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Cross-Polarization/Magic Angle Spinning NMR To Study Glucose Mobility in a Model Intermediate-Moisture Food System

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Theories for the chemical stability of foods cite the role of moisture content or water activity in reactant mobility, though mobility has been variously defined. One theory, based on plasticization by moisture, is limited by a lack of research directly linking the mobility of a matrix to the mobility and reactivity of small solute molecules in foods. A cross-polarization/magic angle spinning technique was developed to study glucose rotational mobility in the solid state over a range of water activities and in matrixes with different glass transition temperatures. Data analysis stressed the significance of separating molecular mobility from relaxation time. Results showed that, in a caseinate matrix, compared to a control, adding glycerol yielded the highest glucose mobility and lowest glass transition temperature (T_g), while adding sorbitol also increased mobility and lowered T_g. Consequently, plasticization by either moisture or these humectants increases the mobility of small solute molecules such as glucose.

KEYWORDS: Spin−lattice relaxation; cross-polarization/magic angle spinning nuclear magnetic resonance; glass transition temperature; humectants; plasticizers; glucose mobility

Water activity, while an important parameter, is not a molecular mechanism that explains how moisture changes in foods result in changes in chemical reactivity. Leading theories for the chemical stability of low- to intermediate-moisture foods all rely on some significance of molecular mobility. From a kinetics perspective, reactant molecules must exhibit a minimum degree of mobility to collide with, orient toward, and react with one another. The significance of the monolayer moisture value (m_0) is based on the minimum amount of water required to provide reactant mobility (*1*). More recently, the glass transition temperature (T_g) has been theorized to be the point of minimum polymer mobility and consequently embedded solute mobility, with plasticization by water explaining increased reactivity with increased moisture content in low- to intermediate-moisture foods (*2*). However, prior research suggested a mechanism other than these for the role of moisture on reaction rates (*5*). The addition of glycol humectants to a model food system allowed very high rates of Maillard browning at water activities as low as 0.20, likely because they could replace moisture as a solvent phase. Theoretically, increased moisture could cause increased reactant dissolution, reactant mobility, and chemical reaction rate. Thus, the solubility of reactants may serve as a mechanism linking moisture changes to mobility and reaction rate.

In citing the significance of molecular mobility for diffusion-limited reactions in low- to intermediate-moisture foods, one significant concern is that theorists have been somewhat ambiguous as to how and for which molecular species mobility should be measured. For some systems, rotational mobility may best predict the limitation of a homogeneously dispersed reactant in high concentrations. For others, translational diffusion based on a Stokes−Einstein diffusion coefficient may limit mobility. For still others, Fickian or non-steady-state bulk diffusivity may best model the diffusion limitation. The significance of plasticization by moisture as a mechanism relies on an assumption that increasing plasticization, lowering the T_g and increasing polymer mobility, results in increased mobility and reactivity of all molecules within the system. However, this has never been directly measured. In the food science field, a method that can measure the degree of mobility for molecules that would be expected to contribute to the reaction rate in solid systems is highly desirable. Additionally, data that link moisture content changes to both the mobility of a reactant and to the reaction rate would contribute to the development of a fundamental mechanism for the role of moisture in governing reaction rates in foods.

Electron paramagnetic resonance (EPR) spectroscopy, also called electron spin resonance (ESR), is a technique that detects the electronic transition moment of a free radical probe in a semisolid matrix. For example, EPR has been employed to study the mobilities of sugars in frozen solutions (*6*, *7*). In these studies, correlations between the occurrence of the glass transition temperature T_g and a change in the mobility of a nitroxide radical probe were sought. However, the free radical

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probes required for any EPR analysis only mimic the mobility of actual solute reactants in foods. Additionally, there is a limit to the sensitivity of this technique in solid or low-moisture systems.

Nuclear magnetic resonance (NMR) may be used in highly mobile semisolid systems in a fashion similar to that of EPR, by measuring the relaxation time of molecular nuclei under an applied magnetic field. Using H^1 NMR, researchers have examined the mobility of water with respect to the T_g (*8*−*12*) and reported that a shift in the relaxation time (T_2) of water coincided with that of the T_g. However, previous researchers found that water mobility was not affected by changes in the T_g of dehydrated carrots (*13*). The mobility of water is of interest to those studying moisture migration in food systems. However, to use water mobility as an indicator of the glass transition temperature (a polymer mobility phenomenon) or, further, as an indicator of reactant molecule mobility and reaction rate may not be valid.

