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DIETARY FIBRES OF APPLE AND APRICOT PASTES: EFFECT OF HEATING ON PLANT CELL WALLS

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A B S T R A C T

Fruit pieces prepared from pastes are now commonly added to breakfast cereals, snack bars and "healthy" confectionery. The objective was to determine the cell wall composition of heat-treated apple paste and apricot paste. The cell walls of apple paste and apricot paste were isolated and sequentially fractionated separately using HEPES, CDTA, Na_2CO_3, 1M KOH, 4M KOH leaving a final residue. The monosaccharide composition of the fractions was consistent with the presence of cellulose, pectic polysaccharides, xyloglucans, heteroxylans and galactoglucomannans. The heat treatment during the apple paste and the apricot paste production did not change the overall amounts of neutral monosaccharides. Heat treatment resulted in the considerable solubilization of pectic polymers in water-soluble and CDTA-soluble fractions compared to Na_2CO_3, KOH and final residue fractions of the fruit pastes. During heat treatment, tissue softening occurs due to the disruption of the constituent cell wall matrix.

Keywords: Apple, apricot, cell wall polysaccharides, pectin, fruit paste, dietary fiber.

INTRODUCTION

The plant cell walls of fruit and vegetables have been studied for many years as they play a key role in human and animal nutrition. The cell walls of edible plants are the major source of dietary fibres. The three-dimensional structure of the plant cell wall is very complex with the major components being polysaccharides. The monosaccharide components that make up the primary cell walls are glucose, galactose, arabinose, rhamnose, fucose, xylose, mannose, galacturonic acid and glucuronic acid (Harris and Smith, 2006). The main polysaccharides of primary cell walls are pectic, hemicellulosic (such as xyloglucans, glucomannans or galactoglucomannans and xylans) and cellulosic polysaccharides in varying proportions (Caffall and Mohnen, 2009; Smith and Melton, 2012).

While the parenchyma cells in the edible tissue of apples are isodiametric (Bain and Robertson, 1950) those of apricot are different with isodiametric cells near the skin progressively elongating as they approach the stone (Archibald and Melton, 1987). Apple cell walls are mainly composed of cellulose, methylesterified pectic polysaccharides, xyloglucans, glucomannans, galactoglucomannans and xylans (Gross and Sams, 1984; Newman *et al*., 1994; Massiot *et al*., 1996; Renard, 2005). The structure of xyloglucan isolated from apple pomace has been ascertained (Watt *et al*., 1999) and the X-ray crystal structure of the component of oligosaccharides has been determined (Watt *et al*., 2000). In addition, the carbohydrate composition of the extracellular polysaccharides of apple suspension cultures have been determined (Reid *et al*., 1999). When processing apples to make apple sauce, the total dietary fibre content decreased; the processing resulted in a loss of insoluble polysaccharides (Colin-Henrion *et al*., 2009), and drying altered the cellular structure (Bai *et al*., 2002). However, the effects of the whole processing procedure for the production of apple paste on cell wall composition is unknown.

Cell walls of apricots, on the other hand, consist of different proportions of cellulose, xyloglucans and pectic polysaccharides (Femenia *et al*., 1998; Kurz *et al*., 2008; Ella Missang *et al*., 2012). Postharvest changes in the

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texture of apricots have been reported (Melton *et al*., 1989) and the quality of the fruit was determined by non-destructive analysis using Fourier transform infrared spectroscopy (FTIR) (Bureau *et al*., 2009). Cell wall composition of apricots seems to be susceptible to heat, since softening taking place during heating (Chitarra *et al*., 1989; French *et al*., 1989). In thermally processed products such as canned apricots, sometimes disintegration of fruit can occur (Ella Missang *et al*., 2011).

Studies on plant cell wall polysaccharides carried out so far aimed at functions as a dietary fibres and their structure and organization of fruit and vegetables. In terms of food quality, attention has been paid to cell wall changes during ripening in order to optimize textural characteristics. However studies on the characteristics of cell wall components, particularly with respect to effect of heat treatment on cell wall polysaccharides are less common. Industrial food products such as breakfast cereals, snack bars and museli are often presented as a healthy and convenient alternative to confectionary, because fruit paste or pieces are incorporated into these foods. Determining the effects of processing is essential for understanding the contribution of dietary fibres on the product and human health. In addition, heat treatment is expected to promote some leaching of polysaccharides from the cell walls, therefore may alter the rheological properties. The cell wall composition of heat treated concentrated apple and apricot fruit purees (referred to as "pastes") were investigated.

