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Thermal transitions in freeze-dried carrot and its cell wall components

D.M.R. Georget, A.C. Smith[1,*], K.W. Waldron

Institute of Food Research, Norwich Laboratory, Colney, Norwich NR4 7UA, UK

Abstract

Differential scanning calorimetry (DSC) was used to measure the glass transition temperature, T_g and a sub-T_g event in freeze-dried carrot material and different carrot cell wall components, at moistures ranging from 0% to 20% (w.w.b.). Two glass transition temperatures were detected in the freeze-dried carrot material associated with two phases: a sugar-rich phase and a cell wall-rich phase. The water distribution in the different phases is also important and sorption isotherms of freeze-dried carrot and different cell wall components were determined and compared with published results produced on sugar, pectins and cellulose. For the insoluble carrot cell wall materials, a single T_g was detected which increased in the order: a Ca^{2+} bound pectin-free residue, an esterified pectin-free residue and a cellulose-rich residue. In all cases T_g increased with decreasing moisture. Additionally, a sub-T_g endothermic event was observed in all the materials which disappeared on rescanning, consistent with results published on ageing of other biopolymers. The T_g of the different cell wall residues was modelled, using the Gordon–Taylor and Kwei equations. The latter showed that water–biopolymers interactions are less prominent in the cellulose-rich cell wall residue, shown by a negative value of q.

Keywords: Carrot; Cell wall; DSC; Cell contents; T_g

1. Introduction

The physical properties of freeze-dried vegetable tissue have been associated with the glass transition temperature, T_g, the values of which have been determined using DSC (differential scanning calorimetry) [1,2]. In these studies, the T_g has been attributed to the solutes or CAS (concentrated amorphous solution). Recently, however, a thermal transition in carrot cell wall has, also, been reported [3], showing that cell walls may also contribute to the thermal properties of freeze-dried vegetable material. In addition, physical ageing has been observed in a number of glassy food polymers [4–6], in which a rearrangement of the polymers occurs towards a more energetically stable conformation during storage. However, no events of this kind have been reported for freeze-dried vegetable tissue and cell wall components. Furthermore, although several studies [7,8] have demonstrated that it is possible to model the T_g of biopolymer mixtures, using empirical equations [9,10], there is little or no information on their applicability to food cell wall materials.

In this paper, thermal transitions of freeze-dried carrot material were examined. The chemical removal of intracellular components followed by the progressive removal of cell wall components was adopted according to the method described by Georget et al. [11]. This technique allowed the study of the con-

*Corresponding author. Fax: +44-1603-507723; e-mail: andrew.smith@bbsrc.ac.uk

[1]Present address: INRA-ENSAM, Laboratoire des Céréates, 34060 Montpellier, France.

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tribution of the insoluble cell wall residues to the thermal properties. Each material was equilibrated at various relative humidities, using saturated salt solutions at room temperature, to generate samples of different moisture contents. DSC was performed on the initial samples, which were then rescanned and results analysed with respect to T_g and a sub-T_g ageing event. T_g results for the different cell wall insoluble residues were fitted to the Gordon–Taylor [9] and Kwei [10] equations. The fitting parameters were compared and interpreted in terms of the properties of the components.

2. Materials and methods

2.1. Materials

Carrots (*Daucus carota* cv. Amstrong) were grown locally. Once harvested, the vegetable was stored at 0°C at 99% relative humidity.

2.2. Sample preparation

2.2.1. Freeze-dried carrot (FDC) material

After storage, the carrots were thoroughly brushed, washed, blotted with absorbing paper, sliced and then immediately immersed in liquid nitrogen. The deep frozen material was freeze-dried (Model 3.5 Birch-over Instruments, Letchworth, Herts, UK), ground using a mortar and pestle and stored in a desiccator over silica gel, in sealed jars.

