Influence of Dehydration Rate on the Vitrification of Corn Protein

Marcos A. Sabino,[1] Ludovic Pauchard,[2] Catherine Allain,[2] Cécile Mangavel,[1] Ming Zeng,[1] Denis Lourdin[1]

[1]*INRA, UR 1268 Biopolymères, Interactions, Assemblages F-44300 Nantes, France*
[2]*CNRS, UMR 7608 Fluides, Automatique et Systèmes Thermiques F-91405 Orsay, France*

Received 16 January 2007; accepted 31 August 2007
DOI 10.1002/app.27374
Published online 11 June 2008 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: This article deals with the physicochemical mechanisms involved during the desiccation of cereals, to understand the basis of their endosperm structure: vitreous endosperm is compact and floury endosperm is porous, giving the endosperm its hard and soft textures, respectively. Dehydration of zein (the most abundant protein in the vitreous region of the corn endosperm) was investigated for water contents ranging from ~ 50% to 10% (wet basis). A system of partial confinement of zein pills was optimized to compare both slow and rapid dehydration on two separate domains of the same sample. Macroscopic observations, porosity analysis, and scanning electron microscopy showed that either a compact or porous structure is obtained, depending on the dehydration rate. The glass transition temperature (T_g) of zein, which varies from 160°C for dry zein to about 15°C for zein with 25% water content, is mainly dependent on water content. A state diagram of the zein/water system, including ice melting, was determined from calorimetric measurements of state and phase temperature changes. The different porous and compact structures observed on vitreous zein are discussed in terms of collapse and dehydration rate. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 1–7, 2008

Key words: zein; corn; dehydration; porous; vitreous; glass transition; collapse

INTRODUCTION

The mechanical properties and structure of cereal grains, like corn grains, influence milling performance. These properties in relation to the natural process of their growth are not fully understood at this time. The endosperm, which is the major part of the corn grain, is mainly constituted of starch and proteins.[1,2] Its macroscopic structure shows a floury part and a vitreous part, which are mechanically soft and hard. The hard part of endosperm is often identified by its visually recognizable features and descriptions such as vitreous (having the nature of glass) and translucent.[3,4] According to the literature, proteins are assumed to play an important role in conferring a compact texture on the corn grains despite their low concentration in regard to starch.[5–8] In the total fraction of proteins, the most abundant in the vitreous region of the corn endosperm is the zein, a prolamin that constitutes 8–10% of the dry weight of corn and comprises 45–50% of the protein in this cereal.[9,10] The solid and compact properties of the grain are acquired during its maturation, which correspond to its dehydration. The average water content in the maize grain is around 50%wb (wet basis) at the beginning of the natural dehydration process, where the main components (starch/proteins) are present in a suspension.[11] The dehydration process, which depends on climatic conditions, takes place for ~ 60 days and the average water content of the grain is 15–25%wb at the end of the process.

In this work, we propose to investigate the desiccation process regarding state changes associated with the glass transition temperature (T_g) of the main protein of the vitreous corn endosperm. As in a previous work focused on the zein porosity,[12] we used model samples, which were pills fabricated from commercial zein, and two rates of isothermal dehydration were applied from a hydration level of about 50%. Although water is not a solvent for zein, it could possibly surround the protein conglomerate or could achieve some degree of flow through this conglomerate state. At lower water content, zein is plasticized by water, which modifies its glass transition temperature (T_g). Accordingly to the T_g/water content curve published by Madeka and Kokini[13] and Lawton,[14] zein are susceptible to undergo a rubber/vitreous state change during an isothermal dehydration. Indeed, the T_g increases from about 20 to 160°C when water content decreases from 25% to 0%. The zein/water system studied in this work is

This article is dedicated to the memory of Dr. Catherine Allain died in 2006.

