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Dynamic mechanical thermal analysis of cell wall polysaccharides extracted from lyophilised carrot *Daucus carota*

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Abstract

Dynamic mechanical thermal analysis (DMTA) was used to determine thermal phase transitions in low water content cell wall polymer mixtures extracted from lyophilised carrot tissue. The cell wall material was sequentially extracted to produce three residues: an extract free from Ca^{2+} pectic polysaccharides, an extract mainly free from pectic polysaccharides and a final residue rich in hemicellulose and cellulose. A single broad transition was found, the temperature of which decreased with increasing water content. The magnitude of the drop in storage modulus (E') at the transition increased with increasing water content, between 0 and 28% (wet weight basis, w.w.b). The values of E' taken at 20°C were similar for all the residues and were typical of a glassy material for the water range 0–20% (w.w.b). Above 20% (w.w.b), E' at 20°C decreased with increasing water content. As more amorphous material was removed during the sequential extraction, the residues became richer in hemicellulose and cellulose, sorption isotherms exhibiting a more hydrophobic behaviour than that of the whole cell wall. This study showed the dominant contribution of the hemicellulose/cellulose domain to the stiffness of lyophilised carrot cell wall at these low water contents.

Keywords: Carrot; Cell wall; Polysaccharides; Dynamic mechanical thermal analysis; Sorption isotherm; Glass transition

1. Introduction

Techniques such as calorimetry and small strain oscillatory deformation measurements together with a polymer science approach provide information on composition/function relationships for plant materials. The physical properties of freeze-dried vegetable tissue have been associated with the glass transition temperature, T_g (Karathanos, Anglea & Karel, 1993; Roos & Karel, 1991), which is an important parameter in governing the mechanical and storage characteristics of dried plant foods. Karmas, Buera and Karel (1992) compared the thermal properties of freeze-dried plant tissue with model systems using differential scanning calorimetry (DSC) and attributed the T_g to the solutes or concentrated amorphous solution (CAS). Lin, Yuen and Varner (1991) investigated the phase transitions of cell wall preparations using DSC and found a T_g which was affected by calcium. Recently the occurrence of T_g in low water content cell wall of carrot using DSC and dynamic mechanical thermal analysis (DMTA) has been reported (Georget, Ng, Smith & Waldron, 1998a; Georget, Smith & Waldron, 1998b). Carrot cell wall comprises complex polymers (principally cellulose, hemicellulose and pectic polysaccharides) and their sequential extraction offers an opportunity to separate their contributions. Aguilera, Cuadros and del Valle (1998) and Wetton (1986) suggested that a technique such as DMTA, being 1000 times more sensitive than DSC, would be a more appropriate method to determine T_g. Sorption isotherms have been shown for fruits and vegetables and their components (Aguilera et al., 1998; Georget et al., 1998b; Sá, Figueiredo & Sereno, 1999) and have been described in more detail for simpler mixtures (Biliaderis, Lazaridou & Arvanitoyannis, 1999; Hartley, Chevance, Hill, Mitchell & Blanshard, 1995; Mousia, Farhat, Blachot & Mitchell, 2000; Tsami, Krokida & Drouzas, 1999).

The aim of this work was to determine the contribution of each category of polysaccharide to the overall thermo-mechanical properties of carrot cell wall. For this purpose, various insoluble cell wall materials were prepared following a sequential extraction of carrot cell wall. These were pressed into uniform blocks and conditioned over various saturated salt solutions and their sorption isotherms determined. Results obtained by DMTA were interpreted in terms of contributions from the carrot cell wall components in relation to their thermal transitions.

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2. Materials and methods

2.1. Material

Carrots (*Daucus carota* cv Amstrong) were grown locally. Once harvested, the crop was stored in an ice bank (0°C, 99% RH). The carrot dimensions were typically 15–20 cm long and 1.5–2 cm diameter.

2.2. Freeze-dried carrot tissues

After storage, the carrots were washed, dried, sliced and frozen in liquid nitrogen. The excess of nitrogen was removed and the frozen material left was freeze-dried (Model 3.5, Birchover Instruments Ltd, Letchworth, Herts, UK). The material obtained represented 7.9% of fresh tissue with a water content typically 12% (wet weight basis, w.w.b). The water content was determined with a Mettler MP 16 moisture balance (Mettler Instruments Ltd., High Wycombe, Bucks, UK). The freeze-dried material was ground using a mortar and pestle and stored in a desiccator over silica gel.

2.3. Cell wall preparation (SIR)

Cell wall material was prepared as described by Parker and Waldron (1995). Approximately, 10 g of dry ground freeze-dried carrot was homogenised with an aqueous solution of sodium dodecyl sulphate (SDS) containing $Na_2S_2O_5$. A few drops of octanol were used to minimise the foaming. The homogenate was filtered and the residue was ball-milled until it was free of intracellular components and starch granules. This procedure was repeated in order to obtain sufficient material for the study.

Enzymes were inactivated using the method of Huber (1991) modified as described by Georget et al. (1998a). Buffered phenol was mixed with the cell wall prepared from freeze-dried carrot, stirred and centrifuged. The buffered phenol cell wall mixture was diluted, filtered and washed with 95% (v/v) ethanol and then washed again with absolute ethanol. The residue was filtered and washed with acetone and left for the acetone to evaporate. This final SDS/phenol buffer insoluble residue (SIR) comprised carrot cell wall material without any intracellular components and with inactivated enzymes.

