.9) containing 0.5% (w/v) SDS and 0.05 M NaH_2PO_4 was used to extract the proteins. Extraction steps were as follows, (1) sample was mixed with 1.4 mL of buffer, vortexed for 10 s, followed by a 5 min shaking at 2000 rpm, and centrifugation at 19,000 RCF. The supernatant was collected in HPLC vials and pellet was used for next extraction step. (2) Pellet was resuspended in 1.4 mL buffer, followed by 30 s sonication, amplitude of 5 μm (Sanyo Soniprep, Tamro, Sweden) and centrifugation at 19,000 RCF to collect proteins in supernatant. (3) Pellet was again resuspended in buffer and sonicated for 30 s + 60 s + 60 s followed by centrifugation at 19,000 RCF. Protein molecular weight and amount of extractable proteins for each extraction step was measured by using an HPLC system (Waters 2690, Milford, USA) equipped with Biosep SEC-4000, column (Phenomenex, USA). Empower Pro software (Waters)