Industrial Crops and Products 72 (2015) 119–124

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Controlling wheat gluten cross-linking for high temperature processing

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ARTICLE INFO

Article history:
Received 2 September 2014
Received in revised form 28 October 2014
Accepted 3 November 2014
Available online 4 January 2015

Keywords:
Wheat gluten
Acid
High-temperature processing
Protein reactions
Protein degradation
Mechanical properties

ABSTRACT

The high temperature blending of wheat gluten with other polymers for the manufacturing of bioplastics is associated with heat induced cross-linking reactions which can increase the viscosity and affect component miscibility. This work showed that a good level of control over the onset of cross-linking can be obtained during heating by adjusting the pH of the environment. Gluten was heated (>100 °C) in buffer solutions of different pH and protein polymerisation was monitored by various methods to assess the extent and nature of aggregation. In a sufficiently acidic environment (pH 2.5–3.0) during heating up to 153 °C, cross-linking was greatly hindered whilst protein degradation was not observed, provided 15 min heating time was not exceeded. Such conditions may well be suited to blend gluten with other polymers at relatively high temperatures in aqueous solvent. When hydrothermally treated gluten samples with large differences in protein cross-linking were compression moulded to produce plastic specimens, little differences in mechanical properties were observed.

1. Introduction

Wheat gluten (WG) is a heterogeneous mixture of proteins which play a vital structural role in bread and pasta. Various characteristics such as excellent gas barrier properties and high strength also make it an attractive candidate for the design of 'green' polymeric materials (Lagrain et al., 2010b). WG can exhibit thermoset characteristics, with the application of heat and pressure leading to protein cross-linking and eventually network formation. Unfortunately, the unique cross-linking ability of WG can also limit its processability. This can be a major drawback to the mainstream production of WG based plastics.

WG consists of two protein fractions, i.e. gliadins and glutenins, which are distinguished by their relative solubilities in aqueous alcohols. Gliadins are single chain peptides with molecular weight (MW) of 30–60 k which are soluble in aqueous alcohol (Delcour et al., 2012; Veraverbeke and Delcour, 2002). Glutenins are large proteins which are insoluble in aqueous alcohols. They consist of single chain subunits covalently linked by interchain disulfide (SS) bonds. They also possess intrachain SS bonds as well as low levels of free sulfhydryl (SH) groups. With a MW of up to several million, glutenins are believed to be the largest proteins in nature.

Heat treatment of WG induces cross-linking of protein chains. Direct oxidation of SH groups leads to additional SS bridges. However, the most important cross-linking mechanism is believed to be an interchange reaction, whereby intramolecular SS bonds are broken and reformed as network building intermolecular SS bonds. Cross-linking starts with the SH containing glutenins. At higher temperatures, gliadins also become incorporated into the network (Lagrain et al., 2008). Although the absolute mechanism of the SH/SS interchange reaction is still somewhat subject of debate, it is widely accepted that in the absence of mechanical stress the chief reaction pathway is an S_N2 reaction involving the thiolate anion as the reactive species (Auvergne et al., 2008; Fernandes and Ramos,

Abbreviations: DHA, dehydroalanine; DTT, dithiothreitol; HPLC, high-performance liquid chromatography; LAL, lysinoalanine; LAN, lanthionine; MW, molecular weight; SDS, sodium dodecyl sulfate; SDSEP, extractable protein with SDS containing medium; SE-HPLC, size-exclusion high-performance liquid chromatography; SS, disulfide; SH, sulfhydryl; TNBS, 2,4,6-trinitrobenzenesulfonic acid; WG, wheat gluten.

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http://dx.doi.org/10.1016/j.indcrop.2014.11.058
0926-6690/

2004). Acidic conditions tend to hinder SH reactivity (Singh and MacRitchie, 2004).

Various other WG cross-linking reactions have also been reported to occur under certain processing conditions. Still, the contribution of non-SS cross-links towards network formation is not fully understood. A study on protein cross-linking during hydrothermal treatment of WG showed basic conditions (pH 8.0) promoted β-elimination of the SS bridge cystine to produce cysteine and the unsaturated amino acid dehydroalanine (DHA) (Rombouts et al., 2010). This further induces the formation of non-reducible cross-links as DHA reacts with cysteine and lysine residues to form lanthionine (LAN) and lysinoalanine (LAL), respectively (Rombouts et al., 2010).

Traditional polymer processing techniques such as high temperature extrusion, injection or compression moulding to manufacture WG based materials can lead to difficulties related to the flow of the protein chains. Water or other plasticisers such as glycerol are commonly added to aid processing. Such addition increases the thermoplastic characteristics not only of WG but also of many other protein materials (Hernandez-Izquierdo and Krochta, 2008). WG is fully hydrated at ~20% moisture content (Weegels et al., 1994) and this greatly improves its processability. However, although the presence of water improves processability, at the same time the possibility to mix in a solvent with other polymers is limited by the occurrence of cross-linking reactions.

