RESEARCH PAPER

Mechanisms of seed ageing under different storage conditions for Vigna radiata (L.) Wilczek: lipid peroxidation, sugar hydrolysis, Maillard reactions and their relationship to glass state transition

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Abstract

Two primary biochemical reactions in seed ageing (lipid peroxidation and non-enzymatic protein glycosylation with reducing sugars) have been studied under different seed water contents and storage temperatures, and the role of the glassy state in retarding biochemical deterioration examined. The viability loss of Vigna radiata seeds during storage is associated with Maillard reactions; however, the contribution of primary biochemical reactions varies under different storage conditions. Biochemical deterioration and viability loss are greatly retarded in seeds stored below a high critical temperature (approximately 40 °C above glass transition temperature). This high critical temperature corresponds to the cross-over temperature \( T_c \) of glass transition where molecular dynamics changes from a solid-like system to a normal liquid system. The data show that seed ageing slows down significantly, even before seed tissue enters into the glassy state.

Key words: Amadori reactions, glass transition, lipid peroxidation, Maillard reactions, seed ageing, seed longevity, Vigna radiata.

Introduction

Orthodox seeds are characterized by their ability to tolerate desiccation and to retain their viability for a long time in the dry state. However, these seeds age during storage and eventually lose their ability to germinate. Several comprehensive reviews have identified free radical-mediated lipid peroxidation, enzyme inactivation or protein degradation, disruption of cellular membranes, and damage to genetic (nucleic acids) integrity as major causes of seed ageing (Priestley, 1986; Smith and Berjak, 1995; Walters, 1998; McDonald, 1999). During the last 20 years, considerable research has been conducted to understand better the physiology of seed ageing, since the primary processes and their interactions involved in seed ageing are not yet fully understood (McDonald, 1999).

Biochemical deterioration during seed ageing has been studied mostly under accelerated ageing conditions using high temperature and high seed water content (McDonald, 1999). Under such storage conditions, seeds typically lose their viability within a few days or weeks. While these studies allowed important progress towards the understanding of seed ageing mechanisms, a major question has been raised whether the mechanisms of seed ageing are the same under such accelerated ageing conditions and under the cool dry conditions where seeds age over many years. A review of the literature suggests that there may be several mechanisms of seed ageing (Walters, 1998). For example, lipid peroxidation and the loss of membrane phospholipids are major causes of seed ageing under accelerated ageing conditions (Priestley, 1986; Wilson and McDonald, 1986; McDonald, 1999). Yet, several studies of long-term storage detected little or no lipid peroxidation and loss of phospholipids from seeds of cucumber (Koostra and Harrington, 1969), rice (Matsuda and Hirayama, 1973), peanuts (Pearce and Abdel-Samad, 1980), soybean (Priestley and Leopold, 1983), and wheat (Petruzzelli and Taranto, 1984).
Under the long-term storage conditions, seeds are likely to be in the glassy state because of the cool storage environment and low seed water content. The extremely high viscosity and low molecular mobility of the seed cytoplasm could prevent or inhibit many deleterious processes (Williams and Leopold, 1989; Sun and Leopold, 1993, 1994, 1997; Leopold et al., 1994; Leprince and Walters-Vertucci, 1995; Sun et al., 1998; Buitink et al., 1998, 2000a). With increasing temperature or seed water content, the solid-like glassy state may soften into the rubbery state or even 'melt' into the liquid state since the glass transition temperature \(T_g\) will fall below the storage temperature. The low viscosity and enhanced molecular mobility in the rubbery or liquid state would permit certain deteriorative reactions to proceed rapidly, which are otherwise retarded in the glassy state. Thus, the major primary process that initiates seed ageing could be different under different storage conditions, depending on the \(T_g\) of seed cytoplasm.

The present study focused on the contributions of lipid peroxidation and non-enzymatic protein glycosylation to seed ageing in a wide range of seed water content and temperature conditions, and the role of the glassy state in retarding biochemical deterioration and thus extending seed survival during long-term storage is examined.