2.4. Sequential extraction of SIR

The above cell wall material was treated 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 CDTA insoluble residue (CIR) was dialysed exhaustively for 10 days and freeze-dried. This procedure enables the removal of ionically (Ca^{2+}) bound pectic polysaccharides (Selvendran & Ryden, 1990). Further extraction 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. This method reduces the β elimination. The treatment with $NaBH_4$ will prevent the peeling of the side chains. Then, the mixture was centrifuged and filtered. After extraction, the residue was dialysed for five days, neutralised and freeze-dried. The Na_2CO_3 insoluble residue (NIR) comprises mainly cell wall material with less esterified pectins than SIR (Jarvis, Hall, Threlfall & Friend, 1981). After extraction with 0.5 M KOH containing 20 mM $NaBH_4$ for 2 h, the 0.5 M KOH insoluble residue (0.5KIR) was dialysed thoroughly for 3–5 days, neutralised and then freeze-dried. The remaining pectic polysaccharides will be solubilised leaving a residue (0.5KIR) rich in cellulose and hemicellulose (Redgwell & Selvendran, 1986; Selvendran, 1985). The final extraction with 4 M KOH containing 20 mM $NaBH_4$ for 2 h was carried out on 0.5KIR. Again, the latter was dialysed for 3–5 days, neutralised then lyophilised to produce the 4 M KOH-insoluble residue (4KIR) containing mainly cellulose (Redgwell & Selvendran, 1986).

All the residues produced above were ground with mortar and pestle and stored in hermetically sealed jars in a desiccator over silica gel.

2.5. Sugar analysis of cell wall residues

The neutral sugars were analysed following the method described by Selvendran, March and Ring (1979) using a Carbo Erba gas liquid chromatograph. The monosaccharides released by acid hydrolysis were reduced and analysed as alditol acetates. The uronic acid content was determined colourimetrically, according to the method of Blumenkrantz and Asboe-Hansen (1973) modified by Selvendran et al. (1979) using a Perkin–Elmer spectrometer.

2.6. Sample pressing and water conditioning

Sufficient water was added to the dry cell wall residues (SIR, CIR, NIR, 0.5KIR) to obtain a water content of 70% (w.w.b). The cell wall residues were mixed with water and sandwiched between two acetate sheets and then loaded into a press consisting of a rectangular mould ring of sides 28 and 22 mm between two male compaction dies which were temperature controlled by four cartridge heaters and an inner cooling system (Georget & Smith, 1995). A force of 35 kN (corresponding to a pressure of 57 MPa) was applied to the upper die with the use of a hydraulic pump. The whole device was heated to 30°C to minimise the water solubilisation of pectic polysaccharides (Georget et al., 1998a). The sample was then left for 15 min in the press before cold water was circulated in the inner cooling system. After 10–15 min cooling, the sample was removed. Approximately, 0.3–0.6 g of material gave a rectangular sheet 28 mm long, 22 mm wide and 0.5–1 mm thick.

Strips 22 mm long and 8 mm wide were cut from the sheet. They were then conditioned for 3–4 weeks over P_2O_5 and the following saturated salt solutions (Young, 1967): LiCl, K_2CO_3, NaCl and KCl to give a water range

Table 1
Sequential extraction of carrot SIR: carbohydrate composition of the different residues

Residues	Yield of SIR (%)	Pectic polysaccharides (%)[a]	Carbohydrate (mol%)									
			Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	UA	UA/NS	Total (μg mg^{-1})
SIR	100	51	3	ND	7	3	2	5	30	50	3	780
CIR	50	46	3	ND	9	4	3	7	39	37	2	829
NIR	39	40	3	ND	9	4	3	7	44	30	2	796
0.5KIR	28	30	2	ND	8	6	3	6	55	20	1	818
4KIR	23	23	2	ND	5	2	3	4	67	17	2	828

[a] Calculated as follows: UA + rhamnose + arabinose + galactose of the residue.

UA: uronic acid; NS: neutral sugars; SIR: SDS/phenol insoluble cell wall; CIR: CDTA insoluble cell wall; NIR: Na_2CO_3 insoluble cell wall; 0.5KIR: 0.5 M KOH insoluble cell wall; 4KIR: 4 M KOH insoluble cell wall; ND: not detected.

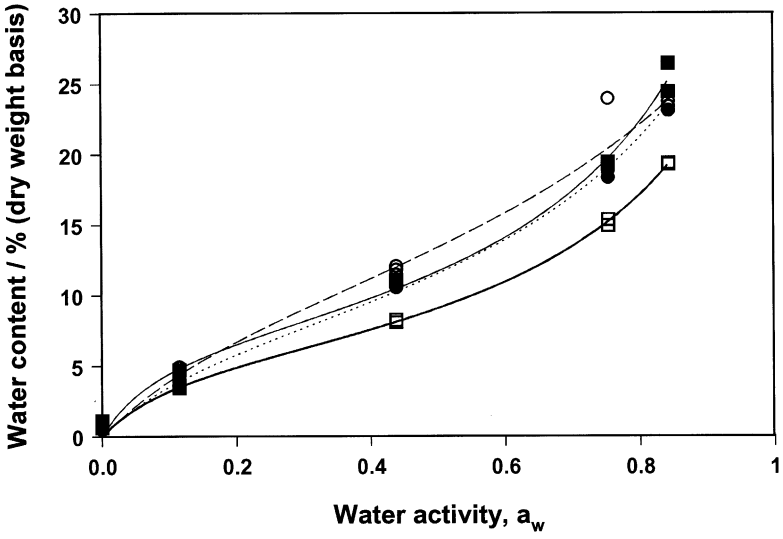

Fig. 1. Sorption isotherms of DMTA specimens: ■—, SIR; ○- - -, CIR; ●……, NIR; □▬▬, 0.5KIR.

0–28% (w.w.b) at room temperature although some specimens were conditioned up to 55% over water.

