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The role of mannitol in affecting the thermal transitions in carrot tissue

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Abstract

Dynamic mechanical thermal analysis (DMTA) and differential scanning calorimetry (DSC) were used to observe thermal events in osmotically manipulated carrot. A glass transition of the concentrated amorphous solution (CAS), recrystallisation of water and ice melting were shown in the tissue by DMTA. DSC showed the role of mannitol as a plasticiser in freeze-dried osmotically manipulated carrot tissue, lowering the cell wall rich and sugar rich phase glass transitions. This cautions against the use of mannitol as an osmoticum for texture measurement since a decrease in stiffness could be due to shift towards a more rubbery state or a reduction of turgor. Processing treatments of air-drying–rehydrating, freezing–thawing or heating–cooling without prior osmotic treatment, induced consistently lower stiffness of carrot tissue compared to that of fresh specimens. The order of stiffness decrease was: fresh > drying–rehydrating ∼ heating–cooling > freezing–thawing. When osmotically manipulated carrot tissue was processed, stiffness decreased in comparison with osmotically treated specimens alone for molarities less than isotonic. Stiffness could not be restored further to any process events after osmotic changes, demonstrating the irreversible effect of processing on stiffness and contrary to some earlier reports that a prior decrease in turgor would enable the mechanical properties to be recovered.

Keywords: Carrot; Glass transition; Processing; Mannitol; Turgor

1. Introduction

Plant material constitutes a major source of food and to the consumers, texture, one of its qualities, is of a particular importance [1]. The texture of plant food is associated with the composition and structure of the cell wall but is also linked to the turgor pressure generated within the cells by osmosis [2]. Since plant foods may lose their textural attributes on processing, it has been envisaged that manipulating plant tissue turgidity by using an external osmoticum may prevent quality deterioration [3–6]. More recently strain oscillatory deformation of plant tissue was studied as a function of osmoticum concentration [7–9]. Mannitol is a commonly used osmoticum, which governs movement of water through the plasma membrane into or out of the cell, increasing or decreasing turgor pressure, respectively. Greenway and Leahy [10] reported that it penetrates the cell wall and the cell membrane and might be metabolised by plant cells. The thermal properties of sorbitol and xylitol [11] have been described as a function of water content. Kim et al. [12] and Yu et al. [13] studied the glass transition of mannitol, the temperature of which was given as 12.6 °C (mid point) [13].

Previous work from our laboratory has reported the thermal properties of carrot using dynamic mechanical thermal analysis (DMTA) and differential scanning calorimetry (DSC) [14–16]. The contribution of the cell wall polymers and the low molecular weight cell contents follows much of the pioneering work on thermal transitions of biopolymers by Slade and Levine [17] and the study of gluten, starch and sugar mixtures by Kalichevsky et al. [18,19]. Given these studies, the role of mannitol, traditionally used in plant science as an osmoticum to manipulate turgor pressure, is examined.

In this work, the thermo-mechanical properties of osmotically manipulated carrot strips were investigated using DMTA. Measurements were performed further to three distinct process events commonly encountered in the food industry: air-drying–rehydrating, freezing–thawing and cooking–cooling. The stiffness from −100 to 20 °C was determined. Results were interpreted in terms of the contribution of the turgor pressure as well as the role of the concentrated amorphous solution (CAS) in measuring the

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stiffness of variously processed carrot tissues and compared with untreated carrot tissues. DSC was also used to assess the role of mannitol in determining the thermo-mechanical properties of freeze-dried carrot tissue.

2. Materials and methods

2.1. Plant materials

Carrots (*Daucus carota* cv. Amstrong) harvested in 1995 and 1996, were grown locally. Once picked, the vegetables were tested directly or stored in a 0 °C room.

2.2. Sample preparation

Carrots were washed thoroughly and strips of tissue were removed from the phloem parenchyma parallel to the longitudinal axis using a slicer. The width and length were accurately measured using a strip cutter designed in our laboratory. The dimensions were typically 20 mm length, 1–2 mm thickness and 7 mm width.