Extensive solid-state NMR studies correlating mobility to the properties and stability of foods have not been performed. However, an excellent collection of NMR applications in the food science field has been previously published (*14*). Schmidt (*15*) studied the mobility of ^{13}C-labeled starch. Using the parameter $T_{1\rho}$ for relaxation rate, which is sensitive to the slower motions inherent to solid systems, she reported a shift in relaxation rate at a moisture content just below the T_g (as measured by differential scanning calorimetry (DSC)). Kalichevsky (*8*) and Ruan (*12*) each used the coincidence of a shift in the T_g and in the relaxation time T_2 in food systems as an indication that low-field NMR can be used to measure the T_g. Using low-field NMR, the primary contributor to the NMR signal would be the relaxation of water, and there is an inherent assumption that T_2 directly correlates not only to water mobility but, by their conclusions, also to polymer matrix mobility. As will be discussed below, there is also an assumption that a shift in the system's relaxation time bears a direct reflection of a shift in the rotational correlation time (or molecular mobility) of the target molecule.

There is a lack of relevant research that has directly determined the mobility of a chemical reactant and correlated this with reaction rate. The following set of experiments uses the solid-state technique of cross-polarization/magic angle spinning (CP/MAS) NMR to determine the mobility of glucose within a caseinate model system over a range of water activities and with added humectants. The model system was chosen to also allow measurement of Maillard browning rate as a function of a_w and humectant type in concurrent experiments, to be reported in a separate paper. It is our hypothesis that both increasing water activity and adding glycerol and sorbitol will cause increased mobility of solid-state glucose in a caseinate model system. The mobility of dispersed solid-state glucose, measured by CP/MAS NMR, is expected to increase due to an increase in the degree of plasticization, as described by $T - T_g$.

MATERIALS AND METHODS

Cross-Polarization/Magic Angle Spinning Theory. The study of solid systems where only limited motion of nuclei occurs requires techniques both to improve the spectral resolution of the relaxation and to match the time frame of the slower motions in the system. CP/MAS NMR accomplishes this by taking advantage of dipolar coupling between magnetized nuclei and adjacent protons. In the technique, the magnetization pulse is applied in the rotating frame, meaning that the magnetic field is aligned parallel to the vector to which the nuclei are aligned (a so-called $\pi/2$ or 90° pulse). This is called spin-locking. Under this condition the precession of the nuclei yields a relaxation rate that is more sensitive to the slower molecular motions. This relaxation rate in the rotating frame is called $1/T_{1\rho}$, or spin−lattice relaxation rate, with "ρ" added to denote the rotating frame. $T_{1\rho}$ is dependent on the rotational correlation time (τ_c) of the excited nuclei as described below.

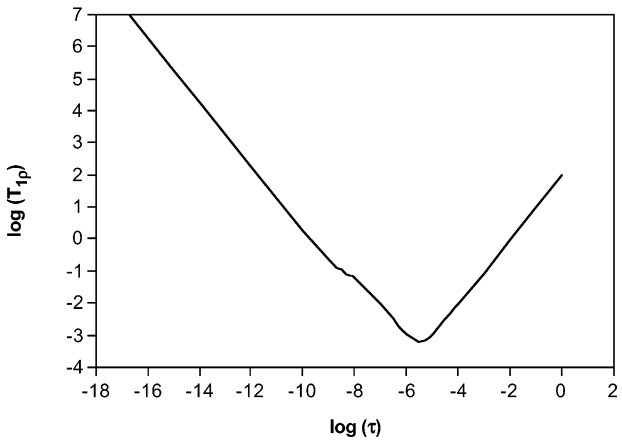

Figure 1. Variation in relaxation time $T_{1\rho}$ as a function of rotational correlation time τ.

Equation 1 describes the relaxation time $T_{1\rho}$ as a function of spectral density (*16*). γ_H = gyromagnetic ratio of H (26.7522×10^7 rad/(s·T)),

$$1/T_{1\rho} = \left(\frac{\mu_0^2 \gamma_H^2 \gamma_C^2 \hbar^2}{4 r_{CH}^6}\right)[4J(\Omega_1) + J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H) + 6J(\omega_H + \omega_C)] \quad (1)$$

γ_C = gyromagnetic ratio of C (6.7283×10^7 rad/(s·T)), μ_0 = magnetic permeability of a vacuum ($4\pi \times 10^{-7}$ H/m), $\hbar$ = Plank's constant (1.054×10^{-34} J·s), Ω = decoupling power (spectrometer-dependent), ω_H and ω_C = Larmor frequencies of ^1H and ^{13}C (spectrometer-dependent), and r_{CH} = bond length of C−H ($\sim 1.09 \times 10^{-10}$ m).

