Effects of different molecular weight water-extractable arabinoxylans on the physicochemical properties and structure of wheat gluten

Yunping Zhu^{1,3} · Fang Li² · Yu Wang³ · Jinlong Li² · Chao Teng^{1,3} ·
Changtao Wang¹ · Xiuting Li^{1,2}

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Abstract The effects of water-extractable arabinoxylans (WEAXs) with different molecular weights on the physicochemical properties and structure of wheat gluten were studied, a transition between β -turns in β -leaves in the structure of gluten has been demonstrated by Fourier transform infrared spectroscopy when WEAX samples were added. The ratio of weakly hydrogen-bonded β -sheets to strongly hydrogen-bonded β -sheets tended to decrease with increasing WEAX content. FT-Raman analysis demonstrated other changes in the structure of gluten concerning β -structures, the conformation of the disulfide bridges, and aromatic amino acid environments; these changes were dependent on the molecular weight of the WEAX. Native WEAXs of different molecular weights that are present as discrete fragments of the cell wall have had a negative effect on the properties of gluten. However, low molecular weight WEAX has helped gluten maintain the left–left conformation of its disulfide bonds. In addition, the gluten elasticity was reduced by the native WEAX to make it softer, but the WEAX low molecular weight impeded this process and improved the gluten network.

Keywords Arabinoxylan · Molecular weight · Wheat gluten · Structure · Dynamic oscillatory rheology

Introduction

Whole wheat foods are important because of their high fiber content. Water extracting arabinoxylan (WEAX) is an important fiber in whole wheat that affects pulp properties and bread qualities because of its water content. The gas bubbles in the dough are stabilized by the viscosity of WEAX (Biliaderis et al. 1995). The latter negatively influences the gluten agglomeration behavior of the wheat dough, the oxidation of the polymers AX and the formation of a crosslinking, which could harm the agglomeration of the gluten because of the ferulated esterification of the polymers AX during the mixing process (Frederix et al. 2004a; Li et al. 2012).

WEAX molecular weight (Mw) has been found to have a substantial influence on the properties of whole wheat dough. Frederix et al. (2004b) have studied the influence of WEAX of different Mw on the agglomeration properties of gluten during pulp-paste and gluten–starch separations. However, at this juncture, the mechanisms of interactions between WEAXs of different Mws and gluten during mixing and cooking/steaming processes are still unclear.

Gluten affects the properties of wheat products, which gives the dough increased water absorption capacity, cohesion, viscosity and elasticity (Noort et al. 2010). The structure of the gluten network is minimized by non-covalent bonds (hydrogen bonds, ionic bonds and hydrophobic interactions). Although members of this class of chemical bonds are less energetic than covalent bonds, non-covalent bonds provide an important influence on protein aggregation and dough structure. Covalent bonds,

✉ Changtao Wang
wangct@th.btbu.edu.cn

✉ Xiuting Li
lixt@th.btbu.edu.cn; li_xiuting@163.com

¹ Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University (BTBU), Beijing 100048, People's Republic of China

² School of Food and Chemical Engineering, Beijing Technology and Business University, No. 33, Fucheng Road, Beijing 100048, People's Republic of China

³ Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology and Business University (BTBU), Beijing 100048, People's Republic of China

such as disulfide bonds, have important effects in cooking in the method of analysis of protein structure (Schofield et al. 1983), Raman and FITR microspectroscopy are two classical methods of analysis, because information on molecular vibrations and gluten structures are given by Raman microscopy and FITR Raman presents benefits, including ease of data acquisition, higher resolution, and the ability to perform confocal measurements (Thygesen et al. 2003). For example, Nawrocka et al. (2016a, b) applied FT-Raman spectra to reveal changes in the structure of seven fiber modified gluten proteins.

We investigated the impact of WEAX on changes in the structural network of gluten formed during heat treatment in a previous study. The objective of the present work was to study the mechanisms of interactions between WEAXs of different Mws and gluten by analyzing the structure and physicochemical properties of wheat gluten. Three natural WEAXs of different Mw and WEAX fragments with different Mw obtained by acid hydrolysis were the subject of the study. The secondary structures and the rheological properties of gluten were also studied by Fourier transform infrared spectroscopy (FTIR), FT-Raman and rheometry.

