

# DETERMINATION OF THE MOISTURE CONTENT OF SEEDS BY NEAR-INFRARED SPECTROPHOTOMETRY OF THEIR METHANOL EXTRACTS<sup>1</sup>

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## ABSTRACT

A rapid, specific, differential spectrophotometric method for determining the water present in methanol extracts of seeds is described. Extracts are prepared by the simultaneous grinding and extracting of the seeds in an enclosed cup by the method of Hart and Neustadt. The absorbance band of water at 1.93  $\mu$  is used. Extracts contain no compounds whose absorbances interfere. Small variations in temperature have no appreciable effect on results. It is necessary that extracts be completely free of suspended material. The standard deviation of the results from those obtained by titration with Karl Fischer reagent is  $\pm 0.24\%$ . The method is more rapid than the Karl Fischer method. No expensive reagent is needed, and the technique required is less exacting.

One of the basic and most accurate methods for determining moisture in seeds (2,3) involves a grinding-extraction procedure which takes place in a completely enclosed cup. Methanol present in the cup extracts the water from the grain as it is being ground. After suspended matter has settled from the extract an aliquot is titrated with Karl Fischer reagent. The method presented here substitutes for the titration a near-infrared spectrophotometric method for determining the water in the extract.

Kaye (5) has suggested that water could be determined by near-infrared spectrophotometry. In recent years this principle has been used in several methods (1,4,7) which have been developed for determining water in various liquids. Keyworth (6) has been able to determine the water content of methanol and other alcohols. He used a differential method, the methanol solvent being the reference. In the method discussed here a similar approach has been used to determine the water content of seeds. The absorption band of water at 1.93  $\mu$  is measured in methanol extracts of seed. The absorption in this region is more intense than that in the more commonly used region around 1.4  $\mu$ . The method is specific for water. It is rapid and easy to perform.

## Materials and Methods

Moisture determinations were made on various types of seeds. They included grains (wheat, corn, oats, barley, grain sorghum, and rice),

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oil-bearing seeds (flaxseed, soybeans, and peanuts), edible beans and a few grasses (alfalfa, fescue, clover, and trefoil) and vegetable seeds (cucumber, radish, onion, beet, and lettuce). Determinations were made of 65 samples ranging in moisture content from 3.44 to 18.54%. Methanol extracts were prepared from each sample by the grinding-extraction procedure (3). An aliquot of each extract was titrated with Karl Fischer reagent and another portion was used for the spectrophotometric determination. It was necessary to centrifuge these extracts or to allow them to stand in tall cylinders until all suspended particles had settled to the bottom. A completely clear liquid could then be pipetted from a cylinder.

A Perkin-Elmer Model 4000A spectrophotometer was used. The quartz absorption cells gave a light path length of 1 cm. The cells were kept stoppered during determinations to prevent absorption of water and evaporation of solvent. The temperature was that of the laboratory,  $78^{\circ} \pm 2^{\circ}\text{F}$ .

## Results

Although methanol absorbs light strongly in the near-infrared, there is a window in the spectrum between 1.7 and 2.0  $\mu$  which permits measurement of the water band at 1.93  $\mu$ . The sample and reference beams of the spectrophotometer were balanced, using methanol from the same lot that had been used in preparing the extracts. The base line corresponding to zero absorbance was traced from 2.0 to 1.8  $\mu$ . The methanol in the sample beam was then replaced by an extract and a tracing of the absorbance made over the 2.0- to 1.8-  $\mu$  region. Since the instrument was stable, it was not necessary to repeat the zero absorbance line between traces. The base line absorbance at 1.8  $\mu$ , where water has no absorption, was subtracted from the peak absorption at 1.93  $\mu$  to obtain the absorbance of the extract. A number of absorbance traces of grain extracts are shown in Fig. 1.

The water content was found by reference to a calibration curve prepared by applying the same procedure to samples of methanol containing known weights of water. The relationship between weights of added water and their corresponding absorbances was found to be linear. The equation for the curve was derived by the method of least squares. Since, in the extraction procedure, the water in the seeds is extracted into 200 ml. of methanol, the water contents of the samples of methanol were expressed in terms of the weights of water added to 200 ml. of methanol. Thus, it was possible to read directly from the curve the percent of water present in the seeds. The weights of water added to 200 ml. of methanol ranged from 0.35 to 2.5 g. The densities

