Characterization of Molecular Mobility in Seed Tissues: An Electron Paramagnetic Resonance Spin Probe Study

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ABSTRACT The relationship between molecular mobility ($\tau_R$) of the polar spin probe 3-carboxy-proxyl and water content and temperature was established in pea axes by electron paramagnetic resonance (EPR) and saturation transfer EPR. At room temperature, $\tau_R$ increased during drying from $10^{-11}$ s at 2.0 g water/g dry weight to $10^{-4}$ s in the dry state. At water contents below 0.07 g water/g dry weight, $\tau_R$ remained constant upon further drying. At the glass transition temperature, $\tau_R$ was constant at $\sim 10^{-4}$ s for all water contents studied. Above $T_g$, isomobility lines were found that were approximately parallel to the $T_g$ curve. The temperature dependence of $\tau_R$ at all water contents studied followed Arrhenius behavior, with a break at $T_g$. Above $T_g$ the activation energy for rotational motion was $\sim 25$ kJ/mol compared to 10 kJ/mol below $T_g$. The temperature dependence of $\tau_R$ could also be described by the WLF equation, using constants deviating considerably from the universal constants. The temperature effect on $\tau_R$ above $T_g$ was much smaller in pea axes, as found previously for sugar and polymer glasses. Thus, although glasses are present in seeds, the melting of the glass by raising the temperature will cause only a moderate increase in molecular mobility in the cytoplasm as compared to a huge increase in amorphous sugars.

INTRODUCTION

The longevity of seeds is determined by the conditions under which they are stored, major factors being temperature and water content. Predictions of the longevity of seeds or the optimum storage conditions that have to be chosen to obtain maximum longevity are valuable assets in maintaining a seed collection. In desiccation-tolerant organisms, such as seeds and pollen, it has been found that the cytoplasm enters into a glassy state when those organisms are stored at low water contents and/or low temperatures (Williams and Leopold, 1989; Leopold et al., 1994; Sun and Leopold, 1994; Leprince and Walters-Vertucci, 1995; Sun, 1997; Buitink et al., 1998b). A glass is a solid-like liquid with an extremely high viscosity (Franks, 1994a). The formation of glasses in seeds is thought to be responsible for the prolonged survival of these tissues in the dry state. It was found that the life span of biological materials such as seeds and pollen was increased profoundly during storage under conditions in which the cytoplasm was brought into a glassy state (Sun and Leopold, 1994; Sun, 1997; Buitink et al., 1998b).

In many glass-forming substances, melting of the glass results in a dramatic increase in translational and rotational motion (Soesanto and Williams, 1981; Roozen et al., 1991; Steffen et al., 1992; Blackburn et al., 1996; Deppe et al., 1996; Champion et al., 1997; Hemminga and Van den Dries, 1998; Van den Dries et al., 1998). It has been known for a long time that stabilization of many macromolecules is greatly enhanced by the presence of aqueous glasses. The shelf life of food materials has been associated with the presence of a glassy state (see Roos, 1995, for a review). In the pharmaceutical industry, it is currently recognized that the presence of an amorphous phase has very important implications for storage of pharmaceutical dosage forms (Hancock and Zografi, 1997). Recently, Hancock et al. (1995) suggested the use of molecular mobility measurements below $T_g$ in the prediction of shelf lives of amorphous drugs, assuming a direct correlation between the molecular mobility and the degradation of the product.

The physical properties of water have been studied in complex biological systems that are able to survive the removal of their water, such as seeds (Vertucci, 1990; Bruni and Leopold, 1992; Konsta et al., 1996) and Artemia cysts (Seitz et al., 1981). The restricted mobility of water at low hydration levels has been attributed to the formation of intracellular glasses (Williams and Leopold, 1989). However, little is known about the viscosity or molecular mobility of molecules other than water in these systems. Detrimental processes associated with aging that take place in the cytoplasm of seeds are likely to be restricted by slow molecular motion of molecules in the cytoplasm. Therefore, characterization of molecular mobility in seeds as a function of temperature and water content might aid in understanding the kinetics of seed aging during storage. Soluble sugars present in the cytoplasm of seeds are thought to be the major component responsible for the formation of intracellular glasses. Comparison of the behavior of intracellular glasses with sugar glasses will shed light on the nature of intracellular glasses.