Materials and methods

Materials

The wheat gluten characteristics marketed were: moisture 6.34%; crude protein, 74.96%; total starch content, 7.51% (dry basis, w/w). Low Molecular Weight AX (LMW AX) (Batch No. 120601b), AX Average Molecular Weight (MMW AX) (Batch No. 40601) and High Molecular Weight AX (HMW AX) (Batch No. 120707a) (> 94 g of arabinoxylan AX/100 g dry matter) were purchased from Megazyme International Ireland Ltd. (Bray, Ireland). All chemical reagents were analytical grade.

Preparation of WEAX fragments of different Mws

A 250-mg portion of HMW AX was added to 25 mL of 0.15 M citric acid solution. The hydrolysis reactions were carried out in a rotary shaker (150 rpm) at 75 °C for 2 h, 4 h, and 6 h and then terminated by adding 31.25 mL of 0.15 M NaOH solution. The WEAX samples hydrolyzed for 2 h, 4 h and 6 h were concentrated and dialyzed to remove the arabinose substituents and oligosaccharides respectively (cutoff Mw of 10,000). The samples inside the dialysis bags were then freeze-dried and used as the WEAX fragments of different Mws.

Reaction of WEAX and gluten

Gluten powder was thoroughly blended with 0% and 5% solutions (w/w, per gram of gluten) of WEAX (three natural WEAX samples and three WEAX fragments with different Mws). A 1.0 g gluten sample and 5.0% WEAX solution were mixed manually in a 50-mL centrifuge tube. As a control sample, 5.0 mL of distilled water was mixed with gluten. Excess water was added to determine the effect of WEAX on gluten without water becoming a limiting factor (Bárcenas et al. 2009; Toyokawa et al. 1989). All samples were freeze-dried, and the freeze-dried gluten was ground and stored in a Ziploc bag at 4 °C until further analysis (Zhu et al. 2017).

High-performance size exclusion chromatography (HPSEC)

The distributions of molar masses of the WEAX fragments with different Mws and three natural WEAX samples were determined by high-performance size exclusion chromatography (HPSEC), which was performed on a Water HPLC system equipped with ACQUITY APC columns and a Waters 2410 RI detector. We can get the parameters, namely, weight (Mw) and number (Mn) average molecular weight and polydispersity index (Mw/Mn) of WEAXs. The column temperature was maintained at 35 °C, and the refractometer was held at 40 °C. The eluent was 100 mM NaNO₃, and the flow rate was 0.5 mL/min. Samples solutions in distilled water (2 mg/mL) were filtered through 0.45 µm syringe filters prior to injection, and the injection volume was 100 µL. The Mw was estimated by comparison to a calibration curve prepared from Dextran T-series standards with known mean Mws (6600, 9900, 26,290, 21,400, 43,500, 124,000, 196,000, 277,000, and 401,000 Da). The formula for determining the Mw is $\text{Log Mw} = 8.13e+0.01 - 4.36e+0.01T^1 + 1.02e+0.01T^2 - 1.21e+0.00T^3 + 7.24e-0.02T^4 - 1.74e-0.03T^5$, where T is the retention time of the WEAX samples under the above experimental conditions (Rakha et al. 2010).

Structure of gluten proteins

Infrared spectra of gluten samples were recorded using an iS50 FT-IR spectrophotometer (Thermo Nicolet Inc., Waltham, MA, USA) equipped with a horizontal multi-reflectance diamond accessory. Spectra were collected in the 4000–400 cm⁻¹ infrared spectral range at room temperature. For each sample, 64 scans at a resolution of 4 cm⁻¹ were averaged, and a minimum of three replicate spectra were obtained. A background spectrum without any sample present was collected before each sample spectrum was collected. The quantity of secondary structures of the

gluten in the gluten dough in the amide I region ($1700\text{--}1600\text{ cm}^{-1}$) of gluten was estimated by using Omnic software (version 8.0, Thermo Nicolet Inc., Waltham, MA, USA). The individual peaks in the amide I region substantially overlapped and were thus resolved with Fourier self-deconvolution by using the method described by others with an enhancement factor of 1.3 and a bandwidth of 30 (Herald and Smith 1992). The second derivative of the Fourier self-deconvoluted spectra was then calculated to determine the positions of the absorbance peaks located in the amide I region. The intensities of the absorbance peaks at 1683 cm^{-1} , 1649 cm^{-1} , 1665 cm^{-1} , 1632 cm^{-1} and 1613 cm^{-1} were manually calculated. The glutamine absorbance peak at 1600 cm^{-1} was used to normalize the other five peaks at different wave numbers. The positions and assignments of amide bands have been summarized by previous authors (Georget and Belton 2006), but the intensity of the peaks at $1683/1649$, $1665/1649$, $1665/1683$, and $1632/1613\text{ cm}^{-1}$ were calculated to evaluate the relative proportion of each secondary structure.

