

Polymer Conformation Structure of Wheat Proteins and Gluten Subfractions Revealed by ATR-FTIR

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ABSTRACT

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The polymer conformation structure of gluten extracted from a Polish wheat cultivar, Korweta, and gluten subfractions obtained from 2 U.K. breadmaking and biscuit flour cultivars, Hereward and Riband, was investigated using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). The results showed the conformation of proteins varied between flour, hydrated flour, and hydrated gluten. The

β -sheet structure increased progressively from flour to hydrated flour and to hydrated gluten. In hydrated gluten protein fractions comprising gliadin, soluble glutenin, and gel protein, β -sheet structure increased progressively from soluble gliadin and glutenin to gluten and gel protein; β -sheet content was also greater in the gel protein from the breadmaking flour Hereward than the biscuit flour Riband.

Gluten is the major storage protein fraction of wheat flour. Gluten proteins are considered to be responsible for the viscoelastic properties of hydrated wheat flour doughs, which are of considerable importance in determining baking quality (Dobraszczyk and Morgenstern 2003). Gluten proteins comprise a highly polydisperse system of polymers, classically divided into two groups based on their extractability in alcohols: gliadins and glutenins. Gliadins are single-chain polypeptides, while the glutenins are multiple-chain polymeric proteins in which individual polypeptides are thought to be linked by interchain disulfide and hydrogen bonds to form large macromolecular aggregates (Tatham et al 1987).

The individual glutenin polymers, HMW glutenin subunits (HMW-GS), have an extended rod-like structure ≈ 50 – 60 nm long made up of three distinct domains: a large central repetitive region that adopts a regular β -turn repeat structure; and N- and C-terminal regions that contain cysteine residues associated with α -helix structure and interchain disulfide cross-linking. It is now widely accepted that variations in the composition of the HMW-GS are responsible for variations in baking quality; in particular, it is the insoluble fraction of the HMW glutenin polymer that is best related to differences in hydrated flour strength and baking quality among different wheat cultivars (Weegels et al 1996a; MacRitchie and Lafiandra 1997).

It has been proposed that the β -turn structure of HMW-GS is organized to give a regular β -spiral structure consistent with dimensions determined by molecular modeling, viscometric analysis, and scanning tunneling microscopy (Shewry et al 2002). It has been suggested that the β -spiral structure of the central repetitive region of the HMW-GS is related to the elasticity of gluten (Pezolet et al 1992; Wellner et al 2005). Initially it was considered that the β -spiral structure could be inherently elastic, acting rather like a spring (Tatham et al 1985). The ease of extensibility of the loose, weakly bonded structure of the β -spiral, as well as its prevalence in other elastic proteins such as spider-silks, elastin, and titin (connectin) indicate that the β -spiral structure is highly involved in the elasticity of proteins, including gluten (Tatham and Shewry 2003). More recently, the loop and train model for gluten elasticity has been proposed (Belton 1999). It involves competition between glutamine residues in the repetitive domains and water for hydrogen bonding, creating mobile loop segments with β -turn structure, and the intermolecular interactions between adjacent HMW-GS to form β -sheets, called “trains”. This model

postulates that elastic energy is stored within the system by the conversion of the loops to trains upon deformation, the elastic restoring force depending on the number of hydrogen bonds, and the length and precise sequence of the amino acids in the HMW-GS (Feeney et al 2003).