Materials and methods

Seed treatment, storage and germination test

Seeds of Vigna radiata (L.) Wilczek (mung bean) were briefly soaked in water for up to 6 h as described previously (Sun et al., 1997). Seeds with loose or damaged testas, which were imbibed rapidly, were discarded. Seeds were selected for ageing experiments when their water content increased to approximately 0.3–0.4 g g\(^{-1}\) DW (g water per g dry weight) during imbibition. Imbibition time for individual seeds to reach this water content varied from 2–6 h, depending on water permeability of the seed coat. Selected seeds were immediately placed in a sealed container at 5 °C for overnight equilibration to ensure uniform rehydration, and then dried back at ambient temperature (24±1 °C) to various water contents ranging from 0.222 to 0.078 g g\(^{-1}\) DW. This range of water contents corresponded roughly to that of relative humidities between 30% and 80%. This brief hydration/dehydration treatment reduced the variation in rates of imbibition and germination due to differences in seed coat characteristics among individual seeds and did not affect seed longevity (Sun et al., 1997). Dried seeds were sealed in laminated aluminium packets for storage. Two series of experiments were carried out to investigate the mechanisms of seed ageing under different conditions. In the first series of experiments, seeds with eight water contents were stored at 33 °C (±0.4 °C). The duration of storage experiment varied from 24 d for seeds with a water content of 0.222 g g\(^{-1}\) DW to 600 d for seeds with a water content of 0.078 g g\(^{-1}\) DW. In the second series of experiments, seeds with a water content of 0.138 g g\(^{-1}\) DW were stored at six different temperatures, ranging from 33–76 °C. The high, non-physiological temperatures were used in this study only for comparison purposes. Seed ageing during storage was regularly monitored. Two replicates of 50 seeds each were imbibed for 3 h and then germinated at 24 °C (±1 °C) for 48 h on moist filter papers in Petri dishes. The percentage of germination and radicle length of germinated seeds were recorded. Seed vigour index was calculated by multiplying percentage germination and the average radicle length of germinated seeds, expressed as a percentage relative to unaged seeds. Seed germination before storage was -99%.

Monitoring of biochemical deterioration during storage

The accumulation of lipid peroxidation products, reducing sugars (e.g. glucose) and Amadori/Maillard products in seed axes was measured during storage. The content of lipid peroxidation products was determined using the TBA reagent (0.25% thiobarbituric acid in 10% trichloroacetic acid). Embryo axes (~20 mg) were homogenized with 1.0 ml phosphate buffer (50 mM, pH 7.2) and centrifuged at 5000 g for 5 min. Aliquots of 0.25 ml supernatant were mixed with 2.0 ml TBA reagent and incubated at 95 °C for 30 min. Samples were cooled and centrifuged at 18 000 g for 10 min. The absorbance of the supernatant was measured at 532 nm and corrected by subtracting the absorbance at 600 nm. Glucose content was determined enzymatically using the glucose kit (Sigma, USA). Isolated axes (~20 mg) were homogenized with 50% ethanol (0.65 ml) and centrifuged at 15 000 g for 5 min. Aliquots of 0.5 ml supernatant were freeze-dried, and redissolved with 20 μl distilled water. For each sample, 1.0 ml glucose assay reagent was added, and the absorbance of the sample was measured at 520 nm after dilution with 3 ml of 0.1 N HCl.

To measure the content of Amadori/Maillard reaction products, embryo axes (~20 mg) were homogenized with 1.2 ml phosphate buffer (50 mM, pH 7.2). Aliquots (200 μl) of 10% streptomycin sulphate (dissolved in 50 mM HEPES, pH 7.2) were added to the homogenate to precipitate nucleic acids. After vortexing and centrifuging at 15 000 g for 15 min, another 200 μl streptomycin sulphate was added, and the suspensions were centrifuged again. Proteins in the supernatant were precipitated with ammonium sulphate (0.55 g ml\(^{-1}\)). After centrifugation, the pellet was redissolved in 3.3 ml phosphate buffer (50 mM, pH 7.2). Seed proteins were further purified using 10-DG columns with the cut-off size of 6–8 kDa (Bio-Rad, Hercules, CA, USA). Extracted proteins were used to measure Amadori reaction products and Maillard reaction products. This procedure minimized the interference of non-protein substances and stabilized protein fluorescence readings (Sun and Leopold, 1995).