The disulfide bridge region ($490\text{--}550\text{ cm}^{-1}$), the tyrosine doublet in an aromatic amino acid environment [$I(850)/I(830)$], and the tryptophan band [$I(760)$] were analyzed by FT-Raman spectra (Sugeta 1975). Structural analyses of the disulfide bridge [percentage distribution of disulfide bridge conformations: gauche–gauche–gauche (SSg–g–g), trans–gauche–gauche (SSt–g–g), trans–gauche–trans (SSt–g–t)] were also conducted using Omnic software. Raman spectra were collected on a BrukerMultiRAM FT-Raman spectrophotometer (Bruker Optics, Germany) equipped with Nd:YAG laser at 1064 nm . Spectra were collected at room temperature with a laser power of 251 mW and spectral resolution of 4 cm^{-1} . High signal-to-noise ratio was assured by averaging 256 scans to generate each spectrum. FT-Raman spectra were plotted as intensity (arbitrary units) against Raman shift in wave numbers (cm^{-1}). The spectra were automatically baseline corrected across the whole range ($3500\text{--}50\text{ cm}^{-1}$) in OMNIC software (version 8.2, Thermo Fischer Scientific Inc., USA). Spectral data from the sample scans were manually normalized against the phenylalanine band at 1003 cm^{-1} using Origin software (Zhou et al. 2014; Nawrocka et al. 2016a).

Dynamic oscillatory tests

Dynamic rheological measurements of gluten with WEAX were determined by using an AR1500ex rheometer (TA Instruments, New Castle, DE, USA) under strain control mode as described by Bárcenas et al. (2009) with some modifications. The measuring system included a cone and a plate (40 mm diameter, 1 mm gap) to eliminate slippage

during tests. A 1.0 g gluten sample was combined with 5.0 mL of 0%, 2.5%, and 5% (w/w, per gram of gluten) WEAX solutions and centrifuged at 2000 g for 10 min , and the supernatant was discarded. After centrifugation, the samples consisted of hydrated gluten and had reached their optimum water-binding capacity. The gluten dough was placed between the plates within 1 h of hydration, and the test was started after 15 min of resting so that residual stresses can relax. The rim of each sample was coated with silicon oil to prevent evaporation during measurements. Strain sweeps at frequency of 1 Hz and a strain of 3% were selected within the linear viscoelastic region. A frequency sweep from 0.1 to 10 Hz was performed at constant strain.

Statistical analysis

The results were statistically analyzed by using SPSS (Systat Software Inc., San Jose, USA). ANOVA was used to determine significant differences between the results, and Duncan's test was used to compare the means that were significantly different at the level of 0.05.

Results and discussion

HPSEC analysis of WEAX

The HPSEC results for Mw distributions of the three natural WEAX samples, namely, their molar mass distribution and their average parameters [weight average molar mass (Mw), number average molar mass (Mn), and polydispersity], are summarized in Table 1. Mw is more dependent on the properties of the macromolecules than other parameters, and it incorporate one of the most important parameters of the polymer. The Mw distribution of the polymer is given by its polydispersity, which defines the Mw of the polymer dispersion, making it easy to discuss the relationship between the properties of the polymer and its microstructure (Rochas and Lahaye 1989). The HPSEC analysis of the three natural WEAX samples proved Mw and Mn values in the range of $1.33 \times 10^5\text{--}2.08 \times 10^5\text{ g/mol}$ and $6.71 \times 10^4\text{--}1.61 \times 10^5\text{ g/mol}$, respectively. The polydispersity of the HMW WEAX was the lowest, indicating that the Mw distribution was more

Table 1 Mw of different natural arabinoxylan samples

Arabinoxylan	Mn (g/mol)	Mw (g/mol)	Polydispersity
LMW AX	67,135	133,199	1.984
MMW AX	140,300	197,188	1.4055
HMW AX	161,318	207,764	1.2879

concentrated and the Mw uniformity was highest. Thus, the HMW WEAX sample was selected to prepare different Mw fragments by acid treatment.

