Degree of Starchy Endosperm Separation from Bran as a Milling Quality Trait of Wheat Grain

Lingyan Kong¹ and Byung-Kee Baik^{1*}

¹US Department of Agriculture, Agricultural Research Service, Soft Wheat Quality Laboratory,
Wooster, Ohio 44691

*Corresponding Author: email, byungkee.baik@ars.usda.gov; phone, 330-263-3891; fax, 330-
263-3651.

Abstract

Easy and clean separation of starchy endosperm from bran during milling could have a significant contribution to the increased flour yield. Starch content and DMSO extractable content of bran were determined as the estimates for remnant endosperm content of bran for 61 and 100 soft red winter (SRW) wheat genotypes grown in 2013 and 2014, respectively. DMSO extractable content was found to be more reliable in the estimation of remnant endosperm content of bran than was starch content with better reproducibility. Flour yields of the 2013 and 2014 SRW wheat varieties tested ranged from 65.1 to 72.4% (w/w), and 63.6 to 73.9% (w/w), respectively. The remnant endosperm content of bran estimated using the DMSO extractable content ranged from 33.9 to 43.6% (w/w) and 35.4 to 47.4% (w/w) for the 2013 and 2014 crops, respectively. The remnant endosperm content was significantly related to flour yield with a correlation coefficient of $r = -0.54$ ($P < 0.001$) from the 2014 crop, which was greater than that for any other grain characteristic including test weight, kernel hardness, and kernel weight. The degree of endosperm separation varied with different wheat varieties, different bran pieces and even different parts of the same piece of bran.

Key words: bran, flour yield, wheat milling

1. Introduction

Flour yield is an important wheat quality trait that not only determines profits for milling industries, but also indirectly affects the quality of end-products. Flour yield is highly heritable and is the primary quality trait evaluated in wheat breeding programs (Souza et al., 2012). Much effort has been devoted to the identification of genes that control the flour yield of wheat (Breseghello and Sorrells, 2006; Breseghello et al., 2005), with no perceivable success. The genetics of flour yield is difficult to precisely define, mainly due to the complexity of the linkage between genetics and the grain characteristics influencing flour yield. A single grain trait is often regulated by multiple genes and consequently is linked to a number of quantitative trait loci (QTLs).

Physical, biochemical and morphological grain characteristics, e.g. test weight, kernel weight, kernel size, and kernel hardness, are known to influence the flour yield of wheat. Test weight, a.k.a. bulk density, is an important index of the density and the soundness of kernels, but is not always a good indicator of flour yield. While some researchers showed that flour yield increased with test weight (Dziki and Laskowski, 2005; Ohm et al., 1998), Lin and Czuchajowska (1997) reported a positive correlation between test weight and flour yield for soft white winter wheat but not for white club wheat. Similar uncertainty was found in other kernel characteristics, including 1000 kernel weight (Dziki and Laskowski, 2005), kernel hardness (Martin et al., 2001; Ohm et al., 1998) and kernel size. Kernel size is presumed to affect milling and baking quality, but the relationship is not definitive. Small kernels tend to be harder in kernel texture than large kernels, and thus negatively affect milling yield (Dziki and Laskowski, 2004; Sutton et al., 1992). Dziki and Laskowski (2004) reported that small kernels exhibited low flour yield with high ash content. Increases in flour yield and protein content corresponding to increased kernel size was reported

by Sutton et al. (1922). Baasandorj et al. (2015) also observed that large kernels had higher flour yields than small kernels. Gaines et al. (1997) disputably reported no significant influence of kernel size on flour yield of soft wheat, although the degree of kernel shriveling showed a significant influence on flour yield. In addition to kernel characteristics, Gaines et al. (2000) reported that soft wheat cultivars with larger starch granules exhibited higher flour yields. They also observed the relationships of lower amylose content and higher total starch content with higher flour yield. In addition, it was found that a decrease in starch granule-bound puroindoline accounts for the increase in flour yield (Gaines et al., 2000).

