Role of Water Mobility on Mold Spore Germination

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A sugar transport defected strain of *Aspergillus nidulans* (biA-1 sorA-2) was tested for spore germination in nutrient media containing various water activity (a_w) values and varying amounts of non-nutritive, nontoxic carbohydrates (L-sorbose and cellulose). Freeze-dried media [containing the same nutrient level but different in sorbose/cellulose (s:c) ratio] were adjusted to $0.75-0.97a_w$ at 25 °C before inoculation. Minimum a_w for germination varied with s:c ratio. Because both sorbose and cellulose were not metabolizable and unable to be transported into the cells, the results reflected the molecular mobility of water. ^{2}H NMR T_2 relaxation time correlated well with spore germination time, and it distiguished the difference between water sorbed to cellulose and water in a solution associated with dissolved sorbose. On the other hand, mold germination time correlated poorly with a_w. It was highly dependent on the s:c ratio. Water mobility was found to correlate better with biological activity than a_w because it differentiated the availability between water in dissolved sorbose and adsorbed water in cellulose.

Keywords: *Aspergillus; mold; spore germination; water activity; hydration; moisture; water mobility; NMR; nuclear magnetic resonance; spoilage*

INTRODUCTION

Water is one of the most important factors governing microbial spoilage in dry and intermediate moisture foods. Water serves many functions that influence living systems, including maintaining the turgor pressure (Prosser, 1994), diluting or washing away any inhibitors (Gottlieb, 1973), weakening spore walls (in germination), and transferring nutrients, oxygen, ions, and other beneficial components to the cell. These are collectively referred as the availability of water. Water activity (a_w) or relative vapor pressure of water (P/P_0) has been traditionally used as an indicator of water availability based on an earlier microbiological study using media adjusted to a given a_w by solute addition (Scott, 1953). The empirical use of a_w has many practical benefits. There are some problems due to the fact that the minimum a_w values for growth (Chirife, 1994), survival (Mugnier and Jung, 1984), spore germination (Lang, 1980; Paik, 1985; Lavoie and Chinachoti, 1995), and toxin production (Northolt and Bullerman, 1982) vary greatly with a number of factors limiting its use. These factors are the composition, including types of solutes used to adjust a_w (Christian, 1981; Chirife, 1994), the mode of water sorption (Labuza et al., 1972), and the processing method to adjust a_w, for example, by solute addition versus by drying (Lavoie and Chinachoti, 1997). As a result, prediction of microbial spoilage in food (and shelf life) using a_w can be subject to errors if variations in formulation and processing are not taken into account.

Recent concepts of the role of water dynamics on microbial activities (in particular on mold spore germination) have been subjects of debate (Slade and Levine, 1991; Chirife et al., 1996). Slade and Levine (1991) proposed that the glassy−rubbery transition should be used as the indicator of changes in molecular mobility in systems and that mold spore germination is more directly dependent on T_g (glass transition temperature) than on a_w. The role of water molecular dynamics is not new, and there has been a need to find a measure of molecular mobility that would serve as a better predictor of microbial activity in food. Proposed parameters include water binding energy calculation (Paik, 1985; Gilbert, 1986), the state of water (Lang, 1980), glass transition (Slade and Levine, 1991), local a_w verse global a_w (Hills et al., 1996a,b), and water mobility measured by nuclear magnetic resonance (NMR) (Lavoie and Chinachoti, 1995). Recently, Chirife and his group (Chirife et al., 1996; Buera et al., 1998) reported that the glass transition concept could not be used to explain their mold germination data, although water dynamics may still play a critical role.

Because glass transition is a mechanical, long-range structural relaxation, it may or may not predict the molecular mobility of small molecules (such as water). There have been reports showing an agreement between T_g and an increase in rotational mobility in simple small solute systems (Hemminga and Roozen, 1993). However, in a more complex system such as paracrystalline polymers (e.g., starch or a protein), molecular inhomogeneity is expected and the motions in various domains result in a wide or a narrow distribution in mobility depending on inter- and intramolecular interactions. For instance, in a solid and glassy state of wheat gluten (2% moisture), the onset temperature of molecular motion measured by $T_{1\rho}(^1H)$ (spin relaxation time in the rotating frame) was found to be at ∼−20 °C, far below ambient temperature (Li et al., 1996). In waxy corn starch containing 9.3% D_2O (i.e., in a glassy state at ambient temperature and $T_g = \sim 80-90$ °C range), 80% of D_2O remained mobile (liquid-like) at 30 °C and decreased to ∼50% mobile at −20 °C (Li et al., 1998). Therefore, it can be concluded that water in a glassy state of starch is highly mobile. These strongly suggest

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Table 1. System Composition

sorbose, g	cellulose, g	OSB[a] solids, g	sorbose/cellulose (s:c)
0	1	0.04	0:1
1	0	0.04	1:0
0.5	0.5	0.04	1:1
0.67	0.33	0.04	2:1
0.33	0.67	0.04	1:2

[a] OSB, orange serum broth.

that molecular mobility can be independent of the glassy−rubbery state of a polymer and that water molecular mobility may not be determined by T_g. In some systems (including cellulose), changes in the molecular mobility have been observed by NMR even though there may not be a glassy−rubbery transition experimentally detected using conventional thermal analytical techniques (Vittadini, 1998).

