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RESEARCH ARTICLE

Zhiying DENG, Jichun TIAN, Yongxiang ZHANG, Cailing SUN, Jiansheng CHEN

Accumulation of lipoproteins in Chinese winter wheat cultivars and their impact on dough mixing characteristics

Abstract The changes in the accumulation of lipoproteins, the relationship between lipoproteins and the dough mixing characteristics of Chinese winter wheat (*Triticum aestivum* L.) were investigated for six cultivars that differ in quality characteristics and was classified into three groups according to their gluten index. All cultivars were grown under the same experimental field conditions, with three replicates. The lipoproteins were found to accumulate during the early stages of grain development. The rate of lipoproteins' accumulation appeared to follow a similar pattern of marked increase during the time from 5 DAA (days after anthesis) to 15 DAA, with a peak at 15 DAA, then quickly decreased for the same group of cultivars. Different patterns appeared from 20 DAA until maturity, but those cultivars with medium quality gluten showed a significant decrease during this period. Significant differences were found in lipoproteins for the six cultivars during grain development on the same days after anthesis. Correlation analysis indicated that lipoproteins from 25 DAA to 30 DAA were positively correlated with dough mixing parameters. However, the correlation coefficients were not statistically significant.

Keywords accumulation, lipoproteins, Chinese winter wheat, dough mixing properties

1 Introduction

Proteins are the important components of wheat (*Triticum aestivum* L.) that contributes to its end-use quality (Weegels et al., 1996). Variations in protein content and composition markedly modify flour quality, especially for bread baking (Weegels et al., 1996; Laflandra et al., 1999; Branlard et al., 2001). Wheat proteins are traditionally separated on the basis of their solubility as albumins, globulins, gliadins, and glutenins (Shewry et al., 1995). Within 10–15 days after anthesis (DAA), the first two fractions reflect only metabolic and structural proteins, and then, they accumulate in the developing starchy endosperm (Gupta et al., 1991; Singh et al., 1991; Gupta et al., 1993). The gliadin and glutenin fractions consist of storage proteins accumulated during the grain-filling period and are used as nitrogen sources during seed germination (Shewry and Halford, 2002).

A fifth fraction, classified as lipoproteins, can be isolated (Marion et al., 1994). The research on lipoproteins in wheat flour began quite early (Blochet et al., 1991). Some lipoproteins are involved in the formation of cell membranes and hydrophobic layers, while some of them play an important role in the transport of fatty acids or their CoA derivatives, such as the lipid transfer protein (LTP) thionins (Douliez et al., 2000). In addition, nonmembrane amphiphil proteins such as puroindolines have a large effect on grain hardness and dough rheological properties (Dubreil et al., 1998; Giroux and Morris, 1998). "Puroindolines from wheat flour are basic proteins that contain five disulfide bridges and a unique tryptophan-rich domain that is involved in lipid recognition and could contribute to the formation and stability of dough foams" (Simeone and Lafiandra, 2003).

Albumins and globulins accumulate from anthesis to approximately 20 DAA and then remain at an almost constant level. Storage proteins accumulate from approximately 6 DAA to the end of grain filling (Gupta et al., 1996; Stone and Nicolas, 1996). Gliadin accumulation occurs earlier in the grain-filling period than glutenin accumulation (Panozzo et al., 2001; Deng et al., 2004). Recently, it was observed that amphiphils accumulated

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Zhiying DENG, Jichun TIAN (✉), Yongxiang ZHANG, Cailing SUN, Jiansheng CHEN
State Key Laboratory of Crop Biology/Group of Quality Wheat Breeding, College of Agronomy, Shandong Agricultural University, Tai'an 271018, China
E-mail: jctian@sdau.edu.cn

during the early stages of grain development and so did the albumins and globulins, although their rate of accumulation did not decrease as much as that of the albumins globulins (Triboi et al., 2003). This pattern of accumulation is in good agreement with their supposed functions in the formation of cell membranes and walls and also as a protein associated with lipid storage in the endosperm (Douliez et al., 2000). Amphiphils can, in part, provide for the intra-and-extra cellular transport of hydrophobic molecules during grain filling. Lipid-protein interactions also play an essential role in lipid functionality during cereal processing. Generally, the functionality of proteins has been researched by reconstitution studies, but the kinetics of lipoproteins accumulation and the way that they affect the dough mixing quality has rarely been reported. Thus, the objectives of this research were to study the lipoproteins' formation and accumulation and further to analyze their relationships with dough mixing quality by using six cultivars known differing in flour quality characteristics.