The content of Amadori reaction products was measured using the nitroblue tetrazolium (NBT) method (Wettlaufer and Leopold, 1991). One ml of NBT reagent (0.5 mM NBT in 100 mM sodium carbonate, pH 10.3) was added to 0.2 mg of extracted axis proteins and incubated at 40 °C in a water bath. The absorbance at 550 nm was recorded after 10 and 20 min of incubation. The increase in absorbance (ΔOD) was used to express the content of Amadori reaction products. The content of Maillard reaction products was determined using the fluorescence method. Extracted seed proteins (0.3 mg ml\(^{-1}\)) were scanned with an excitation wavelength from 270–400 nm and emission wavelengths from 320–500 nm. A new fluorescence maximum was detected at the excitation of 350 nm and emission of 420 nm (Murthy and Sun, 2000). The intensity of the new fluorescence peak was used to express the content of Maillard reaction products in seed proteins.

Determination of glass transition temperature in embryo axes

The glass transition temperature \(T_g\) of embryo axes was determined using differential scanning calorimetry (DSC-131, Setaram, France). Isolated embryo axes were equilibrated over a series of relative humidities from 20–90% at 16 °C for 8–10 d. Axes (~25 mg) with different water contents (0.05–0.28 g g\(^{-1}\) DW) were hermetically sealed in aluminium crucibles and scanned at 5 °C min\(^{-1}\) from ~120 °C to 120 °C. The onset temperature of glass transition was taken as the \(T_g\) of embryo axes.
Results

Effect of water content on seed viability loss and biochemical deteriorations

Seed viability (percentage germination and seed vigour) declined rapidly during storage at 33 °C as seed water content increased (Fig. 1). Germination and vigour data were linearized with the probit transformation and logarithmic transformation, respectively, and were plotted against storage time to calculate the rate constants of seed germination loss and vigour decline (i.e. the slopes of the linear plots). The rate of seed ageing, as expressed by the rate constants of seed germination loss and vigour decline, increased exponentially with increasing water content from 0.078 g g⁻¹ DW to 0.222 g g⁻¹ DW (Fig. 2).

The contents of TBA-reactive products, glucose, Amadori products and Maillard reaction products in embryo axes increased during storage (Figs 3, 4). The accumulation of TBA-reactive products, glucose and Amadori products during storage all followed the ‘square-root-of-time’ kinetics, the plots of their contents against time being linear. The accumulation of Maillard products followed a different pattern, exhibiting a linear increase during storage (i.e. zero-order kinetics). Therefore, the rate constants of lipid peroxidation, glucose accumulation and Amadori reactions were calculated using the square-root-of-time kinetics, and the rate constant of Maillard reactions using the zero-order kinetics. Seed water content affected lipid peroxidation, glucose accumulation, Amadori reactions, and Maillard reactions (Fig. 5). The rate of lipid peroxidation increased with increasing water content up to 0.16 g g⁻¹ DW, but further increases in seed water content inhibited lipid peroxidation (Fig. 5A). Glucose accumulation rate during storage increased significantly with seed water content, which suggested the occurrence of greater sugar hydrolysis from sucrose and oligosaccharides (Fig. 5B). The rate of Amadori product accumulation showed an identical trend to glucose accumulation (Fig. 5C). The rate of Maillard reactions increased very slowly at seed water contents of less than 0.14 g g⁻¹ DW, but increased rapidly as water content increased (Fig. 5D).