NMR measures the rotational motion of water, but microorganisms are more sensitive to the translational motion of water (Hemminga, 1992; Halle and Wennestrom, 1981). Preliminary results showed that microbial growth correlated very well with NMR mobility, possibly better than a_w or moisture content (Lavoie and Chinachoti, 1995, 1997). Chinachoti (1993) showed that by adding sucrose to a hydrated starch system, a_w decreased but NMR water mobility increased (even though the moisture content was maintained constant). Because of the ability of sucrose to compete for water against starch, the starch becomes osmotically dehydrated, causing a redistribution of water. At a higher a_w, so much water is attracted to sucrose that it dissolves into a solution and the liquid water can freely interchange. Thus, addition of a small solute could lead to increasing mobility outside of the starch granules promoting mold germination. This may serve as a possible explanation, for instance, in the case when a much faster germination time was reported in a sucrose medium than in a starch medium both adjusted to the same a_w but different moisture content (Lang, 1980; Lavoie and Chinachoti, 1995). However, it can be argued that various solutes can change the availability of carbon and this affects the germination rate. Therefore, there is a clear need to separate the nutritional factor from the molecular mobility factor.

In this work, the media contained a constant nutrient concentration (on dry basis) but various amounts of other components that did not serve any direct physiological functions, that is, nonmetabolized, nontoxic and nonpenetrating to the cells or spores. Additionally, rather than measuring the glass transition temperature, a direct measure of water molecular mobility by deuterium NMR was used to monitor the short-range molecular dynamic property of the water molecules.

EXPERIMENTAL PROCEDURES

Working System. A mutant strain of *Aspergillus nidulans* (biA-1 sorA-2, ATCC 26454, Rockville, MD) was selected. This particular strain is defective in sugar transport (Elorza and Arst, 1971). This was confirmed experimentally prior to the experiments. Additionally, *A. nidulans* cannot metabolize cellulose (Reese and Downing, 1951). Thus, L-sorbose and cellulose (Sigma, St. Louis, MO) were used as non-nutritive components at various ratios (Table 1), and their effects should be solely from modification of the environmental conditions. By changing the sorbose/cellulose ratio and a_w, there would be a variation in the sorbed water content resulting in a major impact on the physical appearance of samples, the distribution of water among the components, water molecular mobility, viscosity, and other related physical properties of the cell environment. All mixtures were also added with the same amount of nutrients in the form of orange serum broth (OSB; Difco, Detroit, MI), to support growth.

Materials and Methods. *Sample Preparations.* Cellulose (c), sorbose (s), and OSB, which had initial moisture contents of 7, 0.2, and 96% (dry basis), respectively, were mixed mechanically at different ratios (w/w) (Table 1). First, sorbose was completely dissolved with OSB (extra sterile deionized distilled water was added if needed) before cellulose was added into the solution. The hydrated mixtures were quench-cooled and freeze-dried for 3 days at 20 °C shelf temperature and 70 mTorr vacuum. About 0.1 g of the freeze-dried mixtures was placed in a chamber. This chamber was prepared by placing two rectangular layers (microslide size) of Parafilm (cutout center) onto a hot microslide. This chamber allowed the observation of spore germination directly for most cases. The chambers containing samples were then placed in minidesiccators [proximity equilibration cells (PEC); McCune et al., 1981] containing saturated salt solutions with $0.75-0.97a_w$ (Greenspan, 1977) at 25 °C for ∼5 days. The equilibrated samples were analyzed for sorbed water content (moisture sorption isotherm), water mobility using ^2H NMR, and mold spore germination.

Spore Preparations. A. nidulans mutant strain (biA-1 sorA-2) was maintained on orange serum agar slants in a 4 °C incubation chamber and transferred at biweekly intervals. Before the experiment started, the spores were grown in *Aspergillus* agar medium (ATCC Method 627) plates at 37 °C for 3 days. The inoculated agar was then cut into 1-cm^2 pieces. The pieces were lifted upside down and touched gently onto the surface of empty sterile slides (one piece per slide) to transfer only the spores and not the mycelia onto the slides. These slides were air-dried for a few minutes and then pressed onto equilibrated samples (one slide per sample) to transfer the spores (∼3.5×10^5 spores/0.1 g of samples). The inoculated chambers were placed back into the PEC for incubation.

Spore Germination. During incubation at 25 °C, germination of *A. nidulans* conidia was microscopically monitored daily. A spore showing a germinated hyphal length of ≥10 μm was considered to be germinated. A chamber containing ≥20% of such germinated spores was considered to be positive. In most cases, masses of germinated spores could be observed directly from the slides. However, it was more difficult to observe germinated spores in samples containing a high concentration of cellulose and equilibrated at low a_w (<0.91). In these cases, a small amount of sample was suspended on a slide, diluted with water and then observed for germinated spores.

