Wheat Pentosans. II. Estimating Kernel Hardness and Pentosans in Water Extracts by Near-Infrared Reflectance[1]

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ABSTRACT

Wheats of several market classes that varied widely in kernel hardness were extracted with water, methanol-chloroform, ethanol, sodium hydroxide, and various combinations of these solvents. The extracts and their corresponding residues were scanned by a near-infrared spectrometer and the reflectance values log $(1/R)$ at all wavelengths were examined for a relationship to three types of pentosans and for kernel hardness. Water extracts of the whole wheat meals contained a component(s) at 1,366, 1,436, and 2,108 nm that highly correlated with hardness ($r = 0.86$). Attempts to identify the active component(s) by selective treatments of the extracts showed it was likely not a protein, lipid, or pentosan (hemicellulose). Measurement of water extracts significantly improved the near-infrared reflectance estimation of water-soluble, enzyme-extractable, and total pentosans in wheat compared with estimates made on dry whole wheat meal: $r = 0.88$ versus 0.84; 0.89 versus 0.68, and 0.94 versus 0.62, respectively.

Near-infrared reflectance spectroscopy (NIR) is a convenient tool for rapidly measuring the constituents of various food products with acceptable accuracy. Usually, conventional chemical analysis requires an experienced technician to work several hours to obtain reproducible results on a few samples. NIR spectroscopy is particularly useful when large numbers of samples, such as early generation progeny in a breeding program, must be analyzed in a short period of time (Baker 1983, Norris and Hart 1965, Williams 1975). NIR estimation of protein and oil content in corn, soybean, and oat (Hymowitz et al 1974) and fiber in processed cereal food (Baker 1983) was successfully made and found to be suitable for practical analysis.

Hymowitz et al (1974) demonstrated that the grinding time and particle size are critical factors in NIR determination of protein and oil content in soybeans and oats. Watson et al (1976) determined that particle size varied when five classes of wheat were ground on three different types of mills and reported that particle size was not consistently related to NIR prediction of wheat protein. Bread loaf volume, dough mixing time, and water absorption have also been related to NIR determinations with highly significant multiple correlation coefficients by Rubenthaler and Pomeranz (1987).

Currently, endosperm hardness of cereals is receiving widespread attention due to its relationships with milling properties, particle size and resulting water absorption, and dough characteristics, and also as a marketing parameter. With eight collaborating quality testing laboratories, Norris (*unpublished*) developed an NIR method (AACC 1983) for estimating hardness of wheat of different market classes. The biochemical basis of grain hardness of wheat was studied by Simmonds (1974), who demonstrated that water solubles play a role in grain hardness of wheat. The role of crude water extract and purified pentosans in dough and baking properties was extensively studied by Mattern and Sandstedt (1957), who identified the water-soluble portion as a principal factor responsible for determining the mixing requirement of wheat flour. Removal of water solubles from the flour extended the mixing time, and their reincorporation reversed this effect. Udy (1956) indicated that 95% of intrinsic viscosity of the water-soluble fraction is due to polysaccharides, and water-soluble proteins contribute 5% to the total viscosity. A major component of this viscous substance has been found to be pentosans. The objectives of this study were twofold: 1) to investigate the use of NIR for estimating the pentosan content in wheat cultivars of different market classes using water extracts; and 2) to identify the component(s) in water extracts that are responsible for the correlation found between water extracts and dry whole wheat meals for grain hardness. The approach used was to eliminate the major components through enzyme and physical-chemical treatment of the water extracts. A collective objective was to determine if a correlation could be established between pentosan content and grain hardness.

MATERIALS AND METHODS

Wheat Samples

Eighteen wheat genotypes harvested at Lind and Pullman, WA, in 1986 were used in this study. These included six market classes and/or subclasses described in Hong et al (1989). Wheat grains and all dried flour residues were ground in a Udy cyclone mill fitted with a 0.5-mm screen.

Hardness testing and pentosan analysis methods were the same as those described in Hong et al (1989), AACC method 39-70 (AACC 1983), and Hashimoto et al (1987).

