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NMR water mobility in xanthan and locust bean gum mixtures: possible explanation of microbial response

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Abstract

Molecular and structural mobility of xanthan and locust bean gum mixtures (with and without mannitol) were studied in relation to microbial stability. Molecular mobility was measured by solid state ^{1}H and ^{2}H NMR and by ^{2}H high resolution NMR while differential scanning calorimetry and dynamic mechanical analysis were used to investigate structural mobility. The NMR mobile signal was found to increase significantly with increasing moisture content and, at above 30% moisture content, the solid component disappeared with observed structural collapse. Cell survival decreased with moisture content increasing from 6 to 15% corresponding with an increasing mobile proton and deuterium signals (i.e. increased mobility). Presence of mannitol protected the cells from death at increasing moisture content and a relationship with a lower mobile ^{1}H signal (as compared with the control) seems to be present. A strong mannitol–water interaction, leading to a decreased mobility, is suggested to play a role. No evidence of a glassy to rubbery transition was observed from DSC and DMA analyses, suggesting that structural relaxation did not play a significant role in NMR solid-liquid transition, i.e. NMR molecular mobility. © 2002 Published by Elsevier Science Ltd.

Keywords: Xanthan gum; Locust bean gum; Water mobility; NMR; Glass transition; Food stability

1. Introduction

Safety and stability of foods is greatly influenced by water. Water activity (Labuza, McNally, Gallagher, Hawkes & Hurtado, 1972) has been used to describe water availability but it cannot theoretically be related to food stability since it is defined for systems in thermodynamic equilibrium. Its use as an empirical parameter is, however, very useful and widely spread (Franks, 1982, 1991). Use of water activity in food systems has been challenged (Franks, 1982, 1991; Slade & Levine, 1991; Gilbert, 1986) since it has been often found to be composition dependent (Mossel, 1975; Paik, 1985). For example, microorganisms responded differently depending on the solute used to adjust water activity (Chirife, 1994; Lavoie, 1994; Mossel, 1975; Ballesteros, Chirife & Bozzini, 1993).

Search for an alternative parameter to replace water activity in defying water availability and, consequently, food stability has been on-going in recent years. This search was based on the concept that reactions (leading to, for example, food spoilage) are governed by the dynamic processes and molecular mobility (van den Berg, 1991). Glassy to rubbery transition was introduced as a possible factor directly related to food stability (Roos, Karel & Kokini, 1996; Hemminga, Roozen & Walstra, 1993; Slade & Levine, 1991). This approach was based on a rather simplified assumption that structural relaxation is directly related to molecular relaxation. Molecular instability in the glassy state, however, has been observed such as in the case of non-enzymatic browning and enzyme inactivation in glassy polyvinylpyrrolidone (Bell, 1996).

Mobility is defined by the analytical parameters measured according to time frames or frequencies of the methods used. For example, nuclear magnetic resonance (NMR), can detect molecular motions in the picosecond–millisecond range (McBrierty & Packer, 1993), while differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA) detect relaxations over a milliseconds–second range (McBrierty & Packer, 1993). A correlation between structural (glass transition, by DSC) and molecular mobility (as detected by electron spin resonance) has been reported for the case of sugars (Roozen, Hemminga & Walstra, 1991; Hemminga et al., 1993). In more complex,

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heterogeneous food systems, however, a vast discrepancy between molecular and structural mobility can be expected. For example, water molecular mobility was observed in a glassy state of waxy corn starch and glassy water-maltose (Li, Dickinson & Chinachoti, 1998; Hills & Pardoe, 1995) as well as high molecular mobility of diluents (trioctyl phosphate, triphenyl phosphate and tetraxylyl hydroquinone diphosphate) in glassy synthetic polymer (poly(xylylene ether) and BPA polycarbonate; Cauley, Cipriani, Ellis, Roy, Jones & Inglefield, 1991).

One of the means to probe water availability is to investigate microbial cell activity in water-limited environments. Survival of *Rizobium japonicum* in freeze dried xanthan and locust bean gums (1:1 ratio), adjusted to variable water activity has been reported (Mugnier & Jung, 1985). Cell survival was higher at lower moisture contents (<10% dry basis or a_w 0.07–0.11) and mortality rate increased with increasing moisture from 10 to 25% (dry basis). The presence of 14% mannitol (dry basis) in the freeze dried gel was shown to have a protective effect on the cells, increasing their survival at moisture content >17% dry basis ($a_w > 0.3$), as compared with the control. It was hypothesized that mobilization induced by hydrating water was the cause of death and that mannitol altered the process. Water molecular mobility could be responsible for the different microbial responses.

The objective of this work was to analyze system mobility (structural and molecular level) in xanthan and locust bean gum systems in order to evaluate and prove the previously proposed hypothesis on dependence of microbial survival on water mobility.