Effect of temperature on seed viability loss and biochemical deterioration

The effect of storage temperature on seed ageing was examined at a water content of 0.138 g g⁻¹ DW. The rate constants of seed germination loss and vigour decline are shown in Fig. 6 as a function of storage temperature. The rate constants of lipid peroxidation, sugar hydrolysis, Amadori reactions, and Maillard reactions generally

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Fig. 1. Decline in germination (A, B) and vigour (C, D) of mung bean seeds during storage at 33 °C. Water contents are shown in parentheses. Data are the means ±SE. Bars smaller than symbols are not shown.
increased with increasing temperature (Fig. 7). The temperature dependence of lipid peroxidation apparently conformed to the William–Landel–Ferry (WLF) relationship (Fig. 7A, inset). The accumulation of glucose was relatively slow at temperatures below 47 °C, but increased greatly at higher temperatures (Fig. 7B, inset). Amadori and Maillard reactions appeared to follow the Arrhenius relationship (Fig. 7C, D).

**Relationship between seed viability loss and Maillard product accumulation**

Correlation analysis indicated a strong association between loss of seed viability and Maillard reactions (i.e. the accumulation of Maillard products in embryo axes, Fig. 8). All seven sets of water content experiment data could essentially be superimposed on a single curve. The six sets of temperature experiment data showed similar trends, although they did not represent a single curve. Correlations between seed viability loss and other biochemical deterioration parameters were quite complex, and no consistent trend among water content experiments and temperature experiments were observed (data not shown).

**Roles of the glassy state in biochemical deterioration during storage**

The relationship between water content and glass transition temperatures ($T_g$) of isolated axes is shown in Fig. 9. As water content increased, the $T_g$ decreased. At water contents <0.08 g g$^{-1}$ DW, the axis tissue will exist in the glassy state (Fig. 7A, inset). The accumulation of glucose was relatively slow at temperatures below 47 °C, but increased greatly at higher temperatures (Fig. 7B, inset). Amadori and Maillard reactions appeared to follow the Arrhenius relationship (Fig. 7C, D).

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Fig. 4. Accumulation of Amadori products (A, B) and Maillard products (C, D) in mung bean axes during storage at 33 °C. Water contents are shown in parentheses. Data are the means ± SE. Bars smaller than symbols are not shown.

Fig. 5. Effect of seed water content on lipid peroxidation (A), glucose accumulation (B), Amadori reactions (C) and Maillard reactions (D) in seed axes during storage at 33 °C.
glassy (or vitreous) state at ambient temperature. The relationships between seed ageing rate, storage temperature \((T)\) and \(T_g\) are shown in Fig. 10. To interpret both the water content effect and the storage temperature effect in terms of glass transition, the rate constants of seed ageing, as calculated from seed germination loss and vigour decline, were plotted against the value of \(T-T_g\). The kinetics of seed ageing were significantly different at storage conditions below and above \(T_g+40\ °C\) (i.e. \(T-T_g=\pm 40\ °C\)). Below \(T_g+40\ °C\), the rate constants of seed ageing increased very slowly as a function of \(T-T_g\), whereas above \(T_g+40\ °C\), the seed aged rapidly. Although both seed water content effect and storage temperature effect on seed ageing were related to glassy state transition, significant differences between them were observed. Compared with water content, storage temperature had a much greater effect on seed ageing at similar \(T-T_g\) values when storage temperature was above \(T_g+40\ °C\) (Fig. 10).

