A Wide Line NMR R-F Saturation Method to Measure Fat in Moist Samples of Defatted Corn Germ[1]

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ABSTRACT

A wide-line NMR radio-frequency (r-f) saturation technique has been applied to measurement of residual fat (1-6%) in defatted corn germ process samples containing significant amounts (4-11%) of moisture. Since NMR relaxation times for protons associated with the fat differ from those associated with the water, it is possible to use these differences as a basis for discrimination. In process samples where these differences are sometimes marginal, they can often be enhanced by milling the moisture-containing sample at high speed in carbon tetrachloride to alter the relaxation times of protons associated with the fat. The fat content is derived from NMR signals obtained at two r-f levels. At the lower r-f level, energy absorbed is proportional to the number of protons associated with both water and fat. At the higher r-f level, signals from fat are saturated preferentially. This reduction in signal intensity, due to r-f saturation, is proportional to the amount of fat in the sample and can be used as a quantitative measurement. Examples of this methodology for laboratory-prepared and process samples are given. Experimental data for approximately 40 random process samples indicate that the method is accurate to within ± 0.3% fat, absolute, 95% confidence limits.

INTRODUCTION

Within the last seven years, wide-line NMR spectroscopy has been employed successfully to measure the fat content of a variety of oil-bearing seeds and process samples dried to less than 5% moisture content (1-4). These studies indicate that NMR techniques are accurate, rapid and nondestructive. However, from the standpoint of plant process control, it would be advantageous to use NMR spectroscopy to measure the amount of water or oil, or perhaps both, in samples with a minimum of sample preparation. The work described in this paper was directed towards this goal.

In samples which contain two fluid phases (oil and water), several NMR techniques have been devised to determine the amount of one or both hydrogen-containing liquids present. A simple method which takes advantage of differences in the freezing points of the two fluids, has been used by geologists to measure oil and water in geological cores (5). A further example of multiphase analysis is the use of paramagnetic salts to broaden the spectral line of a liquid (6). For example, an analysis of water content is possible by comparing signals from a sample before and after addition of the paramagnetic salt. Recently Zupančič and co-workers (7) introduced a wide-line NMR fast passage technique to measure the oil contents of various seeds without additional sample drying to suppress the NMR water signal. The selectivity of the method is based on the different degrees of mobility of the various hydrogen-containing constituents of plant seeds and the resulting differences in their spin-spin (T_2) and spin-lattice (T_1) NMR relaxation times. In other methods Shanbhag et al. (8) utilized the addition of magnesium perchlorate to convert moisture to water of crystallization which does not give an NMR signal, and Persyn and Rollwitz (9) described quantitative measurements of fat and water via relaxation data derived from pulsed NMR experiments.

The approach described in this paper is a wide-line NMR radio-frequency (r-f) saturation technique devised for the separation of fat and moisture in defatted corn germ process samples. Although intended primarily to measure fat in these samples, it is equally capable of measuring either or both.

Principle

Wide-line NMR spectral characteristics depend on the molecular motion within the sample. For example, fat or oil in corn samples, dried to below 5% moisture, is relatively mobile and behaves like a liquid in the sense that it exhibits a narrow intense signal. In the nonfat matrix the molecular motion of hydrogen nuclei associated with ice-like water, carbohydrates or protein is restricted or hindered and any given nucleus may be in an effective magnetic environment substantially lower or higher than the applied field. Consequently, absorption signals from matrix hydrogen are weak and broad. By measuring the derivative of the absorption signal, the mobile hydrogen