2. Materials and methods

2.1. Sample preparation

Gels made of xanthan and locust bean gum (1:1 ratio by weight, SIGMA Chemicals, St Louis, MO) were prepared following Mugnier and Jung (1985). Two batches (200 ml) of double distilled water (preheated at 70–80°C) were added to 3 g of xanthan gum or 3 g of locust bean gum, respectively. The two batches were stirred for 30 min at 70°C and then cooled to 40°C. Then, 100 ml of double distilled water (control sample) or 100 ml of mannitol solution (5 g/l, for mannitol added sample) was added to each batch. They were then mixed to form a gel that was poured into a tray. In the mannitol added sample, mannitol (Fisher Scientific, Fair Lawn, NJ, USA) was added at 14% (dry basis). Each of the gels was then quench-cooled with liquid nitrogen and freeze-dried (VIRTIS, Gardiner, NY, USA) at 15°C for 48 h. Freeze dried mixtures were then equilibrated at 32°C at different water activities by placing the sample in a sealed container over a saturated salt solution of known a_w (ranging from 0.06 to 0.97 a_w, Greenspan, 1977) or in some cases over deionized distilled water. The saturated salt solutions were prepared either with H_2O or 50% D_2O (samples for ^2H high resolution NMR analysis) or 99% D_2O (samples for ^2H solid state NMR analysis). The samples at $a_w < 0.9$ reached an equilibrium (no further weight change) in 7 days while samples with $a_w > 0.9$ became moldy after 4 days only; in this case, samples were tested before an equilibrium was reached and moisture content was determined independently by vacuum oven method (AOAC method 925.90).

Most data points are in duplicate except for a few higher moisture samples.

2.2. Proton solid state NMR

Samples were packed in a ZrO_2 rotor (7 mm OD Bruker Instruments, Inc., Billerica, MA) by sandwiching 1–3 mg of material in between two spacers (custom made, tri–fluoro–chloro–ethylene). A Bruker ASX 300 spectrometer (Bruker Instruments, Inc., Billerica, MA, USA) was used. A DEPTH pulse sequence (Bendall & Gordon, 1983) was used to minimize contribution to the signal due to extraneous sources such as probe components. The data were acquired with acquisition time of 102.5–9.5 ms (depending on moisture content), 90° pulse width of 5.75 μs, receiver gain of 512, spin rate of 4000 rpm, 64 number of scans and 10–500 KHz spectral width. The FID was exported to WIN-NMR® (Bruker Instruments, Inc., Billerica, MA, USA) software where it was Fourier transformed, phase and baseline corrected. Overlapping spectra were deconvoluted by Peakfit® (Jandel Scientific, San Rafael, CA, USA) analysis. Peak intensity (area) and line width (LW) of the deconvoluted peaks were recorded.

2.3. Deuterium solid state NMR

Approximately 0.5–1 g of samples were packed into glass tubes specific for wide-line probe and analyzed with a ASX 300 (Bruker Instruments, Inc., Billerica, MA, USA) spectrometer. The sample tubes were sealed to avoid water loss during acquisition. The probe was tuned each day of acquisition with perdeuterio-poly(methylmethacrylate). An ECHOCYCLE pulse sequence, was used (Ronemus, Vold & Vold, 1986). Data were acquired with spectrum width of 500 KHz, 90° pulse width of 1.9 μs, acquisition time of 50 ms and a number of scans of 56,000. Recycle delay time (RD) was first set to 1 s to allow for complete relaxation then observe the entire spectrum and subsequently to 1 ms to selectively record only the narrow component. Area of the spectra was measured by integration over a range of 220 KHz centered on the narrow water peak. Area of the wide component was calculated as the relative difference between total and narrow component areas.

2.4. Deuterium high resolution NMR

^2H NMR determination was carried out using a MSL 300 spectrometer (Bruker Instruments, Inc., Billerica, MA, USA). Samples (0.2–0.6 g) were packed into 10 mm

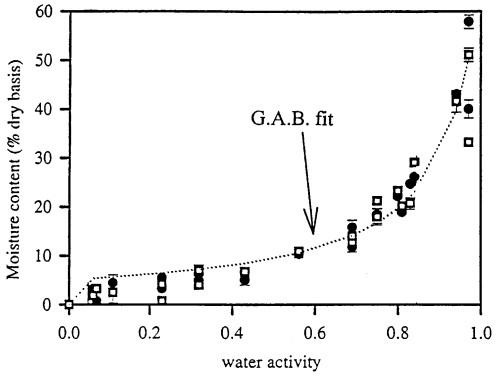

Fig. 1. Water sorption isotherm for xanthan and locust bean gum mixtures (1:1) measured at 32°C. Control (solid circles) and 14% mannitol added (empty squares) samples are shown. Experimental data were fitted to the Guggenheim–Anderson–deBoer (GAB) equation. GAB fits of the two components overlapped.