Preparation of different Mw WEAX fragments by acid treatment

Arabinoxylan (AX) has a linear backbone of β -(1-4)-linked D-xylopyranosyl units with α -L-arabinofuranosyl substituent attached to the backbone through O-2 and/or O-3 (Revanappa et al. 2015). Acid randomly hydrolyzes the glycosidic bonds between adjacent xylose units, cleaving the backbone of AX and concomitantly decreasing the degree of polymerization while increasing the solubility of the polymer and releasing smaller fragments such as monosaccharides and xylooligosaccharides (Santala et al. 2011).

To get hydrolyzed AX samples with more identical Mws, dialysis was used to eliminate the salts and the acidified xylose, arabinose, and oligosaccharides who could interfere with the results. WEAX fragments with different Mws post-treated with citric acid are represented in Table 2, revealing that the Mw of HMW AX decreases with increasing hydrolysis time. The Mn is greatly influenced by the LMW polymers in the system; thus, increasing the content of LMW xyloses, oligosaccharides, and arabinoses would broaden the distribution of the mixture. Dialysis is beneficial for obtaining uniform and pure Mw samples of arabinoxylans. The yield of arabinoxylan with a Mw of 129,000 was 80% and was generated from the commercial arabinoxylan with a Mw of 208,000. Similarly, the yield of arabinoxylans with a Mw of 90,000 and 78,000 were 69.2%, and 50.64%, respectively, where the yield was calculated as the ratio of the mass of the arabinoxylans after freeze-drying to the total mass of the arabinoxylans before hydrolysis.

Effects of WEAX samples of different Mws on the secondary structure of gluten

The effect of addition WEAXs of different Mws on the FTIR spectrum of gluten protein revealed that the amide I and amide III spectral bands were more sensitive to

changes in the secondary structure folding than any of the other spectral features arising from the coupled and uncoupled stretching and bending modes of amide bonds (Buksa 2016). The spectrum of the gluten sample showed a strong absorption band in the amide I ($1700\text{--}1600\text{ cm}^{-1}$) region mainly due to the C=O stretching vibrations of the peptide linkages. In the FTIR experiments, small but reproducible changes were observed in the amide bands of fully hydrated gluten, indicating that the secondary structure changed during treatment (Wellner et al. 2005). In hydrated gluten that contains glutenins and gliadins, which are extremely important to the secondary structure of the protein, the α -helices are present in the N- and C-terminal domains, and the β -turns and intermolecular β -sheets are mainly found in the central repeat domain (Gilbert et al. 2000). Thereby, a “loop and train” model has been proposed and suggests that the terminal domain develop the conversion between β -turns and β -sheets (Georget and Belton 2006). In this model, low moisture conditions resulting to an abnormal structure in the glutenins that is stabilized and tightly arranged by hydrogen linking. Under intermediate moisture conditions, competition between the glutamine side chains and the hydrogen bonds in the water produces loop segments containing β -turns, and the inter-chain interactions form β -sheet regions called trains. The increase in the water content shifts the balance between β -turns and β -sheets toward β -turns. The properties of gluten proteins are mainly determined by the amount and ratio of chain entanglements, hydrogen bonds, covalent bonds, hydrophobic interactions, electrostatic forces, and disulfide bonds in the dough (Zhu et al. 2017). The changes in the secondary structure of the gluten after adding each of the three WEAX fragments with different Mws are given in Fig. 1a–d, and the relative contents of β -sheets and their impacts on the properties of the gluten are shown. The ratio of the intensity of the bands at 1683 and 1649 cm^{-1} corresponds to the ratio of β -sheets to α -helix + random coil. The most significant increase in the β -sheets to α -helix + random coil ratio was observed when the three WEAX fragments with different Mws were added, and the addition resulted in an increase in β -sheets (1683 cm^{-1}) and a decrease in α -helical (1649 cm^{-1}) structures.