Previous studies on protein conformation of gluten using ATR-FTIR showed the presence of β -turn and intermolecular β -sheets (Pezolet et al 1992; Belton et al 1995). These clearly showed that peaks in the Amide I region of the spectrum were able to discriminate the protein secondary structures of gluten proteins such as α -helical structure and random structure, β -turn, and intramolecular and intermolecular β -sheet structure. For mixtures with different gliadin and glutenin proportions, a stronger intermolecular β -sheet structure was observed with the increase of glutenin proportion in these mixtures. The results also showed that β -sheet structure was greater in the hydrated gluten than in solution (Popineau et al 1994). More recently, the conformation of the synthetic and recombinant peptide models of the central repetitive domains of HMW-GS were investigated with FTIR. The β -sheet to turn ratio was related to the length of repetitive domains and the sequence of the amino acid groups (Feeney et al 2003); more intermolecular β -sheet structures were observed with increasing subunit molecular weight. Van Velzen et al (2003) showed that deformation of hydrated flour caused a decrease in the amount of α -helix content and an increase in β -sheet and Wellner et al (2005) showed a conversion of β -turn to β -sheet structures on repeated extension.

One of the problems with these results is that HMW-GS comprise only 10–12% of gluten protein and if, as postulated, changes in sheet-to-turn ratio are due to HMW subunits, then any changes observed will be highly dependent on concentration of HMW-GS. Furthermore, the insoluble fraction of glutenin (the gel protein fraction comprising $\approx 10\%$ of the glutenin fraction) is closely related to flour quality and breadmaking performance (Weegels et al 1996b) and to long-time relaxation processes in gluten (Li et al 2003). Therefore, in view of the low concentration of the proposed active fractions in β -turn to sheet conversion, it was considered necessary to investigate the effect of separation of gluten subfractions on polymer conformation of proteins using ATR-FTIR.

The aim of this study was to investigate the polymer conformation of proteins in flour, hydrated flour, and gluten prepared from wheat cultivars with a range of baking performance using ATR-FTIR. The conformation of gluten subfractions from U.K. breadmaking and biscuitmaking flours was also measured using the same technique. The results obtained in the present study are expected to provide more information on gluten physics and chemistry in terms of variation of the conformation of gluten proteins in flour and separated fractions, and thus to provide a better understanding of their functionality in breadmaking quality.

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MATERIALS AND METHODS

Flours from the U.K. wheat cultivars Hereward (strong, breadmaking) and Riband (weak, biscuitmaking) used in this study were provided by Weston Research Laboratories, Maidenhead, U.K. (now called Allied Technical Centre). A Polish winter wheat cultivar, Korweta, was provided by Instytut Hodowli i Aklimatyzacji Roslin (IHAR), Krakow, Poland. Flour quality parameters are shown in Table I. These cultivars were milled in a Brabender Quadrumat mill to obtain white flour. Protein content was determined by NIR using an Inframatic 8100 (Perten Instruments AB, Sweden).

Equal weights of freeze-dried flour from the Polish flour Korweta and distilled water were frozen separately in liquid nitrogen. The frozen distilled ice was ground with a mortar and pestle. The frozen flour and ground ice water were mixed by hand with a spatula in a bowl surrounded by liquid nitrogen to form a hydrated flour (or undeveloped dough, according to the method of Campos et al [1996]). The preparation conditions were kept constant for all flours studied. The hydrated flour was allowed to stand overnight at 4°C. Gluten was separated from chloroform defatted flours using the Glutomatic 2100. Flour (10 g) from each of the cultivars was mixed with 6 mL of distilled water and washed in a Glutomatic 2100 apparatus under a stream of 6 mL/min of distilled water for 8 min at room temperature (20°C) to produce gluten. After separation, the gluten was frozen in liquid nitrogen and freeze-dried overnight. Gliadin was separated as the supernatant by centrifuging a mixture of gluten and 70% (v/v) ethanol at 10,000 × g for 30 min at room temperature, frozen, and freeze-dried. Soluble glutenin was extracted from the residue with 0.05M acetic acid and centrifuged under the same conditions as above. The insoluble gel layer on the top of the residue was scraped off, frozen, and freeze-dried (Li et al 2003). The freeze-dried fractions of gliadin, acetic acid soluble glutenin, gluten, and gel protein were hydrated with 0.6, 1, 2, and 3 mL/g of distilled water, respectively, to achieve a fully hydrated gel. The hydrated samples were rested for 2 hr in a sealed bottle before being spread on the sample cell of the ATR-FTIR.

