

THE UNIVERSITY OF MANITOBA

NEAR-INFRARED REFLECTANCE

STUDIES ON WHEAT

BY

DONALD PHILIP LAW

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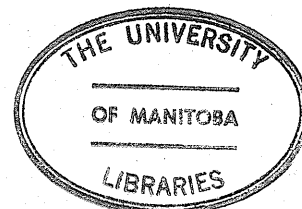
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A B S T R A C T

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Near-Infrared Reflectance Studies on Wheat

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Instruments which use the reflectance of near-infrared radiation are becoming widely accepted within the grain industries. However little information on the basis for their use is available. The main objective of the thesis was to determine the near-infrared absorption characteristics of wheat and its components.

Reflectance spectra of wheat, gluten, starch, pentosans, lipid and water were recorded between 1.0 and 2.5 μ using a Cary 17I spectrophotometer. The spectrum for wheat was strongly influenced by the carbohydrate components. Wheat type had no effect on the position of peaks. Gluten showed major peaks at 1.19, 1.50, 1.73, 1.98, 2.05, 2.18, 2.29 and 2.47 μ . The spectra for starch and pentosans were similar with major peaks occurring at approximately 1.20, 1.45, 1.54, 1.93, 2.09, 2.32 and 2.49 μ . The position of the peaks associated with hydroxyl groups was determined, in part, by the degree of hydrogen bonding. The absorption peaks for liquid water at 1.45 and 1.93 μ occurred at slightly higher wavelengths in wheat, gluten, starch and pentosans. The absorption bands were assigned to various overtone and combination vibrations of C-H, N-H, O-H, and C=O groups.

Experience with commercial near-infrared reflectance grain analysers has shown that the accuracy of the instruments is markedly influenced by variations in the particle size of the prepared samples. Examination of the literature on reflectance

spectrophotometry indicated that the mathematical format of calibration used in these instruments was not theoretically correct. These problems were investigated in relation to the use of near-infrared reflectance spectrophotometry for the determination of moisture in wheat.

Samples of a durum, a hard red spring and a soft white winter wheat were ground on three different mills. The mean particle size of the nine samples obtained (3 wheats x 3 mills), ranged from 170 to 500 μ . Five subsamples taken from each of the above were humidified or vacuum-dried to yield a range of moisture contents from 7.1 to 14.5%. Kubelka-Munk values $[F(R'_{\infty}) = \frac{(1-R^2)}{2R}]$ were recorded at twenty wavelengths between 1.12 and 2.49 μ . After logarithmic transformation, the application of multiple regression analysis to the data yielded an equation $\log m\% = 0.966 + 1.620 \log F(R'_{\infty})_{1.93\mu} - 1.628 \log F(R'_{\infty})_{2.10\mu}$ ($r^2 = 0.98$; C.V. = 1.5%). The validity of the calibration for the moisture range 2.8 to 16.5% was established by predicting the moisture contents of 49 wheats; 32 of which were of a different type and had been ground on a different mill to those used in the calibration.

The method eliminates the susceptibility to particle size of near-infrared reflectance spectrophotometric techniques for the determination of moisture in wheat and provides the basis for the development of instruments which are calibrated with internal standards rather than with samples of known composition.

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I. INTRODUCTION

A rapid method for the analyses of protein and moisture contents of cereals has long been a goal for chemists within the grain industries. The most promising technique so far developed appears to be near-infrared reflectance spectrophotometry. Instruments designed specifically for the analyses of protein, moisture and oil contents in cereals and oilseeds were introduced in 1971. Due to their accuracy, cost and rapidity of analyses, they have gained wide acceptance. A semi-automatic instrument installed at the Grain Research Laboratory, Winnipeg, has enabled the analyses of 2000 samples of wheat for protein content per 8-hour day. Smaller instruments have been introduced at terminal elevators in Canada to assist with the segregation of wheat on the basis of protein content.

