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Variation in polar lipids located on the surface of wheat starch

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ABSTRACT

It is unknown whether starch isolated before dough development has the same surface lipid composition as starch isolated after dough development. The abundance of starch surface polar lipids is related to the physical hardness of the endosperm, but the variation in specific lipid classes and molecular species is unknown. The objective of this study was to determine the variation in polar lipids present on the surface of wheat starch granules. The experimental wheat lines used are, within each set, near-isogenic to each other but vary in endosperm hardness. Starch was isolated using two different processes: a dough and a batter method. Direct infusion electrospray ionization tandem mass spectrometry was used to identify and quantitatively determine the polar lipid species in wheat flour and on starch. Wide ranges in starch surface polar lipid concentrations were observed between the starch isolation methods. Starch isolation method provided a greater source of variation than did wheat kernel hardness. When dough is optimally mixed, lipids originally on the surface of wheat starch are dissociated, whereas in a batter system, starch surface lipids stay associated with the starch surface. The predominant starch surface polar lipids were digalactosyldigylcerol (DGDG), monogalactosyldigylcerol (MGDG) and phosphatidylcholine (PC) polar lipid classes.

1. Introduction

Wheat (*Triticum aestivum* L.) starch constitutes 70–75% of the flour and is composed of three distinctive types of granules: A-type, B-type and C-type. The A-type granules are lenticular in shape with a diameter greater than 16 μm, the B-type granules are spherical in shape with a smaller diameter of 5–16 μm and the C-type granules are spherical and have the smallest diameter of less than 5 μm (Bechtel et al., 1990). During development of the wheat endosperm, A-type granules are synthesized first, beginning at 4 days after flowering. B-type granules begin developing at 10 days after flowering, and C-type granules begin developing at 21 days after flowering (Bechtel et al., 1990).

The internal structures of normal starch granules consist of the high-molecular-weight polymers, amylose and amylopectin. Amylose, the minor component, is a linear polymer of α-(1,4)-linked D-glucopyranosyl units, and amylopectin is a highly branched polymer of α-(1,4)-linked D-glucopyranosyl units with α-(1,6)-branch points. The non-reducing ends of amylopectin and amylose are orientated toward the granule's center, and the reducing ends are toward the surface of the starch granule. Wheat starch granules contain channels that allow some molecules to enter the granule matrix (Kim and Huber, 2008). A-type granules contain two types of channels: larger channels located on the equatorial groove and smaller channels located throughout the granule. B-type starch granules contain only one type of channel: large, void-like channels that are less defined than the channels on the A-type granules (Kim and Huber, 2008). Han et al. (2005) and Lee and BeMiller (2008) found that starch channels are lined with proteins and polar lipids. Han et al. (2005) identified the proteins as 38–40 kDa brittle-1 proteins. Lee and BeMiller (2008) identified the channel lipids in maize starch as lysophosphatidylcholine polar lipids with either palmitic acid (16:0) or linoleic acid (18:2) fatty acid moieties.

The compositional structure of the surface of wheat starch granules plays a crucial role in wheat endosperm hardness.

Abbreviations: Pin-A, puroindoline A; pin-B, puroindoline B; DGDG, digalactosyldigylceride; MGDG, monogalactosyldigylceride; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PA, phosphatidic acid; PS, phosphatidylserine; PG, phosphatidylglycerol; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGMG, digalactosylmonoglycerols; MGMG, monogalactosylmonoglycerol.

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Greenwell and Schofield (1986) identified a molecular marker for endosperm hardness as 15 kDa proteins that were found on the surface of water-washed starch granules from soft wheat. These proteins (called friabilin) were found in greater amounts on water-washed starch from soft wheat than on water-washed starch from hard wheat. The friabilin proteins were absent from water-washed starch from durum wheat (the hardest class of wheat). Jolly et al. (1993) and Morris et al. (1994) further discovered, through N-terminal sequencing, the existence of two proteins, puroindoline A (pin-A) and puroindoline B (pin-B), which together make up friabilin. Besides puroindoline proteins, 30 kDa glycoproteins and 60 kDa starch granule-bound starch synthase enzymes are located on the surface of starch granules (Baldwin, 2001).

As is the case for puroindoline proteins, Greenblatt et al. (1995) found that a pattern existed among polar lipids on the surface of starch granules. Both galactolipids and phospholipids were found, via thin layer chromatography, to be present in greater amounts on water-washed starch from soft wheat than on water-washed starch from hard wheat (Greenblatt et al., 1995). Konopka et al. (2005) found a negative correlation between starch surface lipids (polar and non-polar) and kernel hardness. Finnie et al. (submitted) found that 146 different lipid species were present on the surface of wheat starch granules in total mean amounts ranging from 89 to 482 nmol/g. The major polar lipid species identified were DGDG (36:4), MGDG (36:4), PC (36:4 and 34:2), and LPC (18:2 and 16:0).