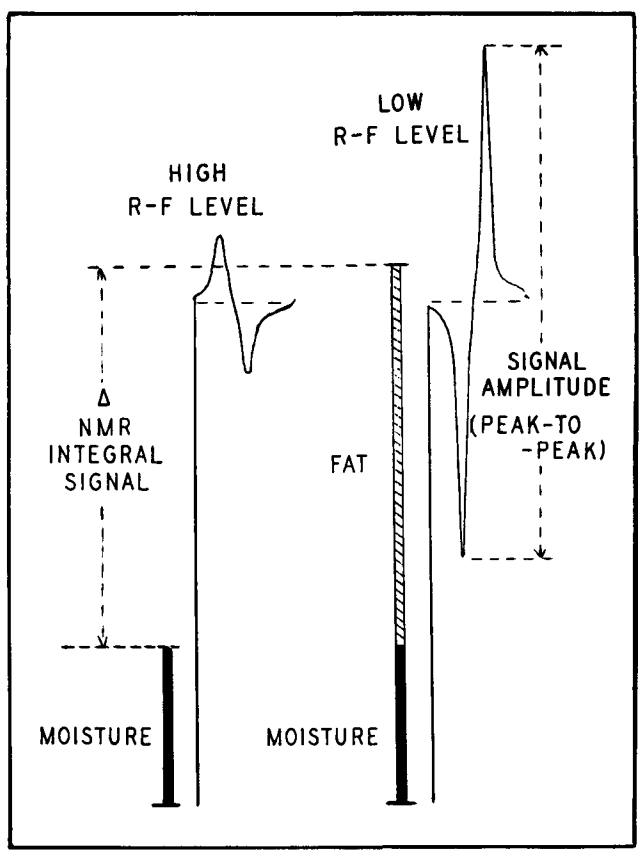

FIG. 1. Typical NMR spectra illustrating the radio-frequency saturation technique.

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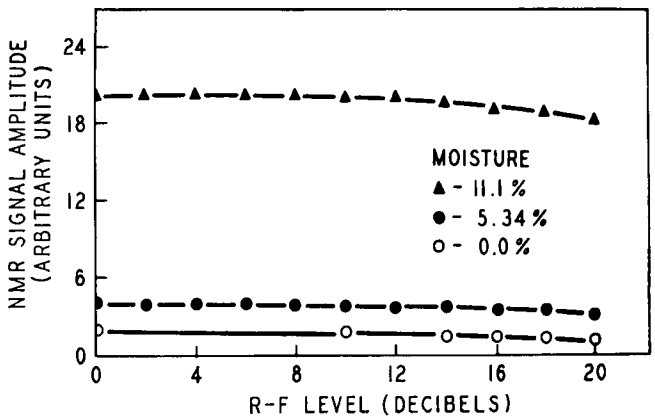

FIG. 2. Effect of radio-frequency level variations on NMR peak-to-peak amplitude signals from laboratory-defatted, re-moistened corn germ flakes.

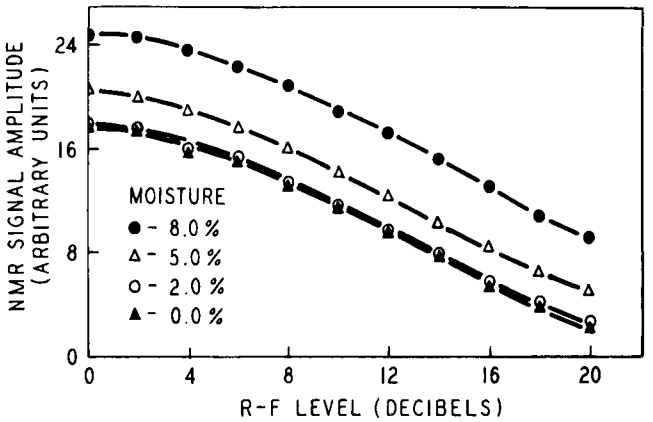

FIG. 4. Effect of radio-frequency level variations on NMR peak-to-peak amplitude signals from 9.2% fat dispersed on corn germ flakes containing 0.0%, 2.0%, 5.0% and 8.0% moisture.

associated with the fat can be analytically separated from hindered matrix hydrogen, since the latter are virtually constant over a narrow range of the magnetic field occupied by the mobile fat signal. This ability to distinguish between the liquid and solid state is of fundamental importance to wide-line applications described in this paper. Also, it must be remembered that the quantitative measurement of oil using wide-line spectroscopy is based on the principle that NMR signals are proportional to the total number of mobile hydrogen nuclei associated with the liquid oil.

For a theoretical NMR spectrum, the area under the absorption mode is proportional to the number of nuclei. This can be seen from the following equation:

$$\text{Area} = C/T \times H_1/r \times N/\left|1 + \gamma^2 H_1^2 T_1 T_2\right|^{1/2} \quad [1]$$

The term C includes nuclear and instrumental constants, T is the absolute temperature, H_1 is the r-f driving field, r is the magnetic field sweep rate, and N is the number of nuclei. The bracketed expression in the denominator is called the "saturation factor" and shows the dependence on the nuclear relaxation times T_1 and T_2.