2.7. Water content determination

The water content before testing was measured gravimetrically by drying to equilibrium in a vacuum oven (Gallenkamp, UK) at 70°C at a pressure of less than 5 mmHg with P_2O_5.

2.8. DMTA measurements

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°C min^{-1}. The transition temperature was defined as the maximum in the peak in tan δ response, although it may also be defined from changes in E' and E'' (Kalichevsky, Jaroszkiewicz, Ablett, Blanshard & Lillford, 1992a).

3. Results and discussion

3.1. Carbohydrate composition

3.1.1. Yield

The yield of SIR represented 2.1% (w.w.b) of the initial fresh weight of carrot root. This value is comparable with results obtained by Massiot, Rouau & Thibault (1988b) and Massiot, Thibault & Rouau (1989) who isolated an alcohol insoluble residue (AIR) and then treated it with a pronase to produce a cell wall material without cytoplasmic proteins. The yield of the latter was 1.8% (w.w.b). This is also consistent with a yield value of 3% (w.w.b) obtained by Ng and Waldron (1997). The yield values for the different cell wall residues, that is, CIR, NIR, 0.5KIR and 4KIR are presented in Table 1 and are comparable with the results reported by Massiot, Rouau and Thibault (1988a) and Ng and Waldron (1997).

3.1.2. Neutral sugars and uronic acid

The compositions in neutral sugars and uronic acid for the sequentially extracted cell wall residues are shown in Table 1. SIR is rich in pectic polysaccharides as indicated by the levels of rhamnose, galactose, arabinose and uronic acid which are similar to those obtained by Massiot et al. (1988a) and Stevens and Selvendran (1984). Pectic polysaccharides constitute 51% of the total cell wall material. Glucose is also present and is likely to originate from cellulose. After hydrolysis with 1 M H_2SO_4, only 13% glucose was released in SIR (Georget et al., 1998a), consistent with data reported by Ng and Waldron (1997).

Further, extraction with CDTA consisted of removing pectic polysaccharides held by Ca^{2+} stabilised chain aggregates (Jarvis et al., 1981). Homogalacturonans are extracted, seen in a decrease in uronic acid content, with a concomitant removal of some branched pectic polysaccharides, as indicated by the decrease in the ratio uronic acid/neutral sugars (UA/NS). The carbohydrate composition of CIR is similar to that reported by Ng and Waldron (1997).

Treatment with Na_2CO_3 resulted in the de-esterification of the pectic polysaccharides (Jarvis et al., 1981). NIR contains 40% of pectic polysaccharides and additionally the amount of uronic acid decreases. This would suggest that more esterified pectic polysaccharides are removed.

Further, the remaining residue was extracted with 0.5 M KOH which yielded a cell wall fraction rich in cellulose as demonstrated by a high amount of glucose. Treatment with 0.5 M KOH induces the cleavage of alkali-labile linkages such as ester linkages (Massiot et al., 1988a,b). These ester cross-links between the galacturonic acid residues and

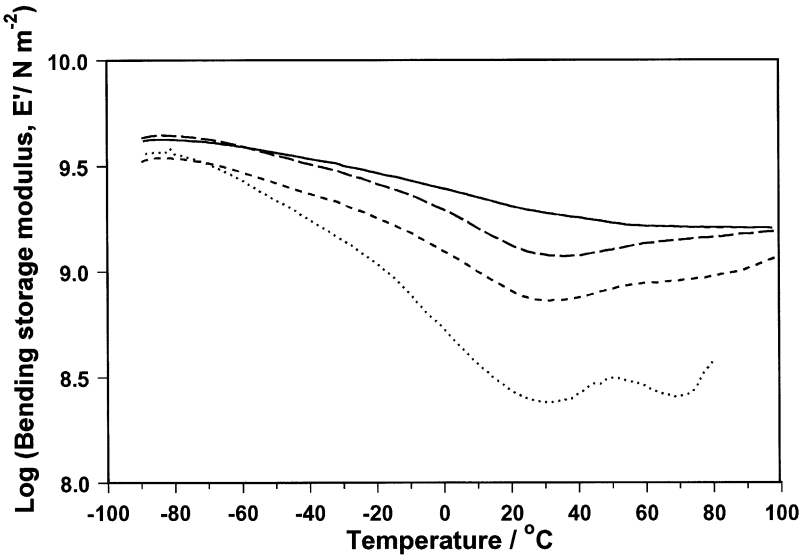

Fig. 2. DMTA E' at 1 Hz of NIR as a function of temperature at various water contents (%, w.w.b): —, 1%; – –, 16%; - - -, 20%; ……, 27%.

hydroxyl groups of sugar residues will be hydrolysed with alkaline solution (Redgwell & Selvendran, 1986). Redgwell and Selvendran (1986) and Selvendran (1985) reported that the polymers solubilised by the above treatments, that is, Na_2CO_3 and 0.5 M KOH, are mainly pectic subtances leaving a residue enriched in cellulose and hemicellulose. However, as observed in Table 1, 30% of pectic polysaccharides are still present in 0.5KIR, implying that these polymers are strongly linked to cellulose and hemicellulose in 0.5KIR. Subsequent extraction with 4 M KOH, resulted in the removal of hemicellulosic materials as observed by the decrease in xylose in 4KIR, also consistent with published results (Massiot et al., 1988a). A significant amount of pectic polysaccharides remains in 4KIR, as reported by Massiot et al. (1988a) and Ng and Waldron (1997) who investigated the cell wall chemistry of carrot. A similar phenomenon was observed in potato (Selvendran & Ryden, 1990) and onion (Redgwell & Selvendran, 1986). Due to the insufficient amount of 4KIR, it was not possible to study the water sorption and to perform DMTA.

