ELSEVIER

Thermochimica Acta 315 (1998) 51–60

thermochimica acta

# Low moisture thermo-mechanical properties of carrot cell wall components[1]

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

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

**Abstract**

Dynamic mechanical thermal analysis (DMTA) and differential scanning calorimetry (DSC) were used to measure phase transitions present in carrot cell wall before and after removal of $Ca^{2+}$ cross-linked pectic polysaccharides, at low moisture (0–20% wet weight basis). A glass transition was detected in the two materials, shifting towards high temperature values with decreasing moisture. Additionally, low temperature relaxations at −50 and 10°C, respectively, were observed and might be assigned to local motions of polysaccharides present in the different cell wall extracts. Stiffness, $E'$, of pressed blocks of materials was similar for both carrot cell wall residues. In comparing the thermo-mechanical properties with commercial biopolymers (citrus pectin, cellulose, their mixture and hemicellulose) the different carrot cell wall residues behaved like a pectin dominant material. They also showed a glass transition higher than that of hemicellulose and cellulose. © 1998 Elsevier Science B.V.

*Keywords:* Carrot; Cell wall; DMTA; DSC; Thermal transitions

## 1. Introduction

Textural attributes of vegetables are predominantly governed by the composition and archestructure of the cell wall [1]. Brett and Waldron [2] and Jackman and Stanley [3] have highlighted the role of cell wall in relation to vegetable texture. Although Carpita and Gibeaut [4] proposed a model to explain the archestructure of cell wall in plant tissue, techniques such as calorimetry and small strain oscillatory deformation measurements utilised recently, can bring additional information on composition/function relationships. A polymer science approach long used in synthetic polymer research [5] has been adopted in earlier studies. Lin et al. [6] investigated the phase transition of cell wall preparation using DSC. Karmas et al. [7] compared the thermal properties of freeze-dried plant tissue with model systems using DSC. Ramana and Taylor [8] studied the rheological properties of carrot cell wall suspensions. In these instances, the cell wall remains the key component responsible for the thermo-mechanical properties. It is of particular interest to relate these properties to the cell wall components.

The present research reports the study of water content on the mechanical and thermal properties of carrot cell wall components. This approach was recently illustrated by Georget and Smith [9] and Georget et al. [10] who studied the mechanical and thermal properties of wheatflakes components and raisin cell wall residues, respectively. The aim has been to determine the contribution of each category of polysaccharide to the overall properties of carrot cell wall. In order to fulfil this objective, cell wall was prepared from carrot using sodium dodecyl sulphate (SDS) and then further extracted with cyclohexane-*trans*-1,2-diamine-N,N,N′,N′-tetraacetate (CDTA). The SDS insoluble residue (SIR) and CDTA insoluble

*Corresponding author. Fax: 01603 507723; e-mail: andrew.smith@bbsrc.ac.uk

[1]Presented at TAC 97, Oxford, UK, 14–15 April 1997.


*PII* S0040-6031(98)00276-7

residue (CIR) were then tested using DSC after conditioning to different moisture contents. Strips were fabricated and equilibrated at different water contents prior to DMTA (Dynamic Mechanical Thermal Analysis). A similar procedure was adopted on commercially produced biopolymers – citrus pectin and cellulose. Results obtained by DSC and DMTA were interpreted in terms of contributions from the carrot cell wall components and compared with data obtained on raisins [10] and also that published on cellulose, wood and citrus pectin.

## 2. Materials and methods

### 2.1. Materials

Carrots (*Daucus carota* cv. Amstrong) were grown locally. Once harvested, the vegetable was stored at 0°C. The carrot dimensions were typically 15–20 cm long and 1.2–2 cm diameter.

Citrus pectin (76% galacturonic acid and 8.6% methoxy) and long fibrous cellulose extracted from cotton lint were supplied by Sigma (Poole, UK) and a mixture to represent their proportions in the cell wall (33% pectin and 67% cellulose [11]).

### 2.2. Sample preparation

#### 2.2.1. Freeze-dried carrot tissues

After storage, the carrots were thoroughly brushed, washed, blotted with absorbing paper and sliced then immediately immersed in liquid nitrogen. The excess of nitrogen was removed and the deep frozen material left in a freeze-dryer (Model 3.5 Birchover Instruments, UK) for over a week. This gave a typical yield of 12% (fresh weight). Further to the freeze-drying stage, the material was ground with a mortar and a pestle, and then kept in a desiccator over silica gel.