Despite considerable efforts to improve wheat milling yield through advances in breeding, cultivation, and milling technologies, the theoretical maximum flour yield has not yet been achieved (Edwards et al., 2010). Our understanding of the biochemical and microstructural characteristics that affect flour yield is still superficial, and measurable grain traits for the effective selection of breeding lines for high flour yield need to be identified to efficiently and effectively predict flour yield.

Easy and clean separation of starchy endosperm from bran during milling would undoubtedly affect milling and flour yield, and is a more important issue for soft wheat than hard wheat, considering the lower tolerance for high bran contamination of products prepared from the former as compared to the latter. Endosperm separation index (ESI), the approximate quantity of endosperm remaining attached to bran, was calculated by subtracting the theoretical bran and germ contents (17% of the grain) from the actual content of bran and known to be related to flour yield (Yamazaki and Andrews, 1982). The ESI, however, suffers from inherent limitations: the use of fixed theoretical bran and germ contents in the calculation of ESI, and dependence of the ESI value on flour yield in the calculation. The endosperm content of bran could be an indicator

of the degree of endosperm separation from bran during milling and eventually be used as an important grain milling trait.

Cell walls have significant impact on the texture of wheat grains and their fractionation process (Jamme et al., 2008). Wheat grain cell walls are composed of many polysaccharides, proteins, and aromatic compounds, which could influence disintegration of the endosperm and eventually affect flour yield. Arabinoxylans, the predominant cell wall polysaccharides, are concentrated between the nucellar epidermis and the outer periclinal aleurone cell walls (Jamme et al., 2008), and play an important role in tissue adhesion and thus affect the separation of cells during milling.

The objectives of the study were to determine the variation in the degree of starchy endosperm separation from bran during wheat milling among soft red winter wheat genotypes, and the significance of the degree of starchy endosperm separation from bran for flour yield.

2. MATERIALS AND METHODS

2.1. Wheat Samples

The study was performed in two experiments involving two different sets of wheat genotypes. For Experiment 1, grains of 61 soft red winter wheat genotypes grown in four regional and state performance trials in 2013 were selected with evenly distributed experimental milling flour yields ranging from 65.1 to 72.4% (w/w). Brans from a subset of 24 varieties with evenly distributed flour yields ranging from 65.1 to 72.2% (w/w) were selected and ground into powders for starch and arabinoxylan content analyses. For Experiment 2, grains of 100 soft red winter wheat varieties grown in 2014 in five different state performance trials were used. Wheat

varieties were selected to represent a wide variation of flour yields ranging from 63.6 to 73.9% (w/w) in preliminary experimental milling.

2.2. *Experimental Milling*

Wheat grain was tempered to 15% moisture for 48 h and milled. Milling was conducted on a modified Brabender Quadrumat Jr. mill (C.W. Brabender Instruments Inc., South Hackensack, NJ) at a controlled temperature of 21°C and relative humidity of 55-60%. Milled products were recovered for sifting on a Great Western sifter box (Great Western Manufacturing Co. Inc., Leavenworth, KS) with screens of 471 and 180 µm openings, and separated into bran, middlings and flour. Flour yield was determined as the percent total flour weight (break flour + middlings) over tempered grain weight.

Brans of 24 genotypes representing a wide variation in flour yield were selected and ground using an Udy Cyclone Sample Mill (UDY Corporation, Fort Collins, CO) fitted with a screen with 0.5 mm openings, and sifted through a sieve of 0.5 mm openings to improve homogeneity of the ground bran. The ground bran was carefully recovered, rigorously blended in jars using a spatula and analyzed for starch and arabinoxylan contents.

2.3. *Grain Characteristics Evaluation*

Grain moisture was estimated using a Near Infrared DA7200 Analyzer (Perten Instruments, Springfield, IL). Softness equivalence was calculated as the percent break flour weight over total flour weight. Test weight of wheat grain was determined according to AACC Method 55-10.01 (AACC, 2002). Kernel weight and hardness were determined using a Perten 4100 Single Kernel Characterization System (SKCS, Perten Instruments, Springfield, IL).

