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Water-dependent thermal transitions in quinoa embryos

Silvia B. Matiacevich [a], Martina L. Castellión [a,b], Sara B. Maldonado [b], M. Pilar Buera [a,*]

[a] *Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Av. Intendente Guiraldes s/n, Ciudad Universitaria, C1428EHA Buenos Aires, Argentina*
[b] *Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Av. Intendente Guiraldes s/n, Ciudad Universitaria, C1428EHA Buenos Aires, Argentina*

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Abstract

The water-dependent thermal transitions of the embryos in the mature seeds of *Chenopodium quinoa* Willd., cv. Baer II were studied by differential scanning calorimetry in order to provide tools for analyzing seed deterioration during storage. For comparative purposes, Guggenheim–Anderson–de Boer (GAB) constants from water sorption isotherms were also obtained from other two cultivars. Glass transition temperatures (T_g), overlapped with lipid melting, could be detected in defatted embryos. Quinoa seeds storage temperature should remain below 0 °C in order to maintain them in a glassy state if relative humidity is higher than 59%. Frozen water was detected in defatted embryos at water contents above 47% (dry basis, d.b.) while protein denaturation occurred even at 5% (d.b.) water content, although at a low extent. The results suggested that protein denaturation, without the requirement of lipid removal, is a potential index to follow seed deterioration during storage.

Keywords: *Chenopodium quinoa*; Thermal transitions; Differential scanning calorimeter (DSC); Proteins

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal native to the Andean regions of South America. As compared to most cereals, quinoa seeds have a higher nutritional value. In fact, protein content represents 14–20% (g/100 g dry basis, d.b.), being particularly rich in essential aminoacids such as lysine and methionine, thus supplying high-quality protein [1]. They also contain large amounts of carbohydrates, fat, vitamins and minerals [2]. Quinoa seeds have an orthodox behavior but previous studies have shown differences in the storage behavior between cultivars of different adaptation groups, i.e. cv. Baer II, original from wet areas of Southern Chile, which belongs to the "sea level" adaptation group and cv. Ollagüe, original from North Chile and Bolivia and belongs to the "Altiplano" adaptation group [3,4]. After 18 months storage at 14% R.H. and 25 °C, cv. Baer II seeds were better preserved than cv. Ollagüe seeds as determined by germination and viability test [3].

In orthodox seeds, deteriorative processes during storage are reported as dependent on their physical state and on solid/water interactions [5]. Under long-term storage conditions, i.e. cool storage environment and low seed water content, intracellular glasses are detected in a number of orthodox seeds [6]. According to Williams and Leopold [7] and Buitink et al. [8] the extremely high viscosity and very low molecular mobility in glassy systems would prevent or inhibit many deleterious processes.

Vitrification of cytoplasm components in orthodox seeds is proposed to be advantageous for germplasm stability and their transitions may affect the seed viability [9]. As storage temperature or water content increases, seeds undergo the glass-to-liquid transition (at the glass transition temperature, T_g), resulting in an increase in molecular mobility. T_g is a function of water content, and whether the seed tissues are in a glassy state or not, it will depend on both seed water content and storage temperature [8,10].

Investigations have led to suggest that the soluble carbohydrates in seeds would induce glass formation as the water content is depressed, thus limiting deteriorative reactions [7,11–14]. A

* Corresponding author. Tel.: +54 11 4576 3366; fax: +54 11 4576 3366.
E-mail addresses: silviam@di.fcen.uba.ar (S.B. Matiacevich), martina@bg.fcen.uba.ar (M.L. Castellión), saram@bg.fcen.uba.ar (S.B. Maldonado), pilar@di.fcen.uba.ar (M.P. Buera).

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high proportion of solids (sugars and other biopolymers) results in a rapid increase in seed cytoplasm viscosity [13]. Equilibrium water content among seeds of different species may vary as it is influenced by cytoplasm composition [15]. Even more, the strength and nature of water binding in seeds is considered to influence the rate of deteriorative reactions [9,16]. Therefore, water sorption characteristics may be a factor in the variation in seed longevity among different species [17] and even among different cultivars although the relationship between seed water-binding properties and storage behavior is yet to be resolved.

