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Change in physicochemical characteristics and molecular weight distribution of glutenin macropolymer induced by postharvest wheat maturation

Y. Yue, X. Liu, J. Wang*, F. Jia, Q. Wang and X. Zhang

College of Biological Engineering, Henan University of Technology, Zhengzhou 450001, China P.R.; jinshuiw@163.com

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Abstract

The present study was aimed to investigate change in physicochemical characteristics and glutenin macropolymer (GMP) molecular weight distribution during postharvest wheat maturation. The freshly harvested wheat was stored under four different conditions (WT1, WT2, WT3 and WT4) for specified times. During maturation, the strengthening of wheat gluten structure was observed by increase in gluten index, swelling index of glutenin and sodium dodecyl sulphate (SDS) sedimentation value. The postharvest maturation resulted in contents increase of glutenin, glutenin/gliadin ratio, and GMP, and the decline of gliadin content. The contents of free sulfhydryl in GMP reduced, and S-S content rose during the whole maturation. Visible aggregates of GMP were observed in SDS-PAGE patterns for WT3 and WT4 samples after 4 weeks of storage. Moreover, in these two samples, polymerisation of gluten proteins into GMP was observed in size-exclusion high performance liquid chromatography patterns during storage. Some low molecular weight glutenin subunits (LMW-GS) incorporated into the aggregate, or combination of high molecular weight glutenin subunits and LMW-GS formed large polymeric glutenins by S-S linkage, and then assembled into GMP in postharvest wheat maturation. GMP polymerisation probably results in the improvement of wheat quality.

Keywords: wheat, postharvest maturation, physicochemical properties, glutenin macropolymer, molecular weight distribution

1. Introduction

Wheat is one of the most important cereals in the world. As a staple food material, wheat foods provide most of energy, protein for people all over the world (Aghababaei *et al.*, 2017). China is the largest wheat producer and consumer in the world. Wheat is particularly widely planted in northern China. Newly harvested wheat has poor milling, processing and nutritional quality. The wheat has a postharvest maturation phenomenon, and postharvest maturation begins after harvest and continues in storage depending on time and conditions of storage. After a series of biochemical and colloidal changes, wheat reaches to the technological and eating maturity during the storage (Tomić *et al.*, 2013). Therefore, the wheat postharvest maturation is an important part of baking and technological quality improvement (Janić *et al.*, 2014).

Protein and starch are the major constituents of wheat wherein gluten proteins play an important role in the production of different wheat and wheat-based products. Wheat flour is unique for forming viscoelastic dough when mixed with water due to the formation of gluten (Lindsay and Skerritt, 1999). Gluten polymers are among the largest and most complex protein networks in nature with molecular weights of more than 10 million (Wieser, 2007). Gluten proteins are storage proteins and divided into two major fractions according to differences in solubility: the monomeric gliadins and the polymeric glutenins. Gliadins and glutenins are responsible for the viscoelastic properties of wheat flour dough. Gliadins contribute to the viscous properties of wheat four dough (Pomeranz, 1988), whereas glutenin fraction has long been considered to have a prominent role in strength and elasticity to the dough (MacRitchie, 1980; Weegels *et al.*, 1996). Viscoelasticities of glutens are governed by glutenin compositions, gliadin/

glutenin ratio, and high molecular weight glutenin subunits (HMW-GS) / low molecular weight glutenin subunits (LMW-GS) ratio. These properties also affect rheological properties and technological quality of wheat flour (Koga *et al.*, 2017; Song and Zheng, 2007). Glutenin subunits form both intra- and inter-chain disulphide bonds, while gliadins are only capable of forming inter-chain disulphide bonds. Differences in the disulphide bonding properties of glutenin subunit impact on their association within the glutenin macropolymer (GMP), and role in establishing gluten structure and subsequent function (Lindsay and Skerritt, 1999). GMP is an important fraction of the glutenins isolated from wheat flour as a sodium dodecyl sulphate (SDS) insoluble gel-layer (Weegels *et al.*, 1996). GMP consists of spherical glutenin particles (Don *et al.*, 2003a,b). There is also great difference of size of GMP, and this variation correlates with a key technological quality parameter (Don, 2005). The importance of GMP in assessing wheat quality and predicting dough properties has been confirmed (Sapirstein and Suchy, 1999). Significant correlations between GMP in flour and quality parameters, such as loaf and physical dough properties, have been observed (Dachkevitch and Autran, 1998; Gupta *et al.*, 1993; Wang *et al.*, 2007).

Storage in favourable condition causes some biochemical and physicochemical changes of the wheat grains. The storage is also an aging process, in which the physicochemical properties, dough viscosity and end-use quality of wheat are improved considerably (Aghababaei *et al.*, 2017). Moreover, the ageing process of these freshly harvested grains was initiated by the control of three factors including storage temperature, moisture content and duration of storage. In China, there are complex and changeable stored-grain ecosystems. To the best our knowledge, there is little information about the mechanism induced quality improvement during postharvest wheat maturation in different stored-grain ecosystem. This study thus aimed to investigate the combined effect of storage temperature, moisture content and storage time on physicochemical characteristics of postharvest wheat maturation, and analysed the composition change of GMP in postharvest wheat maturation.