The function $J(\omega)$ is the spectral density, dependent on the mobility of the excited molecule, or rotational correlation time τ_c. We can assume a Lorentzian shape to the spectral density, which is described in eq 2 (*16*).

$$J(\omega) \approx \frac{2\tau_c}{1 + (\omega\tau_c)^2} \quad (2)$$

A plot of $T_{1\rho}$ against τ_c according to eqs 1 and 2 appears in **Figure 1**. One can see that the dependence of relaxation time $T_{1\rho}$ on mobility τ_c yields two regions. At lower temperatures and longer τ_c, relaxation time will decrease with increasing temperature, and at higher temperatures and shorter τ_c, relaxation time will increase with increasing temperature. For this reason, it is important to determine in which temperature or time-scale region of this curve the nuclear mobility exists. Since τ_c follows an Arrhenius temperature dependence, increasing the temperature will undoubtedly lower τ_c. Measuring the resultant effect of increased temperature on $T_{1\rho}$ is a commonly used technique to reveal the time-scale regions of τ_c depicted in **Figure 1**. In summary, it is important to remember that relaxation time is not identical to rotational correlation time and mobility.

The relaxation rate for the energy transfer along the C−H bond of the ^{13}C-labeled carbon 1 of glucose (the closest protons contribute the most to the dipolarization) is proportional to the contact time for the magnetization. In an analysis of [^{13}C]glucose, where the C−H bond length is not changing, by varying the contact time we can obtain a parameter of cross-polarization time (T_{CH}) that is dependent on molecular mobility as well as the decay of this energy by longitudinal spin−lattice relaxation ($T_{1\rho}$). To observe these parameters, the cross-polarization must occur faster than the spin−lattice relaxation ($T_{CH} < T_{1\rho}$). **Figure 2** illustrates this process of energy transfer via cross-polarization and spin−lattice relaxation. Equation 3 predicts the transfer of energy from X (proton) to Y (^{13}C) and the loss to Z (spin−lattice in the rotating frame) integrated over contact time. In the following

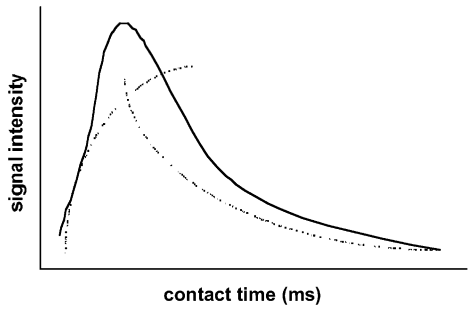

Figure 2. Energy transfer due to cross-polarization and spin–lattice relaxation.

Table 1. Composition of a Model System To Study Mobility

component	amount (dry basis) (%)	amount in control formulation (%)	component	amount (dry basis) (%)	amount in control formulation (%)
sodium caseinate	49.810	74.440 (db)	glucose	16.660	24.810
humectant	33.210		sodium benzoate	0.375	0.747

experiments, this equation was used to calculate $T_{1\rho}$ and T_{CH} from integrated peak areas at multiple contact times.

$$\text{peak area} = \frac{C(e^{-t/T_{CH}} - e^{-t/T_{1\rho}})}{T_{CH}(1/T_{1\rho} - 1/T_{CH})} \quad (3)$$

Model System Preparation. Table 1 shows the model system chosen for the present study, based on that of Labuza et al. (*5*) and representative of high-calorie nutrient bars. Besides their common use as plasticizers in foods, the humectants were chosen for their differing abilities to dissolve glucose. Glycerol is liquid at room temperature, and sorbitol is solid; thus, only glycerol could serve as a solvent for glucose, while both should serve as plasticizers for the caseinate polymer matrix. This is intended to mimic the ability of moisture to also act as a solvent and/or a plasticizer, which are both potential mobility-based mechanisms that could link moisture changes to reaction rate in companion experiments. A control formulation containing no humectant was also studied.

A parent solution was prepared that contained caseinate (Alanate 180, New Zealand Milk Products, Inc., Santa Rosa, CA), β-D-glucose, and sodium benzoate (Sigma-Aldrich Co., Fairlawn, NJ) at a total solids concentration of 9.1%. After dispersion, the pH was adjusted to 8.0 with NaOH, which aided in the dissolution of caseinate. This was also thought to yield a pH of 7.0 in the freeze-dried sample, on the basis of previous work on pH decreases upon freeze-drying food ingredient solutions, although pH was not directly measurable in the solid state (*17*). Glycerol or sorbitol (Sigma-Aldrich) was then dissolved in two portions of the parent solution, while the control solution was not further treated. Aliquots (10 mL) of the three formulations were transferred to 4 cm diameter water activity measurement cups (Decagon Devices, Pullman, WA) for freeze-drying. The cups were immediately placed in a −20 °C blast freezer on a level platform. Samples were frozen, conditioned overnight at −20 °C, and then dried into an amorphous state in a freeze-dryer (Dura-Top MP bulk tray dryer with Dura-Dry MP condenser, FTS Systems, Inc., Stony Ridge, NY) at 100 mTorr over 72 h with the following temperature program: −30 °C for 6 h, −15 °C for 18 h, 0 °C for 24 h, and 25 °C for 24 h. These conditions minimized cracking during drying.