2.2.2. Preparation of the sodium dodecyl sulphate insoluble residue (SIR)

2.2.2.1. Cell wall preparation. A slight modification of the method used by Parker and Waldron [12] was employed. Approximately 20 g of dry ground FDC was mixed with 1.5% aqueous solution of sodium dodecyl sulphate (SDS) containing 5 mM $Na_2S_2O_5$ using an Ystral homogeniser (Ystral GmbH, Dottingen, Germany) for 5 min. A few drops of octanol were used to limit the foaming. The homogenate was filtered through a 100 μm nylon mesh (John Stannier, Manchester, UK) and the residue was ball-milled (Pascall, 0.5 l pot) at 0°C in 0.5% SDS containing 3 mM $Na_2S_2O_5$ for 2 h at 60 rpm to remove the bulk of remaining intracellular contents. After filtering the homogenate through 75 μm nylon mesh, the residue was suspended in cold water containing 3 mM $Na_2S_2O_5$, homogenised for 5 min and refiltered. This procedure was repeated (three times) until the cell wall residue was free of intracellular components and starch granules as assessed by staining with iodine/potassium iodide and by using optical microscopy. The cell wall material was stored as a frozen suspension at −20°C. This procedure was repeated in order to obtain sufficient material for the study. This extraction with SDS solubilises mainly intracellular compounds and only very small amounts of cell wall polymers [13] although cell wall enzymes are not fully inactivated.

2.2.2.2. Inactivation of enzymes of carrot cell wall. The method of Huber [14] was used as modified. Buffered phenol solution was prepared by the addition of 700 ml 500 mM Tris[hydroxymethyl]-amino methane, pH=7.5 to 1.4 kg of phenol. The suspension was stirred and allowed to stand overnight. The upper aqueous phase was removed and the phenol phase was used for the inactivation of cell wall enzymes. The buffered phenol was mixed with the thawed cell wall material described above, and stirred for 45 min. The resulting mixture was centrifuged at 1000 rpm for 30 min at room temperature. Centrifuging the mixture did not lead to the formation of solid pellets. The buffered phenol cell wall mixture was greatly diluted with 95% ethanol and filtered through a 75 μm nylon mesh. The residue was washed with 95% ethanol. This was followed by another wash with absolute ethanol. Further, the residue was washed three times with acetone. The acetone was removed by evaporation at room temperature. The SDS insoluble residue (SIR) so produced consisted of carrot cell wall material free from enzyme activity and intracellular components.

2.2.3. Sequential extraction

2.2.3.1. CDTA extraction. SIR was extracted with 50 mM cyclohexane–*trans*-1,2-diamine-*N*,*N*,*N*′,*N*′-tetraacetate (CDTA, Na^+ salt) pH=6.5 for 8 h at 20°C. After extraction, the residue or CDTA insoluble residue (CIR) was dialysed exhaustively for 10 days and freeze-dried. This procedure leads to the removal of ionically (Ca^{2+}) bound pectic polysaccharides [13].

2.2.3.2. Na_2CO_3 extraction. Extraction of CIR with 50 mM Na_2CO_3 containing 20 mM $NaBH_4$ was carried out for 6 h at 1°C, and then for 2 h at 20°C. The mixture was centrifuged and filtered. After extraction, the residue was dialysed for 5 days and freeze-dried. The Na_2CO_3 insoluble residue (NIR) will comprise mainly cell wall material free from esterified pectins [13].

2.2.3.3. KOH extraction. After extraction with 0.5 M KOH containing 20 mM $NaBH_4$ for 2 h, the KOH insoluble residue (KIR) was dialysed thoroughly for 3–5 days and then freeze-dried. The final residue will be rich in cellulosic material with some hemicellulosic components [13].

Fig. 1 represents schematically the progressive sequential extraction of the different cell wall polysaccharides.