Correspondence to: D. Lourdin (lourdin@nantes.inra.fr).

similar to a colloidal media or suspension. In consequence, it is important to emphasize that zein were not used in a solvated state as opposed to other studies that focused on cast zein.[10]

EXPERIMENTAL

Zein

Corn zein protein was obtained from Fluka Biochemika (Germany), soluble in a 70% ethanol/water solution. Total protein content ($N \times 5.7$), evaluated at 87.6% w/w, was determined after digestion of the samples in concentrated sulphuric acid by an automated ammonia/salicylate reaction.[15] Fractions of protein had a molecular weight of 25–29 kDa (determined by SDS electrophoresis). The molecular or aggregate size of commercial zein determined by quasielastic light scattering (QELS) in solvent media (water/alcohol) revealed two populations of aggregate size whose hydrodynamic radii were centered at 8 and 200 nm. Zein water content was 5.3%, determined by thermogravimetric analysis (TGA), and checked by the Karl Fischer method.

Pills preparation

Zein pills were made with 250 mg of protein powder and were molded at 25°C in a hydraulic press by one compression cycle: (a) 15 s at 100 kg/cm^2; (b) pressure release during 15 s; and (c) 30 s at 120 kg/cm^2. The pills had a diameter of 16 mm and a thickness of 1 mm. The molded zein pills were mechanically very fragile and had a slightly rough surface, whereas the inside had a soft and porous structure. When a drop of water was placed on their surface, it was able to infiltrate the pills.

Hydration/dehydration of zein pills

The pills were completely submerged in distilled water for 1 min until they were swollen and had an approximate water content of 50%wb. They were then immediately placed on a glass slide and covered with a second slide, leaving half of the pill confined and the other half nonconfined, exposed to the air. This device leads to two dehydration rates, slow and rapid, respectively, in the confined and non confined part. Water concentration expressed in relation to the total weight (wet basis) was determined at different times of dehydration by thermogravimetric analysis (TGA). TGA was carried out with a TGA 2050 (TA Instruments, USA) under a nitrogen atmosphere and was properly gauged. The heating scan went from 25°C up to 130°C at a rate of 10°C/min. Samples were left at 130°C for 60 min until total weight balance was obtained.

ATR-FTIR

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy was carried out on a spectrometer (Nicolet Magna IR 550, USA). Spectra in the wavenumber range of 4000–650 cm^{-1} were collected over 64 scans with a resolution of 4 cm^{-1}. The measurements were made at room temperature, on a diamond crystal. Optical alignment of the unit was set to achieve maximum throughput of the infrared beam to the detector. The samples were taken at random from the powder of initial zein, nonconfined part of zein pill and confined part of zein pill. Spectra were analyzed using a standard Grams software program (Galactic Corporation, USA).

Solubility

Thirty milligrams of zein powder samples were dissolved in 3 mL ethanol/water buffer (70% v/v) for 2 h.[16] After a centrifugation at 5000 $\times$ g for 20 min, the supernatants were collected and assayed for their nitrogen content. Zein solubility corresponded to the ratio of this value over the total protein content. Each sample was done in triplicate.

Scanning electron microscopy

The surfaces of dehydrated zein pills were observed after they had been dried and removed from the dehydrated medium. Some samples were also fractured after immersing them in liquid nitrogen for the purpose of analyzing the internal morphology (transversal view) of the pills. The samples were gold-/platinum-coated in a Polaron SC502 Sputter coater (FISON), and a Scanning Electron Microscope Stereoscan 440 (OXFORD) was used for the observations of the surfaces and transversal fracture face of the zein samples. Low voltages (5–10 kV) were used to guarantee minimum radiation damage to the samples.

Differential scanning calorimetry

Thermal analysis was performed with a DSC-121 apparatus (SETARAM, France) calibrated with indium standard. The base line was registered based on an aluminum/aluminum pan. Thermograms shown in the study correspond to the first scan performed from −20°C to +90°C at 3°C/min. Glass transition temperature (T_g) was determined as the midpoint of the sharp heat capacity change at the transition.

