Food Chemistry 213 (2016) 728–734

Contents lists available at ScienceDirect

Food Chemistry

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

Influence of high-molecular-weight glutenin subunit composition at *Glu-A1* and *Glu-D1* loci on secondary and micro structures of gluten in wheat (*Triticum aestivum* L.)

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ARTICLE INFO

Article history:
Received 26 February 2016
Received in revised form 26 June 2016
Accepted 5 July 2016
Available online 6 July 2016

Keywords:
Wheat glutenin subunits
Glu-A1 and *Glu-D1*
Secondary structure of gluten
Micro structure
Dough rheological behaviour

ABSTRACT

As one of critical gluten proteins, high-molecular-weight glutenin subunits (HMW-GS) mainly affect the rheological behaviour of wheat dough. The influence of HMW-GS variations at the *Glu-A1* and *Glu-D1* loci on both secondary and micro structures of gluten and rheological properties of wheat dough was investigated in this study. Results showed that the Amide I bands of the three near-isogenic lines (NILs) shifted slightly, but the secondary structures differed significantly. The micro structure of gluten in NIL 4 (Ax null) showed bigger apertures and less connection, compared to that in Xinong 1330 (Ax1). The micro structure of gluten in NIL 5 (Dx5 + Dy10) showed more compact than that in Xinong 1330 (Dx2 + Dy12). Correlation analysis demonstrated that the content of β-sheets and disulfide bonds in gluten has a significant relationship with dough properties. The secondary structures of native gluten are suggested to be used as predictors of wheat quality.

1. Introduction

Bread wheat (*Triticum aestivum* L.) is one of the major constituents of human diet, because of its various end uses. The gluten proteins are recognised as the crucial components determining bread-making quality of wheat. Based on their solubility in aqueous alcohol, gluten proteins are classified as gliadins or glutenins. The gliadins are monomeric proteins, whereas glutenins, further divided into low- and high-molecular-weight subunits (LMW-GS and HMW-GS), are polymeric proteins in which individual polypeptides are connected by a network formed through intermolecular disulfide and hydrogen bonds (Wieser, 2007). These proteins interact to make dough elastic, enabling it to trap the gas bubbles produced by yeast and allowing bread to rise (Shewry & Halford, 2002). Even though HMW-GS represent only 5–10% of grain protein, allelic variation in HMW-GS composition has been reported to account for up to 70% of the genetic variations for dough properties in wheat (Branlard & Dardevet, 1985; Payne, Holt, Krattiger, & Carrillo, 1988).

The HMW-GS genes are encoded at the *Glu-1* loci on the long arm of group-1 chromosomes (1A, 1B and 1D) (Shewry, Halford, & Lafiandra, 2003). At the *Glu-A1* and *Glu-D1* loci, many alleles have been discovered (McIntosh et al., 2012), including one gene encoding an 'x-type' (Ax or Dx) HMW-GS and one gene encoding a 'y-type' (Ay usually silenced or Dy) HMW-GS (Shewry et al., 2003). HMW-GS have a typical structure with three domains: highly conserved N- and C-terminal non-repetitive domains flanking a long central repetitive domain (Shewry et al., 2003). α-Helices are present at both terminals, whereas β-turns and intermolecular β-sheets are present in the central repetitive domain (Gilbert et al., 2000). It was suggested that secondary and tertiary structures of gluten proteins change under various temperature or additive treatments (Ferrer, Gómez, Añón, & Puppo, 2011; Georget & Belton, 2006; Gómez, Ferrer, Añón, & Puppo, 2013; Kaur, Singh, Kaur, Ahlawat, & Singh, 2014; Nawrocka, Szymanska-Chargot, Mis, Kowalski, & Gruszecki, 2016; Wang et al., 2014). For the secondary structure of gluten proteins, the content of β-sheets and β-turns was found to be positively correlated with the viscoelasticity of dough, while the content of α-helices showed a negative correlation (Georget & Belton, 2006). The number and distribution of cysteine residues differ between x-type and y-type HMW-GS, which affect the structure of glutenin polymers (Lindsay, Tamás, Appels, & Skerritt, 2000). At *Glu-B1* locus, HMW-GS variations with reducing cysteine residues and different length of repetitive domains were reported to affect the secondary and micro

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http://dx.doi.org/10.1016/j.foodchem.2016.07.043
0308-8146/

structures of gluten and thus the rheological properties of wheat dough (Gao et al., 2016). However, the effects of HMW-GS variations at other *Glu-1* loci on the secondary and micro structures of native gluten remain to be investigated.

