

Determination of Water by Nuclear Magnetic Resonance in Hygroscopic Materials Containing Soluble Solids

K. J. PALMER and R. H. ELSKEN
 Western Utilization Research Branch,
 U. S. Department of Agriculture,
 Albany 10, Calif.

Investigation of the influence of soluble solids in determinations of moisture in food products by use of nuclear magnetic resonance shows that the hydrogen nuclei of solutes absorb in a manner indistinguishable from the hydrogen nuclei of water in the high moisture region under conditions of operation of the low-resolution spectrometer described by Shaw and Elsken. Data on several sugar solutions, milk samples, and apple juice concentrate illustrate the effect.

THE METHOD of determining the water content from D_{\max} (2), the maximum value of the derivative of the proton resonance absorption curve, has been found satisfactory for potato tissue but not for fresh apple tissue (3). This discrepancy is due apparently to the contribution to D_{\max} of the hydrogen nuclei present in soluble solids, such as sugars, in the apple tissue.

If the proton line width for a solute is comparable to that for water, as contrasted to the broad line for the insoluble solids, it will be necessary either to determine a calibration curve for each material or to correct the value of D_{\max} for the absorption due to the hydrogen nuclei of the solute. In order to obtain information on the influence of soluble solids on the value of D_{\max} , a formula has been derived which relates the observed value of D_{\max} to the moisture content of the specimen and proton composition of the soluble solids. The formula was derived on the assumption that all protons, regardless of whether they belong to water molecules or soluble solids, contribute equally to D_{\max} . The equation gives, therefore, the maximum value of the correction: a value that applies to those cases in which the resonance absorption peak of the protons of the soluble solids is indistinguishable from the peak produced by the water protons. As the correction is based on a knowledge of the composition of the soluble solids, the practical application is limited. The primary value of the equation is in proving that the nuclear magnetic resonance signals from many soluble solids are, indeed, indistinguishable from that of water under the conditions of operation described by Shaw and Elsken (2).

Analytical Procedure

A formula can be derived which enables the moisture content of a sample containing soluble solids to be calculated,

if it is assumed that the proton absorption line width and frequency observed in the spectrometer for the solids in solution are essentially the same as for water. Because pure water is used as the reference standard and the sensitive volume of the radio-frequency coil is constant and equal to V_0 , it follows that

$$V_0 = \frac{M_w}{\rho_w} = \frac{M_s}{\rho_s} \quad (1)$$

where M_w and M_s are the weight of pure water and solution occupying the sensitive volume of the radio-frequency coil, and ρ_w and ρ_s are their respective densities.

The number of gram-atoms of hydrogen in M_s gram of solution is

$$N_s = \sum_i \frac{n_i M_i}{W_i} = M_s \sum_i \frac{f_i n_i}{W_i}$$

where n_i is the number of hydrogen atoms per molecule and M_i and W_i are the weight in grams and the molecular weight, respectively, of the i th component.

The weight fraction is given by $f_i = \frac{M_i}{M_s}$. The summation includes the water molecules.

D_{\max_0} , the value of D_{\max} extrapolated to zero radio-frequency field, is proportional to the number of contributing hydrogen nuclei in the sensitive volume of the radio-frequency coil (2). It follows that

$$D_{\max_0} = kN$$

where k is a proportionality factor, and therefore

$$\frac{(D_{\max_0})_s}{(D_{\max_0})_w} = \frac{N_s}{N_w} = \frac{M_s \sum_i \frac{f_i n_i}{W_i}}{n_w \left(\frac{M_w}{W_w} \right)}$$

where subscripts s and w are for solution and pure water, respectively. Using Equation 1, this reduces to

$$\frac{n_w \rho_w (D_{\max_0})_s}{W_w \rho_s (D_{\max_0})_w} = \frac{f_w n_w}{W_w} + \sum_i \frac{f_i n_i}{W_i}$$

where the summation is now over all solute molecules, but not water. As f_w is the water content of the solution on a weight basis, solving for f_w gives

$$f_w = \frac{\rho_w}{\rho_s} \times \frac{(D_{\max_0})_s}{(D_{\max_0})_w} - 9 \sum_i \frac{f_i n_i}{W_i} \quad (2)$$

since $n_w = 2$ and $W_w = 18$.