MATERIALS AND METHODS

Materials and chemicals

Apple and apricot pastes: The 38°Brix apple paste and 32°Brix apricot paste were commercial products and were kept stored at -80°C. The pastes were produced by: washing the fruit, destoning (apricots), pulping and screening to remove skin, stalks and seeds. The purees were heated at 95-97°C for 2-3 min to inactivate pectin methylesterase. Then the purees were concentrated in a multi stage evaporator under vacuum to 38° Brix (apples) upto 85°C, or to 32°Brix up to 75°C, (apricots). After screening the pastes were then sterilised at 102-103° C for 2 - 2.5 mins) and asceptically packed. The water content of the pastes was determined gravimetrically.

Chemicals: All reagents used were of analytical grade. Ponceau 2R was purchased from Sigma Chemicals, St Louis, MO; N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid (HEPES) was from Serva, Heidelberg; dithiothreitol (DTT) was from Applichem Chemical Synthesis Services, Darmstadt; *Trans*-1, 2-diaminocyclohexane-*N,N,N', N'*-tetraacetic acid (CDTA) was from Acros Organics, New Jersey, USA. Dialysis tubing (90 mm diameter, molecular weigh cut off of 12 000-14 000 Da) was from Medicell International, England; polytetrafluoroethylene (PTFE) filters (0.22 μm pore size) was from Alltech Deerfield, IL.

Isolation of cell walls from apple and apricot pastes: Cell wall isolation followed the procedure of Melton and Smith (2001). Prior to isolating cell walls from the pastes, a portion was examined microscopically after staining with Ponceau 2R to ensure all the cells were broken open so that any intracellular contents would be removed subsequently.

Apple paste (20 g) or apricot paste (30 g) was mixed thoroughly in 100 ml HEPES buffer (20 mM, adjusted to pH 6.7 with KOH, containing 10 mM DTT, 4°C). The homogenate was centrifuged at 4800 g for 10 min at 4°C and the supernatant decanted and held at 4°C. The pellets were resuspended in 100 ml of HEPES buffer and the mixture was filtered through 11 μm nylon mesh. The filtrate was centrifuged at 4800 g for 10 min and the supernatant was combined with first supernatant. The combined supernatants (the water-soluble fraction, designated as 'HEPES') were dialysed. The retained cell wall material (CWM) was kept at 4°C for further fractionation. A small aliquot of the CWM was freeze dried for analysis. Isolation was done in duplicate for each paste.

Cell wall fractionation: The isolated cell wall materials from the pastes were sequentially fractionated using procedures based on Melton and Smith, 2001. Cell wall fractionation was done in duplicate for each paste.

The cell wall preparations were washed by centrifuging with 50 mM CDTA in 50 mM potassium acetate buffer (pH 6.5). The pellet was then resuspended in CDTA, stirred for 6 h and centrifuged (1000 g, 10min). The pellet was again treated with CDTA (6 h). The extracts were combined to form the 'CDTA fraction'.

The pellet from the CDTA extraction was washed by centrifugation with 50 mM Na_2CO_3 solution containing 20 mM $NaBH_4$. The pellet was then resuspended in Na_2CO_3 solution and stirred for 16 h at 4°C and then at room temperature for 2 h. The suspension was

centrifuged, and then the pellet was resuspended in Na_2CO_3 solution and stirred at room temperature for 2 h. The extracts were combined to form the 'Na_2CO_3 fraction'.

The pellet from the Na_2CO_3 extraction was washed by centrifugation with 1M KOH containing 20 mM $NaBH_4$ and then stirred twice with 1M KOH containing 20mM $NaBH_4$ for 4 h. The extracts were combined to form the '1M KOH fraction'.

The pellet from 1M KOH treatment was treated with 4M KOH containing 20 mM $NaBH_4$ as described for '1M KOH'. The extracts were combined to form the '4M KOH' soluble fraction.

The pellet from 4M KOH treatment was extracted twice by resuspended in water and stirred at room temperature for 1 h, followed by centrifuging. The extracts were combined to form the 'residue wash'. The pellet from residue wash was designated the 'final residue' fraction.

The Na_2CO_3 and KOH fractions were neutralized to pH 6.5 with 1 M acetic acid and concentrated hydrochloric acid (HCl), respectively. The 'residue wash' and 'final residue fraction' were neutralized to pH 6.5 with concentrated HCl.