NMR tubes down to 8–10 mm in height. The probe was tuned each day of acquisition with the sample of higher moisture content analyzed that specific day. A 90° WALTZ pulse sequence (Shaka, Keeler, Frenkiel & Freeman, 1983) was applied using 90° pulse width of 7.25–7.65 ms. The data were acquired with a number of scans of 256, spectral width of 1500–20,000 Hz, acquisition time of 166–12 ms. Recycle delay was set to be 100 ms ($>5T_1$). Samples were run unlocked and spun at 16 rps.

Spin lattice relaxation time (T_1) was determined by inversion recovery (Derome, 1987) with an interpulse spacing (τ) ranging from 50 ms to 5 s depending on the sample relaxation time. Data points were collected over at least eight different τ values and with a recycle time of 100 ms. An FID was obtained and Fourier transformed to obtain a spectrum. Peak height (M_t) was measured and analyzed as a function of τ to fit single or double exponential model (SYSTAT Inc., Evanston, IL, USA).

Spin–spin relaxation time (T_2) was determined with a Carr Purcell Meiboom Gill (CPMG) pulse sequence (Carr & Purcell, 1954; Meiboom & Gill, 1958). The interpulse spacing (τ) was 5–5000 ms range. At least eight different t values were used for each T_2 determination. The acquired FID was Fourier transformed to give a spectrum. Peak height was measured and analyzed as a function of $2\tau n$, where n, number of echoes (two in this case). Single and double exponential fitting was performed using non linear curve fitting program (SYSTAT Inc., Evanston, IL, USA).

2.5. Differential scanning calorimetry (DSC)

A known amount (8–10 mg) of sample was placed in sample pans (stainless steel hermetic, Perkin Elmer, Somerset, NJ, USA) and analyzed on a DSC 100 (Seiko Instruments, Torrance, CA, USA). An empty pan was used as a reference. DSC was calibrated with Indium. The sample and the reference were cooled to −80°C with liquid nitrogen, heated up to 100°C at a heating rate of 2°C/min, cooled immediately to −80°C and rescanned up to 200°C. Glassy to rubbery transitions can be observed as a endothermic baseline shift due to a change in heat capacity of the sample. Samples of equal moisture content were analyzed at least in duplicate.

2.6. Dynamic mechanical analysis (DMA)

DMA analysis were performed using a DMS 110 instrument (Seiko instrument International, Torrence, CA, USA) with three-point bending mode at 1, 5, 10, 20 and 50 Hz frequencies (Hallberg & Chinachoti, 1992). Samples were cut into bars (50 × 12 mm × variable thickness, 0.1–5 mm) with or without previous compression with Carver press at room temperature. Samples were lightly clamped (on both ends) and cooled by liquid nitrogen to −20°C. The samples were tightened and the sample further cooled to −80°C. They were then heated up to the desired temperature at a heating rate of 2°C/min. Samples of equal moisture content were analyzed at least in duplicate.

2.7. Electron scanning microscopy (SEM)

A JEOL 5400 scanning electron microscope (JEOL Technics, LTD, Tokyo, Japan) was used. Samples were equilibrated over saturated salt solutions, quench cooled, cryofractured and freeze dried. The samples were sputter coated with Au/Pd for microscopic observation.

3. Results

3.1. Water sorption behavior

The water sorption isotherms characteristic of xanthan and locust bean gum gels, equilibrated to various water activities at 32°C to obtain their equilibrated moisture contents, are shown in Fig. 1. Moisture content increased with water activity in a sigmoidal fashion for both control and mannitol added (14% dry basis) samples. No difference was found between the control and the sample with mannitol. The water sorption isotherm was fitted according to the Guggenheim Anderson de Boer (GAB) equation (Rizvi, 1986).

$$M/M_0 = (C_g K a_w)/[(1 - K a_w)(1 - K a_w + C_g K a_w)]$$

where M is the equilibrated moisture content (% dry basis), M_0 and K are constants.

The fitting ($R^2 = 0.96$, control sample and $R^2 = 0.94$, 14% mannitol) gave a M_0 of 5.0% (water, dry basis) for the control sample and 5.2% (dry basis) for the mannitol added sample. The GAB equation was not representative of the experimental data at low water activities (<0.1).