In this research, secondary and tertiary structures and micro structure of gluten proteins from three near-isogenic lines (NILs) contrasting in HMW-GS composition encoded by the *Glu-A1* (Ax1 and Ax null) and *Glu-D1* (Dx2 + Dy12 and Dx5 + Dy10) loci were characterised, taking the dough rheological properties of these NILs into consideration, to study the effects of HMW-GS composition on the secondary and micro structures of gluten and rheological properties of wheat dough. Together with the results of HMW-GS composition encoded by *Glu-B1* in our previous study (Gao et al., 2016), the secondary structures of native gluten are suggested to be used as predictive factors for bread-making quality.

2. Materials and methods

2.1. Plant materials

NILs were obtained by crossing Xinong 1330 (Ax1, Bx7 + By9, Dx2 + Dy12) with wheat cultivar Fa 721 (null, Bx7 + By8, Dx5 + Dy10). The detection and selection progress was based on the method described by Gao et al. (2016). Wheat cultivar Xinong 1330 and its two HMW-GS NILs (NIL 4 and NIL 5) were selected for this research. BC_6F_8 and BC_6F_9 plants were planted at Yangling (108° 4′ E, 34° 16′ N), Shaanxi Province of China with two crop cycles in 2010–2011 and 2011–2012. Grains were sun-dried and stored for 60 d before testing and milling. Grains were tempered at 15% (w/w) moisture level for 20 h and milled in a Brabender Quadrumat Senior mill (Brabender OHG, Duisburg, Germany). HMW-GS composition was identified with Chinese Spring (Ax null, Bx7 + By8, Dx2 + Dx12), Guadalupe (Bx13 + By16, Dx5 + Dx10) and Lankao Teaizao (Ax1, Bx7 + By9, Dx2 + Dx12) as controls.

2.2. Extraction and fractionation of gliadin and glutenin

Gliadins were prepared as described by Gao et al. (2016). Glutenins were extracted following the method reported by Gao, Appelbee, Mekuria, Chalmers, and Mather (2012). Gliadins and glutenins were detected by A-PAGE and SDS-PAGE according to Yan, Hsam, Yu, Jiang, and Zeller (2003) and Gao et al. (2012), respectively.

2.3. Separation and identification of gliadin and glutenin

With known quantities of albumin from bovine serum as standard, total amounts of gliadin and glutenin were determined using A-PAGE and SDS-PAGE samples. The gliadins were further illustrated by reversed phase high-performance liquid chromatography (RP-HPLC) according to Daniel and Triboi (2000). The glutenin samples were further prepared according to the method reported by Singh, Shepherd, and Cornish (1991). RP-HPLC analysis following the method described by Vawser and Cornish (2004) was conducted twice for each sample to determine the amounts of HMW-GS and LMW-GS. Subunit peaks were identified according to previously established protocols (Cinco-Moroyoqui & MacRitchie, 2008; Vawser & Cornish, 2004). Based on the RP-HPLC trace, proportion of HMW-GS encoded by three *Glu-1* loci relative to total HMW-GS and HMW/LMW ratio were calculated.

2.4. Near-infrared reflectance (NIR) analysis of grains

A Diode Array 7200 NIR spectrometer (Perten Instrument AB, Sweden) was used for NIR analysis, with 950–1650 nm as collected wavelength range and reflectance as data collection mode. Each sample was tested in duplicate. NIR parameters, such as moisture, protein content, starch content, gluten content, hardness, Zeleny sedimentation and SDS sedimentation, were evaluated.

2.5. Extraction of gluten

Gluten samples were prepared by washing Brabender-milled wheat flour according to Gao et al. (2016). The gluten was kept in water and freeze-dried. Ten mg of ground gluten powder of each sample was immersed in distilled water without stirring and equilibrated at 4 °C for 24 h.

