THE UNIVERSITY OF MANITOBA

NEAR-INFRARED REFLECTANCE
STUDIES ON WHEAT

BY

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF PLANT SCIENCE

WINNIPEG, MANITOBA

November, 1975

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

Law, Donald Philip, M. Sc. The University of Manitoba, February 1976
Near-Infrared Reflectance Studies on Wheat

Major Professor: Dr. R. Tkachuk

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 2.5μ 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 2.47μ. 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 2.49μ. 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 1.93μ 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 500μ. 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 2.49μ. 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.

ACKNOWLEDGEMENTS

I wish to thank my advisor Dr. Russell Tkachuk for his friendship, helpful advice and criticims throughout the course of this study.

Thanks are also due to the Director, Dr. G. N. Irvine and staff of the Grain Research Laboratory, Canadian Grain Commission for their assistance and for the use of laboratory facilities. In particular I wish to acknowledge the competent technical support provided by Mr. F. D. Kuzina.

I am extremely grateful for the financial aid from the Queensland Department of Primary Industries, the Queensland Barley Marketing Board, the Queensland Grain Growers Association, the State Wheat Board, Queensland, and the Central Queensland Grain Sorghum Marketing Board.

The assistance provided by Dr. W. Bushuk during the planning stages of my studies was greatly appreciated.

I am deeply indebted to my wife, Mary Ann, for her encouragement to my graduate study.

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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 2.5μ 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 3.0μ. 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 2.5μ (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

$$E = (v + 1/2)\, hc\nu \qquad (1)$$

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 = Plancks 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

$$E = hc\nu\,[(v + 1/2) - x\,(v + 1/2)^2] \qquad (2)$$

where: x = the anharmonicity constant.

Transitions between the ground state ($v = o$) and second and higher excited states ($v = 2, 3, \ldots.$) 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 rise 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 2.4μ 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 1.94μ to moisture content. Massie and Norris (1965) illustrated the transmittance and reflectance spectra between 0.4 and 2.0μ 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 1.94μ 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 2.2μ, of the band assignments for proteins.

Ellis and Bath (1938b) and Bath and Ellis (1941) assigned the bands at 1.72 and 2.28μ to 1st overtone and combination vibrations arising from $\cdot CH_2\cdot$ groups. Hecht and Wood (1956) made the general assignments of the 1.1 - 1.2μ, 1.7 - 1.8μ and 2.2 - 2.5μ regions to 2nd and 1st overtone and combination C-H vibrations respectively. Fraser (1956) assigned peaks at 2.29 and 2.36μ in wool and 2.30 and 2.35μ in nylon to $\cdot CH_2\cdot$ 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 2.46μ to various overtone and combination vibrations due to $\cdot CH_2\cdot$ groups.

Few studies have been undertaken on band assignment for the methine (·ĊH·) group. Evans, Hibbard and Powell (1951) assigned bands at 1.225 and 1.440μ to overtone and combination vibrations of the methine groups. Fraser and MacRae (1958) proposed that a band at 2.17μ 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 abosrption 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 2.07μ. Korelova (1957) used absorption at 1.475 and 1.570μ 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 1.445μ.

(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 1.725μ 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 2.76μ. 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 1.94μ 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 1.93μ. 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 phenonmenon 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, Kortüm, 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 or 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 Jentzch (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

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

where: n = the refraction index, and

κ = the absorption index.

κ is defined by Lamberts Law

$$I = I_o \exp\,[-4\pi n\kappa d/\lambda o]$$

where: λo = 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 absorption and multiple scattering occur at the surfaces of the particles of which the medium is composed and part of the radiation is returned to the surface (Wendlandt and Hecht, 1966).

The attenuation of diffuse reflection by the absorption within the medium is given by the Bouguer-Lambert Law

$$I = I_o \exp [-\varepsilon \bar{d}]$$

where: ε = molar extinction coefficient; and

$\bar{d}$ = mean penetrated layer thickness.

(b) Dependence on absorption coefficient. The two reflection processes specular and diffuse are opposed to each other. Equation 3 indicates that as the absorption coefficient (κ) increases, the specular reflection approaches unity. In contrast diffuse reflectance requires that the radiation enters the interior of the sample where absorption takes place according to the Bouguer-Lambert Law (Wendlandt and Hecht, 1966).

Kortüm and Vogel (1958) studied the contributions of the separate processes to total reflectance in the case of strong ($KMn0_4$) and weak ($CuS0_4 \cdot 5H_20$) absorbers. They determined reflectance spectra with and without the specular component. Their results confirmed the expectations from theory that the differences in apparent absorbance with the inclusion and exclusion of the specular component were much larger in the case of strong than weak absorbers.

3. The Kubelka-Munk Theory.

(a) General. The Kubelka-Munk Theory is the most widely utilized of the various phenomenological theories that have been proposed for diffuse reflectance (Wendlandt and Hecht, 1966; Kortüm, 1969; Frei and MacNeil, 1973). A statistical approach based on a spherical particle model was developed by Melamed (1963) but the equation is not as readily applied as that from the Kubelka-Munk theory.

Several of the theories of diffuse reflectance, including those of Kubelka-Munk and Melamed were compared mathematically by Simmons (1975). He concluded that the assumption that the Kubelka-Munk remission function is proportional to the absorption coefficient was approximately valid for weakly absorbing powder samples.

For the special case of infinite layer thickness the Kubelka-Munk theory proposes

$$F(R_\infty) \equiv \frac{(1-R)^2}{2R} = \frac{k}{s} \qquad (4)$$

where: $F(R_\infty)$ = the Kubelka-Munk (remission) function,
R = absolute reflectance,
k = the absorption coefficient, and
s = the scattering coefficient.

Infinite layer thickness is usually achieved by a depth of sample of less than 3 mm (Wendlandt and Hecht, 1966; Simmons, 1975). In practice absolute reflectance is not measured, instead sample reflectance is referred to a standard of high reflectance (>95%). In such cases the Kubelka-Munk function is denoted $F(R'_\infty)$ (Wendlandt and Hecht, 1966).

(b) Concentration dependence of the Kubelka-Munk function. The concentration dependence of the Kubelka-Munk function has been discussed in detail by Kortum, Braun and Herzog (1963), Wendlandt and Hecht (1966), Kortüm (1969) and Frei and MacNeil (1973).

The absorption coefficient k in Equation 4 may be replaced by 2.303εc where ε is the extinction coefficient and c the molar concentration of the absorbing substance (Kortum et al, 1963).

$$F(R_\infty) = \frac{2.303\ \varepsilon c}{s} \tag{5}$$

Assuming the validity of the Kubelka-Munk theory a plot of the Kubelka-Munk function versus molar concentration will yield a straight line with a slope proportional to the inverse of the scattering coefficient. Similarly a plot of the logarithm of Kubelka-Munk value against the logarithm of the concentration will yield a straight line with an intercept proportional to minus the logarithm of the scattering coefficient.

The Kubelka-Munk theory is valid only in the case of weak absorption (Wendlandt and Hecht, 1966; Kortüm, 1969). Frei and Frodyma (1965) showed that the useful calibration range could be extended by the use of the semi-emperical relation, $F(R_\infty) = a \log c$, where 'a' is a constant.

Factors which may result in deviations from the Kubelka-Munk theory include the presence of specular reflection, saturation of monomolecular absorbed layers and variation in moisture contents (Kortüm et al, 1963).

(c) Particle size dependence of the Kubelka-Munk function. Particle size may influence the Kubelka-Munk function through its effect on both the absorption and scattering coefficients (Kortüm, 1969).

Kortüm (1969) used relations developed for transmission spectroscopy to show that for values of Kc_0d greater than 0.1, a rapid increase in dependence of the transmission absorption coefficient occurred. (K = natural extinction coefficient of the absorbing molecules; c_0 = the concentration in moles per litre in the particle; and d = mean particle size in centimeters). On the basis that the absorption coefficient in reflectance was related to the absorption coefficient in transmission, Kortüm concluded that the reflectance absorption coefficient should also diminish with increasing values of d and that the change should be more strongly observed at greater extinction modulus (Kc_0) of the molecules.

The effect of particle size on the magnitude of the scattering coefficient has been studied by Kortüm, Braun and Herzog (1963). Using ground didymium filter glass of 1.1, 1.5, 2.4 and 4.2μ diameter, these researchers found that the scattering coefficient in the visible region was inversely proportional to mean particle diameter. For particle diameters approximately equal to the wavelength, the scattering coefficient was dependent on the wavelength; for larger particles there was no such dependency.

4. Applications

(a) General. The use of diffuse reflection spectroscopy as an analytical technique has been reviewed recently by Frei and MacNeil (1973). Until the middle 1960's the application of reflectance spectroscopy was largely limited to the measurement of colour, whiteness, gloss, etc. in the paint, paper and textile industries. Since then the technique has found widespread use particularly in chromatography.

Most of the applications of reflectance spectroscopy have concerned the visible region of the spectrum. The ultraviolet region has been used in the evaluation of thin layer chromatograms while relatively few studies have been conducted using the near-infrared or infrared regions (Frei and MacNeil, 1973).

b. Cereals and oilseeds. Reflectance spectroscopy was introduced to the cereal industry by Kent-Jones and Martin (1950), and Kent-Jones, Amos and Martin (1950). These workers proposed reflectance of flour slurries at 530 nm as an indirect measure of the ash content of flour. Studies on the measurement of flour colour, some of which have included the measurement of bread crumb colour, have been undertaken by Irvine and Anderson (1952), Graza (1959), Kipper (1960), Pomeranz (1960) and Murthy and Dietz (1974).

Reflectance techniques for the measurement of macaroni and spaghetti colour have been published. (Grain Research Laboratory, 1958; Walsh, Gilles and Shuey, 1969). Matz and Larson (1954) attempted to correlate reflectance of semolina at 435 nm with the colour of macaroni and spaghetti made from the semolina.

The use of near-infrared reflectance spectroscopy in the analysis of cereal products was first demonstrated by Hoffmann (1963). He published the relation of reflectance at 1.93 μ to moisture content for a large number of solid substances which included single samples of starch and flour. Near-infrared reflectance instruments designed specifically for the analyses of protein, moisture, and oil contents in cereals and oilseeds were developed in 1971 by the Neotec Instruments Inc. (Rosenthal, 1971).

Similar instruments are now available from other manufacturers.

The Neotec Grain Analyzer requires calibration with samples of known composition and utilizes the relation,

$$X\% = a\Delta O.D._{w} + b\Delta O.D._{p} + c\Delta O.D._{o} + d \qquad (7)$$

Where: X = protein, moisture or oil

$$\Delta O.D._{w} = \log \frac{R_{1.68\mu}}{R_{1.93\mu}}$$

$$\Delta O.D._{p} = \log \frac{R_{2.18\mu}}{R_{2.06\mu}}$$

$$\Delta O.D._{o} = \log \frac{R_{2.31\mu}}{R_{2.06\mu}}$$

a, b, c, d = constants, which are different for each component, and

R = reflectance compared with a Teflon standard.

Several papers have been presented on the accuracy and advantages of these instruments. (Rosenthal, 1971, 1973; Trevis, 1974; Hymowitz, Dudley, Collins and Brown, 1974; Williams, 1975). Williams (1975) quoted an accuracy of analyses as measured by the standard error of the estimates of ± 0.22% protein and ± 0.16% moisture in the case of hard red spring wheat, giving a coefficient of variation of about 1.5% in each case. He also suggested that the variability in particle size and type of particle influenced the accuracy of the method.

III. MATERIALS

A. Spectrophotometer

Spectra were obtained using a Cary 17I spectrophotometer fitted with a lead sulfide detector and model 1711 reflectance accessory. The accessory included a Type 1 (illumination by monochromatic radiation) integrating sphere. The internal surface of the sphere was coated with smoked magnesium oxide (Wendlandt and Hecht, 1966).

Figure 1 shows the optical path of the reflectance attachment. Chopped monochromatic radiation from the beam divider is alternately directed at 30 Hz perpendicularly onto the samples and standard. The radiation is diffusely reflected within the sphere and is measured by a lead sulfide detector in the case of near-infrared radiation. Screens inside the sphere prevent radiation reflected from the sample or standard from falling directly onto the detector (Kortum, 1969). This overcomes the major sources of systematic error found in the majority of commercial integrating spheres (Clarke, 1972).

Dry nitrogen was circulated through the instrument to exclude water vapour.

The wavelength calibration of the instrument was checked using a Hg lamp. The results, listed in Table 1, show the error of the scale was not greater than 1 nm.

Figure 1

Optical diagram for the Cary 17I spectrophotometer fitted with a Type I integrating sphere.