Deuterium High-Resolution NMR Analysis. The media mixtures were adjusted to various a_w using saturated salt solutions made up with 1:1 H_2O/D_2O water. NMR determination was carried out at 46.7 MHz with an MSL 300 spectrometer (Bruker Instruments, Inc., Billerica, MA). Samples (0.2−0.3 g) were packed into 10-mm NMR test tubes by compressing them at the bottom of the NMR tube with a glass plunger, to reach a sample height of 8−10 mm. The probe was tuned each day prior to acquisition with the sample of higher moisture content. At least two duplicated samples were run for each analysis.

A 90° pulse WALTZ sequence (Shaka et al., 1983) was used. Acquisition parameters used were as follows: 90° pulse of 7.45−780 μs; number of scans, 256; spectral width, 1500−200000 Hz; acquisition time, 0.166−0.012 s; and receiver gain, 36−60 depending on moisture content. Recycle delay was set to be $>5T_1$. Samples were run unlocked and spun at 16 rps.

T_2 was determined with a Carr Purcell Meiboom Gill (CPMG) pulse sequence (Meiboom and Gill, 1958) using the parameters described above. The interpulse spacing (τ) ranged from 5 μs to 500 ms. At least eight different τ values were used for each T_2 determination.

Peak height (Mt) was read in WIN-NMR (Bruker Instruments Inc.), recorded and plotted as a function of time ($2\tau n$, where n or number of echoes = 2). Single- and double-exponential fitting of the data was done by nonlinear curve fitting with a Systat (Systat Inc., Evanston, IL) program.

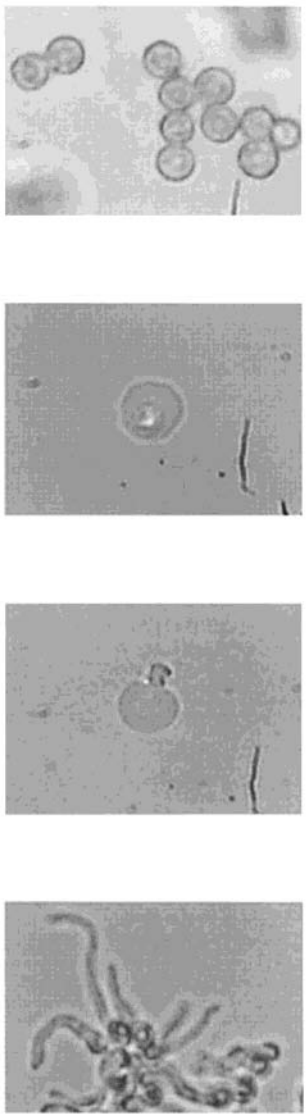

Figure 1. Germination and growth processes of *A. nidulans*, biA-1 sorA-2, at 25 °C in a freeze-dried mixture of sorbose and OSB (1:1 w/v) equilibrated at $0.97a_w$.

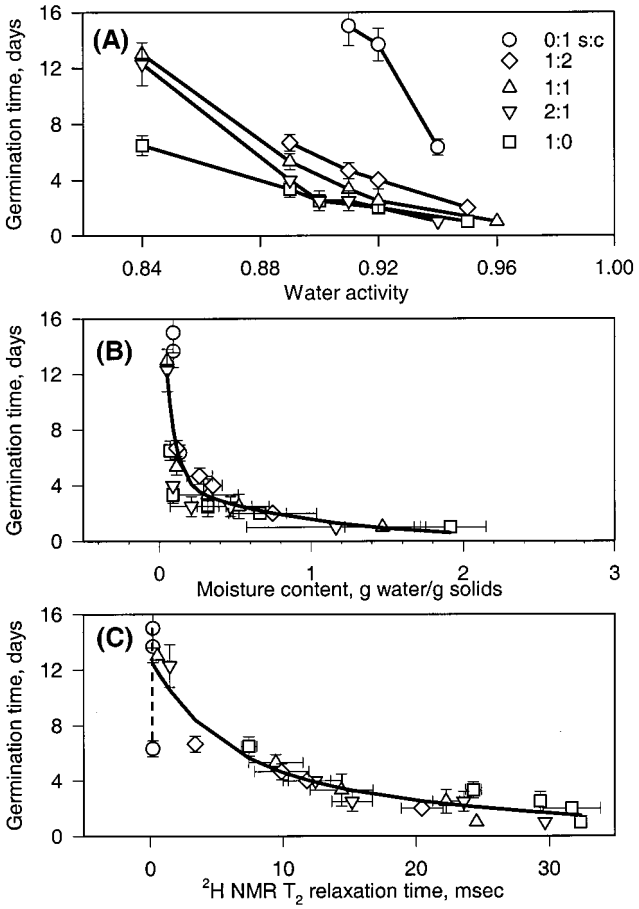

Figure 2. Germination time of *A. nidulans*, biA-1 sor A-2, as a function of water activity (A), moisture content (B), and ^{2}H NMR T_2 relaxation time (C) in a mixture of sorbose (s), cellulose (c), and OSB solids with different s:c ratios at 25 °C.