Monosaccharide composition of cell walls and fractions: The CDTA, Na_2CO_3, 1M KOH, 4M KOH, residue wash fractions and final residue were hydrolysed using trifluoroacetic acid (2M, 121˚C, 1 h). The 'final residue' and CWM were hydrolysed using two stage TFA/sulphuric acid procedure (Harris *et al*., 1988). The monosaccharides were reduced and then acetylated using the method of Blakeney *et al*. 1983. The alditol acetates were determined by GC (Model HP 6890, Hewlett Packard) fitted with a flame ionization detector and dedicated cool on-column capillary inlet using a BPX-70, open tubular fused silica column (25 m long, 0.33 mm internal diameter and 0.25 μm film thickness) (SGE Pty, Melbourne, Australia). Although starch determination tests did not indicate any starch contamination, the glucose content in the CDTA fraction (Table 2) was unexpectedly high therefore the monosaccharide content was calculated with and without glucose for HEPES and CDTA fractions.

Uronic acid (UA) content and determination of the degree of esterification (DE) of pectin: The uronic acid content of all freeze dried fractions (CWM, HEPES, CDTA, Na_2CO_3, 1 M KOH, 4M KOH, residue wash and final residue-cellulose) of apple paste and apricot paste was determined by first hydrolyzing them (Ahmed and Labavitch, 1977), and then determining the liberated UA colorimetrically (Filisetti-Cozzi and Carpita, 1991). The degree of methylation (DM) and acetylation (DA) of HEPES and CDTA fractions were determined as described by Levigne *et al*. 2002.

Statistical analysis: Differences amongst samples were assessed statistically using one-way Analysis of Variance (ANOVA) at the 5% level (Origin Pro 8.0) and pair-wise mean comparisons were performed using Tukey test.

RESULTS AND DISCUSSION

The CWM of apple and apricot yielded 53.1 mg of dry weight / g fresh paste and 64.2 mg dry weight/ g fresh paste, respectively. The water contents of the apple and apricot pastes were 60.9 ± 0.3% and 68.5 ± 0.9%, respectively. The yields of water soluble, HEPES fraction along with other fractions of apple and apricot pastes are given in Table 1. Both pastes showed high yields of HEPES, CDTA and Na_2CO_3 soluble fractions compared to low yields of insoluble fractions suggesting increased solubility of cell wall polysaccharides due to the processing. Notable differences are evident between these results and those obtained for other forms of apple tissues. Percy *et al*. (1998) and Watt *et al*. (1999) showed higher recovery of apple residue cell walls. The apple paste processing method (similar to cooking) would have altered the cell wall structure thus solubilise more pectic polysaccharides and hemicelluloses in HEPES, CDTA and Na_2CO_3 fractions.

Monosaccharide composition of CWMs from apple and apricot pastes: The neutral monosaccharide and uronic acid composition of CWM isolated from apple and apricot pastes are shown in Table 2. Xylose, arabinose, glucose and galactose are the dominant neutral monosaccharides obtained in apples whereas glucose (56.8%) was the dominant neutral monosaccharide followed by arabinose, galactose, xylose and mannose with small amounts of rhamnose and fucose in apricots. The large amount of glucose released by sulfuric acid hydrolysis following TFA hydrolysis (apple - 54.8% and apricot - 63.9%), indicates that a large amount of cellulose was present in CWM. Similarly Gross and Sams (1984) found that arabinose, xylose, glucose and galactose were the predominant neutral monosaccharides in the cell walls of apples. Similar monosaccharide composition was also observed in fresh apricots (Femenia *et al*., 1998).

Table 1 Yields obtained during isolation and fractionation of cell walls from apple and apricot pastes.

Fraction	Dry weight Apple (mg/ g paste)	Dry weight Apricot (mg/ g paste)
HEPES	10.9 ± 0.85	13.8 ± 1.7
CDTA	7.3 ± 2.1	7.3 ± 1.6
Na_2CO_3	11.4 ± 0.6	7.4 ± 0.62
1 M KOH	1.8 ± 0.6	3.2 ± 0.7
4 M KOH	5.8 ± 1.0	4.3 ± 0.7
Residue wash	2.4 ± 0.3	0.6 ± 0.1
Residue	4.3 ± 1.3	3.8 ± 0.6