3.2. Narrow and wide components by ^1H and ^2H solid state NMR

Characteristic ^1H and ^2H solid state NMR spectra at

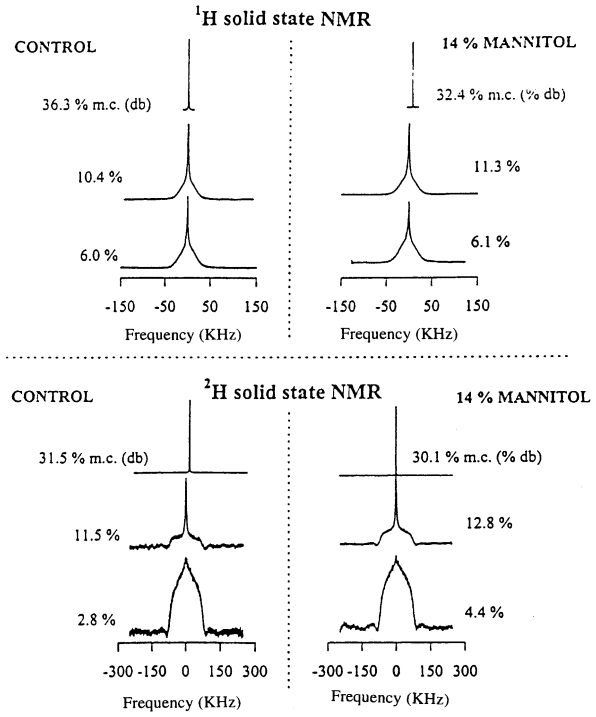

Fig. 2. Series of ^{1}H and ^{2}H solid state NMR spectra at variable moisture content for xanthan and locust bean gum mixtures (1:1) with and without added 14% mannitol (dry basis).

variable moisture contents are shown in Fig. 2. Both ^{1}H and ^{2}H solid state NMR spectra were comprised of a narrow and a wide component. The narrow peaks represented protons and deuterons undergoing rapid motion (Li et al., 1998; Tanner, Hills & Packer, 1991; Capitani, Segre, Attanasio, Bicharska, Focher & Capretti, 1995). The increased intensity of the narrow component with increased moisture content suggested that it was dominated by water (Tanner et al., 1991). The wide component, on the other hand, represented the immobile (rigid) protons or deuterons located on the xanthan and locust bean gums molecules as reported by

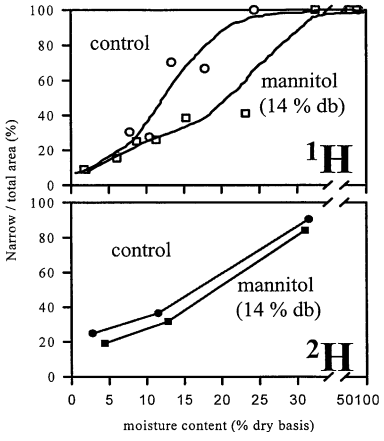

Fig. 3. Percent narrow ^{1}H and ^{2}H components obtained from solid state NMR spectra of xanthan and locust bean gum (1:1) at various moisture contents and 25°C.

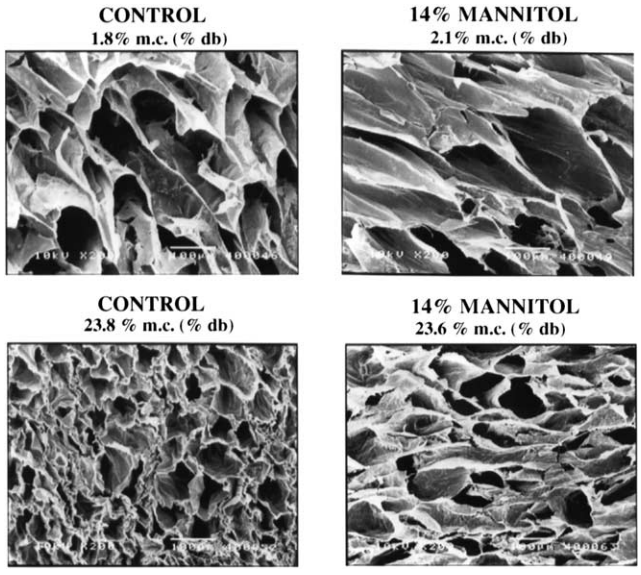

Fig. 4. SEM micrograph for xanthan and locust bean gum mixtures (1:1) with and without.

a number of investigators (Tanner et al., 1991; Capitani et al., 1995; MacKay, Bloom, Tepfer & Taylor, 1982; Li et al., 1998; Yakubu, Ozu & Baianu, 1993).