2.4. *Dimethyl Sulfoxide (DMSO) Extraction of Bran*

DMSO extraction of bran was conducted to estimate the remnant endosperm attached to bran after roller milling. Bran (about 2 g) was weighed into 95% (v/v) DMSO aqueous solution (30 mL) in a 50 mL test tube and incubated in a boiling water bath for 30 min. The test tube was then centrifuged at 1,000 x g. Duplicate aliquots (1 mL) of the supernatant were transferred into pre-weighed test tubes. The DMSO extracts of bran were precipitated by the addition of 95% (v/v) ethanol (3 mL) and centrifugation. The supernatant was discarded, and the precipitate was re-suspended in 95% (v/v) ethanol (3 mL) and centrifuged. This was repeated once more. The precipitate was then dried for 30 min at 85°C and for another 30 min at 115°C. The weight of the DMSO extract of bran was calculated from the dried precipitate weight and expressed as a percentage over the bran weight.

2.5. *Starch Content of Bran*

Starch content of bran was determined using the Megazyme Total Starch Assay Kit (Megazyme International Ireland, Wicklow, Ireland) and procedure according to AACC Method 76.13 with modifications (AACC, 2002). Ground bran (approx. 100 mg) was added to a glass tube and wetted with 0.2 mL of aqueous ethanol (80%, v/v) to aid dispersion. The tube was stirred on a vortex mixer, and 3 mL of thermostable α -amylase reagent was added. The tube was placed in a boiling water bath for 30 min with stirring using a vortex mixer every 10 min. The tube was cooled to 50°C; amyloglucosidase reagent (0.1 mL) was added, and the tube was incubated in a water bath at 50°C for 30 min. Tube content volume was adjusted to 10 mL by the addition of distilled water, and the tube was centrifuged at 3,000 rpm for 10 min. An aliquot (1

mL) of the supernatant was diluted to 10 mL with distilled water. An aliquot (0.1 mL) of the diluted solution was transferred into test tube, and 3 mL of glucose determination reagent was added. The tube was incubated at 50°C for 20 min and the absorbance was read at 510 nm using a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corporation, Columbia, MD). Reagent blanks and glucose controls were used to establish a standard curve for glucose concentration.

For the grains of 100 varieties grown in 2014, starch content of bran was determined by DMSO extraction of starch from bran and subsequent assay of starch for extract. Bran (about 2 g) was weighed into a test tube, DMSO (30 mL) was added, and the tube was heated in a boiling water bath for 30 min and centrifuged at 1,000 x g. Then an aliquot (1 mL) of the supernatant was used for starch content analysis following the same procedure described above for ground bran.

2.6. *Arabinoxylan Content Determination*

Arabinoxylan content of bran was determined using a colorimetric method described by Ramseyer et al. (2011) with modifications. Ground bran (100 mg) was suspended in water (20 mL) and boiled for 30 min in replicate. When boiling, immediately after vortexing, duplicate aliquots (1 mL) from each replicate were transferred into 15 mL screw cap glass tubes with an additional 1 mL of water. Reaction agent was prepared by mixing 110 mL of glacial acetic acid, 2 mL of concentrated hydrochloric acid, 5 mL of 20% w/v phloroglucinol in absolute ethanol, and 1 mL of 1.75% w/v glucose. Reaction agent (10 mL) was added to each tube, and tubes were boiled for 25 min. The tubes were quenched in an ice water bath, and then brought to 23°C. The absorbance of each aliquot was read at 510 and 552 nm. The absorbance reading at 510 nm was subtracted from that at 552 nm to mathematically remove the influence of hexose sugars. A

standard curve was prepared using xylose standard solutions of 0, 5, 10, 50, and 100 mg D-(+)-xylose in 100 mL water. Triplicate standard solutions were used to determine the absorbance following the same procedure as that for ground bran. The total arabinoxylan content was calculated as mg xylose equivalents after conversion using the xylose standard curve (Douglas, 1981).