2.6. FT-Raman Spectroscopy analysis of gluten

According to Ferrer et al. (2011), the parameters related to secondary and tertiary structures of gluten proteins were determined in duplicate for each sample by FT-Raman Spectroscopy. Spectra were recorded at 25 °C with a laser power of 500 mW and a spectral resolution of 6 cm^{-1}. In order to obtain spectra with high signal-to-noise ratios, each spectrum was obtained after collecting and averaging 1000 scans. FT-Raman spectra were performed as intensity (arbitrary units) against Raman shift in wave number units (cm^{-1}). A straight baseline in Amide I region was adjusted in order to calculate the band intensity. With a nonlinear least-squares curve-fitting procedure, including mixed Gaussian-Lorenztian components, the Amide I region was truncated and deconvoluted to calculate the secondary structure components of gluten. The spectra were analysed using the Omnic software package (version 6.1a, Thermo Nicolet Corp). The resulting fitted curve was analysed referring to the band assignment for the secondary structure previously reported in the literature (Kaur et al., 2014; Wang et al., 2014). The targeted structures were β-sheets: 1680–1695 cm^{-1}, β-turns: 1670–1680 cm^{-1}, α-helices: 1650–1660 cm^{-1}, antiparallel β-sheets: 1627–1635 cm^{-1} and intermolecular β-sheets due to protein aggregation: 1613–1620 cm^{-1}. The proportion of the different conformation types to the total area was calculated according to Gao et al. (2016). Based on comparison to Raman data reported in previous researches (Ferrer et al., 2011; Gómez et al., 2013; Navarra, Tinti, Leone, Militello, & Torreggiani, 2009), inter-chain disulfide bands (497 cm^{-1}), tryptophan bands (880 cm^{-1}) and tyrosine bands ($I_{850/830}$) were analysed as the major vibrational motions of the side chains. The inter-chain disulfide bands at 497 cm^{-1} reflect the strength of intermolecular disulfide cross-links. The tryptophan bands at 880 cm^{-1} are used as an indicator of the strength of hydrogen bonding and $I_{850/830}$ is a monitor of the hydrogen bonding of the phenolic hydroxyl group. An increase in $I_{850/830}$ ratio has been reported to reflect a decrease in buriedness of tyrosyl residues in inter- or intra-molecular interactions (Meng, Ma, & Phillips, 2003). Duplicate samples were collected in each year and the mean values are reported.

2.7. Microstructure of gluten samples

Scanning electron microscopy (SEM) was conducted to observe the microstructure of gluten samples. Gluten samples were prepared according to Gómez et al. (2013). Dehydrated samples were coated with gold particles in a sputter coater (Pelco, Redding, California, USA). Images were taken in a JOEL JSM 35 CF SEM (Tokyo, Japan) with a 5 kV acceleration voltage.

2.8. Rheological properties analysis: Farinograph and Extensograph

Standard Farinograph and Extensograph analysis (Brabender GmbH & Co. KG, Germany) were conducted on flour samples of

the three NILs. According to American Association of Cereal Chemists (AACC) Method 54-21 and 54-10, the Farinograph parameters (dough development time and dough stability) and the Extensograph parameters (energy value, extensibility and maximum resistance (R_{max})) were determined.

2.9. Statistical analysis

Significant differences among the three NILs were determined by Student's *t*-test (Spiegel, 1972). And the relationship between the different variables was determined by Pearson correlation analysis using SPSS19.0 (SPSS, Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Variation of HMW-GS composition encoded by Glu-A1 and Glu-D1

The gliadins and glutenins of Xinong 1330 and its two NILs were separated on A-PAGE and SDS-PAGE (Supplementary Fig. S1), respectively. The gliadins and LMW glutenins were further separated by RP-HPLC (Supplementary Figs. S2 and S3) and the variation of gluten composition was investigated. Results show that the gliadins and LMW-GS of the three lines were identical (Supplementary Figs. S1–S3). HMW-GS encoded by *Glu-B1* in all three lines were Bx7 + By9. At *Glu-A1* locus, Xinong 1330 and NIL 5 carry *Ax1*, while the *Ax* gene in NIL 4 is silenced, marked as Ax null. HMW-GS encoded by *Glu-D1* were Dx2 + Dy12 in Xinong 1330 and NIL 4, and Dx5 + Dy10 in NIL 5, respectively. Because of the identical genetic background, wheat NILs contrasting in HMW-GS have been used as ideal materials to study the effects of HMW-GS on functional properties of wheat flour (Jin et al., 2013; Liu et al., 2005; Naeem & MacRitchie, 2005). The contribution of individual subunit encoded by *Glu-A1* and *Glu-D1* to flour functional properties has also been studied with transgenic wheat lines (León et al., 2009). At the *Glu-A1* and *Glu-D1* loci, many allelic variants have been detected (McIntosh et al., 2012). The present study focuses on the effects of two variants at *Glu-A1* locus and two variants at *Glu-D1* locus on wheat gluten structures and rheological properties of the three NILs. The two variants at *Glu-A1* and *Glu-D1* loci account for more than 90% and 84% frequency of HMW-GS alleles in Chinese varieties (Liu et al., 2005), respectively.