RESULTS AND DISCUSSION

Germination. During the incubation, germination of spores was monitored daily using a light microscope until germ tubes of the defined length were formed. At first, the spores slightly increased in size (Figure 1B). Then the cell wall began to break out where the germ tube protruded and elongated (Figure 1C,D).

Germination time (lag time) of the mutant *A. nidulans* conidia was recorded and is listed in Table 2 (also plotted in Figure 2). From Table 2 or Figure 2A, germination time decreased with increasing a_w and sorbose/cellulose (s:c) ratio. Differences in germination time among the samples were found to be highly significant, particularly at lower sorbose content and lower a_w. At $>0.91a_w$, 0:1 s:c resulted in a significantly longer germination time than 1:1 s:c but no significant difference among 1:1, 2:1, and 1:0 s:c samples (99% confidence level, Figure 2A). At $\leq 0.91a_w$, there were significant differences among samples depending on a_w, for example, 1:1 s:c $\neq$ 2:1 s:c = 1:0 s:c at $0.89a_w$ but 1:1 s:c = 2:1 s:c $\neq$ 1:0 s:c at $0.84a_w$.

In a sorbose-free sample (s:c = 0:1), no evidence of germination was observed (over a 60-day period) at $\leq 0.89a_w$, whereas in sorbose-containing samples, germination was observed within 3−13 days. Interestingly, the three samples (0:1, 1:2, and 1:0 s:c) showed about the same germination time (~6 days), but their equilibrated a_w values were 0.94, 0.89, and 0.84, respectively. This obviously showed that germination time and minimum a_w value for germination of the *A. nidulans* mutant spores were highly dependent on solid composition. This was supported by earlier studies. Lang (1980) reported that *Aspergillus* spp. spore germination occurred at lower a_w when sucrose was used than when starch was used, suggesting that the germination was dependent on the "state" or mobility of water (so-called solute and polymer water). Paik (1985) also suggested that the binding energy of water might have contributed to the availability of spore germination.

As mentioned earlier, solutes can influence microbial growth physiologically (e.g., by providing nutrients/toxins and/or functioning as compatible solute) and physically (e.g., modifying the physical properties of the media). In this study, sorbose could not enter into the mutant spores due to a sugar transport defect and could not be accumulated intracellularly (which in turn intoxicates the cells) as normally found in a wild-type strain (Moore, 1981; Elorza and Arst, 1971). This mutant strain was also further tested and confirmed the inability to utilize sorbose as a carbon source. In this test, mutant spores were grown in nitrogen base media with 0% sugar (as a control), 1% sorbose, or 1% glucose at 32 °C. No growth was observed in the 0% sugar. Only a trace of growth was observed in the medium contain-

Table 2. Germination Time of *A. nidulas* at 25 °C as a Function of Water Activity, Moisture Content, and ^{2}H NMR T_2 Relaxation Times of Freeze-Dried Mixtures of Cellulose (c), Sorbose (s), and Orange Serum Broth (OSB) at Different s:c Ratios

		^{2}H NMR experiment		spore germination experiment	
sample (s:c)	water activity	moisture content (g of water/g of solids)	^{2}H NMR T_2 (ms)	moisture content (g of water/g of solids)	germination time (days)
0:1	0.94	0.16 ± 0.03	0.37 ± 0.01	0.13 ± 0.03	6.3 ± 0.6
	0.92	0.12 ± 0.02	0.27 ± 0.01	0.09 ± 0.02	13.7 ± 1.2
	0.91	0.12 ± 0.02	0.26 ± 0.04	0.09 ± 0.02	15.0 ± 1.4
	0.89			0.09 ± 0.00	Ng[a]
	0.84	0.08 ± 0.02	0.18 ± 0.03	0.07 ± 0.02	Ng
	0.81	0.07 ± 0.02	0.14 ± 0.02	0.06 ± 0.02	Ng
	0.75	0.05 ± 0.02	0.13 ± 0.02	0.05 ± 0.02	Ng
1:2	0.95	0.57 ± 0.07	20.45 ± 1.56	0.75 ± 0.29	2.0 ± 0.0
	0.92	0.34 ± 0.13	11.80 ± 1.78	0.35 ± 0.06	4.0 ± 0.0
	0.91	0.23 ± 0.04	9.90 ± 2.04	0.27 ± 0.08	4.7 ± 0.6
	0.89			0.12 ± 0.00	6.7 ± 0.6
	0.84	0.08 ± 0.01	0.38 ± 0.15	0.08 ± 0.01	Ng
	0.81	0.07 ± 0.01	0.31 ± 0.02	0.07 ± 0.01	Ng
	0.75	0.06 ± 0.00	0.14 ± 0.05	0.07 ± 0.00	Ng
1:1	0.96			1.47 ± 0.25	1.0 ± 0.0
	0.92	0.57 ± 0.03	22.25 ± 1.01	0.52 ± 0.09	2.5 ± 0.9
	0.91	0.26 ± 0.02	14.41 ± 2.38	0.32 ± 0.20	3.3 ± 1.2
	0.89	0.20 ± 0.01	9.46 ± 2.04	0.11 ± 0.01	5.3 ± 0.6
	0.84	0.07 ± 0.01	0.56 ± 0.09	0.06 ± 0.00	13.0 ± 0.0
	0.81	0.06 ± 0.00	0.48 ± 0.30	0.05 ± 0.01	Ng
	0.75	0.05 ± 0.00	0.19 ± 0.10	0.05 ± 0.01	Ng
2:1	0.94			1.16 ± 0.59	1.0 ± 0.0
	0.91	0.40 ± 0.14	23.57 ± 0.0	0.47 ± 0.25	2.5 ± 0.7
	0.90	0.22 ± 0.04	15.21 ± 1.53	0.21 ± 0.14	2.5 ± 0.7
	0.89	0.17 ± 0.03	12.44 ± 2.03	0.09 ± 0.01	4.0 ± 0.0
	0.84	0.05 ± 0.00	1.48 ± 0.15	0.05 ± 0.01	12.3 ± 1.5
	0.81	0.04 ± 0.01	0.87 ± 0.08	0.04 ± 0.00	Ng
	0.75	0.03 ± 0.01	0.60 ± 0.42	0.03 ± 0.01	Ng
1:0	0.95			1.91 ± 0.24	1.0 ± 0.0
	0.92	0.43 ± 0.07	31.72 ± 2.19	0.66 ± 0.18	2.0 ± 0.0
	0.90	0.21 ± 0.03	29.32 ± 0.24	0.32 ± 0.07	2.5 ± 0.7
	0.89	0.14 ± 0.00	24.29 ± 0.59	0.09 ± 0.00	3.3 ± 0.6
	0.84	0.05 ± 0.01	7.43 ± 0.59	0.05 ± 0.02	6.5 ± 0.7
	0.81	0.03 ± 0.00	1.91 ± 0.38	0.03 ± 0.01	Ng
	0.75	0.02 ± 0.00	0.84 ± 0.35	0.02 ± 0.01	Ng