Currently, there is a lack in studies on glass transition temperature or other water-dependent thermal transitions in quinoa seeds that could provide adequate information on appropriate storage conditions. In this study, we selected quinoa seeds from cv. Baer II and cv. Chadmo, which belong to the "sea level" adaptation group, and cv. Ollagüe, from the "Altiplano" adaptation group.

The purpose of this study was to analyze water content-dependent thermal transitions in quinoa embryos (cv. Baer II) as part of a comprehensive study on quinoa seed conservation in order to provide tools for predicting quality changes in different cultivars during storage.

2. Materials and methods

2.1. Seed material

Quinoa seeds (Cv. Baer II, cv. Ollagüe and cv. Chadmo) were grown in the experimental greenhouse of the School of Sciences of the University of Buenos Aires in Buenos Aires, Argentina. Seed germinability was 100% (normal germination was assessed according to ISTA [18]). In all experiments, seeds were manually pre-sorted discarding excessively small, large and damaged seeds. Embryos (with protein and lipid storage reserves) were obtained by gently removing perisperm, endosperm and seed coat under a stereoscope. Chopped embryos were defatted by three times soaking for 30 min in 2:1 chloroform: methanol solution (3 ml/g) [7]. Whole seeds, non-defatted embryos or defatted embryos were placed in 1 cm diameter glass vials and stored over saturated salt solutions at several relative humidities (R.H.) into vacuum desiccators for 10 days at 25 °C for seeds and 6 ± 1 °C for non-defatted embryos and defatted embryos. In order to achieve different water contents, the following saturated salt solutions (analytical grade, Mallinckrot, U.S.A.) were employed: LiCl (R.H. 11%); CH_3COOK (R.H. 23%); $MgCl_2$ (R.H. 33%); $MgNO_3$ (R.H. 59%); NaCl (R.H. 75%); KBr (R.H. 85%); KNO_3 (R.H. 96%) [19].

2.2. Model systems

Model sugar systems were prepared with the approximate sugar composition previously reported for quinoa seeds. System A was prepared according to [20] with a relative amount of sugars of: xylose (Merck, Darmstadt, U.S.A.); maltose (Mallinckrot, U.S.A.); glucose and fructose (Anedra, Argentina) (7:5:1:1). The second model system (system B) contained sucrose (Merck, Darmstadt, U.S.A.), glucose, maltose, fructose (30:16:14:2), according to [21]. 15% sugar solutions in the formerly referred proportions were freeze-dried at −110 °C and 4×10^4 mbar (Heto-Holten A/S, CT110 model, Heto Lab Equipment, Denmark). Lyophilized model systems were equilibrated at 23 and 59% R.H. at 6 °C.

2.3. Thermal analysis

After equilibration, embryos or model systems were weighed with a precision of ±0.01 mg, sealed into aluminum pans (40 μl of capacity) and loaded into the differential scanning calorimeter (DSC-Mettler Toledo 822, Switzerland). All experiments were performed in triplicates following the same protocol.

The DSC thermograms were obtained from −140 to 100 °C at a heating rate of 10 °C/min. The enthalpy (ΔH) of the melting transition of water was calculated from the area under the peak. The glass-to-liquid transition temperature (T_g) was determined as the onset of the range where the change in specific heat occurred. All thermograms were analyzed using STARe Software v. 6.1 (Mettler Thermal Analysis).

2.4. Water content

Water content of defatted embryos was determined gravimetrically using a Mettler Toledo Analytical Balance (Switzerland) after the DSC scans. The pans were punctured before dried in an oven at 100 °C for 4 days.

2.5. Sorption isotherms

The measured isotherms were fitted to the GAB equation by using the least square method for minimizing the absolute differences between measured and calculated moisture content values. The GAB model has been considered the best fit model for many food materials over a wide range of water activity [22]. The GAB constants m_0, C and k, were obtained by transforming the GAB equation to the quadratic form:

$$\frac{a_w}{m} = \left(\frac{1}{m_0 Ck}\right) + \left(1 - \frac{2}{C}\right) a_w + \left(\frac{((1/C) - 1)k}{m_0}\right) a_w^2 \quad (1)$$

where m is the moisture content, m_0 the GAB monolayer moisture content (water content needed to cover the entire surface with an unimolecular water layer), C the Guggenheim constant that is related to the heat of sorption of the first layer on primary sites and k is a factor correcting properties of the multilayer molecules with respect to the bulk liquid. C and k are related to the energy of interaction between mono- and multilayer water molecules and they reflect the temperature effect on sorption properties. k is generally near to but less than unity.