2. Materials and methods

Wheat sample and storage

Wheat samples (Luomai18), a hard white winter wheat, were obtained from growing region in Yuzhou, Henan, China. The variety is one of the widely planted varieties in the local area and is suitable for making fermented food such as steamed bread and bread, wheat was harvested in 2017. All samples were free from insect infestation and winnowed and the broken grains were separated. The freshly harvested wheat samples were kept for 3 days under laboratory condition, and then were treated. About 10.0 kg wheat samples were placed in the air tight buckets of uniform size for each treatment. These buckets were stored in constant temperature and humidity incubator under four different conditions (15 °C/50%RH, WT1; 20 °C/65%RH, WT2; 28 °C/75%RH, WT3 and 35 °C/85%RH, WT4) for specified times. Samples were emptied into big boxes to bring back the grains to room temperature before milling. The samples were individually milled by high speed pulveriser, the extraction rate is about 70%. The size of powder should be less than 60 mesh. The lab environment was 25 °C and 45%RH. The conditioned samples were then milled using a laboratory miller (RT-34, Hongquan Pharmaceutical Machinery Ltd., Hong Kong, China) after passing the required storage time.

Determination of wet gluten and gluten index

Wet gluten and gluten index of stored wheat samples were measured in a Glutomatic system (Perten, Stockholm, Sweden) following American Association of Cereal Chemists International approved method of 38.12-02 (AACCI, 2010). Washed gluten was kept in a shaking water bath at 37 °C for different time intervals, and then determination of wet gluten and the gluten index were made according to the standard methods.

Protein analysis

Wheat flour protein content was determined by the Kjeldahl method. Protein content of gliadin, glutenin, GMP was measured using a UV absorption method (Hall, 1996). The UV method was successfully calibrated using a set of known gliadin, glutenin, GMP values determined by the Kjeldahl method.

Isolation of gliadin, glutenin and glutenin macropolymer

Briefly, 1 g of whole wheat flour were suspended in 10 ml of 1 mol/l NaCl solution and magnetically stirred for 30 min (700 rpm) at RT. The suspension was then centrifuged for 10 min at 10,000×*g* at 25 °C. The supernatant was albumin and globulin. The pellet was suspended in 10 ml of 70% ethanol and magnetically stirred for 30 min (700 rpm) at RT. The suspension was then centrifuged for 10 min at 10,000×*g* at 25 °C. The supernatant and the pellet were gliadin and glutenin, respectively.

The wheat flour defatted with petroleum ether was suspended in 1.5% SDS (1.4 g flour in 28 ml) and centrifuged at 10,000×*g* for 20 min at 4 °C (Graveland *et al.*, 1982). The supernatant was discarded and the gel-layer collected as GMP.

Free sulfhydryl groups and disulphide bonds of glutenin macropolymer

Disulphide (SS) and free sulfhydryl (SH) contents of GMP were determined according to the method of Anderson and Ng (2007) with some modifications. GMP was dissolved in 5.0 ml buffer A (buffer A, pH 8.0 containing 8 mol/l urea, 3 mmol/l EDTA, 1% SDS and 0.2 mol/l Tris-HCl), and the mixtures were shaken for 1 h. After that, 1.0 ml buffer B (buffer B, pH 8.0, 10 mmol/l DTNB, 0.2 mol/l Tris-HCL) was added. Then, the mixtures were shaken for 1 h, the suspension was centrifuged for 10 min at 10,000×*g* at 25 °C. Exactly, the absorbance of the supernatant was measured at 412 nm.

Then, 500 mg whole wheat flour were suspended in 10 ml of a SDS solution (1.5%, w/v) and magnetically stirred for 1 h at RT. The pellet was suspended in 5 ml of buffer C (buffer C, pH 9.5 containing 8 mol/l urea, 3 mmol/l EDTA, 1.0% (w/v) SDS, 0.1 mol/l Na_2SO_3 0.5 mmol/l $NTSB^{2-}$ and 0.2 mol/l Tris-HCL) and shaken for 1 h at 25 °C, the suspension was centrifuged for 10 min at 10,000×*g* at 25 °C. the 0.1 ml supernatant was drew, add 0.9 ml buffer D (buffer D, pH 8.0 containing 8 mol/l urea, 3 mmol/l EDTA, 1.0% (w/v) SDS, 0.1 mol/l Na_2SO_3, 0.2 mol/l Tris-HCL), the absorbance of the supernatant was measured at 412 nm.

Absorbance values were converted to level of SH: $A = \varepsilon bc$.

(A is light absorption value (OD); ε (molar absorption coefficients) = 13,600 l/(mol·cm); b is the thickness of cuvette; c is the concentration of the test sample)

Values of the content of disulphide bonds: $SS = (TS - SH) / 2$

(SS is the content of disulphide bonds; TS is the content of the total sulfhydryl groups; SH is the content of free sulfhydryl)

Analysis of SDS-PAGE

GMP was suspended in1.0 ml of sample buffer [containing 0.01 mol/l Tris-HCl (pH 6.8), 10% (w/v) SDS, 10% (v/v) glycerol and 0.1% (w/v) bromophenol blue, 5% (v/v) 2-mercaptoethanol (2-ME)] and left for 1 h. Samples were heated for 5 min at 100 °C and centrifuged for 10 min at 8,000×*g*. GMP proteins were subjected to SDS-PAGE. SDS-PAGE analysis of proteins was performed in a vertical electrophoresis cell using 10% separating gel (pH 8.8) and 5% stacking gel (pH 6.8). GMP proteins on the gel were stained with coomassie brilliant blue R-250 and destained in 7% (v/v) acetic acid.