#### 2.2.2. Cell wall preparation

A slight modification of the method used by Parker and Waldron [12] was carried out. Approximately 20 g of dry ground freeze-dried carrot was mixed with 1.5% aqueous solution of sodium dodecyl sulphate (SDS) containing 5 mM $Na_2S_2O_5$ with an Ystral homogenizer (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, 0.51 pot) at 0°C in 0.5% SDS containing 3 mM $Na_2S_2O_5$, homogenized for 5 min and refiltered. This procedure was repeated (3 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.

#### 2.2.3. Inactivation of cell wall 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 enzyme inactivation. Further to this preparation, the buffered phenol was mixed with the thawed cell wall material described above, and stirred for 45 min. 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 remaining residue was left in a beaker at room temperature allowing acetone to evaporate. The yield was 2.1% (fresh weight). This procedure was repeated to produce sufficient material for the sequential extraction, DSC and DMTA. The produced material (SIR) comprised carrot cell wall material without any intracellular components and free of enzymes activity (Fig. 1) and was tested using DSC and DMTA.

#### 2.2.4. CDTA extraction

The above cell wall material was extracted with 50 mM cyclohexane-*trans*-1,2-diamine-N,N,N′,N′-tetra-acetate (CDTA, $Na^+$ salt) pH=6.5 for 8 h at 20°C. After extraction, the residue was dialysed exhaustively

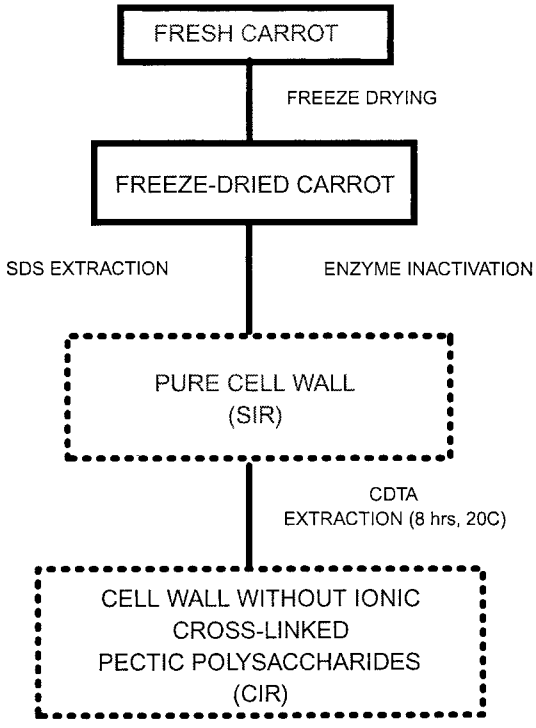


Fig. 1. Sequential extraction of the carrot cell wall.

and freeze-dried. This procedure enables the removal of ionically ($Ca^{2+}$) bound pectic polysaccharides [13] (Fig. 1). This material (CIR) was tested using DSC and used to fabricate specimens for DMTA.

### 2.3. Hot press

Sufficient water was added to the material to obtain 70% w.w.b (wet weight basis) water content prior to moulding. A press was designed in this laboratory [9], consisting of a rectangular mould ring of sides 28 and 22 mm between two male compaction dies which were temperature controlled by 4 cartridge heaters and an inner cooling system. The cell wall material prepared earlier, then mixed with distilled water, was sandwiched between two acetate sheets and loaded between the two dies and the ring. A force of 35 kN was applied to the upper die with the use of an hydraulic pump. The whole device was heated up to 30°C. The sample was then left for 15 mins in the rig before cold water was circulated in the inner cooling system. After 10–15 mins cooling, the sample was removed, a rectangular sheet 28 mm long, 22 mm wide and 0.5–1 mm thick. Strips 28 mm long and 8 mm wide were cut from the plaque. This procedure was repeated for the different insoluble residues (SIR and CIR) obtained during the sequential extraction.

### 2.4. Conditioning at different moisture contents

Specimens either powder (DSC) or strips (DMTA) were conditioned to equilibrium over saturated salt solutions at room temperature in vacuum desiccators for over a 2–3 week period. The following salts were used LiCl ($a_w$=0.113), $K_2CO_3$ ($a_w$=0.432), NaCl ($a_w$=0.753) and KCl ($a_w$=0.843) as well as $P_2O_5$ ($a_w$=0), to give a water range 0–20% (w.w.b).