The cell turgor pressure was manipulated by soaking the strips in different solutions of mannitol (Sigma, Poole, UK). The solutions were of concentrations 0, 0.1, 0.3, 0.8 and 1 M, made up in distilled water. The strips were soaked overnight at room temperature and then tested the following day or further to the different process events.

2.3. Process events

2.3.1. Fluid bed drying–rehydrating

Strips were air-dried using a fluid bed dryer (Johnson Matthey, Royston, UK), blower speed 5.5 and temperature set at 20 °C for 3 h. The dried strips were then left in a desiccator over silica gel for 2 days prior to testing. The specimens were rehydrated in distilled water for 1 h.

2.3.2. Freezing–thawing

Strips were left in a deep freezer at −20 °C for 2 days and thawed at ambient temperature (approximately 20 °C) for 0.5 h prior to testing.

2.3.3. Heating–cooling

The strips were heated in boiling water during a determined time course and then cooled to ambient temperature within 5 min. Stiffness was found to decrease within 5 s during cooking. Hence a heating time of 5 s was used in the heating–cooling experiments.

2.3.4. Freeze-drying and water conditioning for DSC analysis

The 1 M mannitol-treated carrot tissue was freeze-dried (Model 3.5 Birchover Instruments Ltd., Letchworth, Herts, UK), ground with mortar and pestle and then water conditioned *in vacuo* for 3–4 weeks over P_2O_5 and over the following saturated salt solutions: LiCl, K_2CO_3 and NaCl at ambient temperature of 20 °C.

2.4. DMTA measurements

The Polymer Laboratories DMTA was used in the tensile and bending mode at a frequency of 1 Hz and strain $1/\sqrt{2}$ (tensile) and $\sqrt{2}$ (bending). The heating rate was 2 °C min^{-1}. Strips edges were glued to small metal plates which were clamped to the drive-shaft and the frame of the DMTA tensile head, respectively. This is described in more detail elsewhere [14]. Three replicates of each sample were tested.

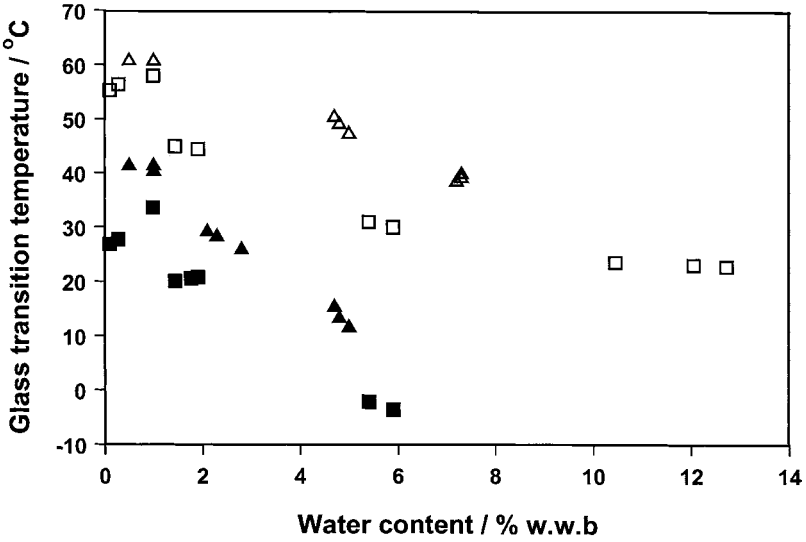

Fig. 1. DSC T_g of freeze-dried carrot osmotically manipulated: (▲) freeze-dried carrot, sugar rich phase; (△): freeze-dried carrot, cell wall rich phase; (■) 1 M mannitol freeze-dried carrot, sugar rich phase; (□) 1 M mannitol freeze-dried carrot, cell wall rich phase.