ATR-FTIR spectra were measured on a Bruker IFS55 spectrometer with a single reflection diamond Golden Gate ATR accessory, using a DTGS detector and a KBr beam splitter to record spectra in the 4000–400 cm⁻¹ range, at room temperature (22 ± 1°C). Opus software v. 2.0 was used to acquire and process the FTIR spectra. Acquisition parameters were 4 cm⁻¹ resolution; 128 scans; scanner velocity 4; 5.0 kHz; gain 8; aperture setting 10.0 mm; acquisition mode single-side fast return; apodization function triangular; phase correction mode Mertz; zero filling factor 2. A small sample portion was placed on the ATR crystal so that complete coverage was achieved. Five replicate spectra were obtained for each sample, rearranging the powder between consecutive acquisitions, to evaluate sample heterogeneity effects (SD < 2%, n = 5). The spectra were normalized to unit length in the 1900–600 cm⁻¹ region using the equation

$$\|V\| = \sqrt{\sum_{i=1}^m V_i^2}$$

$$V_{\text{normalized}} = \frac{V_{\text{nonnormalized}}}{\|V_{\text{nonnormalized}}\|}$$

where V is a vector representing a spectrum with m points or wavenumber values. All spectra were converted into Excel format and aligned before spectral subtraction of the water spectrum for the hydrated flour and hydrated protein samples. A subtraction factor was consistently chosen so that the intensity of the broad water band at 2110–2060 cm⁻¹ (a combination band arising from water alone; that is, clear of other interference bands) was nulled.

The amide I band was closely examined for all spectra, in terms of band intensities relative to that of the 1650 cm⁻¹ band and measured from the baseline of the spectrum normalized as described above. Average values were calculated for each intensity ratio.

RESULTS

Typical ATR-FTIR spectra for hydrated flour and hydrated gluten protein of Korweta flour are shown in Fig. 1. Three peaks can be clearly distinguished in the spectrum for Korweta hydrated flour, which can be assigned to protein components amide I (1580–1720 cm⁻¹), amide II (1480–1580 cm⁻¹), and amide III (1430–1480 cm⁻¹) (Purcell et al 1988; Popineau et al 1994; Mangavel et al 2001). In addition, the peaks at 1170–900 cm⁻¹ are assigned to C–O–C stretching and CO (–COH) stretching of starch, which appeared in the spectrum for hydrated flour but were not observed in the spectrum for hydrated gluten, indicating that the starch had been removed from the gluten proteins during washing. Within the amide peaks, amide I has been defined as mainly arising from amide carbonyl stretching, a combination of amide NH bending and CH stretching that can be used to characterize the protein secondary structures (Fig. 2). A peak at 1650 cm⁻¹ is associated with α -helical and random structure, the shoulder at \approx 1668 cm⁻¹ is associated with β -turns and may also be related to glutamine side chains. Bands at 1612 cm⁻¹ and 1633 cm⁻¹ are assigned to intermolecular and intramolecular β -sheet structure, respectively (Popineau et al 1994; Wellner et al 2005). For the amide I peaks for flour, hydrated flour, and hydrated gluten from Korweta flour (Fig. 2), it was observed that the relative intensities at 1633 cm⁻¹ and 1612 cm⁻¹, compared with that of the 1650 cm⁻¹ band, gradually increased from flour to hydrated flour and finally to hydrated gluten. In contrast, the intensity at 1668 cm⁻¹ (β -turn) decreased from flour to hydrated flour and then to gluten. Furthermore, both flour and hydrated flour seemed to contain a higher contribution from the 1650 cm⁻¹ band, reflecting α -helical structure. The results showed that hydrated gluten had more intermolecular and intramolecular β -sheet structure (1612 and 1633 cm⁻¹), and less β -turn and α -helical structure (1668 and 1650 cm⁻¹) than the corresponding hydrated flour and flour. In addition, hydrated flour showed more intermolecular and intramolecular β -sheet structure and less β -turn structure than the corresponding flour.