3.2. Proportion of HMW-GS encoded by Glu-1 loci relative to the total protein

For Xinong 1330 and NIL 4, there was no significant difference in moisture (Supplementary Table S1). However, there were significant differences among the samples in thousand kernel weight, protein content, starch content, wet gluten content, grain hardness, Zeleny sedimentation and SDS-sedimentation values, indicators of dough strength. The lower protein content in NIL 4, compared to that in Xinong 1330, may be caused by the silence of *Ax* gene in NIL 4, which led to the relatively higher starch content and lower wet gluten content, Zeleny sedimentation and SDS-sedimentation values. For Xinong 1330 and NIL 5, there was no significant difference in moisture, protein content, starch content or wet gluten content. However, NIL 5 showed higher Zeleny sedimentation and SDS-sedimentation values than Xinong 1330 (Supplementary Table S1). Since dough strength can be affected by the expression level of HMW-GS (Vawser & Cornish, 2004), quantitative analysis of HMW-GS, relative to total protein of the three NILs was performed in the current study (Table S2). This analysis showed that NIL 4 represents lower glutenin/gliadin ratio and HMW-GS/LMW-GS ratio than Xinong 1330 and NIL 5. Due to the silent *Ax* gene in NIL 4, the expression level of HMW-GS encoded by *Glu-B1* increased dramatically, compared to that in Xinong 1330 and NIL 5, while the expression level of HMW-GS encoded by *Glu-D1* varies slightly. As the protein content determines the functional property of wheat dough (Uthayakumaran, Gras, Stoddard, & Bekes, 1999), the result verified the lower protein content and lower glutenin/gliadin ratio tested in NIL 4, indicating the poor rheological properties of NIL 4. However, there was no significant difference in the glutenin/gliadin ratio and the HMW-GS/LMW-GS ratio between Xinong 1330 and NIL 5, and the expression level of HMW-GS encoded by *Glu-A1*, *Glu-B1*, and *Glu-D1* represented quite similarly relative to the total amount of HMW-GS in Xinong 1330 and NIL 5 (Supplementary Table S2). The similar expression level of HMW-GS encoded by different *Glu-1* loci between Xinong 1330 and NIL 5 ensured that the differences reported here were due to the HMW-GS inner composition.

3.3. Secondary and tertiary structures of gluten

To quantify the secondary and tertiary structures, reflecting the protein backbone conformation, Amide I bands (1700–1600 cm^{-1}) were analysed in the gluten of each NIL (Fig. 1). The Amide I bands of NIL 4 and NIL 5 (1651 cm^{-1}) show slight shifts towards higher wave numbers, compared to that of Xinong 1330 (1649 cm^{-1}). The shift of the Amide I bands indicates changes in secondary structures of the gluten samples, which is corresponding to the quantitative analysis of the secondary structures in this region (Table 1). The results show that at *Glu-A1* locus, the silent *Ax* gene does not affect the secondary structure content, such as, α-helices, intermolecular β-sheets, β-turns and antiparallel β-sheets, compared to Xinong 1330. However, the β-sheets content in Xinong 1330 is 13.71%, which is different from that in NIL 4 (13.06%). Contrastingly, at *Glu-D1* locus, the α-helix content in Xinong 1330 is significantly higher than that in NIL 5, while β-sheets and β-turns content in NIL 5 are higher than those in Xinong 1330 (Table 1). The information concerning inter-chain disulfide bands, tryptophan bands and tyrosine bands is reported in Fig. 2. The results demonstrate that NIL 5 has the highest intensities of disulfide and tryptophan bands and intensity ratio $I_{850/830}$ among the

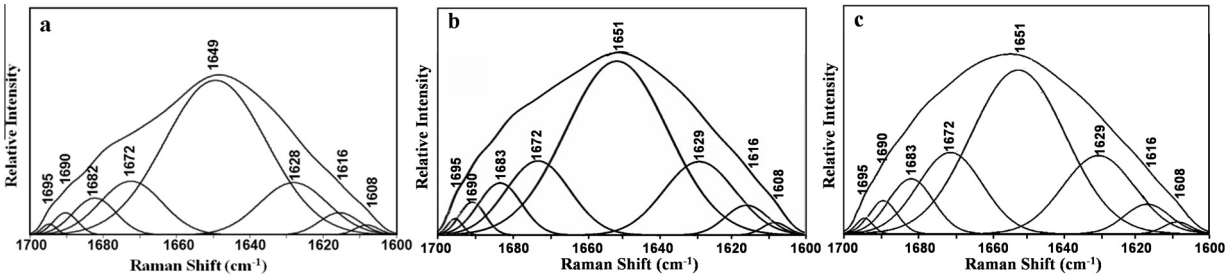

Fig. 1. Amide I band of Xinong 1330 (a) and two near-isogenic lines: NIL 4 (b) and NIL 5 (c).

Table 1
FT-Raman Spectroscopy determination of secondary structure percentages of Xinong 1330 and the two near-isogenic lines.