Spectra lineshape changed from more solid-like to more liquid-like with increasing moisture content. The wide component was observed only in samples of moisture content <25–30% (dry base). The relative intensity of the narrow peak increased with from ~10% at 2% moisture content (dry basis) to 100% at moisture contents >25–30%, (dry basis, Fig. 3). Mannitol addition lowered the amount of narrow proton component, which increased less rapidly with hydration than the control sample in the similar 10–25% moisture range. However, ^{2}H solid state NMR results, showed no significant effect of mannitol on the mobile component (Fig. 3). This discrepancy between ^{1}H and ^{2}H NMR results indicated that cross relaxation might play a role since the effect of mannitol was not observable by ^{2}H NMR. It was possible that close proximity and interaction between mannitol and water caused change in dipole–dipole interaction between liquid and solid protons.

Lineshape analysis of the wide component of the ^{2}H solid state spectra provides additional information about conformation and mobility of the solids (Spiess, 1985). The lineshape observed in xanthan and locust bean gum samples was characteristic of rigid, amorphous systems. No ^{2}H Pake pattern (characteristic of rigid crystalline solids) was observed, indicating that molecules of xanthan and locust bean gum were randomly organized and oriented due to their amorphous nature and that they retained some segmental mobility (Spiess, 1985).

The wide component (Gaussian) of the ^{1}H spectra was 35–40 KHz wide occurred independently of moisture content (5–30% moisture content, dry basis). This indicates that no significant change in the polymer mobility was

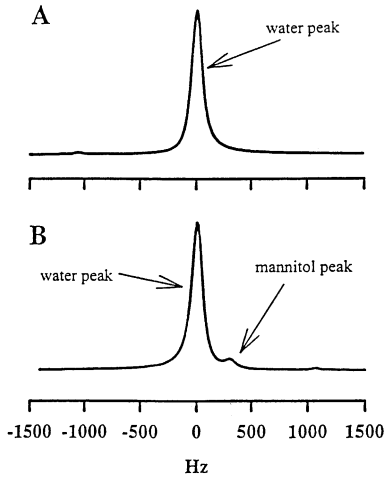

Fig. 5. Narrow peak from ^{1}H solid state NMR spectra for xanthan and locust bean gum mixtures (1:1). (a) control sample (43.8% m.c. db) and (b) sample with 14% (dry basis) mannitol (40.0% m.c. db).

observed in this moisture content range and suggested that the majority of the polymers did not undergo significant rigid solid–mobile solid transition in the 5–30% moisture content range. At higher moisture contents (>30%), the wide component disappeared and the narrow component increased (Fig. 3) indicating that the liquid-like component increasingly dominated the relaxation. It is possible that the polymers underwent some structural transformation (e.g. from a glassy to a rubbery state) that led to significant increase in their mobility. At 23% moisture, the sample physically became a rubbery and collapsed material (Fig. 4). However, control and mannitol added samples did not differ in microstructure (Fig. 4) but showed a significant difference in molecular mobility transition (Fig. 3).

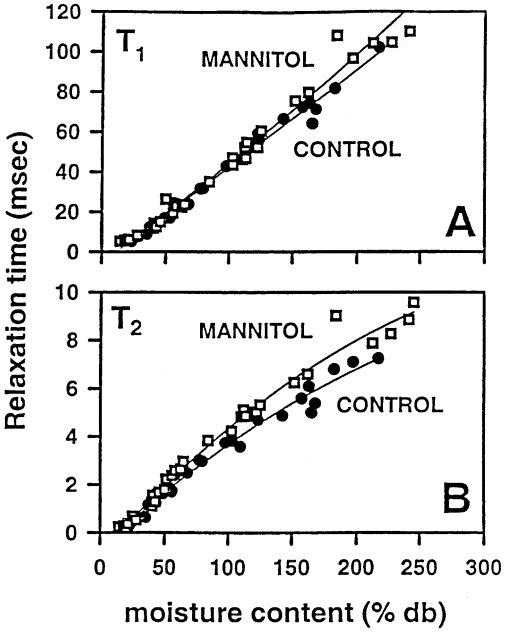

Fig. 6. ^{2}H high resolution NMR T1 (a) and T2 (b) relaxation times for control (solid circles) and sample with mannitol (open squares) as function of moisture content. Solid line = regression curve, dotted line = 95% confidence interval.

3.3. Isotropically reorienting protons

The narrow ^{1}H NMR population showed a small but significant difference in lineshape between control and mannitol added samples. Fig. 5(a) and (b) are spectra at 40% moisture (dry basis) depicting a major peak contributed mainly by water (Fig. 5), as similarly reported by Tanner et al. (1991). An additional but smaller peak (at ~300 Hz) was observed only in samples with mannitol at moisture content (dry basis) >35.0% or 0.94 a_w. This mannitol induced peak was attributed to the solubilized mannitol in the system ('mannitol peak'). This peak (at 300 Hz) was confirmed to belong to mannitol as observed in ^{1}H NMR spectra of mannitol alone (at moisture contents >0.5%, dry basis or $a_w = 0.94$). The fact that this peak was only observed when mannitol was hydrated could be related to an increased mannitol mobility as it became solubilized at a higher moisture content. Dissolved mannitol could compete for water in the system as it becomes hydrated (Chinachoti, 1993; Richardson, Baianu & Steinberg, 1987). Water redistribution between system components may have occurred leading to the formation of a solution surrounding the polymers (Richardson et al., 1987).