RESULTS

Dehydration rate

The variations of water content as a function of dehydration time in the nonconfined and confined

TABLE I
Variation of Water Content, Melting Enthalpy Associated with Freezable Water, and T_g of Zein Sample Variation as a Function of the Dehydration Kinetics Time

	Time (h)	Water content (% total)	ΔH of ice melting (J g^{-1})	Freezable water (% total)	Plasticizing water (%)	T_g (°C)
Nonconfined	1	48	29.7	9	39	–
	2	23	3.1	1	22	16
	3	18	–	–	18	30
	4	16	–	–	16.3	34
	8	13	–	–	12.9	36
	10	12	–	–	12.3	42
	12	11	–	–	11.4	45
	48	9	–	–	8.7	62
Confined	1	36	19.8	6	30	–
	2	36	18.7	5.5	30.5	–
	3	31	17.1	5.1	29	–
	4	28	15.8	4.7	23.3	–
	8	24	11.3	3.6	20.4	$\sim$ 10
	10	20	8.5	2.8	17.7	11
	12	18	2.0	0.85	17.2	15
	18	15	1.4	0.42	14.4	21
	20	13	0.7	0.22	12.5	26
	24	11	–	–	10.7	33
	48	9	–	–	9.3	63

The part of freezable water has been calculated from enthalpy, divided by the enthalpy of pure ice melting (333 J g^{-1}).

areas are given in Table I and shown in Figure 1. As expected, the dehydration rate in the nonconfined area was more rapid than that in the confined area. Because it is difficult to withhold and handle the highly hydrated samples, water contents determined at the beginning of the dehydration are not coherent. In Figure 1, curves are drawn to deduce that $\sim$ 20 h are required to dry the nonconfined area with a level of water content about 10%. In comparison, almost 40 h are necessary to obtain the same level of water content in the confined area.

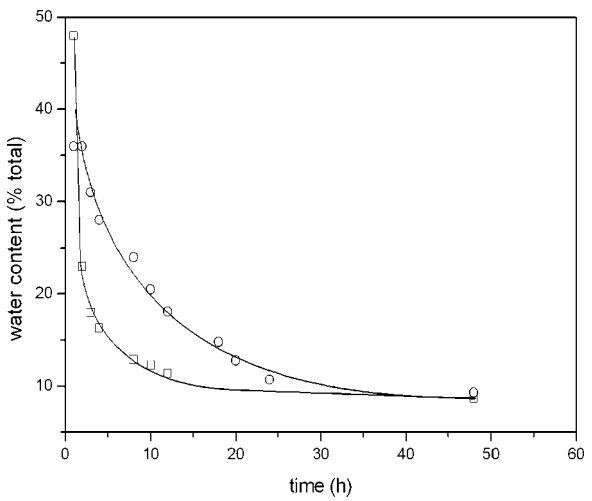

Figure 1 Water content variations over time in different domains of zein pills dehydrated in a semiconfined system (□: nonconfined area; ○: confined area).

Secondary structure and zein solubility

A potential effect of hydration dehydration steps on the secondary structure and solubility of zein was investigated by ATR-FTIR and solubility measurements. The spectra of the initial zein powder, zein pill nonconfined part, and zein pill confined part are shown in Figure 2. Almost all the infrared research on protein has been focused on amide I and amide II bands[17] related to the protein secondary structure. All the samples presented similar bands, thus revealing that zein secondary structure was not affected by any of both dehydration treatments.

The solubility data of samples of initial zein powder and zein after rapid or slow dehydration was

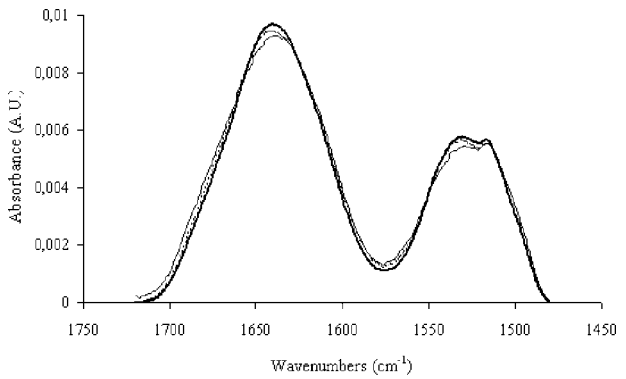

Figure 2 Amide I and II bands of ATR-FTIR spectra for zein powder (——), zein pill nonconfined part (------), and confined part (——) after dehydration.