Experimental Work and Results

To show the effect on D_{\max} due to the hydrogen nuclei of the solute molecules, seven solutions containing between 10 and 70% sucrose by weight were made. D_{\max} values for these solutions were determined by the method of Shaw and Elsken (2) and their water contents were calculated as suggested by these authors. The results are shown in column 3 of Table I. It is evident from a comparison of columns 3 and 5 that the nuclear magnetic resonance method does not give directly the correct value for the moisture content of sugar solutions, as it does in the case of potatoes and starch suspensions (2, 3).

The water content of these sucrose solutions has been calculated with Equation 2 from the observed values of D_{\max} for pure water and sucrose solutions taking n (sucrose) = 22 and W (sucrose) = 342. The results are shown in column 4 of Table I. Column 2 gives the density of the sugar solutions; ρ_w was assumed to be unity. The moisture content determined by the vacuum oven method (60° C. for 30 hours) is given in column 5. The agreement between columns 4 and 5 is seen to be within the experimental error, although the nuclear magnetic resonance results are consistently higher. The agreement between columns 4 and 5 substantiates the assumption that in a low-resolution spectrometer operated as suggested by Shaw and Elsken (2) the hydrogen nuclei of sucrose are indistinguishable from those of water.

Another test of Equation 2 was made

Table I. Nuclear Resonance Absorption Data on Sucrose Solutions

| Sample | ρ_s | % Water | | |
|--------|----------|-------------|------------------|-------------|
| | | Uncorrected | Corrected, f_w | Vacuum oven |
| 1 | 1.038 | 96.4 | 90.6 | 89.94 |
| 2 | 1.081 | 92.6 | 81.0 | 79.8 |
| 3 | 1.127 | 89.1 | 71.7 | 69.8 |
| 4 | 1.176 | 85.3 | 62.1 | 59.8 |
| 5 | 1.235 | 80.4 | 51.4 | 49.4 |
| 6 | 1.292 | 76.5 | 41.7 | 39.04 |
| 7 | 1.359 | 70.8 | 30.2 | 28.01 |

Table II. Moisture Content of Concentrated and Single-Strength Apple Juice

| Sample | Concn., N | ρ_s | % Water | | |
|--------|--------------|----------|------------------|------------------|-------------|
| | | | From D_{max_0} | Corrected, f_w | Vacuum oven |
| 1 | 4 | 1.218 | 80.3 | 51.1 | 51.4 |
| 2 | 4 | 1.218 | 80.9 | 51.7 | 51.3 |
| 3 | 1 | 1.052 | 95.4 | 87.0 | 86.0 |
| 4 | 1 | 1.052 | 95.4 | 86.9 | 85.8 |

by determining the values of D_{max} for concentrated apple juice and this same juice when diluted back to normal strength.

Apple juice, consisting of the clear filtrate from pressed apples, was chosen for study because it contained an appreciable quantity of naturally occurring soluble solids. The amount of soluble solids in the apple juice was evaluated from the vacuum oven data. Because

low-resolution nuclear magnetic resonance spectrometer; the contribution of the hydrogen nuclei of the soluble solids is in proportion to their concentration in both the concentrated and normal-strength apple juice.

In addition to sugar solutes, other constituents such as fats and proteins are expected to give rise to resonance lines which cannot be distinguished in the low-resolution spectrometer from resonance lines given by hydrogen nuclei of water. To determine the contribution of these components to D_{max} , a series of milk samples was used in which both the total solids and their composition were varied. The first five milk samples listed in Table III had nearly the same ratio of fat to total solids and may be considered similar to samples that would be obtained from successive stages in the preparation of evaporated milk. Sample 1 was from homogenized milk and 5 was from regular evaporated milk. Sample 6 is the same as 5, except that 1 gram of lactose per 100 ml. was added. This increment represents the extreme variation of lactose in milk. Samples 7 and 8 were included to study the influence of fat on moisture determination. They are regular skim milk and condensed skim milk, respectively. The percentages of total solids and fat for each of these eight samples are given in Table III.

The nuclear magnetic resonance results obtained from these milk samples are shown graphically in Figure 1. The two points for the skim milk samples do not fall on the straight line through the uncorrected data for the other six samples, because the composition of the solids is different in the two cases.