### 2.5. Moisture content determination

The water content was determined gravimetrically by drying the DSC and DMTA specimens in a vacuum oven (Gallenkamp, UK) at 70°C at a pressure of less than 5 mmHg with a phosphorus pentoxide desiccant over a 16 h period.

### 2.6. DSC (differential scanning calorimetry)

Differential scanning calorimetry runs were performed using an automated Perkin-Elmer DSC7 instrument equipped with a cooling system for scanning below 20°C. Between 8–15 mg of material were accurately weighed into large volume Perkin-Elmer DSC aluminium pans. All runs were made with an empty pan in the reference sample holder. The samples were loaded into the instrument at 20°C and the temperature was then lowered to 0°C. The sample temperature was then raised at a heating rate of 10°C $min^{-1}$. Three replicates were performed. The glass transition temperature $T_g$ was defined as the temperature at which the discontinuity in the slope of the temperature-heat flow occurs [15,16].

### 2.7. DMTA (dynamic mechanical thermal analysis)

The Polymer Laboratories Dynamic Mechanical Thermal Analyser (DMTA) was used in the single cantilever bending mode at a frequency of 1 Hz and strain $\sqrt{2}$ (corresponding to a nominal peak to peak displacement of 23 μm). The heating rate was

$2^{\circ}C\ min^{-1}$. Scans were performed in duplicate. The glass transition $T_g$ was defined as the temperature at which the maximum peak in tan$\delta$ occurs.

## 3. Results and discussion

### 3.1. Effect of water

#### 3.1.1. Sorption isotherm

Fig. 2 shows the water-absorption isotherm of the SIR and CIR. The two cell wall residues have a similar sorption isotherm. At low water activity the moisture content was low, almost equal to 0. They have the typical sigmoid shape as observed by Spiess and Wolf [17] who studied the moisture sorption isotherm for microcrystalline cellulose, for which the moistures were lower compared with SIR and CIR. The polysaccharides other than cellulose (pectin and hemicellulose) present in the residues might contribute to the increase in water intake.

#### 3.1.2. DMTA

The thermo-mechanical properties of biopolymers vary with water content [18] and therefore it is of importance to study the latter in the different carrot cell wall residues. Fig. 3 shows the typical variation of the bending modulus, $E'$, for carrot cell wall material specimens (SIR) which were in the 0–20% (w.w.b) water range at the start of the experiment. The temperature at which $E'$ decreased shifted towards lower values with increasing water content. This confirms the expected role of water having a plasticising effect on the cell wall material and is consistent with results obtained on other biopolymers [18,19]. For example, the low temperature glassy modulus is approximately constant for all water contents of this study whereas the high temperature modulus increased as the water content decreased.

In Fig. 4, tan$\delta$ is plotted against temperature. A maximum peak occured at the $T_g$ which shifted towards lower temperature with increasing water content. The height and width of this peak became smaller and broader, respectively, with decreasing water content. Kalichevsky and Blanshard [19] and Kalichevsky et al. [20] found a similar trend for the tan$\delta$ height and width with decreasing water content in amylopectin–gluten mixtures and amylopectin, respectively.

Additionally, a low temperature transition at $-50^{\circ}C$ present in the 0.8% water specimen also occuring as a shoulder for the other moistures and a shoulder located at $10^{\circ}C$ for the 16% water specimen, were observed. Kelley et al. [21] and Georget et al. [10] detected subglass transitions in wood and raisin insoluble cell wall residues, respectively. This type of low temperature transition, more apparent in low moisture systems, was detected in cellulose [22,23]. This β transition was assigned to the rotation of methylol–water complexes. Later Scandola et al. [24] reported similar

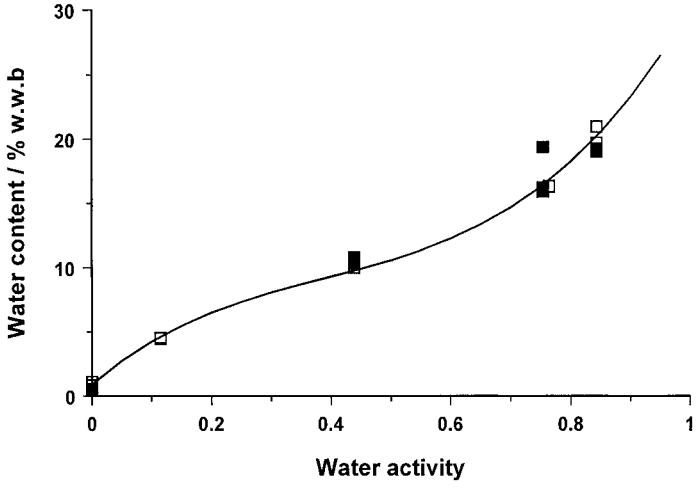


Fig. 2. Sorption isotherm of SIR (□) and CIR (■) DMTA specimens.