Based on the proton line width (LW), a line narrowing was observed with moisture, from ~4000 Hz at 5% moisture content to less than 300 Hz at moisture content >30% (dry basis, data not shown) with and without mannitol. This narrowing suggests a possible increased mobility of liquid-like components with hydration. Other events may also contribute, for example chemical and diffusive proton exchange, cross relaxation and chemical shift anisotropy (Pople, Schneider & Bernstein, 1959; Meiboom & Gill, 1958; Canet & Robert, 1995; Delpuech, 1995). These unknown contributions are complex and further study was done using ^{2}H high resolution NMR relaxation analysis in order to eliminate some of these factors.

3.4. Isotropically reorienting deuterons

Mobility of the isotropically reorienting ^{2}H populations was analyzed for its dependence on moisture content by ^{2}H high resolution NMR. Longitudinal (T_1) and transverse (T_2) relaxation times were measured. Both T_1 and T_2 were found to relax in a single exponential manner ($R^2 > 0.97$) indicating a rapid exchange (Fullerton, Potter & Dornbluth, 1982), but since $T_1 \gg T_2$ the systems were not in the extreme narrowing regime. The values of T_1 and T_2 increased with moisture content [Fig. 6(a) and (b)] indicating higher mobility in samples at higher moisture content. T_1 was found to increase by 20 fold and T_2 by almost 50 fold when the moisture content increased from 18 to 250% (dry basis). The more rapid increase in T_2 with increasing moisture content should eventually lead to a situation where

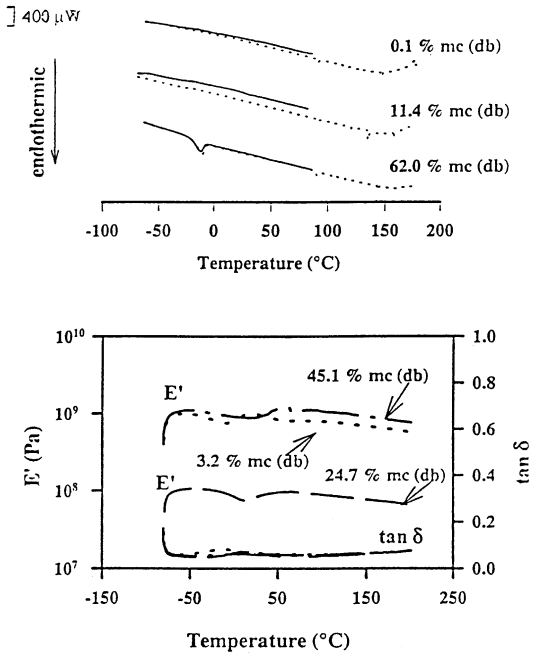

Fig. 7. (a) DSC thermograms (first scan = solid line; rescan = dotted line) of xanthan and locust bean gum mixtures (1:1) as a function of moisture content. (b) DMA thermograms for control samples at variable moisture contents. E'' and tan$d$ for samples at 45.1% (–.–), 24.7% (– – –) and 3.2% (···) moisture content are shown.

$T_1 \cong T_2$ at some (much higher) moisture content. Since some populations of solid deuterons could also contribute to the averaged T_2, further quantitative interpretation is not possible without additional studies.

Mobility of water molecules can be affected by the presence of solids. Restricted mobility was observed in T_1 and T_2. The presence of mannitol in the sample did not affect the values of T_1 relaxation times [Fig. 6(a)] and slightly increased the overall T_2 values in samples of moisture content >50% [dry basis, Fig. 6(b)] that contained solubilized mannitol (above the mannitol solubilization point, $a_w = 0.94$). In the presence of dissolved mannitol, the bulk-like water ('solute water', Lang, 1980) led to the increase in T_2 relaxation times (accompanied by a decreased viscosity). An increased exchange between solids and water was possible (Farhat, Mitchell & Blanshard, 1996). It has been reported that at some very high moisture content, a fast exchange condition might be met (Richardson et al., 1987). Our data showed that $R_2(1/T_2)$ plotted against solids concentration (g solids/mole water) had a linear relationship with a very small increase in R_2 with increasing solids from 0 to 50%, indicating a rapid isotropic orientation (Richardson et al., 1987). In this range, freezable water (~55% dry basis, DSC analysis) was found. Additionally, micorscopic structure collapse (Fig. 4) and material softening were observed. Therefore, it was hypothesized that the presence of excess water surrounding the solids and creating a continuous liquid phase allowed fast exchange among different regions of the sample as well as weakened the physical structure.