Size-exclusion high performance liquid chromatography

The extraction of samples was referred to literature and improved (Labuschagne *et al.*, 2006). 170 mg of wheat flour sample was suspended in 15 ml of a 0.1 mol/l sodium phosphate buffer (pH 6.9) containing 0.5% (w/v) SDS. The mixture was shook at room temperature for 120 min. The suspensions were centrifuged for 15 min at 10,000×*g*. A clear supernatant was obtained after filtration through a 0.45 μm membrane. The protein extracts were loaded on Biosep-SEC-S4000 column with a separation range from 15 to 500 kDa (300×7.8 mm, Phenomenex, Torrance, CA, USA), performed on a liquid Biosep-SEC system. The elution solvent was acetonitrile/water (1:1, v/v) containing 0.1% (v/v) trifluoroacetic acid (v/v) with a flow rate of 0.1 ml/min at ambient temperature for 30 min. Protein elution was monitored at 280 nm.

Statistical analysis

All the data obtained in the study were expressed as the mean of three parallel experiments. Analysis of variance (ANOVA) was performed using the software SPSS16.0 (SPSS Inc., Chicago, IL, USA) with one-way analysis of variance (ANOVA) and Duncan's multiple-range test (Chaudhary *et al.*, 2016). $P<0.05$ was used to define the significance of differences between the samples.

3. Results and discussion

Effect of storage on wet gluten and gluten index of stored wheat

Wheat quality is determined by several variables including: grain physical properties, protein content and composition, and starch content. Among the quality characteristics, storage protein content has been found to be the most important quality feature for wheat (Dowell *et al.*, 2008). Moreover, variation in total protein content alone does not adequately explain the variation in food-making quality, as protein quality is an important factor as well (Bonfil and Posner, 2012; Peterson *et al.* 1986). Wheat gluten proteins including glutenin and gliadin are of immense importance in the food industry as their properties underpin the processing of wheat flour to produce the end foods. Wet gluten content and gluten index (GI) are usually used as the wheat quality traits.

Table 1 shows the effect of storage time on wet gluten content of wheat. No significant differences ($P<0.05$) were found in the amounts of wet gluten as a consequence of storage time for the four samples. In particular, there were no obvious changes in the amounts of wet gluten with storage time for wheat samples (WT1 and WT2) stored in 15 °C, 50% RH and 20 °C, 65% RH. For wheat samples (WT3 and WT4) stored in 28 °C, 75% RH and 35 °C, 85%

Table 1. Change in physicochemical properties of freshly-harvested wheat during the postharvest maturation.[1]

Storage duration/W		WT1	WT2	WT3	WT4
Wet gluten content (%)	0	30.99±0.23a	30.99±0.23a	30.99±0.23a	30.99±0.23a
	2	31.07±0.82a	31.32±0.29ab	31.30±1.31a	30.88±0.34ab
	4	30.57±0.67a	31.18±1.40b	31.03±0.42a	30.13±0.32ac
	6	30.46±0.60a	30.24±0.75a	30.30±0.82a	30.56±0.27ac
	8	29.90±1.05a	31.03±1.12ab	30.60±1.77a	30.29±0.16ac
	10	30.10±1.29a	29.37±0.71ab	29.63±0.45ac	31.05±0.21ab
	12	30.80±0.78a	30.57±0.97ab	30.22±0.47a	30.64±0.41ac
	14	30.70±0.36a	30.67±0.65ac	30.53±0.60a	30.25±0.23ac
Gluten index (%)	0	48.09±0.84a	48.09±0.84a	48.09±0.84a	48.09±0.84a
	2	47.47±1.09b	47.71±2.55b	49.61±1.51ab	50.20±1.01b
	4	48.82±1.75e	50.06±1.06bc	51.26±0.41ab	53.97±0.58c
	6	49.41±1.19c	51.40±1.78cd	53.30±1.05b	63. 89±0.21bd
	8	50.32±0.92e	53.35±1.43d	63.66±1.53c	63. 14±0.48bc
	10	51.77±2.64d	54.75±2.01cd	62.21±0.68bc	62. 73±0.51bd
	12	55.06±1.95e	56.88±0.87cd	63.38±0.59bc	63. 62±0.27d
	14	57.30±1.48e	58.90±2.55e	63.40±0.60cd	63. 06±0.48bd
Sodium dodecyl sulphate	0	29.17±1.04h	29.17±1.04h	29.17±1.04h	29.17±1.04g
sedimentation value (%)	2	29.43±0.60gh	30.33±1.15fgh	30.83±0.76fgh	31.17±0.29fg
	4	30.33±0.58fg	31.33±0.58efg	31.67±1.15efg	32.67±2.00ef
	6	31.50±0.87de	32.0±0.00ef	32.33±0.58ef	34.00±1.00de
	8	32.10±0.36de	32.93±1.01cd	34.33±2.08cd	37.17±0.29bc
	10	33.27±0.46bc	33.83±0.76abc	35.67±0.58abc	38.67±0.58ab
	12	34.17±0.29ab	35.00±0.00ab	36.33±0.76ab	39.50±0.50ab
	14	34.60±0.53a	36.00±0.50a	37.27±0.46a	40.67±1.15a
Swelling index of glutenin	0	2.91±0.12b	2.91±0.12b	2.91±0.12b	2.91±0.12f
	2	2.91±0.10b	2.98±0.11bc	3.05±0.11be	3.21±0.13cf
	4	2.98±0.12b	3.05±0.13c	3.27±0.12e	3.4±0.11bc
	6	3.12±0.11bc	3.24±0.12cd	3.29±0.13ef	3.84±0.14de
	8	3.27±0.10cd	3.29±0.11bc	3.50±0.11bc	3.86±0.12d
	10	3.32±0.12bc	3.34±0.15a	3.52±0.14bc	3.85±0.11def
	12	3.35±0.10ac	3.37±0.10ac	3.52±0.12cd	3.86±0.13cd
	14	3.36±0.11bc	3.37±0.11cd	3.61±0.10bc	3.89±0.15bc

[1] The values in this table are means of triplicates. The means followed by different letters in the same columns within each tested variable are significantly different (LSD, $P<0.05$).