3.2. Water sorption isotherms

The SIR, CIR, NIR and 0.5KIR strips produced by 'hot pressing' were water-conditioned over various salt solutions to give sorption isotherms shown in Fig. 1. These results showed the contribution of the different classes of cell wall polysaccharides compared with previously reported isotherms for the whole cell wall of carrot (Georget et al., 1998b) and apple (Aguilera et al., 1998). The decrease in

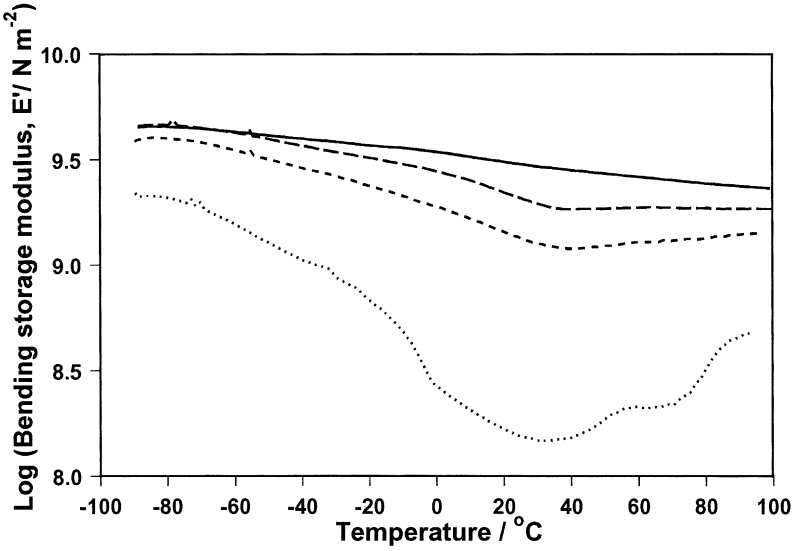

Fig. 3. DMTA E' at 1 Hz of 0.5KIR as a function of temperature at various water contents (%, w.w.b): —, 1%; – –, 8%; - - -, 13%; ……, 28%.

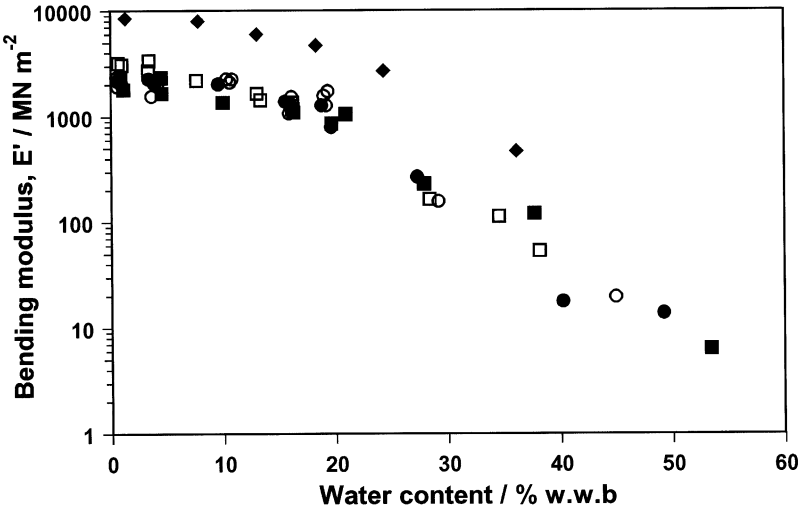

Fig. 4. DMTA E' at 1 Hz determined at 20°C for the different residues as a function of water content: ■ SIR (Georget et al., 1998b); ○ CIR (Georget et al., 1998b); ● NIR; □ 0.5KIR; and Young's modulus for ◆ pine hemicellulose (Cousins, 1978)

amorphous material during the extraction of the carrot cell wall to obtain a cellulose rich residue resulted in a decrease in sorbed water. For similar reasons, Stubberud, Arwidsson, Larsson and Graffner (1996) observed a decrease in sorbed water for tablets of mixtures of PVP (polyvinylpyrrolidone) and MCC (microcrystalline cellulose) as the proportion of PVP was decreased.

3.3. Mechanical and thermal properties of SIR, CIR, NIR and 0.5KIR

3.3.1. Storage modulus (E') measurements

For all the residues, that is, SIR, CIR, NIR and 0.5KIR, the storage modulus, E', was obtained as a function of temperature for various water contents in the 0–55% (w.w.b) water range at the start of the experiment. In Figs. 2 and 3, E' is represented versus temperature for NIR and 0.5KIR, respectively, at different water contents. The temperature at which E' decreased, associated with a T_g, shifted towards lower values with increasing water content. Water had a plasticising effect on the different cell wall residues consistent with T_g results obtained on other biopolymers.

The low temperature glassy modulus was approximately constant for all water contents of this study whereas the high temperature modulus increased as the water content decreased, consistent with results found on amylopectin (Kalichevsky, Blanshard & Marsh, 1993a) and gluten (Kalichevsky, Jaroszkiewicz & Blanshard, 1992b). The high temperature modulus was only low enough to be rubbery at the higher water contents; at low water contents it remained glassy. In some cases at high water content (Fig. 3), the low temperature modulus was lower than that of drier samples, as observed for other systems (Brent, Mulvaney, Cohen & Bartsch, 1997; Kalichevsky et al., 1993a). An upturn in E' was evident above 40°C, attributable to water loss from samples, as described in other systems (Kalichevsky et al., 1993a). In general, progressively removing the pectic polysaccharides from SIR to produce a material rich in cellulose and hemicellulose (0.5KIR) (Table 1) with a small amount of pectic polysaccharides, did not lead to differences in water plasticisation.

