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Raman studies of gluten proteins aggregation induced by dietary fibres

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ABSTRACT

Interactions between gluten proteins and dietary fibre preparations are crucial in the baking industry. The addition of dietary fibre to bread causes significant reduction in its quality which is influenced by changes in the structure of gluten proteins. Fourier transform Raman spectroscopy was applied to determine changes in the structure of gluten proteins modified by seven dietary fibres. The commercially available gluten proteins without starch were mixed with the fibres in three concentrations: 3%, 6% and 9%. The obtained results showed that all fibres, regardless of their origin, caused the same kind of changes i.e. decrease in the α-helix content with a simultaneous increase in the content of antiparallel-β-sheet. The results indicated that presence of cellulose was the probable cause of these changes, and lead to aggregation or abnormal folding of the gluten proteins. Other changes observed in the gluten structure concerning β-structures, conformation of disulphide bridges, and aromatic amino acid environment, depended on the fibres chemical composition.

1. Introduction

Wheat proteins include albumins, globulins, gliadins and glutenins but only the last two participate in the formation of a continuous viscoelastic network within dough. Glutenin polymers are made up of high and low molecular weight subunits which are attached to each other via disulphide bonds (Shewry, Halford, Belton, & Tatham, 2002). The polymers provide strength and elasticity for bread dough development whereas globular proteins, such as gliadins, impart viscosity to dough (Sivam, Sun-Waterhouse, Perera, & Waterhouse, 2012). Gliadins interact with the glutenin polymers via non-covalent hydrophobic interactions and hydrogen bonding. The structure of both proteins is crucial in the breadmaking process, but is also connected with gluten allergenicity. Of these two proteins, gliadins are considered strong food allergens. They cause IgE-mediated allergies such as asthma, atopic dermatitis or celiac disease (Bürk, Melms, Schulz, & Dichgans, 2001). Previous studies (Waga, 2004) have shown three parts of the gluten protein structure which may be responsible for gluten allergenicity. As the first is a short (“toxic”) amino acid sequence, it probably acts as an antibody-binding epitope in immunological reactions. Other structural elements considered as “toxic” are β-turns and disulphide bonds (Waga, 2004).

Studies on limiting or eliminating allergenicity of gluten proteins by biochemical modifications have been carried out for several years. A considerable decrease in the gluten allergenicity was observed after treatment with transglutaminase (Leszczynska et al., 2002; Nakamura et al., 2013), acetic acid (Berti, Dolfini, & Forlani, 2002; Berti et al., 2007) or citric acid (Qiu, Sun, Cui, & Zhao, 2013), causing deamination. Modification can also be regarded as a reduction of the disulphide bridges by thioredoxin (Waga, Kączkowski, & Zientarski, 2003). Although the thioredoxin considerably decreases immunoreactivity of gliadins and does not affect the rheological properties of gluten properties negatively, its high price makes this approach impractical for producing hypoallergenic food on an industrial scale. There were also studies in which antioxidants such as anthocyanins were used to modify gluten proteins with the aim to decrease allergen immunoreactivity (Taddei, Zanna, & Tozzi, 2013; Tozzi, Zanna, & Taddei, 2013).

Dietary fibre preparations rich in antioxidants can be regarded as a good candidates for decreasing gluten allergenicity. In a previous study, changes in the structure of gluten proteins concerning β-turns and disulphide bridges has been evidenced (Nawrocka et al., 2015). The fibre additives are added to bread to increase

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the dietary fibre intake. Since bread is a principal component of western diets it is a convenient way to deliver fibre polysaccharides and antioxidants (Sivam, Sun-Waterhouse, Quek, & Perera, 2010). However, the main problem of dietary fibre addition in baking is a significant reduction of bread quality, which is connected with changes in the structure of gluten proteins. There are a great number of reports confirming a negative effect of dietary fibre supplementation on the rheological properties of bread dough (Miś, 2011; Miś & Dziki, 2013; Peressini & Sensidoni, 2009), but only a few reports correspond to changes in structure of gluten proteins after addition of dietary fibre preparations (Sivam, Sun-Waterhouse, Perera, & Waterhouse, 2013; Sivam et al., 2012). The dietary fibres used in the present study were chosen because of their different sources of origin, and hence different chemical composition. Due to their different chemical composition they could interact with gluten proteins in different ways causing changes in the protein structure.

The objective of this study was to determine changes in the secondary and tertiary structure of gluten proteins mixed directly with dietary fibre preparation without starch. Some changes in the gluten structure are postulated to decrease the allergenicity of gluten proteins (Taddei et al., 2013).

2. Materials and methods

2.1. Materials

Wheat gluten, and sodium chloride were purchased from Sigma–Aldrich and used as received. Chokeberry (CHB), cranberry (CRB), cacao (CAC), carrot (CRR), oat (OAT) and flax (FLX) fibres were purchased from Microstructure (Warsaw, Poland). The carob (CAR) fibre was purchased from Carob General Application (Valencia, Spain). The content of total, soluble and insoluble dietary fibre (DF) were given by the fibre manufacturers and is presented in the previous article (Nawrocka et al., 2015). Double-distilled water was used for all experiments.