* Data expressed as mean ± standard deviation of replicate measurements

Uronic acid, arabinose and galactose together with small amount of rhamnose indicates the presence of branched pectic polysaccharides in the cell walls of apples. The possible side chains of the galacturonic acid of pectic polysaccharides may be galactans, arabinans and arabinogalactons. Xylose and glucose together with galactose and small amount of fucose suggest that CWM also contains xyloglucan. The presence of glucose, galactose and mannose in CWM of apple paste are possibly due to glucomannans and galactoglucomannans. Similar findings were observed by Massiot *et al*. (1996) on fresh apples, except galacturonic acid. The galacturonic acid in water insoluble fraction in the present study was slightly higher than reported by Massiot *et al*. (1996). This difference may be due to the apple paste used in this study was heat treated (2 min at 102^{o}C) after being concentrated at 85^{o}C to a 38 ^{o}Brix paste. Therefore, more pectic polysaccharides might have been solubilized since the cell walls were loosened due to the heat treatment of apple paste. In apricot paste, the uronic acid, arabinose and galactose together with small amount of rhamnose indicate the presence of rhamnogalacturonan (RGs) with arabinans, galactans and arabinogalactans side chains. Xylose, glucose together with galactose and small amount of fucose suggest that CWM contains some xyloglucan. Some of the glucose, galactose and mannose in CWM of apricot paste are possibly due to glucomannans and galactoglucomannans.

Water-soluble HEPES fraction: On TFA hydrolysis of apple paste (Table 3) large amounts of uronic acid (62.2%) together with neutral monosaccharide arabinose, galactose and rhamnose were found indicating that this fraction contains rhamnogalacturonans (RGs). Pectic polysaccharides are likely to be rich in arabinans as indicated by the high recovery of arabinose. Xylose and glucose together with galactose and small amount of fucose (1.7%) suggest that HEPES fraction contains xyloglucans.

The monosaccharide contents obtained for the water soluble fraction in the present study is in agreement with the study carried out by Massiot *et al*. (1996) on apples except for galactose and glucose. Galactose content is lower than the study done by Massiot *et al*. (1996), and glucose which is higher. The low amount of galactose obtained from the present study may be due to the presence of less galactan side chains than arabinan side chains occurring in the RGs backbone in the particular apple variety used in the apple paste. The presence of high glucose can be accounted for, from more cell wall degradation due to the heat treatment in the apple paste rather than attributed to the presence of starch.

The HEPES fraction of apricot paste had arabinose, galactose and followed by rhamnose together with a large amount of uronic acid (79.9%) indicating the fraction contains RGs with arabinans, galactans and arabinogalactans side chains. Pectic polysaccharides are rich in arabinans due the high recovery of arabinose. The reason of solubilizing the arabinan rich pectic polysaccharides in the HEPES fraction is due to the degradation of pectins during heat treatment of the apricot paste as described above. Similar findings were observed by Femenia *et al*. (1998) done in fresh apricots. Apricot paste used for the present study was heat-treated, therefore probably some loosening of cell walls might have been occurred during the heat treatment thus leading to more pectic polysaccharides in the aqueous HEPES fraction.

CDTA and Na_2CO_3 fractions: The CDTA and Na_2CO_3 fractions contained 37.4% and 14.5% (apple); 70.7% and 18.4% (apricot) of uronic acid respectively (Tables 3 & 4). The total yield of monosaccharide component of apple is similar to the results obtained by Renard and

Table 2 Neutral monosaccharide compositions and uronic acid content of the cell walls material (CWM) from apple and apricot pastes✦.

Samples	Neutral monosaccharides (NM) (% w/w) ❖							NM content ‡ (% dry weight)	Uronic acid content ‡ (% dry weight
	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose		
Apple								30.9 (1.93)	27.2 (1.0)
CWM	3.8 (0.2)	5.6 (0.2)	23.4 (0.9)	25.5 (1.0)	5.7 (0.3)	13.2 (1.9)	22.7 (1.5)		
CWM*	4.9 (0.6)	7.3 (0.1)	29.8 (1.0)	32.8 (1.9)	7.7(3.0)	17.4 (2.2)	-		
CWM-TFA insoluble residue	ND	ND	15.1(1.0)	20.8(0.9)	TR	8.9 (0.3)	54.8(2.4)		
CWM-(TFA/H_2SO_4*)	ND	ND	33.7 (2.1)	46.2 (2.6)	TR	19.9 (0.5)	-		
Apricot								28.7 (2.3)	12.4 (1.2)
CWM	2.0 (0.1)	1.8 (0.1)	14.1 (0.1)	9.3 (0.6)	6.2 (0.4)	9.7 (0.6)	56.8 (3.9)		
CWM-(TFA/H_2SO_4*)	TR	ND	ND	TR	36.2 (3.0)	TR	63.9 (4.6)		

✦Cell wall materials were isolated in duplicate and all results are given as an average of duplicate isolations (i.e. total of 4 determinations). ‡Uronic acid content and neutral monosaccharide content are given as percentages based on the dry weight of the isolations. ❖ Percentages were calculated on the total neutral monosaccharides recovered by analysis of GC. TR-trace, ND-not detected. Standard errors are provided in parenthesis. *Percentage of neutral monosaccharides calculated excluding glucose.