An elegant technique for the study of rotational motion is electron paramagnetic resonance (EPR) spectroscopy, which measures the rotational correlation time ($\tau_R$) of spin...
probes dissolved in samples. Continuous-wave (CW) EPR can detect changes in the $\tau_R$ of spin probes ranging from $10^{-12}$ to $10^{-8}$ s and has been applied previously to biological systems (Bruni and Leopold, 1990; Buitink et al., 1998a; Leprince and Hoekstra, 1998). Saturation transfer EPR (ST-EPR) can detect $\tau_R$ on the order of $10^{-7}$ to $10^3$ s and has been successfully applied to determine the $\tau_R$ of spin probes in sugar glasses (Roozen and Hemminga, 1990; Roozen et al., 1991; Hemminga and Van den Dries, 1998; Van den Dries et al., 1998) and organic liquids at low temperatures (Ito, 1983). It has been shown previously that the $\tau_R$ of spin probes provides a unique and simple parameter for the characterization of the state of the cytoplasm of seeds (Buitink et al., 1998a).

For many systems, the temperature dependence of reaction rates or mobility below $T_g$ can be described by the Arrhenius equation (Levine and Slade, 1988; Karmas et al., 1992; Nelson and Labuza, 1994). Above $T_g$, it has been shown that the temperature dependence of viscosity, translational, or rotational relaxation times of model glasses such as sugar systems and polymers cannot be described by an Arrhenius-like relationship. For such systems it turns out that the effect of increasing temperature on relative relaxation times above $T_g$ can be successfully predicted by the Williams-Landel-Ferry (WLF) equation, an empirical equation, the form of which was originally derived from the free volume interpretation of the glass transition (Williams et al., 1955; Ferry, 1980; Soesanto and Williams, 1981; Chan et al., 1986; Roos and Karel, 1991; Steffen et al., 1992; Champion et al., 1997). Fitting of the rotational mobility in pea axes as a function of temperature according to the Arrhenius and WLF equations will enable us to compare mobility in intracellular glasses with that of model systems. In addition, modeling the temperature effect of mobility could aid predictions of shelf life.

This study was performed to obtain insight into how molecular mobility of molecules in the cytoplasm of seed changes, depending on water content and temperature. Different EPR techniques were used to determine the rotational mobility of spin probes in the fast and very slow motional regions. The temperature dependence of $\tau_R$ was assessed in relation to Arrhenius and WLF behavior, and reference was made to other glass-forming substances.

Two to three axes of the same treatment were sealed in a 2-mm-diameter capillary for EPR measurements. After the measurements, the axes were removed from the capillaries and water contents were determined. For samples that were heated above 50°C during the EPR measurements, similar samples equilibrated to the same water content were taken for water content determination. Water contents were analyzed by weighing the samples before and after heating at 96°C for 36–48 h.

**Molecular motion in the fast motional region (CW-EPR)**

Spectra were recorded using a Bruker x-band EPR spectrometer (Bruker Analytik, Rheinstetten, Germany, model 300 E). Rotational correlation times in the fast motional region were determined from the lineshapes of CW-EPR spectra according to the method of Freed and Fraenkel (1963) for isotropic tumbling:

$$\tau_R = 6.5 \times 10^{-4} \Delta B_0 (h_C/h_H)^{1/2} (1)$$

where $B_0$ is the width of the center field component in Teslas, and $h_C$ and $h_H$ are the amplitudes of the central and high field components of the three-line nitroxide radical spectrum, respectively.

**Molecular motion in the slow motional region (ST-EPR)**

At low water contents and temperatures, $\tau_R$ of CP in seed axes becomes slower than $10^{-8}$ s, resulting in the appearance of a powder spectrum (Buitink et al., 1998a). Under these conditions, $\tau_R$ cannot be calculated according to Eq. 1. However, ST-EPR further expands the motional region from $10^{-7}$ to $10^3$ s (Hyde and Dalton, 1979; Van den Dries et al., 1998).