Year	Line	α-Helices	Intermolecular β-sheets	β-Sheets	β-Turns	Antiparallel β-sheets
2010–2011	Xinong 1330	60.33 ± 0.03a	3.44 ± 0.01b	13.71 ± 0.01c	12.83 ± 0.01b	8.69 ± 0.08b
	NIL 4	60.16 ± 0.02a	3.29 ± 0.04b	13.06 ± 0.03b	12.57 ± 0.02b	8.30 ± 0.06b
	NIL 5	48.84 ± 0.04b	4.26 ± 0.08a	17.73 ± 0.01a	16.65 ± 0.01a	11.48 ± 0.04a
2011–2012	Xinong 1330	60.41 ± 0.04a	3.38 ± 0.03b	13.71 ± 0.02c	12.88 ± 0.04b	8.77 ± 0.02b
	NIL 4	60.32 ± 0.02a	3.30 ± 0.04b	13.09 ± 0.01b	12.55 ± 0.03b	8.28 ± 0.04b
	NIL 5	49.01 ± 0.03b	4.13 ± 0.01a	17.62 ± 0.01a	16.52 ± 0.02a	11.60 ± 0.03a

Values followed by a different letter in the same column are significantly different ($P < 0.05$).

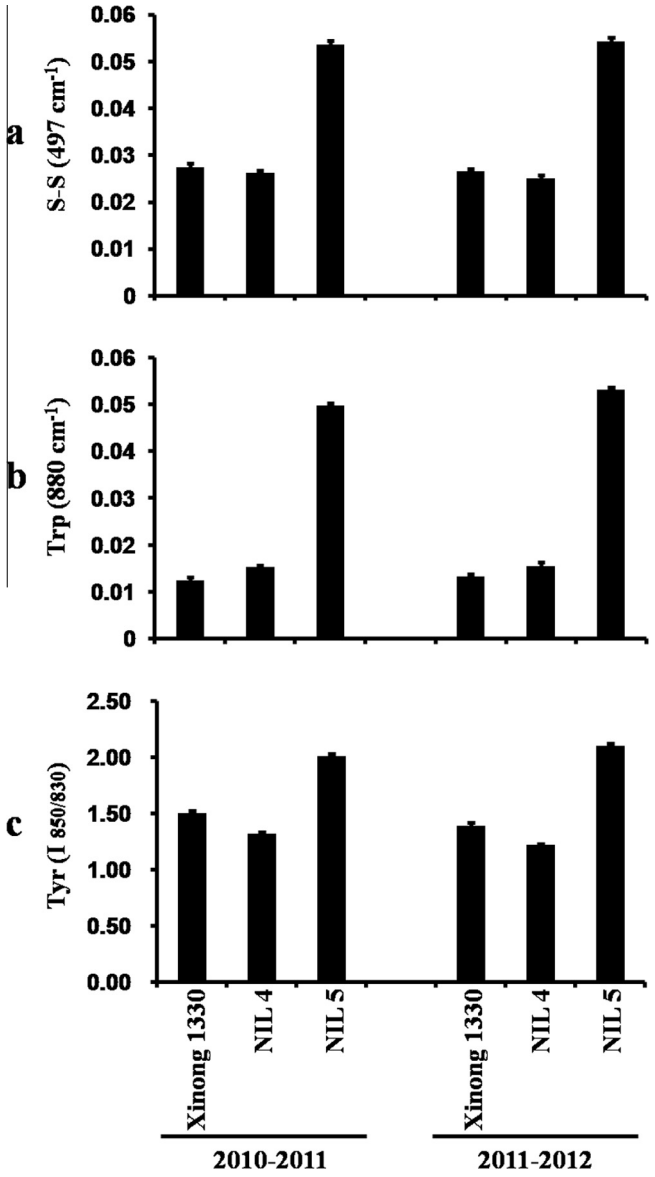

Fig. 2. Side chain vibrations analysis of Xinong 1330 and two near-isogenic lines NIL 4 and NIL 5. (a) Normalized intensity of inter-chain disulfide band appeared at 497 cm^{-1} (b) Normalized intensity of tryptophan band appeared at 880 cm^{-1}. (c) Intensity ratios $I_{850/830}$ indicating tyrosine residues. Normalized intensity values correspond to the mean values of three independent experiments. Vertical bars represent confidence intervals at $p < 0.05$.

three NILs. However, NIL 4 has the lowest intensities of disulfide band and intensity ratio $I_{850/830}$.

Dough rheological properties are influenced by the secondary structures of the gluten. According to Mondher, Barbara, Abdelfattah, Franck, and Mohamed (2005) and Georget and Belton (2006), the content of α-helices is negatively correlated to the viscoelasticity of dough, but the content of β-sheets is positively correlated to this characteristic. The band appearing at 497 cm^{-1}, related to the inter-chain disulfide bonds, and hydrogen bonding at 880 cm^{-1} are the main factors determining dough properties (Belton, 2005; Ferrer et al., 2011; Georget & Belton, 2006; Lindsay et al., 2000). It is also to be noted that except for HMW-GS, disulfide and tryptophan bonds may also exist in gliadins and/or LMW-GS, which may affect dough properties. However, in the current study, the significant differences in secondary structures are mainly caused by HMW-GS as the NILs gave the identical genetic background in gliadins and LMW-GS. Referring to the previous study reporting the variations at *Glu-B1* locus affecting the secondary structures (Gao et al., 2016), the stability of gluten structure was predicted as NIL 5 > Xinong 1330 > NIL 4. Compared to the results reported in Gao et al. (2016), allelic variations at *Glu-A1* represent the least differences in the secondary structures but those at *Glu-D1* represent greater differences, as great as those at *Glu-B1*.