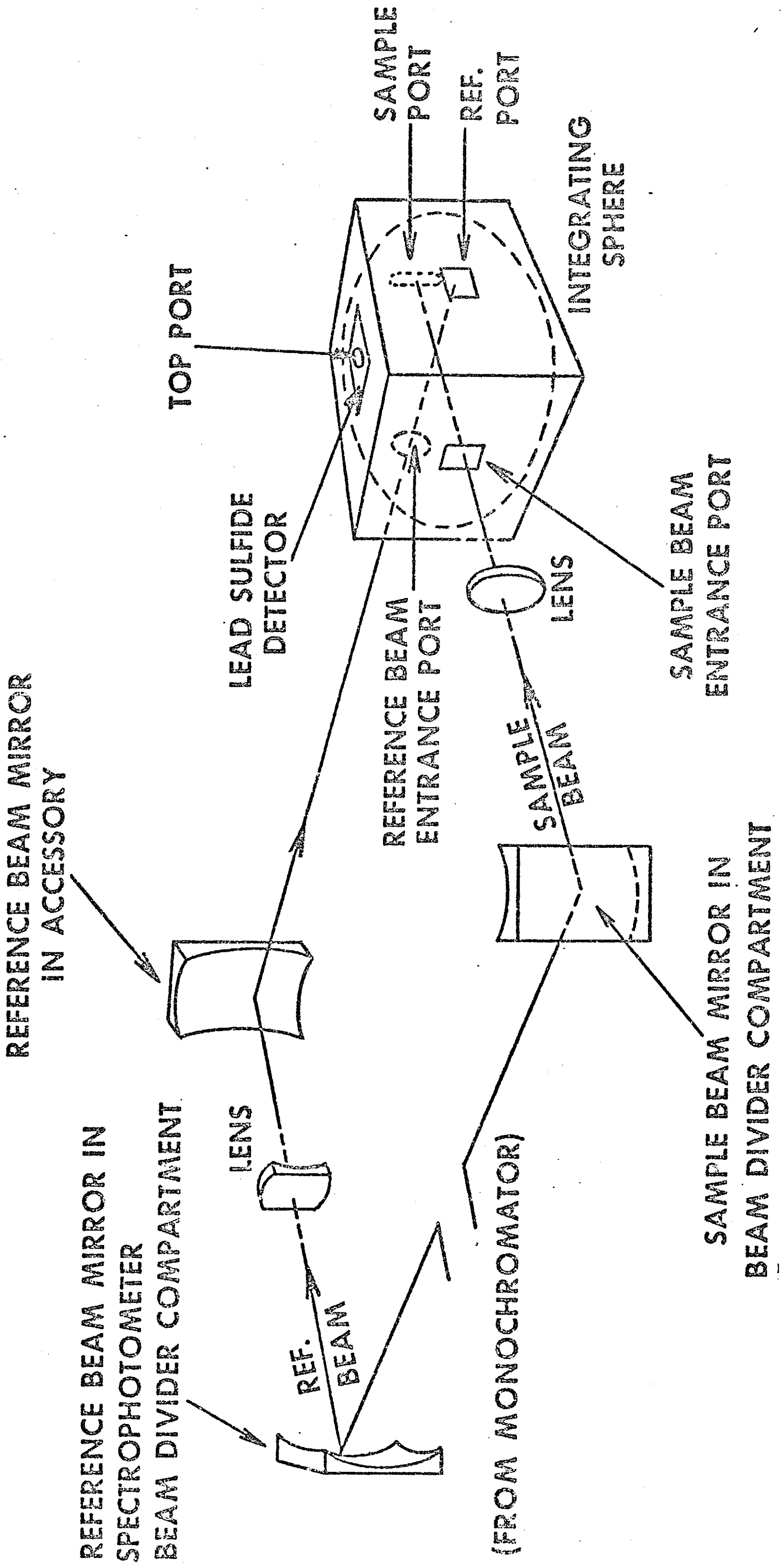

SAMPLE PORT
REF. PORT
TOP PORT
INTEGRATING SPHERE
LEAD SULFIDE DETECTOR
REFERENCE BEAM ENTRANCE PORT
SAMPLE BEAM ENTRANCE PORT
LENS
SAMPLE BEAM
REFERENCE BEAM MIRROR IN ACCESSORY
SAMPLE BEAM MIRROR IN BEAM DIVIDER COMPARTMENT
LENS
REF. BEAM
REFERENCE BEAM MIRROR IN SPECTROPHOTOMETER BEAM DIVIDER COMPARTMENT
(FROM MONOCHROMATOR)

Table 1: Wavelength calibration for Cary 17I spectrophotometer using a Hg lamp.

Wavelength nm	
Pyler and Peters (1950)	Cary 17I
576.96	577
579.07	579
1013.98	1014
1357.03	1358
1367.28	1368
1395.06	1395
1529.52	1530
1692.02, 1694.19	1693
1707.27	1707
1710.99	1711
2325.42	2325

B. Sample Holders

Solid samples were contained within 2 1/8" diameter plastic holders having a capacity of approximately 15 g of ground wheat. The holders were fitted with Infrasil cover glasses.

IV. METHODS

A. Spectra of Wheat and Wheat Components

1. Samples

(a) Wheat. Three samples were used; a durum wheat of 12.8% protein; a hard red spring wheat (HRS), 16.1% protein; and a soft white winter wheat (SWW), 12.3% protein (13.5% moisture basis). All samples were grown in Canada and of milling grade quality. The wheats were ground on a Model CSM-2 Cyclone grinder fitted with a 1.0-mm screen prior to recording the spectra.

(b) Gluten. Glutens were prepared from samples of milling grades of Canadian hard red spring wheat and Canadian durum wheat by the method of Doguchi and Hlynka (1967).

(c) Starch. Prime starch was obtained from a flour milled from a sample of hard red spring wheat by centrifugation and freeze drying. The protein content of the starch was 0.30%.

(d) Pentosans. Soluble and insoluble pentosans were isolated from a flour milled from Canadian hard red spring wheat using the method of Jelaca and Hlynka (1971). The protein content (N x 5.7) of the soluble fraction was 5.1% and the insoluble fraction 4.7%.

(e) Lipid. Lipids were obtained from ground samples of the wheat previously described (1.a.) by extracting overnight in Goldfisch extractors with Skellysolve F95. The lipid content (oven dry basis) of the durum wheat was 2.4%, hard red spring 2.0%, and soft white winter, 2.1%.

(f) Water. Laboratory distilled water was used.

The wheat, gluten, starch and pentosan samples were used on an 'as is' basis, 8 - 12% moisture, and after vacuum-drying at room temperature to approximately 2.0% moisture.

2. Spectra

Spectra were recorded between 1.0 and 2.5µ using smoked magnesium oxide as a reference standard. The instrument was operated in the absorbance mode which resulted in the measurement of apparent absorbance; logarithm (reflectance of standard/reflectance of sample).

The units of apparent absorbance are not presented in the results due to their dependence on the particle size of the samples, the variations in the reflectance of the standard over the wavelength range (see Appendix) and the different sample-holders used. Therefore, their inclusion would be misleading. In terms of absolute apparent absorbance, it was estimated that the maximum values obtained did not exceed 0.9 ± 0.2.

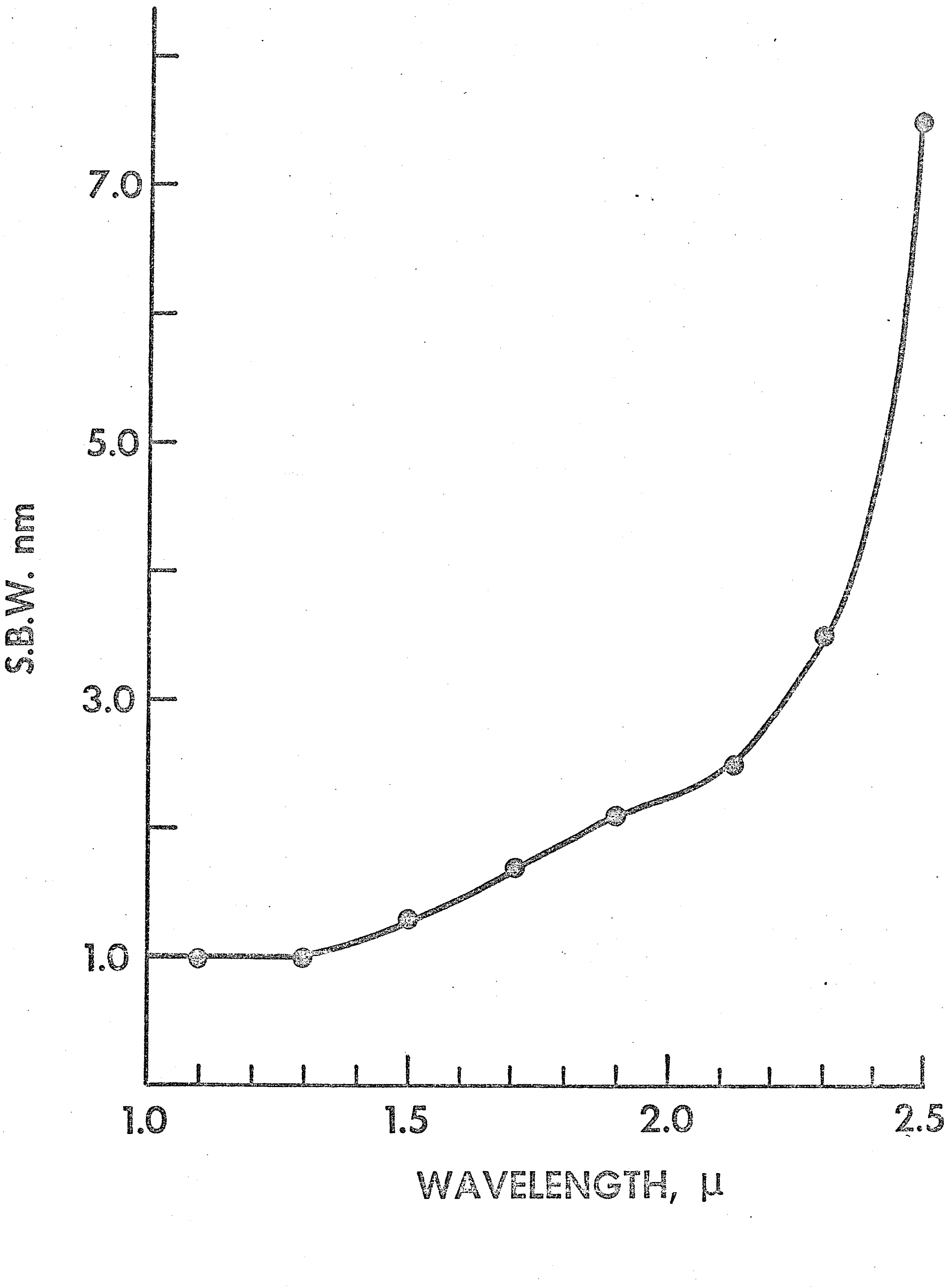
The spectral traces reproduced in this thesis were obtained using a scan speed of 2 nm per sec and chart speed setting of 300 nm per in. For the measurement of the wavelength position of absorption peaks, a scan speed of 0.2 nm per sec and chart speed setting of 60 nm per in. were used.

The solid samples were packed into the sample holders described in Section III B while the lipid and water samples were poured onto the surface of pressings of a mixture of sulfur and polytetrafluoroethylene (Sulflon), (Appendix), and covered with Infrasil glasses.

Figure 2 illustrates the relation of spectral band width to wavelength, applicable to the recordings.

Figure 2

Relation of spectral band width (S.B.W.), nm, to wavelength, μ, for Cary 17I spectrophotometer using smoked magnesium oxide as a sphere coating and reference standard.


S.B.W. nm
7.0
5.0
3.0
1.0
1.0
1.5
2.0
2.5
WAVELENGTH, μ

B. Determination of Moisture Content

1. Samples

(a) Calibration. The three wheats previously described (A.1.a.) were each ground on three mills; a Hobart model 2040 coffee grinder; a Wiley model 1 cutting mill fitted with a 1.0-mm sieve; and a Model CSM-2 cyclone grinder fitted with a 1.0-mm screen. Five subsamples, obtained from each of the above nine samples (3 wheats x 3 mills), were exposed to a range of humidities to produce a range of moisture contents for each wheat grind.

(b) Validation. 49 samples were used to validate the calibration. 32 of the samples represented eight varieties of hard white wheat grown on four sites in Queensland, Australia during 1974. The protein contents of the wheats ranged from 12.1 to 18.0%. These samples were ground using a Christy and Norris cyclone grinder fitted with a 1.0-mm sieve. The ground samples were dried at 100°C for 16 hrs, and allowed to regain moisture. The remainder were samples of hard red spring wheat grown in Canada in 1975. These samples were ground on a Hobart model 2040 coffee grinder.

2. Particle Size Determinations

100 g of the nine ground samples described above (B.1.a.) were sieved through a series of screens by means of a Rotap mechanical shaker. Particle size (root mean square particle diameter) was calculated from the following

$$\sqrt{\bar{d}^2} = \sqrt{\frac{\Sigma n_i d_i^2}{\Sigma n_i}}$$

where: n = percent by weight retained on each sieve, and

d = mean of the apertures of the through and retained screens.

In the case of the fraction which passed through the finest sieve, 63μ, d was assumed to be 31.5μ.

3. Reflectance Measurements

The spectra between 1.0 and 2.5μ of the samples were recorded by the methods previously described (A.2) except that a Sulflon pressing was used as a reference standard. Apparent absorbance values were recorded for wavelengths corresponding to the absorption peaks in the spectra of wheat, starch, and gluten and the valleys in the absorption spectrum of wheat of approximately 10% moisture (1.12, 1.20, 1.31, 1.46, 1.57, 1.66, 1.70, 1.75, 1.78, 1.85, 1.93, 2.01, 2.05, 2.10, 2.18, 2.22, 2.29, 2.31, 2.35, 2.49μ).

After correcting for the effects of the sample holder and cover glass, the recorded data were converted to Kubelka-Munk values using published tables (Hodgman, Weast and Selby, 1957; Kortum, 1969).

4. Oven Moistures

The oven moisture contents of the samples were determined by drying in an air oven at 130°C for one hour. (American Association of Cereal Chemists, 1962). Duplicate determinations were conducted on the samples used for calibration; in other cases single determinations were made.

5. Statistical Analyses

(a) Simple linear regression. A programme provided for the Wang computer at the Canadian Grain Commission was used to evaluate simple linear regressions.

(b) Multiple linear regressions. Programmes DREG and MULREG provided on the Eastern Time Sharing System, Canadian Grain Commission, were used.

V. RESULTS AND DISCUSSION

A. Spectra of Wheat and Wheat Components

The reflectance spectra between 1.0 and 2.5μ for the dried samples of wheat, gluten, starch, soluble and insoluble pentosans and lipid are illustrated in Figures 3 - 8. The spectrum for water is shown in Figure 9. The position of peaks in the spectra of durum and soft white winter wheats and lipids, and of durum gluten were the same as for hard red spring and therefore have been omitted.

The resolution of the spectra is far superior to those published for whole wheat (Massie and Norris, 1965) and those for carbon tetrachloride suspensions of ground wheat obtained by transmission spectroscopy (Norris and Hart, 1965).