Moisture Sorption Isotherms. Moisture sorption isotherms were performed according to the method of Bell and Labuza (*18*) in desiccators that contained saturated salt solutions at constant relative humidities. Just after freeze-drying, the moisture contents of triplicate samples were determined by Karl Fischer (Aquatest CMA by Photovolt, Indianapolis, IN). After 21 days of equilibration, total moisture was calculated as the initial moisture plus the weight difference after storage.

Glass Transition Curve Determination. After preparation, samples were pelletized to a volume of about 8 mm^3 and a weight of 10−15 mg and were hermetically sealed in aluminum pans (parts 900793.901 and 900794.901, TA instruments, Newcastle, DE). Analysis was performed in duplicate on a Perkin-Elmer DSC-7 with a TAC 7 instrument controller (Perkin-Elmer, Norwalk, CT), calibrated with an indium standard and double-distilled water. A scan rate of 10 °C/min yielded thermograms from which a T_g was measured as the onset of an enthalpy shift in the baseline.

Table 2. Experimental Conditions and Resultant Relaxation Times for Three Model System Formulations at Various Temperatures and Water Activities[a]

formulation	sample	a_w	T (°C)	$T_{1\rho}$ (ms)	T_{CH} (ms)
control	A	0.11	25	5.11	0.046
	B	*0.11*	*25*	*3.85*	*0.066*
	B	*0.11*	*35*	*3.72*	*0.054*
	A	*0.33*	*25*	*3.31*	*0.050*
	A	*0.33*	*35*	*1.95*	*0.043*
	A	0.43	25	2.78	0.042
	A	0.65	25	1.33	0.067
	B	*0.65*	*25*	*0.93*	*0.091*
	B	*0.65*	*45*	*0.94*	*0.078*
glycerol	A	0.11	25	0.86	0.089
	B	*0.11*	*25*	*0.68*	*0.080*
	B	*0.11*	*35*	*0.80*	*0.125*
	A	0.33	25	1.17	0.230
	A	0.43	25	1.81	0.337
	A	0.65	25	52.08	2.259
	B	*0.65*	*25*	*2.64*	*0.895*
	B	*0.65*	*45*	*8.85*	*1.740*
sorbitol	A	0.11	25	0.94	0.043
	B	*0.11*	*25*	*1.53*	*0.037*
	B	*0.11*	*35*	*0.98*	*0.039*
	A	0.33	25	0.78	0.047
	A	0.43	25	0.72	0.052
	A	0.65	25	3.05	1.015
	B	*0.65*	*25*	*0.73*	*0.069*
	B	*0.65*	*45*	*1.87*	*0.213*

[a] Results used to determine the temperature effect on relaxation time are italicized and bracketed.

CP/MAS NMR. Sample disks were prepared as above, except replacing all glucose with the labeled isotope D-glucose-*1*-^{13}C. The freeze-dried samples were equilibrated to an a_w of 0.11, 0.33, 0.43, or 0.65 prior to analysis. The complete temperature and a_w conditions are outlined in **Table 2**. Two replicates (A and B) were prepared. Samples A and B were analyzed to determine the effect of increased temperature (and hence mobility) on relaxation times. Sample A was also used to determine the effect of a_w and moisture content on relaxation time.

Variable-contact-time experiments via CP/MAS NMR were performed on a CMX-400 spectrometer (Chemagnetics, Palo Alto, CA) operating at a spin-locking frequency for ^1H of 50 kHz. After data acquisition, integrated peak areas were plotted vs contact time for each sample. Using the software JMP for Macintosh (v. 3.2, SAS Institute, Inc., Cary, NC), eq 3 was fit to the peak area and contact time data by nonlinear regression. The parameters $T_{1\rho}$ and T_{CH} were thereby obtained, which describe two types of relaxation influenced by the rotational mobility of glucose.