2.2. Analysis of pectin content in dietary fibre preparations

Pectin content was determined by the Morris method, modified by Pijanowski, Mrożewski, Horubała & Jarczyk (1973). 20.00 g of the triplicate dietary fibre preparations was weighed into a flask and 30 ml of distilled water was added. The samples were shaken for 15 min, and then filtered into a dry flask. The residue and filter paper were transferred back to the flask. Then 30 ml of water was added and shaken. The extraction was repeated three times. The resulting filtrate was transferred into a 100 ml volumetric flask and topped up with distilled water. 25 ml of the solution was dispensed into a beaker and 50 ml acetone was added. The sample was allowed to stand for 1 hour and then the solution containing the precipitate was filtered through a dry and pre-weighed filter paper. Filter paper and precipitate were dried at 75 °C until reaching a constant weight. Pectin content was calculated from the difference in weight of the filter paper with the sediment after drying and the weight of the dried filter paper taking into account the volume of pectin solution taken, to determine the mass of the sample.

2.3. Analysis of cellulose content in dietary fibre preparations

Cellulose content was determined applying the Kürchner–Hanack method. This method is based on the insolubility of cellulose in water and its resistance to the action of dilute acids and bases. 1 g of the triplicate dietary fibre preparations was weighed into a flask and a 50 ml of mixture of nitric acid (d = 1.4 g/ml, 5 ml), acetic acid (70%, 75 ml) and trichloroacetic acid (2 g) was added. The solution was then boiled in flask with a condenser. The solution was then filtered through a Büchner funnel. The precipitate was washed with hot water until the acid reaction stopped (checked universal indicator paper). The solid was then washed on the filter with about 15 ml of ethyl alcohol. Then the filter paper containing an insoluble residue was dried in oven (100 °C) and measured (Skulmowski, 1974).

2.4. Fourier transform infrared (FT-IR) spectra collection

The FTIR spectra of seven dietary fibres were recorded with a Nicolet 6700 FTIR spectrometer (Thermo Scientific, Madison, WI, USA) equipped with a diamond attenuated total reflectance attachment. The FTIR spectra were recorded between 4000 and 400 cm^{-1} at 4 cm^{-1} intervals. Each spectrum resulted from 128 scans to obtain an optimal signal-to-noise ratio. Each spectrum of the studied dietary fibres was corrected with a linear baseline using OMNIC software (v. 8.2, Thermo Fischer Scientific Inc., Madison, WI, USA).

2.5. Gluten-fibre mixture preparation

The gluten-fibre mixtures were kneaded for 3 min in the vibrating kneader SŻ-1 (Sadkiewicz Instruments, Bydgoszcz, Poland). The fibre contents were 3%, 6% and 9% w/w in relation to the gluten-fibre mixture weight (at the same moisture basis). The 6% content of dietary fibre was used in the previous study (Nawrocka et al., 2015) where the model flour (starch and gluten only) – dietary fibre mixtures were examined. This allows for easy comparison between fibre only effects (present study) and both fibre and starch effects (previous study) on the gluten protein conformation.

The gluten samples were washed out from the gluten-fibre mixtures by using Glutomatic 2200 (Perten InstrumentsHuddinge, Sweden). Next the gluten samples were freeze-dried for 24 h, milled in laboratory grinder and used in FT-Raman measurements.

2.6. Fourier transform Raman (FT-Raman) spectra collection and data manipulation

The FT-Raman spectra were acquired on an FT-Raman module (NXR FT Raman) for a Nicolet 6700 FT-IR bench using an InGaAs detector and CaF_2 beam splitter (Thermo Scientific, Madison, WI, USA). The samples were placed in stainless cubes and were illuminated using Nd:YAG excitation laser operating at 1064 nm. The maximum laser power was 1 W. The spectra were recorded over the range of 3500–150 cm^{-1} and each spectrum was an average of 256 scans at 8 cm^{-1} resolution. The analysed spectra were averaged over five registered spectra. The gluten samples were analysed in powder form.

Spectral data from the sample scans were baseline-corrected, and normalised against a phenylalanine band at 1003 cm^{-1}, using ORIGIN (version 9.0 PRO, OriginLab Corporation, USA). The disulphide bridge region (490–550 cm^{-1}), and aromatic amino acids environment: tyrosine doublet (I(850)/I(830)), tryptophan band (I(760)), and amide I band (1570–1720 cm^{-1}), were analysed. Structural analysis of the disulphide bridges (percentage distribution of disulphide bridge conformations: gauche-gauche-gauche ($SS_{g\text{-}g\text{-}g}$), trans-gauche-gauche ($SS_{t\text{-}g\text{-}g}$), and trans-gauche-trans ($SS_{t\text{-}g\text{-}t}$)) were also conducted using ORIGIN. The Gaussian components in the S–S region were determined on the basis of a second derivative spectrum. The derivative spectrum was obtained using a five-point, two-degree polynomial function. The band profiles of the components for all samples are shown in Table 1 in the Supplementary Material. The S–S bands were assigned to each conformation according to Sugeta (1975).