Comparisons of Fig. 3 with Fig. 4 - 7 indicate that the spectrum for wheat is determined largely by the carbohydrate components with gluten having only a minor effect. The absorption peak at 1.98μ in the spectrum of wheat, which is due to the primary amide group (Elliott, 1952; Fraser, 1955; Goddu, 1960; Krikorian and Mahpour, 1975), was not observed in the undried sample due to the overshadowing effect of the water band at 1.93μ.

The spectrum for gluten (Fig. 4) is similar to the transmission spectrum for porcupine quill published by Hecht and Wood (1956), except for the presence of an intense peak at 1.98μ due to the primary amide group. In the undried samples the intensity of the 1.98μ band was equal to that of the water band at 1.93μ. A weak band at 2.01μ observed by Fraser (1956) 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 the peak in wheat gluten.

Studies with gelatin (Ellis and Bath, 1938b), demonstrated that the intensity of the peak at 1.50μ decreased with increase in moisture content. This effect was assigned to a reduction in the dipole moments of the N-H groups when water was bridged to them. Similar though less significant reductions were noted with the bands at 2.05 and 2.18μ. Whether the same phenomena occurred in gluten was not obvious from the results.

Although the position of peaks in the spectra of the starch, Fig. 5, and pentosan fractions, Figs. 6 and 7, were identical, there were marked differences in their shapes. In the main, these differences were due to variations in the surface characteristics of the samples which determine the degree of resolution obtained in reflectance spectra (Kortüm, 1969). There were notable differences, however, in the first overtone C-H regions. For starch the intensity of the peak at 1.70μ was lower than that at 1.78μ; for the pentosan fraction the order was reversed. These peaks arise mainly from the $\cdot CH\cdot_2$ groups, although on the basis of the results published by Kaye (1954) they must receive a major contribution from $\cdot\dot{C}H\cdot$ groups which absorb in the same region at about one third of 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. The peaks of the acyclic $\cdot CH\cdot_2$ groups have been shown to occur at slightly shorter wavelengths (Wexler, 1968). Whether these differences or differences in the ratio of intensities of .OH to $\cdot CH\cdot_2$ groups can be used in methods for the determination of starch and pentosan contents in cereals warrants further study.

The spectrum for lipid, Fig. 8, is similar to that for castor oil published by Holman and Edmondson (1956). An additional peak at 1.39μ is apparent in the wheat lipid spectrum also the peaks at 1.17, 2.14 and 2.17μ due to cis unsaturation are relatively more intense. The origin of the additional peak is uncertain.

The absorption in the 2.0 - 2.5μ region in the spectrum of water, Fig. 9, is due to an intense absorption band with a peak at 2.70μ (Whetsel, 1969). The peaks at 1.45 and 1.93μ shift to slightly higher wavelengths, 0.005 - 0.010μ, in wheat, gluten, starch and pentosans due to hydrogen bonding. This did not apply to the samples with very low moisture contents.

The contributing groups and assignments for the absorption bands of the spectra of gluten, starch, pentosans and lipid are presented in Tables 2 - 4. These data 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 2.05μ (Kaye, 1954). However due to their minor contribution as well as for simplicity these groups have been omitted from the tables.

While in previous studies on proteins (Ellis and Bath, 1938b; Bath and Ellis, 1941; Fraser, 1956) the bands at 2.05 and 2.18μ 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 (1952) and the studies on primary amides by Krikorian and Mahpour (1975). Weak bands at 1.39μ 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 (Wheeler, 1959; Goddu and Delker, 1960).

The first overtone C-H bands between 1.7 and 1.8μ were poorly resolved in the pentosan fractions compared with starch. The OH peaks at 1.45, 1.55, 1.93 and 2.09μ in the spectra of starch and pentosans were found at slightly longer wavelengths in the undried samples. This was probably due to hydrogen bonding (Wheeler, 1959).

Many of the wavelengths cited have been used in the analysis of cereals and oilseeds both by transmission and reflectance spectrophotometry. Fenton and Crisler (1959) used absorbance at 2.14μ as a measure of cis unsaturation in oils. Absorption at 1.93μ of methanol or carbon tetrachloride suspensions was used by Hart et al (1962), Norris and Hart (1965) and Ben-Gera and Norris (1968b) to determine moisture contents of various grains and soybeans. The near-infrared reflectance grain analyzers utilize absorption at 1.93, 2.18 and 2.31μ to determine moisture, protein and oil contents respectively (Rosenthal, 1973). The results presented provide the basis for investigations of other bands in the near-infrared spectrum which may be useful for the qualitative or quantitative characterization of wheat and its components.

Figure 3

Reflectance spectrum between 1.0 and 2.5μ of a ground sample of hard red spring wheat of 16.1% protein (13.5% m.b.) and approximately 2.0% moisture.

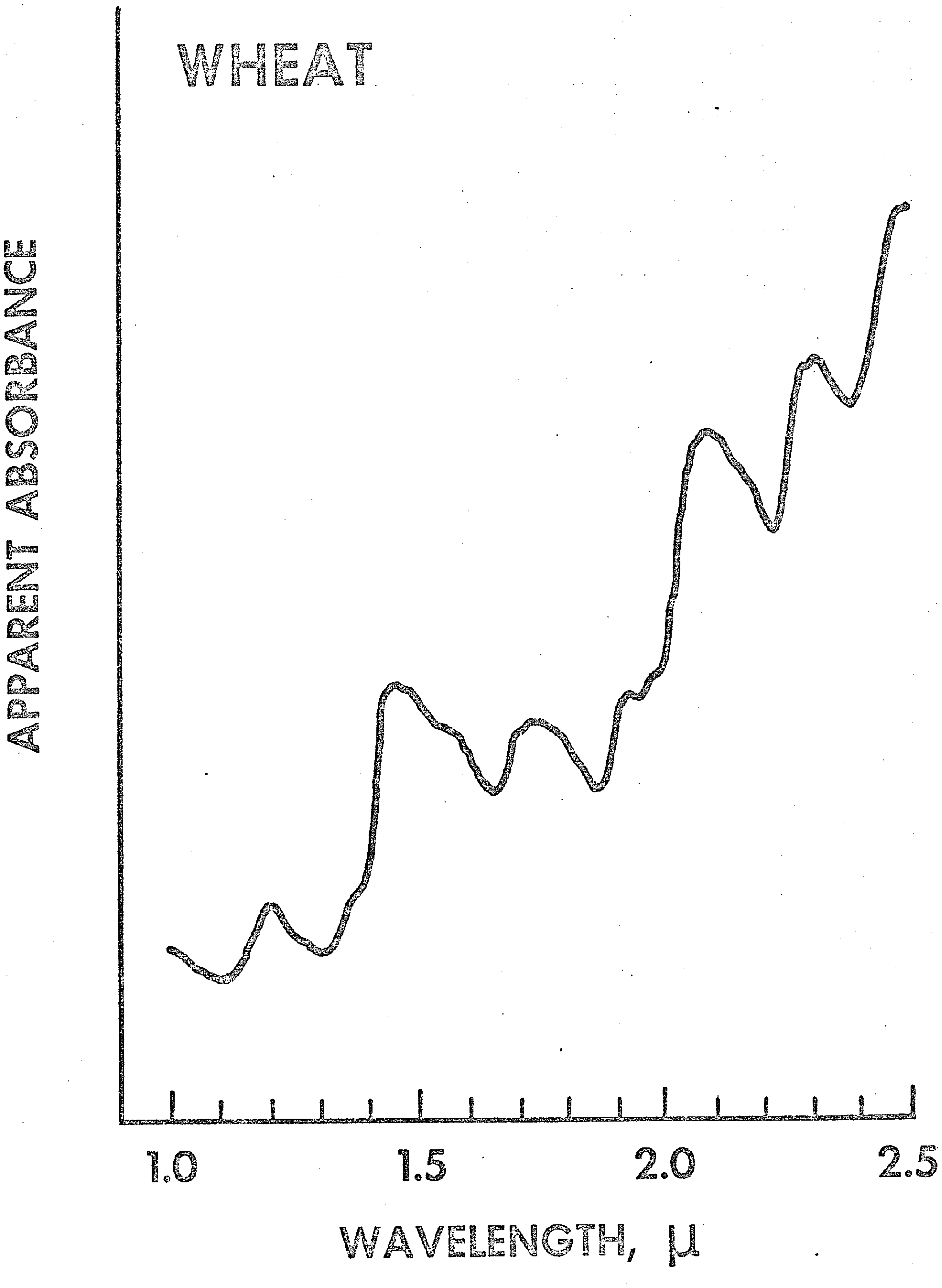

WHEAT
APPARENT ABSORBANCE
1.0
1.5
2.0
2.5
WAVELENGTH, μ

Figure 4

Reflectance spectrum between 1.0 and 2.5μ of gluten, approximately 2.0% moisture, washed from a sample of hard red spring wheat.

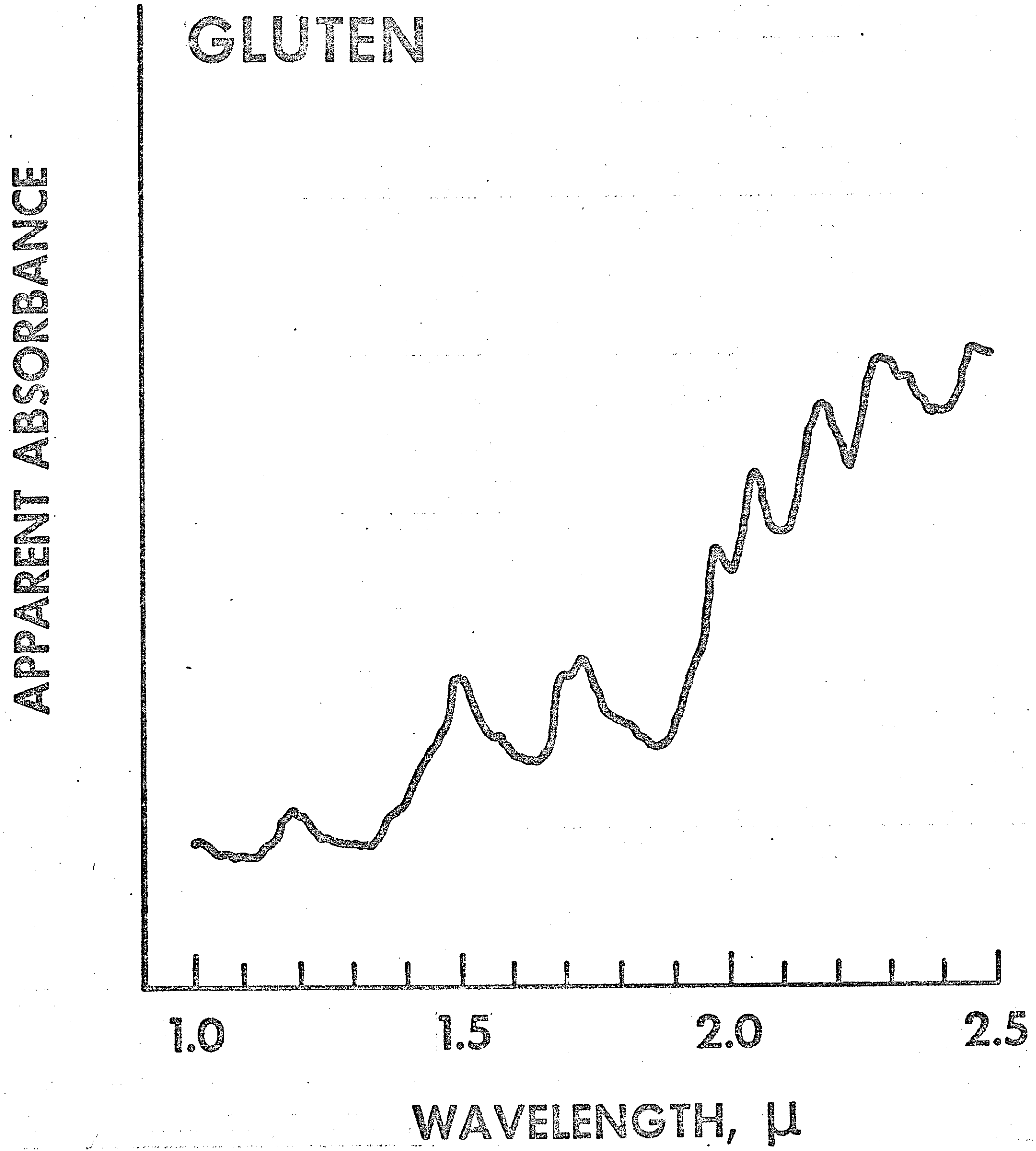

GLUTEN
APPARENT ABSORBANCE
1.0
1.5
2.0
2.5
WAVELENGTH, μ

Figure 5

Reflectance spectrum between 1.0 and 2.5μ of starch, approximately 2.0% moisture, washed from a sample of hard red spring wheat.