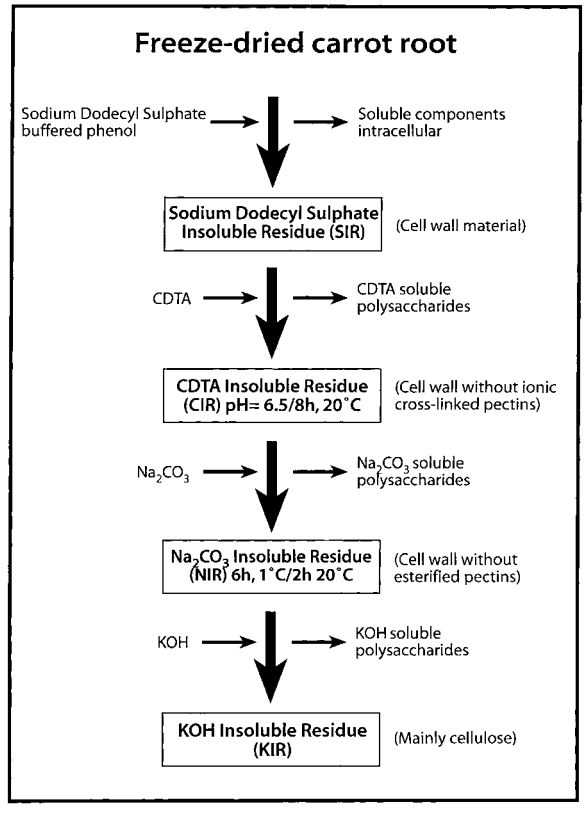

Fig. 1. Sequential extraction of the different carrot cell wall residues.

2.3. Conditioning at different relative humidities

All the materials were ground using a mortar and pestle, prior to water conditioning. Specimens were conditioned to equilibrium in vacuo at room temperature for a 2–3 week period over P_2O_5 (a_w=0) and the following saturated salt solutions, LiCl (a_w=0.113), K_2CO_3 (a_w=0.432), NaCl (a_w=0.753) and KCl (a_w=0.843) [15] to give water contents of 0–20%, wet weight basis (w.w.b.).

2.4. Moisture content determination

The water content was measured gravimetrically by drying in a vacuum oven (Gallenkamp, UK) at 70°C at a pressure of less than 5 mmHg with P_2O_5 over a 16 h period.

2.5. DSC

An automated Perkin-Elmer DSC 7 instrument equipped with a cooling system for scanning below 20°C was used. Between 8 and 15 mg of material were accurately weighed into large volume Perkin-Elmer DSC aluminium pans, using a Cahn 2000 recording electrobalance with a sensitivity of 0.1 μg [16]. All scans were made with an empty pan in the reference sample holder. The samples were loaded into the instrument at room temperature (20°C) and the temperature was then lowered. The heating rate used was 10°C min^{-1}.

2.6. Algebraic fitting

Gordon–Taylor [9] and Kwei [10] equations were fitted to the experimental results using a data regression package (Fig P for Windows 1.2, Biosoft, Cambridge, UK).

3. Results and discussion

3.1. Sorption isotherms of the different materials

The sorption isotherms of FDC and the different cell wall residues are shown in Fig. 2. At low water activity, the moisture content was almost equal to 0.

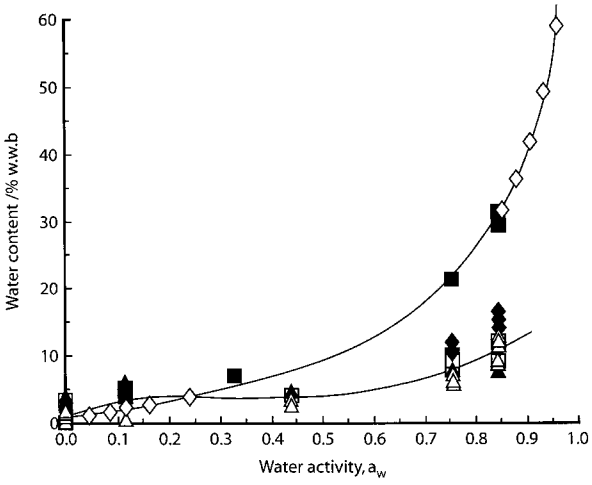

Fig. 2. Sorption isotherms of freeze-dried carrot (■), SIR (◆), CIR (△), NIR (□), KIR (▲) and sucrose (◇) [18].