RH, contents of wet gluten decreased slightly with storage time. As shown Table 1, the change pattern of GI values in different storage environments is different. GI gradually increased for WT1 and WT2 samples in the whole storage stage. During the initial storage phage for WT3 and WT4 samples, GI increased significantly ($P<0.05$). The GI reached to the highest values (63.66% and 63.89%) in WT3 and WT4 samples stored for 8 weeks and 6 weeks, respectively. WT4 sample reached the highest GI in the shortest time. GI is a measurement of wheat protein that provides a simultaneous determination of gluten quality and quantity (AACCI, 2010). The GI value expresses the weight percentage of the wet gluten remaining on a sieve after automatic washing with salt solution and centrifugation. The initial increase in gluten index indicated the improvement in gluten quality during postharvest wheat maturation (Elizabet *et al.*, 2014). In this period, polymerisation and macromolecular redistribution of gluten proteins occurred and then progressively increases and as a consequence increase the proportion of gluten retained on the sieve (Aja *et al.*, 2004; Elizabet *et al.*, 2014).

Swelling index of glutenin in stored wheat

Table 1 presents the changes in swelling index of glutenin (SIG) of wheat flour for the four samples in postharvest maturation. SIG for all wheat samples rose with storage time. Significant increase in SIG for WT4 was found during maturation compared with the other three samples. At 12 weeks of storage, SIG was 3.86, while SIGs of WT1, WT2 and WT3 were 3.35, 3.37 and 3.52, respectively. SIG for WT4 nearly reached the highest value at 6 weeks of storage, and then slowly rose with storage time. However, time of SIGs reaching the highest value for other three samples was late than WT4. The SIG can predict of wheat quality, the dough characteristics and the food quality, quickly and simply. SIG was generally used to assess the suitability as a screening procedure for wheat gluten strength. The insoluble glutenin was the protein fraction most responsible for the SIG value and gluten strength (Wang *et al.*, 2007).

SDS sedimentation value of stored wheat

Sedimentation tests were generally used to characterise wheat flours and aimed to predict wheat processing, gluten quality and end-product qualities (Oelofse *et al.*, 2010). It measures the sedimentation volume of an acidified suspension of wheat flour. It is a relatively low-cost, less time-consuming test that requires low manpower, is inexpensive and requires no elaborate laboratory equipment compared with other quality tests. It has proved to be a reliable, highly reproducible quality test that generally gives a good indication of the end-use quality of wheat (Carter *et al.*, 1999) especially in cases where the wheat has a low to medium protein content. Change in the SDS-SV of wheat under four different storage conditions was shown in Table 1. According to table1, at 12th week of storage, the SDS-SV for WT1, WT2, WT3 and WT4 increased by 5.43, 6.83, 8.1 and 11.5 ml, respectively. Besides, SDS-SV for WT4 reached 37.17 ml at 8th week of storage. It meant that SDS-SV reached a maximum value for wheat stored in condition of higher temperature and higher moisture in short time compared to wheat stored under lower temperature and lower moisture. It is reported that, as a function of heating time when dry gluten was heated at 80 °C, the SDS-SV initially increased until it reached the maximum value, and further heat treatment resulted in decreased SDS-SV (Veraverbeke *et al.*, 1997). The SDS-SV was positively related to the baking quality of wheat flour (Carter *et al.*, 1999). The baking quality and gluten quality are mainly governed by storage protein content and composition (Taenzler *et al.*, 2002). SDS-SV reflected not only differences in protein content, but also differences in protein quality (Carter *et al.*, 1999).

