Characterization of the Oxygen-17 Nuclear Magnetic Resonance Water Mobility Response Surface

S. J. RICHARDSON, M. P. STEINBERG, R. E. DE VOR and J. W. SUTHERLAND

ABSTRACT
This study uses factorial design techniques to investigate the Oxygen-17 (17O) Nuclear Magnetic Resonance (NMR) relaxation rate response of a corn starch-water system as affected by three experimental conditions: concentration, time between sample preparation (hydration) and measurement and storage temperature. It was found that neither time (between 0.1 and 20 hours) nor storage temperature (room temperature and low temperature) had a significant effect on the 17O NMR relaxation rate response. The only variable which affected the 17O NMR relaxation rate was the concentration of corn starch in water (10-70%, w/w). Mathematical modeling of the dependence of the 17O NMR response on concentration is discussed in detail.

INTRODUCTION
CHARACTERIZATION of sorbed water and its mobility in food components and systems has been a subject of longstanding investigation (Vail and Bailey, 1940; Bull, 1944; Nemethy and Scheraga, 1962; Eisenberg and Kauzmann, 1969; Labuzka, 1975; Leung and Steinberg, 1979; Lang and Steinberg, 1983). One of the most successful and relatively recent techniques for such characterization is Nuclear Magnetic Resonance (NMR) spectroscopy, specifically Oxygen-17 (17O) NMR (Koenigm et al., 1975; Halle and Wennerstrom, 1981; Halle et al., 1981; Liutis, 1984; Richardson et al., 1986). 17O NMR transverse relaxation rate (R2) measurements directly and non-invasively monitor the molecular motions of the water molecule (Richardson et al., 1986), and can thus be used to study the mobility of the states of water in food systems.

As with any analytical technique, the effects of the experimental conditions on the measured response are of extreme importance. The major question of interest here is how do the major experimental variables influence the 17O NMR response in both magnitude and direction. The answer to this question is of utmost importance to the design of future experiments, the control of conditions required by the experiment, the reliability of the data obtained and the scientific interpretation of those data.

A Factorial design is a testing structure which is particularly suited to answer the above question (Lal et al., 1980; DeVor, 1985). Factorial designs are frequently performed and statistically analyzed to measure the effects of one or more independent variables on a response (Box et al., 1978). They allow for the identification of both the magnitude and direction of the effects of the experimental conditions on the desired response in the form of a mathematical model. These modeling techniques allow the investigator to determine, with very few trials, detailed information concerning the nature of the response surface under the experimental conditions of interest (Box et al., 1978). Examples of this are the application of Factorial designs to the development of complicated food formulations (Thalheimer and Rusch, 1970; Chow et al., 1983), as well as, the use of Factorial designs to elucidate the nature of the response surface in Process Optimization studies (Cochran and Cox, 1957; Montgomery, 1961; Lah et al., 1980; Motycka et al., 1984; Oh et al., 1985).

The purpose of this work was to employ Factorial design techniques to characterize the unknown 17O NMR R2 response surface as affected by three independent experimental variables: concentration, time between sample preparation (hydration) and measurement and temperature of storage.

MATERIALS & METHODS
Sample analysis and preparation
The corn starch was commercial grade, Argo brand (Best Foods, Inc., Englewood cliffs, NJ). Moisture content was 8.5% as determined by vacuum oven method (AOAC, 1980) using 60°C and 29 g in Hg vacuum for 24 hr. Determinations were made in triplicate. Protein content (N x 6.25) was determined by the macro-Kjeldahl method (AOAC, 1980) to be 0.31%.

Thirteen corn starch samples, ranging in concentration from 10 to 70%, weight/weight, were prepared by adding the calculated amount of distilled water and hand mixing for 2.5 min. The specific moisture contents studied were statistically selected based on the requirements of the Factorial design (Table 1). The calculated moisture contents were verified by the vacuum oven method.

NMR measurements
A laboratory assembled NSF-250 Multinuclear NMR-Spectrometer of the Oldfield and Meadows (1978) design operating at 34 MHz 17O NMR resonance frequency was used. Single pulse experiments were done in triplicate at 20°C upon sample equilibration from storage temperature, which required about 5 min. Pulse width was 45 microseconds, beta delay was 55 microseconds, and recycle time was 0.21 sec. Spectra were stored in a 16K point array which provided adequate resolution.

The 17O NMR transverse relaxation rate (R2) response was determined by measuring the line widths at half-height (\(\text{f}_{1/2}\)) of each spectrum at each concentration and design condition. To correct for any residual magnetic field inhomogeneity, the net line broadening (\(\Delta f\)) was calculated by subtracting the line width of liquid water (\(\text{f}_{\text{H}_{2}\text{O}}\)).