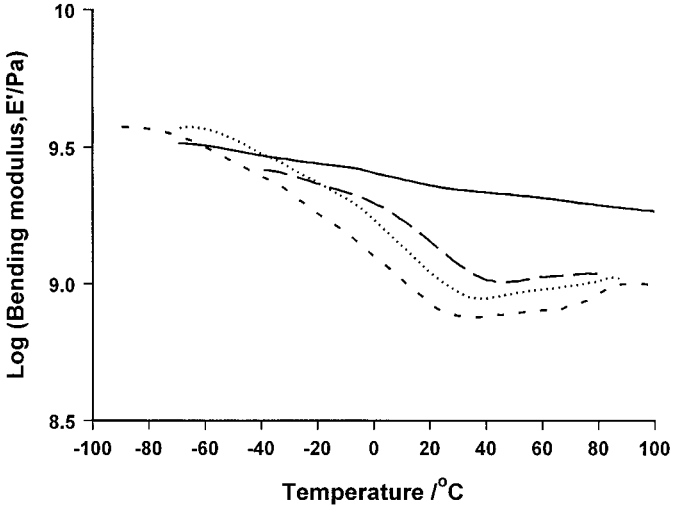


Fig. 3. DMTA $E'$ of SIR at (———) 0.8, (—— ——) 10, (· · ·) 16.3, (- - -) 19.6% (w.w.b).

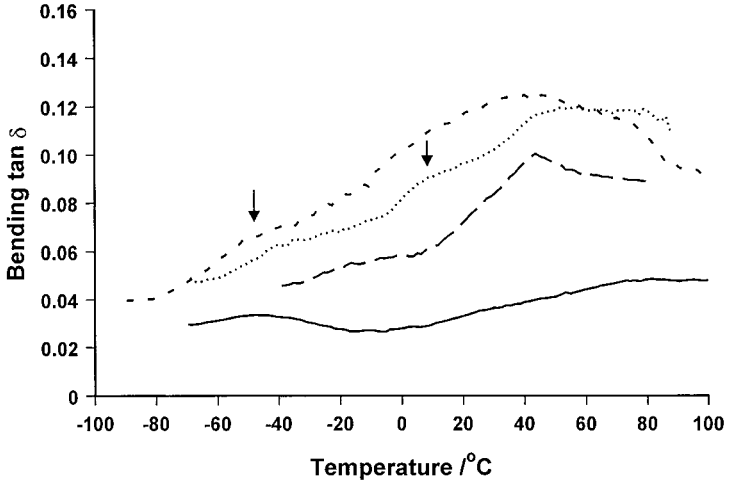


Fig. 4. DMTA tan$\delta$ of SIR. Legend, see Fig. 3. Arrows indicate the low temperature transitions.

results on polysaccharides i.e amylose, dextran and pullulan and associated the low temperature transition to local motions of the polysaccharide backbone. Kalichevsky et al. [20] reviewed the low temperature transitions in biopolymers and suggested that more transitions may be observed due to the complexity of the systems. With regard to the SIR, the $-50^{\circ}$C relaxation peak present at each water content, might be attributed to local motions of the polysaccharides. Its precise assignment to any categories of biopolymer present in the SIR is uncertain.

Similar results were observed for the CIR. In Figs. 5 and 6, the Log bending modulus, $E'$, and the tan$\delta$ for various moistures, respectively, are represented. The $T_g$ shifted towards high temperature values with decreasing water content as determined for the SIR. Interestingly, at a given temperature and moisture, the $E'$ values for SIR were significantly