[a] Ng, no germination after 60 days.

ing 1% sorbose after 4 days, but a significant amount of mycelia (10-fold higher) was observed in the medium with 1% glucose. The mycelia that grew in the medium with 1% sorbose were further transferred onto plate count agar to test for survivability after the sorbose exposure to determine whether it was because of the intracellular accumulation of sorbose or lack of nutrient availability for spores to grow. Growth was observed after 2 days (32 °C), indicating that sorbose did not enter and entoxify the mutant spores. The mutant spores were also inoculated onto pure cellulose equilibrated against water, and no evidence of germination was observed after 1 month. This confirmed the results found in the literature that *A. nidulans* does not grow on cellulose (Reese and Downing, 1951). Thus, the influences of both cellulose and sorbose were mainly on the external environment surrounding the cells by modifying the physical properties of the media. Therefore, the differences observed in Figure 2A were due to the modifications of the outside environment of the spores.

Moisture Sorption Isotherm. Moisture sorption isotherms of the mixtures of cellulose (c), sorbose (s), and orange serum broth solids (OSB) at different ratios of s:c and of the controls are shown in Figure 3. Depending on the amount of sorbose present, the sorption isotherm of the mixtures varied greatly. When graphed over an entire a_w range, they gave a sigmoidal plot for the cases of cellulose and cellulose−OSB mixture but an exponential shape for other cases. Similarly to

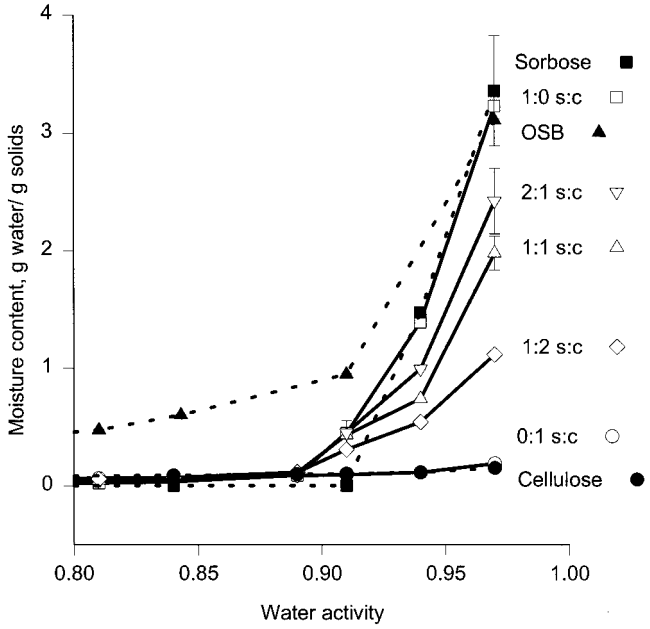

Figure 3. Moisture sorption isotherms at 25 °C of experimental components, pure cellulose (c), sorbose (s), and OSB. Mixture of s:c ratios all contained 4.0% OSB on a dry weight basis.

freeze-dried solute−polymer systems (Chinachoti and Steinberg, 1984), the amount of water sorbed by sorbose