ATR-FTIR scans on the gluten subfractions showed gliadin, glutenin, and gel protein, fractionated from typical U.K. breadmaking and biscuitmaking flours, Hereward and Riband. In the ATR-FTIR spectra for the hydrated gluten subfractions of Hereward

TABLE I
Flour Analytical Data for Three Wheat Cultivars^a

	Hereward	Riband	Korweta
Moisture (%) ^b	13.4	13.2	13.6
Protein (N × 5.7) % ^c	10.7	9.7	11.0
Water absorption % ^d	61.1	55.6	54.3
Starch damage % ^e	35	15	30
Hagberg Falling No. ^f	517	338	480
Ash % ^g	0.54	0.62	0.48

^a Flour analyses at Weston Research Laboratories (Allied Technical Centre), Maidenhead, UK.

^b ISO method 712 (1998)

^c Protein content determined by NIR (Inframatic 8100, Perten Instruments AB, Sweden), calibrated against the Dumas method.

^d Water absorption obtained by Approved Method 54-21 (AACC International 2000).

^e Rapid flow analyzer (Alpkem RFA 300) calibrated against method of Farrand (1964).

^f Determination of falling number by Approved Method 56-81B (AACC International 2000).

^g Ash determined by Approved Method 08-01 (AACC International 2000).

(Fig. 3), it was observed that the gliadin and soluble glutenin fractions had weaker and very similar relative intensities for β -sheet content (1633 cm^{-1} and 1612 cm^{-1}) and relatively greater β -turn intensity at 1668 cm^{-1} . In the insoluble gel protein fraction, β -turn and α -helical structures were at levels similar to those in the whole gluten but the strongest relative intensity was at $>1650\text{ cm}^{-1}$, indicating that gel protein had the highest proportion of intramolecular and intermolecular β -sheet structure. Interestingly, the whole gluten protein, being a mixture of gliadin, soluble glutenin, and gel protein fractions, had a β -sheet conformation intermediate between gliadin, glutenin, and gel protein extracted from Hereward flour. This is similar to the stress relaxation behavior of gluten fractions observed by Li et al (2003), where the long-time relaxation behavior of gliadin and glutenin were very similar, and that for gluten was intermediate between gliadin, glutenin, and gel protein.

The spectra for gluten fractions of Riband flour were very similar to those of Hereward flour (not shown). Differences were found in the amounts of intermolecular and intramolecular β -sheet structure in gel proteins between Hereward and Riband (Fig. 4). The hydrated gel protein of Hereward flour had more β -sheet structure than that of Riband; the increase in β -sheet was more pronounced between hydrated gels than between freeze-dried gels and the freeze-dried gels generally had lower amounts of β -sheet and greater amounts of β -turn structure.

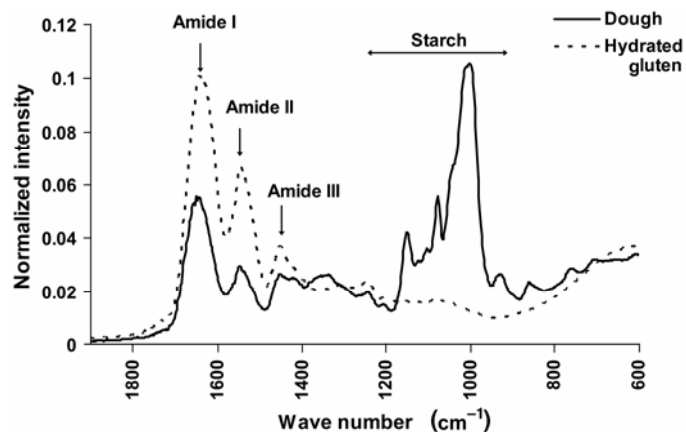


Fig. 1. Normalized ATR-FTIR spectra for Korweta hydrated flour and hydrated gluten.