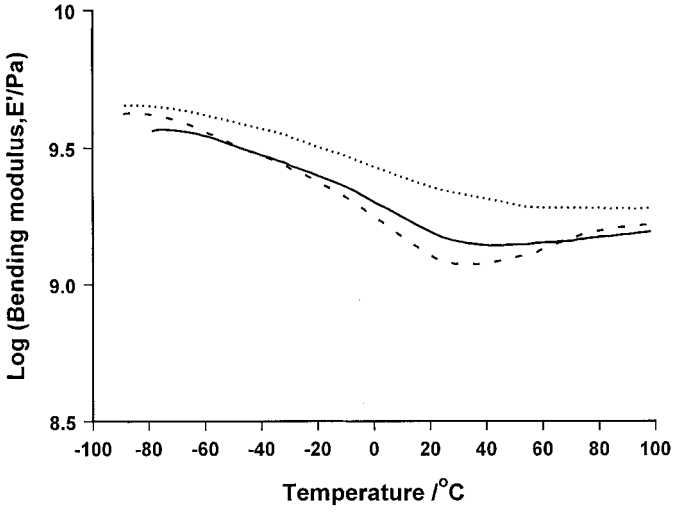


Fig. 5. DMTA $E'$ of CIR at (···) 10.8, (———) 16.1, (- - -) 19.2% (w.w.b).

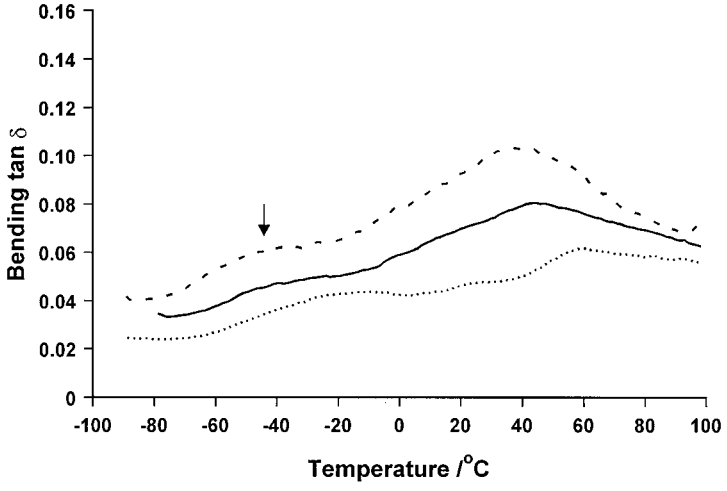


Fig. 6. DMTA tan$\delta$ of CIR. Legend, see Fig. 5. Arrow indicates low temperature transition.

lower than that for CIR (Fig. 3 and Fig. 5). This indicates that CDTA-soluble pectic polymers and $Ca^{2+}$ might have a direct role in determining wall stiffness. Additionally, the peaks in tan$\delta$ separated more clearly. The $T_g$ peak was lower than that of SIR (Fig. 4 and Fig. 6) which might indicate the difference in heterogeneity between SIR and CIR. A shift of the $-50^{\circ}C$ relaxation peak towards high temperature values with increasing moisture was observed. Scandola et al. [24] found a similar effect which also confirms that this low temperature transition involves the contribution of water although the effect was not so evident in the SIR.

### 3.1.3. DSC

In Figs. 7 and 8, results obtained for SIR and CIR conditioned at different moistures are shown. A major discontinuity of the heat flow was observed at the $T_g$

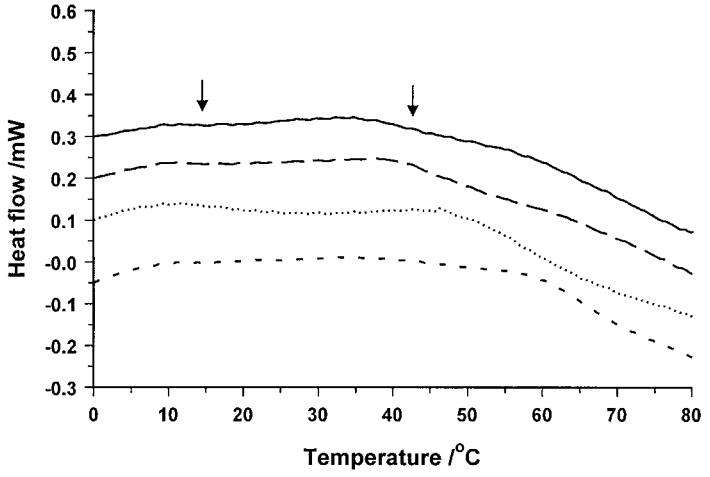


Fig. 7. DSC scans of SIR at (———) 16.4, (——— ———) 12.2, (···) 4.2, (- - -) 1.7% (w.w.b). Arrows indicate the 10°C transition and the glass transition $T_g$.