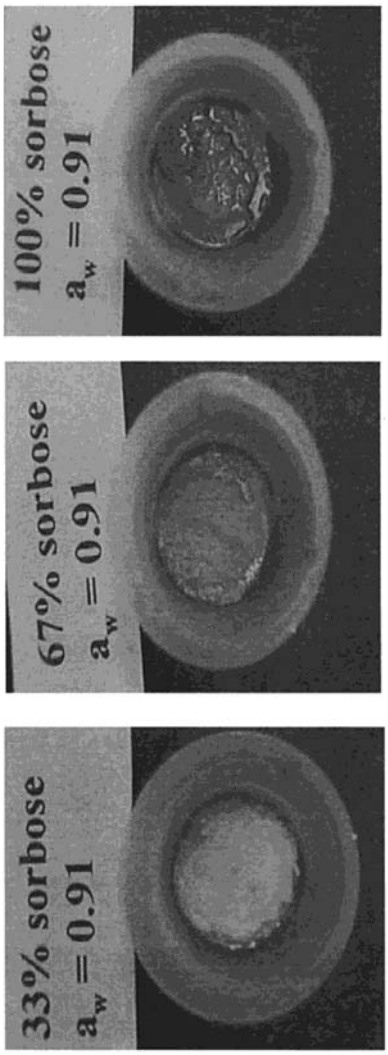

Figure 4. Effect of sorbose content on physical appearance of freeze-dried mixtures of cellulose (c), sorbose (s), and OSB (at different s:c ratios) equilibrated at $0.91a_w$.

at $\geq 0.91a_w$ dramatically increased, and at $0.91a_w$ solubilization of sorbose occurred (visually observed). Cellulose and OSB solids sorptive ability was much less significant than that of sorbose, in particular at $\geq 0.91a_w$. This has been known to be due to the higher solubility of smaller solutes (Chinachoti and Steinberg, 1984). The sorption isotherms of the s:c mixtures behaved very similarly to that of sorbose; that is, more water was associated with sorbose (a small solute) than with cellulose (a polymer).

Upon sorbing water, the samples' physical appearance also changed. It was observed that at $0.91a_w$, sorbose went through a phase transition from crystals to semisolid and to completely liquid, so that the mixture with a higher amount of sorbose content (1:0 s:c) was more visibly liquid-like compared to lower sorbose contents (Figure 4). For example, at $0.91a_w$, the 1:0 s:c (sorbose rich) sample (Figure 4A) was more wet or liquid-like than the 1:2 s:c sample (Figure 4B) and the 2:1 s:c sample (Figure 4C).

It was obvious that sorbose played an important role in determining the equilibrated moisture content and physical appearance (and microstructure) of the mixtures. This effect is likely to lead to a significant change in the molecular dynamics, which plays a significant role in mold germination.

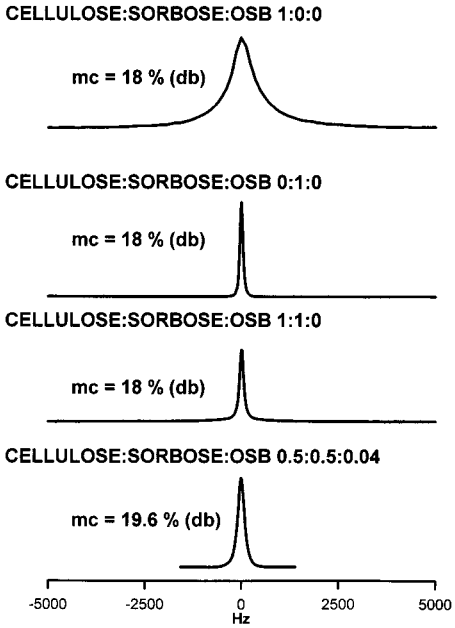

Figure 5. ^{2}H NMR spectra of cellulose, sorbose, cellulose/sorbose (1:1), and cellulose/sorbose/OSB (0.5:0.5:0.04).

^{2}H NMR. The deuterium NMR spectra, from which the line width was measured, are shown in Figure 5 for cellulose, sorbose, and mixture samples (line width for pure water was 29.8 Hz or $T_2 = 5.3$ ms). The line width of the spectrum for cellulose, 520 Hz, was found to be wider than those of pure sorbose (180 Hz), a cellulose/sorbose/OSB (1:1:0) mixture (200 Hz), and a cellulose/sorbose/OSB (1:1:0.04) mixture (200 Hz), all at similar moisture contents (Figure 5). This indicated that the addition of sorbose decreased the line width of a system (increased mobility) effectively and that the presence of OSB had very little or no effect on the line width.

Figure 6 represents the plots of ^{2}H NMR T_2 relaxation time (mobility) against a_w, moisture content, and percent sorbose. It can be observed that ^{2}H NMR T_2 relaxation time increased with moisture and sorbose contents. In Figure 6A, T_2 relaxation time increased with a_w in a similar fashion as the moisture content, which was earlier shown in the water sorption data (Figure 3A). This suggests that T_2 might be directly proportional with the amount of water present in the sample.