2.7. *Light and Fluorescent Microscopies*

Bran pieces from the dorsal parts of wheat grains were examined using a reflected light microscope with Optem™ zoom lenses (Qioptiq Inc., Rochester, NY). Bran specimens were prepared for microtome and light/fluorescent microscope observations according to the procedures by Santarem et al. (1997) and Wu et al. (2011). Bran pieces were infiltrated and fixed with 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M potassium phosphate buffer of pH 7.4 for 2 h at 23°C, and then overnight at 4°C. The specimens were then dehydrated in an ethanol series (50-100% ethanol for 10 min each) and critical point dried. Dehydrated specimens were embedded in Spurr's resin (Sigma-Aldrich, St. Louis, MO). Cross-sectional slices 1 µm thick were obtained using a Sorvall JB-4 Microtome (Highland Scientific, Stanwood, WA), and optionally stained with 0.5% toluidine blue for a few minutes and washed with water. Stained specimens were observed using an Olympus BX41 optical microscope (Olympus Corporation, Center Valley, PA). Unstained specimens were observed using a Leica DM IRB inverted fluorescence microscope (Leica Microsystems Inc., Buffalo Grove, IL), with UV fluorescent filters set at 340-380 nm and 425 nm excitation and emission wavelengths, respectively. The exposure time was set at 380 ms for all specimens.

2.8. *Scanning Electron Microscopy*

The inner surfaces and edges of bran pieces were observed using a Hitachi S-3500N scanning electron microscope (Hitachi America Ltd., Tarrytown, NY). Bran specimens were infiltrated, fixed and dehydrated following the same procedure described for the light microscope in the previous section. The dehydrated specimens were mounted onto SEM stubs using double-sided carbon tape and were coated with gold prior to observation.

2.9. *Statistical Analyses*

Starch content, arabinoxylan content, and DMSO extractable content analyses were replicated. Data were analyzed for least significant difference (LSD, $P < 0.05$), analysis of variance (ANOVA) and Pearson's linear correlation using the Statistical Analysis System 9.3 (SAS Institute Inc., Cary, NC).

3. Results and discussion

3.1. *Experiment 1: Estimating the Degree of Endosperm Separation in 2013 Crops*

Sixty-one soft red winter wheat varieties grown in 2013 were selected with evenly distributed experimental milling flour yields ranging from 65.1 to 72.4% (w/w). In a subset of 24 wheat varieties, starch content of bran as an estimate of remnant endosperm content of bran ranged from 25.1 to 31.9% (w/w) and showed significant genotypic differences, but failed to show a relationship with flour yield (Fig. 1A), possibly due to the small number (24) of varieties included in the test and the poor reproducibility of bran starch content assay. The arabinoxylan content of 24 varieties ranged from 9.1 to 11.7% (w/w), but showed no significant differences among varieties, nor a relationship with flour yield (Fig. 1B). There were relatively large differences between replications in starch and arabinoxylan assays of bran, possibly due to the

lack of homogeneity of the ground bran. Relatively heavy starch granules released from endosperm cells during grinding tend to be segregated from light and fluffy bran particles, making it extremely difficult to obtain homogenous ground bran.

To achieve a more reliable estimation for the remnant endosperm content of bran, DMSO extraction was employed with an increased amount of ground bran (2 g). Aqueous DMSO (95%, v/v) solubilizes starch as well as lipids, proteins, and polysaccharides, but leaves bran cell walls composed of cellulose. With DMSO extraction, bran became translucent and free of endosperm cells, indicating that endosperm cells were removed from bran and dissolved. The DMSO extractable content of 61 SRW wheat varieties ranged from 33.9 to 43.6% (w/w) and showed a significant correlation ($r = -0.30$, $P < 0.05$) with flour yield (Fig. 1C). Kernel hardness and test weight, however, exhibited no significant relationship with flour yield in 61 SRW wheat varieties (Table 1). The DMSO extraction method appears to yield a reliable estimate of the remnant endosperm content of bran with relatively low standard deviations of $<1.39\%$ (w/w).