In ordinary quantitative wide-line NMR spectroscopy H_1 is selected to minimize the effect of the saturation factor. In the current investigation H_1 values are deliberately selected to alter the degree of saturation and thus the area or amplitude of the NMR absorption signal.

The measurement of fat in relatively dry samples is comparatively simple. However, the problem becomes more complicated as the moisture content increases. At higher moisture levels, the water is no longer tightly bonded to the nonfat matrix. Consequently, NMR absorption signals from water are no longer weak and broad; in fact, they become much narrower and exhibit strong signals much like those from fat. Thus, the problem becomes one of not only separating signals from the solid and liquid phases but of distinguishing between the relatively narrow signals from water and fat. Fortunately, for most of the samples described in this paper, the NMR relaxation times associated with the fat and water are different and it is possible to use these differences as a basis for discrimination. In process samples where these differences are sometimes marginal, they can often be enhanced by milling the moisture-containing sample at high speed in carbon tetrachloride to alter the relaxation times of the protons associated with the fat.

In our method the fat content of a sample is derived from NMR integral or peak-to-peak signals obtained at two r-f levels (Fig. 1). At the lower r-f level, the energy absorption is proportional to the number of protons associated with both the water and fat. At the higher level, signals from fat are saturated preferentially. Consequently, the reduction in signal intensity, due to r-f saturation, is proportional to the amount of fat in the sample and can be used as a quantitative measurement. The moisture content of the same sample can be derived from the second NMR measurement since the signal intensity is proportional to the water content.

Instrumentation

The Varian Model PA-7 Analyzer (10) used in these experiments was equipped with a Model V4221 electronic integrator and a 40 ml r-f probe. Two r-f attenuators (40 and 60 dB) were employed; their specific use is indicated in

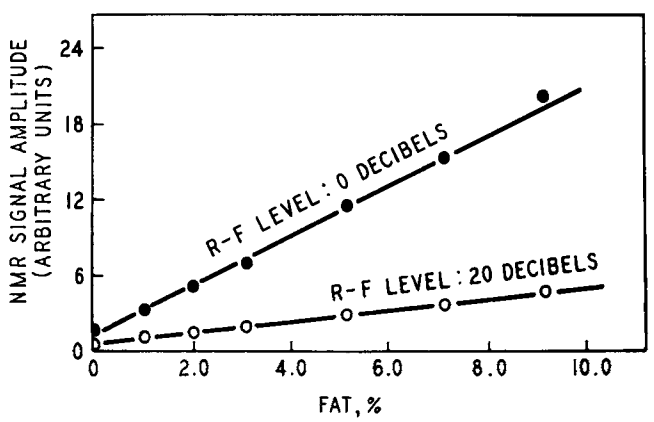

FIG. 3. Relationship between NMR peak-to-peak amplitude signals and fat contents for laboratory-dried, defatted corn germ containing added fat; two radio-frequency levels (low, 0 dB and high, 20 dB).

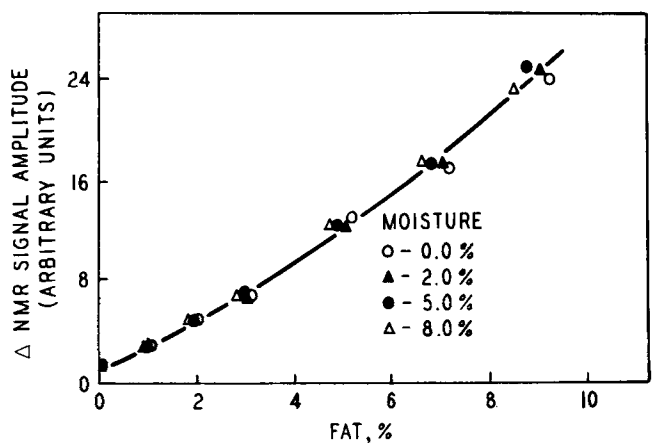

FIG. 5. Relationship of NMR differential peak-to-peak amplitude signals versus fat contents for laboratory-defatted corn germ flakes containing added moisture and fat.