ST-EPR is based on the diffusion and recovery of saturation between different parts of the powder spectrum in competition with field modulation (Hemminga, 1983). For ST-EPR measurements the second harmonic quadrature absorption signal was detected under the following conditions: field modulation amplitude 0.5 mT, microwave power 100 mW, and field modulation frequency 50 kHz (Hemminga et al., 1984). The phase was set with the self-null method (Thomas et al., 1976).

ST-EPR spectra can be well characterized by independent lineshape parameters, such as the line-height ratios $L/L$ and $C/C$ (see Fig. 1 for details). Using reference material with known viscosity, $\tau_R$ values are usually obtained in an empirical way. We used spectra of CP in anhydrous glycerol to construct a calibration curve (Hemminga and Van den Dries, 1998). Because the viscosity for anhydrous glycerol is known over a broad temperature range, $\tau_R$ of CP in glycerol can be obtained from the modified Stokes-Einstein equation (Roozen et al., 1991):

$$\tau_R = \frac{\eta V/\kappa_v T}{k} + \tau_0 \quad (2)$$

where $\tau_R$ is the rotational correlation time, $\eta$ is the solvent viscosity, $\kappa_v$ is Boltzmann’s constant, $V$ is the volume of the rotating molecule, $T$ is the absolute temperature, $\tau_0$ is the zero viscosity rotational correlation time, and $k$ is a dimensionless slip parameter. The slip parameter was assumed to be temperature-independent (Hemminga and Van den Dries, 1998).

Anhydrous glycerol, containing 1 mM CP, was cooled to −150°C, and after equilibration for 30 min, spectra were recorded at 3°C increments. For each temperature the values of the lineshape parameters $L/L$ and $C/C$ were calculated. From the curves representing the lineshape parameters of CP in glycerol against $\tau_R$, the values of $\tau_R$ of CP in the seed axes were obtained by interpolation of the corresponding lineshape parameters.

**DSC**

The glass transition temperature is conventionally the temperature at which a change in the heat capacity can be detected by DSC. Two to three pea axes of different water contents were hermetically sealed into aluminum caps.
RESULTS AND DISCUSSION

Rotational motion of CP in pea axes in relation to temperature and water content

ST-EPR spectra of CP in pea axes (0.09 g water/g dry weight) at different temperatures are displayed in Fig. 1. It has been shown previously that to exclusively obtain spectra of the spin probe in the cytoplasm, a nitroxide spin probe of high polarity has to be selected. More apolar spin probes have the tendency to partition into the lipid phase with drying (Buitink et al., 1998a; Golovina et al., 1998). With the use of a broadening agent such as potassium ferricyanide, the CP signal was completely removed from the intercellular spaces and is therefore exclusively of intracellular origin. Although CP is expected to be present in a heterogeneous environment consisting of a mixture of ions, sugars, and proteins, its signal in dry seeds appears to be a single component spectrum (Fig. 1). Only at high temperatures may a small second component appear in the spectrum (Fig. 1). Baselines were determined as the onset of the temperature range over which the change in specific heat occurred. All analyses were performed with Perkin-Elmer software.

The τ_R of CP in pea axes was derived from spectra as shown in Fig. 1. With increasing temperature, the line height ratios (L''/L and C'/C) decreased, indicating an increase in rotational motion. The saturation transfer in the central part of the spectrum is more extensive than that in the low field region, especially at higher temperatures. If the motion were isotropic, then the two peak height ratios would give the same correlation time, because overall rotation would modulate all of the spectral anisotropies, giving rise to saturation transfer throughout the entire spectrum (Marsh, 1980). However, there appears to be some motional anisotropy around the z axis, which modulates the anisotropy of the g-tensor in the x-y plane, giving rise to saturation transfer in the central part of the spectrum. Because this effect complicates the interpretation of the results from the C'/C measurements, only the τ_R derived from the line-height ratio L''/L will be utilized in the analysis of the ST-EPR spectra.

