High-Speed NIR Segregation of High- and Low-Protein Single Wheat Seeds

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ABSTRACT

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Wheat breeders need a nondestructive method to rapidly sort high- or low-protein single kernels from samples for their breeding programs. For this reason, a commercial color sorter equipped with near-infrared filters was evaluated for its potential to sort high- and low-protein single wheat kernels. Hard red winter and hard white wheat cultivars with protein content >12.5% (classed as high-protein, 12% moisture basis) or < 11.5% (classed as low-protein) were blended in proportions of 50:50 and 95:5 (or 5:95) mass. These wheat blends were sorted using five passes that removed 10% of the mass for each pass. The bulk protein content of accepted kernels (accepts) and rejected kernels (rejects) were measured for each pass. For 50:50 blends, the protein in the first-pass rejects changed as much as 1%. For the accepts, each pass changed the protein content of accepts by ≈0.1%, depending on wheat blends. At most, two re-sorts of accepts would be required to move 95:5 blends in the direction of the dominant protein content. The 95:5 and 50:50 blends approximate the low- and high-protein mixture range of early generation wheat populations, and thus the sorter has potential to aid breeders in purifying samples for developing high- or low-protein wheat. Results indicate that sorting was partly driven by color and vitreousness differences between high- and low-protein fractions. Development of a new background specific for high- or low-protein and fabrication of better optical filters for protein might help improve the sorter performance.

Breeding for either high-or low-protein content is a goal of wheat breeding programs because protein composition and quality are related to wheat end-use quality (Huebner et al 1999; Baenziger et al 2001). Methods have been developed to help breeders rapidly screen wheat for protein content and other desirable traits. One method, PAGE, is being used in screening wheat cultivars for γ-gliadin 45, which is strongly linked with good cooking quality of pasta (Bushuk 1998). The method, however, is destructive and obtains information from bulk wheat samples. Another method is near-infrared (NIR) transmittance or reflectance spectroscopy. Protein and other seed constituents related to end-use quality have absorptions in the NIR region. Thus, protein content could be calibrated against specific NIR wavelengths or wavelength regions. The technique is nondestructive and allows protein measurements from single kernels, which is potentially useful to breeders for their selection programs. Williams (1979) developed a method based on NIR reflectance spectroscopy for screening early generations of wheat simultaneously for protein and hardness. NIR reflectance of wheat ground by a burr mill was calibrated against Kjeldahl protein and hardness (as measured by particle size index [PSI]), and against protein only for wheat ground by an impeller-type mill. For burr-milled wheats, protein was predictable to within 0.7% and PSI to within less than two units. Protein was predictable to within 0.31% for impeller-ground wheat.

Delwiche (1995) showed the feasibility of measuring protein content on individual wheat kernels using NIR transmittance (850–1,050 nm). Abe et al (1996) used the same technique but developed models using combinations of selected wavelengths. Models that used spectra averaged from four different directions yielded the least standard error of prediction, showing that shape effects could be minimized by spectral averaging. NIR reflectance is easier to adapt to real-time analysis than transmittance; thus, Delwiche (1998) developed this method for protein measurement of single wheat kernels. Reflectance spectra (at 1,100–1,500 nm) from individual kernels, oriented crease-side-down, were used to develop calibration models for single wheat classes, for classes pooled according to color, and for all five U.S. wheat classes. Delwiche obtained standard errors of performance of 0.46–0.72% protein, depending on the modeling technique. Stepwise analysis of multiple linear regression models identified eight important wavelengths for protein detection: 1,106, 1,138, 1,156, 1,170, 1,186, 1,200, 1,306–1,318, and 1,500–1,504 nm. Velasco and Möllers (2002) used NIR reflectance spectroscopy as a screening tool for segregating populations of rapeseed for protein. Law (1985) reviewed the use of NIR reflectance for estimating barley quality parameters (including protein) with respect to malting in breeding programs.

Currently, breeders do not have a means to select single seeds with desirable nonvisible traits such as high protein content for propagation. Screening large populations of early generation (up to F4) material requires much time and resources, causing screening for protein and hardness to be delayed to later generations (Williams 1979). End-use quality tests typically begin on F5, whereas milling and baking evaluations begin in the F7 wheat population (Baenziger et al 2001).

