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Modification of cell-wall polymers of onion waste III. Effect of extrusion-cooking on cell-wall material of outer fleshy tissues

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Abstract

The polymers of onion cell walls are known to be modified by heating, but there is little information on the effects of extrusion-cooking. This work investigates the effects of extrusion-cooking on the physico-chemical characteristics and microstructure of cell walls of onion waste in relation to cell-wall chemistry. Cell-wall material from white fleshy outer scale leaves of waste onions was extruded at a range of moisture contents, barrel temperatures and screw speeds through a co-rotating twin-screw extruder. Extrusion-cooking had little effect on the carbohydrate composition of cell-wall material. However, it resulted in an increase in the solubility of pectic polymers and hemicelluloses, and this was accompanied by an increase in swelling of the cell-wall material. The degree of solubility of the pectic polysaccharides was largely dependent on the barrel temperature, and involved depolymerisation. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Extrusion-cooking is a process used widely in the food industry in the manufacture of snacks, crackers and breakfast cereals. It makes use of the combination of temperature and shear to modify the microstructure of the material being processed. Although starches and proteins have primarily been exploited in the extrusion process, beans and cell-wall material have also been treated (Ralet, Della Valle & Thibault, 1993; Edwards, Becker, Mossman, Gray & Whitehand, 1994; Gourgue, Champ, Guillon & Delort-Laval, 1994).

Dietary fibre has received increased attention in recent years. Some researchers have reported that extrusion-cooking causes no significant changes in soluble and insoluble cereal and vegetable fibre (Varo, Laine & Koivistoinen, 1983; Artz, Warren & Villota, 1990), while others have reported a reduction (Fonal, Soral-Smietana & Szpendowski, 1987) or increase in fibre content (Theander & Westerlund, 1987) or a redistribution from insoluble to soluble fibre (Bjorck, Nyman & Asp, 1984; Gourgue, Champ, Guillon & Delort-Laval, 1994). Nyman, Palsson and Asp (1987) considered a number of thermal processes other than extrusion and found a constant or increased dietary fibre on a dry matter basis and also a redistribution from insoluble to soluble fibre in many cases.

Onion waste, comprising mainly brown skin and the outer two fleshy scale leaves, is the major by-product after industrial peeling of onions (450 000 tons produced annually in Europe, mainly from the UK, Holland and Spain). Valorisation of the waste, particularly exploitation for the profitable production of food-grade products, would benefit both onion producers and processors. The waste has been identified as a potential source of dietary fibre (Ng, Smith & Waldron, 1998). The principal objective of this investigation has been to investigate the use of extrusion-cooking over a range of conditions for modifying the physico-chemistry (e.g. solubilisation) of onion cell-wall material, and to relate this to changes in microstructure.

2. Materials and methods

2.1. Materials

Onion waste (*Allium cepa* L.) derived from the white outer fleshy scale leaves was obtained from the British Onion Producers Association (BOPA, Spalding, UK) and stored in an Ice Bank (0°C, 99% RH). Unless otherwise stated, all chemicals were of AnalaR grade.

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2.2. Preparation of cell-wall material (CWM)

Onion waste was macerated in a food processor (Magimix 3000) and passed twice through a pin mill (Model 160 Z, Alpine, West Germany). The homogenate was filtered through a 35 μm nylon mesh and the residue was suspended in deionised water and re-filtered. The procedure was repeated until the cell-wall residue was virtually free of onion flavour. The residue was further washed with industrial ethanol (99.9%) and acetone before being dried in a fluid bed drier (Johnson Matthey, Royston, UK, blower speed 8 for 45 min per 250 g CWM wet weight at 30°C).

2.3. Extrusion-cooking

CWM was passed through a K-Tron feeder (K-Tron Soder, Niederlenz, Switzerland) at a rate of 0.6 kg/h into an APV Baker MPF 19-25 co-rotating and intermeshing twin screw laboratory extruder (APV Baker, Peterborough, UK) with a 3 mm circle die. The barrel bore diameter was 19 mm and the length-to-diameter ratio was 25:1.