3.2. Experiment 2: Estimating the Degree of Endosperm Separation in 2014 Crops

A preliminary analysis of the grain characteristics of 300 soft red winter wheat varieties harvested in 2014 revealed that test weight, SKCS kernel hardness, SKCS kernel weight and softness equivalence were not significantly correlated with flour yield. SKCS kernel weight had the strongest influence on flour yield among these grain characteristics. A subset of 100 varieties was selected with evenly distributed flour yields and SKCS kernel weights for further analysis.

The remnant endosperm content determined using the DMSO extraction method exhibited a significant correlation ($r = -0.54$, $P < 0.001$) with flour yield (Fig. 2A). Starch content of the bran determined by extracting starch from bran using DMSO and then assaying the extract also

238 showed a correlation with flour yield ($r = -0.46$ $P < 0.001$) (Fig. 2B). Better homogeneity of
239 DMSO extract than that of ground bran improved the reproducibility of starch assay, likely
240 contributing to the significant relationship of bran starch content with flour yield in addition to
241 the increased number of varieties. The correlations between grain characteristics and flour yield
242 in 100 SRW wheat varieties are summarized in Table 2. Test weight, SKCS kernel hardness,
243 softness equivalence, and remnant endosperm content of bran showed significant correlations
244 with flour yield. Test weight is a measure of grain bulk density and is indicative of grain
245 soundness and plumpness, serving as an important grading factor. The correlation coefficient
246 between test weight and flour yield was nominal ($r = 0.23$, $P < 0.05$) (Fig. 2C), indicating that
247 high test weight is desirable for, but not an effective predictor of, high flour yield. Kernel
248 hardness was significantly correlated positively with flour yield ($r = 0.41$, $P < 0.001$) (Fig. 2D)
249 and negatively with remnant endosperm content of bran ($r = -0.50$, $P < 0.001$), indicating that
250 the hard kernels of SRW wheat tend to give clean separation of bran during milling and
251 increased flour yield. Softness equivalence is the proportion of break flour over total flour, and is
252 heavily dependent on kernel hardness (Finney and Andrews, 1986). Softness equivalence
253 showed a significant correlation with SKCS kernel hardness ($r = -0.85$, $P < 0.001$). Kernel
254 weight failed to show a significant relationship with flour yield, but was correlated with SKCS
255 kernel hardness ($r = -0.43$, $P < 0.001$) and remnant endosperm content of bran ($r = 0.21$,
256 $P < 0.05$). The remnant endosperm content of bran showed a significant relationship with flour
257 yield, having a markedly higher correlation coefficient ($r = -0.54$, $P < 0.001$) than other grain
258 characteristics including test weight, kernel hardness and kernel weight (Table 2), thus indicating
259 that the degree of endosperm separation from bran during milling is a greater contributor to, and
260 better predictor of, flour yield than other grain characteristics. An even stronger correlation

between remnant endosperm content of bran and flour yield ($r = -0.67$, $P < 0.001$) was observed for 25 SRW varieties with kernel weights ranging from 37 to 41 mg.

3.3. Microscopic View of Bran

The scanning electron microscopic (SEM) images of a bran piece in Fig. 4 show the endosperm separation from bran by experimental milling. An edge view (Fig. 3A) of bran shows multiple layers of pericarp and seed coat (p), and aleurone layer (a), with a significant amount of endosperm (e). The extent of endosperm separation from bran can vary as seen in Fig. 3B to 3D. The bran piece shows a large amount of remnant endosperm (Fig. 3B), and also a relatively clean separation of endosperm as is evident in the center region of Fig. 3C. In some parts of the bran, the aleurone layer had completely separated from the pericarp and seed coats layer, exposing a fibrillar cell wall surface (Fig. 3D). The aleurone layer of grain is presumed to play an important role in endosperm separation from bran during milling, since this is where the separation of endosperm and bran takes place. The edge view of aleurone cells (Fig. 3A) shows the loss of cell content, possibly during milling or specimen preparation for SEM. On the interface between the aleurone layer and the starchy endosperm, residual fragments (r) of the peripheral endosperm cell walls remain attached to the aleurone layer. The peripheral endosperm cells are similar in size to the aleurone cells. Endosperm cells consist of spherical bodies (s) (approx. 5–20 μm in diameter) in a destructured cementing matrix. The spherical bodies are starch granules, and their separation from the protein matrix was probably caused by the dehydration process during specimen preparation.