Biochemical deterioration in embryo axes as a function of \(T-T_g\) at different water contents and storage temperatures is presented in Fig. 11. The temperature at 40±45 °C above the \(T_g\) also appeared to be a critical point. Storage temperature had a greater effect on seed ageing at similar \(T-T_g\) values when compared to seed water content. However, the relationship between biochemical deterioration and \(T-T_g\) was more complex for water content experiments except for Maillard reactions (Fig. 11D). Lipid peroxidation and sugar hydrolysis were almost arrested at temperatures below \(T_g\) (water content <0.08 g

![Fig. 6. Rate constants of seed ageing as a function of storage temperature for mung bean seeds. Water content was 0.138 g g⁻¹ DW for all treatments. The inset shows the correlation between rate constants based on seed germination and vigour. The unit of measure for rate constant: germination, probit d⁻¹; vigour, d⁻¹.](image)

![Fig. 7. Effect of storage temperature on lipid peroxidation (A), glucose accumulation (B), Amadori reactions (C), and Maillard reactions (D) in seed axes. Water content was 0.138 g g⁻¹ DW all treatments. Insets show rate constants on the logarithmic scale.](image)
g⁻¹ DW) (Fig. 11A, B). At intermediate water contents (0.10–0.16 g g⁻¹ DW), lipid peroxidation, sugar hydrolysis and Amadori reactions increased with increasing $T-T_g$ value. At water content >0.16 g g⁻¹ DW, lipid peroxidation decreased with increasing $T-T_g$ value.

**Discussion**

*Seed ageing pathway and Maillard reactions*

Maillard reactions are a series of complex reactions that occur following an initial simple non-enzymatic attack on amino groups of proteins and nucleic acid/protein complexes by reducing sugars or aldehydes. The AGE products (i.e. advanced glycosylation end-products) from Maillard reactions occur in both accelerated and naturally aged seed tissues (Wettlaufer and Leopold, 1991; Sun and Leopold, 1995). It was recently found that lipid peroxidation and sugar hydrolysis (formation of reducing sugars) were coupled to the Maillard reactions during seed storage (Murthy and Sun, 2000). It was hypothesized that Maillard reactions are the ultimate pathway of seed ageing with, possibly, different primary biochemical reactions depending on seed species and storage conditions. This study provides strong experimental support for this hypothesis.

The content of Maillard products increased in seeds during storage at all moisture and temperature conditions. When seed vigour was plotted against the account of Maillard products (Fig. 8), all seven data sets of moisture experiments were identical, while six data sets of temperature experiments demonstrated a close trend, although many temperature points were above those to which seeds are usually subjected (Fig. 8). These data suggest a close relationship between the rate of seed ageing and Maillard reactions. In contrast, no consistent trend was observed between seed viability loss and other biochemical parameters, indicating that the contribution by various primary reactions to the Maillard reactions vary under different moisture and temperature conditions.

An important question is whether the close correlation between Maillard reactions and seed ageing reflects a cause–effect relationship. Figure 8 show that the loss of seed vigour was associated with the initial accumulation of Maillard products in seed axes, and Maillard products continued to accumulate even after seed vigour was lost. There are several lines of evidence suggesting that seed vigour is sensitive to Maillard reactions in axes. Maillard reactions generally involved four steps: (1) non-enzymatic condensation, (2) Amadori re-arrangement, (3) degradation of Amadori products, and (4) the formation of the AGE products (Lee and Cerami, 1989; Sun and Leopold, 1995). After Amadori rearrangement (step 2), the reactions are irreversible and the functions of affected proteins or reactions are the ultimate pathway of seed ageing with, possibly, different primary biochemical reactions depending on seed species and storage conditions. This study provides strong experimental support for this hypothesis.

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enzymes are lost. The carbonyl intermediates from step 3 can initiate additional non-enzymatic attack on proteins and nucleic acid/protein complexes. Therefore, the continued Maillard product accumulation is not unexpected even after seed death. In model systems, non-enzymatic glycosylations reduce the activities of Cu-Zn-superoxide dismutase (Taniguchi et al., 1989), ribonuclease (Eble et al., 1983), Na+/K+ ATPase (Garner et al., 1987), and glucose-6-phosphate dehydrogenase as well as lactate dehydrogenase (Wettlaufer and Leopold, 1991). In mung bean seeds, several antioxidant enzymes, glutathione reductase, catalase, and ascorbate peroxidase, are very sensitive to Maillard reactions (Murthy et al., 2002). The contribution of Maillard reactions to seed ageing is likely to be through the chemical modification of macromolecules during storage, which gradually reduces metabolic capability, the ability of the metabolic events to limit the free radical damage and to repair the damage during germination. As a result, they lead to a decline in seed vigour during storage and eventually seed death. Non-enzymatic glycosylation was also associated with DNA damage (Lee and Cerami, 1989).