3.5. Structural relaxation by thermal analysis.

DSC thermograms detected an ice melting transition (Fig. 7) in samples of moisture contents >55% (dry basis). However, no evidence for glassy to rubbery transition (no baseline shift) was observed by DSC and by dynamic mechanical analysis (DMA) since no significant thermal events typical of glass transition (Chinachoti, 1994; Vodovotz & Chinachoti, 1996) were observed in any of the samples analyzed. It was concluded that the freeze dried and rehydrated mixtures of xanthan and locust bean gum (with and without mannitol) did not exhibit a glassy to rubbery transition under the conditions used. This supported an earlier finding by Gidley, Cooke and Ward-Smith (1993) that these polysaccharides did not have glassy to rubbery transition in the range 5–150°C.

The presence of structural collapse without detection of glassy to rubbery transition could appear incongruous. This could be explained by the fact that gums are flexible, heterogeneously oriented molecules. Upon water absorption, the different regions of the macromolecules are expected to undergo a gradual, heterogeneous transition over a wide temperature range (McBrierty & Packer, 1993). If these macromolecules increased in molecular mobility, this event occurred as a multitude of small steps undetectable by thermal analysis (Quinn, Kampff, Smyth & McBrierty, 1988; Cauley et al., 1991; Roy & Inglefield, 1990) but observable at a molecular level at shorter time frames (NMR analysis; Quinn et al., 1988; Myth, Quinn & McBrierty, 1988; Coyle, Martin & McBrierty, 1996; Li et al., 1998; Cauley et al., 1991).

4. Discussion

The objective of this work was to investigate the changes in molecular and structural mobility in xanthan and locust bean gum mixtures and to correlate these mobility parameters with microbial survival data (from Mugnier & Jung, 1985) clarifying the role of molecular and structural mobility in food safety.

4.1. Structural and molecular mobility

Structural mobility changes, as measured by glassy to rubbery transition were not observed (by thermal analysis) in the xanthan and locust bean mixtures (with and without mannitol) between −80 and 200°C (moisture content range 0–165% dry basis) although significant molecular mobility changes were found. This experimental evidence indicates that glassy to rubbery transition may not be an effective parameter to predict molecular dynamics in heterogeneous systems and, therefore, it should not be used to predict microbial survival (Buera, Jouppila, Roos & Chirife, 1998). This evidence strongly suggested that averaged and structural properties (glass transition) of a system do not read the system molecular dynamics (Hills, Manning,

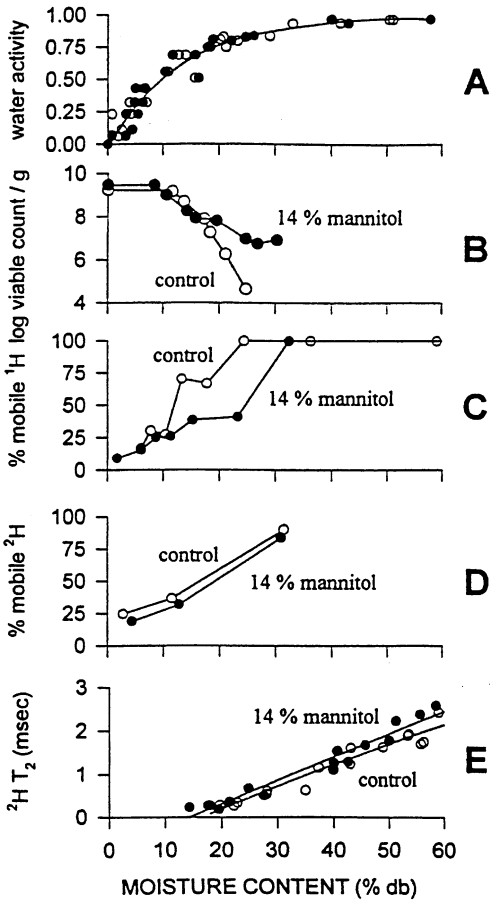

Fig. 8. Summarized chart for freeze dried xanthan and locust bean gum mixture with and without 14% mannitol (dry basis; 32°C). Changes in water activity (a), ^{2}H NMR T_2 relaxation times (e), ^{1}H and ^{2}H mobile component [solid state NMR, (c) and (d), respectively] and survival data of *R. Japonicum* [(b), data from Mugnier & Jung, 1985] as function of moisture content.

Ridge & Brocklehurst, 1996, 1997; Franks, 1982, 1991; Lavoie, 1994). Changes observed by thermal analysis are long range transformations that are far in a timescale prospective from phenomena occurring at molecular level as demonstrated by our NMR studies.

