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The effect of cross-linking additives on the structure and properties of glassy wheat gluten material

Kevin Bruyninckx[a,*], Koen J.A. Jansens[b,c], Jan A. Delcour[b,c], Mario Smet[a]

[a] Polymer Chemistry and Materials Division, KU Leuven, Chemistry Department, Celestijnenlaan 200F, B-3001 Leuven, Belgium
[b] Laboratory of Food Chemistry and Biochemistry, KU Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium
[c] Leuven Food Science and Nutrition Research Centre (LFoRCe), KU, Leuven

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ABSTRACT

High-temperature compression molding of wheat gluten, an industrial co-product of the manufacture of wheat starch, yields a rigid, glassy gluten material. The improvement of the mechanical properties of this type of material by thiolated additives has been attributed to their simultaneously occurring reducing and/or cross-linking impacts. In this study, three additives with different cross-linking functionalities but which lack reducing properties are tested for their ability to cross-link the gluten network and improve its mechanical properties: glutaraldehyde (DAL), neopentyl glycol diacrylate (DAC) and a retro Diels–Alder based thermally activated cross-linker. The effect of cross-linking on fully reduced disulfide free gluten (MG) was also studied. Both DAL and DAC were able to cross-link (modified) gluten protein during mixing as well as during the molding step while TAC was active during molding. All additives were more effective at cross-linking the MG network than untreated gluten likely due to its higher level of available thiol groups and its less constrained network. However, there was no straightforward link between increased cross-link density and improved mechanical properties. Overall, we postulate that increasing the level of cross-linking in gluten protein can only benefit the mechanical properties when favorable secondary interactions and physical entanglements in the network are retained.

1. Introduction

Wheat (*Triticum aestivum* L.) gluten is an industrial co-product of the manufacture of wheat starch. Bioplastic made thereof is a promising substitute for petroleum-based materials. Various ways of processing gluten protein can lead to different bioplastics. Gluten films are arguably the most well-studied and can be obtained by solution casting. Thermomolding is a more industrially-friendly method. It yields a gluten rubber or a glassy gluten material when molded with high or low levels of plasticizer respectively (Lagrain et al., 2010). A well-known method to improve the mechanical properties of polymer based materials is to increase its network density via the introduction of (extra) cross-links in the polymer network. For gluten rubbers, failure strain decreases whereas tensile modulus and strength increase with increasing cross-link density, an effect which is in line with the classical rubber theory (Eroğlu, 1998). With increasing molding temperature, tensile modulus and strength of gluten rubbers improve. This has been attributed to an increased cross-link density (Cuq et al., 2000). Moreover, there are numerous examples of cross-linking agents used to improve the mechanical performance and the moisture resistance of gluten rubbers. Aldehyde-based cross-linkers (formaldehyde (Hernández-Muñoz et al., 2004a,b; Marquié, 2001; Sun et al., 2007), glyoxal (Hernández-Muñoz et al., 2004b; Marquié, 2001; Zhang et al., 2007b), cinnamaldehyde (Balaguer et al., 2011), glutaraldehyde (Hernández-Muñoz et al., 2004b; Marquié, 2001; Reddy et al., 2008; Sun et al., 2007)) but also alkoxysilanes (Zhang et al., 2007a) and diisocyanates (Huang et al., 2004) have been investigated.

The present study deals with rigid glassy gluten bioplastic, a material distinctively different from gluten rubbers. With increasing molding temperature, the flexural modulus of glassy gluten material does not change whereas flexural strength and failure strain increase (Jansens et al., 2013c; Ye et al., 2006). This increase

Abbreviations: DAC, diacrylate cross-linker (neopentyl glycol diacrylate); DAL, dialdehyde cross-linker (glutaraldehyde); DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); DTT, dithiothreitol; MG, modified gluten; NMR, nuclear magnetic resonance; rDA, retro Diels–Alder; SDS, sodium dodecyl sulfate; SDSEP, the level of protein extractable in SDS containing medium; SDSEPred, the level of protein extractable in SDS containing medium under reducing conditions; SH, thiol; TAC, thermally activated cross-linker; TCEP-HCl, tris(2-carboxyethyl) phosphine hydrochloride.

* Corresponding author.
E-mail address: Kevin.Bruyninckx@chem.kuleuven.be (K. Bruyninckx).

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in toughness has also been linked to an increased cross-link density (Jansens et al., 2013c). Alkaline pretreatments of gluten protein in 70% ethanol, which strongly affects cross-linking during mixing and thermomolding, improves the strength of bioplastic made from the resulting gluten samples. Moreover, it seems that the type of cross-links (disulfide versus non-disulfide) in the sample does not impact the mechanical properties (Jansens et al., 2013a). However, the resulting glassy gluten bioplastic is still quite brittle and also displays poor water resistance, problems which can possibly be solved by increasing the cross-link density. However, cross-linking additives for rigid gluten-based materials – the case of interest of this study – have received considerably less attention than those for gluten films and rubbers. Woerdeman et al. (2004) were the first to report improvements in the mechanical properties of glassy gluten when a trithiol was used which was suggested to act both as a reducing and as a cross-linking agent. Dicharry et al. (2006) thiolated various poly(vinylalcohol)s (PVAs) and used these as additives to obtain rigid gluten-PVA blends with improved mechanical properties. A more extensive study on the influence of mono- and polythiol additives on the gluten network was conducted by Jansens et al. (2013b) and raised questions concerning the importance of the cross-linking effect of these additives while emphasizing the importance of disulfide reduction and structural reorganization of the gluten protein. Subsequent research reinforced the hypothesis that besides cross-linking conformational changes induced by thiolated additives are at least as important for the improvement of the mechanical properties (Jansens et al., 2014). Moreover, our group modified wheat gluten via a procedure which eliminates all cross- links in the gluten sample via disulfide reduction to obtain modified (cross-link free) gluten (MG). The resulting MG plates display mechanical properties which outcompete those when using untreated gluten even if they have hardly any cross-links. This effect was attributed to enhanced intermolecular secondary interactions and increased molecular entanglements (Bruyninckx et al., 2015).