Primary biochemical reactions under different storage conditions

Temperature and moisture content are the most important factors affecting the rate of seed deterioration. A wide range of seed water contents and storage temperatures was used to determine the relationship between advances in seed ageing and several possible primary biochemical deterioration processes, including lipid peroxidation and sugar hydrolysis (i.e. glucose accumulation). At low moisture contents, enzymatic reactions are expected to play little role in seed ageing (Priestley, 1986). However, even at low moisture contents (<0.08 g g⁻¹ DW), mung bean seeds contained a considerable amount of compounds (e.g. reducing sugars and aldehydes) that were able to initiate the Maillard reactions during storage (Fig. 3). Although the content of such compounds did not increase significantly, seed ageing during storage could well be due to the slow Amadori and Maillard reactions. As seed moisture content increased from 0.08 g g⁻¹ DW to 0.14 g g⁻¹ DW, lipid peroxidation and sugar hydrolysis increased similarly (Figs 3, 5A, B). Thus, one would not expect a significant change in the relative contribution of lipid peroxidation and sugar hydrolysis to the Amadori and Maillard reactions. At moisture contents around 0.16 g g⁻¹ DW, lipid peroxidation increased sharply, almost 5-fold; whereas sugar hydrolysis still occurred relatively slowly. As seed moisture content increased further, lipid peroxidation was drastically reduced and sugar hydrolysis increased rapidly (Fig. 5A, B). These data indicate that the products of lipid peroxidation are a dominating driving force of the Amadori and Maillard reactions. At moisture contents around 0.16 g g⁻¹ DW, whereas reducing sugars from sugar hydrolysis might become a dominating driving force at higher moisture contents. Storage temperature also affected lipid peroxidation and sugar hydrolysis differently. For example, lipid peroxidation was more sensitive to temperature increases than was sugar hydrolysis between 30 °C and 50 °C (insets of Fig. 7A, B). These data demonstrated that primary biochemical reactions varied at different storage conditions.

The sharp decrease in the rate of lipid peroxidation at high moisture contents was not unexpected, since water could act as a buffer between oxidation-generated free radicals and target molecules, suppressing the autocatalytic chain reaction in lipid peroxidation. The dependence of lipid peroxidation on moisture content and temperature has been well known from earlier studies on food materials (McDonald, 1999). At moisture contents <0.06 g g⁻¹ DW lipid oxidation mainly occurs by auto-oxidation. At intermediate moisture contents between 0.08 and 0.12 g g⁻¹ DW, auto-oxidation is retarded and enzyme-mediated
lipid peroxidation becomes likely. The products of lipid peroxidation may vary under different seed moisture and temperature conditions, which are also expected to affect the Amadori and Maillard reactions. The presence of different primary reactions coupled to the Amadori and Maillard reactions offers a good explanation of the fact that no consistent association between lipid peroxidation and seed ageing was observed in several studies (Priestley and Leopold, 1983; Powell and Harman, 1985; Kalpana and Madhava Rao, 1994).

The ‘square-root-kinetics’ of seed deterioration observed here was described previously by Pikal and Rigsbee (1997) who studied protein stability during dry storage. From their numerical analyses, they proposed that the ‘square-root-kinetics’ is due to the presence of intermediate degradation steps. Sugar hydrolysis, lipid peroxidation and Amadori reactions are all complex processes that consist of many reaction steps.