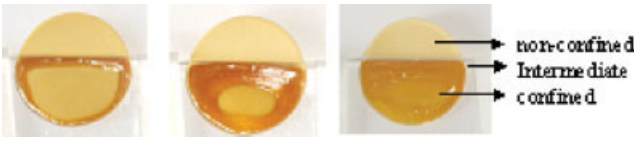

Figure 3 Pictures of the zein pills during the drying process in a semiconfined system at 25°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

measured. There are many hydroxyl and amide/acid moieties present in zein, and crosslink reactions would occur resulting from the plasticizing effect of water, leading to the polymer insolubility.[16,18] The solubility in ethanol/water of hydrated/dehydrated zein has been found at 89% ± 1% and 94% ± 6% for non confined and confined domains, respectively. Considering the uncertainty of the measure, it is comparable to the solubility of initial zein of 87% ± 11%. The results suggest that the structure of zein is not deformed after dehydration treatment, which is supported by the results from the infrared analysis.

Macroscopic and microscopic structure of semiconfined zein pills

The images in Figures 3 and 4(a) illustrate the structure changes that were observed during the slow and rapid drying processes, respectively, in the confined and nonconfined domains of the system. At the end of drying, the pill dehydrated rapidly in the nonconfined domain was completely opaque with yellowish coloration and a slightly rough superficial aspect. On the contrary, the pill dehydrated in the confined domain was practically translucent with a flat and shiny surface. During dehydration, vitrification of the pill occurs radially in the confined area of the sample. This is probably due to a densification of the pill, which slowly liberates space between its surface and the sheets of glass and allows dehydration.

Figure 4(b–d) shows the surface morphology of different areas analyzed by SEM. The confined part dehydrated leads to a flat structure with a quasi total absence of microcavities or pores [Fig. 4(b)]. In the opposite, nonconfined part leads to a highly porous structure [Fig. 4(c)], and the interconnection of pores has been demonstrated by drop imbibition measurements.[12] In the SEM microphotographs [Fig. 4(d)], corresponding to the intermediate area, a dramatic change is observed from one area to another (it is important to mention that these microphotographs are not overlapping and that they clearly show the discontinuous change in appearance between nonconfined and confined domains). The internal structure observed on fractured samples was similar to the surface, i.e. porous or compact, which demonstrate the relative homogeneity of the sample.

Calorimetric experiments

DSC experiments performed on nonconfined and confined domains of zein pills are shown in Figure 5(a) and Figure 5(b), respectively. Each thermogram corresponds to a sample taken from a pill at one of the drying times that varied from 1 to 48 h (one pill for each dehydration time). Two main events de-