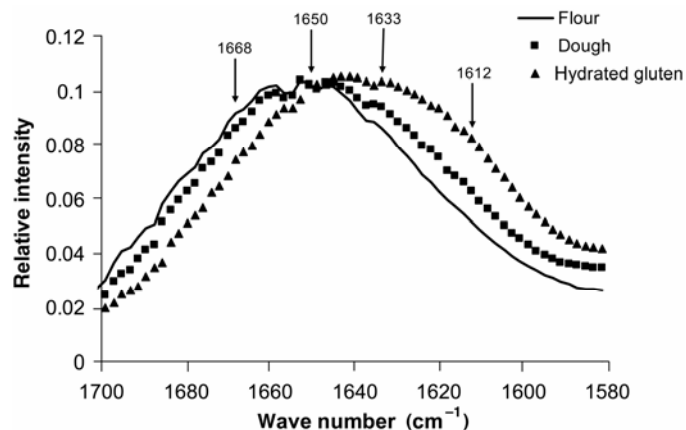


Fig. 2. Amide I region for normalized ATR-FTIR spectra of Korweta flour, hydrated flour and hydrated gluten. Assigned bands: 1668 cm^{-1} β -turn; 1650 cm^{-1} α -helical structure; 1633 cm^{-1} intramolecular β -sheet; 1612 cm^{-1} intermolecular β -sheet.

DISCUSSION

In the present study, the conformation of proteins in flour, hydrated flour, and gluten in its hydrated state was investigated by ATR-FTIR techniques. The results clearly showed that the conformations of proteins varied between flour, hydrated flour, and gluten. The ATR-FTIR spectra in Fig. 2 showed that hydrated flour had a much greater amount of β -sheet structure than the corresponding flour. This indicates that hydration of flour produces more β -sheet structure, which is considered to be related to elasticity in dough and gluten (Belton 1999; Tatham and Shewry 2003). Furthermore, the results in the present study also showed that gluten in its hydrated state had much more β -sheet structure compared with that in the corresponding hydrated flour and flour. This phenomenon suggests that gluten proteins in their isolated state are able to form more β -sheet structure at the expense of β -turn structure. The isolation and hydration of gluten by washing and mixing leads to a considerable increase in β -sheet and decrease in β -turn structure. A difference in the amount of β -sheet structure between gluten protein isolated by mixing and centrifugation was also observed by Wellner et al (2005).

The conformations for gluten fractions shown in Fig. 3 observed in the present study are in agreement with previous work (Popineau et al 1994; Mangavel et al 2001). The soluble glutenin

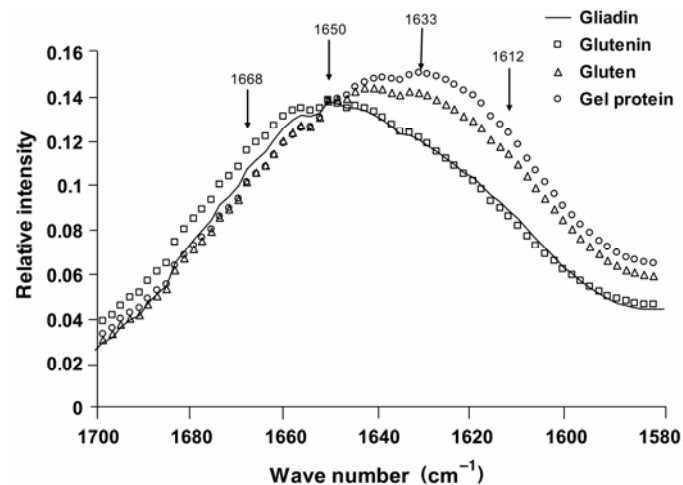


Fig. 3. Normalized ATR-FTIR spectra for hydrated Hereward gluten fractions: gliadin, soluble glutenin, gluten, and gel protein.