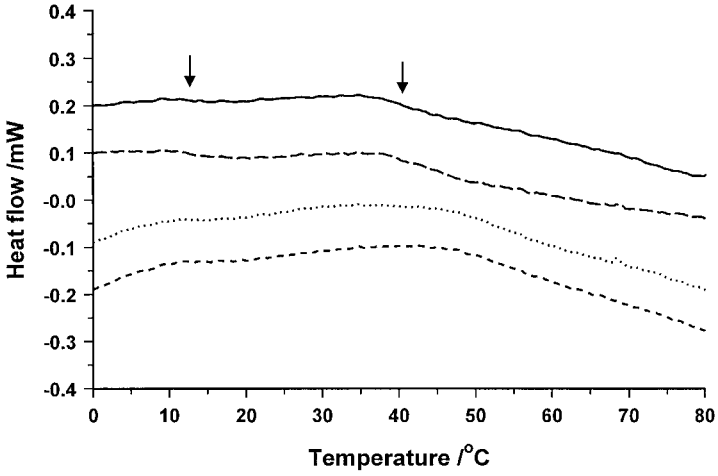


Fig. 8. DSC scans of CIR at (———) 10.5, (—— ——) 7.9, (···) 2.4, (- - -) 0.4% (w.w.b). Arrows, see Fig. 7.

which correlates with DMTA data and also shifted to low temperature values with increasing water content. This was observed immediately after rescanning. Lin et al. [6] studied the glass transition of cell wall extracted from soybean hypocotyl, using DSC and found a $T_g$ of 53°C. Although their system was water saturated, they related the $T_g$ to the pectic phase of the cell wall extract. In relation to the present study, this would indicate that the $T_g$ of cell wall is less affected by water at high levels. In low moisture cell wall residues, water plays a major role as a plasticizer. In addition to the $T_g$, the low temperature transition at 10°C was detected as in the DMTA study. Its origin remains uncertain as observed previously.

### 3.2. Comparison of the stiffness between different systems

Fig. 9 shows the variation of the bending modulus with water content determined at room temperature

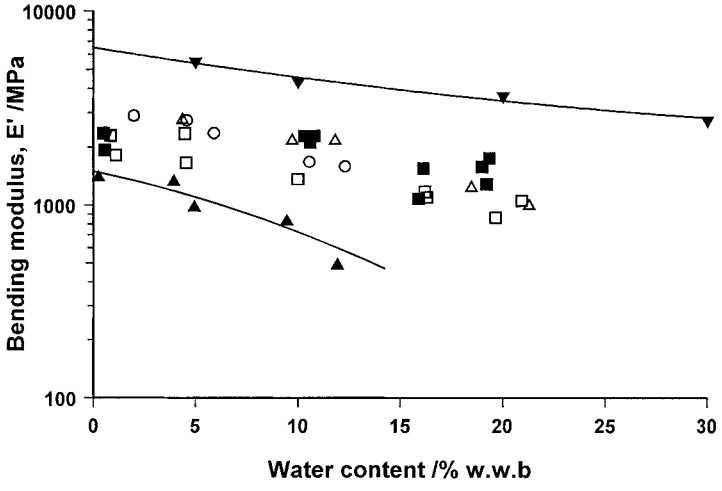


Fig. 9. DMTA $E'$ determined at 20°C, for SIR (□), CIR (■), citrus pectin (△), cellulose (▲), 67% cellulose and 33% citrus pectin (○) and wood [21] (▼).

(20°C) for SIR and CIR. The stiffness decreased with increasing water content (0–20% w.w.b), consistent with previous observations on single components [18,20] and mixtures [9]. Removal of the ionic cross-linked pectic polysaccharides between SIR and CIR had relatively little effect on a Log scale. Stiffness of SIR is within a similar range to that determined on raisin cell wall water insoluble residues (WIR) [10] although the CIR was not studied in this work. As a comparison, data determined on hemicellulose [21], cellulose, pectin and their mixture are included in Fig. 9. The modulus of pectin and its mixture with cellulose is comparable to that of SIR and CIR. It is also comparable to values of 4000–5000 MPa determined by Coffin and Fishman [25] on solvent – cast citrus pectin films. These data taken together indicate that the SIR and CIR behave like a pectin dominant mixture.