RESULTS

Glass Transition Curve. The glass transition curves are displayed in **Figure 3** as functions of both moisture content and a_w. The T_g curve of the glycerol formulation was the lowest over the a_w range studied. The sorbitol formulation gave a T_g curve about 40−60 °C higher than this, while the control sample showed the highest T_g curve, about 40 °C higher than that of sorbitol. At room temperature, all samples were in the rubbery state, with the exception of the control formulation at $a_w = 0.11$. The Gordon−Taylor model described very well the plasticizing effect of increasing moisture content in comparison to a dry

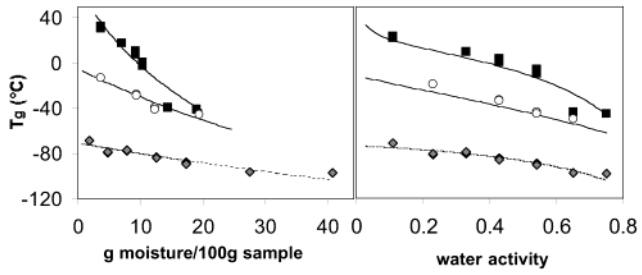

Figure 3. Glass transition curve of model system formulations expressed vs moisture content and a_w: (□) control, (◆) glycerol, (●) sorbitol. Lines represent the Gordon–Taylor model.

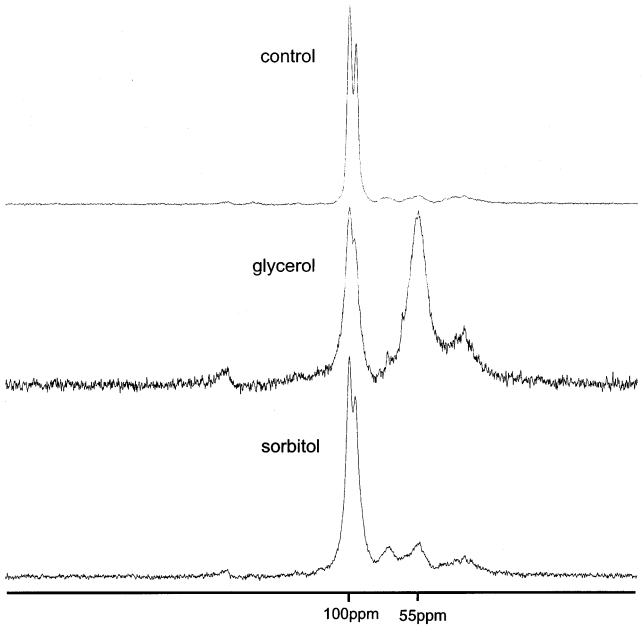

Figure 4. ^{13}C spectra for the three model system formulations. Samples were stored at $a_w = 0.11$ for 3 weeks.

control formulation. The predicted T_g curves using this model are included in **Figure 3**.

Rotational Mobility. The CP/MAS spectra for each of the three formulations containing labeled [^{13}C]glucose appear in **Figure 4** ($a_w = 0.11$ and 0.1 ms contact time). The distinct large double peak at approximately 95 ppm fits the predicted chemical shift for carbon 1 on the glucose molecule (*19*). The peak split is presumably due to the presence of the α and β isomers of D-glucose. The third peak, which appears in the glycerol spectrum at 55 ppm, is most likely for a Maillard reaction product, since glycerol samples showed significant browning during the storage period. High-a_w control samples also showed a peak at 55 ppm (spectra not shown). The typical effect of contact time on peak area is illustrated in **Figure 5**. Complete results are summarized in **Table 2**.

Temperature Effect on $T_{1\rho}$. Figure 1 is central to our interpretation of the relative relaxation times of the control, sorbitol, and glycerol formulations over the range of a_w. Since according to eq 1 and **Figure 1** there can be two correlation times associated with the same relaxation time, it is important to determine in which time-scale region of the curve the measured glucose molecules exist. An increase in mobility, or moving left along the *x*-axis of **Figure 1**, can be reflected in the relaxation time parameters $T_{1\rho}$ and T_{CH} as a decrease, increase, or in some cases negligible change in relaxation time.

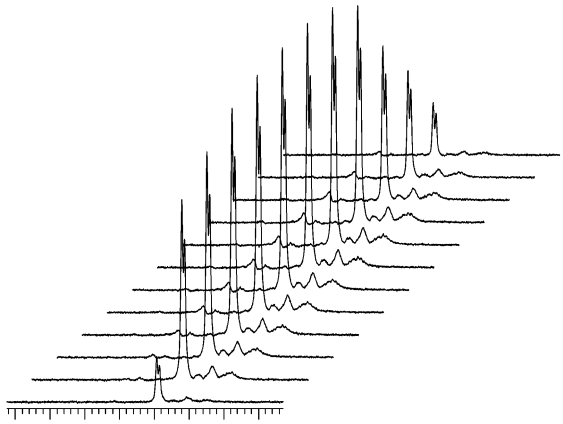

Figure 5. NMR spectra over multiple contact times for the control sample at $a_w = 0.33$ and 25 °C. The prominent peak is at a chemical shift of 97 ppm.