TABLE I

Instrument Parameters and Sample Weights

Fig. No.	Sample wt., g	Model PA-7 spectrometer						Model V4221 integrator			
		Time constant, sec.	R-f[a] Attenuation, dB	Sweep time, min	Sweep amplitude, gauss	Sensitivity	Modulation amplitude gauss	Wt. setting	Readout multiplier	Threshold, mv	Signal multiplier
2	15.0	0.5	Varying	0.5	2.0	10	1.0	---	---	---	---
3	15.0	0.5	0, 20	0.5	1.0	20	0.5	---	---	---	---
4	15.0	0.5	Varying	0.5	1.0	20	0.5	---	---	---	---
5	15.0	1.0	0, 12	1.0	0.5	10-50	0.2	---	---	---	---
6	8.0	2.0	-6, +14	2.0	1.0	10	0.2	3000	5	0.2	5
7	8.0	2.0	0[b]	2.0	5.0	5	2.0	2000	10	0.5	5

[a]40-dB Attenuator: range: -20 (high attenuation) to + 20 (low attenuation) dB.
[b]60-dB Attenuator; range: 60 (high attenuation) to 0 (low attenuation) dB.

Table I. The notations -20 + 20 dB and 60 - 0 dB for high and low attenuation, respectively, were used by the manufacturer during development of the present attenuator.

The analysis of an individual sample is comparatively simple. A weighed and properly prepared sample contained in a 40 ml glass sample cell is placed in the magnet unit. The proper instrument parameters are selected and after the start switch is energized the spectrometer will trace out the peak-to-peak signal amplitude and readout the NMR integral signal.

Initially, during our investigation, the electronic integrator was not commercially available so all quantitative measurement were made utilizing NMR signal amplitudes obtained from the derivative curves displayed on the strip chart recorder. Use of amplitude measurements instead of NMR signal integrals is valid for those sample types whose absorption spectra exhibit a relatively constant line width. As this is not always true, the integral is the preferred method of signal measurement.

MATERIALS AND METHODS

Laboratory-Prepared Samples

Approximately 600 g of commercially dried and defatted corn germ flakes from the Argo plant extraction process were ground to pass 20 mesh. Aliquots of this material were laboratory extracted with carbon tetrachloride for 24 hr using large Soxhlet extractors. Subsequently, a second extraction was made using diethyl ether. Traces of solvent and moisture were removed by drying the material overnight in vacuo at 60 C. NMR spectra from the doubly-extracted sample exhibited a small residual mobile hydrogen signal; we subsequently learned that it was residual fat which could not be extracted without further reduction in sample particle size.

Portions of this material were used to prepare three types of samples: (a) defatted corn germ flakes (commonly referred to as spent flakes) containing 0-11% moisture, (b) dry flake containing 0-9% added fat, and (c) samples containing known amounts of both fat and moisture at concentrations commensurate with the plant process. For the first type, portions of the dry defatted flake were remoistened by exposure to a humid atmosphere. By varying the holding times a moisture range of 0-11% was obtained. Moisture contents were determined by 4 hr drying in vacuo at 100 C. For the second group of samples crude corn oil was dispersed as a carbon tetrachloride solution on the dry defatted flake. Solvent and any sorbed water was removed by drying the samples overnight in an air oven at 60 C. Fat contents were obtained from 16 hr Soxhlet extractions using carbon tetrachloride. Later, the third sample series was prepared from portions of the second type, by wetting samples at room temperature to adjust the moisture levels to 2.0%, 5.0% and 8.0%. Moisture contents were estimated from the gains in sample weights as a result of moisture sorption.

Plant Process Samples

Two series of approximately 40 random samples were taken at different times from the plant process, for NMR examination using the differential signal methodology. The first series was examined before the electronic integrator became commercially available and the second afterwards. Prior to NMR examination, all samples were ground to pass 20 mesh, blended and stored in sealed containers to preserve samples integrity.

The fat contents of the first series were determined by 16 hr extraction using carbon tetrachloride. As was mentioned previously, the method failed to remove all the fat; consequently, the second series was analyzed using a