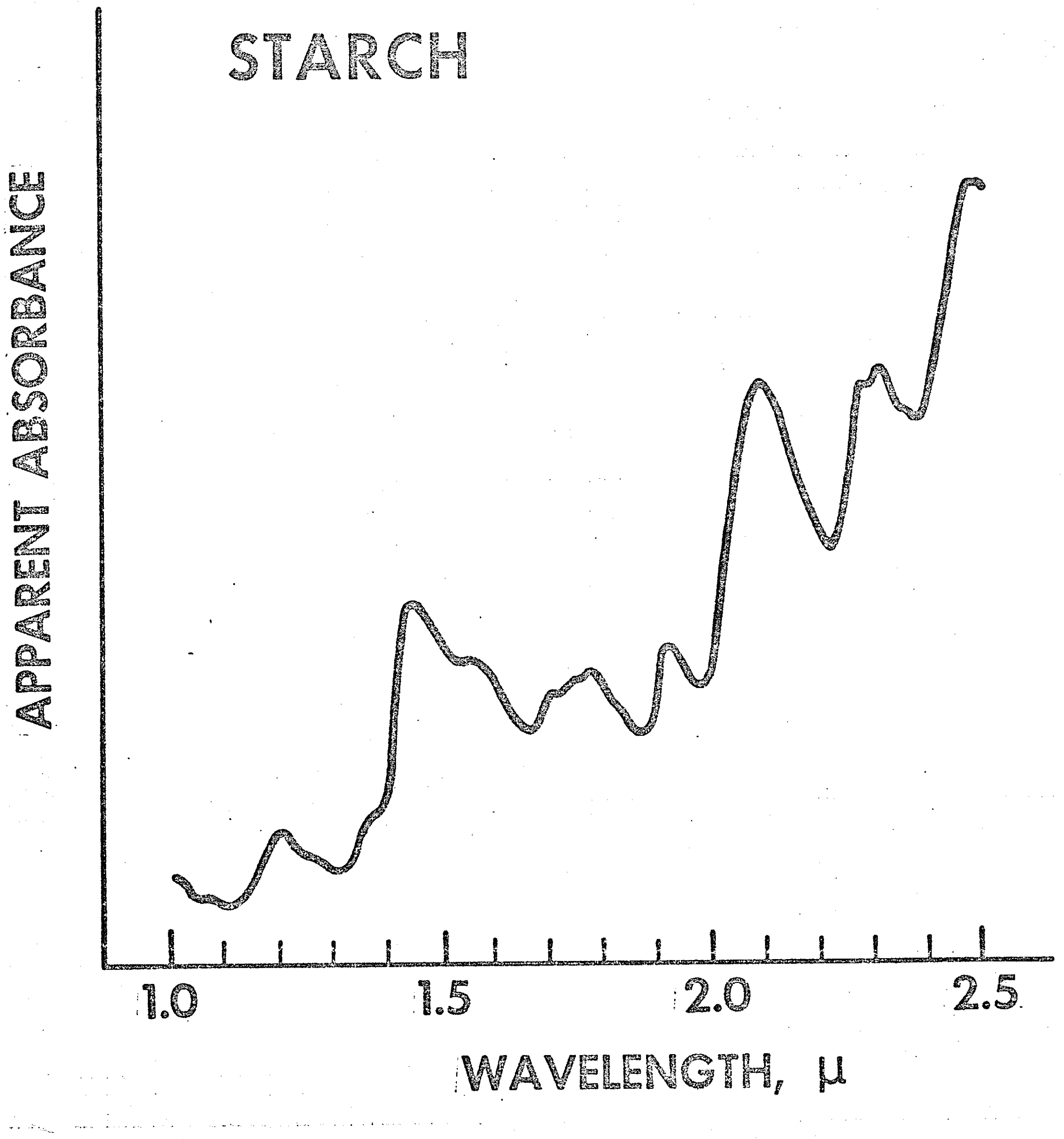

STARCH
APPARENT ABSORBANCE
1.0
1.5
2.0
2.5
WAVELENGTH, μ

Figure 6

Reflectance spectrum bewteen 1.0 and 2.5μ of water-soluble pentosans, approximately 2.0% moisture, obtained from a sample of hard red spring wheat.

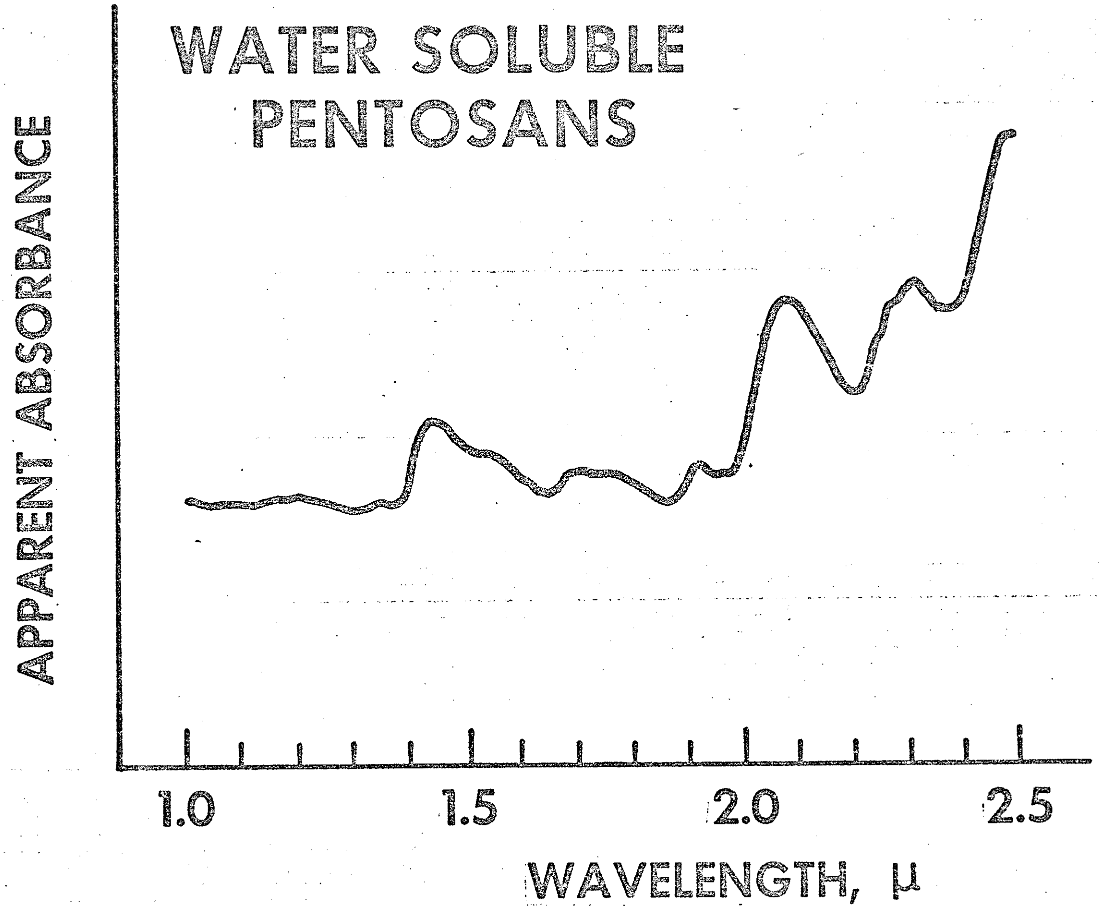

WATER SOLUBLE PENTOSANS
APPARENT ABSORBANCE
1.0
1.5
2.0
2.5
WAVELENGTH, μ

Figure 7

Reflectance spectrum between 1.0 and 2.5μ of water-insoluble pentosans approximately 2.0% moisture, obtained from a sample of hard red spring wheat.

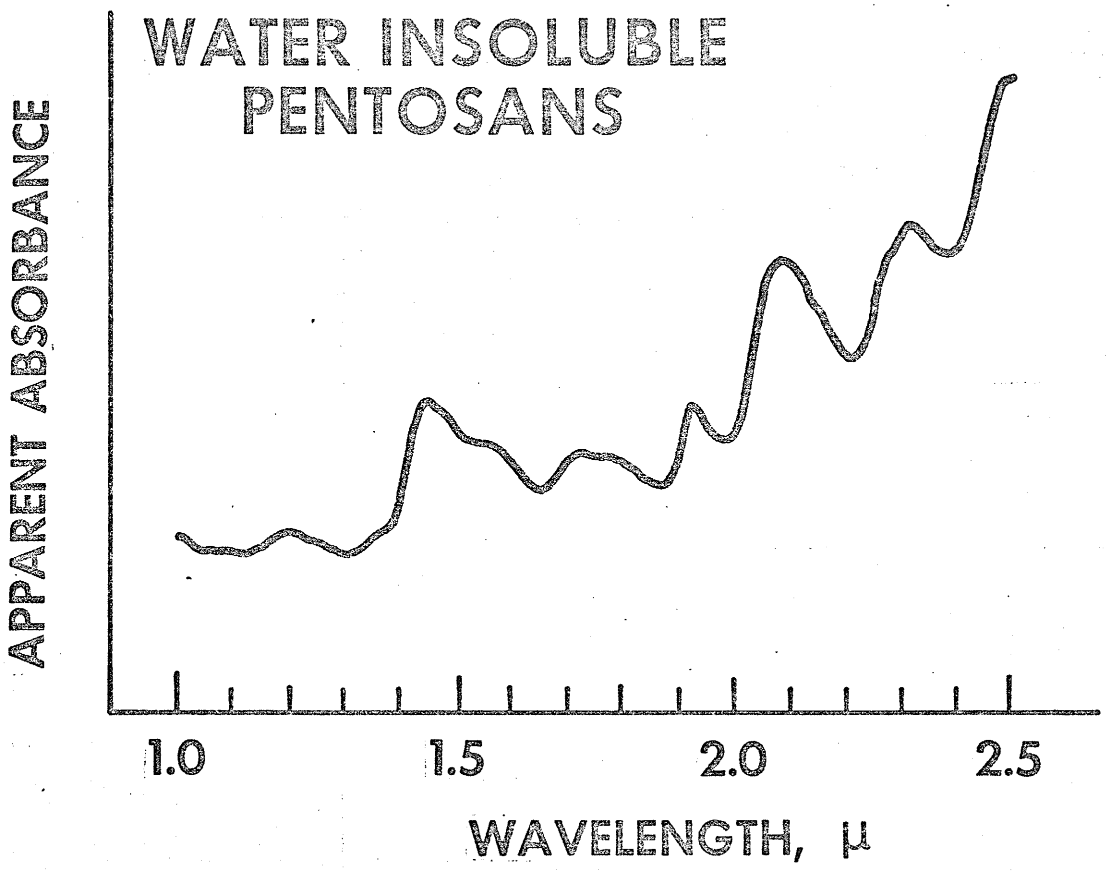

WATER INSOLUBLE
PENTOSANS
APPARENT ABSORBANCE
1.0
1.5
2.0
2.5
WAVELENGTH, μ

Figure 8

Reflectance spectrum between 1.0 and 2.5μ for lipid extracted from hard red spring wheat and adsorbed on Sulflon.

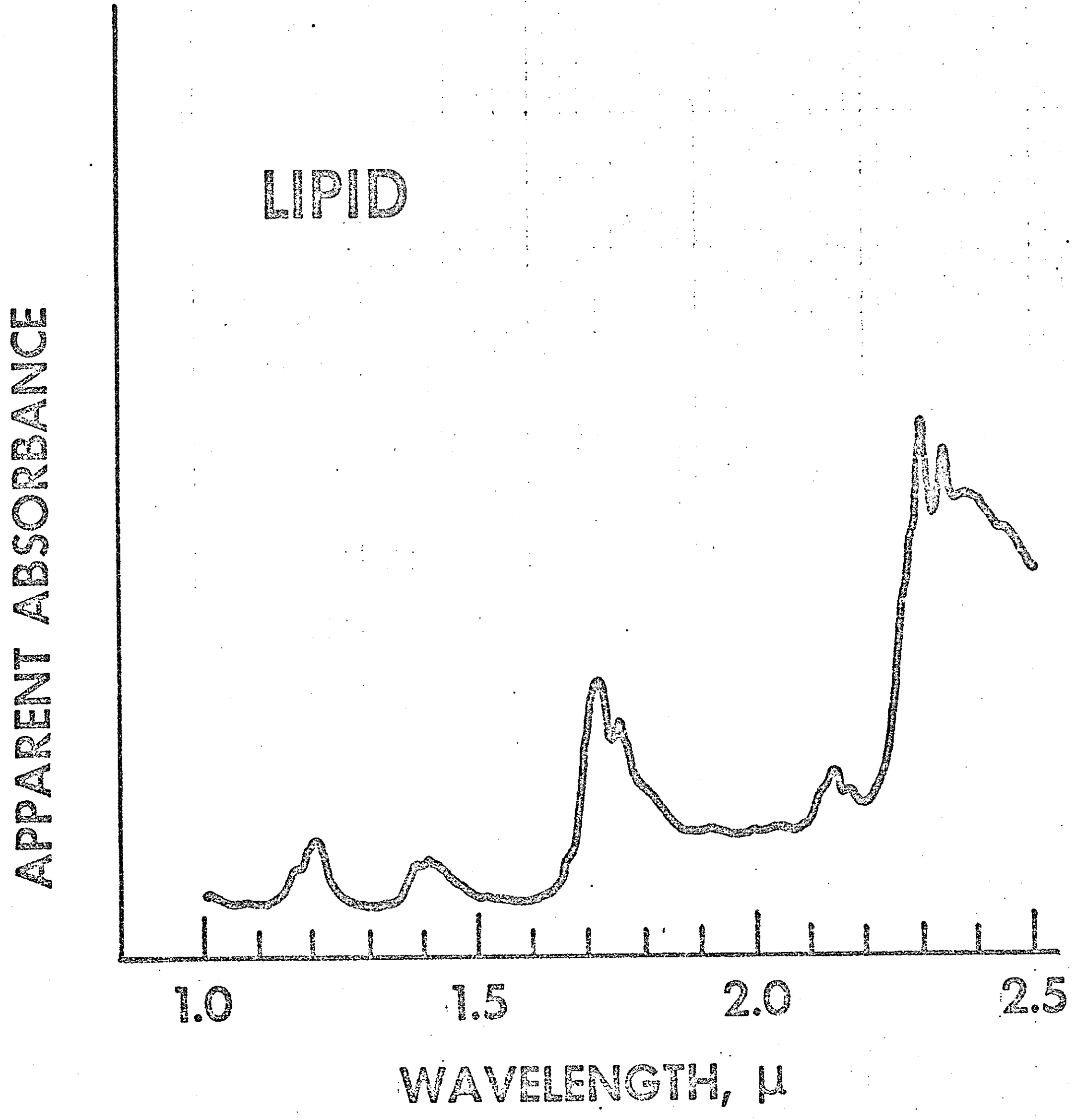

LIPID
APPARENT ABSORBANCE
1.0
1.5
2.0
2.5
WAVELENGTH, μ

Figure 9

Reflectance spectrum between 1.0 and 2.5μ of water adsorbed on Sulflon.