The temperature dependence of τ_R of CP is shown in Fig. 2 for pea axes. The different curves represent pea axes with decreasing water contents from left to right. The arrows denote the onset of T_g as measured by DSC. Below T_g, τ_R followed a linear behavior with temperature. Around T_g, a change occurred in the relationship between τ_R and temperature. Above T_g, the τ_R increased more sharply with temperature from 10^-4 s to ~10^-5 s over the next 50°C temperature increase. At temperatures ~50°C above T_g, the increase in τ_R with temperature leveled off.

The increase in mobility of CP in pea axes above T_g is not as dramatic as found for glycerol or other glass-forming sugars (Williams et al., 1955; Soesanto and Williams, 1981; Chan et al., 1986; Roozen and Hemminga, 1990; Roozen et al., 1991). For 20% wt sucrose-water mixtures, τ_R increased by about four orders of magnitude in the first 20°C above T_g (Roozen and Hemminga, 1990). In a maltoheptaose glass, dry or stored at 33% relative humidity, the τ_R increased by

![FIGURE 1](image1.png)  
**FIGURE 1** ST-EPR spectra of CP in pea axes with a water content of 0.09 g water/g dry weight, recorded at different temperatures.

![FIGURE 2](image2.png)  
**FIGURE 2** τ_R of CP in pea axes as a function of temperature. τ_R was calculated from the line-height ratio L''/L. The different curves represent pea axes with different water contents (g water/g dry weight): 0.048 (♦), 0.07 (▼), 0.085 (△), 0.12 (●), 0.16 (○). Arrows indicate the onset T_g as measured by DSC at a scanning rate of 10°C/min.
three orders of magnitude over the same temperature interval (Roozen et al., 1991). In comparison, the increase in $\tau_R$ for CP in pea axes from $T_g$ to 20°C above $T_g$ was only a factor of 6 (Fig. 2). Thus, although glasses are present in seeds, the effect of melting the glass by raising the temperature will not dramatically increase the mobility of molecules in the cytoplasm. Considering the survival of seeds in their natural habitat, the relatively small change in mobility above $T_g$ might render them fairly insensitive to fluctuations in the environmental conditions that drive their glassy state.

During drying of pea axes at 25°C, $\tau_R$ of CP in the cytoplasm increased from $7 \times 10^{-11}$ s at 2.2 g water/g dry weight to $10^{-4}$ s at 0.07 g water/g dry weight (Fig. 3). Upon further drying $\tau_R$ remained constant. The inset shows the decrease in $\tau_R$ during drying determined according to Eq. 1, where the water contents were sufficiently high to obtain a sharp three-line spectrum. The rotational mobility decreased when the tissues were dried below 2.2 g water/g dry weight. The dotted line indicates the range of magnitude in which $\tau_R$ cannot be measured with either methods. Nonetheless, it can be seen that between 0.3 and 0.2 g water/g dry weight, $\tau_R$ strongly increases. This sharp increase in $\tau_R$ coincides with the water content range in pea axes at which the water remains unfrozen as measured by DSC (Vertucci, 1990).

The change in $\tau_R$ upon drying is on the order of more than seven orders of magnitude. The slow molecular mobility at low water contents is thought to have a protective effect on the structural and functional stability of enzymes and other molecules in the cytoplasm (Burke, 1986; Leopold et al., 1994; Leprince and Walters-Vertucci, 1995). Indeed, the considerable decrease in molecular mobility that we found with drying argues in favor of this hypothesis. The preservation of the cellular components in the dry state is likely to prolong the survival of the seeds in the dry state (Leopold et al., 1994; Sun and Leopold, 1994; Sun, 1997; Buitink et al., 1998a,b).