Extracts

Standard water extract. Whole wheat meals of the 36 samples were weighed to 5 g and placed into 50-ml plastic centrifuge tubes. Distilled water (25 ml) was added to the tubes. Water solubles were extracted by shaking tubes at room temperature for 2 hr with a rotary shaker. After extraction, the tubes were centrifuged for 15 min at 4,080 relative centrifugal force (rcf). The supernatant was drained from the tubes and stored in a cold room (5 ± 1°C) overnight.

Water extract of defatted flour. Wheat meals were defatted by a methanol-chloroform mixture, dried, and reground. These samples (5 g) were then extracted with 25 ml of distilled water for 2 hr at room temperature. After centrifugation for 15 min at 1,000 rcf, the supernatant was scanned with an NIR spectrometer (model 500, Technicon) in the transflectance mode.

"Protein free" water extracts. Water extracts (20 ml) in a 50-ml Erlenmeyer flask were heated over boiling water to precipitate the water-soluble proteins. Coagulated proteins were removed by filtration through Whatman No.2 filter paper, and the filtered solution was used for NIR scanning.

Water extract treated with pectinase. Water extract (24 ml) was treated with 1 ml of 5% pectinase solution, obtained from Sigma Chemicals, and the pH was adjusted to 4.0 with acetic acid. Samples were incubated in a water bath at 35°C for 1 hr

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with frequent stirring and then scanned with NIR.

Water extract treated with pentosanase. For pentosanase treatment, 1 ml of 25% Veron HE (Rohm Tech., New York, NY) was added to 24 ml of substrate water extract. Samples were incubated for 2 hr at 32°C before NIR scanning. Sodium acetate buffer adjusted to a pH of 4.5 with acetic acid was used in making the pentonase stock solution.

Water extract treated with limpet juice. Limpet powder, acetone form, obtained from Sigma Chemicals, was dissolved in distilled water at 35°C to approximately 2,500 ppm. A 1-ml sample of this stock solution was added to 24 ml of water extract and incubated for 1 hr at 35°C before NIR scanning.

Water extract treated with protease. Standard whole wheat meal (5 g) was mixed with 25 ml of a 0.2% protease (papaya, from Sigma Chemicals) solution in a 50-ml plastic centrifuge tube, and the protein was extracted for 16 hr with gentle shaking in a rotary shaker at room temperature. After extraction, the sample was centrifuged for 15 min at 1,000 rcf. The supernatant was drained from the tube and stored overnight in a cold room (5°C) before scanning.

Ethanol extract of water-extracted flour. Water-extracted residues were dried to about 9% moisture level at room temperature. These pellets were ground in a Udy cyclone sample mill and used as flour for alcohol extraction. Ethanol (25 ml) was mixed with 5 g of flour and gently extracted for 16 hr at room temperature with a rotary shaker (90 shakes per minute). This suspension was centrifuged 15 min at 1,000 rcf, and aliquots of the supernatant were scanned with NIR.

Sodium hydroxide extract solution. Meal residues that had been extracted by water and ethanol were used for an NaOH extraction after being dried to 9% moisture. After ethanol extraction and drying, flours were weighed to 5 g and mixed with 0.2% NaOH solution and extracted for 1 hr with a rotary shaker at room temperature. After extraction, samples were centrifuged for 15 min at 1,000 rcf, and aliquots of the supernatant were scanned with NIR.

Flour Residue Samples

Flours extracted with methanol-chloroform. Methanol and chloroform were mixed in a 3-to-1 ratio by volume and used for defatting whole wheat flour samples. Samples (10 g) were mixed with 50 ml of methanol-chloroform solution. This mixture was gently shaken by hand to disperse the sample and extracted for 1 hr, stirring every 10 min. After extraction, the mixture was filtered (Whatman No. 1), dried, and humidified to about 9% moisture. The residue was reground before scanning.