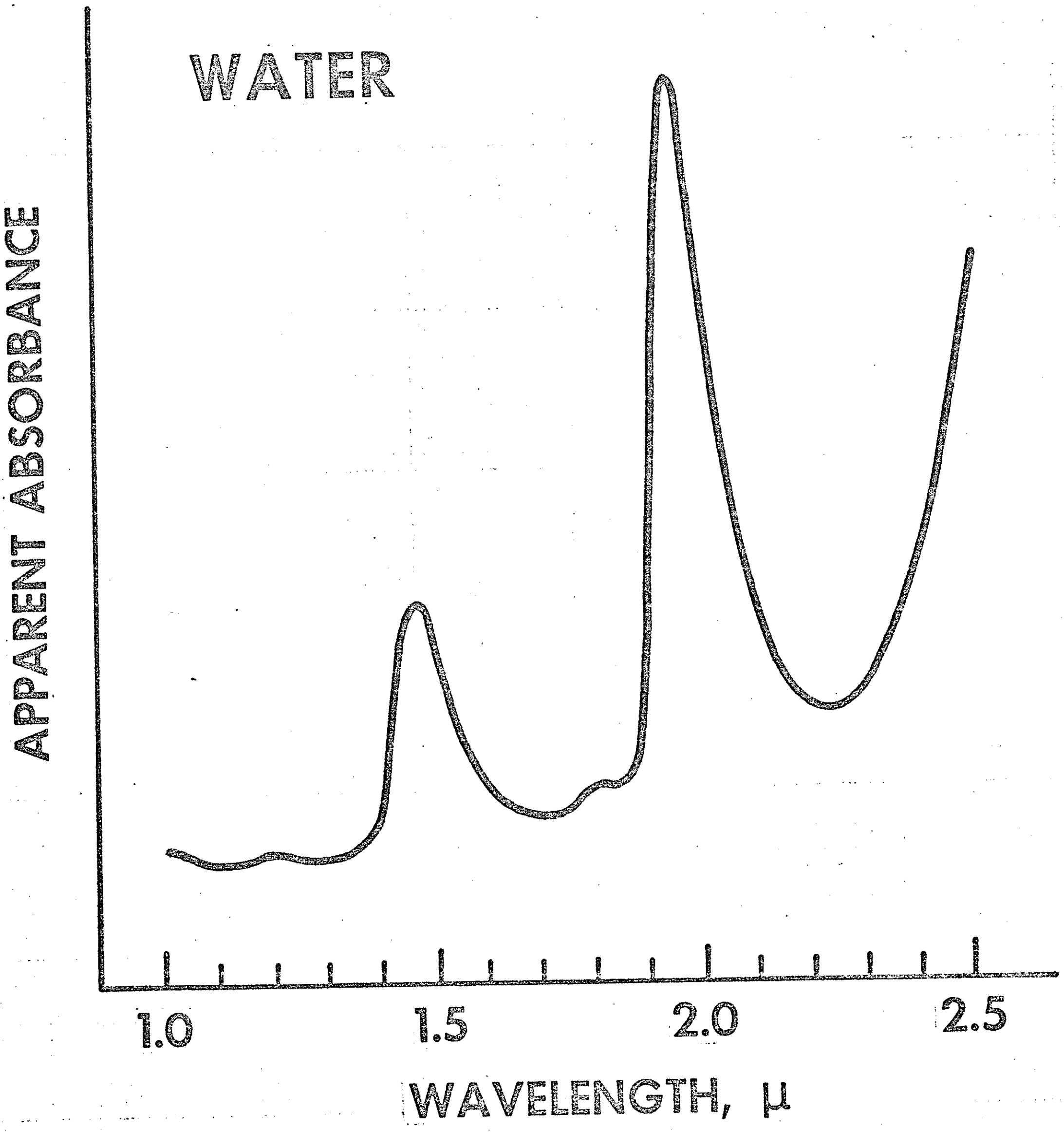

WATER
APPARENT ABSORBANCE
1.0
1.5
2.0
2.5
WAVELENGTH, μ

Table 2: Contributing groups and assignments of near-infrared bands of gluten

Wavelength µ	Contributing Group	Assignment	References
2.47, 2.34, 2.30, 2.29	$\cdot CH \cdot_2$	C-H Combination	1, 2, 3, 4
2.18	$\cdot NHCO \cdot$	C=O + Amide III	3, 5, 6
	$\cdot COHN_2$	C=O + Amide III Combination	6, 7
2.05	$\cdot NHCO \cdot$	N-H + Amide II Combination	3, 5, 7
	$\cdot COHN_2$	N-H + Amide III Combination	6, 7
1.98	$\cdot CONH_2$	N-H + Amide II Combination	6, 7, 8
1.79	H_2O	O-H Combination	1
1.76, 1.73, 1.70	$\cdot CH \cdot_2$	C-H 1st Overtone	1, 5, 9, 10
1.57, 1.50	$\cdot NH \cdot$	N-H 1st Overtone	1, 5, 9
1.46	$\cdot CONH_2$	N-H 1st Overtone	11
1.39	$\cdot CH \cdot_2$	C-H Combination	12, 13
1.19	$\cdot CH \cdot_2$	C-H 2nd Overtone	5, 10

References:

1. Ellis and Bath (1938b)
2. Glatt and Ellis (1951)
3. Fraser (1956)
4. Larose (1961)
5. Hecht and Wood (1956)
6. Krikorian and Mahpour (1975)
7. Elliott (1952)
8. Fraser (1955)
9. Bath and Ellis (1941)
10. Kaye (1954)
11. Goddu (1960)
12. Wheeler (1959)
13. Goddu and Delker (1960)

Table 3: Contributing groups and assignments of near-infrared bands of starch and pentosan fractions

Wavelength μ	Contributing Group	Assignment	References
2.49, 2.32, 2.29	$\cdot CH\cdot_2$	C-H Combination	1, 2
2.09[a]	$\cdot OH$	O-H Combination	2, 3
1.93[a]	H_2O	O-H Combination	4, 5
1.78[b], 1.72[b], 1.70[b]	$\cdot CH\cdot_2$	C-H 1st Overtone	4, 6
1.54[a], 1.45[a]	$\cdot OH$	O-H 1st Overtone	3, 7, 8
1.37	$\cdot CH\cdot_2$ (?)	C-H Combination (?)	6, 9
1.20	$\cdot CH\cdot_2$	C-H 2nd Overtone	4, 6

[a] Peaks shift to slightly higher wavelengths in undried samples (0.005 - 0.010μ).

[b] Peaks poorly resolved in pentosan fractions.

References:

1. Glatt and Ellis (1951)
2. Miller and Willis (1956)
3. Nikitin (1950)
4. Kaye (1954)
5. Goddu (1960)
6. Goddu and Delker (1960)
7. Korelova (1957)
8. Mitchell et al (1957)
9. Wheeler (1959)

Table 4: Contributing groups and assignments of near-infrared bands of wheat lipid

Wavelength μ	Contributing Group	Assignment	Reference
2.34, 2.31	$\cdot\mathrm{CH}\cdot_2$	C-H Combination	1
2.17, 2.14	$\cdot\mathrm{HC{:}CH}\cdot$	C-H Combination	1
2.07	$\cdot\mathrm{OH}$	O-H Combination	1
1.76, 1.72	$\cdot\mathrm{CH}\cdot_2$	C-H 1st Overtone	1
1.41	$\cdot\mathrm{OH}$	O-H 1st Overtone	1
1.39	$\cdot\mathrm{CH}\cdot_2$ (?)	C-H Combination (?)	2, 3
1.21	$\cdot\mathrm{CH}\cdot_2$	C-H 2nd Overtone	1
1.17	$\cdot\mathrm{HC{:}CH}\cdot$	C-H 2nd Overtone	1

References:

1. Holman and Edmondson (1956)
2. Wheeler (1959)
3. Goddu and Delker (1960)

B. Determination of Moisture Content

The particle sizes of the samples used in the calibration are listed in Table 5. Assuming that grain hardness decreased in the order, durum, HRS, SWW, particle size generally followed hardness. An exception was the SWW sample ground on the Hobart mill where the particle size result appears to be too high. A possible explanation is that the distribution in particle size was wider in this sample than the other two samples ground on the same mill (Fig. 10). Figure 10 shows that while the particle sizes of the Hobart and Wiley mills were similar, there were marked differences in their distribution.

Figure 11 illustrates the relation of the Kubelka-Munk value at 1.93μ to oven moisture for the nine samples treated separately. The parameters and correlation coefficients of the regression lines are listed in Table 6. With the exception of the Wiley SWW sample, there was good agreement with the Kubelka-Munk theory. This is in contrast to the findings of Hoffmann (1963), that showed a curvilinear relation for a single sample of flour used in the study. Hoffmann used a much larger range of moisture contents, 0 - 25%. The variation in the slopes and intercepts indicates that no single regression is applicable to a single mill or wheat type.

The application of multiple regression analyses to the combined data both in 'as is' and 'common log' format resulted in the following equation being the most significant on the basis of the lowest coefficient of variability.

$$\log m = 0.966 + 1.620\ (\pm 0.038) \log x_1 - 1.628\ (\pm 0.040) \log x_2 \qquad (8)$$

where: m = percent moisture

x_1 = $F(R'_\infty)$ at 1.93μ; and

x_2 = $F(R'_\infty)$ at 2.10μ.

The relation of the predicted moisture content to oven moisture content is illustrated in Figure 12. The coefficient of variability of 1.5% is the same as that quoted for the determination of moisture by the Neotec Grain Analyzer when a single wheat type and mill were used (Williams, 1975).

The accuracy of the calibration (Equation 8) is illustrated in Figure 17. The most notable feature is the accuracy below 7.1% and above 14.5%, which were the lowest and highest moisture contents used in the calibration. It is obvious that the 45° line is not the line of best fit for the Australian samples and that there is a bias towards underestimation by the reflectance method. (Mean difference, $\bar{D} = -0.14$). Similarly, there is a tendency for overestimation on the Canadian samples ($\bar{D} = +0.16$). These differences, however, are not of sufficient magnitude to detract from the adaptability of the method.

When the data used for the calibration (Equation 8) was partitioned on the basis of mill type, three regressions were obtained each with a lower coefficient of variation than that of Equation 8; namely, Hobart 0.9%, Wiley 1.0%, and Cyclone 1.0% compared with 1.5% for Equation 8. The equations were as follows:

Hobart: $$\log m = 0.970 + 1.725\ (\pm 0.040) \log x_1 - 1.750\ (\pm 0.044) \log x_2 \tag{9}$$

Wiley: $$\log m = 0.964 + 1.885\ (\pm 0.056) \log x_1 - 1.917\ (\pm 0.056) \log x_2 \tag{10}$$

Cyclone: $$\log m = 0.965 + 1.448\ (\pm 0.033) \log x_1 - 1.451\ (\pm 0.042) \log x_2 \tag{11}$$

where x_1 and x_2 are as previously defined.

In contrast, partitioning on the basis of wheat type, yielded equations which were similar to Equation 8 in terms of regression coefficients and coefficients of variation. The relations corresponding to Equations 9 - 11 are illustrated in Figures 13 - 16.

The lower coefficients of variation as well as the differences in the regression coefficients suggest that Equation 8 may not overcome all of the variation introduced by the use of different mills and that an improved calibration might be obtained if the type of mill was standardized. However the use of the Hobart calibration (Equation 9) to predict the moisture contents of the Canadian samples resulted in lower accuracy when compared with Equation 8; $\bar{D} = +0.40$ vs. $\bar{D} = +0.16$. This aspect requires further investigation.

In all four calibrations the regression coefficients of x_1 are approximately equal to those of x_2. This suggests equations of the form, $\log m = a + b \log (x_1/x_2)$ would be equally applicable to the data. This was shown to be the case. However the equations are not presented as the format applies unnecessary restrictions to the calibration and offers few practical advantages.

The results demonstrate that the Kubelka-Munk theory is applicable to the determination of moisture in wheat by reflectance spectrophotometry, and that the resulting method is independent of wheat type and only slightly influenced by method of sample preparation.

Table 5: Particle size (root mean square particle diameter), microns, of samples used for moisture calibration

	MILL TYPE		
Wheat Type	Hobart	Wiley	Cyclone
Durum	500	485	199
HRS	443	435	184
SWW	481	377	170

Table 6: Parameters of the regression equations $F(R'_{\infty})_{1.93\mu} = a + bm$ for samples used for calibration (n=5)

Sample	Intercept a	Slope b	Correlation Coefficient
Hobart			
Durum	0.20	0.18	0.99
HRS	0.06	0.14	0.99
SWW	0.27	0.06	0.99
Wiley			
Durum	0.22	0.14	1.00
HRS	0.00	0.07	0.98
SWW	0.32	0.02	0.91
Cyclone			
Durum	0.00	0.08	1.00
HRS	0.00	0.06	1.00
SWW	0.08	0.03	1.00

Figure 10

Particle size distributions of the samples used for the calibration of the reflectance method. Areas under the curves were normalized.