**Modeling of molecular mobility in pea axes**

If the rate of reactions were controlled by the mobility of molecules in the cells, characterizing the temperature dependence of molecular mobility would aid in predicting shelf life. In addition, it allows us to compare the behavior of rotational motion in intracellular glasses with that of sugar and polymer glasses. The temperature dependence of reaction rates is often described by the Arrhenius equation:

$$k = k_0 \exp(-E_a/RT)$$

(3)

where $k$ is the rate constant at temperature $T$, $k_0$ is a preexponential factor, $R$ is the ideal gas constant, and $E_a$ is the activation energy. Fig. 4 shows Arrhenius plots of $\tau_R$ of CP in pea axes at two water contents. The arrows indicate the onset of $T_g$ as measured by DSC. The temperature dependence of $\tau_R$ of CP in pea axes followed Arrhenius behavior below $T_g$, with a break in the plot at $T_g$. Above $T_g$, Arrhenius behavior with a higher activation energy was found compared to below $T_g$. At temperatures $\sim 40–60^\circ C$ above $T_g$, the data deviated from Arrhenius behavior, as became apparent from the deviation of the data points from a straight line. Activation energies from the slopes of the Arrhenius plots below $T_g$ and for the first 40–60°C above $T_g$ for pea axes containing different water contents are summarized in Fig. 5. For $\tau_R$ of CP in pea axes above 0.07 g water/g dry weight, activation energies below $T_g$ were lower (7–11 kJ/mol) than above $T_g$ (25 kJ/mol), as generally expected (Levine and Slade, 1988; Karmas et al., 1992; Nelson and Labuza, 1994). This indicates that the rotational motion below $T_g$ changes at a slower rate than above $T_g$. Below 0.07 g water/g dry weight, activation energies above $T_g$ decreased substantially (Fig. 5). The activation energy values of rotational motion of CP in pea axes below $T_g$ are in the same range as those found for glassy sugar systems (Roozen et al., 1991).

**FIGURE 4** Arrhenius plots of the temperature dependence of $\tau_R$ of CP in pea axes. The $\tau_R$ was calculated from the line-height ratio $L'/L$. The different curves represent pea axes with different water contents (g water/g dry weight): 0.07 (△), 0.12 (○). Arrows indicate the onset $T_g$, as measured by DSC at a scanning rate of 10°C/min. Lines represent linear regressions. Values represent the activation energy expressed as kJ/mol.

**FIGURE 3** $\tau_R$ of CP in pea axes as a function of water content at 25°C. Values of $\tau_R > 10^{-6}$ s were calculated from the $L'/L$ ratio of ST-EPR spectra. For $\tau_R < 10^{-6}$ s, Eq. 1 was used with the EPR spectra. The inset shows the decrease in $\tau_R$ during drying from high water contents.
The data points were derived from the Arrhenius plots as shown in Fig. 4.

For model glasses, such as sugar-water systems and polymers, it has been shown that the temperature dependence of viscosity or mobility above \( T_g \) cannot be described by an Arrhenius-like relationship (Williams et al., 1955; Ferry, 1980; Soesanto and Williams, 1981; Chan et al., 1986; Roos and Karel, 1991; Steffen et al., 1992; Champion et al., 1997). Instead, it can be described by the WLF equation (Peleg, 1992), and other values have been applied to molten glucose (Williams et al., 1955), amorphous sucrose-water systems (Chan et al., 1986), amorphous sucrose and lactose powders at low moisture (Roos and Karel, 1991), and concentrated solutions of mixed sugars (Soesanto and Williams, 1981). However, several problems are associated with the use of the average coefficients in the WLF equation (Peleg, 1992), and other values have been used to obtain a better fit (Ferry, 1980; Slade and Levine, 1991; Champion et al., 1997).

The \( \tau_R \) values for CP in pea at different water contents were fitted to the WLF equation (Fig. 6 A). The curves of \( \tau_R \) from CP in pea axes with water contents higher than 0.07 g water/g dry weight followed approximately the same relationship (Fig. 6 A). To obtain a reasonable fit, the WLF constants had to be changed considerably by decreasing \( C_1 \) and increasing \( C_2 \) compared to the universal constants. This becomes apparent from Fig. 6 B, in which the \( \tau_R \) of CP in pea axes is compared to the \( \tau_R \) of CP in glycerol, fitted with the universal constants. These data were also obtained using ST-EPR spectroscopy (Van den Dries et al., 1998; Buitink et al., 1998a). The curve fitted to the data in Fig. 6 A was calculated using \( C_1 = 3.4 \) and \( C_2 = 150 \). In samples with water contents below 0.07 g water/g dry weight, there was a further decrease in \( C_1 \) or increase in \( C_2 \) needed to obtain a good fit.