2.5. DSC measurements

An automated Perkin Elmer DSC 7 instrument equipped with a cooling system for scanning below 20 °C was used. It was calibrated from the melting point of indium. The heating rate was 10 °C min^{-1}. The glass transition temperature T_g was defined as the temperature at which the midpoint in the slope of the temperature–heat flow occurs, as detailed earlier [15]. Three replicates of each sample were analysed.

3. Results and discussion

It was observed that the volume of carrot strips increased monotonically with increasing turgor or decreasing mannitol concentration, 0.42 M mannitol corresponding to an isotonic solution [16]. A value of 0.5 M has been used by Greve et al. [6] when studying carrot tissue and 0.42 M mannitol corroborates with the value used by Carpita [20] who investigated carrot cell suspensions.

3.1. DSC analysis of freeze-dried mannitol-treated tissue

Fig. 1 shows the DSC T_g of the sugar rich phase and the T_g of the cell wall rich phase of 1 M mannitol-treated carrot tissue, freeze-dried then conditioned over water range 0–15% (wet weight basis, w.w.b.). As a comparison, results obtained previously [15] on untreated freeze-dried carrot tissue were superimposed. It demonstrates clearly that reducing the turgor by soaking carrot tissue in 1 M mannitol significantly affects the T_g of both phases. It depresses the two T_gs by 10–20 °C proving the role of mannitol as a plasticiser. It is also interesting to note that as a comparison the T_g of the sugar rich phase is within the envelope of T_g val-