Despite the widespread popularity of these instruments, the principles of the technique are not widely understood. This is due to the lack of published information on the absorption of near-infrared radiation by cereals and their components, as well as on the use of reflection spectroscopy for the analysis of complex mixtures involving variability in surface characteristics.

A study was conducted to establish the characteristics of the near-infrared absorption of wheat and its components. This thesis illustrates the spectra between 1.0 and 2.5 μ of wheat, gluten, starch, soluble and insoluble pentosans, lipid and water. In addition, the absorption peaks were assigned to various overtone

and combination vibrations of C-H, N-H, O-H and C=O groups.

The thesis also presents a method for the determination of the moisture content in wheat. Detailed evaluation of the commercial near-infrared reflectance grain analyzers demonstrated that the accuracy of the instruments in the analysis of wheat was determined largely by the degree of uniformity of the particle size distribution of the ground sample. This distribution may be influenced by the method of sample preparation, grain type, variety, and degree of weathering (Williams, 1975). Examination of the literature on the quantitative application of reflectance spectroscopy indicated that the mathematical treatment of the reflectance measurements used in the instruments was not based on any of the accepted theories.

The Kubelka-Munk theory of diffuse reflectance was applied to reflectance measurements made on samples of durum, hard red spring and soft white winter wheats which had been ground on three different mills and adjusted to five different moisture levels. Multiple regression analyses of the data yielded a method for the determination of moisture in wheat, which was independent of wheat type and only slightly affected by the method of sample preparation.

II. LITERATURE REVIEW

A. Near-Infrared Spectroscopy

1. General

Reviews on near-infrared spectroscopy and its applications have been published by Ellis (1929) Lauer and Rosenbaum (1952), Kaye (1954, 1955, 1960), Wheeler (1959), Goddu (1960), McCallum (1964) and Whetsel (1966, 1969).

The near-infrared region is usually considered to cover the section of the electromagnetic spectrum between 0.7 and 3.0 μ . As such, the region involves low energy electronic transitions and overtones and combinations of hydrogenic stretching and bending vibrations. The fundamentals of the stretching and bending vibrations occur in the regular infrared. For reasons of instrumentation the upper limit of the near-infrared is often considered to be 2.5 μ (Whetsel, 1969).

In comparison with the regular infrared, the near region is considered to have limited value in qualitative analytical work; however, it has proved to be extremely valuable for detecting and determining functional groups which contain unique hydrogen groups (Goddu, 1960). Colthup-type charts showing position and range of bands for various molecular groups have been published by Kaye (1954), and Goddu and Delker (1960).

2. Theory

This treatment of theory is based on those presented by Wheeler (1959) and Whetsel (1969).

The absorption of radiation of a definite wavelength by a molecule results in an increase in the frequency of vibration of the bond between a pair of atoms. To the first approximation, a molecular vibration is analogous to a simple harmonic oscillator. All energy changes within molecules are quantized and there is a definite frequency associated with the quantized change in vibrational energy. This change in energy will result from the absorption of light of a definite frequency or wavelength.

From quantum mechanical considerations, the vibrational energy of a strict harmonic oscillator is given by

$$E = (v + 1/2) h c \nu \quad (1)$$

where: E = the vibrational energy

v = the vibrational quantum number; which can assume integer values of 0, 1, 2, 3,

ν = the wave number of the fundamental vibration (cm^{-1}),

h = Planck's constant, and

c = the speed of light.

In the case of a true harmonic oscillator, selection rules forbid transitions between two adjacent energy levels and interactions between different oscillators. However, as molecular vibrations are not true harmonic oscillators, transitions between non-adjacent energy levels and interactions between vibrations occur. The energy levels are not equally spaced as suggested by Equation 1

but given by

$$E = h\nu \left[\left(v + \frac{1}{2} \right) - x \left(v + \frac{1}{2} \right)^2 \right] \quad (2)$$

where: x = the anharmonicity constant.