Flour Samples After Various Extraction Methods

After water extraction, all residues were dried and reground for scanning. After methanol-chloroform extraction, samples were extracted again with water and the flour pellets dried at room temperature and reground before scanning. After extraction with water and ethanol, the samples were extracted again with NaOH (0.2%), dried, and reground for scanning. For all the samples (extracts and flour residues) prepared by the methods described above, the log $(1/R)$ was obtained every 4 nm in the wavelength range from 1,100 to 2,400 nm. A liquid cell in the transflectance mode was used for the extracts. Calibrations were made in relation to hardness scores obtained on the original wheat meals using AACC method 39-70 (AACC 1983) and pentosan data obtained by the orcinol-HCl method (Hong et al 1989) using the Technicon best-fit (three wavelength) combination search program. Statistical analysis was made with software furnished with the Technicon model 500 instrument.

RESULTS AND DISCUSSION

Flour Samples After Extraction with Various Solvents

Hardness scores of 19–70 and the contents of three types of pentosans (water-soluble, enzyme-extractable, and total) for the 36 samples were used as reference chemical data for analysis with NIR data. Whole wheat meal residues of the 36 samples (18 cultivars × 2 locations) that were extracted with the various solvents as given in Table I were reground and scanned with NIR.

Correlations for hardness score were the same ($r = 0.98$) in the samples of the original untreated checks as for those that had been extracted with water and the Met-Chl, but with a shift in wavelengths. Flours that were extracted with Met-Chl/water, water/ETOH, and water/ETOH/NaOH tended to have slightly lower correlation coefficients and an associated increase in the standard error of estimate. However, the flour samples still exhibited significantly high correlation coefficients with hardness after these various extraction treatments which suggests an important role of insoluble substances (including high molecular weight protein) in expressing grain hardness.

The three best wavelength combinations for estimating water-soluble pentosan produced lower correlations ($r = 0.78$ vs. 0.84)

TABLE I
Best Wavelengths (λ), Multiple Regression Correlation Coefficients (*r*), and Standard Errors of Estimate (SEE) for Hardness and Pentosans in Whole Wheat Flour Residues After Extraction by Various Solvents

	Hardness Score			Pentosan								
				Water-Soluble			Enzyme-Extractable			Total		
Flours Extracted by	λ	*r*	SEE	λ	*r*	SEE	λ	*r*	SEE	λ	*r*	SEE
Water	1,730 1,982 2,024	0.98	4.5	2,080 2,136 2,346	0.78	0.08	1,100 1,324 1,338	0.81	0.07	1,100 2.332 2,346	0.70	0.45
Met + Chl[a]	1,352 1,688 1,730	0.98	4.0	1,422 2,066 2,290	0.81	0.08	1,128 1,408 1,464	0.78	0.08	1,198 1,254 2,206	0.81	0.40
Met + Chl/water	2,066 2,080 2,345	0.95	6.5	1,394 1,898 1,954	0.80	0.08	1,254 1,898 1,940	0.74	0.08	1,912 1,954 2,276	0.81	0.37
Water/EtOH	1,912 2,038 2,234	0.96	5.9	1,674 2,024 2,192	0.81	0.08	1,128 1,156 1,212	0.82	0.07	1,128 1,282 1,394	0.76	0.41
Water/EtOH/NAOH	1,114 1,170 1,646	0.93	7.6	1,660 1,786 2,318	0.80	0.08	1,198 1,240 2,024	0.82	0.07	1,674 1,688 1,716	0.78	0.40
Original untreated flour	1,422 1,436 2,094	0.98	3.9	1,912 2,024 2,206	0.84	0.07	1,618 1,758 1,800	0.68	0.15	2,360 2,374 2,444	0.62	0.87

[a]Methanol + chloroform.

in the extracted flours than those of the untreated check flours. However, enzyme-extractable and total pentosan contents of the check flour expressed a lower relationship with log (1/*R*) spectra than that of any of the extracted flours. Barton (1987) suggested glucose interference in estimating hemicellulose in forage materials with NIR. This could be the case with the check flour considering the improved relationships with total pentosan in the analyzed defatted and/or water-extracted samples. Zawistowska et al (1985) identified the role of carbohydrates and lipids in aggregation of glutenin proteins in wheat, and it seems likely that water or Met-Chl extraction would change the physical-chemical nature of these components, which may result in ultimate changes in NIR absorption spectra.