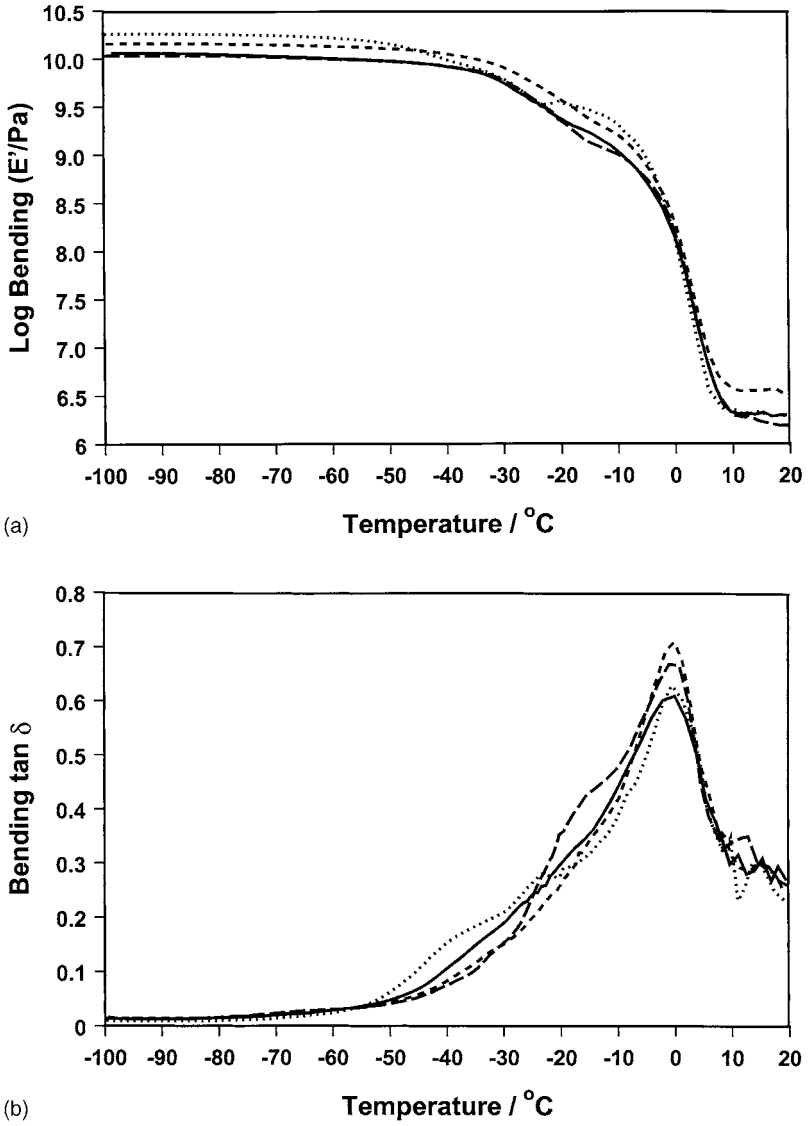

Fig. 2. (a) DMTA bending E' below 20 °C for osmotically manipulated carrot strips: (—) fresh; (– – –) 0 M mannitol; (- - -) 0.3 M mannitol; (. . .) 1 M mannitol. (b) DMTA bending tan δ below 20 °C for osmotically manipulated carrot strips.

ues for sucrose [21], but greater than that for mannitol alone [13].

3.2. Effect of turgor on the stiffness of carrot tissue

In Fig. 2a, bending storage modulus (E') is represented against temperature (−100 to 20 °C) for different mannitol concentrations. Below −30 °C, a glassy plateau was observed. Anglea et al. [7] studied low temperature transitions of osmotically manipulated potato strips using an oscillatory plate rheometer. Their modulus range (3×10^{10} to 6×10^{10} Pa) agrees with that of the present study (1.2×10^{10} to 2×10^{10} Pa). It is noteworthy that the glassy E' increases with increasing mannitol concentration. There would be a reinforcement of the matrix by the sugar glass, consistent with the work of Kalichevsky et al. [19] on gluten and fructose. A reduction in stiffness, which is defined as the glass transition, T_g, of the CAS is detected. This CAS comprises mainly intracellular solutes such as sugars and water-soluble cell wall components. Above T_g, bending modulus decreased by two orders of magnitude at 0 °C. This corresponds to the melting of ice after which the stiffness reached a plateau of 0.5–1 MPa.

In Fig. 2b, the bending $\tan\delta$ is plotted for carrot strips variously manipulated at low temperature. Results show the occurrence of T_g of the CAS, seen as a shoulder, located at −40 °C. This feature becomes greater for the 1 M specimen and less visible for the 0 M sample. Furthermore, between the T_g and the T_m (temperature of ice melt), the recrystallisation of the unfrozen water occurs (−25 °C), noticeably for the 0 M carrot strip. Similar results were observed at low temperature using the tensile mode.

At 20 °C, the tensile storage modulus (E') decreased with increasing mannitol concentration (Fig. 3). This increase in stiffness with increasing turgor was observed by Pitt and Chen [22], and Jackman et al. [23], who studied the rheology of apple tissue and the viscoelastic properties of tomato tissue at various turgor pressures, respectively. It is noteworthy that stiffness was not linearly related to osmoticum concentration as also reported by Ramana and Taylor [8]. It clearly shows that tissue manipulated with 0.42 M mannitol behaved differently to the fresh specimens. This kind of discrepancy was previously observed by Ramana and Taylor [8] and could be explained by the plasticising effect of mannitol as indicated using DSC on 1 M mannitol-treated freeze-dried carrot tissue (Fig. 1).

3.3. Effect of processing on the stiffness of carrot tissue

At 20 °C, the tensile storage modulus (E') decreased when carrot tissues underwent any processing event (Fig. 3). Willis and Teixeira [5] observed that air-dried celery specimens at water activity, $a_w = 0.25$, resulted in ultra structural damage of the cell wall. They determined the modulus of elasticity and found it to be zero. They attributed this absence of rigidity to the damage of the cell wall and the middle lamella. Based on these observations, processing plant tissue resulted in loss of cell integrity. This also agreed with results obtained by Shipman et al. [4] who found a decrease in the apparent modulus of elasticity when pieces of celery stalks were air-dried. Ahmed et al. [24] also found that the stiffness of frozen–thawed or boiled–cooled carrot discs was significantly lower than that of fresh material.