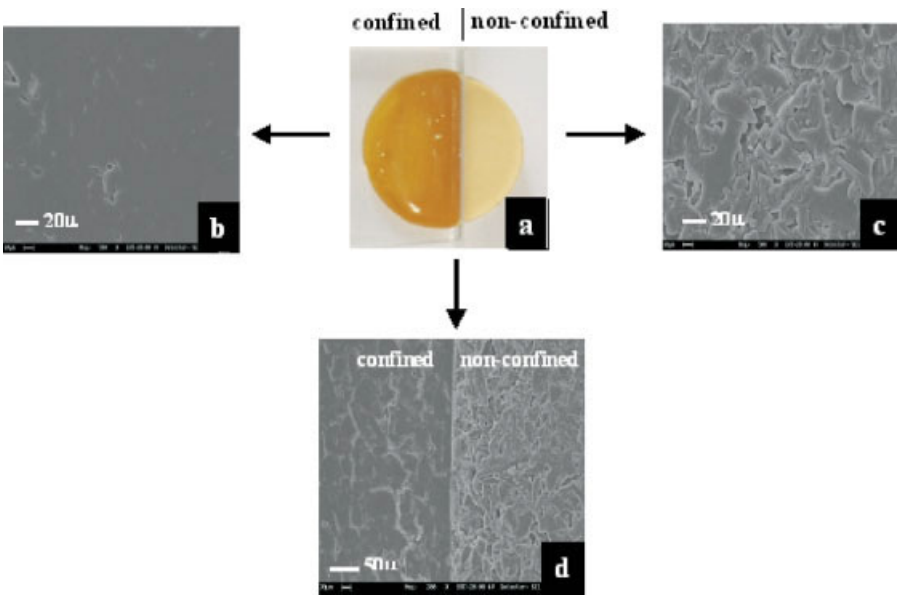

Figure 4 Scanning electron micrographs of surface external morphology of zein pills dehydrated in a semiconfined system at 25°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

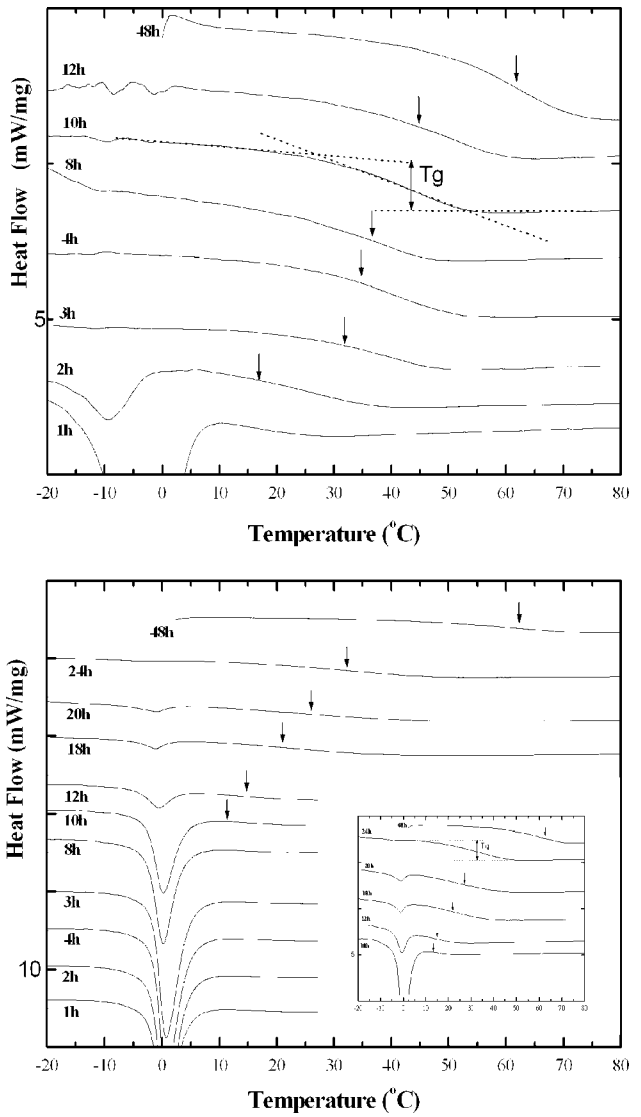

Figure 5 (a, b) DSC thermograms performed on the nonconfined area (a) and on the confined area (b) of zein pills during dehydration at 25°C. Dehydration time corresponding to each thermogram is indicated on the figure. T_g are indicated by arrows and its graphic determination is shown on two examples.