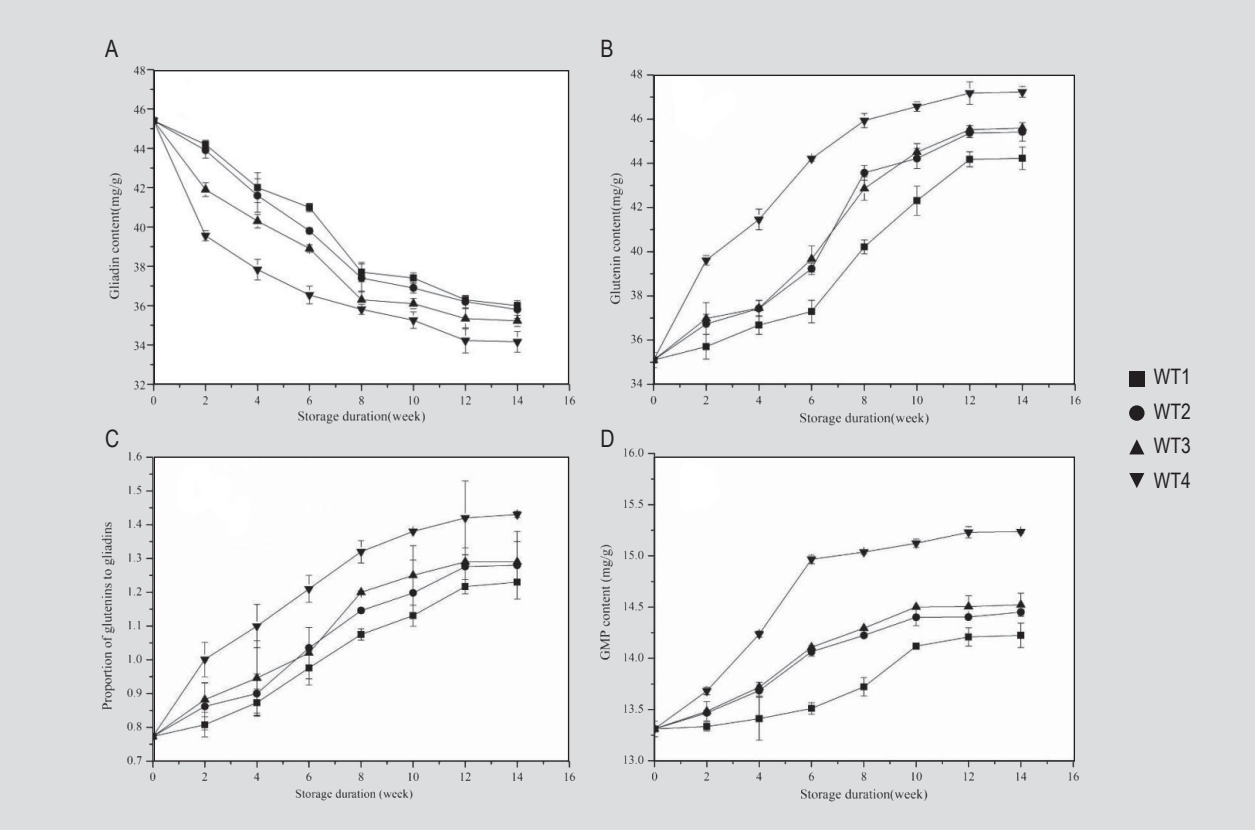

Figure 1. Changes in content of different protein fractions during postharvest wheat maturation. (A) gliadin; (B) glutenin; (C) ratio of glutenin and gliadin; (D) glutenin macropolymer (GMP). The values in this figure are means of triplicates.

Change in content of gluten protein fractions

During wheat postharvest maturation, the obvious changes in content of gliadin, glutenin and SDS-insoluble glutenin (GMP) were found (Figure 1). The content of gliadins for the four wheat samples decreased with storage time (Figure 1A). After storage for 12 weeks, gliadin content began to keep in relative constant level. The content of glutenin and GMP, proportion of glutenins to gliadins increased during storage (Figure 1B-D). It is shown that during postharvest maturation, the components of gluten protein changed continuously. Content of proteins in wheat flour does not play the key determinant role in flour performance; rather, flour quality is determined by certain protein sub-fractions deduced from gluten (Anjum *et al.*, 2007). These wheat gluten proteins can be classified into two main sub-groups: gliadins and glutenins (Osborne, 1907). Gliadins confer viscosity and extensibility properties to the dough, whereas glutenins is the major components that impart strength and elasticity to the dough (Wieser, 2007). The variation of size and composition of GMP was correlation with a key technological quality parameter of wheat (Don, 2005). It is very interesting to study the change of polymer content, polymer size, polymer including subunit composition in postharvest maturation. Significant increase (P<0.01) in GMP content for WT4 was found in initial 6 weeks of storage compared with the other three wheat samples. And then there were no obvious alteration in GMP content with long time of storage. It was in consonance with change in SIG and SDS-SV mentioned above. The temperature and drought had an important effect on the aggregation of gluten proteins in the developing wheat grain. Particularly, drought caused the early onset of the rapid polymerisation of gluten proteins (Carceller and Aussenac, 2001). A significant change in protein solubility of wheat kernel samples kept at higher temperature was observed when stored 4 and 30 °C for 270 days (Wilkes and Copeland, 2008). The profiling and identification of gluten proteins revealed that the formation of GMP was an increase in the content of high molecular weight glutenin subunits or conversion of gliadin fractions during postharvest wheat maturation.

The content of free sulfhydryl groups of glutenin macropolymer during the wheat postharvest maturation

About the structure of the GMP, a number of models have been proposed for the polymeric structure of glutenin. Most of structural models proposed the backbone of GMP was composed of HMW-GS only or HMW-GS and LMW-GS, and in which, the GMP contained both inter- and intra-chain disulphide bonds, as well as non-covalent interactions (Lindsay *et al.*, 1999). Differences in the bonding properties of glutenin subunits were thought to account for differences in the physiochemical properties of polymeric glutenin, which, in turn, accounted for variation in the functional properties of dough. Effect of storage duration, temperature, and relative humidity on the contents of SH groups and disulphide bonds of GMP was shown in Figure 2. The content of SH groups in GMP for the four wheat samples decreased during postharvest maturation (Figure 2A). Significant reduction (P<0.05) in the content of free SH was found for WT4 during the initial 6 weeks of storage. The contents of free SH for the other three samples slowly reduced during the whole maturation. Contrary to the content of free SH, the contents of disulphide bonds (S-S) of GMP rose from 0.142 to 0.173 mmol/g (WT1), 0.185 mmol/g (WT2), 0.194 mmol/g (WT3) and 0.251 mmol/g (WT4) during the total period of storage. A significant increase (P<0.05) in the content of S-S bond was noticed for WT4 during the initial 6 weeks of storage. The prolongation of storage time resulted in slow increase in the content of