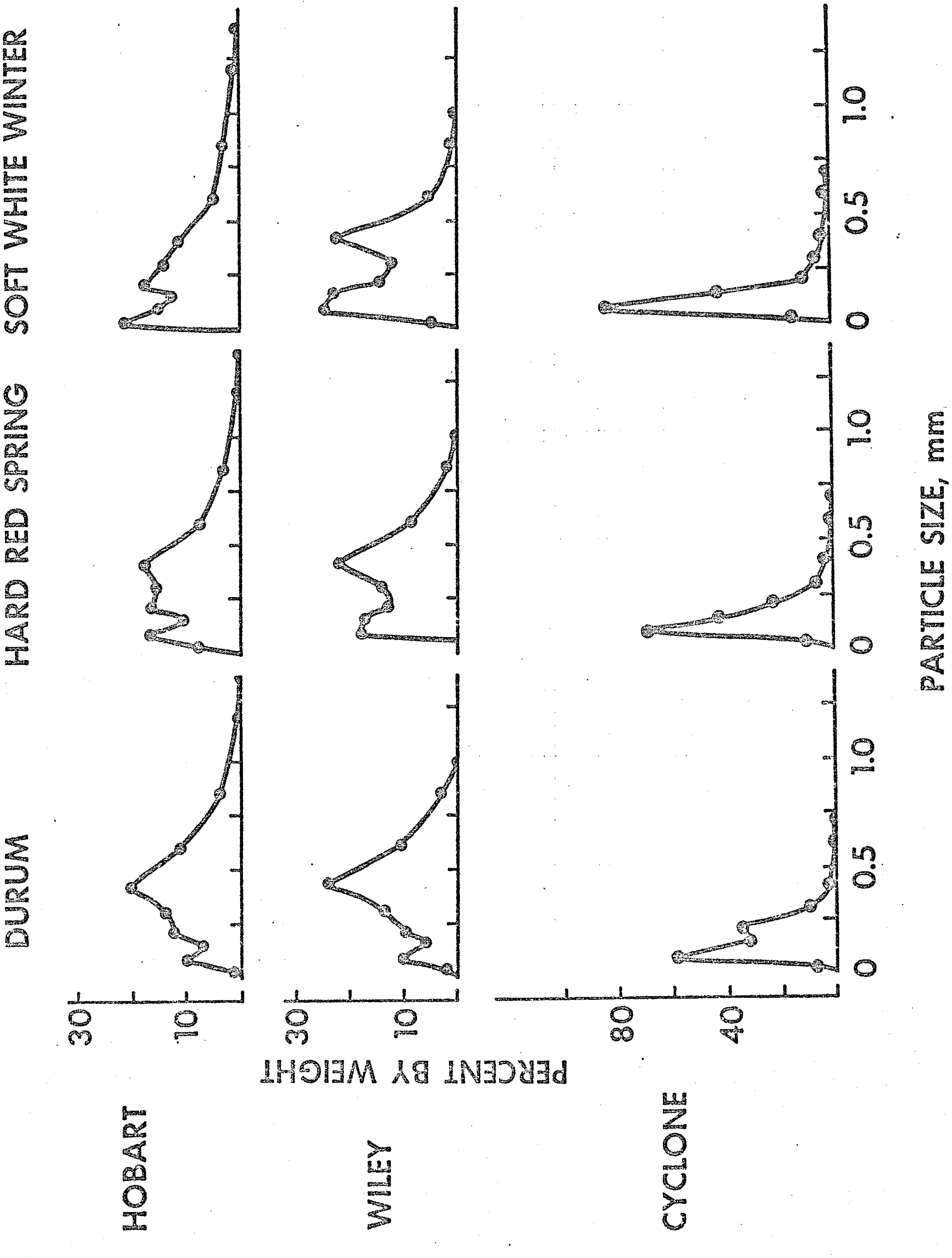

DURUM
HARD RED SPRING
SOFT WHITE WINTER
HOBART
WILEY
CYCLONE
PERCENT BY WEIGHT
30
10
30
10
80
40
0
0.5
1.0
0
0.5
1.0
0
0.5
1.0
PARTICLE SIZE, mm

Figure 11

Relation of $F(R_\infty)$ at 1.93μ to moisture content for samples representing 3 mills x 3 wheat types. D = durum, H = hard red spring, S = soft white winter. ———————— Hobart; — — — — — — Wiley; ------------ Cyclone.

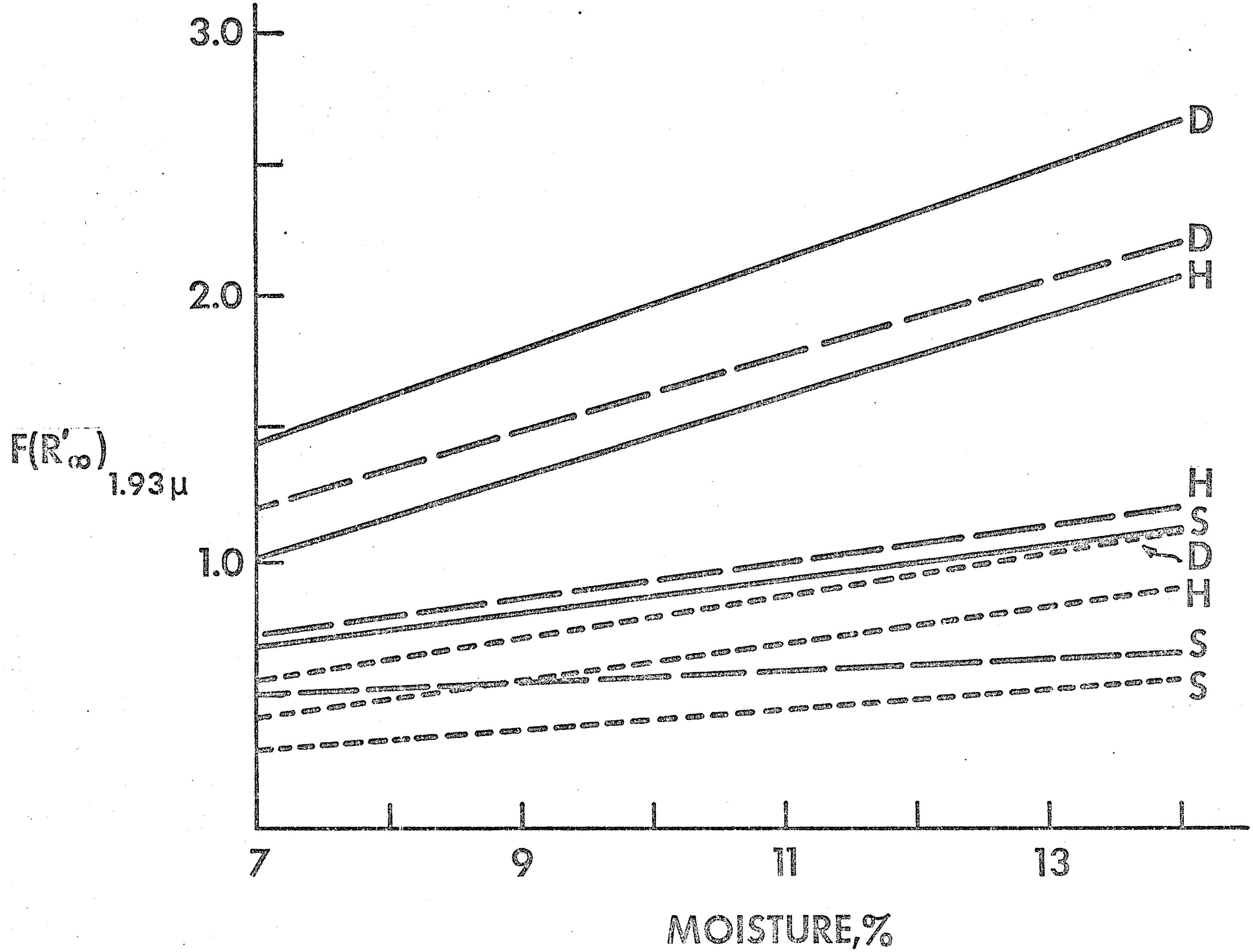

3.0
2.0
1.0
$F(R'_{\infty})_{1.93\mu}$
7
9
11
13
MOISTURE,%
D
D
H
H
S
D
H
S
S

Figure 12

Relation of moisture content determined by relfectance to moisture content determined by the oven method for all samples used to derive the equation. $x_1 = (F'_\infty)$ at 1.93μ ; $x_2 = F(R'_\infty)$ at 2.10μ.

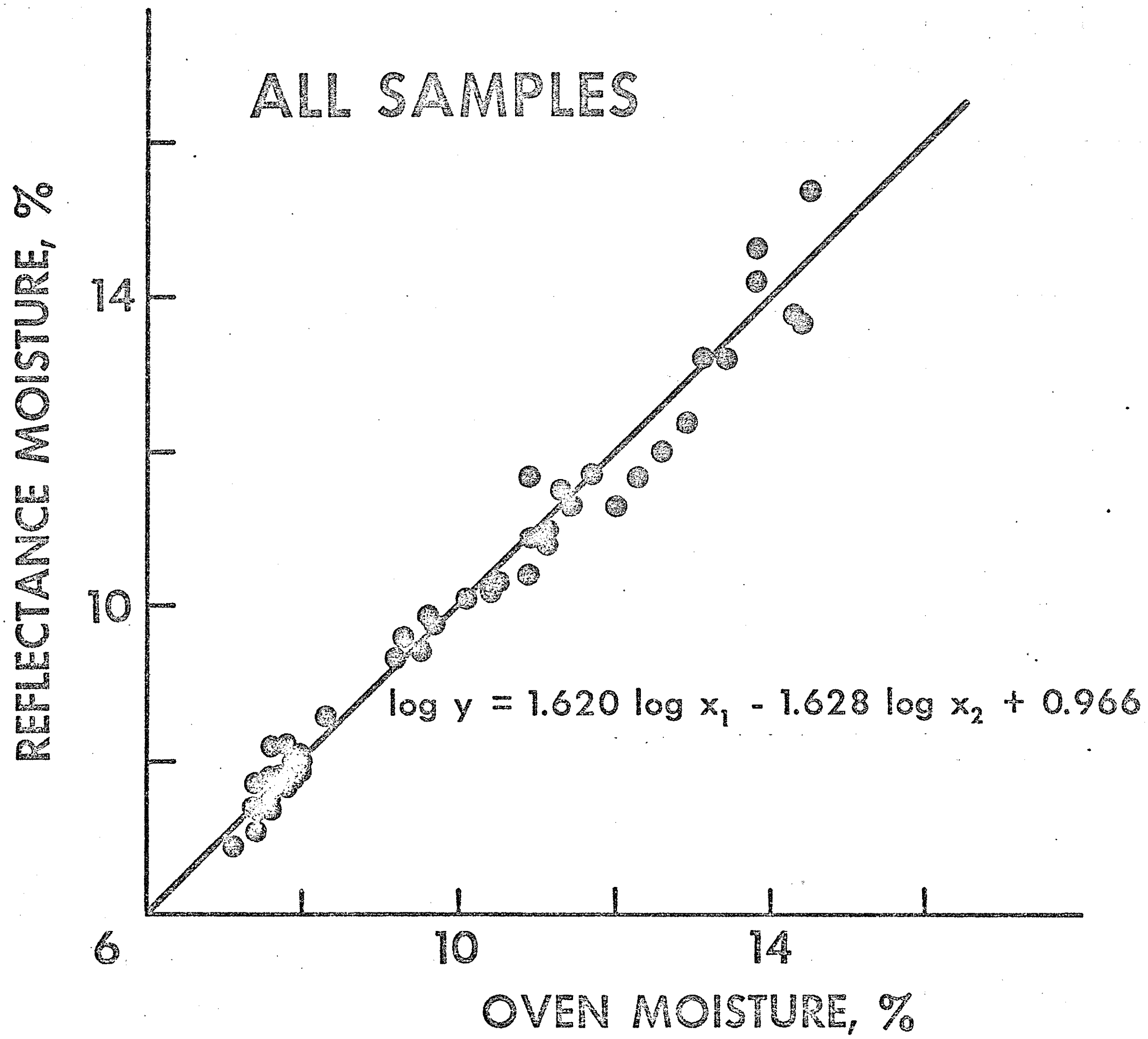

ALL SAMPLES
REFLECTANCE MOISTURE, %
14
10
6
10
14
OVEN MOISTURE, %
log y = 1.620 log x₁ - 1.628 log x₂ + 0.966

Figure 13

Relation of moisture content predicted from $\log m = 1.620x_1 - 1.628x_2 + 0.966$ to moisture content determined by the oven method for Australian, ●, and Canadian, o, samples.

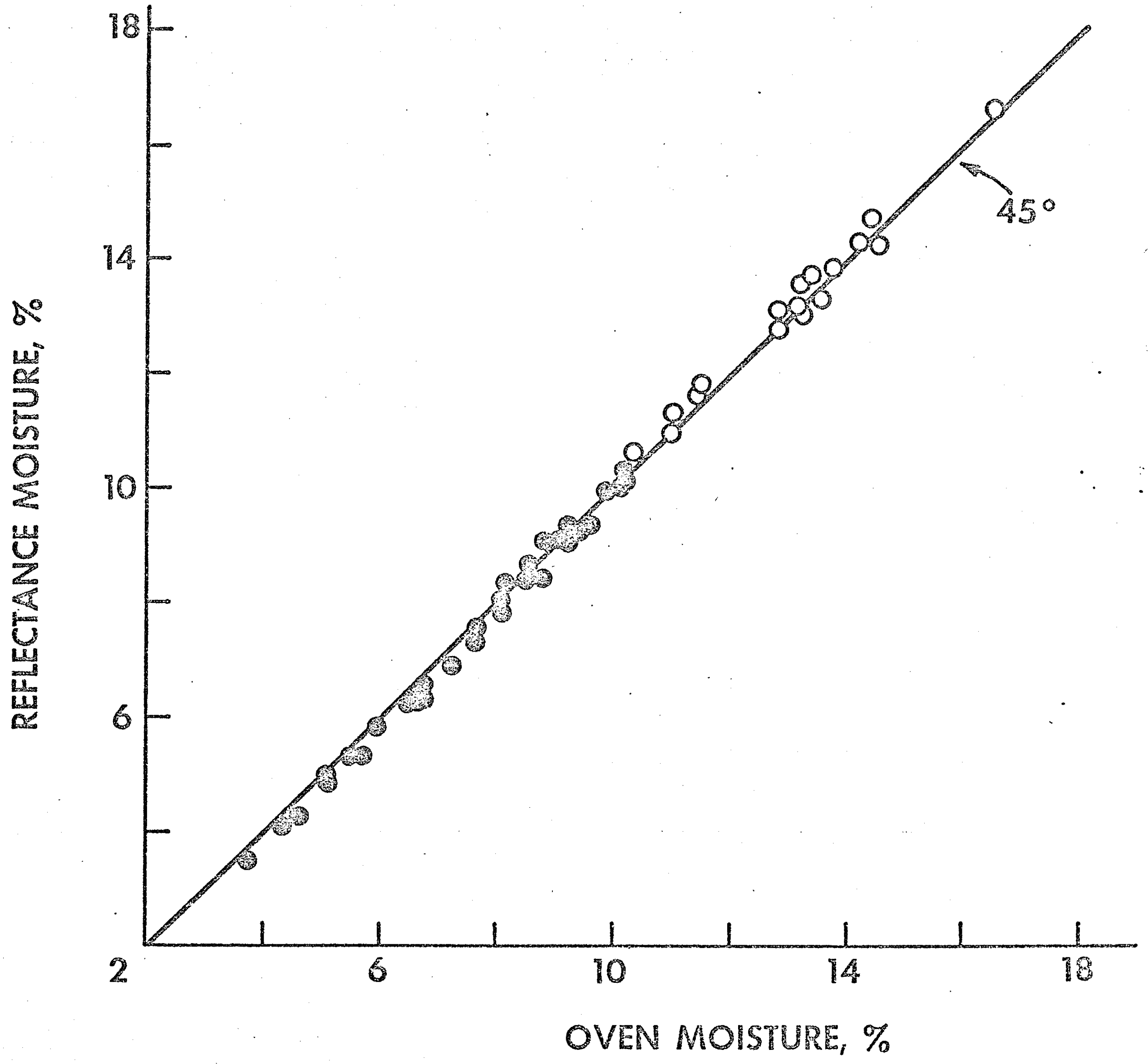

18
14
10
6
2
6
10
14
18
45°
REFLECTANCE MOISTURE, %
OVEN MOISTURE, %

Figure 14

Relation of moisture content determined by reflectance to moisture content determined by the oven method for the Hobart-ground samples used to derive the equation, $x_1 = F(R'_\infty)$ at 1.93μ; $x_2 = F(R'_\infty)$ at 2.10μ.