The screw configuration comprised: 95 mm, feed screw; 19 mm, 60° forwarding paddle; 57 mm, feed screw; 19 mm, 60° forwarding paddles; 47.5 mm, feed screw; 19 mm, 60° forwarding paddles; 19 mm, feed screw; 19 mm, 60° forwarding paddles; 19 mm, single lead screw; 33.25 mm, 60° forwarding paddles; 19 mm, single lead screw; 33.25 mm, 60° forwarding paddles; 19 mm, single lead screw; 38 mm, 60° forwarding paddles; 19 mm, single lead screw. The extruder included four heating and cooling zones. The die pressure was monitored using a Dynisco (Alton, UK; 0–5000 psi) pressure transducer.

Steady-state extrusion conditions were assumed to have been reached when there were no detectable drifts in product temperature at the die and percentage torque for at least 5 min. Samples were then dried in a forced-air oven (Gallenkamp, Loughborough, UK; Hotbox oven with fan size 1) at 28°C for 2 days, and were then ground in a coffee blender (Janke and Kunkel, GMBH and Co. KG, Germany) to particle size < 1 mm.

Specific mechanical energy (SME) was calculated using the equation:

$$\text{SME (kJ/kg)} = \frac{\text{Torque (\%)}}{100} \times \frac{\text{Screw speed (rpm)}}{\text{Screw speed maximum (rpm)}} \times \frac{\text{Motor power (kJ/s)}}{\text{Throughput (kg/s)}}$$

The three extruder-cooking regimes were: (i) feed moisture adjusted to 40% (sample I), 60% (sample II) and 80% wet weight basis (wb) (sample III) with barrel temperature constant at 100°C and screw speed maintained at 100 rpm; (ii) barrel temperature set at 100 (sample II), 120 (sample IV), 140 (sample V) and 160°C (sample VI) with feed moisture constant at 60% wb and screw speed maintained at 100 rpm; (iii) screw speed set at 100 (sample V) and 250 rpm (sample VII) with feed moisture constant at 60% wb and barrel temperature maintained at 140°C.

2.4. Microscopic analysis

Samples of the CWM and extrudates were fixed in 3% glutaraldehyde (Agar Scientific Ltd., Stansted, UK) in 0.05 M cacodylate buffer (TAAB Lab., Reading, UK; pH 7.4). The samples were dehydrated in an ethanol series with 3 changes in 100% (v/v) ethanol (Fisons, Leicester, UK), and then infiltrated with the acrylic resin LR White (London Resin Co. Ltd., Reading, UK). The samples were transferred to gelatine capsules containing fresh resin, which was polymerised for 24 h at 60°C. Sections, 1-2 μm thick, were cut with glass knives, dried down onto glass slides and stained with 1% toluidine blue (Aldrich, Poole, UK) in 1% (v/v) borax (BDH, Poole, UK; pH 11). The samples were examined under a light microscope.

2.5. Moisture content

The moisture content (wb) of each sample (0.5 g) was determined for CWM and extrudates immediately after extrusion, by an infrared balance (Mettler Instruments Ltd, PM 200, Buckinghamshire, UK) at 135°C for 30 min.

2.6. Expansion and bulk density

The expansion ratio of each sample was calculated by dividing the average cross-sectional area of the fresh extrudate by the cross-sectional area of the extruder die-nozzle orifice. The bulk density was calculated as the mean of 3 measurements by weighing approximately 150 g of extruded product and measuring its length and average diameter using vernier callipers (Kirby, Ollett, Parker & Smith, 1988)

2.7. Water extraction

Dry, milled extrudates (100 mg) were suspended in water (10 ml; pH 5.1) and stirred for 2 h at 20°C. The insoluble residues were recovered by filtration through centrifugation at 12 000 g for 30 min. The water-soluble polymers (WSP) were filtered through GFC (Whatman) filter paper. Aliquots of the residue and WSP were freeze-dried prior to analysis.

2.8. Sugar analysis

Cell-wall neutral sugars and uronic acids were analysed as described previously (Ng & Waldron, 1997a). All analyses were carried out in duplicate, and the standard deviations of the data were less than 2%. Sugars were released from cell-wall material by dispersing it in 72% (w/w) H_2SO_4 (Fisons) for 3 h followed by dilution to 1 M and hydrolysing for 2.5 h at 100°C (Selvendran & O'Neill, 1987). All samples were analysed in duplicate. Neutral sugars were reduced with $NaBH_4$ and acetylated by the