pendent on water content can be observed on the thermograms. An endothermic peak appears at a constant temperature close to 0°C, independent of water content. It is attributed to the melting of freezing water probably containing minor soluble components as salts. The peak intensity decreases with drying time until complete disappearance, after 2 and 20 h in the nonconfined and confined domains, respectively. The quantity of freezable water has been evaluated from the melting enthalpy given in Table I, divided by the enthalpy of ice melting, which is 333 J g^{-1}. This is possible under the supposition that zein contain a negligible quantity of soluble component, and melting enthalpy of freezable phase is not different to the melting enthalpy of pure ice. In addition to ice melting, the thermograms show a sharp heat capacity change corresponding to a glass transition, which clearly appears when the water content is less than 20%. The heat capacity increment ΔC_p, previously determined, was 0.17 J g^{-1} $°C^{-1}$ for the zein containing 10% water.[19] This value is lower than the ΔC_p observed for gluten proteins,[20] which ranged from 0.29 to 0.47 J g^{-1} $°C^{-1}$ for similar water content. As water content exceeding 20% wb, the melting endothermic peak overlaps with the ΔC_p variation. The T_g reported in Table I and Figure 6 are in the order of 10–20°C for zein with 20%wb water content and increases rapidly with decreasing water content, at about 60–65°C for zein with 8–9%wb water content. This behavior is due to the plasticization of zein by water, previously observed. From the Figure 6, it appears that the T_g values of the zein samples measured during rapid dehydration are about 10–15°C higher than the T_g values measured during the slow dehydration. For comparison, the T_g measured previously by Lawton[14] and by Madeka and Kokini[13] has also been reported in the Figure 6. It appears that there is large difference between the T_g values measured by Lawton and that measured by Madeka and Kokini, which are about 15–20°C lower. On the one hand, the T_g values of samples obtained by rapid drying are close to the values previously published by Lawton. On the other hand, the T_g values of samples resulting from a slow drying are close to the values published by Madeka and Kokini. These observations suggest that the repartition of water between plasticizing

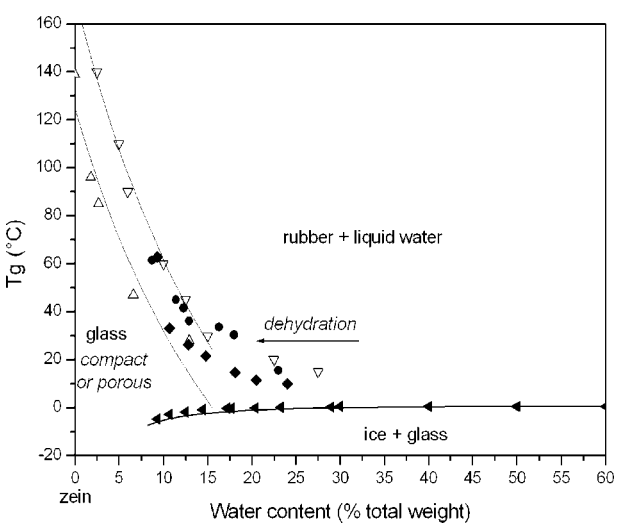

Figure 6 T_g versus water content of all zein samples subjected to slow dehydration (◆) and rapid dehydration (●). Data from Madeka and Kokini[13] (△) and data from Lawton[14] (▽) are also included. Ice melting temperatures are also reported (◀).

and nonplasticizing sites is dependent on the rate of hydration/dehydration process. The melting temperature of freezing water in zein samples during the slow drying is also reported.

The T_g (solid lines) calculated from the Gordon–Taylor equation[21] from Lawton's and Madeka and Kokini's results are also reported in Figure 6:

$$T_{g(\text{blend})} = \left(w_1 T_{g_1} + k w_2 T_{g_2}\right) / (w_1 + k w_2)$$

where w_1 and w_2 are weight fractions, T_{g_1} and T_{g_2} are the glass transition temperatures of the polymer and plasticizer, respectively, and k is a constant. The T_g of water is reported to be –135°C.[22] The T_g of the dry zein, determined to be 172 and 125°C, and the constant k, determined to be 4.94 and 4.90, fit to the values of Lawton, and Madeka and Kokini, respectively. The calculated curves show a relative agreement between experimental and predicted T_g below 10–15%wb water content. For higher water content, the large difference between predicted and experimental T_g values is probably due to the part of freezing water, which does not act as plasticizer for the zein. Because of the presence of nonplasticizing water in most of the samples studied, the Gordon–Taylor equation has not been used to fit our experimental results.