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Although WLF behavior is expected for glass-forming substances above \( T_g \), we found that the Arrhenius equation can well describe the temperature dependence above \( T_g \) for the first 50°C (Fig. 4). Deterioration kinetics in complex food systems (Karmas et al., 1992; Nelson and Labuza, 1994) and aging kinetics in pollen (Buitink et al., 1998b) were also found to follow Arrhenius behavior both above and below \( T_g \). Apparently, kinetics in complex systems such as food materials and seeds can be described by the Arrhenius equation for the first 50°C above \( T_g \). The activation energy of relaxation kinetics increases by a factor of \( \sim 3 \) when the pea axes are brought to conditions in which the intracellular glass melts. Interestingly, a comparable change in activation energy for aging kinetics around \( T_g \) has been shown for cattail pollen (Buitink et al., 1998b).

Mobility at any temperature depends primarily on the free volume present (Ferry, 1980). Some information regarding the free volume of the system can be derived from the
The implication of a higher amino acids, sugars, and proteins, that are responsible for are composed of many different molecules, such as ions, amino acids, sugars, and proteins, that are responsible for the increase in free volume because of imperfect packing. The implication of a higher $C_2$ constant is that the difference in thermal expansion coefficient between the glassy and liquid states would be smaller than that for other glass-forming substances.

When spin probe techniques are used, the rotational mobility of probes in glassy systems is not only determined by the free volume of the host system, but also by the specific interaction of the spin probe with the chain molecules. Hydrogen bonds are often the most important interaction (Roozen and Hemminga, 1990). The deviating behavior of rotational mobility of the spin probe in intracellular glasses compared to sugar glasses can therefore also be mediated by the spin probe’s interaction with the surroundings. To learn which of the two factors is determining the rotational mobility, the $\tau_R$ values at the $T_g$ (measured by DSC) of CP in pea axes with different water contents were plotted (Fig. 7). With decreasing water content, the onset $T_g$ from pea axes increases from $-50^\circ$C at 0.26 g water/g dry weight to 90°C when they are completely dry, as measured by DSC. The $\tau_R$ is constant at $T_g$, on the order of $10^{-4}$ s (Fig. 7). These results would imply that it is indeed the free volume that is the major factor in influencing the rotational mobility. If hydrogen bonding would affect the rotational mobility, it would be unlikely that $\tau_R$ at $T_g$ would remain constant, considering the wide range of water contents (0.01–0.20 g water/g dry weight) and temperatures ($-50^\circ$C to 90°C) studied (Fig. 7).

The use of the WLF and other models in predicting temperature dependence of viscosity allows the establishment of state diagrams that show isoviscosity states above $T_g$ as a function of water content. Such diagrams may be used in the evaluation of changes in water content on relaxation times at constant temperature in establishing critical temperatures for the stability of amorphous materials (Roos, 1995) or may possibly aid in predictions of seed longevity. Slade and Levine (1991) demonstrated that parallel to the $T_g$ curve of sucrose, isoviscosity lines can be found. Fig. 8 shows a state diagram in which isomobility lines are drawn above the $T_g$ curve. Isomobility lines were found to be parallel to the $T_g$ curve.

**Implications for seed storage**

The presence of intracellular glasses in seeds is thought to be of considerable significance for the storage longevity of the seeds (Leopold et al., 1994; Sun, 1997; Buitink et al., 1998b). The role of intracellular glasses in longevity is derived from the dramatic increase in viscosity or decrease in the molecular mobility of molecules in other glass-forming substances, thus decreasing the rate of detrimental reactions (Soesanto and Williams, 1981; Roozen et al., 1991; Steffen et al., 1992; Blackburn et al., 1996; Deppe et al., 1996; Champion et al., 1997; Hemminga and Van den Dries, 1998; Van den Dries et al., 1998). Detrimental processes associated with aging that take place in the cytoplasm of seeds are likely to be restricted by slow molecular motion of molecules in the cytoplasm. The low rotational mobility

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**FIGURE 7** $\tau_R$ of CP in pea axes of different water contents at $T_g$. $\tau_R$ was calculated from the line-height ratio $L'/L$. $T_g$ was determined as the onset of the second-order transition measured by DSC at a scanning rate of 10°C/min. $T_g$ for the different water contents ranged from $-50^\circ$C at 0.26 g water/g dry weight to 0.01 g water/g dry weight.