However, the relationship between T_2 and moisture content (Figure 6B) indicated that T_2 was not entirely moisture dependent; significant discrepancies were observed due to the variable sorbose content. This suggests that the deuterium T_2 NMR mobility increased not only with water content but also with the sorbose content; that is, sorbose greatly influenced the molecular mobility of water (Figure 6C). It seemed that T_2 of the samples would reach an asymptotic level at high moisture content range (>0.5 g of water/g of solids).

Vittadini (1998) studied ^{17}O NMR in comparison with ^{2}H NMR on the same system as the present study and found that the ^{17}O NMR T_2 relaxation time plotted similarly against moisture content gave a curve, regardless of s:c ratio. This difference in ^{2}H and ^{17}O NMR (the former being both sorbose- and water-dependent and the latter being only water-dependent) could be due to the rapid exchange between the solid protons and the aqueous deuterons experienced in the ^{2}H NMR experi-

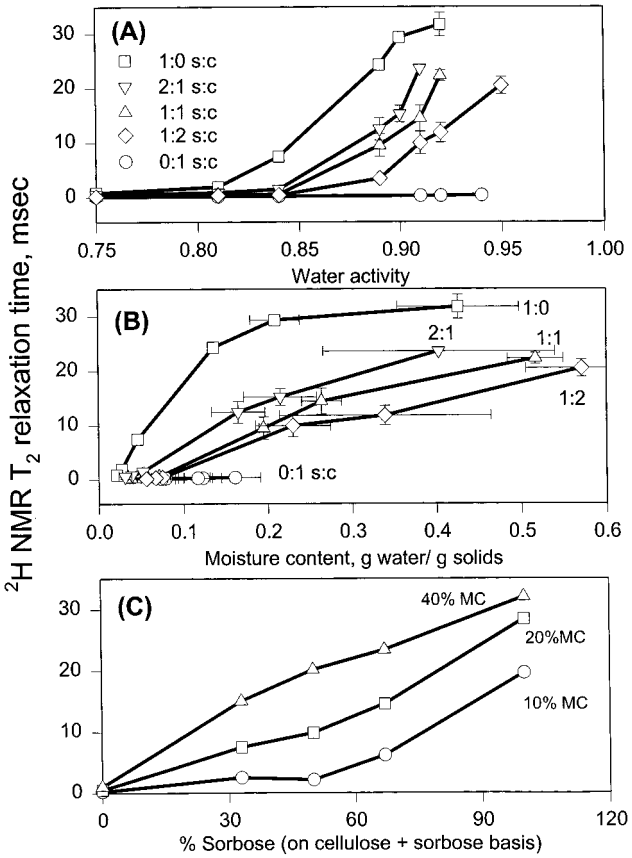

Figure 6. Relationship between ^{2}H NMR T_2 relaxation time and water activity (A), moisture content (B), and percent sorbose (C) of mixture of cellulose (c), sorbose (s), and OSB solids (OSB) at different s:c ratios.

ment (thus, the deuterium NMR signal also included some contributions of the solids). This process was more likely in higher sorbose samples and at higher a_w. Additionally, the experiments were performed at different moisture ranges, that is, <60% moisture range for ^{2}H NMR and >40% moisture range for ^{17}O NMR.

When associated with a small solute (such as sorbose), the water molecules in a solution are allowed to rapidly interchange within the NMR time frame (Chinachoti, 1993). It has been earlier proposed (Lang and Steinberg, 1983) that water associated with small solutes, for example, NaCl and sucrose, is different in molecular dynamic state and more "free" (so-called "solute water") than water associated with polymers, for example, starch and casein (so-called "polymer water"). The dependence of NMR observed T_2 on water and sorbose contents (dependent on the nuclei used) suggested that the water molecules experienced different environments (becoming more bulk water or "solute" water), which in turn might influence mold activity.

Water sorption behavior in solute−polymer mixtures has been studied in detail by Chinachoti and co-workers (Chinachoti and Steinberg 1984, 1985, 1986a,b, 1988, 1989; Chinachoti et al., 1988; Chinachoti, 1990; Chinachoti and Stengle, 1990) over the range $0.33-0.97a_w$. Given that there is negligible interaction between a solute and a polymer, the amount of water sorbed by a solute−polymer mixture can be predicted or calculated by assuming that each component sorbs its compliment amount of water. A hygroscopic solute (e.g., sugar) is dissolved in a solution at a saturation a_w, and sorption of water at above this a_w leads to a solution of sugar. In the present work, free sorbose contributed to the increased association with water at $a_w \geq 0.91a_w$. As shown in Figure 3, the dissolution of sorbose occurred at $\geq 0.91a_w$ when the amount of water sorbed mainly by sorbose sharply rose, indicating the presence of bulk water in the sorbose−water solution within the mixtures. This was evident also in the ^{2}H NMR line-narrowing effect (Figure 5) when one may suggest that the water in cellulose is more bound (broad NMR spectrum) than that in sorbose (narrow NMR spectrum). In our earlier study on sucrose−starch systems (Chinachoti and Stengle, 1990), ^{17}O NMR line width also sharply narrowed with added sucrose, strongly indicative of bulk water when sucrose was dissolved in a solution; a strong correlation between the ^{17}O NMR intensity and calculated "solute" water sorbed by sucrose, was observed. ^{17}O NMR could not measure water sorbed more strongly to starch at $\leq 0.93a_w$ (Chinachoti and Stengle, 1990); adsorbed water in ~0−20% was concluded to be less mobile that bulk water in a solution. Thus, one may apply the same analogy to the present sorbose−cellulose systems; that is, water associated to dissolved sorbose is more free or bulklike, and water in cellulose is less mobile (and only becomes mobile or free at $a_w < 0.93$. Therefore, addition of sorbose at a given a_w leads to a higher amount of water sorbed, and this added water is bulklike (evident in NMR line-narrowing effect, Figure 5).