Table 3 Neutral monosaccharide compositions and uronic acid content of the fractions extracted from the apple paste cell walls✦.

Fraction	Neutral monosaccharides (NM) (% w/w) ❖							NM content‡ (% dry weight)	Uronic acid content‡ (% dry weight
	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose		
HEPES	5.2 (0.2)	1.7 (0.1)	42.4 (2.7)	6.5 (0.3)	2.4 (0.1)	17.5 (1.4)	24.3 (1.7)	22.9 (2.3)	62.2 (2.2)
HEPES*	7.0 (0.8)	2.3 (0.3)	55.9 (2.9)	6.7 (0.8)	3.2 (0.3)	22.7 (1.3)	-	11.6 (1.6)	-
CDTA	5.4 (0.2)	2.8 (0.1)	26.2 (0.5)	11.5 (0.3)	3.0 (0.05)	12.3 (0.3)	38.8 (2.2)	10.3 (1.7)	37.4 (1.8)
CDTA*	9.2 (0.9)	4.7 (0.5)	40.8 (2.0)	19.6 (1.7)	5.2 (0.5)	20.8 (0.4)	-	6.3 (0.4)	-
Na_2CO_3	6.7 (0.4)	3.6 (0.1)	44.5 (1.4)	18.2 (0.2)	2.8 (0.1)	16.8 (0.6)	7.4 (0.1)	4.4 (0.6)	14.5 (0.3)
1M KOH	2.6 (0.3)	7.3 (0.7)	17.8 (0.7)	31.4 (2.3)	3.4 (0.3)	13.8 (1.0)	23.7(2.8)	31.2 (2.2)	49.1 (1.5)
4M KOH	1.5 (0.2)	7.9 (0.3)	10.2 (1.7)	30.3 (1.4)	4.6 (0.7)	12.7 (0.9)	32.8 (2.6)	48.9 (3.2)	14.2 (2.3)
Residue wash	5.8 (0.6)	3.9 (0.1)	37.8 (3.2)	24.8 (1.6)	0.8 (1.0)	15.2 (0.9)	11.6 (0.7)	29.3 (2.0)	53.3 (3.8)
Final residue	3.4 (0.9)	5.9 (0.2)	25.6 (0.9)	30.9 (1.1)	1.4 (0.04)	14.6 (0.6)	18.1(0.3)	26.2 (1.1)	16.5 (2.1)
Final residue-(TFA/H_2SO_4*)	4.8 (0.1)	TR	8.0 (0.9)	4.2 (0.8)	TR	13.9 (1.2)	69.0(2.2)	-	-

✦Cell walls were isolated in duplicate and all results are given as an average of duplicate fractionations (i.e. total of 4 determinations). ‡Uronic acid content and neutral monosaccharide content are given as percentages based on the dry weight of the fractions. ❖ Percentages are calculated on the total neutral monosaccharides recovered. TR-trace. Standard errors are provided in parenthesis. *Percentage of neutral monosaccharides calculated excluding glucose.

Thibault (1993). The neutral monosaccharide composition of CDTA fraction from apples consisted of glucose and arabinose and followed by galactose and xylose with small amounts of rhamnose, mannose and fucose. The high amount glucose in the CDTA fraction is unusual compared with the results on fresh apples obtained by O'Neill (2001) and Renard and Thibault (1993) where a small amount of glucose was detected in the CDTA apple fraction. The neutral monosaccharide composition of CDTA fraction from apricots on the other hand was predominantly arabinose (59.6%) and galactose (14.6%) followed by rhamnose, glucose, xylose and mannose and small amount of fucose.