3.4. Microstructure of the gluten network

The microstructure of the gluten network of the three NILs is shown in Fig. 3. It is observed that the apertures of NIL 4 (Fig. 3b) are significantly larger than those of Xinong 1330 (Fig. 3a) and NIL 5 (Fig. 3c). Gluten of NIL 5 in Fig. 3c has more cross-links than that of Xinong 1330 in Fig. 3a, followed by that of NIL 4 in Fig. 3b, which is consistent with the results of disulfide bonds determined by FT-Raman Spectroscopy analysis. The degree of connectivity for NIL 5 is greater than that for Xinong 1330, but both Xinong 1330 and NIL 5 showed higher levels than NIL 4. This could be explained by the absence of Ax glutenin subunits, which decreases the protein content but increases the starch content. Thus, the apertures of NIL 4 are filled with more starch granules. Glutenin subunits are stabilised into a gluten network by inter-chain disulfide and hydrogen bonds (Shewry, Tatham, Forde, Kreis, & Miflin, 1986), and HMW-GS are suggested to form two types of network when gluten is extended (Belton, 2005). In one type of network, the HMW-GSs act as a chain extender and link together via head-to-tail disulfide bonds to form a linear chain (Pirozi, Margiotta, Lafiandra, & MacRitchie, 2008). The gluten network of Xinong 1330 (Fig. 3a) was observed to carry more subunits of the first type, as it is more linear than that of NIL 5. In the other type, the HMW-GS act as chain brancher by linking through intermolecular disulfide bonds (Köhler, Keck-Gassenmeier, Wieser, & Kasarda, 1997) to promote a highly cross-linked gluten network, as observed in Fig. 3c for NIL 5. This can be explained by the cysteine number of the HMW subunit Dx5. Compared with typical x-type glutenin subunits containing four cysteines (three at the N-terminal and one at the C-terminal), subunit Dx5 has five cysteines, with an additional one at the N-terminal end of the repetitive domain (Anderson et al., 1989). This extra cysteine residue was found to be linked with cysteine residues in the C-terminal domain of other HMW-GS by inter-chain disulfide bonds (Lutz, Wieser, & Koehler, 2012). As suggested by Köhler et al. (1997), the Dx5 HMW-GS forms one or three inter-chain disulfide in its

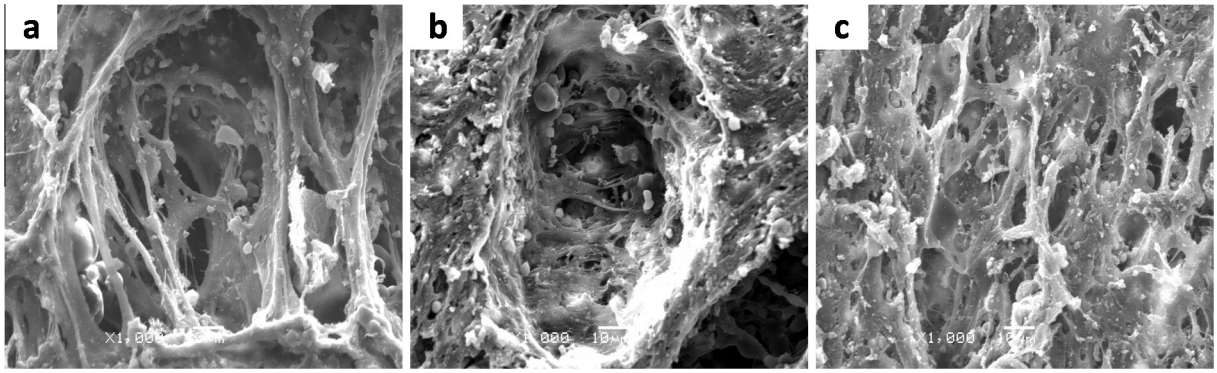

Fig. 3. Scanning electron microscopy of gluten extracted from Xinong 1330 and two near-isogenic lines. (a) Xinong 1330, (b) NIL 4, (c) NIL 5 Magnification: 1000×. Bars: 10 μm.