Transitions between the ground state ($v = 0$) and second and higher excited states ($v = 2, 3, \dots$) give rise to first, second, and higher overtone bands at wave numbers slightly less than two, three or more times the wave number of the fundamental vibration.

Combination bands arise when changes in vibrational energy levels of two or more bonds occur simultaneously. Absorption occurs at wave numbers slightly lower than the sum of the wave numbers of the fundamental vibrations.

As the probability of transition between two energy levels becomes progressively less as Δv increases, the intensities of the bands decrease with increase in v . Simple binary combination bands have intensities of the same order of magnitude as first overtone bands.

3. Applications

(a) Cereals. Transmittance spectra between 1.0 and 2.4 μ for carbon tetrachloride suspensions of wet and dry wheat have been published (Norris and Hart, 1963). The authors studied the relation of optical density at 1.94 μ to moisture content. Massie and Norris (1965) illustrated the transmittance and reflectance spectra between 0.4 and 2.0 μ for whole grain wheat, oats, corn and rice in

connection with optimum energy sources for grain drying. Except for the assignment of bands at 1.45 and 1.94 μ to water, these studies provided no information on the groups responsible for the various absorption bands.

(b) Proteins. Absorption in the near-infrared region by proteins and other compounds containing peptide bonds has been studied extensively by transmission spectroscopy (Ellis and Bath, 1938b; Bath and Ellis, 1941; Elliott and Ambrose, 1950; Ambrose and Elliott, 1951a, 1951b; Elliott, 1952; Elliott, Hanby and Malcolm, 1954; Sutherland, Tanner and Wood, 1954; Fraser 1955, 1956; Hecht and Wood, 1956; Fraser and MacRae, 1958; Hermans and Scheraga, 1960; Larose, 1961; Susi and Ard, 1966). This work as well as studies on primary and secondary amides by Goddu (1969), and Krikorian and Mahpour (1975) have resulted in a comprehensive understanding, particularly in the N-H combination region, 1.9 to 2.2 μ , of the band assignments for proteins.

Ellis and Bath (1938b) and Bath and Ellis (1941) assigned the bands at 1.72 and 2.28 μ to 1st overtone and combination vibrations arising from $\cdot\text{CH}_2$ groups. Hecht and Wood (1956) made the general assignments of the 1.1 - 1.2 μ , 1.7 - 1.8 μ and 2.2 - 2.5 μ regions to 2nd and 1st overtone and combination C-H vibrations respectively. Fraser (1956) assigned peaks at 2.29 and 2.36 μ in wool and 2.30 and 2.35 μ in nylon to $\cdot\text{CH}_2$ group vibrations. Larose (1961) in studying the degradation of nylon by HCl assigned bands at 1.21, 1.42, 1.74, 1.76, 2.30, 2.34, 2.36 and 2.46 μ to various overtone and combination vibrations due to $\cdot\text{CH}_2$ groups.

Few studies have been undertaken on band assignment for the methine ($\cdot\dot{\text{C}}\text{H}\cdot$) group. Evans, Hibbard and Powell (1951) assigned bands at 1.225 and 1.440 μ to overtone and combination vibrations of the methine groups. Fraser and MacRae (1958) proposed that a band at 2.17 μ that persisted after complete deuteration of proteins was due to combination vibrations of C=O and the α -carbon hydrogen.

(c) Polysaccharides. There have been no extensive studies of absorption by polysaccharides in the near-infrared region. Nikitin (1950) studied the effect of hydrogen bonding in cellulose on the free hydroxyl peaks at 1.44, 1.91 and 2.07 μ . Korelova (1957) used absorption at 1.475 and 1.570 μ in a study of hydrogen bonding in cellulose, while Mitchell, Bockman and Lee (1957) determined the acetyl content of cellulose acetate by the reduction in hydroxyl absorbance at 1.445 μ .