In order to determine whether the hydrogen nuclei of the solids are contributing to D_{max} in a manner indistinguishable from those of water, it is necessary to know the chemical formulas for the fats and proteins in milk solids. To simplify the calculation, an empirical

formula for a fat molecule of average composition was calculated in the following way. From the fatty acid content of cow's milk (4) an average composition of $C_{15}H_{30}O_2$ was calculated. On the assumption that three of these average fatty acids are esterified to glycerol, an empirical formula representing the average composition of milk fat was calculated to be $C_{48}H_{92}O_6$.

As all milk proteins have nearly the same ratio of hydrogens to total weight, the composition of the protein fraction in milk solids was assumed to be the same as casein. The empirical formula for casein was calculated from the elemental analysis data for casein given by Sherman (4). The formula derived in this way was $C_{16}H_{25}O_5N_4$. The empirical formula for lactose is $C_{12}H_{22}O_{11}$.

The composition of whole milk solids as given in several sources was averaged. The values used in the following calculations were: lactose, 53.6%; protein, 38.2%; and ash, 8.25%. The fat content was known for each sample and is given in Table III.

Table III. Composition of Milk Samples

| Sample | Fat, % | Total Solids Including Fat, % |
|--------|--------|----------------------------------|
| 1 | 3.35 | 11.79 |
| 2 | 4.33 | 15.38 |
| 3 | 5.78 | 20.50 |
| 4 | 6.47 | 22.80 |
| 5 | 7.37 | 26.10 |
| 6 | 7.31 | 27.00 |
| 7 | 0.305 | 9.01 |
| 8 | 0.331 | 13.60 |

With the above information Equation 2 can be applied to the milk data; the results are shown as open circles in Figure 1. The corrected data are in excellent agreement with the moisture contents determined by the vacuum oven method and, furthermore, the corrected data on skim milk as well as on whole milk are on the same straight line. The slight overcorrection shown in Figure 1 may be due to the fact that a small percentage of the hydrogen nuclei of the solids are more restricted in their motion than are the hydrogens of water and consequently have a broader absorption line.

It would be interesting to know for which compounds and under what conditions a correction to D_{max} needs to be applied. A complete answer to this question will require a more extensive investigation, but the following preliminary results on sugar-water-gelatin systems are of interest in this connection.

Two gels were made, one containing 5% gelatin and 30% sucrose and one containing 10% gelatin and 60% sucrose. After the components were mixed each

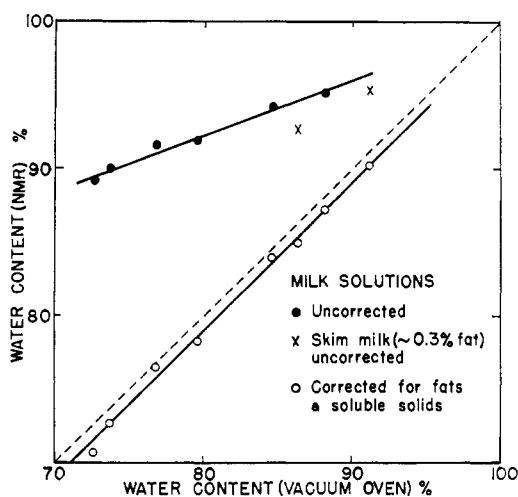


Figure 1. Water content of milk solutions

the soluble solids content of apple juice is predominantly sugars with a small amount of soluble carbohydrates, it was assumed, in order to simplify the calculation, that the composition of the soluble solids is that of a hexose sugar. The value of D_{max_0} was corrected by Equation 2 on the basis of this assumption and the results are given in Table II. It is apparent from these data that the moisture content calculated with the aid of Equation 2 is in very good agreement with the vacuum oven results. It follows, therefore, that all hydrogen nuclei in apple juice contribute equally to D_{max_0} when the latter is observed in the

solution was, in turn, poured into the spectrometer sample holder and D_{\max} determined several times over a period of 24 hours. During this period both solutions formed a stiff gel, but the value of D_{\max_0} remained essentially constant. The only effect noted was a decrease in relaxation time, T_1 , as the viscosity increased, an effect discussed by Bloembergen, Purcell, and Pound (7).