The optical microscopy images of wheat bran cross-sections are illustrated in Fig. 4A to 4D. The specimens were stained with toluidine blue which binds to nucleic acids and proteins. The

large unstained globular and oval shapes clearly indicate the presence of multiple layers of starchy endosperm cells, whereas only tiny starch granules are scattered in the protein-rich aleurone cells. An overall view of a fairly representative bran piece (Fig. 4A) indicates that the degree of endosperm separation from bran varies even within the same piece of bran. Fig. 4B and 4C show close views of a region of bran with a considerable amount of remnant endosperm and a region of bran with relatively clear separation of endosperm, respectively. Several layers of endosperm cells stacking up to almost 10 times the thickness of the aleurone layer were also observed in Fig. 4D. Bran pieces separated from the dorsal part of a wheat kernel of two wheat varieties showing large differences in flour yield were examined under the optical microscope to determine the thickness of residual endosperm on bran. The two varieties showed no significant difference in the thickness of endosperm remaining attached to bran.

Fig. 4E and 4F shows autofluorescence images of unstained bran slices with UV excitation and emission. Autofluorescence was generated by aromatic components in cell walls, especially ferulic acid, the most abundant phenolic acid in cereal grains (Adom et al., 2003), which fluoresces naturally under UV light (Pussayanawin et al., 1988). Ferulic acid is often covalently bound to arabinoxylan (Gélinas and McKinnon, 2006). Therefore, the localization and distribution of ferulic acid and arabinoxylan are similar, but not identical, in barley and wheat, since there might be variable amounts of ferulic acid side groups on arabinoxylan (Jääskeläinen et al., 2013). In wheat, the arabinoxylan in aleurone cell walls was feruloylated, while arabinoxylan feruloylation was less abundant in endosperm cell walls (Jääskeläinen et al., 2013). The aleurone cell walls appear to be enriched with autofluorescing substances, while the endosperm cells emit very weak autofluorescence (Fig. 4F). However, no apparent difference in

autofluorescence was observed between bran pieces from wheat varieties of high and low flour yields.

4. Conclusions

DMSO extractable content of bran was a more reliable estimate of the remnant endosperm content than was starch content, and could be used to determine the degree of starchy endosperm separation from bran during milling. The remnant endosperm content of bran as an index of the degree of endosperm separation from bran varied widely from 33.9 to 43.6% (w/w) in 61 SRW wheat varieties grown in 2013, and from 35.4 to 47.4% (w/w) in 100 SRW wheat varieties grown in 2014. The remnant endosperm content of bran exhibited a significant relationship with flour yield with a greater correlation coefficient than test weight, kernel hardness and kernel weight, suggesting that the degree of starchy endosperm separation from bran contributes significantly to flour yield and should be considered in the development of soft wheat varieties with improved flour yield potential. The degree of endosperm separation from bran varies with different wheat varieties, different bran pieces and even different portions of the same piece of bran as observed using optical and scanning electron microscopy.

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Legends for figures

Fig. 1. Relationship of starch (A) and arabinoxylan (B) contents of bran with flour yield in 24 soft red winter wheat varieties harvested in 2013. Both correlations are not significant.

Relationship between remnant endosperm content of bran (C) and flour yield of 61 soft red winter wheat varieties harvested in 2013 ($r=-0.30, P<0.05$).

Fig. 2. Relationships of remnant endosperm content ($r=-0.54, P<0.001$) (A), starch content of bran ($r=-0.46, P<0.001$) (B), test weight ($r=0.23, P<0.05$) (C), and SKCS kernel hardness ($r=0.41, P<0.001$) (D) with flour yield of 100 soft red winter wheat varieties harvested in 2014.