Addition of water and energy to wheat flour produces a unique viscoelastic dough capable of retaining gases during fermentation and proofing. When dough is mixed to optimum (i.e., with sufficient hydration and work applied), the gliadin and glutenin proteins interact and form a continuous protein macropolymer (gluten) surrounding the starch granules (Pomeranz, 1988; Singh and MacRitchie, 2001). Few research articles have documented the interaction between gluten proteins (gliadin and glutenin) and the starch granule surface as it relates to flour functionality (Hoseney et al., 1971; Larsson and Eliasson, 1997; Sandsted, 1961). Larsson and Eliasson (1997) used *in vitro* modification of the starch granule surface (heat treatment, absorption of wheat protein and absorption of phosphatidylcholine) and found that these treatments affected the rheological properties of the dough, thereby indicating the importance of the starch granule surface components in dough functionality. Because the starch granule surface components appear to interact with the other components in dough, their abundance on the starch granule surface, their composition or both could vary depending on whether the starch was isolated before or after dough development (i.e., before or after interactions).

The results presented in Finnie et al. (submitted) were derived from starch isolated with a batter method, thus representing the starch granule surface polar lipids absent of dough development. If the starch granule-gluten interactions are mediated or affected by the starch granule's surface composition, the polar lipids on the surface of the starch granules might not be present in the same abundance or ratios after dough development. Additionally, the results of Greenblatt et al. (1995) indicate that the abundance of the starch granule surface polar lipids is related to the physical hardness of the endosperm (implying a relationship between puroindoline proteins and polar lipids). Results of Greenblatt et al. (1995) indicated a genetic source of variation in the relative abundance of starch granule polar lipids but did not indicate how the specific classes and molecular species of the polar lipids were affected by puroindoline protein expression. Therefore, the objective of this study was to quantitatively determine the variation in polar lipids present on the surface of wheat starch granules. Variation in the starch granule surface polar lipids was evaluated as resulting from genetic variation (from differences in wheat endosperm hardness) or induced by the starch isolation processes. Genetic variation studies were conducted using three pairs of near-isogenic wheat lines that varied in their puroindoline expression and kernel endosperm hardness. Induced variation studies were conducted using two distinctly different starch isolation processes: a dough-ball method (Wolf, 1964), representing starch after experiencing dough development, and a batter method, representing starch that had not experienced dough development.

2. Experimental

2.1. Wheat samples

A series of unique experimental wheat lines (Table 1) were collected that vary in their starch granule surface components. The experimental wheat lines used are, within each set, near-isogenic to each other but vary in their grain endosperm hardness. One set was derived from the hard cultivar Hi-Line (PI 549275), the second set was derived from the hard cultivar Bobwhite, and the final set was derived from the soft cultivar Alpowa (PI 566596) (Hogg et al., 2005; Morris and King, 2008).

The wheat samples derived from Hi-Line and Bobwhite were grown near Bozeman, MT at Montana State University Post Agronomy Farm. The Alpowa-derived samples were grown near Pullman, WA by the USDA-ARS Western Wheat Quality Laboratory. The wheat was grown in replicated plots in 2007. Once the wheat lines were harvested and cleaned, single kernel hardness was determined with the Single Kernel Characterization System 4100 (Perten Instruments North America, Inc., Springfield, IL). To provide enough wheat for the experiments, the two field replicates were bulked into one sample (hardness values were compared to ensure no combinations of multiple wheat lines).

2.2. Milling

The wheat lines were milled into straight-grade flour with a Bühler experimental mill per AACC International Approved Method 26–31 (AACC International, 2008). Modifications included a short tempering time (20 min) for the Hi-Line and Bobwhite derived samples instead of the specified 16–24 h time. The 20-min tempering was short enough to ensure that the water did not penetrate into the endosperm but provided enough time for the bran to become plastic and not break into small particles, which could contaminate the flour. The Alpowa-derived samples were tempered (24 h) per AACC International 26–31. All wheat samples were tempered to 14% moisture content, and the wheat was milled at a reduced feed rate of 100 g/min.

Table 1
Cultivar and corresponding experimental line, puroindoline haplotype, molecular change and SKCS hardness value of the wheat samples.

Cultivar/experimental line	Puroindoline haplotype	Molecular change from wild-type[†]	Hardness (SKCS)[§]
Alpowa	*Pina-D1a/Pinb-D1a*	–	31
Alpowa/hard-nil	*Pina-D1a/Pinb-D1e*[*]	Pinb nullTrp-39 to stop	68
Hi-Line	*Pina-D1a/Pinb-D1b*	Pinb Gly46 to Ser	82
HGAB18	*Pina-D1a*[**]*/Pinb-D1a*[**]	–	9
Bobwhite	*Pina-D1b/Pinb-D1a*	Pina null	84
BW2	*Pina-D1a*[**]*/Pinb-D1a*	–	24

[†] Wild-type defined as *Pina-D1a/Pinb-D1a* puroindoline haplotype.
[§] SKCS single kernel characterization system hardness index value.
[*] Indicates puroindoline gene modified through backcross breeding method.
[**] Indicates puroindoline gene modified through transgenic method.