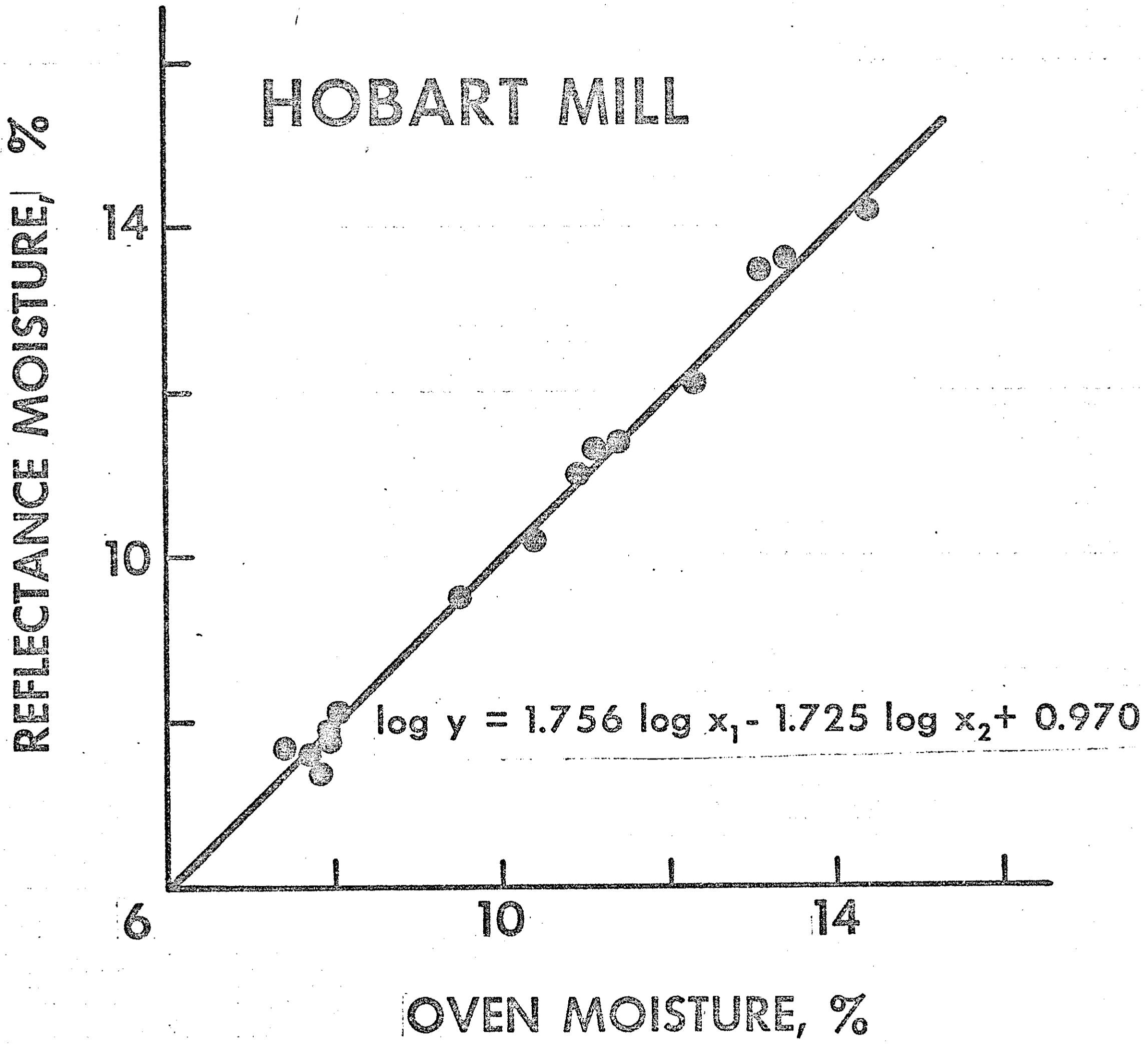

HOBART MILL
log y = 1.756 log x₁ - 1.725 log x₂ + 0.970
REFLECTANCE MOISTURE, %
14
10
6
10
14
OVEN MOISTURE, %

Figure 15

Relation of moisture content determined by reflectance to moisture content determined by the oven method for the Wiley-ground samples used to derive the equation. $x_1 = F(R'_\infty)$ at 1.93μ; $x_2 = F(R'_\infty)$ at 2.10μ.

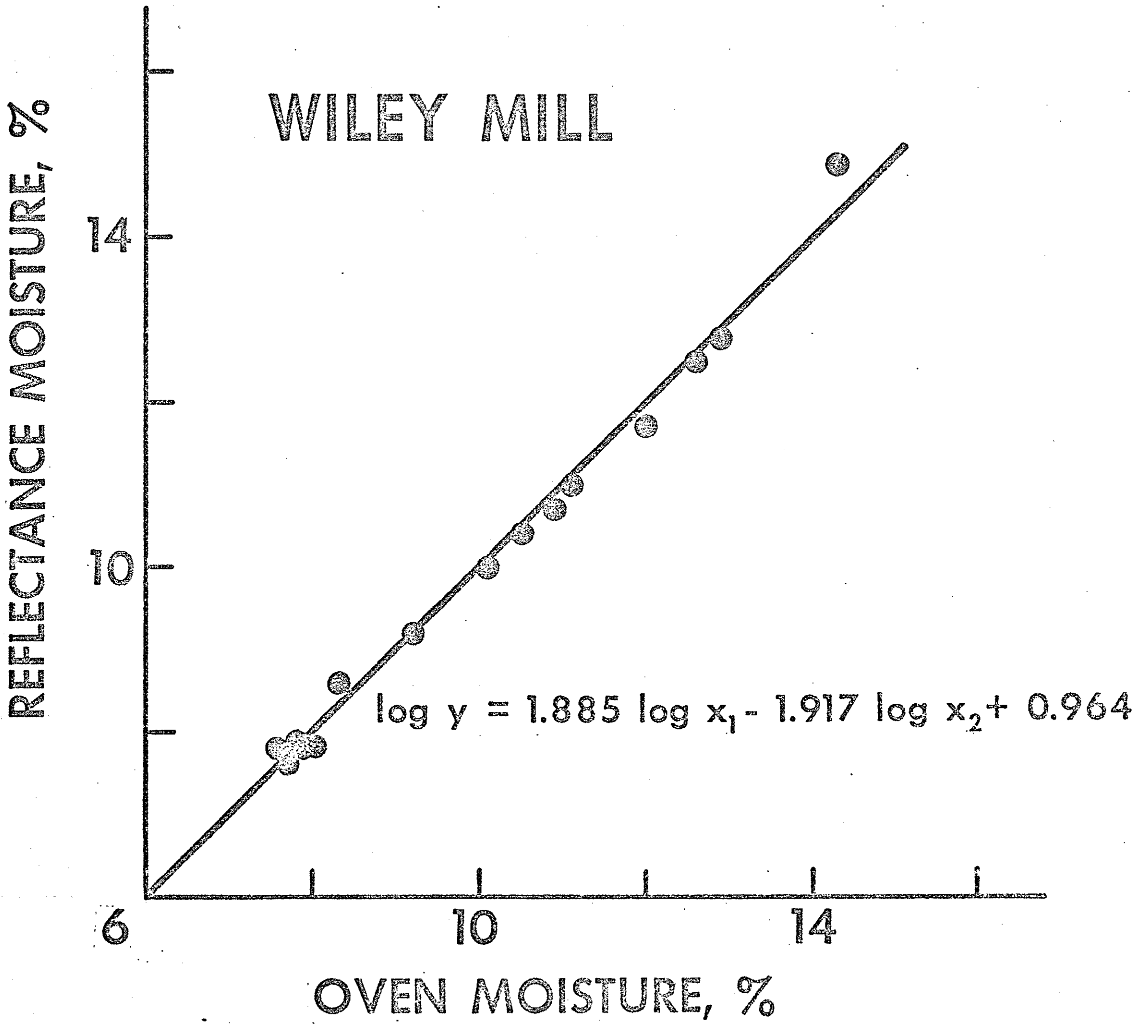
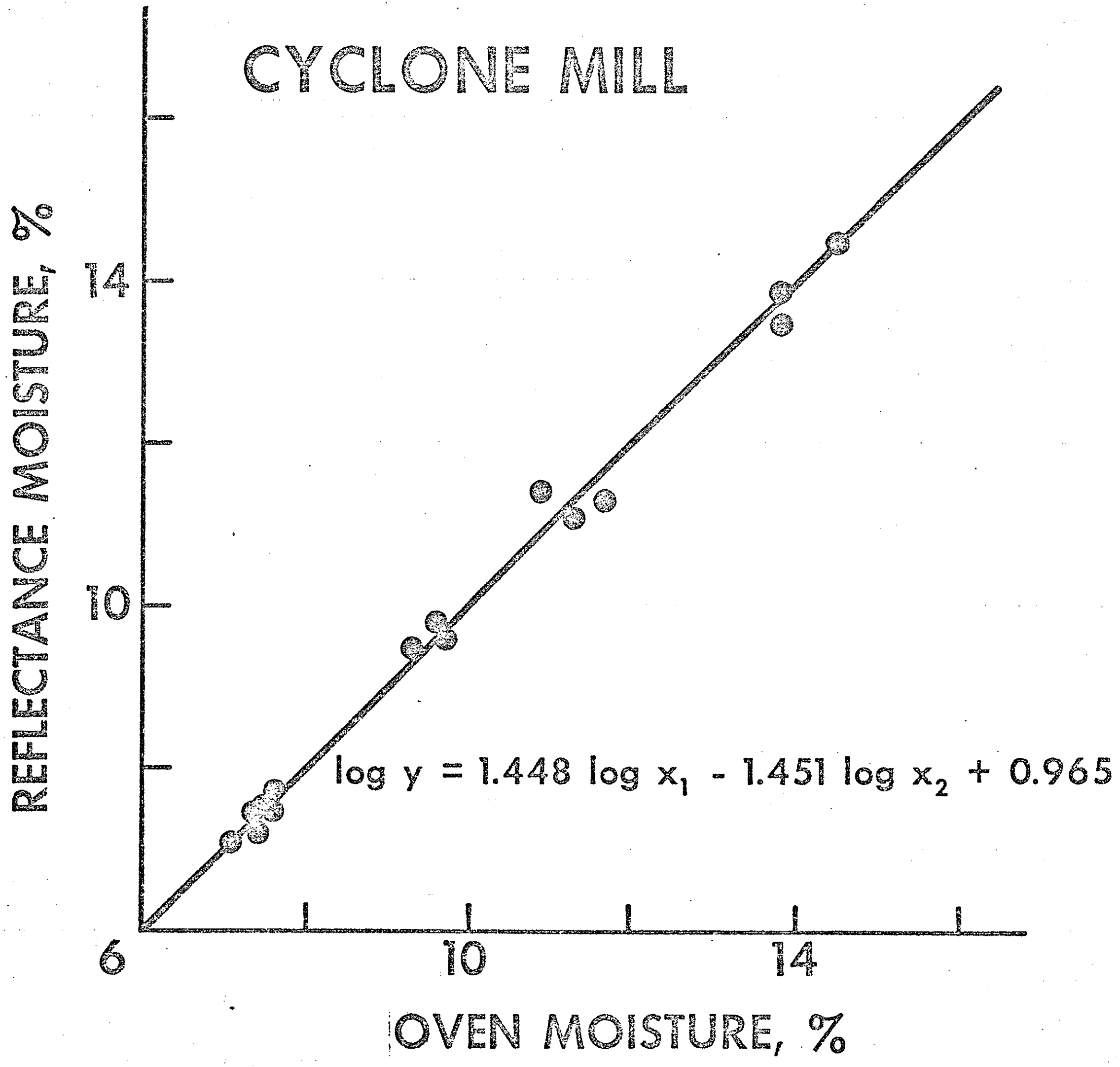

WILEY MILL
REFLECTANCE MOISTURE, %
14
10
6
10
14
OVEN MOISTURE, %
log y = 1.885 log x₁ - 1.917 log x₂ + 0.964

Figure 16

Relation of moisture content determined by reflectance to moisture content determined by the oven method for Cyclone-ground samples used to derive the equation. x_1 = log at 1.93μ; x_2 = $F(R'_\infty)$ at 2.10μ.