The quality of the fit of the GAB model was assessed from the value of the relative percent deviations modulus (%E):

$$\%E = \frac{100}{n} \sum \frac{|m - m_i^*|}{m} \quad (2)$$

where n is the number of measurements, m the measured moisture content and m_i^* is the calculated moisture content. According to [22], a good fit is obtained when $\%E < 5$.

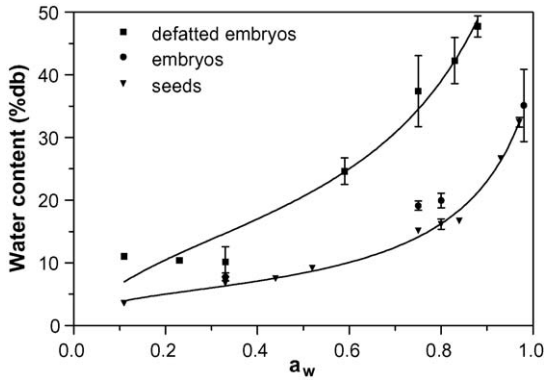

Fig. 1. Sorption isotherms of seeds, non-defatted embryos and defatted embryos at 25 °C. Average values of three experimental results, bars indicate respective standard deviations (S.D.).

Table 1
Monolayer moisture content[a] and constants values[b] of Eq. (1) and goodness of fit[c] as applied to the experimental adsorption isotherms of quinoa grains

Sample	m_0[a] (%d.b.)	k[b]	C[b]	%E[c]
Defatted embryos, cv. Baer II	11.7	0.87	12	5
Seeds, cv. Baer II	5.0	0.873	23	3
Seeds. cv. Ollagüe	4.5	0.87	52	3.2
Seeds. cv. Chadmo	4.7	0.88	56	3.4
Quinoa seeds[d]	8.67	0.7	15.3	0.43

[a] Moisture content needed to cover the entire surface with a unimolecular layer (m_0).
[b] Factor correcting properties of the multilayer molecules with respect to the bulk liquid (k). Guggenheim constant (C).
[c] Relative percent deviation modulus (%E).
[d] Data from Tolaba et al. [28], undetermined genotype.

3. Results and discussion

3.1. Sorption isotherms

Sorption characteristics are essential for the evaluation of the influence of relative humidity on glass transition since water is the most important plasticizer in biological systems [23,6]. When analyzing seed storage conditions, as plasticizing of intracellular glasses in seeds depends on water content and temperature, Vertucci and Roos [9,24], Sun [6] and Pukacka et al. [25] suggest the existence of differences in water sorption between defatted and non-defatted seed tissues. In order to ascertain this assumption, water sorption isotherms were obtained separately from whole seeds, non-defatted embryos and defatted embryos (Fig. 1). Isotherms were identical for whole seeds and non-defatted embryos, indicating that the equilibrium water content value achieved was the same for both systems at a given R.H. The shape of the isotherm of defatted embryos was similar to the others but water content was higher than in seeds and non-defatted embryos at the same R.H. According to Vertucci and Roos [9], the moisture content of a seed at a given R.H. decreases as lipid content increases because lipids are inaccessible to water but they contribute to the dry weight. In this way, we suggest that the differences in water adsorption between quinoa defatted embryos on the one hand and non-defatted embryos and seeds on the other could be explained by differences in lipid content [9,26,25].

Sorption isotherms of two other quinoa cultivars (cv. Chadmo and cv. Ollagüe) were also determined for comparative purposes. The isotherms were fitted by the GAB model (Eq. (1)), which has a wide range of applicability (adequate fit up to water activity (a_w) = 0.9). There are no previous data regarding GAB parameters of defatted quinoa embryos. The compilation of GAB parameters (m_0, k and C) and %E in different quinoa genotypes is shown in Table 1. The values for %E [22] were less than 5 in all cases, which reflected a very good agreement between experimental and calculated values. The constant k for all analyzed materials was lower than unity, as it is in many food materials [27]. The m_0 value for cv. Baer II seeds was similar to the other two quinoa cultivars (cv. Chadmo and cv. Ollagüe) analyzed in this study. The obtained m_0 values were lower and the C values were higher than those reported by Tolaba et al. [28]. Considering that the differences in these values are mainly due to differences in seed composition [29,15] and the accumulation of storage reserves in the embryo is particularly dependent on environmental factors as reviewed by Triboi and Triboi-Blondel [30], we suggest that GAB parameters depend mostly on the environmental conditions of the plant rather than on the genetic diversity. Consequently, in the present study, GAB parameters were found to be similar among seeds of different genetic origin, obtained in the same experimental conditions and different to those reported previously by [28] (although these authors did not identify the quinoa cultivar employed in their study).