This result is coherent with previously reported data (Georget and Belton 2006), indicating that the secondary structure of gluten is dominated by α -helices. Katyal et al. 2017 earlier reported the similar results for Indian wheat varieties. The positive correlation of IM- β -sheets and AP- β -sheets and negative correlation of α -helix with dough stability was observed. Indian durum wheat (PDW291) gluten demontred the prevalence of higher proportion of IM + AP- β -sheets and lower of α -helix indicating their affects on dough and noodle characteristics (Kaur et al. 2015). All those results showed that the dough with a

Table 2 Mw and polydispersity of hydrolyzed arabinoxylan products (WEAX fragments from acid treatment of the HMW AX)

Time (h)	Mn (g/mol)	Mw (g/mol)	Polydispersity
2	76,953	129,489	1.6827
4	53,587	90,484	1.6885
6	44,512	78,478	1.7631

Fig. 1 Effect of natural WEAXs on the secondary structure content of gluten: **a** 1683/1649; **b** 1665/1649; **c** 1665/1683; **d** 1632/1613

higher proportion of β -sheets and a lower proportion of α -helix had greater gluten strength. Furthermore, the decrease in the α -helix content is related to the amount of WEAX sample added. If a larger quantity of WEAX is added, the α -helix content will be reduced to a greater degree. The ratio of β -sheets to α -helices + random coil was highest when 5% of the WEAX solution prepared from the MMW AX (MMW AX) was added. As shown in Fig. 1B, the values of the 1665/1649 ratio are indicators of the ratio of β -turns to α -helices + random coil.

There were no obvious regular patterns in the effects of the three natural WEAXs with different Mws on the ratio of β -turns to α -helices. As shown in Fig. 1c, when adding the three natural WEAXs with different Mws, the values of 1665/1683, representing the ratios of β -turns to β -sheets, decreased compared to the control sample, indicating a shift in the equilibrium between β -turns and β -sheets. This may be allied to the qualities of the AX, which can absorb many times their weight in water and influence the properties of the gluten in the dough, including absorbing the

water in their micro environment, produce the water content in the gluten to decrease (Döring et al. 2017). According to the “loop and train” model, as the water content decreases, the balance between β -turns and β -sheets lag toward β -sheets. This hypothesis was validated by measuring the strength of the hydrogen bonds (Fig. 1d), and a greater percentage of weakly hydrogen-bonded β -sheets were present than strongly hydrogen-linked β -sheets. A significant difference in the ratio between the control gluten and WEAX-gluten was observed. The content of weakly hydrogen-bonded β -sheets decreased and that of strongly hydrogen-bonded β -sheets increased, indicating that the decrease in the water content in the gluten entail not only the content of β -sheets to increase but also increase the strength of their hydrogen bonds.

Changes in environments surrounding aromatic amino acids

Raman bands corresponding to the oscillations of two amino acids, tryptophan (TRP) and tyrosine (TYR), convey information about the hydrophobic environment (TRP band at 760 cm^{-1}) and hydrogen link at 850 and 830 cm^{-1} (tyrosine doublet) (Nawrocka et al. 2016a). The TRP band at 760 cm^{-1} has been suggest to be an indicator of the power of the hydrogen bonds and the hydrophobicity of the indole ring (Linlaud et al. 2011). The addition of the three natural WEAX samples and the three WEAX fragments with different Mws destored the intensity of the TRP band at 760 cm^{-1} (Fig. 2a). No significant difference was spotted between the normalized Raman intensities of the LMW arabinoxylan and MMW arabinoxylans compared with that of the control sample. However, remarkable modifications in the severity of the TRP band for HMW arabinoxylans was observed, indicating the generation of a hydrophilic environment. WEAX is known as a hydrophilic polysaccharide as it contains a large number of

hydroxyl groups; it thus forces the hydrophobic regions in gluten aggregate to decrease surface tension. These results are in consistent with the results of Nawrocka et al. (2015), who studied the interactions between gluten proteins and fiber preparations in gluten dough. Subsequently, an obvious increase in the intensity of the band 760 cm^{-1} was observed when HMW AX that had been hydrolyzed for 2 h (Mn approximately 77,000) was added, indicating an increase in the hydrophilicity of the tryptophan. However, this increase has not yet been spotted with lower-molecular-weight AX samples (Mn approximately 54,000) (Table 2). Although, the disulfide links are assumed to be the main bonds responsible for the formation of the gluten network during dough mixing, tyrosine bonds (hydrogen bonds) also take part in this process (Wieser 2007; Nawrocka et al. 2016b).