N-terminal domain, enabling it to act as a chain brancher to promote a highly cross-linked glutenin network, which dramatically increases dough strength and improves baking properties (Darlington et al., 2003; Lafiandra, D'Ovidio, Porceddu, Margiotta, & Colaprico, 1993; Tatham, Field, Keen, Jackson, & Shewry, 1991). For NIL 5, the gluten network was better distributed than those of Xinong 1330 and NIL 4, which could be attributed to the higher content of disulfide bonds and β-sheets reported above. As suggested in the previous researches (Belton, 2005; Georget & Belton, 2006), both disulfide bonds and β-sheets act synergistically, which gave greater stability of gluten polymers. Consequently, it can be predicted from the results of microstructure that NIL 5 presents greater stability than other wheat lines.

3.5. Quality properties of grain and rheological properties of dough

Based on the NIR analysis, there were dramatic variations for Zeleny sedimentation and SDS sedimentation among the three NILs. NIL 5 had the highest Zeleny and SDS sedimentation values, while NIL 4 had the lowest Zeleny and SDS sedimentation values (Supplementary Table S1). Based on correlation among bread-making quality assessment indices (Najafian, 2012), it was demonstrated that SDS-sedimentation volume is the most reliable and practical index in evaluation of wheat grain quality. According to the NIR data, it is predicted that the contribution ranks of HMW-GS encoded by the *Glu-A1* and *Glu-D1* loci to bread-making quality are Ax1 > Ax null and Dx5 + Dy10 > Dx2 + Dy12, which is consistent with the results reported previously (Jin et al., 2013; Liu et al., 2005).

Both Farinograph and Extensograph measurements were conducted to analyse the rheological properties of the three NILs (Fig. 4). For the Farinograph, it was demonstrated that NIL 5 had the longest dough development time and strongest dough stability. However, NIL 4 gave the shortest dough development time and Xinong gave the poorest dough stability. For the extensograph, NIL 5 showed the greatest energy value, extensibility and R_{max}, which confirmed the inference based on the secondary and micro structures of the gluten network. Moreover, NIL 4 had the lowest value in all parameters among the three NILs, except for the R_{max} in 2011–2012. Consistent with the prediction derived from the NIR data, the contribution to dough development time and extensibility of HMW-GS at the *Glu-A1* and *Glu-D1* loci were ranked as Ax1 > Ax null and Dx5 + Dy10 > Dx2 + Dy12.

3.6. Correlation analysis of the secondary structures and dough rheological properties

The correlation coefficients between the secondary structures and dough rheological properties are listed in Table 2. It shows that

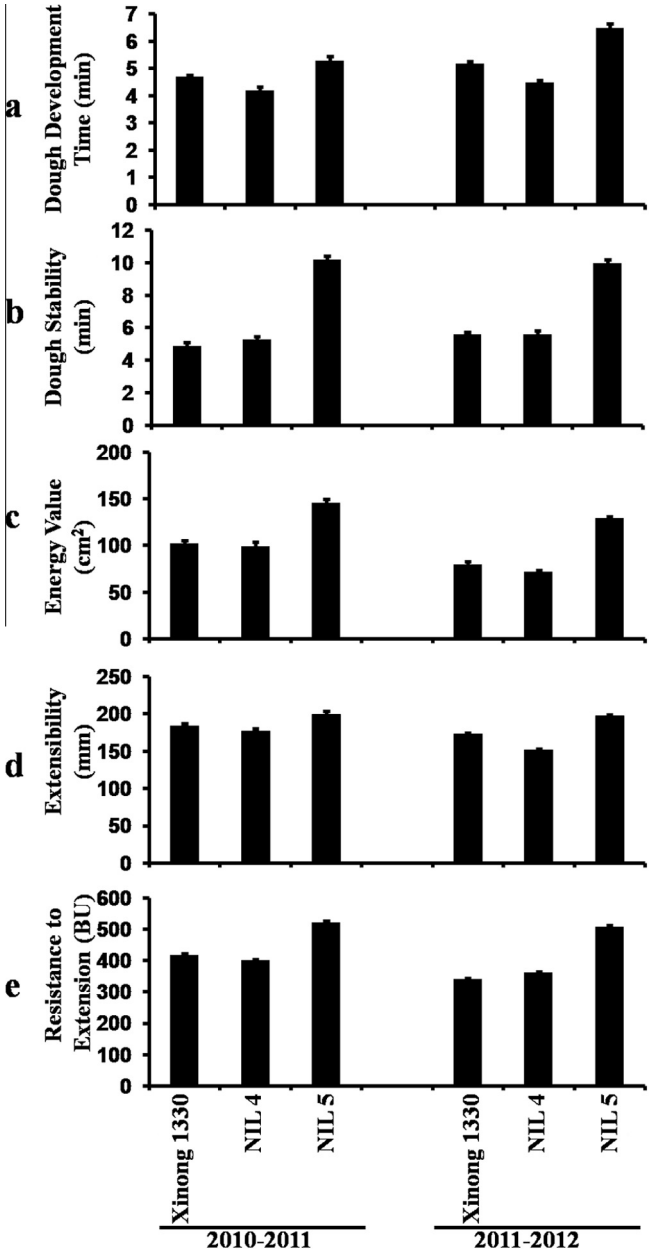

Fig. 4. Rheological properties of dough determined by Farinograph and Extensograph for Xinong 1330 and two near-isogenic lines. (a) Dough development time. (b) Dough stability. (c) Energy value of dough relaxed in 135 min. (d) Extensibility of dough relaxed in 135 min. (e) R_{max} of dough relaxed in 135 min.