Table 1
Conditions used for extrusion cooking

Sample	Barrel temp. (°C)	Torque (%)	Feed moisture (% wb)	Screw speed (rpm)	Die pressure (kPa)	Melt temp. (°C)	Final moisture (%wb)	Yield (g/min)	SME (kJ/kg)
I	100	65	40	100	318	96	29	10.5	1634
II	100	60	60	100	294	97	45	22.6	700
III	100	28	80	100	193	98	70	40.0	185
IV	120	65	60	100	318	118	38	23.4	733
V	140	40	60	100	196	138	40	22.2	475
VI	160	30	60	100	147	160	45	20.6	384
VII	140	20	60	250	69	138	49	23.0	574

method of Blakeney, Harris, Henry and Stone (1983) using 2-deoxyglucose (Sigma, Poole, UK) as an internal standard. Alditol acetates were quantified by gas chromatography.

Uronic acids were determined colorimetrically by a modification of the method of Blumenkrantz and Asboe-Hansen (1973) in which each sample was dispersed in 72% (w/w) H_2SO_4 for 3 h at room temperature, diluted to 1 M H_2SO_4, and hydrolysed for 1 h at 100°C.

2.9. Gel-filtration chromatography

Molecular weights were determined as described previously (Ng & Waldron, 1997b). Samples were eluted with imidazole buffer (1 M, pH 7, containing 0.02% sodium azide) at a flow rate of 10 ml/h and collected by a LKB Bromma 2111 multirac fraction collector (15 min per fraction; LKB, St. Albans, UK). The collected fractions (2.5 ml) were assayed for total carbohydrate using the phenol/sulphuric acid method of Dubois, Gilles, Hamilton, Rebers and Smith (1956). Samples were dissolved in 1 ml of buffer and dialysed against buffer before being applied to the column. Dextrans (2 mg in 0.5 ml, Sigma) of molecular size approximately 72 000 and 2 000 000 Da, and sucrose (2 mg, Sigma) of size approximately 342.3 Da were used for calibration. The bed volume of the column (V_t) was 280 ml, and the void volume (V_0) was 27 ml.

2.10. Water-solubility index (WSI) and water-absorption index (WAI)

The water-solubility index (WSI) and water-absorption index (WAI) of the extrudates were measured by the method of Anderson, Conway, Pfeifer and Griffin Jr. (1969). A 2.5 g sample was dispersed in 25 g of distilled water, taking special care to break up any lumps using a glass rod. After stirring for 30 min, the dispersions were rinsed into tared centrifuge tubes, made up to 32.5 g and then centrifuged at 3000 g for 10 min. The supernatants were decanted for determination of their solids content and the sediments were weighed to determine the WAI. The indices are defined by

$$\mathrm{WAI} = \frac{\text{Weight of sediment}}{\text{Weight of dry solids}}$$

$$\mathrm{WSI}\ (\%) = \frac{\text{Weight of dissolved solids in supernatant}}{\text{Weight of dry solids}} \times 100$$

The reported WAI and WSI values were the averages of two measurements each.

3. Results and discussion

3.1. Extrusion

The extruder response may be compared with results using other feed materials. Increasing the screw speed from 100 (sample V) to 250 rpm (sample VII) decreased torque, increased specific mechanical energy (SME) and decreased die pressure (Table 1) as also observed in mixtures of corn meal and soy fibre (Hu, Hsieh & Huff, 1993). The decrease in torque is due to the decrease in the fill of screw in a starve-fed extruder. Decreased die pressure as the screw speed increases is normally due to reduced viscosity at the extruder die (Fletcher, Richmond & Smith, 1985). Increasing the screw speed increases both shear rate in the extruder and also the extrudate temperature, both of which will decrease the viscosity and hence the die pressure.

Increasing the barrel temperature from 100 to 160°C (samples II, IV, V and VI) decreased the die pressure as a result of viscosity decrease in the die and barrel with simultaneous reductions in torque and SME (Table 1). Similar arguments apply to increases in moisture content of 40% wb (sample I), 60% wb (sample II) and 80% wb (sample III) which decreased the viscosity in the extruder barrel and die and hence die pressure, torque and SME. These observations are in agreement with studies on maize (e.g. Kirby et al., 1988) and with the findings of Arora, Zhao and Camire (1993) on potato peel.