Extract Solutions

Water extracts, water extracts after methanol-chloroform extraction of lipids, and water extracts with water-soluble proteins removed were scanned by NIR. The best three wavelength combinations and their correlation to grain hardness and pentosan(s) level were determined from the NIR spectra (Table II) using a wet cell in the transflectance mode. NIR spectra at 1,366, 1,436, and 2,108 nm gave highly significant correlation coefficients between hardness ($r = 0.86$) and the plain water extract.

Removing the bulk of the water-soluble proteins by boiling, centrifuging, and filtering the supernatant lowered the correlation and raised the standard error of estimate with hardness from that obtained with plain water extracts ($r = 0.86$ to 0.80 and 11.0 to 12.2), respectively. Other experiments to remove protein from the extracts using trichloroacetic acid (not reported here) precipitate and protease enzymes (which follow) gave similiar results. We believe it is clear that protein has no role as a quantitative factor in grain hardness in water extracts.

The best wavelengths for the three types of pentosans and their correlation coefficients are also given in Table II. In general, correlations were slightly stronger for the three types of pentosans when the lipids were removed with the methanol/chloroform. These results may indicate that the lipids have a small interference role of interaction within the spectrum that masks the pentosans.

Water extracts of defatted flour (Met-Chl/water) demonstrated good estimations of the three type of pentosans. The best wavelengths for the three types were 1,408, 1,954, and 2,010 nm, which had correlation coefficients of $r = 0.91$–0.93. Removal of the protein from the extract weakened the predictive fit of all three types of pentosans, but it did not reduce the quantitative relationship to an insignificant level. However, it was interesting to note that 2,100 nm (2,094–2,108 nm) was the optimum wavelength for hardness, water-soluble and enzyme-extractable pentosans. This wavelength was recognized for protein and damaged starch estimation in wheat flour by Osborne et al (1982). Hoseney (1986) suggested cross-linkage of esterified phenolic acid (ferulic acid) between arabinoxylan and protein molecules in the oxidative gelation process of the water extracts. Accordingly, heat coagulation of protein and removal of it from the water extract could also remove protein-bound pentosan and result in the poorer estimation of pentosans compared with other treatments. Prediction equations for the three types of pentosan from water extracts of defatted wheat meals are presented in Table III. Also included is the best prediction equation for hardness using plain water extracts.

The work described in Tables I and II failed to indicate the composition of the extractable component(s) in the water solubles that were measured by NIR and that correlated with wheat hardness. Precipitation of the protein and the lipid extraction of the flour prior to preparing water extracts (Table II) lowered the relationship from 0.86 to 0.80. This small change suggested that the associated components were not proteins or lipids.

Another approach involved treatment of the water extracts with specific enzymes—pentosanase, pectinase, limpet juice, and proteinase. None of these enzyme treatments substantially altered the ability of predicting hardness and/or pentosans of wheat when compared with a water extract without enzyme treatment (Table IV).

The pentosanase (Veron HE) had a small effect on correlation coefficients involving hardness, water-soluble pentosans, and enzyme-extractable pentosans. Veron HE treatment substantially reduced the correlation of total pentosans from 0.94 to 0.73. Pectinase, the other hemicellulose enzyme, had less influence on predictability of hardness and the pentosans than pentosanase.

Protease did less to modify the predictable relationship of hardness than the earlier precipitation method (Table II). It did,

TABLE II
Three Best Wavelengths (λ), Multiple Regression Correlation Coefficients (*r*), and Standard Errors of Estimate (SEE) for Hardness and Pentosan Content in Different Water Extract Solutions

	Hardness Score			Pentosan								
				Water-Soluble			Ezyme-Extractable			Total		
Method of Extraction	λ	*r*	SEE	λ	*r*	SEE	λ	*r*	SEE	λ	*r*	SEE
Water	1,366 1,436 2,108	0.86	11.0	1,814 2,038 2,094	0.88	0.07	1,520 1,898 2,108	0.89	0.09	1,296 1,478 2,500	0.94	0.39
Met-Chl[a]/Water	1,394 1,912 2,038	0.80	13.0	1,408 1,954 2,010	0.92	0.06	1,408 1,954 2,010	0.93	0.08	1,408 1,954 2,010	0.91	0.45
Water, no protein	1,870 1,954 2,024	0.80	12.2	1,618 1,814 2,052	0.84	0.07	1,632 1,870 2,164	0.73	0.13	1,590 2,010 2,094	0.80	0.52

[a]Methanol-chloroform.