Because D_{\max_0} is unaffected by gelation of the sugar-gelation solutions, it is concluded that the experimental method used by Shaw and Elsken for determining the moisture content of hygroscopic materials may give a value for D_{\max_0} which includes the contributions from the hydrogen nuclei of both water and soluble solids in liquid samples and samples as rigid as a stiff gel.

Conclusions

The observed value of D_{\max} obtained under the conditions described by Shaw and Elsken (2) is influenced by

soluble solids which contain hydrogen nuclei. In all cases investigated, the hydrogen nuclei of the soluble solids contributed to D_{\max} in direct proportion to their concentration. In order to calculate the moisture content of a sample that contains soluble solids it is necessary, therefore, either to make a calibration curve or to use Equation 2. The latter method requires that the soluble solids content be known, while the former method requires that the soluble solids composition be the same for all standard and unknown samples. The uncorrected data for the two skim milk samples shown in Figure 1 illustrate what may occur when the solids composition varies. In specific cases it may be necessary to have separate calibration curves for each variety of a product as well as for different maturity grades.

It is also evident from the data presented in Figure 1 that for these particular samples the nuclear magnetic resonance results change by only 4.7% when the moisture content changes by 14.5%;

consequently the percentage change in D_{\max} , the value measured in the nuclear magnetic resonance spectrometer, is only about one third as much as the percentage change in water content.

Acknowledgment

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Literature Cited

- (1) Bloembergen, N., Purcell, E. M., and Pound, R. V., *Phys. Rev.* **73**, 679 (1948).
- (2) Shaw, T. M., and Elsken, R. H., *Anal. Chem.* **27**, 1983 (1955).
- (3) Shaw, T. M., and Elsken, R. H., *J. AGR. FOOD CHEM.* **4**, 162 (1956).
- (4) Sherman, H. C., "Food Products," pp. 73-4, Macmillan, New York, 1948.

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CROP STORAGE

Feedstuffs Values and Chemical Changes in Spontaneously Heated Alfalfa Pellets

W. S. RULIFFSON, MAX MILNER,
and H. L. MITCHELL
Kansas State College,
Manhattan, Kan.

Alfalfa pellets from fresh alfalfa hay have become a major feed ingredient. Recently serious losses have occurred, due to spontaneous heating in grain elevators. A comprehensive analytical study, showing progressive deterioration from sound through brown to black pellets, indicated that spontaneous heating is initiated and sustained by sugar-protein interactions of the Maillard type, accompanied by rapid utilization of sugars, slower destruction of fat, and increases in ash, total nitrogen, and crude fiber. In preliminary and intermediate phases, sharp reduction of carotene and regular increases in fluorescence occur. Fat acidity increases rapidly only with onset of advanced browning. Riboflavin values are influenced by fluorescence due to browning products. Retention of nitrogen in heated pellets is attributed to melanoidins resistant to thermal destruction and nutritive utilization. Decomposition is advanced in terms of color, chemical deterioration, and nutritive losses before flaming combustion.

SPONTANEOUS HEATING IN STORED HAY resulting in the production of brown to black residues appears to be a common occurrence (4). Impairment of the feeding value of alfalfa hay for livestock due to spontaneous heating has been noted (3). Recently, spontaneous heating to the point of ignition in commercial alfalfa pellets was brought to the attention of the authors.

Alfalfa pellets are prepared by extrusion pelleting of chopped, artificially dried alfalfa. This product has become

a major feed ingredient and is handled and stored in considerable quantities by methods common to bulk grain.

It appeared that the physical and chemical factors which play a part in the spontaneous heating of other bulk granular agricultural materials might also be involved in the spontaneous heating of this product. It was also of interest to compare the chemical changes occurring in pellets at progressive stages of heating with those in soybeans, concerning which considerable information is available (7).

Materials and Methods

Samples of alfalfa pellets at various stages of deterioration were obtained from a commercial elevator bin in which spontaneous heating of pellets occurred in November 1954. This bin had been filled with 1100 tons of bulk pellets about 3 months prior to this event. One of the authors (MM) inspected the material shortly after the advanced heating had commenced and while salvage operations were in progress. The pel-