Table 1—Definition, levels and 17O NMR differential relaxation rate responses for the Central Composite Design in Fig. 1

<table>
<thead>
<tr>
<th>Natural variables</th>
<th>17O (\Delta R_2) NMR response</th>
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<tbody>
<tr>
<td>Conc. mole water</td>
<td>Storage time (hr)</td>
</tr>
<tr>
<td>4.14</td>
<td>3</td>
</tr>
<tr>
<td>4.14</td>
<td>17</td>
</tr>
<tr>
<td>23.02</td>
<td>3</td>
</tr>
<tr>
<td>23.02</td>
<td>17</td>
</tr>
<tr>
<td>10.76</td>
<td>10</td>
</tr>
<tr>
<td>10.76</td>
<td>10</td>
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<tr>
<td>10.76</td>
<td>10</td>
</tr>
<tr>
<td>10.76</td>
<td>10</td>
</tr>
<tr>
<td>31.61</td>
<td>10</td>
</tr>
<tr>
<td>2.22</td>
<td>10</td>
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<tr>
<td>10.76</td>
<td>1.414</td>
</tr>
<tr>
<td>10.76</td>
<td>10</td>
</tr>
<tr>
<td>10.76</td>
<td>1.414</td>
</tr>
</tbody>
</table>

\(\text{f}_{\text{H}_{2}\text{O}} = 25 \pm 1°C \text{ and } \text{f}_{\text{H}_{2}\text{O}} = 25 \pm 1°C\)

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from that of the sample \(e_{0,\text{ref}}\). The net or differential relaxation rates \(\Delta R_2 (\text{sec}^{-1})\) were calculated \(\pm 0.5\%\) from the following standard net line broadening equation \(\text{(Dewar, 1973)}\):

\[
\Delta R_2 (\text{sec}^{-1}) = 0.24 \Delta y_0 (\text{sec}^{-1})
\]

Experimental design

The critical nature of sample preparation and experimental conditions in water binding studies are discussed by D'Arcy and Wear (1986). The three conditions of interest in this study were: (1) concentration of solids, (2) time between sample preparation and \(\text{H}_2\) NMR measurements, and (3) temperature of storage. The structure of the experimental design was intended to elucidate the effect of measurement time (0.1-10 hr) and temperature (room and low temperature) on the \(\text{H}_2\) NMR relaxation rate response to various corn starch-water concentrations (10-70%, weight/weight). In order to characterize the surface of the \(\text{H}_2\) NMR response, a model of degree two or higher is required because of the possible curvature in the true response surface due to time and concentration. In most cases, the second order model:

\[
y = B_0 + \sum_{i=1}^{k} B_i x_i + \sum_{i<j}^{k} B_{ij} x_i x_j + \sum_{i=1}^{k} B_0_i x_i
\]

provides an adequate approximation (Montgomery, 1976). A preferred Factorial design for fitting such a second-order model is the Central Composite Design (CCD). These designs consist of a 2\(^k\) factorial (or fractional factorial) block augmented by 2\(^k\) axial points and \(n_0\) center points. The CCD can be made rotatable by the choice of the axial point, \(F\), where \(F = (F_{\alpha}^2)\), \(F\) is the number of points used in the factorial portion of the design; it can be made orthogonal or uniform precision by the choice of the number of \(n_0\) (Montgomery, 1976).

In this study \(k=2\); concentration \((x_1)\) and time \((x_2)\). \(F = 4\), \(\alpha = 1.414\) and \(n_0 = 5\) (for uniform precision). The CCD for \(k=2\) is shown in Fig. 1, and the design in Table 1. The CCD was performed for both room temperature \((25 \pm 1^\circ\text{C})\) and low temperature \((1.25 \pm 1.0^\circ\text{C})\) conditions. The order in which each trial was performed was randomized (Steel and Torrie, 1980). CCA analysis of the quadratic regression was done by a computer (Sutherland, 1985).

RESULTS & DISCUSSION

TWO STATISTICAL ANALYSES were employed to evaluate the Factorial design results: (1) fit analysis of the second order CCD model for the concentration and time variables under each storage condition, separately, and (2) paired t-test analysis for the two storage conditions.

The second order model \(\text{(Eq. (2))}\) was employed with the data from the CCD to estimate the true response surface of the \(\text{H}_2\) NMR differential relaxation rate results for each temperature condition. For \(k=2\), Eq. (2) becomes:

\[
y = B_0 + B_1 x_1 + B_2 x_2 + B_{12} x_1 x_2 + B_4 x_1^2 + B_5
\]

where \(x_1\) is concentration, \(x_2\) is time between sample preparation and NMR measurement and \(B_0, B_1, B_2, B_{12}, B_4, \text{and } B_5\) are the regression coefficients. This model allows evaluation of the effect of concentration and of time between sample preparation and \(\text{H}_2\) NMR measurement under each temperature condition.