2 Methods

2.1 Materials

Six winter wheat cultivars having different bread-making qualities were used. They were classified into three groups according to their basic characteristics, that is, Gaocheng8901 (Gao8901) and PH3259 (strong gluten), Youmai2 (YM2) and Youmai3 (YM3) (medium strength gluten), and 97-4 and 101 (weak gluten) (Table 1).

2.2 Experimental design

The six cultivars were grown at the experimental fields at Tai'an Research Farm of Shandong Agricultural University, in 2002–2003. The randomized plot area was 12 m^2, with three replicates. Total nitrogen ($0.89\pm0.05\ g\cdot kg^{-1}$), available phosphorus ($45.37\pm0.02\ mg\cdot kg^{-1}$), and available potassium ($80.35\pm0.01\ mg\cdot kg^{-1}$) were contained in the soil from depths of 0 to 20 cm.

2.3 Plant sampling and protein extraction

At anthesis, single heads (about 100) with a uniform size were marked. Kernel samples were taken at intervals of 5 days after anthesis until harvest. Grains harvested from about 100 whole heads were treated by liquid nitrogen. The individual grains freeze dried and were then milled using Perten 3100 mill (Perten instruments, AB. Sweden).

The extraction method was similar to those described by Marion et al. (1994) and Triboi et al. (2000), but some modifications were made such that the lipid bound proteins, which are designated lipoproteins, were extracted from 0.300 g of whole meal. During each extraction step, the samples were continuously stirred and shaken in an oscillating water incubator. Soluble and insoluble fractions were separated by centrifugation at $8000\times g$ for 20 min at the extraction temperature. First, the albumins and globulins were removed by extracting at 20°C with 7 mL of $0.05\ mol\cdot L^{-1}$ NaCl, $0.05\ mol\cdot L^{-1}$ sodium phosphate buffer at pH 7.8, and then centrifuged. The supernatant was discarded. Lipoproteins were extracted at 20°C from the pellet using 7 mL of 2% (v/v) TritonX-114, $0.1\ mol\cdot L^{-1}$ NaCl, and $0.05\ mol\cdot L^{-1}$ sodium phosphate buffer at pH 7.8. The supernatant was kept refrigerated at 4°C for further study.

2.4 Protein content determination

The supernatant solution containing lipoproteins was measured for protein content by the auto-Kjeldahl instrument using the procedures, as described in method AACC 46-11 (AACC International, 1983).

2.5 Physical-chemical analysis

Mature grains were milled to flour using Brabender Quadrumat Senior mill (Brabender OHG. Duisburg, Germany) (flour extraction rate = 70%). Physical dough tests were performed using Brabender Farinograph (Brabender OHG. Duisburg, Germany) with a 50-g mixing bowl, following the AACC 54-21 (AACC International, 1983) method. The mixing properties of the flour were also tested on Mixograph (Lincoln, Nebraska, U.S.A.) with a

Table 1 Basic characteristics in cultivars

cultivar	hardness index	protein content/%	gluten index/%	SDS-SE/mL	types of HMW-GS		
					1A	1B	1D
Gao8901	85	15.4	94.3	47.8	1	7 + 8	5 + 10
PH3259	82	15.2	81.08	45.2	Null	14 + 15	5 + 10
YM2	70	12.3	75.9	30.8	Null	7 + 9	5 + 10
YM3	73	11.0	62.7	25.9	1	14 + 15	2 + 12
101	50	9.3	25.6	18.2	Null	7 + 8	2 + 12
97-4	52	9.5	41.6	17.8	1	7 + 8	2 + 12

Note: SDS-SE represents sodium dodecyl sulfate-sedimentation volume. HMW-GS represents high molecular weight glutenin subunit.