The modulus, E' determined at 20°C as a function of water content is given in Fig. 4 for all the residues. The stiffness decreased with increasing water content (0–55% w.w.b), consistent with previous observations on single components (Kalichevsky et al., 1992b; Kalichevsky et al., 1993a) and more complex systems (Georget & Smith, 1995; Kelley, Rials & Glasser, 1987). As stated earlier (Georget et al., 1998b) the first step of the sequential extraction consisted of removing Ca^{2+} bound pectic polysaccharides, using CDTA. This showed little difference in E' between SIR and CIR. During the next step, that is, the extraction with Na_2CO_3, the rest of the ion linkages were disrupted and some esterified pectic polysaccharides were cleaved. Again further to extraction with 0.5 M KOH, more strongly esterified pectic polysaccharides were removed, leaving 0.5KIR, which comprises cellulose, hemicellulose and a small amount of pectic polysaccharides (Table 1). Since SIR and 0.5KIR have similar moduli (Fig. 4), the removal of pectic polysaccharides had little effect on the thermomechanical properties of cell wall over 0–55% (w.w.b) water content range. Above 28% (w.w.b) water content, the scattering of the stiffness values was greater which might result from the non-equilibrium sorption of water. As a comparison, data (Cousins, 1978) reported on hemicellulose extracted from pine are plotted in Fig. 4. It shows that stiffness values of SIR, CIR, NIR and 0.5KIR are lower than those of hemicellulose.

The comparison between published results on biopolymers such as pectin, hemicellulose and cellulose (Georget

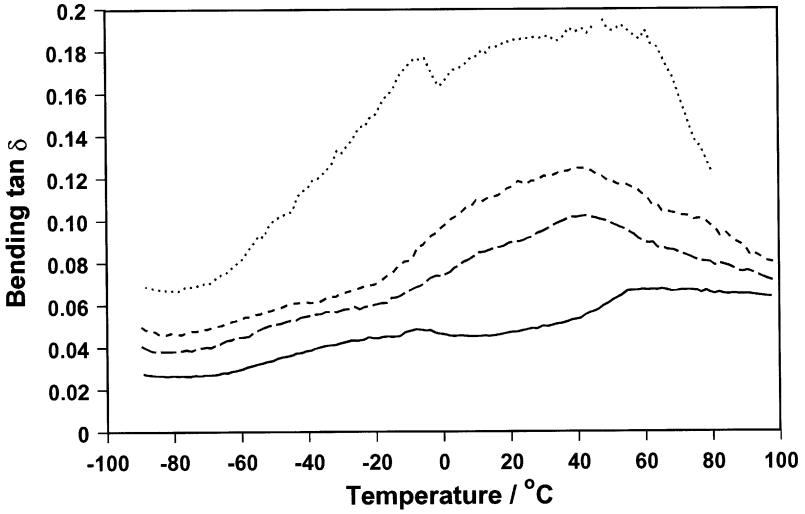

Fig. 5. DMTA tan δ at 1 Hz of NIR as a function of temperature at various water contents. Legend see Fig. 2.

et al., 1998b) taken individually and the different cell wall residues, is not straightforward. Considering the composition of the various carrot cell wall residues, the cell wall from lyophilised carrot behaved like a cellulose dominant system with the contribution of hemicellulose, the latter being closely linked to cellulose. With regard to the pectic polysaccharides, their removal had little effect on the stiffness particularly at water contents below 28% (w.w.b). However the approach of extracting sequentially the various polysaccharides of carrot cell wall did not remove all pectic polysaccharides encountered in 0.5KIR (Table 1), which may still have an effect on the thermo-mechanical properties of the residue.

3.3.2. Damping factor, tan δ

The tan δ represents the ratio between the loss modulus, E'' (viscous component) and the storage modulus, E' (elastic component) and since E' and E'' are sensitive to sample geometry (Rodríguez-Pérez, Rodríguez-Llorente & De Saja, 1997; Vodovotz, Hallberg & Chinachoti, 1996) the study of tan δ is preferred. The latter responds in a systematic way to the volume fraction of any given relaxing phase whereas E'' does not (Wetton, 1986). In Figs. 5 and 6, tan δ is plotted against temperature for NIR and 0.5KIR, respectively. A major peak occurred which shifted towards low temperature values with increasing water content. It is interesting to note the occurrence of a peak at 0°C for water contents at 27–28%, as also observed in SIR and CIR. A similar phenomenon, attributed to ice melting, was found in bread at water content >30% (Vodovotz et al., 1996), extruded cereal melts at water content >29% (Brent et al., 1997), amylopectin/fructose mixtures at water content >27% (Kalichevsky, Jaroszkiewicz & Blanshard, 1993b) and processed wheat flakes at water content >31% (Georget & Smith, 1996). This shows the existence of phase separation and also demonstrates (Brent et al., 1997) that there is a

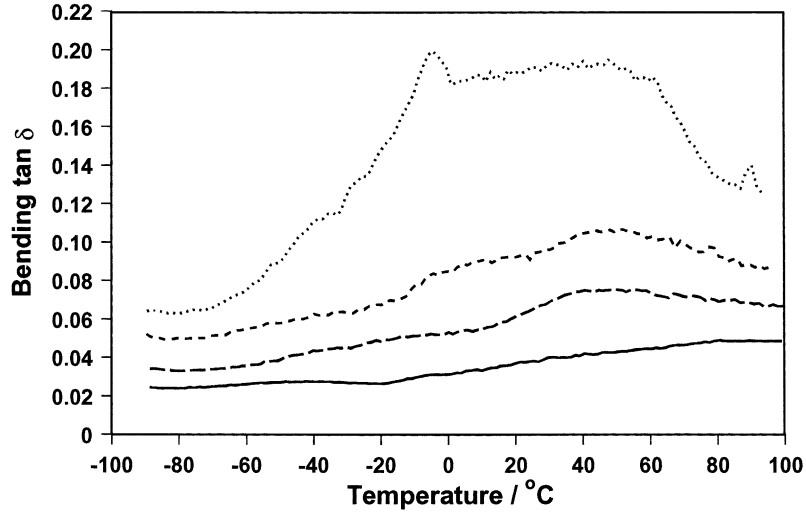

Fig. 6. DMTA tan δ at 1 Hz of 0.5KIR as a function of temperature at various water contents. Legend see Fig. 3.