We propose use of high-speed single-kernel sorting to rapidly move early populations of wheat toward the desired high- or low-protein trait and thus advance the elimination of nonpromising populations in terms of end-use quality. The method, based on reflected NIR energy, does not require grinding, thus each kernel would be conserved. This would be an advantage because early generation wheat populations are typically available in limited quantities, and the probability of selecting the desirable trait is small. For example, in an F3 population from a cross between a high- and low-protein wheat, a high-protein line could only be recovered ≈2–5% of the time within random selections from the cross (J. Martin, *personal communication*). The high-speed sorter should able to sort wheat for protein from a few hundred grams to a few hundred bushels.

The objective of this study was to evaluate the potential of a commercial color sorter, equipped with infrared sensors and filters, for segregating high-and low-protein wheat kernels.

MATERIALS AND METHODS

Sorter

A ScanMasterII 200 DE high-volume color sorter equipped with near-infrared optical filters and detectors (Satake-USA, Houston, TX) was used in these experiments. This sorter has been described previously for red and white wheat segregation using red and green filters (Pasikatan and Dowell 2002). For protein sorting, the dual peak-filter 920/1,660 nm was chosen because these wavelengths have stronger absorption for protein than those of other

available filters. The 920 ± 3 nm filter passed reflected energy around that wavelength to a silicon sensor, whereas the 1,660 ± 7 nm filter passed reflected energy around that wavelength to an indium-gallium-arsenide (InGaAs) sensor. The 920 nm and 1,660 nm filters have a full-width, half-maximum bandwidth of 20 ± 5 and 45 ± 9 nm, respectively. The sorter has 10 inclined, parallel channels with 3-mm radius grooves that singulate seeds before each is viewed by front and rear charged coupled device (CCD) cameras (Fig. 1). Seeds in which optical signals exceeded the set threshold are rejected by air ejectors. Only two of the 10 channels were used at feed rate of 20/kg/hr/channel to allow longer uniform feeding time (•40 sec) for the small (1.2 kg) wheat samples.

Wheat Blends

Early generation wheats are only produced in small quantities; that precluded their use in these replicated sorting experiments. Instead, their protein content and distribution were approximated using blends from wheat cultivars. Hard white (HDWH) and hard red winter (HRW) wheat cultivars that are common in the Midwest and have protein content of either >12.5% (classed as high-protein) or <11.5% (classed as low-protein) were selected. These protein ranges were based on the classification of U.S. Wheat Associates (2001, 2002); wheat with protein range of 11.5–12.5% is classed as medium-protein wheat. Wheats were cleaned and graded using a dockage tester (Carter Day Co., Minneapolis, MN). Protein variance of 2.25–5.26 within a wheat field had been observed by Bramble (2001). A wider protein variance could be assumed for these wheat samples because they came from different fields in a county. Because protein content was measured from bulk samples, a
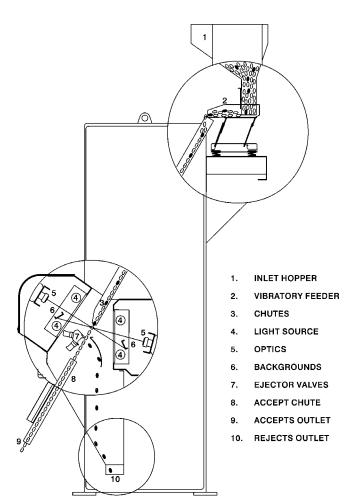

Fig. 1. Side view and cut-away sections of high-volume color sorter. Computer screen is in front.

1. INLET HOPPER
2. VIBRATORY FEEDER
3. CHUTES
4. LIGHT SOURCE
5. OPTICS
6. BACKGROUNDS
7. EJECTOR VALVES
8. ACCEPT CHUTE
9. ACCEPTS OUTLET
10. REJECTS OUTLET

high-protein wheat with wide variance might have low-protein kernels in it, or vice versa. Therefore, to approximate the assumption of 100% high-protein or low-protein kernels before mixing, the low-protein kernels from high-protein wheat and high-protein kernels from low-protein wheat were sorted out. Two-pass sorting that rejected 10% mass for each pass was used.