The Na_2CO_3 fraction contained arabinose (44.5%) as the predominant neutral monosaccharide followed by xylose, galactose and glucose in apple paste (Table 3). In apricot paste, Na_2CO_3 fraction contained arabinose (60.2%) as the predominant neutral monosaccharide followed by galactose, xylose, rhamnose and small amount of fucose (Table 4). The total uronic acid content of the CDTA and Na_2CO_3 fractions is lower than HEPES fraction in both apple paste and apricot paste. This may be due to the heat treatment of the apple paste which induced the changes of the pectic polysaccharides, solubilizing the most uronic acid and arabinan side chains in HEPES fraction (Ng and Waldron, 1997; Ng *et al*., 1999). The apple monosaccharide composition indicates that CDTA and Na_2CO_3 fractions contain rhamnogalacturonan. The high amount of arabinose extracted in CDTA (26.2%) and Na_2CO_3 (44.5%) fractions indicates that the pectic polysaccharide branches have more arabinans than galactans side chains as observed in apple by O'Neill (2001). Xylose, glucose together with galactose and a small amount of fucose suggest that CDTA and Na_2CO_3 fractions contain some xyloglucan. Some glucose, galactose and mannose in CDTA and Na_2CO_3 fractions of apple paste are possibly due to galactoglucomannans.

In apricots, the monosaccharide contents of CDTA and Na_2CO_3 fractions indicate that these fractions contain rhamnogalacturonan with branches of arabinans, galactans and arabinogalactans. The high amount of arabinose extracted in CDTA and Na_2CO_3 fractions indicate that the pectic polysaccharide side chains have more arabinans than galactan side chains. This indicates that more branched side chains are extracted by Na_2CO_3 solution. Xylose and glucose together with galactose and small amount of fucose suggest that CDTA and Na_2CO_3 fractions contain xyloglucans. A small amount of glucose, galactose and mannose in CDTA and Na_2CO_3 fractions of apricot paste is possibly due to glucomannans and galactoglucomannans. The monosaccharide components of CDTA and Na_2CO_3 fractions were generally in agreement with the study carried out by Femenia *et al*. (1998) on fresh apricot (CDTA and Na_2CO_3 fractions) except for xylose and arabinose in Na_2CO_3 fraction. These differences may be due to the heat treatment of apricot paste resulted in disrupting linkages between polysaccharides thus solubilizing more polysaccharides in Na_2CO_3 fraction.

1M KOH fraction: In apple paste 1M KOH fraction, uronic acid was found to be present in large amounts (49.1%) when compared with the CDTA (37.4%) and Na_2CO_3 (14.5%) (Table 3). Whereas in apricots the 1M KOH fraction uronic acid content was lower compared with the CDTA (70.7%) and Na_2CO_3 (18.4%) (Table 4). The monosaccharide composition indicates the apple paste 1M KOH fraction contains RGs with arabinans, galactans and arabinogalactan side chains. Similar side chains were also observed by Watt *et al*. 1999. Xylose, glucose together with galactose and a small amount of fucose suggest that 1M KOH fraction contains some xyloglucan. Some of the glucose, galactose and mannose in 1M KOH fraction of apple paste are possibly due to galactoglucomannans.

In apricot paste, the total neutral monosaccharide was found to be 28.7%. The neutral monosaccharide composition of arabinose, galactose and rhamnose along with uronic acid indicated the 1M KOH fraction contains RGs (Table 4). Interestingly the ratio of arabinose : uronic acid and galactose : uronic acid are almost equal (2 : 1), indicating equal amounts of arabinan and galactans side chains present in pectic polysaccharides. The heterogeneity amongst the pectic polysaccharides was evident from differing ratios of neutral sugars (arabinose and galactose) both to each other and to the uronic acid component in CDTA, Na_2CO_3 and 1M KOH fractions (Femenia *et al*., 1998). The presence of xylose, glucose and galactose together and small amounts of mannose and fucose suggest that 1M KOH fraction contains xyloglucan and some glucomannan and galactoglucomannan.

4 M KOH fraction: Apple 4M KOH fraction was notable for xylose and glucose hence xyloglucan was released and some branched pectins indicated by uronic acid, arabinose, galactose while mannose is present at much

Table 4 Neutral monosaccharide compositions and uronic acid content of the fractions extracted from the apricot paste cell wall material✦.

