NEAR INFRARED DIFFUSE REFLECTANCE SPECTRA OF WHEAT AND WHEAT COMPONENTS[1]

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ABSTRACT

Near infrared diffuse reflectance spectra between 1.0 and 2.5 μ were recorded for wheat, gluten, starch, pentosans, lipid, and water. The spectral absorption bands were assigned to various overtone and combination vibrations of C-H, N-H, O-H, and C=O bonds. The spectrum for wheat was determined largely by the carbohydrate components. The type of wheat had no effect on the position of reflectance peaks. Major peaks in the gluten spectrum occurred at 1.19, 1.50, 1.73, 1.98, 2.05, 2.18, 2.29, and 2.47 μ. The absorption peak at 1.98 μ is characteristic of gluten and is due to the high concentration of primary amide groups in gluten. The spectra for starch and pentosans were similar, with major peaks at 1.20, 1.45, 1.54, 1.93, 2.09, 2.32, and 2.49 μ. The position of peaks associated with hydroxyl groups was determined, in part, by the degree of hydrogen bonding. The lipid spectrum was characterized by intense absorption due to $\cdot CH \cdot_2$ groups. Bands at 1.17, 2.14, and 2.17 μ were due to the C-H vibrations associated with *cis* double bonds, while absorption at 1.41 and 2.07 μ was due to ·OH groups. Liquid water had absorption peaks at 1.445 and 1.928 μ, while water in undried wheat, gluten, starch, and pentosan samples exhibited absorption at slightly longer wavelengths. This was probably due to hydrogen bonding.

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Instruments which use near infrared reflectance spectroscopy for the analyses of cereals and oilseeds for protein, moisture, and oil contents are now widely used. Several papers have been presented on the advantages and accuracy of these instruments (1–6); however, little attention has been given to the fundamental background of the technique.

Except for low resolution spectra between 1.0 and 2.0 μ for whole grain (7), no reflectance spectra have been published for wheat and its components. Transmission spectroscopy has been used to study near infrared absorption of various proteins (8–21), carbohydrates (22–25), lipids (26), and bound and free water (8, 27–29). However, apart from the allocation of absorption bands at 1.45 and 1.94 μ to water (7,28), no assignments have been made for the near infrared bands of wheat and its components.

This paper presents reflectance spectra between 1.0 and 2.5 μ for wheat, gluten, starch, soluble and insoluble pentosans, lipid, and water. The observed absorption bands were assigned to various overtone and combination vibrations of C-H, N-H, O-H, and C=O bonds.

MATERIALS AND METHODS

Methods

Protein Content. All protein contents (N × 5.7) were determined by the Kjeldahl method.

Moisture Content. Determined by drying 2-g samples at 130°C for 1 hr.

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Samples

Wheat. Sound samples of Canada Western (C.W.) amber durum, C.W. red spring, and Canada Eastern (C.E.) white winter wheat were used with protein contents of 12.8, 16.2, and 12.3%, respectively (13.5% moisture basis). The wheat was ground on a Model CSM-2 Cyclone grinder fitted with a 1.0-mm screen.

Gluten. Glutens were prepared from flour milled from C.W. red spring and C. W. amber durum wheat by the method of Doguchi and Hlynka (30). Protein contents of glutens were: from red spring, 82.6%, and from durum, 86.4% (dry basis).

Starch. Prime starch was isolated from a C.W. hard red spring wheat straight-grade flour by centrifuging a flour slurry, washing the prime starch residue twice with water, and then freeze-drying to remove the water. Protein content of the starch was 0.34% (dry basis).

Pentosans. Soluble and insoluble pentosans were isolated from C.W. red spring wheat flour by the method of Jelaca and Hlynka (31). The soluble and insoluble pentosans contained 5.9 and 5.5% protein (dry basis).

Lipid. Crude lipid fractions were obtained from the three wheat types described above by extracting the ground samples overnight in Goldfisch extractors with petroleum ether (Skellysolve F95) and weighing the vacuum-dried lipid. Lipid contents of the wheats were: durum, 2.4%, red spring, 2.0%, and white winter, 2.1% (dry basis).