DISCUSSION

The simple experimental device, confined non-confined media, allows simultaneous modulation of isothermal dehydration rate and observation of the structure of zein pill. The microscopic structure of the material, resulting from the slow or rapid dehydration, is compact or porous, respectively. Solubility and FTIR measurements did not provide evidence of molecular changes during hydration/dehydration treatment. This result invalidates the possibility of chemical modification, which appears in some proteins as gluten reticulation (wheat proteins). The state diagram in Figure 6 has been drawn from the calorimetric analysis of the zein–water system during its slow dehydration. In addition to the decrease of T_g, from 160°C for dry zein to about 15°C for zein with 25% water content, already described in previous work, there is a water demixing from about 10% water content. The consequence is the coexistence of the zein in its rubbery state and free water at ambient temperature. The free water (or nonplasticizing water), which acts as a swelling agent of the protein, maintain the ''structure'' by filling microscopic pores. From this state, the isothermal drying from 50% to 9% water content leads to a rubbery to glassy state change of the zein. Then, it is the rate of dehydration, which governs the porosity of the final material by a phenomenon that looks to collapse,

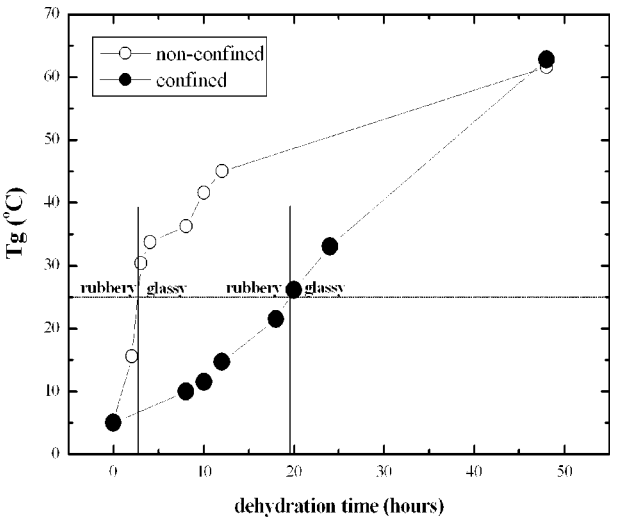

Figure 7 T_g changes of zein as a function of dehydration time in the two areas of the semiconfined system. During slow or rapid dehydration, the rubber glassy change is crossed after ~ 18 or 3 h, respectively.

related in previous studies.[23–26] Because the viscosity decreases above T_g, the collapse phenomenon induces the loss of structure, the reduction of pore size, and volume shrinkage of material. These phenomena are due to a critical viscosity (around 10^6 – 10^8 Pa s) where the material is no longer able to support its own weight or capillary forces.[27] The collapse is also a time-dependent phenomenon whose rate is linked to the difference between experiment temperature and glass transition ($T - T_g$).[24] In this study, complementary experiments have been realized to observe the zein collapse by thermomicroscopy. They have shown that a temperature above T_g in watertight conditions does not lead to the collapse, whatever be the duration of the experiment. The collapse is only observed when water is extracted very slowly and hydration level maintains a T_g below experimental temperature as long as possible. Figure 7 shows zein T_g changes as a function of dehydration time in the confined and nonconfined areas. During the rapid dehydration, the zein is maintained 3 h above T_g, which lead to the porous structure. At the same temperature, during the slow dehydration, the zein is maintained about 18 h above T_g, which lead to the compact structure. The collapse rate was not directly measured in this study; however, it is expected to be continuously modified by the T_g increase due to protein dehydration.