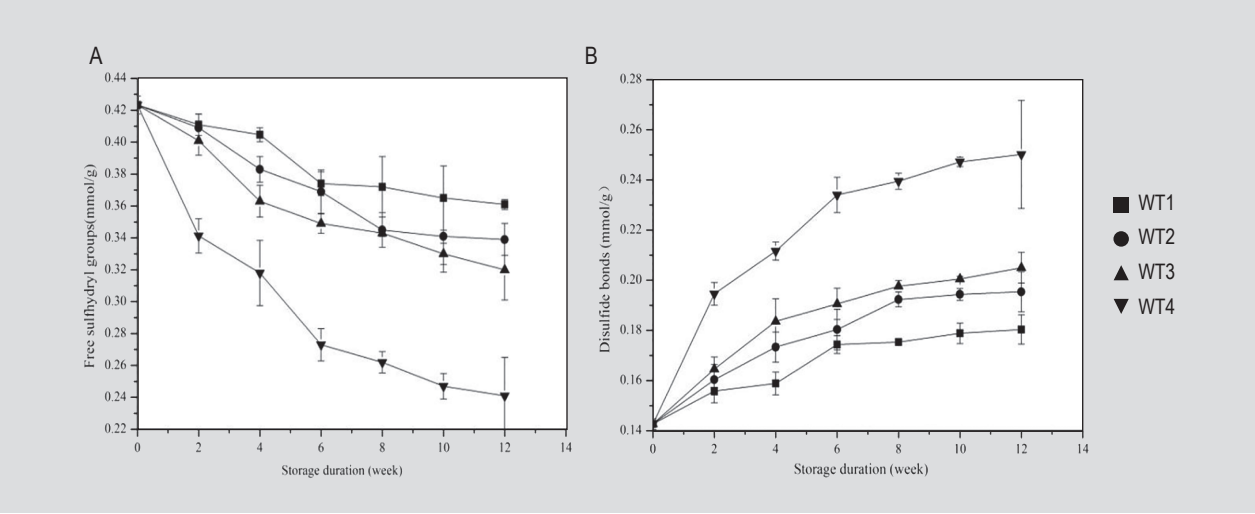

Figure 2. Changes in free sulfhydryl groups and disulphide bonds of glutenin macropolymer (GMP) during wheat postharvest maturation (mmol/g GMP). (A) is free sulfhydryl groups, and (B) is disulphide bonds.

S-S bond. It was in agreement with the GMP contents. It was suggested that the oxidisation of free SH into SS bond in GMP during the wheat maturation. In addition, some of HMW-GS and LMW-GS in glutenins may incorporate into the previous GMP through the SH-SS exchange during the postharvest wheat maturation. Therefore, larger particle of GMP formed. The rearrangement of GMP during wheat maturation in the field occurred to some extent, which resulted from SH/SS exchange and resulted in the increased number of SS bonds (Johansson *et al.*, 2013). According to our results, these mechanisms probably continued during the wheat postharvest maturation process as reported by Tomić *et al.* (2013). Moreover, the increase in SIG, SDS sedimentation value and GMP content could be explained as a result of strengthen in structural linkage within GMP.

Subunits composition of glutenin macropolymer

To understand the effect of postharvest maturation on composition and structure of GMP, SDS-PAGE was carried out to analyse the change in subunits of GMP. The SDS-PAGE profile of GMP under reducing conditions was shown in Figure 3. The GMP showed the presence of both HMW-GS (between 85 and 120 KDa) and LMW-GS. A typical difference among these four wheat samples was the presence of a new high molecular weight band at approximately 145 KDa in the upper region for WT4 after 4 weeks of postharvest maturation. Corresponding to this trend, some of molecular weight bands below 20 KDa lost in this period (Figure 3D). At the same time, colour of some molecular weight bands between 85 and 120 KDa turned darker (Figure 3D). Although no significant change in number of molecular weight bands was observed for WT1, WT2 and WT3, some bands in the LMW region were lost while some new bands appeared in the HMW region (Figure 3A-C). Moreover, some bands in the HMW region gradually turned darker in colour with storage time. It was suggested that the change of GMP in wheat grains was obvious under the high temperature and humidity storage condition. The reason may be that some of LMW-GS were incorporated into the aggregate, or that different HMW-GS and LMW-GS form more complex polymeric structures by S-S linkage that were assembled into GMP. In conclusion, and the assemble process of GMP depended on storage temperature, storage time and moisture content.

Gluten protein distribution patterns by size-exclusion high performance liquid chromatography analysis

Size-exclusion high performance liquid chromatography (SE-HPLC) can be used to analyse the size or molecular weight distribution of gluten proteins. Samples with smaller particle size are eluted out earlier than larger ones in SE-HPLC (Cornec *et al.*, 1994). The extractability of glutenin in SDS solutions presents a good indication of the cross-linkage degree (Hayta and Schofield, 2004). According to Figure 4, the SE-HPLC profiles of gluten proteins followed the same chromatogram pattern from the four samples. The peaks mainly included four fractions at a particular interval of elution time, the peaks F1 and F2 corresponded with HMW glutenin polymers and LMW glutenin polymers. It was well known that the polymeric and oligomeric glutenin fractions (F1 and F2) mainly contributed to GMP formation (Van Herpen *et al.*, 2008). The third peak (F3 fraction)