After the two-pass sorting, wheats were blended into 95:5 and 50:50 proportions by mass, which approximated the expected minimum and maximum ratio of high-protein and low-protein fractions of early generation wheat populations. Two wheat blends (Betty-Betty and Jagger-Jagger) approximated the properties of wheat from the same cultivar with different protein contents. The other two blends (Heyne-Lakin and Prowers-2137) approximated the properties of wheat from different cultivars with different protein contents (Table I). Each sample for a wheat blend weighed 1.2 kg.

Tests of Backgrounds

A background is a strip of colored material with spectral properties that provide contrast between color or reflectance signals from acceptable and rejectable products. Using a white background, signals for the InGaAs sensor could be balanced for both front and rear views, but those for the silicon sensor could be balanced only for the front view. Therefore, the threshold for the 920-nm signal was only used for the front view, and the amount of wheat rejected by this threshold was limited to •20% mass for each pass. This allowed 920 nm to contribute to sorting, but not so much as to introduce significant error due to extraneous absorptions and partial signal imbalance for the rear view. The 920-nm signal imbalance for the rear view using the white background indicated that a much lighter background was needed. Backgrounds made from styrene sheets 0.38-mm thick (Evergreen Scale Models, Kirkland, WA) were tested to check whether they would improve signal quality, and thus sorting. The same test procedures and 50:50 wheat blends were used for the styrene background sorting experiments.

Initial tests used invisible UV ink (Theatre Effects, Hagerston, MD) to coat and tag high- or low-protein wheat. Wheat was soaked in this ink for 1 hr and air-dried until it reached its pre-soaking moisture content. Coated kernels are not visible in white light, but fluoresce under UV light, thus allowing determination of coated kernels in either accepted wheat (accepts) or rejected wheat (rejects). These tests showed that single-pass sorting was inadequate in segregating high- from low-protein wheat, or vice versa; it would require rejection of >50% wheat mass to completely reject 5% coated wheat. Therefore, we decided to use a five-pass sorting procedure with threshold set to reject 10% mass of the incoming wheat for each pass. A completely randomized design was used for the sorting experiments, with four wheat blends and two thresholds (high-protein threshold [HPT] and low-protein threshold [LPT]) in a factorial arrangement of treatments. Three 1.2-kg samples (one for each replicate) were provided for each combination of blend and threshold, and each sample was subjected to five passes. Blends with 50:50 and 95:5 (or 5:95) high- and low-protein wheat proportions were grouped and sorted separately. The small sample size (1.2 kg) would not allow sampling without replacement of subsamples of accepts for protein analysis for each pass. Protein analysis requires •75-g samples. Thus, two representative subsamples (75–120 g) were taken from the accepts using a Boerner divider (Seedburo, Chicago, IL) and measured for protein; after protein measurements, these were remixed with the accepts for the next pass. Rejects were not subsampled because of their small amounts (75–120 g). The protein content of rejects was measured from the total rejects for each pass. The bulk protein content of subsamples was measured using an Inframatic 9100 (Perten-USA, Springfield, IL). It was set at 12% moisture content basis and to display a reading for each of two presentations of a single subsample. Tests of significance and comparison of means were conducted using repeated measures analysis of variance with the procedure PROC MIXED (SAS Institue, Cary, NC) (Littell et al 1996).