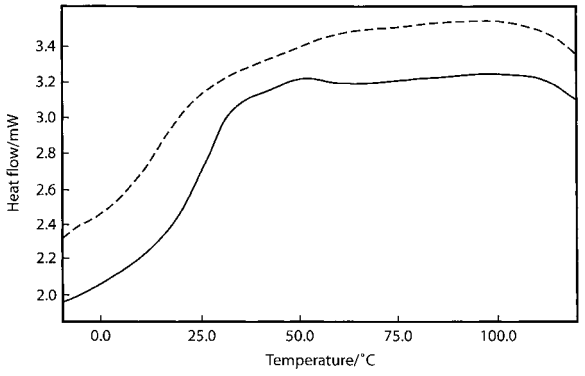

Fig. 3. DSC scans of freeze-dried carrot at 5% moisture (w.w.b.) showing first scan (———) and second scan (- - -).

Results obtained for FDC are comparable to those reported for dried carrot [17] and for sucrose [18] which is the principal sugar in carrot [19]. SIR, CIR, NIR and KIR sorb considerably less water than FDC above a water activity value of 0.3. Tsami et al. [20] studied the sorption isotherms of pectins of different molecular weights, pectin/sucrose gels and sucrose. They observed a high level of sorbed water, particularly in pectin surcose gels and sucrose and attributed this to the dissolution of sugars. This explanation will also apply to the sorption isotherm of FDC. With regard to the progressively extracted cell wall residues, the SIR isotherm is comparable to that of pectin [20]. This is because SIR and KIR comprise 51% and 30% pectic polysaccharides, respectively (Georget et al., unpublished data). A large portion of the pectic and other components are removed by the sequential extraction of SIR in CDTA, Na_2CO_3 and KOH to leave a cellulose-rich residue [13], which has an isotherm similar to that of microcrystalline cellulose (MCC) [21]. Hence, as more amorphous cell wall components are removed during the extraction sequence, less water is absorbed by the insoluble residue (Fig. 2). This decrease of sorbed water as a function of the progressive removal of the cell wall polysaccharides is consistent with results communicated by Stubberud et al. [22] who studied the sorption isotherm of polyvinyl pyrrolidone (PVP) and microcrystalline cellulose (MCC). The increase of amorphous fraction by milling MCC or adding PVP resulted in an increase of the amount of sorbed water.

3.2. Physical ageing

3.2.1. Freeze-dried carrot (FDC)

Fig. 3 shows the DSC scan for FDC conditioned at 5% water content (w.w.b.). A major discontinuity of the heat flow was observed at the T_g (24°C) consistent with DSC scans reported on freeze-dried horseradish [23] and freeze-dried grape [24]. An endothermic peak was also observed at 50°C. After a rescan, this thermal event disappeared and a second smaller T_g occurred at 50°C. The major T_g, originally at 24°C, shifted towards low temperature values (∼15°C), after rescan. Similar results were obtained for other moisture contents of this study (not shown).