During the fabrication of DMTA strips, cellulose samples were more brittle than samples produced from the different carrot cell wall residues, pectin and its mixture with cellulose. This would indicate a greater difference in fracture properties. Work is in progress to produce the cellulose-rich KOH – insoluble residue (KOHIR). It is interesting that a KOH insoluble residue rich in cellulose was fabricated from raisin cell wall and was comparable in stiffness to that of WIR [10].

### 3.3. Glass transition $T_g$

In Fig. 10, the glass transition temperature determined by DSC and DMTA for SIR and CIR is represented. It shows that the two techniques produced comparable results. Kalichevsky et al. [18] studied the glass transition of amylopectin and its behaviour with water. They found a 10°C difference between the DMTA and DSC. They suggested that the DSC $T_g$ would be expected to be at a lower temperature than the tan$\delta$ as it is effectively a static technique, whereas the tan$\delta$ peak is obtained at a frequency of 1 Hz.

Fig. 10 also shows that by chelating $Ca^{2+}$ and consequently removing the $Ca^{2+}$ bound pectic polysaccharides, $T_g$ values were not significantly changed. The converse might have been expected since Lin et al. [6] reported the effect of $Ca^{2+}$ on the $T_g$ of water - saturated extracted cell wall. They found that upon the addition of $Ca^{2+}$, the $T_g$ values were increased. They explained this result by the increase of $Ca^{2+}$ ionic linkages amongst pectic polysaccharides. Other work by Matsuura and Eisenberg [26] on synthetic ionomers showed also the increase of the $T_g$ on addition of ions.

DSC scans were performed on pectin specimens and failed to show a glass transition behaviour, confirming the results of Gidley et al. [27]. $T_g$ temperatures could not be precisely identified, using DSC or

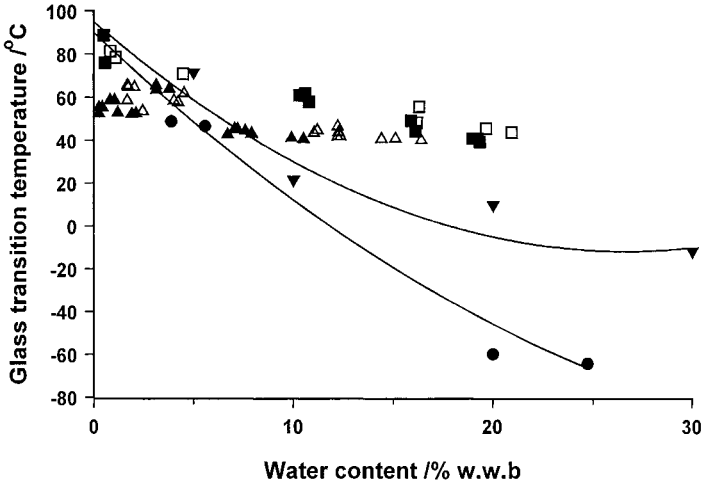


Fig. 10. $T_g$ as a function of moisture content for: i) DSC. SIR (△), CIR (▲), cellulose [28] (●) ii) DMTA. SIR (□), CIR (■) and hemicellulose [21] (▼).

DMTA, for the commercial biopolymers. As a comparison, data for cellulose [28] and hemicellulose [21] are included in Fig. 10.

The $T_g$ of SIR and CIR was greater than that published for cellulose and hemicellulose, over the water range of the study. It is noteworthy that a $T_g$ value of 20°C for cellulose corresponds to a moisture content of 10%. Below this moisture at the same temperature, cellulose is in a glassy state. This correlates with the present results plotted in Fig. 9 where the stiffness determined at 20°C below 10% moisture is the order of 1000 MPa, typical of a glassy material. At 20°C and moisture contents above 10%, specimens begin to behave like a non-glassy or rubbery material, consistent with the limited data of Fig. 9. This demonstrates that the presence in the cell wall of polymers other than cellulose and pectin, may be responsible for defining the glass transition $T_g$. More work is needed since cellulose may include a crystalline phase which will affect the $T_g$ [29].

## 4. Conclusion

The present study showed that the stiffness of carrot cell wall decreased with increasing temperature and increasing water content. Removal of $Ca^{2+}$ cross-linked pectic polysaccharides, had relatively little effect on the thermo-mechanical properties of the cell wall residues, and the stiffness was comparable to that of commercial pectin and a pectin/cellulose mixture. The glass transition of the different carrot cell wall residues was greater than that published for cellulose and hemicellulose. Two sub-glass transitions were detected and were assigned to local motions of polysaccharide backbone which were observed in other biopolymers.