RESULTS AND DISCUSSION

Tests Using a White Background

For the 50:50 high- and low-protein wheat blends, the protein content of the accepts was significantly influenced by blends ($P < 0.0001$), threshold ($P < 0.0001$), blend × threshold ($P < 0.005$), threshold × pass ($P < 0.0001$), and blend × threshold × pass ($P = 0.0011$). Table II shows the effect of blend × threshold × pass interaction on protein content of accepts. In general, across wheat blends, HPT (level that defines the mass of high-protein kernels to be rejected) effectively lowered the protein content of the accepts, and LPT (level that defines the mass of low-protein kernels to be rejected) effectively increased the protein content of the accepts. The number of passes before the protein content of the accepts changed significantly from that of the original blend varied, depending on wheat blend. For example, for HPT, it was the second pass (P2) for Betty-Betty (HDWH) blend, whereas it was the third pass (P3) for Prowers-2137 (HRW) blend. This might have been caused by protein content differences between wheat cultivars in the blend (Table I), kernel protein distribution, and the amount of bran protein in each cultivar. Protein concentration increases from the center of the kernel to the bran layer (Morris et al 1946; Normand et al 1965; Kent 1966; Kent and Evers 1969; Piot et al 2000). However, protein may be lower in the outermost layer if such layer is high in true bran constituents (Normand et al 1965). Variations in bran proportion may, therefore, account for differences in grain protein content among wheat cultivars (Vogel et al 1976). Kernel size variation may also affect sorting because smaller kernels are richer in proteins than large kernels (% basis) (Pomeranz and MacMaster 1968).

The wheat blend Jagger-Jagger was the most difficult to sort; it changed significantly in protein content of accepts only on the fifth pass. The Jagger samples (both high and low protein) were the darkest among the blends, and it is likely that color was interfering with the sorting. The protein content difference (PCD) between the original blend and fifth-pass accepts was 0.32–0.79% for all wheat blends (Table II). This meant that protein content could be increased or decreased by ≈0.1% for each pass for 50:50 blends. Across wheat blends, the fifth-pass accepts did not reach the protein content of the original high-protein sample for LPT (Fig. 2). The same was true for the HPT and the original low-protein sample. However, for three out of four blends, protein content of first-pass HPT rejects was higher than that of the fifth-pass LPT accepts (Fig. 2, Table III). And for two out of four blends, the protein content of first-pass LPT rejects was lower than that of the fifth-pass HPT accepts. Signal differences between high- and low-protein kernels were highest at first pass, thus the protein content differences between the original sample and the first-pass rejects were the highest compared with the succeeding passes. These results suggested that first-pass rejects for both HPT and LPT (assuming adequate amounts) could be used by breeders instead of fifth-pass accepts to move their breeding populations to as much as 1% change in protein content. Unlike the 50:50 wheat blends, where the reference protein content was that of the original wheat blend, the 95:5 (or 5:95) high- and low-protein wheat blends used the protein content of the 95% wheat portion as the reference. The measure of sorting effectiveness is the pass at which protein content of accepts approached that of the 95% wheat portion. For HPT, the 95% wheat portion is the low-protein wheat; for LPT, the 95% wheat portion is the high-protein wheat.

TABLE I
Wheat Blends in Sorting Experiments

Wheat Blends (HP-LP)[a]	High-Protein Wheat			Low-Protein Wheat			Protein Content Difference (%)
	Origin	Crop Year	Protein Content[b] (%)	Origin	Crop Year	Protein Content[b] (%)	
HDWH blends							
Betty HP-Betty LP	Finney, KS	2001	14.48 (0.12)	Republic, KS	2000	11.88 (0.12)	2.60
Heyne HP-Lakin LP	Republic, KS	2001	14.64 (0.11)	Brown, KS	2001	10.31 (0.06)	4.33
HRW blends							
Jagger HP-Jagger LP	Finney, KS	2001	14.38 (0.12)	Harvey, KS	2000	11.45 (0.08)	2.93
Prowers HP-2137 LP	Colorado[c]	2001	14.53 (0.18)	Kansas[c]	2001	11.43 (0.09)	3.10

[a] Hard white (HDWH) and hard red winter (HRW) wheat cultivars. HP, high-protein; LP, low-protein.
[b] 12% moisture basis; values are mean and standard deviation (in parentheses) of 12 measurements. Protein values measured by Inframatic 9100 protein analyzer after the initial two-pass sort.
[c] Composite from different counties.