3.2. Thermal transitions

In previous investigations, frozen water was detected in quinoa embryos and seeds (Cv. Ollagüe, cv. Sajama and cv. Baer II) equilibrated at and above 98% R.H. (corresponding to water contents about 30–35 g water/100 g solids), for which the ice melting transition started at −30 °C [31]. In this study, frozen water was detected in cv. Baer II defatted embryos as endothermic peaks (with the same onset temperature of −30 °C) at and above R.H. 85% (corresponding to a water content of 48 ± 2 g water/100 g solids), as shown in Fig. 2. At a given R.H. defatted embryos were capable of adsorbing a higher amount of water than non-defatted embryos and seeds (Fig. 1). The relative water content required to detect frozen water, which is a critical point determining the degree of injury in cryopreserved systems, was higher in the defatted systems, due to the decreased amount of dry weight in them, but keeping the same degree of interaction between water and non-lipid solids.

Thermal transitions, which were independent on water content, were observed in the range of −80 to −10 °C (data not shown) and they were mainly attributed to lipid melting transitions. These thermal events were in accord to those reported in corn embryos [7], *Cuphea* seeds [32] and quinoa embryos [31], three species with high lipidic content.

Glass transition temperatures could not be accurately detected in the non-defatted embryos because the melting transitions of lipids extended over a wide temperature range where the

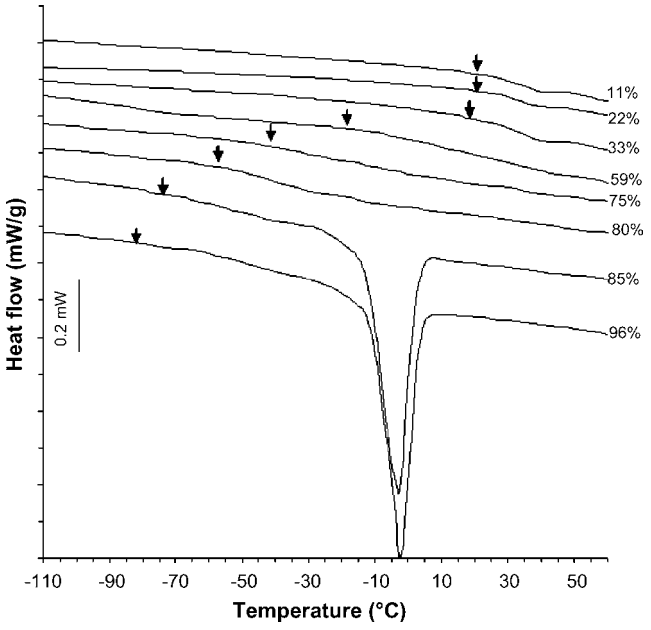

Fig. 2. Thermograms obtained by DSC from quinoa defatted embryos equilibrated at different relative humidities (R.H.). Scan rate, 10 °C/min. Baselines were adjusted to produce the stacking effect. Thermograms were normalized to the corresponding sample size. The R.H. is indicated on the right of each curve. Arrows indicate the onset of glass transition temperature (T_g). Endothermic peak around 0 °C (ice melting) was observed at and above 84% R.H., indicating the presence of frozen water.

glass transition was expected. When embryos were defatted, the thermal endothermic events attributable to lipid melting either disappeared or diminished significantly and glass transitions could be analyzed (Figs. 2 and 3). The increase in equilibrium water content led to a lower glass transition temperature due to the plasticizing effect of water. Molecular mobility below T_g is