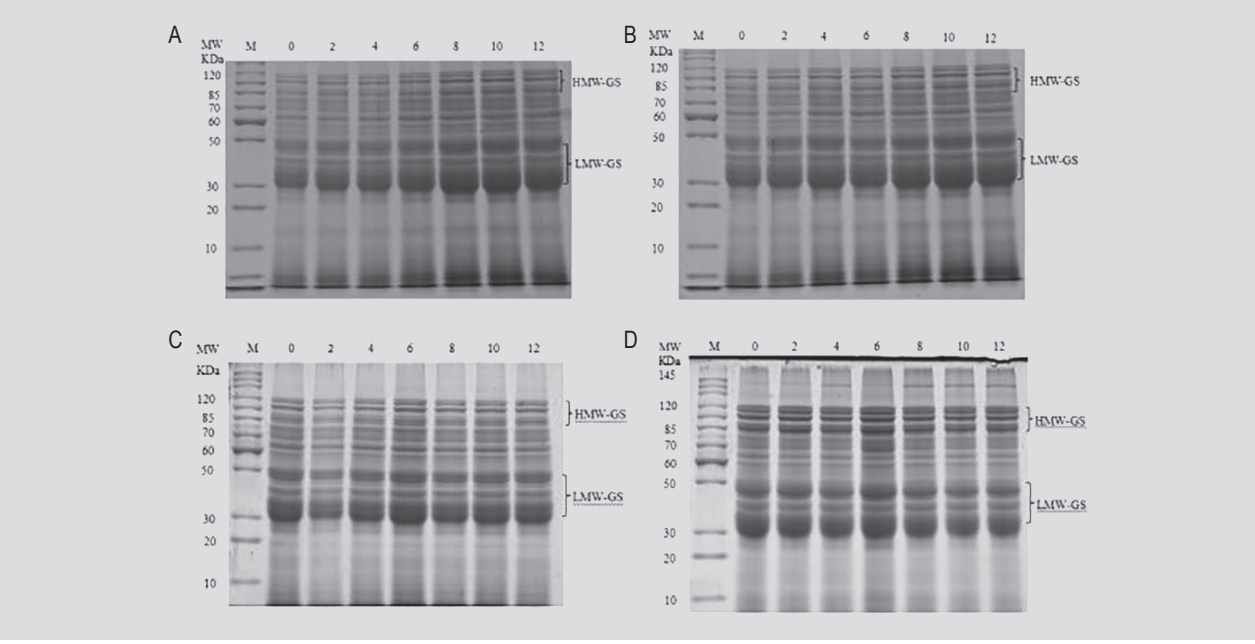

Figure 3. SDS-PAGE pattern of glutenin macropolymer of wheat during postharvest maturation. A, B, C and D represent WT1, WT2, WT3 and WT4, respectively.

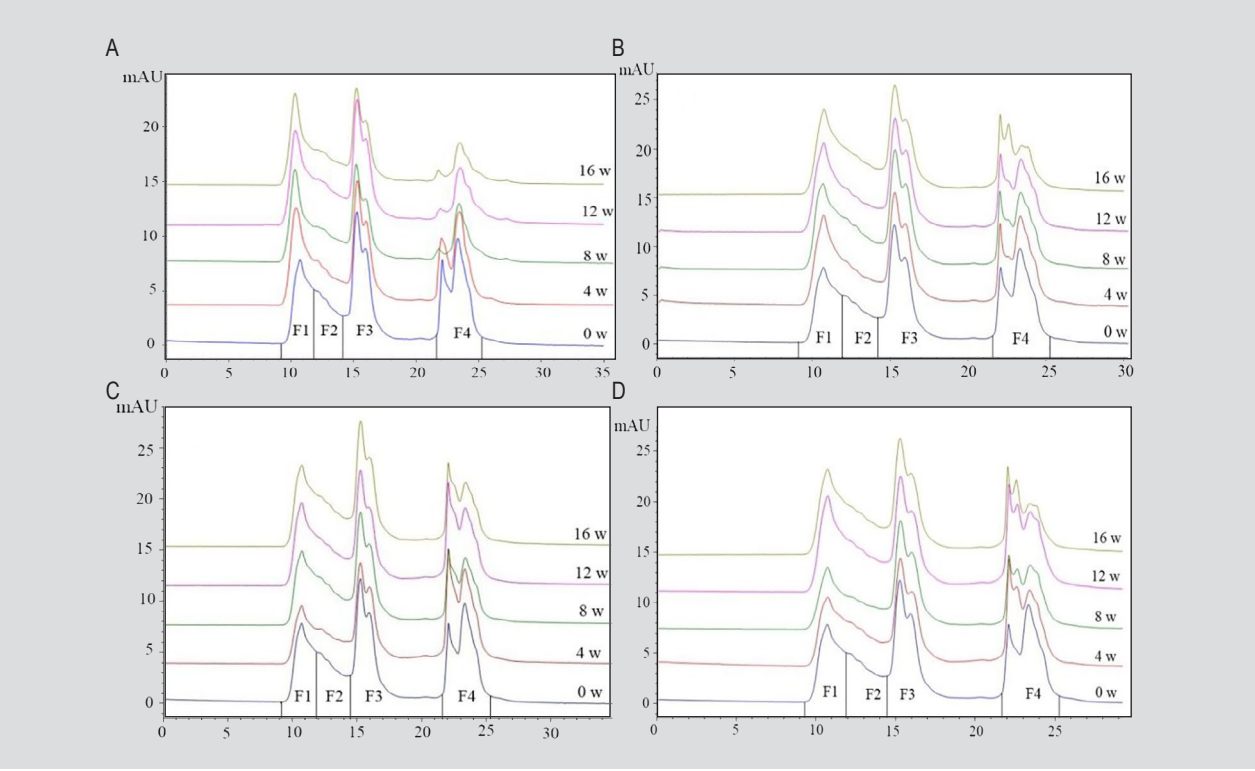

Figure 4. Molecular weight distribution of protein during postharvest maturation by size-exclusion high performance liquid chromatography. A, B, C and D represent WT1, WT2, WT3 and WT4, respectively.