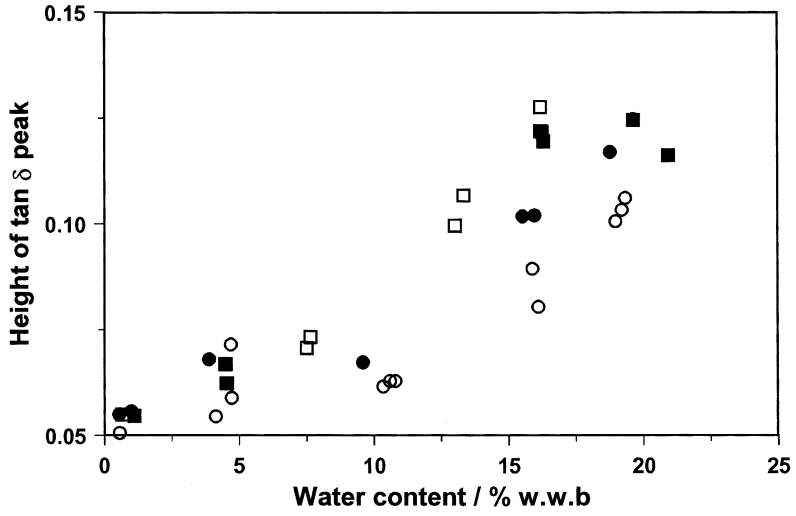

Fig. 7. Height of tan δ peak at 1 Hz of different residues as a function of water content: ■, SIR; ○, CIR; ●, NIR; □, 0.5KIR.

limit, which is 27–29% for the present study, for maximum plasticisation of the biopolymer mixtures by water.

The height of the major peak in tan δ increased when the water content increased for the different carrot cell wall residues (Fig. 7). Several examples of this behaviour can be found in the literature. For instance, this phenomenon was observed in blocks of compacted bread for water content <30% using DMTA (Vodovotz et al., 1996). The authors suggested that this increase was due to the increase in degree of heterogeneity in the T_g. A similar effect was reported by Kalichevsky and Blanshard (1992) who studied gluten, amylopectin and their mixture. Water increases the mobility of these biopolymers but may also in some way reduce self-association (by hydrogen bonding), thus reducing cross-linking and increasing the volume of the relaxing phase. Brent et al. (1997) found a similar effect in extruded cereal melt and postulated that the tan δ breadth was due to the broad molecular weight distribution resulting in a distribution of relaxation times. This might be applicable to the different carrot cell wall residues.

It is also interesting to note that for any particular water content, there was an increase in tan δ height as the carrot cell wall was sequentially extracted to produce a cellulose rich material (Table 1). In a related study, crystallinity was found to increase from 12 to 18% during sequential extraction to give the cellulose-rich residue (0.5KIR) (Georget, Cairns, Smith & Waldron, 1999). Rodríguez-Pérez et al. (1997) observed an increase in tan δ height with increasing crystallinity for various polyolefins. The CIR had the lowest tan δ height when compared with the other residues which may be due to the removal of Ca^{2+} cross-linked pectic polysaccharides since Ca^{2+} bridges have been reported to increase the ratio of E'' to E' (tan δ) (Miyoshi, Takaya, Williams & Nishinari, 1996).

The transition temperatures, determined by DMTA from the maximum in tan δ, for the residues (Fig. 8) were not

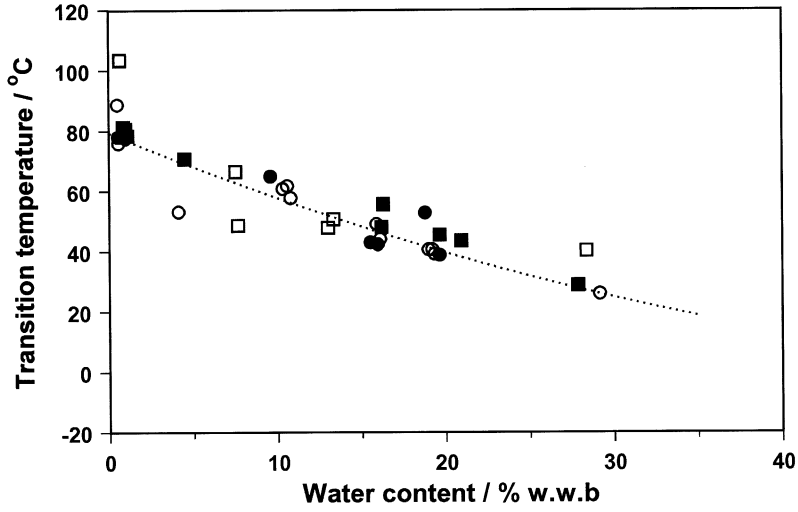

Fig. 8. DMTA transition temperature at 1 Hz of cell wall residues as a function of water content: ■, SIR; ○, CIR; ●, NIR; □, 0.5KIR.

significantly changed by chelating Ca^{2+} and consequently removing the Ca^{2+}-bound pectic polysaccharides from SIR, or by further extraction with Na_2CO_3 and 0.5 M KOH. This demonstrates that hemicellulose and cellulose rather than the pectic polysaccharides determine the T_g of cell wall. However, as observed previously by the sugar analysis and elsewhere (Massiot et al., 1988a), branched pectic polysaccharides were detected and were still present in 0.5KIR.