Table 2
Pearson correlation coefficients between different secondary structures and dough rheological properties.

	Inter-molecular β-sheets	β-Sheets	β-Turns	Antiparallel β-sheets	S-S (497 cm^{-1})	Trp (760 cm^{-1})	Tyr ($I_{850/830}$)	Dough development time	Dough stability	Energy value	Extensibility	R_{max}
α-Helices	−0.987**	−0.990**	−0.997**	−0.990**	−0.998**	−0.996**	−0.965*	−0.781	−0.994**	−0.905*	−0.790	−0.932**
Intermolecular β-sheets	1.000	0.996**	0.995**	0.991**	0.990**	0.972*	0.976**	0.768	0.977**	0.925**	0.829*	0.934**
β-Sheets		1.000	0.998**	0.999**	0.995**	0.980**	0.984*	0.819*	0.982**	0.906*	0.826*	0.919*
β-Turns			1.000	0.997**	0.999**	0.989**	0.976**	0.802	0.991**	0.907*	0.811	0.924**
Antiparallel β-sheets				1.000	0.996**	0.983**	0.985**	0.839*	0.982**	0.897*	0.824*	0.913*
S-S (497 cm^{-1})					1.000	0.993**	0.979**	0.802	0.988**	0.913*	0.817*	0.936**
Trp (760 cm^{-1})						1.000	0.954**	0.841*	0.993**	0.879*	0.758	0.918*
Tyr ($I_{850/830}$)							1.000	0.798	0.940**	0.924**	0.892*	0.934**
Dough development time								1.000	0.784	0.576	0.633	0.617
Dough stability									1.000	0.864***	0.736	0.889*
Energy value										1.000	0.933**	0.976**
Extensibility											1.000	0.870*

* and ** indicate significant correlations at $P < 0.05$ and $P < 0.01$, respectively.

the secondary structures of gluten are significantly correlated with the dough rheological parameters. Of them, the content of α-helices is negatively correlated with intermolecular β-sheets ($r = -0.987$), β-sheets ($r = -0.990$) and antiparallel β-sheets ($r = -0.990$), which could be explained by the transformation between α-helices and β-sheets under certain conditions (Georget & Belton, 2006). Different from the results based on the HMW-GS variation at the *Glu-B1* locus, the content of α-helices was also negatively correlated with β-turns and antiparallel β-sheets. As the correlation analysis was derived from three wheat lines, it needs to be further studied with more variations at the *Glu-1* loci. The contents of β-sheets, antiparallel β-sheets and disulfide bonds are correlated with parameters from the Farinograph and Extensograph, which indicated that they played a crucial role in both dough stability and extensibility. Similar with the previous research (Gao et al., 2016), the content of disulfide bonds is significantly correlated with dough stability. Results of the current research further confirm the effects of β-sheets and cysteine number on dough properties. Together with the results based on the variations at the *Glu-B1* locus (Gao et al., 2016), a conclusion can be drawn that the content of β-sheets and disulfide bonds in gluten determining dough quality can be used as indicators for wheat quality.

4. Conclusion

The three wheat NILs studied currently provided the identical genetic background to compare the secondary and micro structures of gluten and wheat processing quality difference caused by variations in HMW-GS composition. The results demonstrated that the different HMW-GS compositions encoded by *Glu-A1* and *Glu-D1* affected the proportion of secondary and micro structures of gluten and the rheological properties of wheat dough. The absence of HMW-GS encoded by *Glu-A1*, and HMW-GS encoded by *Glu-D1* with an extra cysteine residue, gave distinguishing patterns of the microstructure of gluten. Among the three near-isogenic lines, NIL 5 with Dx5 + Dy10, possessed the highest content of β-sheets and disulfide bonds, and gave the greatest dough strength and viscoelasticity. The content of β-sheets and disulfide bonds in gluten determining dough quality can be used as indicators for wheat quality.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgements

This research was supported by projects "National Natural Science Foundation of China" (31501300), "Chinese Universities Scientific Fund" (2452015009), "Agriculture Technology Innovation Program" (2015NY086) and "Coordinator Innovative Engineering Project" (No. 2014KTZB02-01-01) of Shaanxi Province.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.07.043.

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