These trends correspond to three regions in **Figure 1**, the right-hand low-mobility region, the center moderate-mobility region, and the left-hand high-mobility region. The terms "high" and "low" mobility are arbitrary, and are only meant to infer a relative mobility across the range of conditions studied. By increasing the temperature experimentally, the rotational mobility of the nuclei was also increased according to the Arrhenius relationship. One was thereby able to determine in what region of **Figure 1** the glucose existed at a low a_w (0.11) and a higher a_w (0.65). As summarized in **Table 2**, the three formulations each existed in different mobility regions of **Figure 1**. As an example, an increase in temperature for sample A of the control formulation resulted in a decrease in $T_{1\rho}$ at both $a_w = 0.11$ and $a_w = 0.65$. Thus, according to **Figure 1**, sample A was in the right-hand or low-mobility region over the entire range of a_w. Any decrease in $T_{1\rho}$ for the control formulation over this range of a_w could be interpreted as an increase in mobility. The mobility in the glycerol formulation corresponded to the left side of **Figure 1**. Thus, for the glycerol formulation we can conclude a trend in $T_{1\rho}$ opposite than for the control formulation. That is, any increase in $T_{1\rho}$ signifies an increase in mobility over all a_w. In the sorbitol formulation, the effect of temperature on $T_{1\rho}$ was unique, and highlights the importance of avoiding the use of relaxation time as a direct indicator of rotational correlation time. Shown in **Table 2**, at a low a_w (0.11) a decrease in $T_{1\rho}$ occurred with an increase in temperature, while at a high a_w (0.65) an increase in $T_{1\rho}$ occurred with an increase in temperature. This behavior shows that, over the entire range of a_w, sorbitol spanned both the left and right sides of **Figure 1**. Therefore, the dependence of $T_{1\rho}$ on mobility at low a_w is the inverse of that at high a_w.

Water Activity Effect on $T_{1\rho}$. Given these trends for increases in temperature (and thus solid-state glucose mobility), we can correctly interpret the effect of a_w on $T_{1\rho}$. These results are summarized for all three formulations in **Figure 6**. As expected, sample A of the control formulation yielded a decrease in $T_{1\rho}$ over the entire range of a_w, signifying an increase in mobility. The $T_{1\rho}$ in the glycerol formulation showed an increase over the entire range of a_w, and this also signified an increase in mobility. Last, the sorbitol formulation showed a decrease in $T_{1\rho}$ up to an a_w of 0.43, at which point a crossover occurred and $T_{1\rho}$ increased. Consequently, in the sorbitol formulation, mobility also increased consistently with a_w.

Temperature Effect on T_{CH}. Similar results occurred when using the relaxation time T_{CH} as a parameter indicative of mobility. According to **Table 2**, a decrease in T_{CH} was indicative

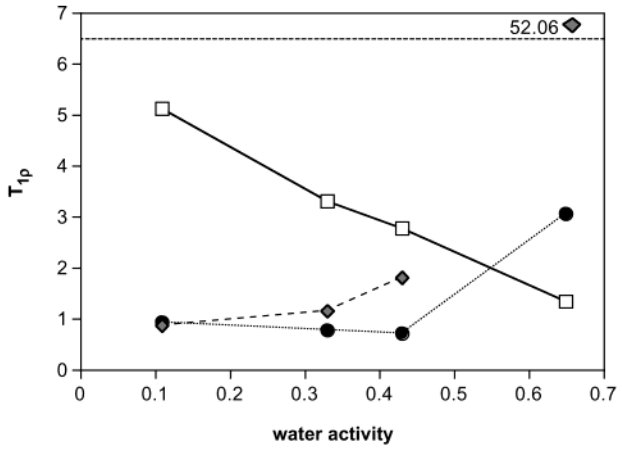

Figure 6. Effect of water activity on $T_{1\rho}$ in the (□) control formulation, (◆) glycerol formulation, and (●) sorbitol formulation. Lines are included only for ease of reading.