Fraction	Neutral monosaccharides (NM) (% w/w) ❖							NM content‡ (% dry weight)	Uronic acid content‡ (% dry weight)
	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose		
HEPES	9.5 (0.2)	0.64 (0.07)	62.9 (1.2)	3.3 (0.1)	3.2 (0.1)	13.7 (0.3)	6.8 (0.3)	18.7 (2.3)	79.9 (3.6)
CDTA	8.3 (0.5)	1.6 (0.09)	59.6 (3.7)	5.4 (0.3)	4.3 (0.7)	14.6 (0.9)	6.2 (1.6)	13.6 (1.4)	70.7 (4.1)
Na_2CO_3	5.7 (0.2)	1.8 (0.04)	60.2 (1.9)	8.1 (0.1)	2.9 (0.01)	16.4 (0.4)	4.7 (0.1)	12.3 (2.6)	18.4 (3.3)
1M KOH	2.2 (0.06)	5.3 (0.1)	16.9 (0.2)	32.4 (0.5)	5.8 (0.1)	16.8 (0.4)	20.6 (1.1)	28.7 (1.9)	8.1 (1.1)
4M KOH	1.3 (0.01)	4.4 (0.1)	3.5 (0.1)	22.2 (0.4)	15.9 (0.3)	18.7 (0.3)	33.9 (0.6)	32.9 (1.0)	ND
Residue wash	9. 4(0.2)	0.6 (0.05)	49.4 (0.8)	6.5 (0.1)	4.5 (0.1)	12.1 (0.3)	17.5 (0.5)	15.0 (1.5)	26.5 (1.5)
Final residue	1.2 (0.06)	0.5 (0.1)	8.9 (0.1)	13.0 (0.7)	8.2 (0.1)	18.7 (0.1)	49.5 (0.7)	7.5 (0.8)	3.0 (0.6)
Final residue-(TFA/H_2SO_4*)	7.8 (0.1)	TR	4.3 (0.6)	7.5 (0.2)	TR	1.0 (0.4)	79.4 (1.6)		

✦Cell wall materials were isolated in duplicate and all results are given as an average of duplicate fractionations (i.e. total of four determinations). ‡Uronic acid content and neutral monosaccharide content are given as percentages based on the dry weight of the fractions. ❖ Percentages are calculated on the total neutral monosaccharides recovered. TR-trace, ND-not done. Standard errors are provided in parenthesis.

lower levels than in the relevant apricot fraction.

Apricot 4M KOH fraction was notable for no uronic acid and high mannose suggesting strong alkali dissociates mannan or glucogalactomannan adhering to the surface of cellulose fibrils. Xylose and glucose suggest xylocose is also released by the strong alkali. The branch pectin extracted was minimal.

Interestingly, the galactose content is much higher than the amount of arabinose, indicating pectic polysaccharides extracted using 4M KOH solution contains more galactan than arabinan side chains.

Residue wash: The residue washes are minor fractions (Table 1) and while containing neutral sugars they are notable for higher uronic acid content and associated arabinose suggesting tightly bound highly branched pectin has been released (Table 3). These results are in agreement with a study done by O'Neill (2001) in fresh apples who obtained predominantly neutral monosaccharides as arabinose followed by xylose and galactose in residue wash fraction. In apricots (table 4), based on the xylose and glucose contents, a small amount of xyloglucan is present. The ratio of uronic acid : galactose (2.1 : 1) and ratio of uronic acid : arabinose (1 : 1.8) indicates that pectic polysaccharides in the residue wash fraction are highly branched with more arabinans than galactans and arabinogalactans.

Final residue: The final residue is expected to be largely cellulose with smaller amount of very strongly adhering cell wall polysaccharides and indeed in this case. The very high levels of glucose in the TFA insoluble material indicates cellulose is the dominant while they contain small amounts of associated xyloglucan and branched pectins (Table 3 & 4). The apricot fraction is notable for its mannan content. Similar neutral monosaccharides were also obtained by Percy *et al*. (1996 and 1998) on fresh apple tissues, in different concentrations depending on the maturity of the fruit.

In apple paste, the ratio of uronic acid : galactose (1 : 1.1) and ratio of uronic acid : arabinose (0.6 : 1) indicates that the pectic polysaccharides are less highly branched in final residue. Pectic polysaccharides were also found in final residue from cell wall materials of mature fresh apple (Percy *et al*., 1996; O'Neill, 2001).

In apricot paste, the presence of uronic acid along with arabinose, galactose and a small amount of rhamnose indicate that the pectic polysaccharides were associated with the cellulose (Table 4). The ratio of uronic acid: galactose (1 : 6.2) and ratio of uronic acid : arabinose (1 : 2.9) indicates that pectic polysaccharides are indeed highly branched in final residue. The uronic acid content is very low compared to other fractions. Similarly it has been reported that the total pectic polysaccharide contents of final residue fraction from carrots (Ng *et al*., 1997) and winter squash (Ratnayake, Melton, Hurst, 2003) decreased on cooking. Cooking may induce changes which are due to β-

Table 5 Degree of esterification of HEPES and CDTA fractions of apple paste and apricot paste and commercial citrus pectin.