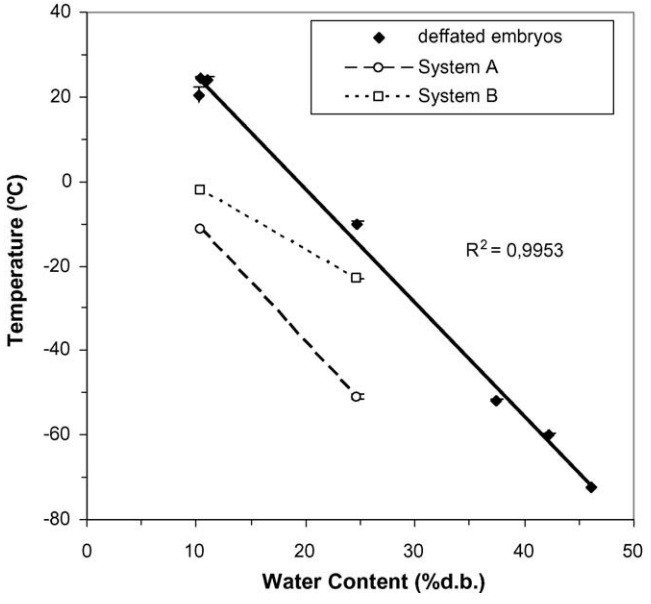

Fig. 3. Relationship between water content and glass transition temperature (T_g) for cv. Baer II embryos and sugar model systems. System A: xylose; maltose; glucose; fructose. System B: sucrose; glucose; maltose; fructose. R^2 indicates the goodness of fit for quinoa embryos. Bars indicate standard deviation (S.D.).

suggested to be a key factor influencing the storage stability of biological tissues because it could control the rate of detrimental reactions that reduce storage life [6,8,10]. As shown in Fig. 3, defatted embryos were very close to their T_g values at 25 °C at R.H. values up to 33%, but their very low T_g values indicated high molecular mobility at and above 59% R.H. On the other hand, very low water contents have proven to be detrimental in seed conservation [33,34]. Thus, in order to improve quinoa seed stability, the variable that could be adjusted is storage temperature. According to these results, when samples are stored close to 59% R.H., storage temperature should be set below 0 °C in order to maintain them in a glassy state.

Sugars (mainly sucrose) are considered as mayor vitrifying agents in plants [35,36]. Sugar composition was reported for genotypically undetermined quinoa seeds [20,21]. Therefore, two model sugar systems (A and B) were prepared with the approximate sugar compositions reported with the aim of comparing the sugar-related thermal transitions with thermal transition obtained of quinoa cultivars used in this study. In order to analyze the behavior of the components in the glassy or supercooled media, the glass transition temperatures of sugar model systems (A and B) were determined and compared to those of the defatted embryos. As shown in Fig. 3, the glass transition temperatures observed in both model systems were lower (about −11 °C for systems A and −2 °C for system B) than the values obtained for defatted embryos (24 °C) at the same R.H. (22%). This was probably due to the presence of high molecular weight biopolymers in the latter.

In defatted embryos equilibrated in the range 59–96% R.H., an endothermic transition was observed with onset temperature at 32 ± 2 °C and midpoint at 45 ± 2 °C, which were independent on water content. This endothermic peak disappeared completely upon rescanning the sample, suggesting that it was an irreversible event. A similar transition is reported in seeds of quinoa (cv. Baer II and cv. Ollagüe) [31] but no explanation was provided. Appelqvist et al. [37] observed similar events for various polysaccharides interpreting them in terms of water-carbohydrate interaction. These observations indicate that this transition requires further investigation.

Endothermic peaks which disappeared on subsequent rescans were observed in non-defatted and defatted embryos at 60–90 °C. Leprince and Walters-Vertucci [38], Sun et al. [39] and Sanchez del Angel et al. [40] reported similar transitions in bean, mung bean and corn seeds, respectively. These peaks were considered typical of protein denaturation phenomena by these authors. The enthalpy changes due to protein denaturation, measured as ΔH, may be associated with molecular structure alterations because of protein unfolding and are the consequence of a combination of endothermic reactions (i.e. disruption of hydrogen bonds) and exothermic reactions (i.e. disruption of hydrophobic interactions) [40]. As shown in Fig. 4 for cv. Baer II, the area of the endothermic transitions (ΔH) and their onset temperature (T_m) changed considerably as water content was modified. The same pattern was observed for cv. Ollagüe (data not shown). As increasing water content, ΔH increased while T_m diminished, this is in accord with a previous report for bean [38].