TABLE II
Protein Content (12% moisture) of Accepts for Each Pass of Four Wheat Blends and Two Thresholds

Blends (50:50 ratio) and Thresholds[a]	Pass[b]						PCD[c]
	P0	P1	P2	P3	P4	P5	
Betty-Betty							
HPT	13.41a	13.34a	13.19b	12.93c	12.87c	12.62d	0.79
LPT	13.12a	13.19a	13.37b	13.46b	13.47b	13.61c	0.49
Heyne-Lakin							
HPT	12.94 a	12.82a	12.68ab	12.66b	12.54b	12.49b	0.46
LPT	12.47a	12.56a	12.72b	12.75b	12.93c	13.02c	0.55
Jagger-Jagger							
HPT	12.96a	13.03a	12.94a	12.90a	12.83a	12.65b	0.32
LPT	12.89a	12.94a	13.00a	13.09a	13.10a	13.24b	0.35
Prowers-2137							
HPT	12.97a	13.03a	12.92a	12.74b	12.67b	12.62b	0.35
LPT	12.57a	12.63a	12.77ab	12.85ab	12.95b	13.03b	0.46

[a] HPT, high-protein threshold (sensitivity setting that rejects high-protein kernels). LPT, low-protein threshold (sensitivity setting that rejects low-protein kernels).
[b] P0, original blend before sorting (reference protein content); P1…P5, first, second, third, fourth, and fifth pass, respectively. Satake white background. Values followed by the same letter in the same row are not significantly different ($P < 0.05$). Standard error 0.054.
[c] Protein content difference between original blend and fifth-pass accepts.

Except for Heyne-Lakin, Jagger-Jagger, and Prowers-2137 HPT that approached the protein content of the 95% wheat portion after the second pass, all wheat blends approached the protein content of the 95% wheat portion after the first pass (Table IV). This indicated that removal of 10% wheat mass removed nearly all the 5% high- or low-protein wheat portion. Initial tests that used kernels coated with UV invisible ink showed that a few high-protein kernels were still with the low-protein majority, or vice versa. However, because these kernels constitute <5% of the bulk, they do not markedly affect the averaging done by the bulk protein analyzer. The difference in number of passes before the protein content approached that of the dominant wheat portion might be attributed to protein content differences between wheat cultivars in the blend and the amount of protein in the bran layer.

In early generation wheat population, where high-protein wheat might constitute the lesser portion (2–5%), high-protein wheat could be separated by rejecting 10% of the mass using HPT followed by little-at-a-time rejections of low-protein wheat. Alternatively, rejecting <10% mass in more than two passes might be done to ensure that high-protein kernels are separated. In these experiments, a 1.2-kg sample was single-pass sorted in ≈1 min using two-channel feeding at 20 kg/hr/channel. This same sample would take only 24 sec for 10-channel feeding. Typical 2.3-kg (5 lb) bags of early generation wheat

seeds would take <1 min/pass to process with this feed rate. For color sorting, faster processing and better sorting results could be achieved with feed rates of 44–61 kg/hr/channel (Pasikatan and Dowell 2001). In theory, these feed rates should work as well for protein sorting.

Tests Using Styrene Background

For the wheat blends Betty-Betty, Heyne-Lakin, and Prowers-2137, the styrene background enhanced the signal differences between high- and low-protein kernels, compared with the previous white background used. However, signals for both high- and low-protein Jagger kernels were the same for 1,660 nm. There was a slight difference in the 920-nm signals for the high- and low-protein kernels, but this was not enough to give a good sort because 920 nm has weak protein absorption. Possibly because of its whiteness, styrene gave a good contrast for high-protein (which is darker) but not for low-protein kernels. Thus, the sorter always yielded high-protein rejects despite use of LPT. For the white background, the 920-nm signal imbalance favored LPT. However, because sorting was governed primarily by the weaker signal (920 nm), sorting was less accurate. Because of near balance using the styrene background, the 920-nm signals could not bear the brunt of sorting. Because the sorter would not sort for both HPT and LPT, Jagger-Jagger blends were excluded from the tests.
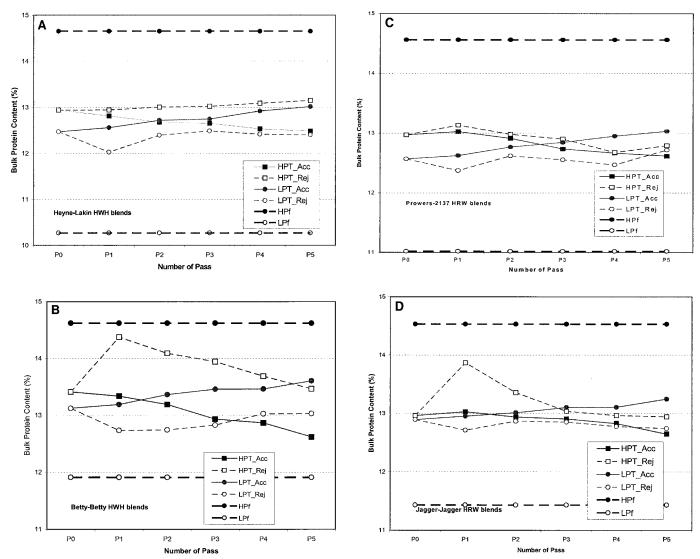