The transition temperatures for SIR, CIR, NIR and 0.5KIR were lower than the T_g of other biopolymers such as amylopectin (Kalichevsky et al., 1992a), hemicellulose (Kelley et al., 1987) and galactans (Mitsuiki, Yamamoto, Mizuno & Motoki, 1998). It has been demonstrated by Bizot, Le Bail, Leroux, Davy, Roger and Buléon (1997), in the context of starch oligomers, that branching depressed T_g and the branching was described as an internal plasticisation. The different residues produced in the present study consist of a very complex mixture of pectic polysaccharides, hemicellulosic polysaccharides and cellulose which in turn will have various degree of branching and crystallinity. Salmén and Back (1977) described a reduction in T_g with increasing crystallinity for cellulose. These factors could considerably depress T_g when compared with the values of these biopolymers taken individually.

4. Conclusion

The sequential extraction of lyophilised carrot cell wall removed the pectic polysaccharides to leave a residue rich in cellulose and hemicellulose. However, a significant amount of pectic polysaccharides survived 4 M KOH treatment. The sorption isotherms of the progressively extracted cell wall residues were determined and showed that less water was sorbed when the pectic polysaccharides were removed. DMTA revealed the existence of a single broad transition, similar for the different cell wall residues, which shifted towards lower temperatures with increasing water content.

Removal of the pectic polysaccharides had little effect on the storage modulus of the cell wall residues at 20°C, which would indicate that the stiffness of the cell wall is mainly governed by cellulose/hemicellulose in the water content range 0–55% (w.w.b).

Faced with the complexity of the residues even after extraction with 4 M KOH, the study of some binary or even ternary mixtures of the pure cell wall polymers would serve to define better the basic transitions and stiffness values.

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References

Aguilera, J. M., Cuadros, T. R., & del Valle, J. M. (1998). Differential scanning calorimetry of low-moiture apple products. *Carbohydrate Polymers*, *37*, 79–86.

Biliaderis, C. G., Lazaridou, A., & Arvanitoyannis, I. (1999). Glass transition and physical properties of polyol-plasticised pullulan-starch blends at low moisture. *Carbohydrate Polymers*, *40*, 29–47.

Bizot, H., Le Bail, P., Leroux, B., Davy, J., Roger, P., & Buléon, A. (1997). Calorimetric evaluation of the glass transition in hydrated, linear and branched polyanhydroglucose compounds. *Carbohydrate Polymers*, *32*, 33–50.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.

Brent Jr., J. L., Mulvaney, S. J., Cohen, C., & Bartsch, J. A. (1997). Thermomechanical glass transition of extruded cereal melts. *Journal of Cereal Science*, *26*, 301–312.

Cousins, W. J. (1978). Young's modulus of hemicellulose as related to moisture content. *Wood Science and Technology*, *12*, 161–167.

Georget, D. M. R., & Smith, A. C. (1995). Mechanical properties of wheat-flake components. *Carbohydrate Polymers*, *28*, 305–311.

Georget, D. M. R., & Smith, A. C. (1996). The effect of sugars on the mechanical properties of processed cereals. *Journal of Thermal Analysis*, *47*, 1377–1389.

Georget, D. M. R., Ng, A., Smith, A. C., & Waldron, K. W. (1998a). The thermomechanical properties of carrot cell wall material. *Journal of the Science of Food and Agriculture*, *78*, 73–80.

Georget, D. M. R., Smith, A. C., & Waldron, K. W. (1998b). Low moisture thermo-mechanical properties of carrot cell wall components. *Thermochimica Acta*, *315*, 51–60.

Georget, D. M. R., Cairns, P., Smith, A. C., & Waldron, K. W. (1999). Crystallinity of lyophilised carrot cell wall components. *International Journal of Biological Macromolecules*, *26*, 325–331.

Hartley, L., Chevance, F., Hill, S. E., Mitchell, J. R., & Blanshard, J. M. V. (1995). Partitioning of water in binary biopolymer mixtures at low water content. *Carbohydrate Polymers*, *28*, 83–89.

Huber, D. J. (1991). Acidified phenol alters tomato cell wall pectin solubility and calcium content. *Phytochemistry*, *30*, 2523–2527.

Jarvis, M. C., Hall, M. A., Threlfall, D. R., & Friend, J. (1981). The polysaccharide structure of potato cell walls: chemical fractionation. *Planta*, *152*, 93–100.

Kalichevsky, M. T., & Blanshard, J. M. V. (1992). A study of the effect of water on the glass transition of 1:1 mixtures of amylopectin, casein and gluten using DSC and DMTA. *Carbohydrate Polymers*, *19*, 271–278.

Kalichevsky, M. T., Jaroszkiewicz, E. M., Ablett, S., Blanshard, J. M. V., & Lillford, P. J. (1992a). The glass transition of amylopectin measured by DSC, DMTA and NMR. *Carbohydrate Polymers*, *18*, 77–88.

Kalichevsky, M. T., Jaroszkiewicz, E. M., & Blanshard, J. M. V. (1992b). Glass transition of gluten 1: gluten and gluten–sugar mixtures. *International Journal of Biological Macromolecules*, *14*, 257–266.

Kalichevsky, M. T., Blanshard, J. M.V., & Marsh, R. D. L. (1993a). Applications of mechanical spectroscopy to the study of glassy biopolymers and related systems. In J. M. V. Blanshard & P. J. Lillford, *The glassy state in foods* (p. 143). Nottingham: Nottingham University Press.