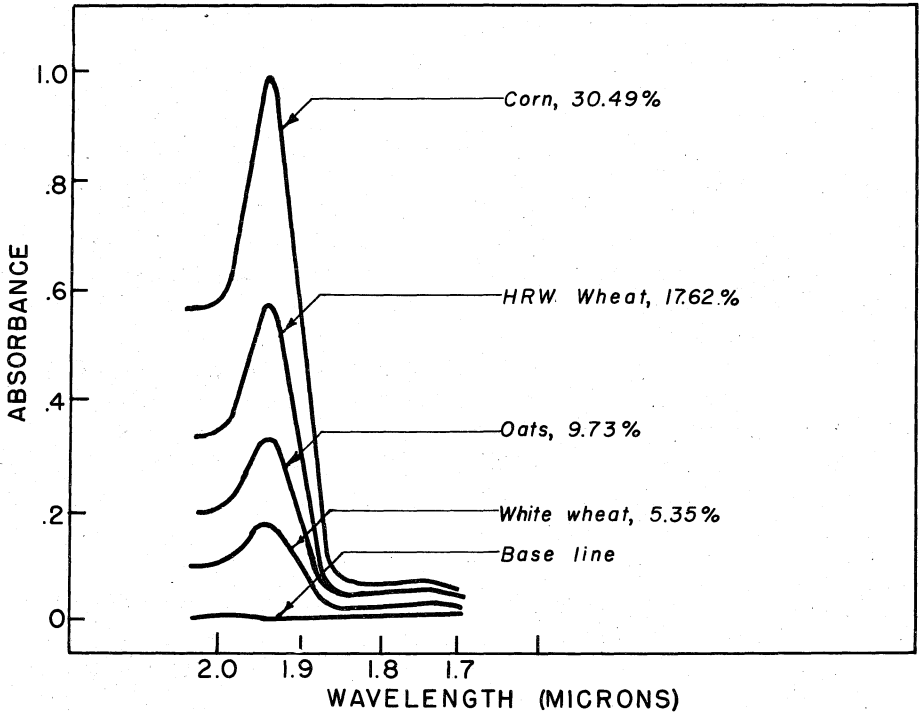


Fig. 1. Spectrograms of methanol extracts of grain with specified moisture content.

of the resulting solutions may be considered as constant and the water concentrations as proportional to the weights of water added. The data are consistent with Beer's law.

Kaye's work (5) indicates that there are a few chemical groups which cause absorption peaks in the region from about 1.96 to 2.00  $\mu$ . Primary amino, amide, and phenolic hydroxyl groups are among them. Since some of these groups might be present in seed extracts, experiments were carried out to determine whether they were interfering with the determinations for water. One hundred milliliters of extract from each of four different seeds were evaporated to dryness on a steam bath in a stream of nitrogen gas. The residues were held in a desiccator over phosphorus pentoxide for 3 days and redissolved in 100 ml. of methanol. Traces of their absorbances showed no deviation from the zero base line between 2.0 and 1.8  $\mu$ . Since the moisture contents of all of the samples, as determined by the spectrophotometric method, were in as good agreement with the corresponding

results obtained by the Karl Fischer method as these four samples, it was concluded that there was no interference from these groups.

Since it was not feasible to control the temperatures of the cells while in the spectrophotometer, some indication of the effect of temperature was desired. An approximate indication of this effect was obtained by cooling both a reference and a sample extract to 20° F. below room temperature and making rapid determinations. The time elapsing between recording of temperature and finish of determination was less than 2 minutes. Comparisons were made between these results and those obtained at room temperature. Determinations were also made when the reference was 20° F. cooler than the extract, and vice versa. The results are given in Table I.

TABLE I  
EFFECT OF TEMPERATURE ON THE ABSORBANCE OF SEED EXTRACTS

SAMPLE NO. AND KIND	TEMPERATURE		ABSORBANCE	CHANGE IN ABSORBANCE PER DEGREE
	Reference	Extract		
	°F	°F		
19—Corn	76	76	0.586	
19—Corn	55	55	0.580	0.00029
20—Soybeans	77	77	0.454	
20—Soybeans	55	55	0.446	0.00036
19—Corn	55	79	0.558	
19—Corn	79	55	0.626	
20—Soybeans	55	81	0.416	
20—Soybeans	81	55	0.480	

Data indicate that, as long as reference and sample extract are at the same temperature, a change in temperature of 10° F. produces a change in the moisture content determination of 0.1% or less. However, if reference and sample extract are not at the same temperature, serious error may result.

The effect of suspended particles on absorbance readings was also studied. It was found that suspended matter in extracts of soybeans and corn was especially slow in settling. Portions of extracts of corn and soybeans were clarified by centrifuging. Other portions of the same extracts were allowed to stand in hydrometer jars for 5 hours. At the end of this time a barely perceptible cloudiness still remained in the extracts. Some of this partially clarified liquid was pipetted out of each jar. Absorbances of the clarified extracts were compared with those of the partially clarified. Results are given in Table II. The differences in absorbance due to scattering correspond to differences in moisture content ranging from 0.07 to 0.73%.