3.2.2. Different cell wall residues

In Figs. 4 and 5, DSC results are shown for NIR and KIR, at 4% and 5% water content (w.w.b.), respectively. Again an endothermic event was detected during the first scan. During the second scan, a discontinuity in the slope of the heat flow, associated with a glass transition was identified. Similar results were obtained for SIR and CIR (not shown) and for the other moistures. Thiewes and Steeneken [4] and Yuan and Thompson [5] observed a sub-T_g event in glassy potato starch and waxy starch and derivatives, respectively. This endotherm has been described in synthetic polymers [25–27] as the enthalpy of relaxation. As glassy materials are in a nonequilibrium state and

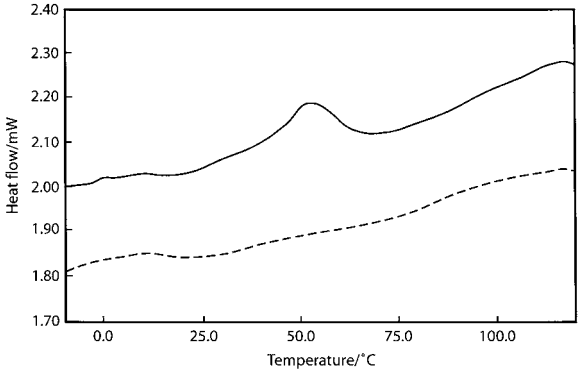

Fig. 4. DSC scans of NIR at 4% moisture (w.w.b.) showing first scan (———) and second scan (- - -).

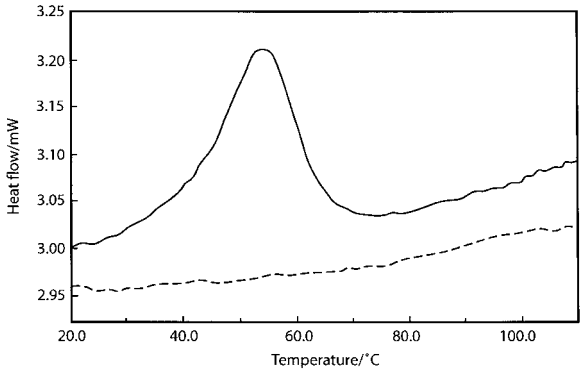

Fig. 5. DSC scans of KIR at 5% moisture (w.w.b.) showing first scan (———) and second scan (- - -).

relax towards a more stable conformation, the latter is commonly referred to as physical ageing. However Appelqvist et al. [6] associated this thermal event with water–polysaccharide interactions. In the present study, FDC, SIR, CIR, NIR and KIR show this feature systematically. During the conditioning of specimens at different relative humidities, a concomitant ageing process takes place. This study shows the occurrence of a sub-T_g event in freeze-dried vegetable and different cell wall components as in other biopolymer systems. Given the complexity of the composition of the different cell wall residues, it is noteworthy that a single T_g is detected. This shows the homogeneity and the miscibility present in SIR, CIR, NIR and KIR.

3.3. Glass transition temperature, T_g

3.3.1. Comparison between FDC, SIR, CIR, NIR and KIR

In Fig. 6, the two T_gs observed on the thermograms of FDC are plotted against moisture content. As a comparison, data for sucrose [2] have been added. The two transitions may be associated with two phases present in the freeze-dried carrot material: a sugar-rich phase and a cell wall-rich phase. These findings might be compared with those of Kalichevsky et al. [7,28] who reported the presence of two phases and two T_gs in amylopectin/fructose mixtures. The sorption isotherm results (Section 3.1) suggest that there is an unequal partitioning of water between the phases, as discussed by Farhat et al. [29]. Interactions between water and the sugar-rich phase are probably greater than those between water and the cell wall-rich phase. In the present work the DSC rescan suggests that water is directed to the sugar-rich phase resulting in a shift of the T_g of this phase to lower temperatures (Fig. 3).