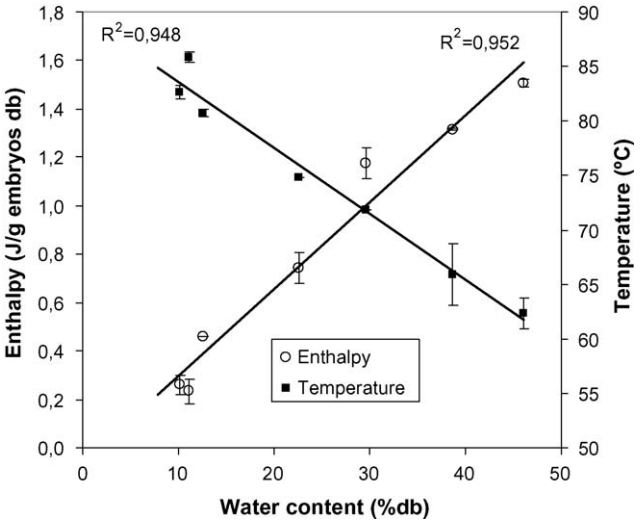

Fig. 4. Water content effect on protein denaturation temperature (onset) and normalized enthalpy for protein denaturation obtained by DSC in defatted embryos of cv. Baer II. Bars indicate standard deviation (S.D.). R^2 indicate the goodness of fit. The same pattern was observed for deffated embryos of cv. Ollagüe.

Both the presence of frozen water and the decrease of protein denaturation temperature would be indicatives of seed state when deteriorative reactions were not restricted. When the endothermic peak related to protein denaturation was compared between cultivars, both onset and peak temperatures of cv. Baer II were higher than those of cv. Ollagüe (Fig. 5). When either non-defatted embryos or whole seeds were compared, cv. Baer II showed a higher protein denaturation temperature than cv. Ollagüe (data not shown).

Although isotherms between cultivars are similar, we observed in previous investigations, that cv. Ollagüe seeds contained a higher content of frozen water than cv. Baer II seeds [31]. In addition, a lower resistance to protein denaturation by effect of high temperature in cv. Ollagüe would be an indicator of their differential storage behavior.

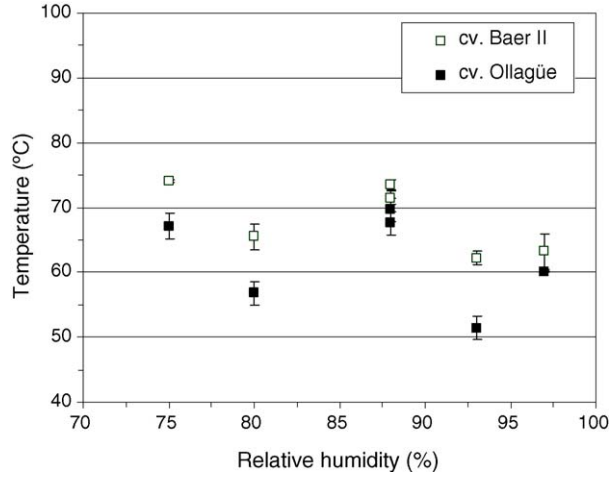

Fig. 5. Protein denaturation temperature (onset) obtained by DSC for cv. Baer II and cv. Ollagüe embryos equilibrated at different relative humidities (%). Bars indicate standard deviation (S.D.).

As explained before, glass transitions were difficult to observe since they were overlapped with other thermal phenomena. The inhibition of molecular mobility at low water content was better reflected in the restrictions for water crystallization, and for low protein denaturation. Frozen water was detected in defatted embryos of cv. Baer II at water content above 47%, while protein denaturation occurred even at 5% water content, although at a low extent. The increase in water content was also reflected in the increased extent of protein denaturation and in the decrease of the temperatures at which this transition occurred. Sun et al. [39] report that thermal stability of seed proteins exhibit a strong dependence on the T_g of intracellular glass, attributing an important role of the glassy state in protein stabilization. As the transitions corresponding to protein denaturation were of similar characteristics in whole seeds, in non-defatted embryos and in defatted embryos, the analysis of protein denaturation could be performed without a previous step of lipid removal. Besides, a different pattern of protein denaturation temperature was observed among cultivars of different storage behavior. Therefore, we propose the analysis of protein denaturation as a potential index to analyze seed deterioration during storage.

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