## Acknowledgements

The authors wish to thank the Biotechnology and Biological Sciences Research Council for funding.

## References

[1] J.P. van Buren, J. Text. Stud. 10 (1979) 1.
[2] C. Brett, K. Waldron, Physiology and Biochemistry of Plant Cell Walls, Chapman and Hall, London, 1996, p. 222.
[3] R.L. Jackman, D.W. Stanley, Trends Food Sci. Tech. 6 (1995) 187.
[4] N.C. Carpita, D.M. Gibeaut, Plant J. 3 (1993) 1.
[5] Y.H. Roos, Phase transitions in foods, Academic press, San Diego, USA, 1995, p. 157.
[6] L.S. Lin, H.K. Yuen, J.E. Varner, Proc. Natl. Acad. Sci. 88 (1991) 2241.
[7] R. Karmas, M.P. Buera, M. Karel, J. Agric. Food Chem. 40 (1992) 873.
[8] S.V. Ramana, A. Taylor, J. Sci. Food Agric. 60 (1992) 39.

[9] D.M.R. Georget, A.C. Smith, J. Therm. Anal. 47 (1996) 1377.
[10] D.M.R. Georget, M. Guardo, A. Ng, A.C. Smith, K.W. Waldron, Thermochim. Acta 294 (1997) 71.
[11] J.P. Van Buren, in: R.H. Walter (Ed.), The Chemistry and Technology of Pectin, Academic Press Inc., San Diego, USA, (1991) p. 5.
[12] M.L. Parker, K.W. Waldron, J. Sci. Food Agric. 68 (1995) 337.
[13] R.R. Selvendran, P. Ryden, in: P.M. Dey, J.B. Harbone (Eds), Methods in Plant Biochemistry, Carbohydrates, vol. 2, Academic press, London, 1990, p. 552.
[14] D.J. Huber, Phytochemistry 30 (1991) 2523.
[15] J.H. Flynn, Thermochim. Acta 8 (1974) 69.
[16] M. Karel, S. Anglea, P. Buera, R. Karmas, G. Levi, Y. Roos, Thermochim. Acta 246 (1994) 249.
[17] W.E.L. Spiess, W.R. Wolf, in: R. Jowitt, F. Escher, B. Hallström, H.F.T. Meffert, W.E.L. Spiess, G. Vos (Eds.), Physical Properties of Food, Applied Science Publishers, Barking, UK, 1983, p. 68.
[18] M.T. Kalichevsky, E.M. Jaroszkiewicz, S. Ablett, J.M.V. Blanshard, P.J. Lillford, Carbohydr. Polym. 18 (1992) 77.
[19] M.T. Kalichevsky, J.M.V. Blanshard, Carbohydr. Polym. 19 (1992) 271.
[20] M.T. Kalichevsky, J.M.V. Blanshard, R.D.L. Marsh, in: J.M.V. Blanshard, P.J. Lillford (Eds.), The Glassy State in Foods, Nottingham University Press, Nottingham, UK, 1993, p. 133.
[21] S.S. Kelley, T.G. Rials, W.G. Glasser, J. Mater. Sci. 22 (1987) 617.
[22] M. Kimura, J. Nakano, J. Polym. Sci.: Polym. Lett. Ed. 14 (1976) 741.
[23] S.A. Bradley, S.H. Carr, J. Polym. Sci.: Polym. Phys. Ed. 14 (1976) 111.
[24] M. Scandola, G. Ceccorulli, M. Pizzoli, Int. J. Biol. Macromol. 13 (1991) 254.
[25] D.R. Coffin, M.L. Fishman, J. Agric. Food Chem. 41 (1993) 1192.
[26] H. Matsuura, A. Eisenberg, J. Polym. Sci. 14 (1976) 1201.
[27] M.J. Gidley, D. Cooke, S. Ward-Smith, in: J.M.V. Blanshard, P.J. Lillford (Eds.), The Glassy State in Foods, Nottingham University Press, Nottingham, UK, 1993, p. 308.
[28] H. Batzer, U.T. Kreibich, Polym. Bull. 5 (1981) 585.
[29] W.J. MacKnight, F.E. Karasz, J.R. Fried, in: D.R. Paul, S. Newman (Eds.), Polymer Blends, vol. 1, Academic Press, New York, USA, 1978, p. 200.