Water. Laboratory deionized water was used.

The wheat, gluten, starch, and pentosan samples were used on an as-is basis, *i.e.*, 8–12% moisture, and also at 2% moisture after they were dried in a vacuum desiccator at room temperature.

Spectra

Diffuse spectra were recorded between 1.0 and 2.5 μ with a Cary 17I prism-grating double monochromator fitted with a Model 1711 reflectance attachment containing Spectrosil W fused quartz optical lenses, and a lead sulfide detector. The spectrophotometer was used in the double beam mode for all the measurements. The interior of the instrument was flushed with dry nitrogen to minimize interferences due to atmospheric moisture and carbon dioxide. A mercury lamp was used to check the calibration of the spectrophotometer wavelength readings (32). Smoked magnesium oxide was used as a reference standard and as the interior coating in the integrating sphere.

Solid samples were contained in black plastic holders (2-1/8 in. diameter) faced with Infrasil optical windows (1/16 in. thick). Holder capacity was approximately 15 g. Lipid and water samples were adsorbed onto pressed disks made with a Carver press; the disks were made from a mixture of sulfur and powdered polytetrafluoroethylene which had an absolute reflectance greater than 95% (33). For these two samples only, a reference disk made from sulfur and powdered polytetrafluoroethylene was used.

Spectra were recorded in apparent absorbance units: log (standard reflectance ÷ sample reflectance). The actual units for apparent absorbance are not given in the figures because the absorbance is dependent on particle size and physical nature of the samples being examined. Generally, the maximum absorbances recorded (as illustrated in the figures) are in the vicinity of 0.8 apparent absorbance units relative to smoked magnesium oxide.

In recording the spectra from 2.5 to 1.0 μ, the slit width varied automatically from approximately 2.6 to 0.2 mm. A further approximate tenfold reduction in slit width (0.26 to 0.02 mm) did not improve the resolution of the absorption peaks.

RESULTS AND DISCUSSION

The reflectance spectra between 1.0 and 2.5 μ for the dried samples of wheat, gluten, starch, soluble and insoluble pentosans, and lipid are illustrated in Figs. 1 and 2. The spectrum for water is shown in Fig. 3. Spectra for durum and white winter wheats and their constituents are not described in this report, as they were found to be similar to the red spring counterparts.

The spectra obtained for ground wheat showed additional and sharper absorption peaks as compared with the spectra published for whole wheat (7), or for those obtained by transmission spectroscopy for carbon tetrachloride suspensions of ground wheat (28). The differences in spectra may be due to a variation in the sample particle size, and/or the high resolution and lower stray light of the Cary 17I instrument used in the present study.

Examination of Fig. 1 indicates that the spectrum for ground wheat is determined largely by the carbohydrate component with gluten having only a minor effect. The 1.98 μ absorption peak in the ground wheat spectrum is due to the primary amide group (13,16,34,35), and was not observed in the undried sample because of the overshadowing effect of water and starch bands at 1.93 and

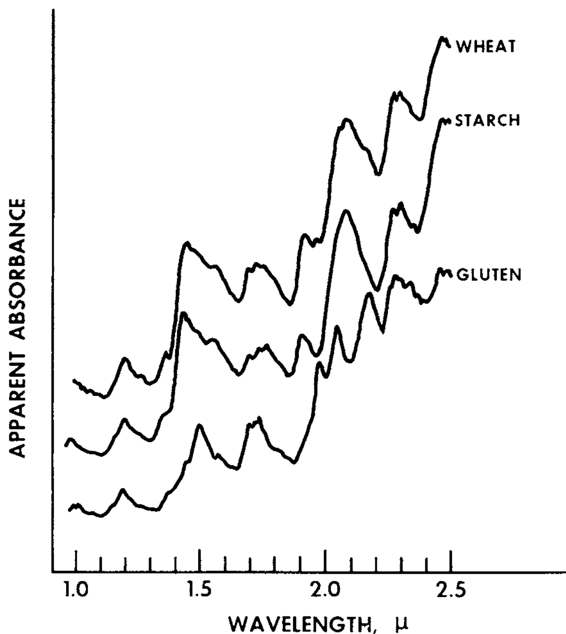

Fig. 1. Reflectance spectra for vacuum-dried ground red spring wheat and vacuum-dried starch and gluten isolated from red spring wheat.