Fig. 3. SEM images of bran: A, edge; B, remnant endosperm; C, inner surface; and D, inner surface without the aleurone layer. a, e, and p indicate aleurone layer, endosperm cell, and multiple layers of pericarp and seed coat, respectively. r and s point to residual fragments of the peripheral endosperm cell walls and spherical bodies in the destructured cementing matrix, respectively.

Fig. 4. Optical microscopy (A to D) and autofluorescence (E & F) images of bran cross-sections of 1 μm thickness: A, a representative cross-section, and B to D, the cross-sections of the dorsal part of wheat bran. The optical micrographs were obtained after staining specimens with toluidine blue. The autofluorescence images were captured using UV excitation at 340-380 nm and emission at 425 nm without staining.

Table 1

Pearson correlation coefficients for grain characteristics and remnant endosperm content estimated using DMSO extractable content of 61 soft red winter wheat samples harvested in 2013^a

	Softness Equivalence (%)	Kernel Hardness ^b	Test Weight (lb/bu)	Remnant Endosperm of Bran (% w/w)
Flour yield (%)	0.18ns	-0.23ns	0.07ns	-0.30s
Softness Equivalence (%)		-0.46ss	-0.67ss	-0.06ns
Kernel Hardness			0.45ss	-0.12ns
Test Weight (lb/bu)				-0.07ns

^as, correlations significant at $P<0.05$; ss, significant at $P<0.001$; ns, not significant at $P<0.05$.

^bKernel hardness measured by NIR DA7200 Analyzer.

Table 2

Pearson correlations between grain characteristics and remnant endosperm content estimated using DMSO extractable content of bran for 100 soft red winter wheat genotypes harvested in 2014^a

	Test Weight (lb/bu)	SKCS Kernel Hardness ^b	SKCS Kernel Weight ^b (mg)	Softness Equivalence (%)	Remnant Endosperm of Bran (%, w/w)
Flour yield (%)	0.23s	0.41ss	0.00ns	-0.53ss	-0.54ss
Test Weight(lb/bu)		0.35ss	0.00ns	-0.40ss	-0.13ns
SKCS Kernel Hardness			-0.43ss	-0.85ss	-0.50ss
SKCS Kernel Weight (mg)				0.12ns	0.21s
Softness Equivalence (%)					0.37ss

^as, correlations significant at $P < 0.05$; ss, significant at $P < 0.001$; ns, not significant at $P < 0.05$.

^b Kernel hardness measured by Perten Single Kernel Characterization System.

Fig. 1. Relationship of starch (A) and arabinoxylan (B) contents of bran with flour yield in 24 soft red winter wheat varieties harvested in 2013. Both correlations are not significant. Relationship between remnant endosperm content of bran (C) and flour yield of 61 soft red winter wheat varieties harvested in 2013 ($r=-0.30, P<0.05$).

Fig. 2. Relationships of remnant endosperm content ($r = -0.54$, $P < 0.001$) (A), starch content of bran ($r = -0.46$, $P < 0.001$) (B), test weight ($r = 0.23$, $P < 0.05$) (C), and SKCS kernel hardness ($r = 0.41$, $P < 0.001$) (D) with flour yield of 100 soft red winter wheat varieties harvested in 2014. Starch content of bran was determined by DMSO extraction of starch from bran and subsequent assay of starch for extract.

Fig. 3. SEM images of bran: A, edge; B, remnant endosperm; C, inner surface; and D, inner surface without the aleurone layer. a, e, and p indicate aleurone layer, endosperm cell, and multiple layers of pericarp and seed coat, respectively. r and s point to residual fragments of the peripheral endosperm cell walls and spherical bodies in the destructured cementing matrix, respectively.

Fig. 4. Optical microscopy (A to D) and autofluorescence (E & F) images of bran cross-sections of 1 μm thickness: A, a representative cross-section, and B to D, the cross-sections of the dorsal part of wheat bran. The optical micrographs were obtained after staining specimens with toluidine blue. The autofluorescence images were captured using UV excitation at 340-380 nm and emission at 425 nm without staining.