3.4. Effect of turgor on the stiffness of variously processed carrot tissue

The tensile storage modulus, E', at 20 °C is plotted for the osmotically manipulated carrot tissue strips, variously processed, in Fig. 3. Freezing–thawing, heating–cooling and

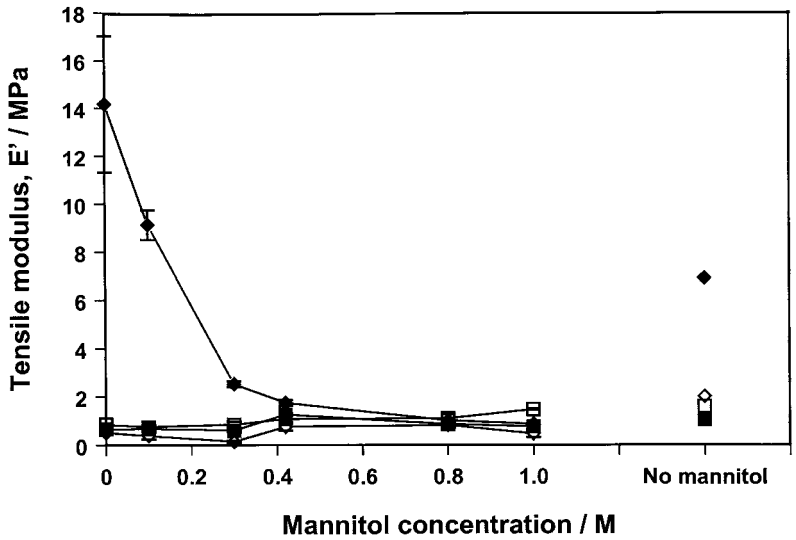

Fig. 3. DMTA tensile E' taken at 20 °C for mannitol-treated carrot tissues prior to various processes. Data for no mannitol treatment also shown. Error bars represent the standard error of the mean of three replicates: (◆) unprocessed; (■) frozen–thawed; (□) air-dried–rehydrated; (◇) heated–cooled.

air-drying–rehydrating resulted in a substantial decrease of tissue stiffness in comparison with untreated specimens below the isotonic mannitol concentration of 0.42 M. However, osmotically manipulating prior to processing had little effect on stiffness. Again Fig. 3 demonstrates that the stiffness of fresh material is affected more than isotonic tissue by these processes. Of the different processes, air-drying–rehydrating resulted in the stiffest and heating–cooling gave the least stiff tissue. In contrast, Greve et al. [6] and Ramana et al. [9] minimised the texture loss of heated specimens by osmotically modifying the tissues, carrot and potato, respectively.

In order to determine whether turgor is a reversible phenomenon in processed plant tissue, strips were soaked in 0.8 M mannitol to generate plasmolysed cells. Tissue strips were then processed then soaked in 0.1 M mannitol after processing to maximise any turgor contribution post-processing. The values were comparable (data not shown), demonstrating that turgor could not be restored and secondly, the cell wall is likely to be affected. The latter would impart to the treated plant tissue a decrease in stiffness.

4. Conclusion

By using DMTA, the thermal events observed in osmotically manipulated carrot tissue were a glass transition of the CAS (−40 °C), recrystallisation of water (−25 °C) and ice melting (0 °C). A reinforcement of the matrix was detected upon the increasing concentration of mannitol.

DSC showed the role of mannitol as a plasticiser in osmotically manipulated freeze-dried carrot tissue and cautions against the use of mannitol as an osmoticum for carrot tissue since a decrease in stiffness could be due to shift towards a more rubbery state or reduction of turgor.

Further to air-drying–rehydrating, freezing–thawing or heating–cooling without prior osmotic treatment, the stiffness of carrot tissue was consistently lower than that of fresh specimens. The order of stiffness decrease was: fresh > drying–rehydrating ∼ heating–cooling > freezing–thawing.

When osmotically manipulated carrot tissue was processed, stiffness decreased in comparison with osmotically treated specimens for molarities less than isotonic. Stiffness could not be restored further to any process events after osmotic changes, demonstrating the irreversible effect of processing.

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