TABLE II  
EFFECT OF SUSPENDED PARTICLES ON ABSORBANCE READINGS

SAMPLE	EXTRACT COMPLETELY CLARIFIED		EXTRACT PARTIALLY CLARIFIED	
	Absorbance	Moisture Content Indicated	Absorbance	Moisture Content Indicated
		%		%
Soybeans	0.240	8.09	0.256	8.62
Soybeans	0.434	14.45	0.436	14.52
Soybeans	0.238	8.02	0.262	8.75
Corn	0.438	14.55	0.460	15.28

Nephelometric determinations made on portions of the same extract which had been allowed to stand for different lengths of time indicated that 2 days' time was sufficient to clarify extracts even of soybeans and corn. For other seeds 4 or 5 hours' time is sufficient.

### Conclusions

Moisture determinations were made by the Karl Fischer method and by the near-infrared absorption method on all of the 65 samples. Considering the Karl Fischer determinations as standard and expressing near-infrared determinations as a function of that standard re-sults in the equation

$$Y = 1.0080X - 0.0561(1)$$

as the best-fitting linear function by the method of least squares. Figure 2 shows a plot of these data with the graph of equation 1. The standard deviation from regression of the spectrophotometric determinations is  $\pm 0.24\%$ . The standard deviation obtained in preparing the calibration curve was  $\pm 0.05\%$ . In making determinations by the Karl Fischer method, results are considered satisfactory if duplicate determinations differ by no more than 0.2%.

The data presented above show that near-infrared spectrophotometry may be used to measure accurately the moisture in seeds. The method is more rapid in terms of time actually spent in a determination than the Karl Fischer method. No expensive reagent is needed and the technique required is less exacting. The Perkin-Elmer Model 4000A spectrophotometer was used because it was readily available. There is no reason why a simpler and less expensive instrument, which is capable of measuring absorbance at the two specified wave lengths, could not be used satisfactorily.

The method should be applicable to the determination of water in softer and more easily extractable materials, such as dried fruits and vegetables, where a less rugged grinder could be used in the extraction.

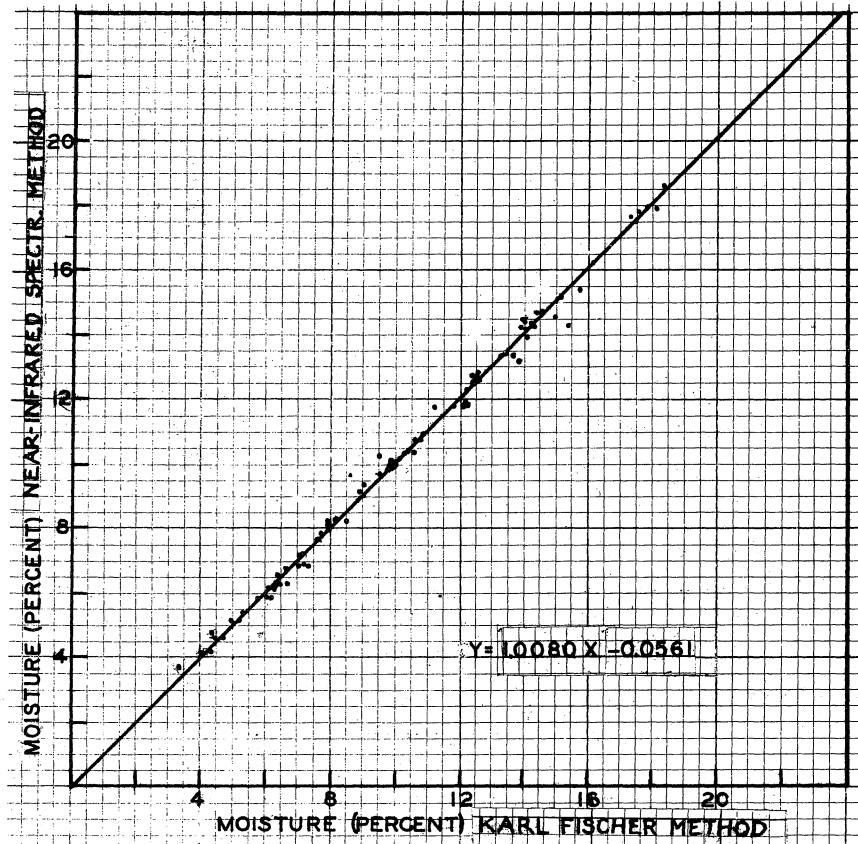


Fig. 2. Moisture content of seeds determined by the Karl Fischer method and by near-infrared spectrophotometry.

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