Molecular mobility observed by NMR increased with increasing moisture content. The influence of the solids on the motion of water molecules over the entire range of moisture content studied (0–240% dry basis) and narrowing limit conditions were never met. At moisture contents below 5% (dry basis), 90% of the protons and 80% of the deuterons were highly immobile (solid state NMR). The solid signals (^{1}H and ^{2}H) were detected at moisture up to 25–30% moisture content (corresponding to the point in the isotherm concavity upward). Above this moisture content, the structural collapse was observed (without a glass transition detected by DSC or DMA). At this point, all ^{1}H and ^{2}H were highly mobile and liquid like (bulk water region of the WSI).

Presence of 14% (dry basis) mannitol in the mixture did not significantly alter the microstructure and the water sorption behavior of the system but it did alter the NMR mobility of water. Mannitol lowered the mobile proton and deuterium signals at <25% moisture; at higher moisture contents (bulk water region) mannitol solubilized causing a redistribution of water that favored the formation of a liquid phase. As moisture increased to this range (≥55%, dry basis) fast exchange condition and freezable water were observed in both samples.

4.2. 'Mobility' and microbial survival

Fig. 8 shows relative changes in a_w, *R. japonicum* viable count percent mobile ^{1}H and ^{2}H (total area basis, form solid state NMR) and relaxation time (from high resolution ^{2}H NMR) as function of moisture content.

Samples of moisture content up to 6% (dry basis) contained >80% of solid protons and >75% solid deuterons [Fig. 8(d) and (e), respectively). In these conditions *R. japonicum* survival was at its highest. As moisture content increased from 6 to 25% (dry basis), the cells viability decreased significantly (more drastically in the control than in the mannitol containing sample). In this range an increased mobile proton and deuterium was observed (^{1}H and ^{2}H solid state NMR) which was accompanied by a significant increase in T_2 relaxation time [Fig. 8(c)–(e)]. The almost identical relationship between water activity and moisture content mixtures without or with 14% mannitol (dry basis) indicated that the different microbial response was not due to differences in water activity. The rapid increase in liquid ^{1}H and ^{2}H components as well as T_2, coincides with the rapid decrease in viable cell counts.

Above 17% moisture content (dry basis) mannitol reduced death rate of the cells. These results suggested that the reduced mobility induced by the strong mannitol–water interaction had a protective effect on the cells. The more mobile water facilitated cell death in the control sample while the strong association of water with mannitol seems to have a protective effect on the cells. Further studies are needed to understand the metabolic response of the cell.

Higher (but still retarded) water molecular mobility (control sample) might allow initiation but not the completion of metabolic reactions leading to cells death. Other explanations could be found in a possible role of mannitol as nutrient and/or osmoprotectant (Crowe, Crowe, Carpenter & Aurell Winstrom, 1987; Crowe et al., 1988). Unfortunately the range of moisture content studied by Mugnier and Jung (1985) did not cover the high moisture content range where cell growth is expected. In this region (above 50% moisture content range), mannitol increased the mobility of water molecules (higher T_2) compared to the control sample. A better correlation between cells growth and higher water mobility could be suggested but it could not be verified in this study. This occurrence was previously reported in *Staphylococcus aureus* growth studies (Lavoie & Chinachoti, 1995, 1997; Lavoie, 1994).

5. Conclusions

Structural mobility or glass transition (thermal analyses) can be difficult to quantitate or it does not exist in gums. On the other hand, proton and deuterium NMR data showed a major molecular mobility increase as moisture increased above 6% moisture content (dry basis) reaching a liquid like behavior above 30% moisture content (dry basis). In this study, NMR was able to detect the molecular dynamic transition in samples where structural transition was either not existing or not detectable by DSC and DMA. Mannitol was strongly associated with water but, above its solubilization point, caused a slight increase in mobility (T_2). Survival of *R. japonicum* in such a system decreased with moisture content increasing from 6 to 15% corresponding with an increasing mobile proton and deuterium signals (i.e. increased mobility). Mannitol protected the cells from death at increasing moisture content and a relationship with a lower mobile ^{1}H signal (as compared with the control) seems to be present. Strong mannitol–water interaction, leading to a decreased mobility, was suggested to play a role.

The NMR techniques used in this work allowed only determination of rotational water mobility, while microorganisms are expected to be more sensitive to translational molecular motions, considering that the cell environment interaction is a dynamic process based on translational exchange of nutrients, waste products, dissolved oxygen etc. The data reported in literature that related translational mobility to bacterial survival showed a good correlation between these two parameters (Hills et al., 1996, 1997; Kou, 1998). In the future the use of gradient field NMR in these type of studies could provide a better insight of the problem.

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