2.09 μ. This difference between undried and dried samples of ground wheat is shown in Fig. 4.

The spectrum for dried gluten (Fig. 1) is similar to the transmission spectrum for porcupine quill published by Hecht and Wood (18), except for the presence of

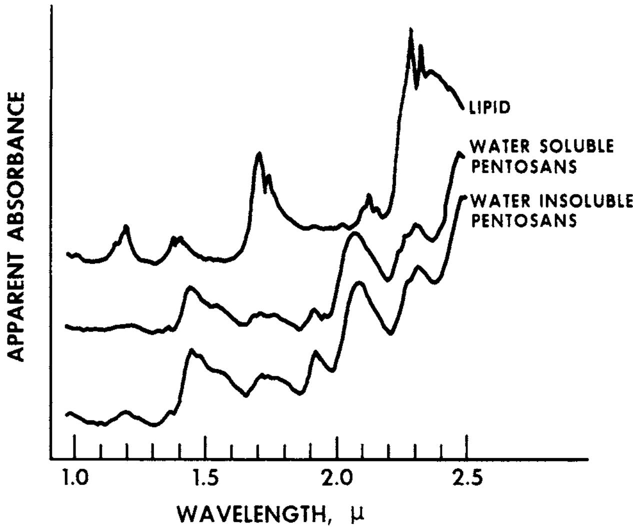

Fig. 2. Reflectance spectra for vacuum-dried crude lipid, water-soluble and water-insoluble pentosans extracted from red spring wheat.

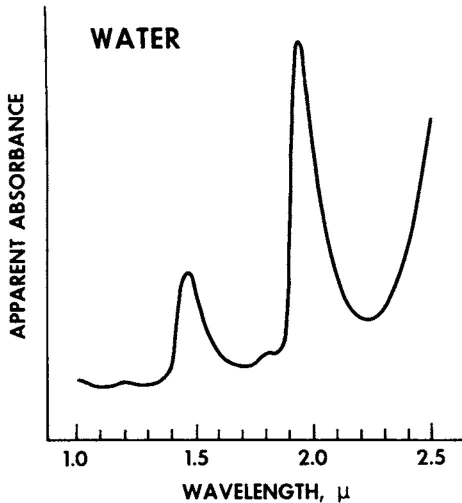

Fig. 3. Reflectance spectrum for laboratory deionized water adsorbed onto a pressed disk made from a mixture of pulverized sulfur and powdered polytetrafluoroethylene.

an intense peak at 1.98 μ due to the primary amide group. In the undried gluten samples, the intensity of the 1.98 μ band was equal to that of the water band at 1.93 μ. A weak band at 2.01 μ observed by Fraser (17) in all peptide-containing polymers except silk was not present in the gluten spectrum. This may have been due to overshadowing by the primary amide band, inadequate resolution, or the absence of this peak in wheat gluten.

Studies with gelatin (8) demonstrated that the intensity of the peak at 1.50 μ decreased with increase in moisture content. This effect was assigned to a reduction in the dipole moments of the N-H groups when bridged with water. A similar decrease in peak intensity, although less significant, was noted at the 2.05 and 2.18 μ bands. Whether the same phenomenon occurred in gluten was not obvious from the results.

Although the positions of peaks in the spectra of the starch (Fig. 1) and pentosan fractions (Fig. 2) were identical, there were marked differences in their shapes. These differences are probably due to variations in the surface characteristics of the samples which partly determine the degree of resolution obtained in reflectance spectra (36). In the first overtone C-H regions for starch, the intensity of the peak at 1.70 μ was lower than that at 1.78 μ; for the pentosan fractions, the intensity ratio was reversed. These peaks arise mainly from the $\cdot CH\cdot_2$ groups, although on the basis of the results published by Kaye (37) they must receive a major contribution from ·CH· groups which absorb in the same region at about one-third the intensity. Interpretation is further complicated in that the $\cdot CH\cdot_2$ groups in pentosans are mainly alicyclic due to the pyranose structure of the anhydro-xylose molecules, whereas the $\cdot CH\cdot_2$ groups in starch are acyclic. In contrast to the alicyclic $\cdot CH\cdot_2$ groups, the peaks of the acyclic $\cdot CH\cdot_2$ groups have been shown to occur at slightly shorter wavelengths (38).