The tyrosine doublet [$I(850)/I(830)$] is a right indicator of hydrogen bonding involving the phenolic hydroxyl group. A lowering in the value of the ratio reflects an increase in how profound the phenolic hydroxyl group is buried, which suggests TYR residues could be engaged in the intermolecular and intramolecular interactions (Nawrocka et al. 2016a). As shown in Fig. 2b, a decrease in the ratio relative to that of the control was observed for the three natural WEAXs with different Mws, indicating that WEAX causes the TRY residues to be more buried based on the possible involvement of the TYR residues in the intermolecular and intramolecular interactions. A significant decrease in this value was observed when HMW AX that had been hydrolyzed for 2 h (Mn approximately 77,000) was added since that AX sample increases how buried the TYR residues are to a greater extent than other the AX samples. Moreover, the burial tendency and the aptitude to form hydrogen bonds are related to the Mw of the arabinoxylans. To this end, we found that after 4 h of acid hydrolysis (the average Mw of the arabinoxylans was less than 47,000 g/mol), the addition of the sample had no effect on the tyrosine environment. These results echoed a previous discovery that significant alterations in microenvironment of tyrosine were linked with polysaccharide–protein interaction (Zhou et al. 2014). Whether tyrosine is exposed or buried is more dependent on its microenvironment. According to Zhou et al. 2014, addition of 2.5% Konjac glucomannan affects conformational behaviour of gluten, thus polarity around tyrosine residues was decreased and the hydrophobicity was increased.

Fig. 2 Effect of WEAX on the tertiary structure content of gluten: **a** 760, **b** 850/830

Changes in the conformations of the disulfide (S–S) bridges

Gluten proteins contain approximately 2% cysteine, which participates in S–S formation and is important for the structure and functionality of gluten. Most cysteines are

present in their oxidized form and are concerned in either intrachain or interchain disulfide bonds. The disulfide S–S stretches are visible in the $500\text{--}550\text{ cm}^{-1}$ region, which allows the disulfide band to be used to study the structural changes in the protein. Analysis of the disulfide region ($500\text{--}550\text{ cm}^{-1}$) of the spectra showed bands located at 510 cm^{-1} (SSg–g–g), 525 cm^{-1} (SSg–g–t), and 540 cm^{-1} (SS_t–g–t) (Zhou et al. 2014). In the control spectrum, the S–S band is prominent and is located at approximately 510 cm^{-1} , indicating that these bonds are primarily in the g–g–g conformation, which is a stable conformation, followed by the g–g–t conformation, and the t–g–t content was relatively low (Fig. 3a). Similar results were obtained in a previous study on gluten removed from commercial flour by washing (Nawrocka et al. 2015). From Fig. 3a, analysis of the percentage distribution of the conformations of the disulfide bridges showed that the number of S–S bonds in the g–g–t conformation decreased, especially with the addition of HMW AX, but the number of S–S bonds in the t–g–t conformation remained unchanged. The number of S–S bonds in the g–g–g conformation increased relative to the control sample when the three natural WEAXs (LMW

AX, MMW AX, HMW AX) were added, indicating that the WEAX is beneficial to maintaining the g–g–g conformation of the S–S bonds in the gluten protein. The g–g–g conformation is more stable than the g–g–t and t–g–t conformations. No significant effect was observed on the S–S band located at approximately 540 cm^{-1} (SS_t–g–t); this is unlike the 520 cm^{-1} band (SS_g–g–t) where a significant effect on the S–S conformation was observed (Fig. 3b). When WEAX that had been hydrolyzed for 6 h (Mw approximately 78,000) was added, the ratio of the intensity of the gauche–gauche–gauche conformation (540 cm^{-1}) and the gauche–gauche–trans conformation peaks (520 cm^{-1}) was almost 1:1. These results suggest that the observed transformation of the disulfide bridges from a more stable conformation (g–g–g) to a less stable conformation (g–g–t) led to weakening of the gluten network when LMW WEAX was added. Weakening of the protein structure could be caused by the limited access of the gluten proteins to water because the dietary fiber preparations absorb considerable amounts of water (Nawrocka et al. 2016a).