was gliadin, while other peak (F4) represented albumin and globulin fractions. The shape and area of F4 had no obvious change in storage for the four samples. Decrease in area of F4 for the samples was noticed during storage. In contrast, the areas of F1 and F2 increased during the wheat postharvest maturation. At 16 weeks of wheat storage, the relative proportion of F3 was quiet low in comparison to F1 and F2. The relative proportion of SE-HPLC peaks' area % eluted fractions is summed up in Figure 5. The change of F1, F2 and F3 was observed at 4 weeks, 8 weeks and 12 weeks of storage. The percentage yield of large polymeric protein significantly increased at 4 weeks (30.74, 35.35, 34.99 and 39.35, respectively). Afterward, it continued to increase slowly until 16 weeks of storage. The F3 decreased significantly at 4 weeks of storage, and then slowly declined. The increase in small polymeric protein was observed with storing time. All samples reached their maximum at 16 weeks of storage in F1 and descend to minimum in F3. Moreover, significant difference among these four fractions was also observed for the four samples at the same time of storage. At 16 weeks of storage, F1 exhibited the lowest area (33.793) in WT1, while the highest area of F3 (19.544). Nevertheless, F1 of WT4 sample exhibited an increment from 27.85 to 45.54,F3 showed a decrement from 26.05 to 12.98. These revealed that the presence of higher proportion of polymeric glutenins and lower proportion of monomeric gliadins. It is indicated that the content of large polymeric protein and small polymer protein increased, and content of monomeric gliadins decreased during the wheat postharvest maturation.

4. Conclusion

The physicochemical properties of freshly-harvested wheat were greatly modified during the postharvest maturation at four different conditions of storage (WT1, WT2, WT3 and WT4). During wheat postharvest maturation (in initial storage period), rearrangement or polymerisation of GMP was observed resulted from interaction of free SH and S-S bond. This led to the great increase of GI, SIG and SDS-SV, indicating that the quality of freshly-harvested wheat improved significantly during maturation. The rearrangement or polymerisation of GMP was also found in the increase in the contents of glutenin, ratio of glutenin and gliadin, and GMP, and the decline of gliadin content of wheat during storage. Moreover, polymerisation of GMP was indicated in SDS-PAGE profiles and SE-HPLC patterns, especially for WT3 and WT4 samples. The results indicated that some HMW-GS only or HMW-GS and LMW-GS of glutenins were assembled into larger GMP by S-S linage. The change in structure and subunit composition of glutenin maybe associated with the quality improvement of wheat during the postharvest maturation. The present study provided useful information about the modification of the structure and composition of GMP of the postharvest wheat maturation under the selected storage conditions.

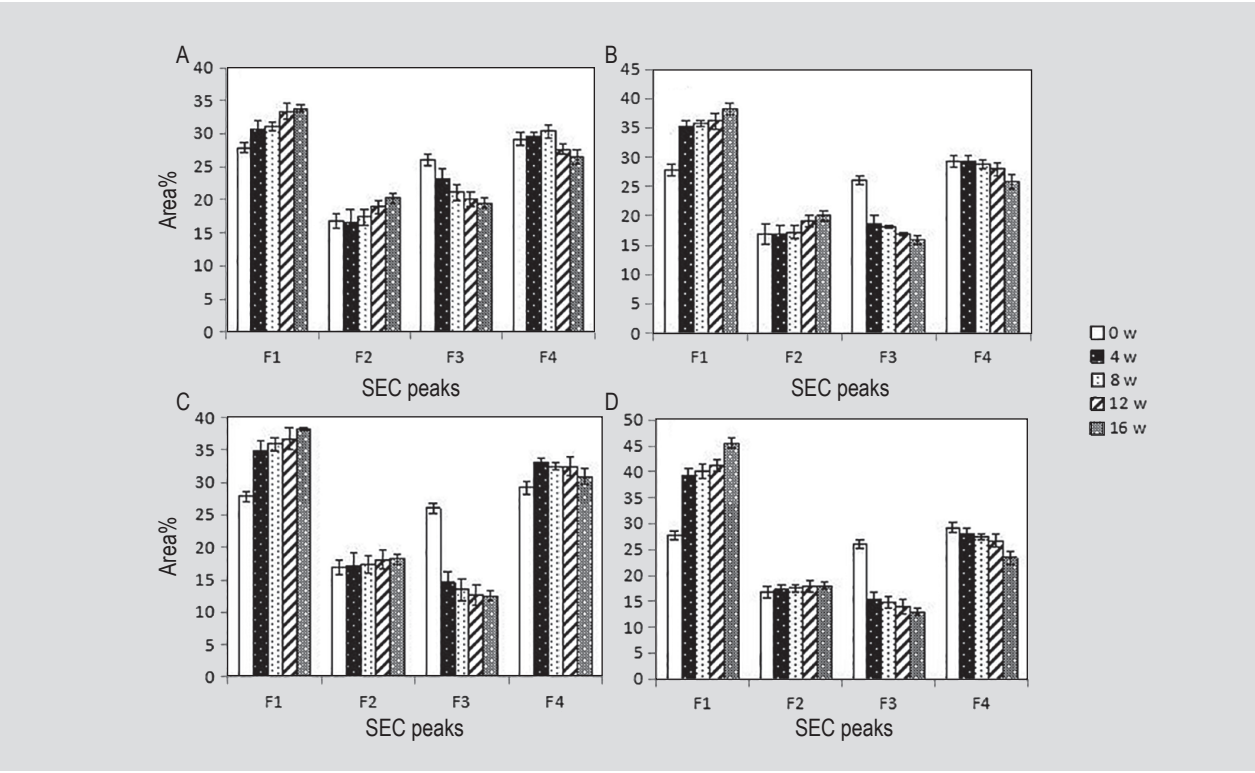

Figure 5. Relative area % of peaks obtained using size-exclusion high performance liquid chromatography. A, B, C and D represent four storage conditions of WT1, WT2, WT3 and WT4, respectively.

The present study confirmed the necessity of postharvest wheat maturation in order to make the technological quality improvement. In order to draw more reliable conclusions, further investigations should be made covering a wider range of wheat varieties from different gluten strength and different production years.

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