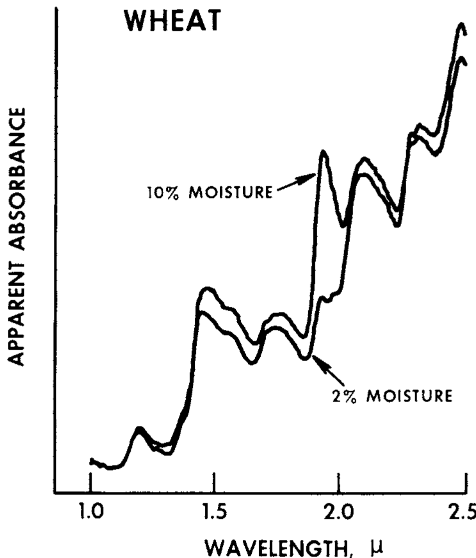

Fig. 4. Reflectance spectra for ground red spring wheat containing 2 and 10% moisture.

Further studies will be necessary to show whether these differences in wavelength, or intensity ratios, of $\cdot OH$ to $\cdot CH\cdot_2$ groups can be used to determine starch and pentosan contents in cereals.

The spectrum for lipid (Fig. 2) is similar to that for castor oil published by Holman and Edmondson (26). An additional peak at 1.39 μ is apparent in the wheat lipid spectrum; also the peaks at 1.17, 2.14, and 2.17 μ due to *cis* unsaturation are relatively more intense. The origin of the additional peak is uncertain.

The spectrum for liquid water shows major peaks at 1.45 and 1.93 μ (Fig. 3). These peaks are overtone-combination, and combination bands, respectively, arising from fundamental O-H bands at 2.86, 3.05, 4.71, and 6.08 μ (29). The intense absorption by water starting at approximately 2.3 μ and increasing toward longer wavelengths is due to intense intra- and inter-molecular bands for O-H at 2.55, 2.86, and 3.05 μ. The peaks at 1.45 and 1.93 μ shift to slightly longer

TABLE I
Contributing Groups and Assignments of Near Infrared Bands of Gluten

Wavelength μ	Contributing Groups	Assignment	References
2.47, 2.34, 2.30, 2.29	$\cdot CH\cdot_2$	C-H Combination	(8, 17, 41, 42)
2.18	·CONH·	C=O + Amide III[a]	(17, 18, 35)
	·CONH· + $\cdot CONH_2$	C=O + Amide III Combination	(13, 35)
2.05	·CONH· + ·CONH·	N-H + Amide II Combination	(13, 17, 18)
	·CONH· + $\cdot CONH_2$	N-H + Amide III Combination	(13, 35)
1.98	$\cdot CONH_2$	N-H + Amide II Combination	(13, 16, 35)
1.79	H_2O	O-H Combination	(8)
1.76, 1.73, 1.70	$\cdot CH\cdot_2$	C-H 1st Overtone	(8, 9, 18, 37)
1.57, 1.50	·NH·	N-H 1st Overtone	(8, 9, 18)
1.46	$\cdot CONH_2$	N-H 1st Overtone	(34)
1.39	$\cdot CH\cdot_2$	C-H Combination	(39, 40)
1.19	$\cdot CH\cdot_2$	C-H 2nd Overtone	(18, 37)

[a]Amide absorption bands (43):

Band	*Wavelength*		*Band Description*
Amide A	3.05 μ	Fermi resonance	N-H stretching
Amide B	3.24 μ	Fermi resonance	2 × Amide II
Amide I	6.05 μ		C=O stretching
Amide II	6.38 μ		N-H in-plane bending, C-N stretching
Amide III	7.70 μ		C-N stretching, N-H in-plane bending

wavelengths, *i.e.*, an increase of 0.005–0.010 μ, in wheat, gluten, starch, and pentosans, owing to hydrogen bonding. Similar shifts were not observed in samples with very low moisture contents.