TABLE III
Prediction Equations for Three Types of Pentosans Using Water Extracts of Defatted Flours and Hardness Scores from Water Extracts of Whole Wheat Meals

Components	Equation Coefficients and Wavelengths[a]	*r*	SEE
Pentosan			
Water-soluble	Pw = 128.042 − 111.718 (1,408 nm) + 83.837 (1,954 nm) − 178.628 (2,010 nm)	0.92	0.06
Enzyme-extractable	Pe = 105.55 − 44.613 (1,408 nm) + 85.217 (1,954 nm) − 198.852 (2,010 nm)	0.93	0.08
Total	Pt = 257.549 + 827.59 (1,408 nm) + 403.776 (1,954 nm) − 1,283.774 (2,010 nm)	0.91	0.45
Hardness score			
Water extract	Hw = 9,083.3 − 61,166.8 (1,366 nm) − 25,312.9 (1,436 nm) + 45,638.8 (2,108 nm)	0.86	11.0

[a]Pw = water-soluble pentosans, Pe = enzyme-extractable pentosans, Pt = total pentosans, and Hw = grain hardness from water extracts.

TABLE IV
Best Wavelengths (λ), Multiple Regression Correlation Coefficients (*r*), and Standard Errors of Estimate (SEE) for Hardness Score and Pentosan Level Estimated with Water Extracts Treated with Specific Enzymes

Enzyme Treated	Hardness Score			Pentosan								
				Water-Soluble			Enzyme-Extractable			Total		
	λ	*r*	SEE	λ	*r*	SEE	λ	*r*	SEE	λ	*r*	SEE
Water (no treatment)	1,366 1,436 2,108	0.86	11.0	1,814 2,038 2,094	0.88	0.07	1,520 1,898 2,108	0.89	0.09	1,296 1,478 2,500	0.94	0.39
Pentosanase	1,464 2,080 2,486	0.82	11.7	1,352 1,464 2,108	0.84	0.07	1,506 2,150 2,192	0.80	0.07	1,296 1,800 1,926	0.73	0.71
Pectinase	1,870 2,038 2,108	0.87	10.0	1,702 1,814 2,080	0.83	0.07	1,618 1,940 2,052	0.77	0.07	1,772 1,814 2,150	0.84	0.35
Protease	1,128 1,492 1,548	0.87	10.0	1,184 1,730 2,164	0.76	0.08	1,142 1,730 2,164	0.71	0.08	1,576 2,136 2,444	0.83	0.36
Limpet juice	1,436 1,534 1,576	0.81	12.0	1,352 1,506 1,562	0.86	0.07	1,282 1,548 2,024	0.80	0.07	1,464 1,940 2,094	0.86	0.32

however, reduce the fit with each of the pentosan test data.

The limpet juice, which contained β-glucurondase, sulfatase, and a trace of pentosanase, was ineffective in digesting out any components that affected wheat hardness and/or pentosan content measured from water extracts.

CONCLUSIONS

Water extracts of whole wheat flours contain a component(s) that is highly correlated to wheat hardness by the log $(1/R)$ at 1,366, 1,436, and 2,108 nm. Selective treatments demonstrated that the active component(s) was not a protein, lipid, or pentosan (hemicellulose). A test for fermentable sugars (not reported here) also proved negative. A water-extraction step improved significantly the estimation by NIR of water-soluble pentosans, enzyme-extractable pentosans, or total pentosans compared with estimates made on dry whole wheat flour ($r = 0.88$ vs. 0.84; 0.89 vs. 0.68; and 0.94 vs. 0.62, respectively).

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