10-g sample bowl according to the method AACC 54-40 (AACC International, 1983).

2.6 Data analysis

SPSS11.0 was used to compare the difference of amphiphil means and correlation analysis.

3 Results

3.1 Lipoproteins accumulation

The lipoproteins had begun to accumulate 5 days after anthesis (Table 2). Initially, similar changes in accumulation were found for different cultivars, and a peak appeared at about 15 DAA, but the accumulation pattern was different. For cultivars with strong gluten (Gao8901 and PH3259), the accumulation in lipoproteins content trended upward from 5 DAA to 15 DAA, dropped at 20 DAA, but then markedly resumed increasing from 20 DAA on. The amount of lipoproteins accumulated was about 0.9 μg per kernel per day in Gao8901. PH3259 also increased slightly up to 30 DAA.

Mean comparison tests were produced by considering the six cultivars having different qualities. Significant differences in lipoprotein content were found in different cultivars within the same days after anthesis (Table 2). These results indicated that the total accumulation of lipoproteins at different development stages could be related to the cultivars.

The accumulation of lipoproteins in YM2 and YM3 cultivars per kernel per day increased by 0.28 ‰ and 0.47 ‰, respectively, from 5 DAA to 15 DAA. Peaks appeared at 15 DAA, dropped quickly, and remained stable when approaching maturity.

In contrast with strong gluten cultivars, the rate and amount of accumulation of lipoprotein proteins for the weak gluten cultivars were markedly greater up to 15 DAA. Although the amount was very small at the beginning, the accumulation rate per day was about 72 μg. The accumulation pattern for 101 increased first to a plateau, maintained a period, then declined, and finally increased slightly at maturation, which is similar to that for albumin and globulin (Deng et al., 2004).

3.2 Correlation between lipoproteins content and dough rheological parameters

Table 3 indicates that lipoproteins from different stages showed different correlations with Farinograph and Mixograph parameters. From 15 DAA to 20 DAA, a great number of coefficients appeared negative between lipoproteins and dough rheological characteristics except for MTI. While from 25 DAA to 30 DAA, lipoproteins are positively correlated with major Farinogram and Mixogram parameters. All these correlation coefficients did not appear to be statistically significant.

These results indicated that lipoproteins at different stages had different effects on dough mixing parameters and also suggested that there was an optimal lipoproteins time, which would avail to dough mixing characteristics.

4 Discussion

In the present study, lipoproteins were extracted at different development stages of wheat kernels prior to storage protein synthesis. The accumulation rates and totals of lipoproteins followed a similar initial trend in the same groups, with a peak occurring at 15 DAA. However, the total accumulation rates appeared to be different in cultivars. Perhaps this difference is caused by the genotype or the interactions between lipoproteins and other proteins. These need to be further studied.

It was observed that the formation of lipoproteins apparently began earlier than 5 DAA, as the albumins-globulins did, but the accumulation rates for lipoproteins did not decrease as much as that for the albumin and globulin fractions (Deng et al., 2004). Perhaps this is because the latter are a relatively homogeneous group of proteins, while the lipoproteins appear diverse, containing many proteins such as lipid-bound proteins, lipid-transferable proteins, puroindolines, and so on. These proteins, perhaps, have different accumulations during grain development, which will affect the lipoproteins accumulation. Therefore, the lipoproteins can be separated into fractions by electrophoresis, and then each fraction will be researched, which will be a good method to study the accumulation of lipoproteins. This work is to be further studied by high-performance capillary electrophoresis

Table 2 Comparisons of lipoproteins of cultivars in the same days after anthesis (%)

cultivar	5 DAA	10 DAA	15 DAA	20 DAA	25 DAA	30 DAA
PH3259	0.336E	0.391E	0.416F	0.346F	0.562C	0.583C
Gao8901	0.431D	0.435D	0.630D	0.560C	0.881A	0.898A
YM2	0.473C	0.636C	0.753C	0.470D	0.433E	0.487E
YM3	0.499B	0.716A	0.967A	0.671B	0.564C	0.578D
101	0.697A	0.660B	0.940B	0.941A	0.709B	0.846B
97-4	0.076F	0.160F	0.487E	0.399E	0.544D	0.579D

Note: Different capital letters in a column represent significance at 0.01 probability level.