Kalichevsky, M. T., Jaroszkiewicz, E. M., & Blanshard, J. M. V. (1993b). A study of the glass transition of amylopectin–sugar mixtures. *Polymer*, *34*, 346–358.

Karathanos, V., Anglea, S., & Karel, M. (1993). Collapse of structure during drying of celery. *Drying Technology*, *11*, 1005–1023.

Karmas, R., Buera, M. P., & Karel, M. (1992). Effect of glass transition on rates of nonenzymatic browning in food systems. *Journal of Agricultural and Food Chemistry*, *40*, 873–879.

Kelley, S. S., Rials, T. G., & Glasser, W. G. (1987). Relaxation behaviour of the amorphous components of wood. *Journal of Materials Science*, *22*, 617–624.

Lin, L.-S., Yuen, H. K., & Varner, J. E. (1991). Differential scanning

calorimetry of plant cell walls. *Proceedings of the National Academy of Science of the USA*, *88*, 2241–2243.

Massiot, P., Rouau, X., & Thibault, J. F. (1988a). Isolation and characterisation of the cell-wall fibers of carrot. *Carbohydrate Research*, *172*, 217–227.

Massiot, P., Rouau, X., & Thibault, J. F. (1988b). Characterisation of the extractable pectins and hemicelluloses of the cell wall of carrot. *Carbohydrate Research*, *172*, 229–242.

Massiot, P., Thibault, J. F., & Rouau, X. (1989). Les pectines et les fibres de carotte. *Industries Alimentaires et Agricoles*, *106*, 727–732.

Mitsuiki, M., Yamamoto, Y., Mizuno, A., & Motoki, M. (1998). Glass transition properties as a function of water content for various low-moisture galactans. *Journal of Agricultural and Food Chemistry*, *46*, 3528–3534.

Miyoshi, E., Takaya, T., Williams, P. A., & Nishinari, K. (1996). Effects of sodium chloride and calcium chloride on the interaction between gellan gum and konjac glucomannan. *Journal of Agricultural and Food Chemistry*, *44*, 2486–2495.

Mousia, Z., Farhat, I. A., Blachot, J. F., & Mitchell, J. R. (2000). Effect of water partitioning on the glass-transition behaviour of phase separated amylopectin–gelatin mixtures. *Polymer*, *41*, 1841–1848.

Ng, A., & Waldron, K. W. (1997). Effect of cooking and pre-cooking on cell-wall chemistry in relation to firmness of carrot tissues. *Journal of the Science of Food and Agriculture*, *73*, 503–512.

Parker, M. L., & Waldron, K. W. (1995). Texture of Chinese water chesnut: involvement of cell wall phenolics. *Journal of the Science of Food and Agriculture*, *68*, 337–346.

Redgwell, R. J., & Selvendran, R. R. (1986). Structural features of cell-wall polysaccharides of onion *Allium cepa*. *Carbohydrate Research*, *157*, 183–199.

Rodríguez-Pérez, M. A., Rodríguez-Llorente, S., & De Saja, J. A. (1997). Dynamic mechanical properties of polyolefin foams studied by DMA techniques. *Polymer Engineering Science*, *37*, 959–965.

Roos, Y., & Karel, M. (1991). Plasticizing effect of water on thermal behavior and crystallization of amorphous food models. *Journal of Food Science*, *56*, 38–43.

Sá, M. M., Figueiredo, A. M., & Sereno, A. M. (1999). Glass transitions and state diagrams for fresh and processed apple. *Thermochimica Acta*, *329*, 31–38.

Salmén, N. L., & Back, E. L. (1977). The influence of water on the glass transition temperature of cellulose. *TAPPI*, *60*, 137–140.

Selvendran, R. R. (1985). Developments in the chemistry and biochemistry of pectic and hemicellulosic polymers. *Journal of Cell Science Supplement*, *2*, 51–88.

Selvendran, R. R., & Ryden, P. (1990). Isolation and analysis of plant cell walls. In P. M. Dey & J. B. Harborne, *Methods in Plant Biochemistry-Carbohydrates* (p. 553). , 2. London: Academic Press.

Selvendran, R. R., March, J. F., & Ring, S. G. (1979). Determination of aldoses and uronic acid content of vegetable fiber. *Analytical Biochemistry*, *96*, 282–292.

Stevens, B. J. H., & Selvendran, R. R. (1984). Structural features of cell-wall polysaccharides of the carrot *Daucus carota*. *Carbohydrate Research*, *128*, 321–333.

Stubberud, L., Arwidsson, H. G., Larsson, A., & Graffner, C. (1996). Water solid interactions II. Effect of moisture sorption and glass transition temperature on compactibility of microcrystalline cellulose alone or in binary mixtures with polyvinyl pyrrolidone. *International Journal of Pharmaceutics*, *134*, 79–88.

Tsami, E., Krokida, M. K., & Drouzas, A. E. (1999). Effect of drying method on the sorption characteristics of model fruit powders. *Journal of Food Engineering*, *38*, 381–392.

Vodovotz, Y., Hallberg, L., & Chinachoti, P. (1996). Effect of aging and drying on thermomechanical properties of white bread as characterized by dynamic mechanical analysis (DMA) and differential scanning calorimetry (DSC). *Cereal Chemistry*, *73*, 264–270.

Wetton, R. E. (1986). Dynamic mechanical thermal analysis of polymers and related systems. In J. V. Dawkins, *Developments in polymer characterisation* (p. 182). London, UK: Elsevier.

Young, J. F. (1967). Humidity control in the laboratory using salt solutions — a review. *Journal of Applied Chemistry*, *17*, 241–245.