The results of the two trials to be fitted (room temperature and low temperature) are shown in Table 1. The regression coefficients (Table 2) were tested for significance with a 95% confidence interval (Steel and Torrie, 1980). For both temperature conditions only \(B_0, B_1, \text{and } B_2\) regression coefficients were significant. This indicates that the only statistically significant experimental variable was concentration and that time from 0.1-10 hr had no effect on the \(\text{H}_2\) NMR response.

The second order polynomial approximation of the true but unknown response surface employing all six \(B\) through \(B_5\) of the regression coefficients (Table 2) was used to develop contour plots of the predicted response surface over the range of variables tested. The contour plots for the room temperature and low temperature conditions are shown in Fig. 2 and 3, respectively. Each plot shows the \(\text{H}_2\) NMR differential relaxation rate response as a function of concentration and time. These full second order model plots visually reflect the trends seen in the regression coefficients that the \(\text{H}_2\) NMR response is affected only by the concentration and not by the time between sample preparation and instrument measurement.

The Analysis of Variance (ANOVA) for each temperature condition (Table 3 for room and Table 4 for low-temperature) shows that the full second order model \(\text{(Eq. (3))}\) was

![Fig. 1—Central composite design (CCD) display for two variables \((k=2)\): concentration \((x_1)\) and time \((x_2)\). CCD coordinates correspond to the coded variables defined in Table 1.](image1)

![Fig. 2—Response surface contours for \(\text{H}_2\) NMR relaxation rate response for room temperature conditions in the regions tested as a function of concentration and time. Levels of variables correspond to the natural variables given in Table 1.](image2)
Table 3—Analysis of variance for the room temperature condition under the full second-order model [Eq. (3)].

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sums of squares</th>
<th>Mean squares</th>
<th>F</th>
</tr>
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<tbody>
<tr>
<td>Model</td>
<td>6</td>
<td>977,920.49</td>
<td>162,896.75</td>
<td>402.51*</td>
</tr>
<tr>
<td>Residual</td>
<td>7</td>
<td>2,834.51</td>
<td>404.93</td>
<td></td>
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<tr>
<td>Lack of fit 1</td>
<td>3</td>
<td>2,768.49</td>
<td>922.83</td>
<td>55.90*</td>
</tr>
<tr>
<td>Pure error 1</td>
<td>4</td>
<td>66.02</td>
<td>16.51</td>
<td></td>
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<tr>
<td>Total</td>
<td>13</td>
<td>980,755.00</td>
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* Significant at the 1% level: R² = 0.99711; R²corr = 0.99355.

Table 4—Analysis of variance for the low temperature storage condition under the full second-order model [Eq. (3)].

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sums of squares</th>
<th>Mean squares</th>
<th>F</th>
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<tr>
<td>Model</td>
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<td>981,727.99</td>
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<td>770.24*</td>
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<tr>
<td>Residual</td>
<td>7</td>
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<tr>
<td>Lack of fit 2</td>
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<td>1,461.98</td>
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<tr>
<td>Pure error 2</td>
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<tr>
<td>Total</td>
<td>13</td>
<td>983,721.00</td>
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</table>

* Significant at the 1% level: R² = 0.99849; R²corr = 0.99955.

highly significant (F = 402.51), there was a large lack of fit (F = 55.90) for the model under both storage temperature conditions.

Thus, the key result of this first statistical analysis was that the 17O NMR transverse relaxation rate response was independent of the time variable under the range tested in this experiment, under each storage condition. Therefore, the true response surface data can be used to determine whether the effect of concentration on the 17O NMR response can be modeled.

Since only regression coefficients B₀, B₁, and B₄ were found to be significant (Table 2) the full second-order model [Eq. (3)] reduces to:

\[ \hat{y} = B_0 + B_1x_1 + B_4x_1^2 \]  

(4)

for each temperature condition. In order to determine the suitability of this model to describe the behavior of the 17O NMR relaxation response against concentration, a least squares fit was done on a computer (Sutherland, 1985). The ANOVA for each temperature condition is given in Tables 5 and 6. The model for room temperature 17O NMR response accounted for 0.99659 of the total variation (Table 5). The R² corrected value, which is calculated as \((\text{Model SS} - \text{Mean SS})/\text{Total SS - Mean SS})\) and describes the actual variation accounted for by the model when corrected for the mean, was 0.99240 (Table 5). The model for low temperature 17O NMR response accounted for 0.99822 of the total variation and had an R² corrected value of 0.99606 (Table 6). The ANOVA (Tables 5 and 6) indicated that although the second order model [Eq. (4)] was highly significant, a large lack of fit was evident for the model under each temperature condition.