**FIGURE 8** State diagram of pea axes. The solid circles represent the onset $T_g$ measured by DSC at a scanning rate of 10°C/min. Lines represent the temperature/water content combinations at which $\tau_R$ is equal. $\tau_R = 5 \times 10^{-3}$ s (○), $\tau_R = 2 \times 10^{-3}$ (▲), $\tau_R = 1 \times 10^{-4}$ (□). Lines are fourth-order polynomial regressions.
of CP in dry pea axes, when the cytoplasm is in a glassy state, indicates that formation of a glassy matrix is indeed beneficial to the preservation of molecules during storage in the dry state.

The significance of glasses in seeds can be assessed by comparing the changes in mobility of molecules as a function of temperature and relating them to those found previously for other glass-forming substances. In particular, comparison with sugar glasses will ascertain whether the formation of intracellular glasses can be attributed to the soluble sugar present in the cytoplasm, as previously suggested (Williams and Leopold, 1989; Leopold et al., 1994). Intracellular glasses behave in a manner somewhat similar to that of other glass-forming substances in that the temperature dependence of the rotational motion follows WLF behavior above 0.07 g water/g dry weight, which gives rise to isomobility lines parallel to $T_g$. However, the rate of change of the rotational motion with temperature is not as large as seen in sugar glasses. Thus, although glasses are present in seeds, the effect of melting the glass by raising the temperature will not have a tremendous effect on changes in mobility of molecules in the cytoplasm. Nonetheless, although the difference in activation energy is relatively small below and above $T_g$, the change in activation energy warns us not to simply extrapolate kinetic data obtained by aging seeds or pollen above $T_g$ (brought about by high humidity and temperature) to aging conditions below $T_g$ (Franks, 1994b; Buitink et al., 1998b; Duddu and Dal Monte, 1997). Considering the different kinetics between sugar glasses and intracellular glasses, sugars may aid in the formation of the intracellular glass in seeds, but other molecules will also participate in the formation of intracellular glasses (Leopold et al., 1994; Leprince and Walters-Vertucci, 1995).

Below 0.07 g water/g dry weight, mobility starts to deviate from the general behavior. The activation energy above $T_g$ decreases with decreasing water content, and the temperature dependence of $\tau_R$ of CP in pea axes changes for each water content. It is interesting to note that below this water content, the storage behavior of pea seeds also changes (Vertucci et al., 1994). Instead of an increase in shelf life with decreasing water contents, the shelf life decreases. The factors that cause the change in kinetics of mobility and aging rates are unknown. It has been suggested that the shelf life of foods and seeds or pollen is associated with the Brunauer–Emmett–Teller monolayer, derived from isotherms (Labuza et al., 1970; Labuza, 1980; Buitink et al., 1998b). Removal of this structural water may create holes in the cytoplasm, resulting in increased mobility (Buitink et al., 1998a) and increased aging kinetics. Alternatively, removal of the last water changes the hydrogen-bonding properties of molecules, thereby changing the behavior of the spin probe, which could account for the increase in activation energy at these low water contents (Roozen et al., 1991).

CONCLUDING REMARKS

A spectroscopic method was successfully employed to characterize the rotational motion of small spin probes incorporated into cells of biological materials. The results indicate that intracellular glasses can be formed in dry seeds, but that soluble sugars are not the only determining factor in the glass formation. The complex composition of the intracellular glass is suggested to be responsible for the moderate increase in mobility when the glass is melted, compared to other glass-forming substances. Our work shows that the use of a physical approach in obtaining detailed molecular information will be crucial to predicting the stability of food and biological materials.

This work was financially supported by the Netherlands Technology Foundation (STW) and was coordinated by the Life Sciences Foundation.

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