CYCLONE MILL
REFLECTANCE MOISTURE, %
14
10
6
10
14
OVEN MOISTURE, %
log y = 1.448 log x₁ - 1.451 log x₂ + 0.965

VI. SUMMARY

The main objective of these thesis was to accumulate some basic information on the near-infrared absorption characteristics of wheat and wheat components. This information was not available and is fundamental to realizing the full potential of near-infrared spectroscopy in the analysis of cereals.

Diffuse reflection spectra between 1.0 and 2.5μ were recorded for wheat, gluten, starch, pentosans, lipid and water using a cary 17I spectrophotometer. The instrument was fitted with an integrating sphere which was coated with smoked magnesium oxide. Smoked magnesium oxide was also used as a reference standard. The absorption bands were assigned to various overtone and combination vibrations of C-H, N-H, O-H and C=O groups.

The spectrum for wheat was largely determined by the carbohydrate components. The type of wheat had no effect on the position of peaks. Major peaks in the spectrum of gluten occurred at 1.19, 1.50, 1.73, 1.98, 2.05, 2.18, 2.29 and 2.47μ. The spectrum for gluten was similar to transmittance spectra published for other proteins except for the intense absorption at 1.98μ due to the high concentration of primary amide groups in gluten.

The spectra for starch and pentosans were similiar with major peaks occurring at approximately 1.20, 1.45, 1.54, 1.93, 2.09, 2.32 and 2.49μ. The position of the hydroxyl peaks was influenced by moisture content. This change in position is probably due to differences in degree of hydrogen bonding. Differences in the relative intensities of peaks warrant further investigation as a basis for a method for the determination of starch and pentosans in wheat.

The spectrum for lipid was characterized by intense absorption due to $\cdot CH \cdot_2$ groups. Bands at 1.17, 2.14 and 2.17μ were due to the C-H vibrations associated with cis double bonds while absorption at 1.41 and 2.07μ was due to ·OH groups. The bands at 1.445 and 1.928μ in liquid water were found at slightly higher wavelengths in the undried wheat, starch and pentosan samples. This was probably due to hydrogen bonding.

These results provide the basis for investigations of quantitative methods which may be useful in cereal chemistry. Complete exploitation of the near-infrared region in the analysis of wheat will depend on further studies on the relative intensities and relations of the various absorption bands as well as the development of suitable instrumentation and techniques for rapid analyses.

Variations in the particle size of samples have been shown to have a marked influence on the accuracy of near-infrared reflectance grain analyzers. Accurate results are achieved by limiting the samples used to calibrate the instruments to a single type or variety, standardizing the method of grinding and limiting subsequent analyses to samples of the same type or variety which have been prepared on the same mill as those used for the original calibration. These requirements place major limitations on the use of the method.

This problem was investigated in relation to the determination of moisture in wheat. Examination of the literature indicated that the mathematical format of the calibration equation used in the reflectance grain analyzers was not based on any of the accepted reflectance theories.

A durum, a hard red spring and a soft white winter wheat were ground on each of three mills; a Hobart coffee grinder, a Wiley Model I, fitted with a 1.00-mm sieve and a Model CSM-2 cyclone grinder fitted with a 1.00 mm screen. The nine samples obtained (3 wheats x 3 mills) ranged in mean particle size from 170 to 500μ. Five subsamples withdrawn from each sample were vacuum-dried or humidified to yield a range of moisture contents. The range was 7.1 to 14.5%. Kubelka-Munk values ($F(R'_\infty) = (1-R)^2/2R$) were recorded at twenty wavelengths bewteen 1.12 and 2.49μ. These wavelengths corresponded to the peaks in the spectra of wheat, starch and gluten and valleys in the absorption spectrum of wheat of approximately 10% moisture.

After logarithmic transformation of the data, the application of multiple regression analysis 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 using the equation to predict the moisture contents of 49 wheats; 32 of which were of different type and had been ground on a different mill from those used in the calibration.

The method eliminates the susceptibility to particle size variation of the 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 samples of known composition.

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VIII. APPENDIX

DIFFUSE REFLECTANCE STANDARDS FOR THE NEAR-INFRARED

INTRODUCTION

On commencing investigations into the near-infrared reflectance characteristics of cereals using a double beam spectrophotometer it was necessary to select a material of high diffuse reflectance for use as a reflectance standard.

Examination of the literature showed that the compounds most intensively studied as reflectance standards for the near-infrared were those suitable for the visible region, namely, magnesium oxide, smoked and as a pressed powder, and pressings of barium sulfate (Middleton and Sanders, 1953; Sanders and Middleton, 1953; Gier, Dunkle and Bevans, 1954; Jacquez, McKeehan, Huss, Dimitroff and Kuppenheim, 1955; Edwards, Gier, Nelson and Roddick, 1961; Grum and Luckey, 1968; Morren, Vandermeesch and Antoine, 1972). However, the results indicated that both compounds were unsuitable due to the presence of water peaks, instability and low reflectance. Other materials which had been suggested as suitable for reflectance standards or as coatings for integrating spheres for the near-infrared region were alumina (Sklensky, Anderson and Wickersheim, 1967), polytetrafluoroethylene powder (Hoffmann, 1963), and flowers of sulfur (Kronstein, Kraushaar and Deacle, 1963; Blevin and Brown 1965; Egan and Hilgeman, 1975).

This paper reports on the relative diffuse reflectance of the above compounds and recommends the use of pressings of a mixture of sulfur and polytetrafluoroethylene (Sulflon) as working reflectance standards for the 0.8 - 2.5μ region.

MATERIALS AND METHODS

Reflectance Measurements

Reflectances were recorded from 0.8 to 2.5μ using a Cary 17I spectrophotometer fitted with a lead sulfide detector and a Model 1711 diffuse reflectance accessory containing an integrating sphere. Smoked magnesium oxide was used as a sphere coating and as a reference standard (Wendlandt and Hecht, 1966). The optical arrangement of the sphere was $R_{o,d}$.

The materials studied, their source and description are listed in Table A. The smoked MgO was applied to the surface of an Infrasil cover glass (5 cm diameter). The coated glass was then backed with a polished aluminum block (5 cm sq.) containing a 3.8 cm x 2 mm well which had previously been smoked with MgO.

MgO powder, $BaSO_4$, Al_2O_3 and sulfur samples were contained within acrylic "white reflectance holders"[1]. $BaSO_4$ was packed according to the instructions supplied by the manufacturer, 2g/cm^3, the remainder were hand compacted to achieve a smooth or uniform surface. All pressings were made using a Carver laboratory press and a 5.6 cm die. Sulfur and TFE were mixed by grinding together in a Krups grinder for one minute. All samples were used "as received" basis, except $BaSO_4$ which was dried overnight at 200°C. Infrasil cover glass was used with all samples.

RESULTS AND DISCUSSION

The results of the reflectance measurements are listed in Table B. The values for smoked MgO are those published by Morren et al (1972). All other results are relative to these. The values for smoked MgO are higher up to 1.2μ than those published by

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[1] supplied by Eastman Kodak Company, New York.

Sanders and Middleton (1953) however they are similar over this range to those published by other workers (Edwards et al, 1961; Grum and Luckey, 1968). At longer wavelengths, the values are lower than those of Sanders and Middleton (1953) and Edwards et al (1961) but higher than those of Gier et al (1954) and Grum and Luckey (1968). As several of the results exceed 100%, the values used for smoked MgO must be higher than absolute reflectance values. This is particularly true for the values below 1.4μ.

The reflectance of MgO powder was lower than for smoked MgO over the entire range of wavelengths. The results for $BaSO_4$ are lower up to 1.80μ, than those presented in previous publications (Grum and Luckey, 1968; Morren et al, 1972) and intermediate over the remainder of the range. In contrast to previous findings (Morren et al, 1972) a drying treatment increased the reflectance of $BaSO_4$ by 1.0-3.5%; the largest increase being at the longest wavelength.

Of the TFE powders studied, B and H exhibited the highest reflectances. The results support the conclusion that TFE-H has an aboluste reflectance of greater than 95% over the range 1.0 - 2.0μ (Hoffmann, 1963). The reflectance over the visible range is reported to be 1.0 to 1.5% lower than the common $BaSO_4$ powder (Hoffmann, private communication). Above 2.0μ the reflectance of all TFE powders decreased sharply due to an absorption band at 2.14μ. This was probably due to the presence of C-H groups, indicating incomplete fluoridation.

The pressure used for making the pressing had a marked influence on the reflectance values for all TFE powders. Typical results are illustrated in Table B. These results were consistent with those of Schatz (1966) which showed a decrease in reflectance with increase in pressure for MgO, $BaSO_4$: Al_2O_3 and other oxides. There was a noticeable increase in specular reflection of the TFE pressings with increase in pressure.

The reflectance of Al_2O_3 relative to smoked MgO was similar to that published by Schatz (1966) with Al_2O_3 having higher reflectances over the entire range.

Of all materials studied, sulfur showed the best reflectance characteristics. It was superior to smoked MgO and $BaSO_4$ at all wavelengths. At shorter wavelengths, sulfur was equal to TFE-B, TFE-H and Al_2O_3; at longer wavelengths it was markedly superior to all compounds. The results are in contrast to those of Kronstein et al (1963) and Blevin and Brown (1965) which showed the reflectance of sulfur flowers was lower than smoked MgO for wavelengths less than 1.9μ. Compaction using pressures up to 82,700 k Pa had no effect on the reflectance values.

As pressings of sulfur were friable, they were unsuitable as reference standards. Mixtures of TFE and sulfur were tested. It was found a mixture of 2.5 parts sulfur and one part TFE yielded a pressing with a homogeneous, smooth surface and which was easy to handle. In addition the excellent reflectance properties of sulfur were retained except for a 0.5 to 1.0% loss in the 1.9 - 2.5μ region (Table B). The type of TFE did not appear to influence the reflectance or physical properties. Alteration of sphere geometry to include the specular component had no effect on the reflectance values and the reflectance of the pressings was unchanged after three months storage.

Erb (1975) recently listed the ideal properties of relfection standards as transportable, stable, homogeneous smooth surface, diffuse reflecting, spectrally non-selective, non-transparent, nonfluorescent and easy to handle. While Sulflon pressings may not be ideal in terms of spectral non-selectivity, they are superior or equal to the other near-infrared reflectance standards in all properties listed by Erb (1975) and as such will be extremely valuable to workers in the field of near-infrared reflectance spectroscopy.

ACKNOWLEDGEMENTS

Samples of TFE-A, B, C and 8 were donated by E. J. du Pont de Nemours, New York. Dr. K. Hoffmann, Hoechst, Germany donated the TFE-A.

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TABLE A. Source and description of materials used in reflectance study

Compound	Source	Description
Smoked magnesium oxide	Mg ribbon; Matheson, Coleman and Bell, Ohio.	See Text
Powder magnesium oxide	J.T. Baker, New York	"Ultrex", No. 4903
Barium sulfate ($BaSO_4$)	Eastman-Kodak, New York	White reflectance standard
Alumina (Al_2O_3)	J.T. Baker, New York	"Ultrex", No. 4901
Polytetrafluoroethylene TFE		
A	E.I. du Pont de Nemours, New York	Round particles, 35μ diameter
B	" " " " " " "	Round particles, 25μ diameter
C	" " " " " " "	Stringy particles, size approx. 30μ
8	" " " " " " "	Round particles, 600μ diameter
H	Hoechst, Germany	Properties unknown
Sulfur	Fisher Scientific, New York	Sublimed, lab. grade

TABLE B. Reflectance values for materials based on data for smoked MgO published by Morren et al (1972)

Wavelength	MgO		$BaSO_4$	TFE Pellet[a]					Al_2O_3	Sulfur	Sulflon Pellet[b]
μ	Smoked	Powder		A	B	C	8	H			
0.8	98	95	97.5	98	102	96.5	98	101	101.5	101.5	101.5
0.9	97.5	96	98.5	99.5	101	96.5	98	102	102	101	101
1.0	97	94.5	97	98.5	100	95	97	100	101.5	100.5	100.5
1.1	97	91.94	97	98.5	100	95.5	96	100	101	100.5	100.5
1.2	96	93	95.5	97.5	98.5	94.5	95	99.5	100.5	100	100
1.3	96	93	95.5	97.5	97.5	94.5	95	100	100.5	100.5	100.5
1.4	94.5	91	92	96	98	93	94	98.5	98	99.5	99.5
1.5	95.5	92	92	96.5	98	93.5	94.5	99	99	100.5	100.5
1.6	95	91.5	92.5	95.5	98	93	94	98.5	99	100	100
1.7	94	90	91	95	97	92	92.5	97.5	98	99.5	99.5
1.8	94	90	90	95.5	97	92.5	92.5	97.5	98	100	99.5
1.9	93	89	84.5	95	97	93	93	98	96.5	100.5	100
2.0	92.5	88	85	93.5	95	91	91.5	96.5	97	100	99.5
2.1	92.5	88	86.5	91	92	88.5	88.5	93	97	100	99
2.2	92	87.5	86	93	94	90	90	96	97.5	100.5	100
2.3	91	86	84.5	91	92	88.5	89	93	97	99.5	98.5
2.4	90	85	81.5	87.5	89	85.5	85.5	91	96.5	99.5	98.5
2.5	87	81.5	77.5	86.5	88	85	85	90	94.5	98	97

[a] 6890 k Pa

[b] 34470 k Pa

TABLE C. Effect of pressure on the reflectance of TFE-B powder

Wavelength μ	Pressure, k Pa 6,890	34,470	82,734
0.8	102	98.5	92
1.0	100	97.5	89.5
1.2	98.5	96.5	86
1.4	98	95.5	82.5
1.6	98	95	80
1.8	97	94.5	76.5
2.0	95	92	72
2.2	94	91	69
2.4	89	85	61