Fig. 2. Bulk protein content of accepted and rejected kernels for each pass, for high- and low-protein thresholds, for the four wheat blends used (white background). HPT_Acc, kernels accepted by high-protein threshold; HPT_rej, kernels rejected by high-protein threshold; LPT_Acc, kernels accepted by low-protein threshold; LPT_rej, kernels rejected by low-protein threshold; HPf, high-protein fraction of the blend; LPf, low-protein fraction of the blend.

For the 50:50 wheat blends, the protein content of accepts was significantly influenced by blends ($P < 0.0001$), threshold ($P < 0.0001$), blend × threshold ($P < 0.0001$), blend × pass ($P = 0.0003$), threshold × pass ($P < 0.0001$), and blend × threshold × pass ($P = 0.0074$). Generally, a slight improvement in sorting was observed,

as expressed in protein content difference between the original blend and fifth-pass accepts (Table V). This could be attributed to the use of bichromatic thresholds as permitted by the near balance of 920/1,660 nm signals.

Heyne-Lakin and Betty-Betty wheat blends have vitreous and nonvitreous fractions. Mostly vitreous fractions were rejected by the HPT and mostly nonvitreous fractions rejected by the LPT. Vitreous kernels are higher in protein content than starchy or non-vitreous kernels (Dexter et al 1989). One of the wavelengths identified by Dowell (2000) for classifying vitreous from nonvitre-ous kernels by NIR reflectance was 1,670 nm, a protein absorption wavelength in the bandwidth of the 1,660 nm filter used. Prowers wheat (high-protein) has a slightly darker color than 2137 (low-protein). However, high-protein Jagger had no visible difference in color or vitreousness with low-protein Jagger. Both were dark brown red and darker than the other three blends. Thus, results for the styrene background suggest that secondary optical differences (color or vitreousness) related to protein were contributing to sorting. In the absence of these differences, sorting by means of primary differ-ences (in protein) would be harder, as shown with the Jagger-Jagger blends. These indicated that a new background, which is both light and enhances well the signal difference between low- and high-protein wheat, as well as filters with stronger and more specific absorption to protein, would be needed to further improve protein sorting.

The discovered potential of the styrene background and 920/1,660 nm filters for vitreousness sorting would enable segregation of vitreous from nonvitreous kernels for different studies or for concentrating vitreous fractions for rapid measurement of vitre-ousness of a wheat sample.

TABLE III
Protein Content (12% moisture) of Original Unsorted Sample and First-Pass Rejects for Four Wheat Blends and Two Thresholds

Blends (50:50) and Thresholds[a]	P0[b]	P1R[c]	PCD[d]
Betty-Betty			
HPT	13.41	14.40	0.99
LPT	13.12	12.70	0.42
Heyne-Lakin			
HPT	12.94	12.92	0.02
LPT	12.47	12.11	0.36
Jagger-Jagger			
HPT	12.96	13.90	0.94
LPT	12.89	12.71	0.18
Prowers-2137			
HPT	12.97	13.13	0.16
LPT	12.57	12.37	0.20

[a] HPT, high-protein threshold (sensitivity setting that rejects high-protein kernels). LPT, low-protein threshold (sensitivity setting that rejects low-protein kernels).
[b] P0, original blend before sorting (reference protein content). Satake white background.
[c] P1R, first-pass reject.
[d] Protein content difference between original blend and the first-pass rejects.