CONCLUSIONS

The same case as many other biopolymers, the dehydration process of zein leads to a change from a rubbery state to a vitreous state. Contrary to starchy

products, the structure of the vitreous state is highly dependent on dehydration rate. The appearance of the porous or compact structure is dependent on the rate of the retirement of freezable water. The compact appearance of zein pills following a slow dehydration presents similarities with the vitreous endosperm appearance of maize grain. Therefore, this vitreous aspect of endosperm could be due to the important presence of zein in this region of the endosperm of the maize grain, which undergoes a slow drying process during its final stage of maturation.

The authors wish to express their sincerest thanks to Dr. Heriberto Pfeiffer, a post-doctoral researcher at the Laboratoire Génie des Materiaux of the Ecole Polytechnique at the Université de Nantes (France), for his technical support and assistance in obtaining the SEM micrographs presented in this work. The authors would also like to thank Joelle Davy for her technical assistance in obtaining calorimetric results.

References

1. Bielinski, D.; Pysklo, L.; Dul, J.; Slusarski, L.; Janowska, G.; Lewandowicz, G. Macromol Symp 2003, 194, 233.
2. Robutti, J. L.; Hoseney, R. C.; Wassom, E. Cereal Chem 1974, 51, 173.
3. Pomeranz, Y. Cereal Chemistry 1974, 51, 545.
4. Sene, M.; Thevenot, C.; Hoffmann, D.; Benetrix, F.; Causse, M.; Prioul, J. L. Theor Appl Genet 102, 591, 2001.
5. Duvick, D. Cereal Chem 1961, 38, 374.
6. Seckinger, H. L.; Wolf, M. J. Cereal Chem 1967, 44, 669.
7. Piot, O.; Autran, J. C.; Manfait, M. J Cereal Sci 2000, 32, 57.
8. Buitink, J.; Leprince, O. Cryobiology 2004, 48, 215.
9. Landry, J.; Delhaye, S.; Damerval, C. Cereal Chem 2004, 81, 153.
10. Shukla, R.; Cheryan, M. Ind Crops Prod 2001, 13, 171.
11. Watson, S. A.; Ramstad, P. E. Corn: Chemistry and Technology; American Association of Cereal Chemist: St Paul, MN, 1991.
12. Sabino, M. A.; Pauchard, L.; Allain, C.; Colonna, P.; Lourdin, D. Eur Phys J E 2006, 20, 29.
13. Madeka, H.; Kokini, J. L. Cereal Chem 1996, 73, 433.
14. Lawton, J. W. Cereal Chem 1992, 69, 351.
15. Wall, L. L.; Gehrke, C. W. J—Assoc Off Anal Chem 1975, 58, 1221.
16. Lawton, J. W. Cereal Chem 2002, 79, 1.
17. Coleman, C. E.; Larkins, B. A. In Seed Proteins; Shewry, P. R., Casey, R., Eds.; Kluwer: Dordrecht, 1999; p 109.
18. Selling, G. W.; Sessa, D. J.; Palmquist, D. E. Polymer 2004, 45, 4249.
19. Chanvrier, H.; Della Valle, G.; Lourdin, D. Carbohydrate Polymer 2006, 65, 346.
20. Noel, T. R.; Parker, R.; Ring, S. G. Int J Biol Macromol 1995, 17, 81.
21. Gordon, M.; Taylor, J. S. Cereal Chem 1952, 81, 153.
22. Sugisaki, M.; Suga, H.; Seki, S. Bull Chem Soc Jpn 1968, 41, 2591.
23. Levi, G.; Karel, M. Food Res Int 1995, 28, 145.
24. Levine, H.; Slade, L. Hydrate Polym 1986, 6, 213.
25. Roos, Y. H.; Karel, M. J Food Sci 1991, 56, 38.
26. Brinker, C. J.; Scherer, G. W. Sol–Gel Science: The Physics and Chemistry of Sol–Gel Processing; Academic Press: London, 1990.
27. Allain, C.; Amiel, C. Ann Chim Sci Des Materiaux 1992, 17, 91.