**Dependence of ageing mechanisms on seed glass transition**

The glassy state of dry seeds plays an important role in seed longevity. The high intracellular viscosity of the glassy state could retard molecular mobility and thus slow down seed deterioration during storage. This hypothesis is well supported by a number of experimental studies. The $T_g$ in several seeds corresponds to the maximum temperature for long-term survival during storage, and the loss of glassy state is associated with the rapid decline in seed viability under accelerated ageing conditions (Sun and Leopold, 1993, 1994; Leopold et al., 1994; Sun, 1997). Certain deteriorative reactions that occur during seed storage, such as protein denaturation and the formation of free radicals and reducing sugars are significantly inhibited during storage when seeds are in the glassy state (Sun and Leopold, 1995, 1997; Sun et al., 1998). The $T_g$ of seeds is a function of moisture content, and whether the seed is in a glassy state or not depends on seed moisture content and storage temperature. As storage temperature or moisture content increases, the seed will undergo the glass-to-liquid transition, resulting in an increase in molecular mobility. Over a range of seed water content and storage temperature conditions, ageing rates of several pollen and seed species have been correlated with molecular mobility (Buitink et al., 1998, 1999, 2000a, b). Molecular mobility appears to be a key factor influencing the storage stability of biological tissues because it controls the rate of detrimental reactions that reduce storage life.

This study examined the effect of glass transition on the loss of seed viability, lipid peroxidation, sugar hydrolysis, Amadori and Maillard reactions. It was observed that there is a high critical temperature at ~40 °C above the $T_g$. The rates of primary biochemical reactions (e.g. lipid peroxidation and glucose formation) progressed very slowly.

![Fig. 11. Biochemical deterioration in seed axes as a function of the $(T-T_g)$ at different water contents (open symbols) and temperatures (closed symbols). Rate constants for (A) lipid peroxidation; (B) glucose accumulation; (C) Amadori reactions; (D) Maillard reactions.](https://academic.oup.com/jxb/article-abstract/54/384/1057/631142)
below this high critical temperature, but increased rapidly above it (Fig. 11A, B). The rapid increase in the Maillard reactions and seed ageing corresponded to the same high critical temperature (Figs 10A, B, 11C, D). These data demonstrated that the rate of seed ageing slowed down significantly even before seed tissue entered into the glassy state. The high critical temperature in mung bean axes varied from 1.13 $T_g$ to 1.18 $T_g$ (in Kelvin). The nature of this high critical temperature may be related to the qualitative change in the dynamics of the glass-forming systems near the so-called cross-over temperature ($T_c$) above $T_g$. The cross-over temperature of ionic and van der Waals glass-forming liquids is known to be around 1.15 to 1.20 $T_g$ (Sokolov, 1996). Using the ST–EPR technique, Buitink et al. (2000c) reported a significant increase of molecular mobility at a temperature 50–60 °C above the $T_g$ in pollen and seed tissues, and hypothesized that such a high critical temperature relates to the stability of dry organisms. The high critical temperature observed in this study (Figs 10, 11) was similar to the one related to the change in molecular mobility. [Note that the $T_g$ value obtained from the EPR measurement was typically 15–20 °C lower than the DSC measurement (see Buitink et al., 1998). Also note that the $T_g/T_g$ in Buitink et al. (2000c) was calculated based on temperatures in Celsius and was reported to be >1.5 $T_g$ for pollen and seed tissues. When converted to temperatures in Kelvin, the $T_g/T_g$ value from their EPR study was 1.16–1.20 $T_g$.] This study has provided evidence that ageing-related biochemical reactions in seed tissues are retarded markedly at temperatures below the $T_c$ and increased rapidly above it (Figs 10, 11).

In conclusion, the contributions of primary biochemical reactions to Amadori and Maillard reactions vary under different storage conditions. The occurrence of kinetic changes in molecular mobility at a high critical temperature well above $T_g$ corresponds to the increase in primary biochemical reactions that contributed to Maillard reactions and seed ageing. Biochemical reactions and seed ageing slow down significantly below $T_c$, before seed tissues enter into the glassy state.

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