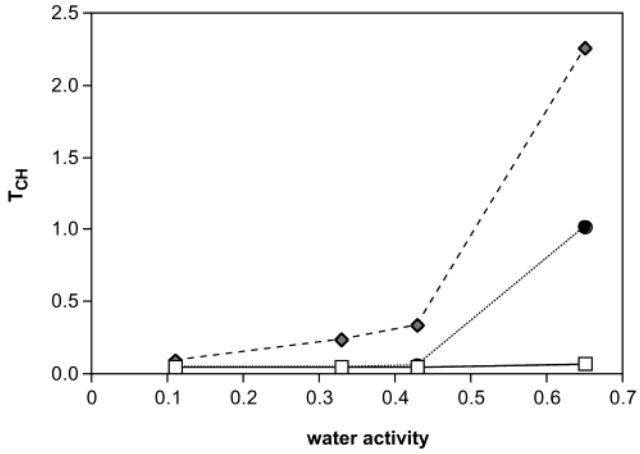

Figure 7. Effect of water activity on T_{CH} in the (□) control formulation, (◆) glycerol formulation, and (●) sorbitol formulation. Lines are included only for ease of reading.

of an increase in glucose mobility in the control formulation. A crossover in T_{CH} was again witnessed when the sorbitol formulation was compared at low a_w and high a_w. Likewise, for the glycerol formulation, an increase in glucose mobility results in an increase in T_{CH}.

Water Activity Effect on T_{CH}. The relative effects of a_w on T_{CH} are similar to those of $T_{1\rho}$, although with somewhat less sensitivity in the control and sorbitol formulations (**Figure 7**). However, there is no discernible change in T_{CH} in the control formulation over the entire range of a_w. Note again that $T_{1\rho}$ and T_{CH} are sensitive to different types of molecular motions, and it is not unexpected that changes in certain molecular motions may not be detectable. Calculating both parameters gives greater confirmation and more sensitivity in finding overall trends.

For the sorbitol formulation, there is no change in the T_{CH} over the range of $a_w = 0.11-0.43$, followed by an increase up to an a_w of 0.65. This may be indicative of the crossover that was expected from the results in **Figure 6**. The glycerol formulation yielded more sensitive changes in T_{CH}, with an increase over the entire range of a_w, signifying an increase in mobility, as previously explained. Again, these changes are in relaxation rate and not molecular mobility. The molecular mobilities are derived from these relaxation rates in the following sections, and because of the existing differences in

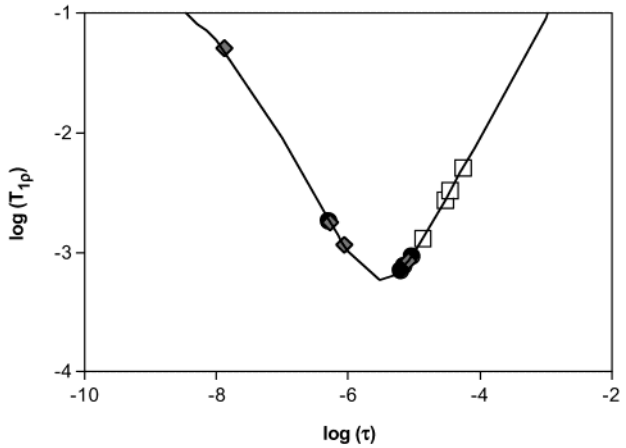

Figure 8. Relative mobilities of three model system formulations across a range of a_w from 0.11 to 0.65: (□) control formulation, (◆) glycerol formulation, (○) sorbitol formulation, (---) theoretical values according to eq 1.

the mobility ranges already discovered above for the three formulations at high and low a_w (according to **Figure 1**), no comparisons among the three formulations can yet be made solely by using **Figures 6** and **7**.

Rotational Correlation Time Calculation. Once these time-scale regions of τ_c were determined, specific values could be calculated according to eq 1. These results are summarized in **Figure 8**. The range of mobilities for each formulation matched those predicted in the previous sections, with glycerol exhibiting the highest mobility over the entire a_w range, sorbitol the second highest, and the control formulation the lowest. The calculated correlation times are plotted vs a_w and moisture content in **Figure 9**. Correlation time decreased with increasing a_w as described previously. Most significantly, the mobility of the glucose was greater in both the sorbitol and glycerol formulations in comparison to the control formulation at equal moisture contents. Therefore, the different mobility ranges for each of the formulations (glycerol > sorbitol > control) were not due solely to a humectant effect, whereby at equal a_w the addition of glycerol and sorbitol increased the moisture content.

DISCUSSION

Comparing the results of the T_g curve (**Figure 3**), which can be used to define the degree of plasticization at 25 °C, with the glucose mobility curve (**Figure 9**), we see that plasticization of the caseinate matrix correlated to an increase in solid-state glucose mobility, that is, that glucose mobility among the three systems followed the same pattern as the glass transition temperature, or polymer mobility. Glycerol yielded the lowest T_g over the entire range of a_w and showed the greatest mobility of glucose (lowest τ_c); this was followed by sorbitol, and finally the control. Additionally, not only did the addition of the two plasticizing humectants cause an increase in solid-state glucose mobility at equal moisture contents and equal a_w values, but an increase in moisture content (caused by an increase in a_w) also caused an increase in glucose mobility and a decrease in the T_g within each of the three formulations. Thus, the plasticizing effect of moisture was also shown to correlate to a change in the mobility of solid-state glucose.