In order to investigate the cause of this large lack of fit, analysis of the residuals (observed response - predicted response) associated with the model [Eq. (4)] was done. The assumptions underlying the statistical model expressed by Eq. (4) are that the errors associated with the true response (Y) are normally and independently distributed with constant variance (Box et al., 1978). However, when the residuals were plotted against the predicted response (not shown) it was revealed that the experimental errors associated with the 17O NMR R₂ response were larger at both the lower and higher concentrations than at the intermediate concentrations. In an effort to stabilize the variance of this model, a log transformation was applied to the model [Eq. (4)] (Box et al., 1978):

\[ \ln \hat{y} = B_0 + B_1x_1 + B_4x_1^2 \]  

(5)

The log transformation did decrease the lack of fit (Tables 7 and 8), however, the F ratios were still significant at the 1% level. Analysis of the residuals still revealed that the experimental errors associated with the 17O NMR R₂ response were larger at the low and high concentrations than at the intermediate concentrations.

The experimentally observed 17O NMR differential relaxation rate response and the second order and log transformation predicted responses plotted against starch concentration for the room and low temperature storage conditions are shown in Fig. 3.
4 and 5, respectively. One can visually observe the location of the lack of fit in the second order (Equation 4) and log transformation (Eq. (5)) models. The lack of fit in the second order model is most apparent at the lower starch concentrations, whereas the log transformation model fits better at lower concentrations but over estimates the response at higher concentrations.

It must be noted that a large portion of the residual lack of fit of the two models may be attributable to the estimate of pure error used in the models. The pure error in the ANOVA Tables (Tables 3, 4, 5, 6, 7 and 8) reflect the pure error associated with the replicated center point of the CCD design, \( n_0 \). As discussed, the experimental errors associated with the \(^{17}O\) NMR relaxation time response are larger at both the lower and higher concentrations than at the intermediate concentrations (at \( n_0 \)). Thus, if the pure error would be estimated from the sum of squares of the difference between the individual response and the average over the entire concentration range, the lack of fit would decrease significantly for both the second order and log transformation models.

This lack of fit in the second order and log transformation models indicates that, even though these mathematical models were able to explain a significant portion of the variability in the data, further refinement of the model should be possible, either by taking into account additional unidentified variables and/or by obtaining a better estimate of the pure error, as previously discussed.

Recently a mechanistic model for the interpretation of the dependence of the NMR relaxation rates on concentration has been investigated (Kumosinski and Pessen, 1982; Richardson et al. 1986). The Kumosinski model based on charge repulsion or charge fluctuation effects attempts to physically account for the non-linearity of the concentration dependence of the NMR relaxation rates (Kumosinski and Pessen, 1982). The theory and application of this mechanistic model to the corn starch-water system is currently being investigated.

**Paired t-test**

The paired t-test was employed to determine whether there was a statistically significant difference at the 95% confidence level between the \(^{17}O\) NMR differential transverse relaxation rate (\( \Delta R_2 \)) response under the two storage temperature conditions. The paired t-test analysis, done with a computer (Sutherland, 1985), yielded a calculated t-value equal to 0.147 (null hypothesis: \( \mu_{LT} - \mu_R = 0 \); alternative hypothesis \( \mu_{LT} - \mu_{RT} \neq 0 \); with a confidence interval of 0.5185 to 0.5079 for the true mean. From the standard t-tables (Steel and Torrie, 1980) to \( 0.05 \) equals 0.443; therefore, we fail to reject the null hypothesis at the 5% significance level and conclude that there is no significant difference between the room and low temperature storage conditions. Therefore, we concluded that there is no significant difference between the room and low storage temperature \(^{17}O\) NMR relaxation responses for the 0.1-20 hr interval tested.

**CONCLUSIONS**

UNDERSTANDING what conditions affect the \(^{17}O\) NMR relaxation rate both in magnitude and direction is critical to the design and interpretation of future \(^{17}O\) NMR experiments. The results of this study showed that the \(^{17}O\) NMR relaxation rate response was not affected by the experimental conditions: (1) time between sample hydration and measurement, and (2) storage temperature. However, it was affected by corn starch concentration, i.e., moisture content.

Further studies are required to determine whether the other food constituents, such as proteins and sugars, would be affected in the same way by these variables.

**REFERENCES**


texture is desired and shape is not important, the cake should be baked with a low air velocity and a high temperature (Fig. 10A). When a flat cake suitable for decoration as shown in Fig. 10B is desired, baking should be done at a high air velocity and a low temperature.

CONCLUSION

THE HEATING PERFORMANCE of the forced convection oven could be expressed by the apparent heat transfer coefficient. The time needed to bake sponge cakes could be reduced by increasing air velocity and/or air temperature; both factors affect the apparent heat transfer coefficient. There was a strong relation between baked sponge cake characteristics and changes in air velocity or air temperature. Sponge cake with good texture but of variable shape can be baked with a low air velocity and a high temperature. Flat cakes suitable for decoration should be baked with a high air velocity and a low temperature.

REFERENCES


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