Generally, absorption in the near infrared region is due to overtones and combinations of C-H, N-H, and O-H stretching vibrations. The associated fundamental absorption bands occur in the regular infrared region. The contributing groups and assignments for the absorption bands of the spectra of gluten, starch, pentosans, and lipid are presented in Tables I – III. These data

TABLE II
Contributing Groups and Assignments of Near Infrared Bands of Starch and Pentosan Fractions

Wavelength μ	Contributing Groups	Assignment	References
2.49, 2.32, 2.29	$\cdot CH\cdot_2$	C-H Combination	(41, 44)
2.09[a]	·OH	O-H Combination	(23, 44)
1.93[a]	H_2O	O-H Combination	(34, 37)
1.78[b], 1.72[b], 1.70[b]	$\cdot CH\cdot_2$	C-H 1st Overtone	(37, 40)
1.54[a], 1.45[a]	·OH	O-H 1st Overtone	(23, 24, 25)
1.37	$\cdot CH\cdot_2$ (?)	C-H Combination (?)	(39, 40)
1.20	$\cdot CH\cdot_2$	C-H 2nd Overtone	(37, 40)

[a]Peaks shift to slightly longer wavelengths in undried samples (0.005–0.010 μ).
[b]Peaks poorly resolved in pentosan fractions.

TABLE III
Contributing Groups and Assignments of Near Infrared Bands of Wheat Lipid

Wavelength μ	Contributing Groups	Assignment	References
2.34, 2.31	$\cdot CH\cdot_2$	C-H Combination	(26)
2.17, 2.14	·HC:CH·	C-H Combination	(26)
2.07	·OH	O-H Combination	(26)
1.76, 1.72	$\cdot CH\cdot_2$	C-H 1st Overtone	(26)
1.41	·OH	O-H 1st Overtone	(26)
1.39	$\cdot CH\cdot_2$ (?)	C-H Combination (?)	(39, 40)
1.21	$\cdot CH\cdot_2$	C-H 2nd Overtone	(26)
1.17	·HC:CH·	C-H 2nd Overtone	(26)

refer to the groups responsible for the major absorption within each band. Other groups may contribute to the intensity of absorption, for example, the primary amine groups at 1.50, 1.97, and 2.05 μ (37); however, due to their minor contribution, and for simplicity, these groups have been omitted from the tables.

While, in previous studies on proteins (8,9,17) the bands at 2.05 and 2.18 μ have been assigned solely to peptide bond combination vibrations, on the basis of the large glutamine plus asparagine content of gluten, the primary amide is also included as a contributing group in these bands. This assignment is consistent with the suggestion by Elliott (13) and the studies on primary amides by Krikorian and Mahpour (35). Weak bands at 1.39 μ which have not been reported previously in proteins or lipids have been assigned tentatively to combination C-H vibrations arising from $\cdot CH \cdot_2$ groups (39,40).

The first overtone C-H bands between 1.7 and 1.8 μ were poorly resolved in the pentosan fractions as compared with starch. The O-H bands at 1.45, 1.55, 1.93, and 2.09 μ in the spectra of starch and pentosans were found at slightly longer wavelengths in the undried samples. This was probably due to hydrogen bonding (39).

Many of the wavelengths cited have been used in the analysis of cereals and oilseeds both by transmission and reflectance spectroscopy. Fenton and Crisler (45) used the absorbance at 2.14 μ as a measure of *cis* unsaturation in oils. Absorption at 1.93 μ of methanol or carbon tetrachloride suspensions was used to determine moisture contents of various grains and soybeans by Hart *et al.* (27), Norris and Hart (28), and Ben-Gera and Norris (46). The near infrared reflectance grain analyzers use absorption at 1.93, 2.18, and 2.31 μ to determine moisture, protein, and oil contents, respectively (2). Further exploitation of the near infrared region will depend on the critical examination of the relative intensities and relations of the various absorption peaks as well as the development of suitable instrumentation and techniques for rapid and accurate analyses.

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