Table 3 Correlation coefficients between lipoproteins and dough rheological parameters

coefficient	5 DAA	10 DAA	15 DAA	20 DAA	25 DAA	30 DAA
PT	−0.100	−0.232	−0.455	−0.373	0.578	0.402
PV	0.324	0.320	−0.158	−0.244	−0.264	−0.240
PW	0.284	0.263	−0.182	−0.268	−0.236	−0.205
PI	−0.073	−0.194	−0.486	−0.422	0.481	0.315
TV	0.258	0.206	−0.247	−0.300	0.111	0.044
TW	0.060	−0.032	−0.414	−0.406	0.309	0.182
PS	−0.283	−0.397	−0.180	−0.081	0.649	0.526
DTT	0.004	−0.156	−0.470	−0.362	0.500	0.358
DST	−0.010	−0.174	−0.373	−0.265	0.651	0.496
MTI	−0.130	−0.125	0.294	0.396	−0.176	−0.045
BT	0.012	−0.151	−0.418	−0.312	0.574	0.427
FQN	0.012	−0.151	−0.418	−0.312	0.574	0.427

Note: PT, PV, PW, PI, TV, TW, PS, DDT, DST, MTI, BT, and FQN represent midline peak time, midline peak value, midline peak width, midline peak integral, midline 8-min time value, midline 8-min time width, right peak slope of midline, dough development time, dough stability time, mixing tolerance index, breakdown time, and Farinogram quality number, respectively.

(HPCE) in our laboratory. In addition, the accumulation trend of YM2 was very similar to that of YM3 because they share the same parents.

It is well known that the storage proteins, gliadins, and glutenins play a critical role in governing wheat flour properties, including baking quality (Dachkevitch and Autran, 1989; Gupta et al., 1993; Popineau et al., 1994; Ciaffi et al., 1996; Gupta et al., 1996; Triboi et al., 2000). Although lipoproteins contribute to the spreading and stability of lipid and lipid-protein films, the role of lipoproteins in dough mixing properties is not well known except for the work done with the puroindolines of mature kernels, as reported by Dubreil et al. (1998). In the current study, the correlation coefficients between lipoproteins and major dough mixing properties appeared not to be significant. The lipoproteins, to some extent, appeared to not strongly affect the Farinograph and Mixograph properties. At present, the influencing mechanism has been unknown in wheat, and the components of lipoproteins have been individually unthinkable for the effect. Perhaps the amount of lipoproteins would influence their distribution between structural and storage proteins. Therefore, a larger number of samples with different quality properties, including grain hardness, should be used to further study the mechanism between lipoproteins and dough mixing properties. In addition, exchange and reconstitution studies between lipoproteins and base flours with different characteristics would be another good method to study this mechanism.

5 Conclusions

The study on the effects of using different types of wheat on lipoproteins formation and accumulation gave new insights into the accumulation of lipoproteins. Lipoproteins accumulated in the grain during the early stages, that is, the cell division stage. The initial accumulation trend of lipoproteins in the same type of cultivars was similar. However, the total accumulation amount of lipoproteins produced during grain development was significantly different among cultivars. Therefore, the type of cultivars might affect the rates and total accumulation of lipoproteins during grain filling. As for the cultivars studied, the relationships between lipoproteins and dominating dough mixing properties appeared not to be statistically significant.