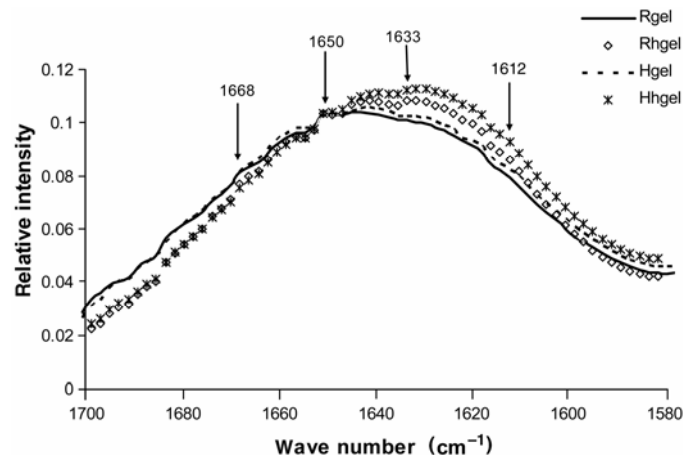


Fig. 4. Normalized ATR-FTIR spectra for freeze-dried and hydrated gel proteins obtained from Hereward and Riband: Rgel, freeze-dried Riband gel protein; Rhgel, hydrated Riband gel protein; Hgel, freeze-dried Hereward gel protein; Hhgel, hydrated Hereward gel protein.

fraction was richest in β -turn structure, which has been related to the amount of HMW subunits present in glutenin (Parchment 2001) but had much less β -sheet structure. Gliadin and glutenin had very similar β -sheet content, which was much lower than both gluten and gel protein. Gel protein had the highest proportion of intramolecular and intermolecular β -sheet structure. The β -sheet content of gluten was intermediate between gliadin, glutenin, and gel protein. Gel protein content has been closely related to flour quality and breadmaking performance (Weegels et al 1996b; Singh and MacRitchie 2001) and able to form a polymer network structure, observed in stress relaxation measurements (Li et al 2003). More recently, Wellner et al (2005) found that during relaxation of gluten, the ratio of β -sheet to β -turn decreased in the same timescale as in the stress relaxation measurements. It is suggested that the presence of β -sheet structure gives a long-time relaxation process, which is typical of the relaxation of a polymer network structure. Therefore, it is proposed that gliadin and soluble glutenin molecules mainly form β -turn structures and insoluble glutenins in gel protein form a β -sheet structure, which is the main component that contributes to the formation of a network structure and elastic properties to gluten protein. In a previous study, the long-time relaxation properties of gluten were a composite of gliadin, glutenin and gel protein, with gel protein contributing mainly to the long-time relaxation process and gliadin and gluten contributing to the shorter time relaxation processes (Li et al 2003). A more pronounced peak in the long-time relaxation process was observed for the gel protein from Hereward flour, indicating more polymer network structure in Hereward dough than in Riband. In this study, more β -sheet structure was also observed for the gel protein from Hereward flour than that from Riband under the same hydration conditions. This difference in the polymer conformation and network structure may account for the variation in baking performance between the two flours. It remains a challenge to explore the relationships between polymer conformation, relaxation properties, elasticity, and breadmaking performance of different wheat cultivars in more detail.

CONCLUSIONS

The conformation of flour proteins varied in flour, hydrated flour, and gluten. Protein in the hydrated flour had more β -sheet structure than that measured in flour. Gluten proteins had increased β -sheet structure and less β -turn than that in hydrated flour and flour. In gluten protein fractions, gliadin and soluble glutenin fractions had lower amounts of β -sheet content and relatively greater amounts of β -turn. Insoluble gel protein had the greatest β -sheet content. The β -sheet conformation of gluten was intermediate between gliadin, glutenin, and gel protein, similar to long-time stress relaxation behavior. Gel protein was the main component contributing to β -sheet structure in gluten.

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