Fig. 3 The S–S region of the spectra of the gluten samples. **a** Three natural WEAXs, **b** WEAX fragments with different Mws from acid treatment

Dynamic oscillatory tests

The oscillatory frequency sweep measurements of all samples were conducted in their linear viscoelastic range. The dynamic shear storage modulus (G') is a measure of the energy recovered per cycle of deformation and represents the solid or elastic character of the material. The loss modulus (G'') is an estimate of the energy dissipated as heat per cycle of deformation and is regarded as an indicator of the viscous properties of the material. The loss tangent ($\tan\delta = G''/G'$) indicates the elastic and viscous properties of the material (Porter and Johnson 1966). The rheological properties of gluten made with natural WEAXs, WEAX fragments, and the control (without WEAX) were determined. The typical rheological characteristics and the changes in G' and G'' for the samples prepared with the three natural WEAXs with different Mws showed that $G' > G''$ throughout the tested range and the modulus frequencies were dependent on the WEAX; the

gluten samples showed characteristics of a solid viscoelastic material (Fig. 4a) (Tang et al. 2014). The values of both G' and G'' for gluten made with natural WEAXs were significantly lower than similar values for control gluten, indicating that WEAX can reduce the viscoelasticity of gluten and prevent the formation of a gluten protein network. Similar results were obtained by other studies (Wang et al. 2004); WEAX has been reported to have a dilution effect on the gluten network and interfere in the attraction between particles. Our results confirm that the rheological properties of gluten might be linked to the Mw and viscosity of the WEAX. In addition, the $\tan\delta = G''/G'$ for gluten made with WEAXs with different Mws was significantly higher than those of the gluten samples made without WEAX, and the sample prepared with HMW arabinoxylan had the highest $\tan\delta$ value. Thus, gluten containing WEAX was relatively more viscous than it was elastic compared to gluten without WEAX; this result indicates that during gluten network development, WEAX

Fig. 4 Mechanical spectrum of gluten doughs with added WEAX samples with different Mws: **a** three natural WEAXs; **b** WEAX fragments with different Mws from acid treatment; **c** curves of $\tan\delta$

after adding the three natural WEAXs; **d** curves of $\tan\delta$ after adding WEAX fragments with different Mws from acid treatment

inhibition produces more crosslinking than the control (Porter and Johnson 1966). Theoretically, WEAX reduces the elasticity of gluten because WEAX interacts with gluten by noncovalent interactions (hydrogen bonding and hydrophobic interactions) causing the gluten to exhibit lower extensibility and a lower rate of aggregation (Zhu et al. 2017). Moreover, WEAX increases the viscosity of the system, limiting gluten hydration and thus interfering with the ability of gluten to form a network (Zhang et al. 2016). The rheological characteristics and changes in G' and G'' for each gluten sample mixed with either unhydrolyzed (0 h) or acid-hydrolyzed (2 h, 4 h, and 6 h) WEAX or the control (without WEAX) are presented in Fig. 4b. The addition of unhydrolyzed HMW AX was associated with reductions in the elastic (G') and viscous (G'') moduli compared to the control gluten, indicating that WEAX limits the ability of gluten to form a network. However, both G' and G'' increased with the addition of acid-hydrolyzed HMW AX compared with the addition of unhydrolyzed HMW AX to the gluten, indicating an improvement in the viscoelastic properties of the gluten. A hydrolysis reaction time of 2 h produced the optimum level of hydrolysis since no significant differences were observed at 4 and 6 h. The $\tan\delta$ decreased in the presence of the acid-hydrolyzed HMW AX compared with when the unhydrolyzed HMW AX sample was added, proving that the relative contribution of the solid character (G') was increased by adding acid-hydrolyzed HMW AX. This result implies that acid hydrolysis generally leads to a more elastic gluten system. An increase in G' and a decrease in $\tan\delta$ indicated that the quality of the gluten had improved because increased elasticity prevents gas cell breakage during fermentation (Liu et al. 2017)

Conclusion

The present study attempts to explain the different WEAX-gluten weight interactions responsible for the rheological properties and the microstructure of the gluten paste. WEAXs of different molecular weights have an effect on the rheological properties, secondary structure, conformation of disulfide bridges, and aromatic amino acid environments of the gluten protein. The Fourier Transform Infrared Spectroscopy (FTIR) results showed that the addition of three WEAX fragments with different significant Mw caused a β -turn to β -leaf transition due to the decrease in the water content of the β -turn gluten. FT-Raman results showed that WEAX had evolved a hydrolyzed environment for 2 h (about 77,000 Mn) and that the phenolic hydroxyl on ferulic acid could form new H bonds with the phenyl groups of the Tyr residues not dissociated. In addition, high molecular weight WEAX

had the most detrimental impact on agglomeration behavior, but WEAX of low molecular weight may hinder this process. WEAX's low molecular weight helped gluten maintain the original left–left–left conformation of its disulfide bonds, improved the gluten network and increased the viscoelasticity of gluten.

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