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References

AACC International (1983). Approved Methods of the American Association of Cereal Chemists, 8th ed. The Association: St. Paul, MN

Blochet J E, Kaboulou A, Compoint J P, Marion D (1991). Amphiphilic proteins from wheat flour: specific extraction, structure and lipid binding properties. In: Bushuk W, Tkachuk R, eds. Gluten proteins. St Paul, Minnesota: American Association of Cereal Chemists, 314–325

Branlard G, Dardevet M, Saccomano R, Lagoutte F, Gourdon J (2001). Genetic diversity of wheat storage proteins and bread wheat quality. Euphytica, 119: 59–67

Ciaffi M, Tozzi L, Lafiandra D (1996). Relationship between flour protein composition determined by size-exclusion high-performance

liquid chromatography and dough rheological parameters. Cereal Chemistry, 73: 346–351

Dachkevitch T, Autran J C (1989). Prediction of baking quality of bread wheats in breeding programs by size-exclusion high-performance liquid chromatography. Cereal Chemistry, 66: 448–456

Deng Z Y, Tian J C, Liu X P (2004). Accumulation regularity of protein components in wheat cultivars with different HMW-GS. Acta Agronomica Sinica, 30: 481–486 (in Chinese)

Douliez J P, Michon T, Elmorjani K, Marion D (2000). Structure, biological and technological functions of lipid transfer proteins and indolines, the major lipid binding proteins from cereal kernels. Journal of Cereal Science, 32: 1–20

Dubreil L, Meliande S, Chiron H, Compoint J P, Quillien L, Branlard G, Marion D (1998). Effect of puroindolines on the breadmaking properties of wheat flour. Cereal Chemistry, 75: 222–229

Giroux M J, Morris C F (1998). Wheat grain hardness results from highly conserved mutations in the friabilin components puroindolines a and b. Proceedings of National Academic Science, 95: 6262–6266

Gupta R B, Khan K, MacRitchie F (1993). Biochemical basis of flour properties in bread wheats. Ⅰ. Effects of variation in the quantity and size distribution of polymeric protein. Journal of Cereal Science, 18: 23–41

Gupta R B, Masci S, Lafiandra D, Bariana H S, MacRitchie F (1996). Accumulation of protein subunits and their polymers in developing grains of hexaploid wheats. Journal of Experimental Botany, 47: 1377–1385

Laflandra D, Masci S, Blumenthal C S, Wrigley C W (1999). The formation of glutenin polymer in practice. Cereal Foods World, 44: 572–578

Marion D, Nicolas Y, Branlard G, Landry J (1994). A new and improved sequential extraction procedure of wheat protein. In: Proceedings of international meeting on heat kernel proteins molecular and functional aspects. Viterbo: Univerita Degli Studi Della Tuscia, 197–199

Panozzo J F, Eagles H A, Wootton M (2001). Changes in protein composition during grain development in wheat. Australian Journal of Agricultural Research, 52: 485–493

Popineau Y, Cornec M, Lefebvre J, Marchylo B (1994). Influence of high Mr glutenin subunits on glutenin polymers and rheological properties of glutens and gluten subfractions of near-isogenic lines of wheat Sicco. Journal of Cereal Science, 19: 231–241

Shewry P R, Halford N G (2002). Cereal seed storage proteins: structures, properties and role in grain utilization. Journal of Experimental Botany, 53: 947–958

Shewry P R, Sayanova O, Tatham A S (1995). Structure, assembly and targeting of wheat storage proteins. Journal of Plant Physiology, 145: 620–625

Simeone M C, Lafiandra D (2003). Analysis of puroindoline b and GSP genes in rye. Proceedings of the tenth international wheat genetics symposium, Italy, 521–523

Singh N K, Shepherd K W, Langridge P, Gruen L C (1991). Purification and biochemical characterization of triticin, a legumin-like protein in wheat endosperm. Journal of Cereal Science, 13: 207–219

Stone P J, Nicolas M E (1996). Varietal differences in mature protein composition of wheat resulted from different rates of polymer accumulation during grain-filling. Australian Journal of Plant Physiology, 23: 727–737

Triboi E, Abad A, Michelena A, Lloveras J, Ollier J L, Daniel C (2000). Environmental effects on the quality of two wheat genotype. 1. Quantitative and qualitative variation of storage proteins. European Journal of Agronomy, 13: 47–64

Triboi E, Martre P, Triboi-Blondel A M (2003). Environmentally-induced changes in protein composition in developing grains of wheat are related to changes in total protein content. Journal of Experimental Botany, 54: 1731–1742

Weegels P L, Hamer R J, Schofield J D (1996). Critical review: functional properties of wheat glutenin. Journal of Cereal Science, 23: 1–18