Protein degradation is also a problematic factor at high temperatures as this leads to inferior mechanical properties. Consequently, to optimise WG materials, degradation phenomena should be identified and avoided (Jansens et al., 2013a). In this paper, we have assessed the reactivity of WG upon heating in superheated buffers. Temperature, time and acidity have been varied in order to map out the conditions required for the onset of the formation of SS linkages, DHA derived cross-linking and protein chain hydrolysis.

2. Materials and methods

2.1. Materials

WG (protein content 72%, as is basis) was obtained from Tereos Syral (Aalst, Belgium). Protein content was determined on a Dumas protein analysis system (EAS Variomax N/CN, Elt Gouda, The Netherlands) using an adaptation of the AOAC Official Method (AOAC, 1995). A conversion factor of 5.7 was used to calculate protein from nitrogen content. All chemicals, solvents and reagents were from Sigma–Aldrich (Steinheim, Germany) unless specified otherwise and were at least of analytical grade.

2.2. Hydrothermal treatment of gluten

WG powder (1.0 g) was added to 6.0 ml of buffers of different pH (made up by adding different quantities of 0.1 M citric acid to 0.2 M sodium phosphate) in glass tubes which were then sealed and shaken thoroughly to give homogeneous dispersions. Sample tubes were submerged in a preheated oil bath for various times and temperatures. A thermocouple and data logger showed that the actual temperature inside the glass tubes increased during the first 10 min of heating and then remained isothermal at typically about 15 °C lower than the oil bath set temperature. Temperatures stated hereafter refer to the oil bath set temperature. Following removal from the oil bath, the samples were frozen in liquid nitrogen and water was removed by freeze-drying. Samples were ground in a laboratory mill (IKA, Staufen, Germany) and sieved (250 μm).

2.3. Protein extractability in SDS containing medium

Changes in protein size distribution and extractability in sodium dodecyl sulfate (SDS) containing medium were evaluated with size-exclusion high-performance liquid chromatography (SE-HPLC) as described in Jansens et al. (2011). In each case, samples (1.0 mg protein) were added to sodium phosphate buffer (1.0 ml, 0.05 M, pH 6.8) containing 2.0% (w/v) SDS. Samples were extensively vortexed, shaken (60 min, 150 rpm) and centrifuged (10 min, 10,000 × *g*). Protein extractability under reducing conditions was determined using the same procedure but now with the addition of urea (2.0 M) and 1.0% (w/v) dithiothreitol (DTT) to the sodium phosphate buffer. To prevent re-oxidation, reduced samples were prepared under a nitrogen atmosphere. SE-HPLC was carried out on an LC-2010HT system (Shimadzu, Kyoto, Japan). Extracts were filtered and loaded (60 μl) on a BioSep SEC-S4000 column (300 × 7.8 mm, Phenomenex, Torrance, CA, USA) using acetonitrile/water (1:1, v/v) containing 0.05% (v/v) trifluoroacetic acid. The flow rate was 1.0 ml/min and the column temperature was 30 °C. Protein elution was monitored at 214 nm. The amount of protein extractable in SDS containing medium (SDSEP) was calculated from the area under the HPLC elution curves and expressed as a percentage of the peak area of unheated WG extracted under reducing conditions. All samples were prepared and analysed in triplicate.

2.4. Amino acid analysis

Samples (16 mg protein) were heated in sealed reaction tubes in 1.0 ml 6.0 N HCl for 24 h at 110 °C to liberate individual amino acids. Amino acids were subsequently separated and quantified by high performance anion exchange chromatography with integrated pulsed amperometric detection using a Dionex (Sunnyvale, CA, USA) BioLC system as described by Rombouts et al. (2009). Cysteine could not be determined as it is degraded during the acid hydrolysis. However, in WG cysteine is almost exclusively present as the more stable cystine which could be determined.

2.5. Determination of dehydroalanine content

Samples (100 mg protein) were heated in sealed reaction tubes (12.0 ml) in 0.5 ml 1.5 N HCl at 110 °C for 120 min to liberate dehydroalanine as pyruvic acid. After clarification, the pyruvic acid concentration was determined enzymatically according to a Megazyme (Bray, Ireland) procedure based on the stoichiometric conversion of pyruvic acid into D-lactic acid by D-lactate dehydrogenase in the presence of reduced nicotinamide-adenine dinucleotide as described by Rombouts et al. (2011).

2.6. Primary amine analysis

Samples (9.0 mg protein) were suspended in 1.0 ml 0.4 M sodium phosphate buffer (pH 8.0). 2,4,6-Trinitrobenzenesulfonic acid (TNBS) solution (1.0 ml, 0.5% v/v) was added and the reaction tubes were sealed and left in the dark for 4 h at 50 °C. Sulfuric acid solution (8.0 ml, 50% v/v) was then added to quench the reaction and samples were shaken for 1 h at room temperature and then left to stand 30 min at 50 °C. Each sample (1.0 ml) was diluted with 3.0 ml of the same sulfuric acid solution and the absorbance was measured at 345 nm. The free amino group concentration was calculated using a molar absorption coefficient of 14,600 $lmol^{-1}$ cm^{-1} for trinitrophenyl lysine (Adlernissen, 1979; Tropini et al., 2004).