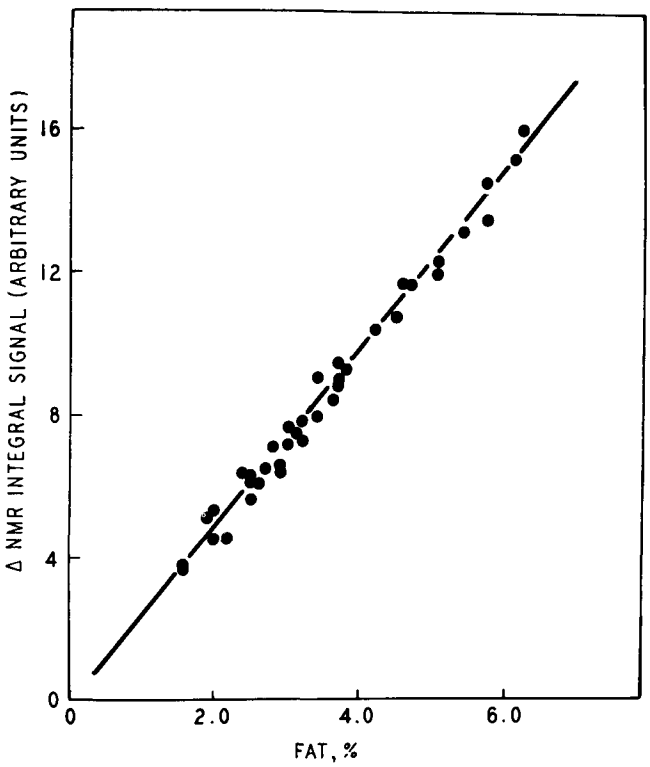

FIG. 6. Differential NMR integral signals versus fat contents for random plant process samples of defatted corn germ flakes milled in carbon tetrachloride.

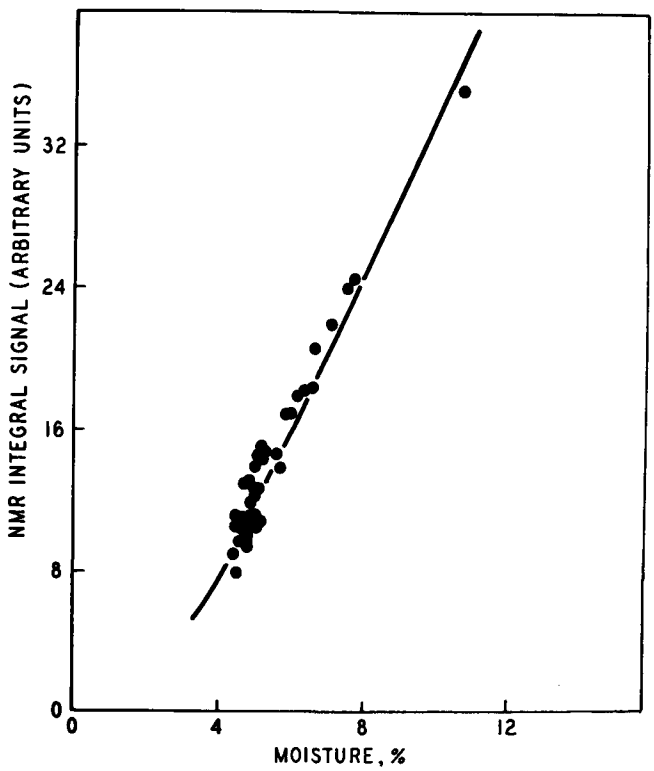

FIG. 7. NMR integral signals versus moisture contents for random process samples of defatted corn germ flakes milled in carbon tetrachloride.

referee fat extraction method which was a modification of the original procedure.

Referee Fat Extraction Procedure

For the referee gravimetric analyses, 5 g samples were milled in carbon tetrachloride at 45,000 rpm for 3 min using a modified Virtis disintegrator. Immediately after grinding, the slurry was transferred to a filter paper and the filtrate recovered in a tared oil extraction flask. The original filter paper and residue were wrapped in a second filter paper and extracted for 16 hr with the previous extract in a Soxhlet extractor. After extraction, excess solvent was evaporated off on a steam bath and final traces of solvent were removed in vacuo at 100 C for 60 min. Oil content was determined gravimetrically by weighing the cooled flask.

NMR Measurements

In a series of experiments the laboratory-prepared and the first 40 plant process samples were transferred to 40 ml NMR samples cells and examined. Instrument parameters for individual experiments are shown in Table I.

For the second series of 40 plant samples, 8 g sample portions were transferred to NMR cells and 15 ml of carbon tetrachloride were added. Each sample-containing NMR tube was transferred to the Virtis disintegrator. After 3 min milling at 45,000 rpm, the shaft and blade were rinsed with enough solvent to bring the sample depth to 1 3/4 in. and the tube sealed with a polyethylene stopper. Later the milled samples was examined instrumentally at two r-f levels. From the difference in the two integral signals the fat content was estimated.