Source/ fraction	Degree of esterification (%)	
	Degree of methylation (DM)	Degree of acetylation (DA)
Commercial citrus pectin	60.8 ± 0.11^{a}	0.36 ± 0.01^{a}
Apple HEPES fraction	63.2 ± 1.8^{b}	2.6 ± 0.6^{b}
Apple CDTA fraction	65.1 ± 2.8^{c}	4.7 ± 1.0^{c}
Apricot HEPES fraction	60.1 ± 3.0^{d}	8.7 ± 1.2^{d}
Apricot CDTA fraction	58.3 ± 1.9^{e}	8.3 ± 1.1^{d}

elimination in the pectin backbone. Cooking also resulted in increase in the uronic acid content in water soluble and CDTA fractions and decrease in residue fraction, indicating that heat treatment was solubilizing uronic acid rich polysaccharides moiety. It is likely that highly branched pectic polysaccharides with high degree of cross linking were retained in the final residue (Ng *et al*., 1997).

Degree of esterification of uronic acid of apple paste and apricot paste: The HEPES and CDTA fractions of apple paste and apricot paste were analyzed for the degree of esterification. The fractions obtained from the alkaline treatments were not analyzed for degree of esterification because methyl and acetyl substituents have already been removed by the alkali (Melton and Smith, 2001). The DM of commercial citrus pectin (60.8%) was within the product specification range (57.5-62%) (Table 5). The DM of HEPES and CDTA fractions of apple are 63.2% and 65.1%, respectively (Table 5). The DA of HEPES, CDTA fractions and commercial pectin are 2.6%, 4.7%, and 0.36% respectively. Renard and Thibault (1993) found that fresh apple had a DM (80%) and DA (6%) which are higher than the values obtained in the present study. This may be due to the heat treatment of apple paste. Pectin methylesterase (PME) activity can be enhanced during the heat treatment as the temperature rises thus leading to a reduction in the pectin esterification (Klein *et al*., 1994).

According to the findings of Klein *et al*. (1994) apples at harvest, the DM of water soluble and a CDTA fraction were 80% and 60%, respectively. Further they found that heat treatment caused 30% drop in the DM in the water soluble fraction. Therefore, it is more likely that the low values for the DM is due to the heat treatment of apple.

The DM of HEPES and CDTA fractions of apricot paste are 60.1% and 58.3%, respectively (Table 5). The DA of HEPES and CDTA fractions are 8.7% and 8.3%, respectively. Femenia *et al*. (1998) observed that the DM of apricot changed over the ripening development stages, DM dropped from 87% to 60%. Though the values of DM of HEPES and CDTA fractions of apricot pastes are comparable to the literature values (80-60%) (Femenia *et al*., 1998), the degree of esterification is likely to have been reduced by the heat treatment. Reduction of degree of esterification was also observed in cooked carrot (Ng *et al*., 1997), taro (Quach *et al*., 2000) and winter squash (Ratnayake *et al*., 2003; Ratnayake *et al*., 2004). The de-esterification could be the result of enhanced pectin PME activity or degradation due to chemical reactions and physical forces experienced during processing (Melton *et al*., 1989).

CONCLUSION

The yields from the isolation and fractionation of apple and apricot pastes were lower in the 'final residue' containing cellulose compared to the earlier fractions. This could be due to the heat treatment which weakened the cell walls leading in solubilizing cell wall polysaccharides in early water- (HEPES), CDTA- and Na_2CO_3- soluble fractions. In addition biological differences in developmental stages also play a major role in cell wall compositions. This investigation showed that the apple and apricot cell walls contained relatively high levels of neutral sugars such as arabinose, xylose, galactose and glucose. Rhamnose, mannose and fucose were also found to be present in small amounts. Apple and apricot pastes showed higher level of uronic acid in HEPES fraction than the other fractions as pectic polysaccharides had been converted to a water-soluble forms due to the heat treatment. Concomitantly there was a decrease in the uronic acid content in final residue fraction. The pectic polysaccharides include rhamnogalacturonan I with highly branched side chains of arabinans and somewhat less galactans and arabinogalactans. The 4M KOH-soluble fraction of apricot paste had higher galactan side chains than arabinans. The heterogeneous nature of the pectic polysaccharides was observed in all fractions. The likely

polysaccharides in the cell walls of the apple and apricot pastes are rhamnogalacturonanans, galactans, arabinogalactans, cellulose, xyloglucans, glucomannan and galactomannan.

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