Studies involving non-thiolated cross-linking additives to improve rigid gluten material are scarce. Recently, Diao et al. (2014) used the macromolecular cross-linker polyethylene-alt-maleic anhydride (PEMA) to improve the properties of rigid gluten material. By making a gluten-PEMA blend, flexural strain and stress of the resulting rigid material was significantly improved (Diao et al., 2014). Increasing the level of cross-linking and in this way improving the network quality is still considered to be a valuable route for improving the mechanical properties of any amorphous rigid material. However, the importance of introducing cross-links with additives for improving the mechanical properties of both an untreated as well as a (partially) reduced gluten matrix remains unclear.

In this paper, three distinctively different cross-linking additives were studied: glutaraldehyde (a dialdehyde), neopentyl glycol diacrylate (a diacrylate) and a retro Diels–Alder based thermally activated cross-linker, respectively abbreviated as DAL, DAC and TAC. The thermally activated cross-linker, synthesized using the Diels–Alder cyclo-addition, undergoes a retro Diels–Alder reaction at a specific temperature (which was determined via temperature controlled nuclear magnetic resonance (NMR) studies), releasing an activated double bound which can engage in cross-linking reactions (Gandini, 2013). Therefore, as all three of these additives react with nucleophilic amino acid in the gluten network (most likely cysteine and lysine), they allow studying the effects of cross-linking at different stages of the bioplastic production process. DAL and DAC react both during mixing and during thermomolding whereas TAC only reacts during thermomolding. All three additives are tested on gluten and on modified (cross-link free) gluten (MG). The latter is studied in an effort to also examine the effect of an increased level of cross-linking on an already improved network. All three cross-linking additives were mixed with gluten and MG via solvent-mixing and the mixtures were compression molded. The effects of the additives on the molecular properties were analyzed by determining the protein extractability in sodium dodecyl sulfate (SDS) containing medium, the level of free sulfhydryl (SH) groups in the network and the water absorption of the resulting molded samples. Furthermore, the obtained network characteristics were related to the mechanical properties which were obtained via a three point flexural test and uniaxial compression test.

2. Materials and methods

Wheat gluten with a protein content of 77.8% (dry basis) and a moisture content of 5.6% was obtained from Tereos Syral (Aalst, Belgium). The moisture content was determined according to the AACC Approved Method 44-19 (AACC, 2000). Protein content (N x 5.7) was determined using an adaptation of the AOAC Official Method to an automated Dumas protein analysis system (EAS Variomax N/CN Elt, Gouda, The Netherlands) (AOAC, 1995).

Glutaraldehyde (50 wt% in water) and neopentyl glycol diacrylate further referred to as DAL and DAC respectively, were used as received from Sigma–Aldrich (Steinheim, Germany). All other chemicals, solvents, and reagents were from Sigma-Aldrich unless specified otherwise and at least of analytical grade.

2.1. Synthesis of tris(2-carboxyethyl) phosphine hydrochloride (TCEP-HCl)

TCEP-HCl was synthesized as previously reported. In short, hydroxymethyl was replaced in tetrakis(hydroxymethyl) phosphonium chloride with acrylonitrile (Vullo, 1966). The obtained tris(2-cyanoethyl) phosphine was then hydrolyzed to TCEP-HCl (Burns et al., 1991).

2.2. Preparation of cross-link free wheat gluten

Cross-link free wheat gluten was prepared as described in Bruyninckx et al. (2015). In short, cross-link free gluten, further referred to as modified gluten (MG), was prepared by slowly adding gluten (50 g protein) to 750 ml 50% (v/v) aqueous 1-propanol containing 3.0 g of TCEP-HCl while stirring vigorously. Next, the pH of the mixture was adjusted to pH 6.0 with 1.0 M sodium hydroxide and the suspension was shaken for 3 h at 60 °C. Subsequently, the excess and reacted reducing agent were removed by dialysis against 0.01% acetic acid for 3 days. The sample was then freeze-dried and the resulting powder was ground in a laboratory mill (IKA, Staufen, Germany) and sieved to pass a 250 μm sieve. The moisture content of the sample was then adjusted to 7.0% by adding appropriate amounts of crushed ice, which itself was prepared by sprinkling water in a mortar with liquid nitrogen and grinding the resulting mixture to a fine ice powder with a pestle. Finally, to homogenize it, the sample was shaken overnight with a head-over-head shaker.

2.3. Synthesis of thermally activated cross-linker (TAC)

TAC was synthesized as depicted in Fig. 1 and described below.

2.3.1. Synthesis of 3a,4,7,7a-tetrahydro-4,7-epoxyisobenzofuran-1,3-dione (1)

Maleic anhydride (10.0 g, 0.10 mol), toluene (300 ml) and furan (20.0 ml, 0.28 mol) in a round-bottom flask were magnetically stirred at room temperature for 48 h under argon atmosphere. The precipitated product was collected by vacuum filtration, washed with toluene. After drying in vacuo, the product was used with-