TABLE IV
Protein Content (12% moisture) of Accepts for Each Pass of Four Wheat Blends and Two Thresholds

Blends (95:5) and Thresholds[a]	Pass[b]						P95[c]
	P0	P1	P2	P3	P4	P5	
Betty-Betty							
HPT	12.14a	11.99b	12.01b	12.01b	11.99b	11.98b	11.92b
LPT	14.27a	14.42b	14.41b	14.39b	14.41b	14.42b	14.40b
Heyne-Lakin							
HPT	11.08 a	11.02a	10.93b	10.92b	10.95b	10.93b	10.90b
LPT	14.30a	14.49b	14.43b	14.48b	14.47b	14.49b	14.53b
Jagger-Jagger							
HPT	11.75a	11.64a	11.61ab	11.58b	11.51b	11.53b	11.52b
LPT	14.20a	14.38b	14.34b	14.33b	14.35b	14.36b	14.40b
Prowers-2137							
HPT	11.26a	11.15a	11.10b	11.09b	11.05b	11.05b	11.08b
LPT	14.22a	14.42b	14.36b	14.33b	14.35b	14.35b	14.49b

[a] HPT, high-protein threshold (sensitivity setting that rejects high-protein kernels). LPT, low-protein threshold (sensitivity setting that rejects low-protein kernels).
[b] P0, original blend before sorting; P1…P5, first, second, third, fourth, and fifth pass, respectively. Satake white background. Values followed by the same letter in the same row are not significantly different ($P < 0.05$). Standard error 0.071.
[c] Protein content of the 95% portion of the blend (reference protein content).

TABLE V
Protein Content (12% moisture) of Accepts for Each Pass for Four Wheat Blends and Two Thresholds

Blends (50:50) and Thresholds[a]	Pass						PCD[c]
	P0	P1	P2	P3	P4	P5	
Betty-Betty							
HPT	13.41a	13.31a	12.96b	12.83c	12.74c	12.53d	0.88
LPT	13.12a	13.20a	13.39b	13.45b	13.53b	13.69c	0.57
Heyne-Lakin							
HPT	12.94a	12.71b	12.61b	12.56b	12.47c	12.33c	0.61
LPT	12.47a	12.60b	12.85c	12.87c	13.00d	13.09d	0.62
Prowers-2137							
HPT	12.97a	12.98a	12.86b	12.72c	12.68c	12.53d	0.44
LPT	12.57a	12.74b	12.89c	12.96c	13.05c	13.23d	0.66

[a] HPT, high-protein threshold (sensitivity setting that rejects high-protein kernels). LPT, low-protein threshold (sensitivity setting that rejects low-protein kernels).
[b] P0, original blend before sorting (reference protein content); P1…P5, first, second, third, fourth, and fifth pass, respectively. Styrene background. Values followed by the same letter in the same row are not significantly different ($P < 0.05$).
[c] Protein content difference between original blend and fifth-pass accepts.

CONCLUSIONS

A high-speed color sorter equipped with near-infrared filters was evaluated for its potential to segregate high- and low-protein wheat. For wheat with 50:50 proportions of high- and low-protein wheat, obtaining a nearly pure high- or pure low-protein portion in the final accepts would require more than five re-sortings of accepts. Measured from the accepts, the potential change in protein content for each pass rejecting 10% mass was ≈0.1%, depending on the thresholds used and wheat blends sorted. However, changes in protein content by as much as 1%, relative to the original blend, could be obtained from the first-pass rejects instead of the fifth-pass accepts because signal differences between high- and low-protein kernels were highest at first pass. Wheat blends with 95:5 or 5:95 proportion of high- and low-protein wheat could be moved toward the direction of the dominant wheat portion by re-sorting the accepts. While results showed the potential of the sorter for rapidly segregating high- and low-protein wheat, the same results indicated that the sorting was partly driven by vitreousness and color differences. Development of high- and low-protein specific backgrounds that will enhance signal differences between high- and low-protein kernels and the fabrication of dual-peak filters with more specificity and stronger absorption to protein might improve sorting based mainly on protein differences. Also, further tests should be conducted with naturally occurring blends of high- and low-protein kernels. The study showed also that the sorter has potential for vitreousness sorting. With appropriate backgrounds, the same sorting concept could be used to rapidly sort seeds based on starch, oil, and other major constituents.

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