Fig. 6 also shows the T_gs for the different cell wall residues. Three distinct regions are discerned: firstly, the sugar-rich phase from FDC (and published data on sucrose), secondly, the cell wall-rich phase from FDC, and SIR and CIR, and thirdly, NIR and KIR. The second T_g determined in FDC and associated with a cell wall-rich phase, is comparable to that of SIR and CIR. This finding confirms its origin in the cell wall material. The T_g of NIR and KIR is significantly

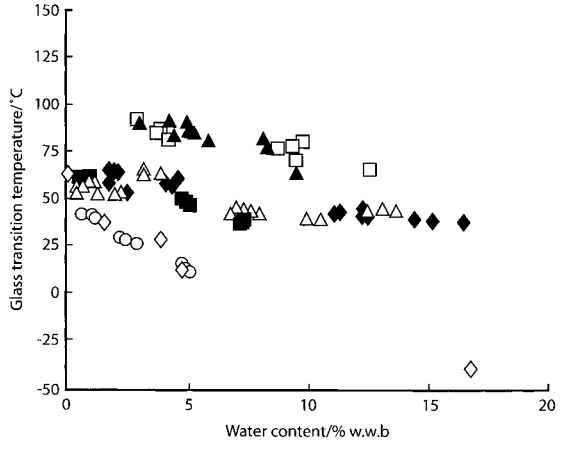

Fig. 6. T_g as a function of moisture content for (1) freeze-dried carrot: sugar-rich phase (○), cell wall-rich phase (■); (2) different cell wall residues: SIR (◆), CIR (△), NIR (□), KIR (▲); (3) sucrose (◇) [2].

higher than that of SIR and CIR. As described previously (Section 3.1), during the production of NIR and KIR, the esterified pectins are progressively removed and the remaining residue is richer in cellulose with some hemicellulosic material [11]. Because of this, fewer amorphous polysaccharides are present in NIR and KIR. The crystallinity is therefore higher in these samples and this increases the T_g, as reported for amylopectin [30].

3.3.2. Gordon–Taylor and Kwei prediction of the T_g of the different cell wall residues

The prediction of the T_g of a multicomponent system, has been reviewed by Roos [31]. Empirical equations have been applied to biopolymers [8,32] and food systems [7,16]. The Gordon–Taylor [9] equation predicted the plasticising effect of water in amylopectin [7] and proteins [16] and is as follows:

$$T_g = \frac{w_s T_{gs} + K w_w T_{gw}}{w_s + K w_w}, \quad (1)$$

where w_s is the solid fraction, T_{gs} the T_g of the solid, w_w the water fraction, T_{gw} the T_g of water and K is a constant. The latter is the ratio of the volume expansion coefficients of the components at T_g or the ratio of the heat capacity increments [33]. Addition of the term $q w_s w_w$, to the Gordon and Taylor equation gives the Kwei [10] equation below:

$$T_g = \frac{w_s T_{gs} + K w_w T_{gw}}{w_s + K w_w} + q w_s w_w, \quad (2)$$

where q is a constant and the term $q w_s w_w$ is representative of the number of specific interactions present in the mixture, such as hydrogen bonding [10,34].

Because the T_{gs} value for dry SIR, CIR, NIR and KIR were not measured, Eq. (1) was rearranged for the simultaneous evaluation of T_{gs} and K with a linear regression routine for the Gordon–Taylor fitting:

$$T_g = T_{gs} + K \frac{w_w}{w_s}(T_{gw} - T_g). \quad (3)$$

A T_{gw} value of -135°C was used for pure water [35]. In Fig. 7, curve fitting using Gordon–Taylor [9] and Kwei [10] equations is plotted superimposing the experimental data for SIR, CIR, NIR and KIR. Results with regard to the constants, K and q are presented in Table 1. In general, the two models give different values of K. By definition, K is always positive [33]