RESULTS AND DISCUSSION

Laboratory-Prepared Samples

The NMR signal amplitude-moisture correlation for laboratory-defatted corn germ samples exhibited a high degree of correlation. The shape of the curve indicated that a portion of the sorbed water is bound to the nonfat matrix and that detection below 3% moisture would be difficult at room temperature. The effect of r-f level variations on NMR signal amplitude for three samples (0.0%, 5.3% and 11% moisture), selected from the previous NMR-moisture correlation, is demonstrated in Figure 2. The effect was slight. The small decrease in peak-to-peak signal amplitude for samples containing 0.0% and 5.3% moisture resulted from saturation of the residual fat which remained after extraction. Even at 11% moisture the effect of increased r-f level was minimal when allowance is made for the residual fat. The relationship between NMR signal amplitudes and fat contents at two different r-f levels (0 dB, low; 20 dB, high) are shown in Figure 3 for the series of dried spent flake samples containing 0-9% added fat. High degrees of correlation were observed for both r-f levels between fat content and peak-to-peak amplitude signals. A high degree of correlation also exists (Fig. 3) between fat contents and the difference in peak-to-peak signals at 0 and 20 dB r-f levels. From these data the residual fat content of the original defatted dry sample was estimated at approximately 0.5%.

To study further the extent to which moisture might contribute to differential signal amplitude measurements, r-f saturation curves were run on selected samples containing 9% added fat and 0-8% moisture. R-f saturation data are shown in Figure 4, and it is obvious that the saturation effect from an increased r-f level was similar irrespective of the moisture level. These data suggested that a differential peak-to-peak signal amplitude approach to measuring fat in the presence of moisture was valid.

To further test the validity of this approach, samples containing 0-9% fat and 0-8% moisture were examined differentially and these results are shown in Figure 5. While these data establish the feasibility of a differential signal approach for measuring fat contents, it must be remembered that they were obtained for an ideal sample system and do not necessarily represent plant process samples with respect to possible variations in NMR relaxation times.

Plant Process Samples

To further evaluate our NMR method, a series of 40 random samples, representing several months of operation, was taken from the Argo plant and examined instrumentally. The relationship between fat content and NMR differential peak-to-peak signal amplitude measurements was obtained. With the exception of two samples, a satisfactory relationship was observed. The spread in analytical data was greater than for the laboratory-prepared samples; in general, lower differential signal amplitudes were observed, probably due to variations in NMR relaxation times. The binding of fat to the nonfat matrix was suggested as a possible cause for the spread in our data after examining two samples which originally exhibited widely different NMR differential signals and yet contained the same amount of solvent-extractable fat. Furthermore, when the extracted fat and defatted residue from each of these respective samples were recombined and re-examined as a slurry in carbon tetrachloride, similar NMR differential signals were obtained. This experiment suggested an alternate approach to improve the NMR-fat correlation. If the sample could be dispersed in carbon tetrachloride so as to alter the relaxation times of the protons associated with the fat, the problem of fat binding and the resultant variations in relaxation times could be circumvented.

Thus, a second similar series of 40 samples were taken at random from the plant process. Sample-aliquots were dispersed in carbon tetrachloride by high-speed milling in the Virtis disintegrator and examined instrumentally. The NMR instrumentation was improved by the addition of an electronic integrator. Later, the 40 dB attenuator was replaced with a 60 dB unit to provide increased r-f range. The correlation between NMR integral signal differentials and fat contents for samples containing 1-6% fat and 4-11% moisture is shown in Figure 6. An excellent correlation was evidenced; from computer analysis of the data a correlation coefficient of 0.993 was calculated; and the accuracy was estimated at ± 0.32% fat, absolute (95% confidence limits).

The data in Figure 7 show the applicability of the NMR technique to measure moisture. Over a moisture range of 4-11% NMR integral signals were linearly related to moisture content with a calculated correlation coefficient of 0.971. The accuracy of the method was estimated at ± 0.56% for 95% of the data, which is within the limits required for the process measurement.

While this study has been limited to defatted corn germ, the feasibility of this NMR approach is demonstrated and can probably be extended to other types of defatted material.

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