In contrast to the glycerol formulation, in which the glycerol could serve as a solvent, the sorbitol formulation did not contain an additional solvent phase beyond moisture. Despite this, the

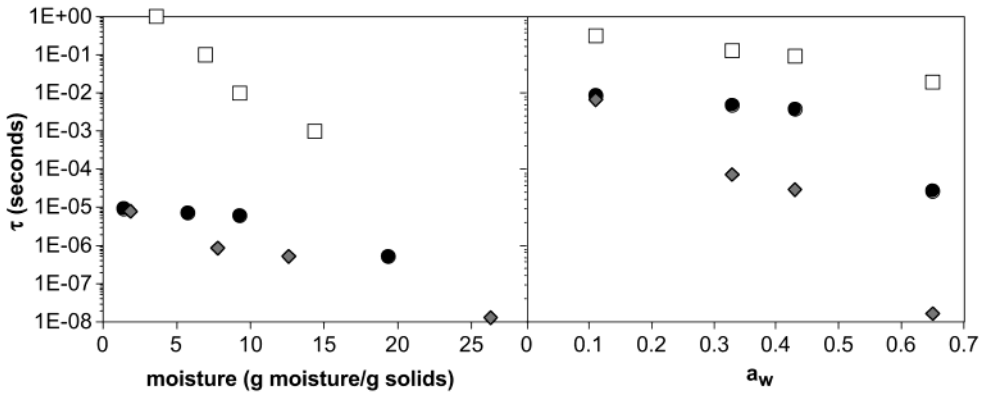

Figure 9. Semilog plot of the effect of moisture content and a_w on the rotational correlation time of the (□) control formulation, (◆) glycerol formulation, and (○) sorbitol formulation.

sorbitol formulation still showed an increase in glucose mobility in comparison to the control at equal moisture contents. Therefore, the increased mobility of glucose in the glycerol formulation is only partially due to the presence of the added solvent glycerol phase. One must also consider the effect that glycerol imparts on the caseinate matrix as a plasticizer. By an identical mechanism, the addition of moisture through exposure to higher a_w could also be expected to cause increased glucose mobility not just because of the additional solvent phase, but also because of the plasticization of the caseinate matrix. In conclusion, these results support the assertion that plasticization will affect the mobility of small solute molecules embedded within.

Simatos (*20*) measured the mobility of a spin-label probe, TEMPO, using EPR spectroscopy. A critical a_w existed at which the probe demonstrated a partitioning into a dissolved and a solidlike state. This critical a_w might represent the moisture content correlating to T_g, though this concept had not been introduced in foods at that time. In general, over a range of a_w, one would expect the increased adsorption of moisture to dissolve and render more mobile a greater proportion of glucose molecules in the aqueous phase.

Therefore, there is a significant point that must be stressed prior to using these results to confirm that plasticization may also be linked to the reactivity of small solutes embedded within the matrix. The technique of CP/MAS NMR yields the net mobility of those glucose molecules that exhibit longer time frame motions. This can be thought of as the glucose that is partitioned into the undissolved or solid state. Whether there is sufficient mobility of these undissolved glucose molecules to allow reactivity has not yet been shown. Instead, it may be that only the more mobile dissolved partition of glucose would be able to participate in chemical reactions. To further examine the role of increased moisture as both a plasticizer and a solvent, additional experiments are needed that would explore the effect of plasticization of the caseinate matrix on the mobility of the *dissolved* or more highly mobile glucose molecules, in contrast to the above experiments on *undissolved* or solid-state glucose. Regardless, the above results are significant for uniquely finding a direct link between plasticization of a polymer and the mobility of small reactant molecules within a food system.

In conclusion, CP/MAS NMR is a very useful tool for directly measuring the mobility of slow-motion solute reactants within a low- to intermediate-moisture food. Compared to a control formulation, the addition of glycerol imparts the greatest mobility to solid-state glucose, followed by sorbitol and last a control formulation with no added humectant. The mechanism by which the mobility of solid-state glucose increased was strongly suggested to be plasticization by sorbitol, glycerol, and moisture. Having established a direct link between polymer matrix mobility and solute molecule mobility in a food system, additional research can unravel the specific mechanism for the role of moisture, perhaps acting as a plasticizer, on the changes of chemical reactivity in low- to intermediate-moisture foods.

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