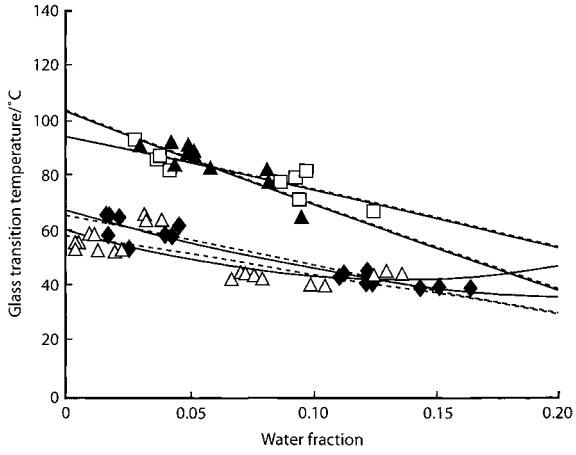

Fig. 7. T_g as a function of water fraction (w_w) for SIR (◆), CIR (△), NIR (□) and KIR (▲). The Gordon–Taylor [9] (- - -) and Kwei [10] (———) fittings are also plotted.

and therefore the Kwei prediction may not be applicable to SIR and CIR. Higher values of K have been found for hemicellulose, lignin [8], polyanhydroglucose [36] and corn starch [37] (Table 1). As K reflects the plasticising effect of water [7], a small plasticising effect of water on SIR and CIR would result in a low value of K as observed in Table 1. For NIR and KIR, K equals 1. As reported previously, q is associated with hydrogen bonds [10] and positive and negative values were obtained for NIR and KIR, respectively. Lin et al. [34] discussed the physical meaning of K and q for poly(vinyl cinnamates). They suggested that the term $q w_s w_w$ is related to contributions to backbone stabilisation by heterocontacts and homocontacts. In the present study, heterocontacts and homocontacts are represented by biopolymer–water and biopolymer–biopolymer/water–water interactions, respectively. When $q>0$, interactions between water and cell wall polymers are strong, as observed in NIR. This might be explained by the presence in NIR of some amorphous material interacting with water. When $q<0$, water–water and polymer–polymer interactions are favoured. This leads to immiscibility between water and polymer as observed in KIR. Again, the latter is rich in cellulose with some hemicellulosic material and intra hydrogen linkages are more likely to be present.

The T_{gs} values of SIR, CIR, NIR and KIR are also presented in Table 1. Values for NIR and KIR are

Table 1
Fitting parameters of Gordon–Taylor (Eq. (1)) and Kwei (Eq. (2)) equations

Material	Gordon–Taylor			Kwei			
	K	T_{gs} (°C)	r^{2a}	K	T_{gs} (°C)	q	r^{2a}
SIR	0.83	65	0.90	−0.39	67	−325.1	0.90
CIR	0.63	58	0.51	−0.54	60	−388.7	0.54
NIR	0.82	94	0.78	1	94	42.7	0.79
KIR	1.44	103	0.75	1	103	−99.8	0.75
Polyanhydroglucose [36]	4.00–6.67						
Starch [7,37]	5.60–5.13	243					
Lignin [8]				10	200	585	
Hemicellulose [8]				13	200	355	

[a] Coefficient of determination.

similar and greater than those for SIR and CIR. It should be noted that T_{gs} values calculated, using the Gordon–Taylor and Kwei equations are lower than these reported for other biopolymers [7,8,16,38].

4. Conclusion

The present study showed that sorption isotherms of FDC and the various cell wall components were comparable with results published for sugar, pectin and cellulose. By using DSC, a sub-T_g endothermic event was observed in freeze-dried carrot material and various cell wall extracts, consistent with ageing results published for other biopolymers. Two glass transitions were detected in FDC, associated with a sugar-rich phase and a cell wall-rich phase, respectively, the T_g of the cell wall-rich phase being greater than that of the sugar-rich phase. There might be an unequal water partitioning between the two phases, on the basis of the sorption isotherm studies.

The single T_g of the insoluble cell wall material shifted to higher temperatures both with decreasing moisture content and as amorphous polysaccharides were progressively removed to leave a cellulose-rich residue. The Gordon–Taylor equation predicts low T_{gs} values and K is also lower than values reported for other biopolymer systems. The Kwei parameters seem less reliable although the q parameter equations for two of the different cell wall residues showed that interactions between water and biopolymers decreased as the different amorphous components were removed to leave a cellulose-rich residue.

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