Correlation of Germination Time and Other Parameters. The amount of liquid water and water mobility in samples with various s:c ratios was related to germination time. Total moisture contents for all samples were somewhat related to germination time (Figure 2B) in two phases. The curve showed a steep drop in germination time at a lower moisture content range (near 0%) and a leveling out as moisture content increased above ~0.2 g of water/g of solids. In the very low moisture content region, there was only a small difference in moisture content (0−0.2%) but a wide variation in germination time (3−12 days). This suggested that spore germination was very sensitive to this region of moisture content. In a higher moisture range, the opposite was found. The moisture content when the break on the curve occurred (~0.2 g of water/g of solids) roughly coincided with the steep increase in water sorbed by added sorbose at $\geq 0.91a_w$ (Figure 3). It was possible that samples with <0.20 g of water/g of solids mostly were samples containing no dissolved sorbose and those with >0.20 g of water/g of solids contained dissolved sorbose. Therefore, the change in the germination time relationship (in Figure 2B at ≥0.2 g of water/g of solids) was likely due to the increase in bulklike water associated with sorbose in a solution.

To verify this point, T_2 relaxation time was measured and correlated with the spore germination time (Figure 2C). There were two sets of behavior observed. In sorbose-containing samples, the relationship followed a single-exponential fitting (solid line $r^2 = 0.94$), showing germination time dependence on T_2 relaxation time. Among these sorbose-containing samples, the relationship was found to be independent of the sorbose content (s:c) ratio. In sorbose-free samples (open circles, Figure 2C), a steeper linear line was observed, suggesting a different relationship. This deviation was clearly related to the lack of sorbose and its associated water. According to some investigators [e.g., Duckworth (1981) and Lang and Steinberg (1983)], the presence of small solutes

leads to solvation and a drastic increase in water mobility and bulklike state ("solute" water; Lang and Steinberg, 1983). The strong dependence of germination time on T_2 relaxation time was also found by others (Lavoie and Chinachoti, 1995) in the case of *A. niger* in sucrose−starch systems. The data suggested that mold germination responded differently to bulklike water in a solution ("solute" water) as opposed to water in hydrated cellulose ("polymer" or "multilayer" water). It is speculated that when cellulose is hydrated beyond $0.97a_w$, bulk or "capillary" water starts to appear and mold spore germination and T_2 relaxation time dramatically increase.

Therefore, it was concluded that the molecular dynamic properties of water provide valuable information that can describe and predict the availability of water to mold spore germination. It is important to note that there is usually no sharp transition between the germination time and the growth process. In fact, by the time hyphae are normally observed, growth has already begun. It is, therefore, reasonable to state that the dynamic property of water (mobility) not only influences the hydration of the spore walls, which triggers necessary physiological responses for germination, but also allows nutrients to migrate and be utilized by the cells. Additional work especially on hyphal growth is being investigated. Although it is the translational mobility of the nutrients and water molecules that would contribute most significantly in this biological process, NMR rotational mobility of water was observed to correlate quite well with spore germination in this study.

Conclusions. The purpose of this work was to identify the relationship between germination time and the water mobility or state in various substrates. The germination time of the L-sorbose resistant strain of *A. nidulans* (biA-1 sorA-2) was observed to be highly dependent on solid composition and a_w, but correlation was best with ^{2}H NMR T_2 relaxation time (independent of solid composition). Liquid water in a solution with sorbose (relatively high molecular mobility) clearly facilitated spore germination, whereas water in cellulose was far less mobile ($<0.97a_w$) and showed a different effect on spore germination behavior. Water NMR mobility is a parameter to describe mold germination at a molecular level when a_w failed to predict mold germination with changing solid composition. Using a_w to monitor microbial safety is dependent on food composition (minimum a_w varies with systems). In this case, NMR was found to serve as a better indicator of water states and their different availabilities to mold spore germination. Even when in this case no specific solute effects are present, a_w showed a marked discrepancy in predicting germination among different media, but ^{2}H NMR T_2 relaxation further differentiated the bulklike water in a solution from the less-mobile, adsorbed water on cellulose in availability. Further investigation on how this can be further applied in real food systems is underway.

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