(d) Lipids. Holman and Edmondson (1956) published near-infrared spectra and band assignments for a large number of fatty acids and related substances. Near-infrared methods were subsequently used by Holman, Ener, and Edmondson (1959) and Fenton and Crisler (1959) to determine cis unsaturation in natural fats and oils, hydrogenated oils and shortenings. Absorption at 1.725 μ has been used by Ben-Gera and Norris (1968a) to determine the fat content in meat products. The use of the near-infrared region in the determination of oil content of cereals and oilseeds is discussed in Section II B3.

(e) Water. The near-infrared region has been used extensively for studies on the structure of water as well as the determination of moisture contents (Whetsel, 1969). In dilute carbon tetrachloride solution, water has four absorption bands; 1.39, 1.90, 2.70 and 2.76 μ . In most other media, the bands are shifted to longer wavelengths due to various types of hydrogen bonding (Goddu, 1960).

Elliott and Ambrose (1950) suggested that absorption at 1.94 μ might be applicable to the determination of water in proteins. This was confirmed by Hecht and Wood (1956). Kaye (1954) demonstrated the application of the region to the determination of water in hydrocarbons, alcohols and carboxylic acids. Methods applicable to agricultural products have been published by Hart, Norris and Golumbic (1962), Norris and Hart (1965) and Ben-Gera and Norris (1968a, 1968b). In general these methods embodied the use of methanol extracts or suspensions of the material in methanol or carbon tetrachloride prior to measurement of absorption at 1.93 μ . The determination of water by near-infrared reflectance techniques is discussed in Section II B3.

B. Reflectance Spectroscopy

1. General

Reflectance spectroscopy is the investigation of the spectral composition of surface-reflected radiation with respect to its angularly dependent intensity and the composition of the incident radiation (Kortüm, 1969).

The first measurements of accurate reflection data are credited to Hardy in the 1920's following his development of a recording reflectometer (Wendlandt and Hecht, 1966). Hardy (1938) reported: "The first few months of operation of this instrument were very exciting. We measured the colour of everything in sight and then went out to look for more samples. The instrument was found to act with such rapidity that it generally took less time to make a measurement than it did to decide whether such a measurement was likely to be significant."

Three classes of reflectance spectroscopy have developed - specular, diffuse and internal reflection. This thesis considers mainly diffuse reflectance. Specular reflection is considered in sufficient detail to explain its interference with the phenomenon and measurement of diffuse reflectance. For information on specular and internal reflection spectroscopy the reader is referred to textbooks by Wendlandt and Hecht (1966), Harrick (1967) and Kortüm (1969). Additional information on diffuse reflectance spectroscopy may be found in publications by Judd (1966). Wendlandt and Hecht (1966), Wendlandt (1968), Kortüm (1969) and Frei and MacNeil (1973).

Whereas specular reflection may be explained by Fresnel law, no rigorous theory exists to explain attenuation due to both

the absorption and scattering involved in diffuse reflectance (Wendlandt and Hecht, 1966, Kortum, 1969). However, many phenomenological theories have been developed; the most widely utilized is that proposed by Kubelka and Munk (1931) and modified by Kubelka (1948).

2. The Nature of Reflected Radiation

(a) Specular and diffuse reflection. The reflection of radiant energy at boundary surfaces involves two distinct processes. The first is specular reflection which is also known as regular or mirror-type reflection. The second, diffuse reflection, is characteristic of reflection from matt surfaces. Studies undertaken by Jentzsch (1912) indicate that both processes, specular and diffuse, always occur and that the relative proportion of each depends on the nature of the reflecting medium.

Regular reflection is described by Fresnel equations which in the case of perpendicular incident reduce to

$$R = \frac{(n-1)^2 + n^2 \kappa^2}{(n+1)^2 + n^2 \kappa^2} \quad (3)$$

where: n = the refraction index, and

κ = the absorption index.

κ is defined by Lamberts Law

$$I = I_0 \exp [-4\pi n \kappa d / \lambda_0]$$

where: λ_0 = the wavelength in a vacuum, and

d = layer thickness.

Diffuse reflection arises from penetration of a portion of the incident radiation into the interior of the sample. Partial