3.2. Physical properties

The physical characteristics of each extrudate are shown

Table 2
Physical properties of extrudates

Sample	Diameter (mm)	Length (mm)	Bulk density (g/cm^3)	Expansion ratio
I	2.5	25.5	1.2	0.8
II	2.5	25.5	1.2	0.8
III	3.1	28.4	0.7	1.0
IV	1.5	56.6	1.5	0.5
V	2.5	23.5	1.3	0.8
VI	1.7	47.2	1.4	0.6
VII	2.5	17.9	1.7	0.8

in Table 2. Increasing the screw speed (samples V and VII) resulted in extrudates with similar axial expansion, and higher bulk density. Extrusion studies using starch material have normally shown a greater radial expansion and lower bulk density of extrudates with increasing screw speed (Owusu-Ansah, Van de Voort & Stanley, 1984; Fletcher et al., 1985), although Launay and Lisch (1983) observed that radial and axial expansion were inversely related.

Increasing the moisture content (samples I, II and III) increased diameter and induced a slight decrease in the bulk density of the extrudate as also occurs in starchy materials (Fletcher et al., 1985; Alvarez-Martinez, Kondury & Harper, 1988). The axial expansion also increased whereas the converse was observed for maize (Alvarez-Martinez et al., 1988). However, no significant change of these physical characteristics was observed with the increase of temperature (samples II, IV, V and VI), whereas increased axial and radial expansion and lowered bulk density were observed in starchy materials (Fletcher et al., 1985; Alvarez-Martinez et al., 1988).

3.3. Light microscopy

Onion cell-wall material (CWM) contained complete cells and wall fragments of all the principal tissues of onion scales. The major fraction consisted of walls from parenchyma cells and there were lesser amounts of abaxial epidermis with attached cuticle and components of the vascular system. All components of the cell-wall sample stained strongly and evenly with toluidine blue (Fig. 1(a)).

The CWM was extruded using regimes of increasing moisture, barrel temperature and screw speed. It should be noted that the fresh extrudates were immersed in an aqueous fixative as part of the embedding procedure, so that the appearance of the cell-wall material in the micrographs reflects not only extrusion-induced changes but also the subsequent response of the extruded material to an aqueous environment. For example, cell-wall linkages modified during extrusion may then allow the cell-wall material to swell on hydration.

CWM was extruded at 40% wb (sample I), 60% wb (sample II) and 80% wb (sample III) moisture contents at fixed barrel temperature and screw speed. Slight swelling of the parenchyma wall material occurred in these samples, (Fig. 1(b)–(d)) accompanied by a loss of staining from the bulk of the wall so that the middle lamella region became distinct. Little swelling was observed in the epidermal tissue, some of which retained its cuticle.

CWM was extruded at barrel temperatures of 100°C (sample II), 120°C (sample IV), 140°C (sample V) and 160°C (sample VI) at fixed moisture content and screw speed. From the slight swelling noted in Sample II at 100°C (Fig. 1(c)), barrel temperatures of 120°C (Fig. 1(e)) and 140°C (Fig. 1(f)) resulted in considerable swelling of the parenchyma-derived cell-wall material, accompanied by an increase in water-solubility index and a decrease in the water-absorption index of the extrudates (Table 3). Much of the material extruded at 160°C was solubilised during the embedding procedure and these were the only conditions to affect the integrity of the epidermal layer; the structure of the residue was diffuse, poorly-stained and the middle lamella material was concentrated into dark-staining spots (Fig. 1(g)).

There was little difference between the appearance of cell-wall material extruded at a screw speed of 100 rpm (sample V, Fig. 1(f)) and 250 rpm (sample VII, Fig. 1(h)) at fixed moisture content and barrel temperature. Both samples contained a considerable volume of swollen parenchyma-derived cell-wall material as in Sample IV (Fig. 1(e)) interspersed with intact epidermal layers.

3.4. Water-solubility index (WSI) and water-absorption index (WAI)

Increasing the barrel temperatures (samples II, IV, V and VI) increased WSI (%) and decreased WAI (Table 3). With increasing moisture (samples I, II and III) and barrel temperature at 100°C, WSI decreased and WAI increased slightly, as observed for maize (Kirby et al., 1988). The specific mechanical energy (SME) for samples of different feed moistures (samples I, II and III) showed a close correlation with WSI (%), but not with samples of different barrel temperatures (samples II, IV, V and VI). A general relationship between WSI and SME for maize extrudates produced with different barrel temperatures, moistures and screw configurations has been shown earlier (Kirby et al., 1988).

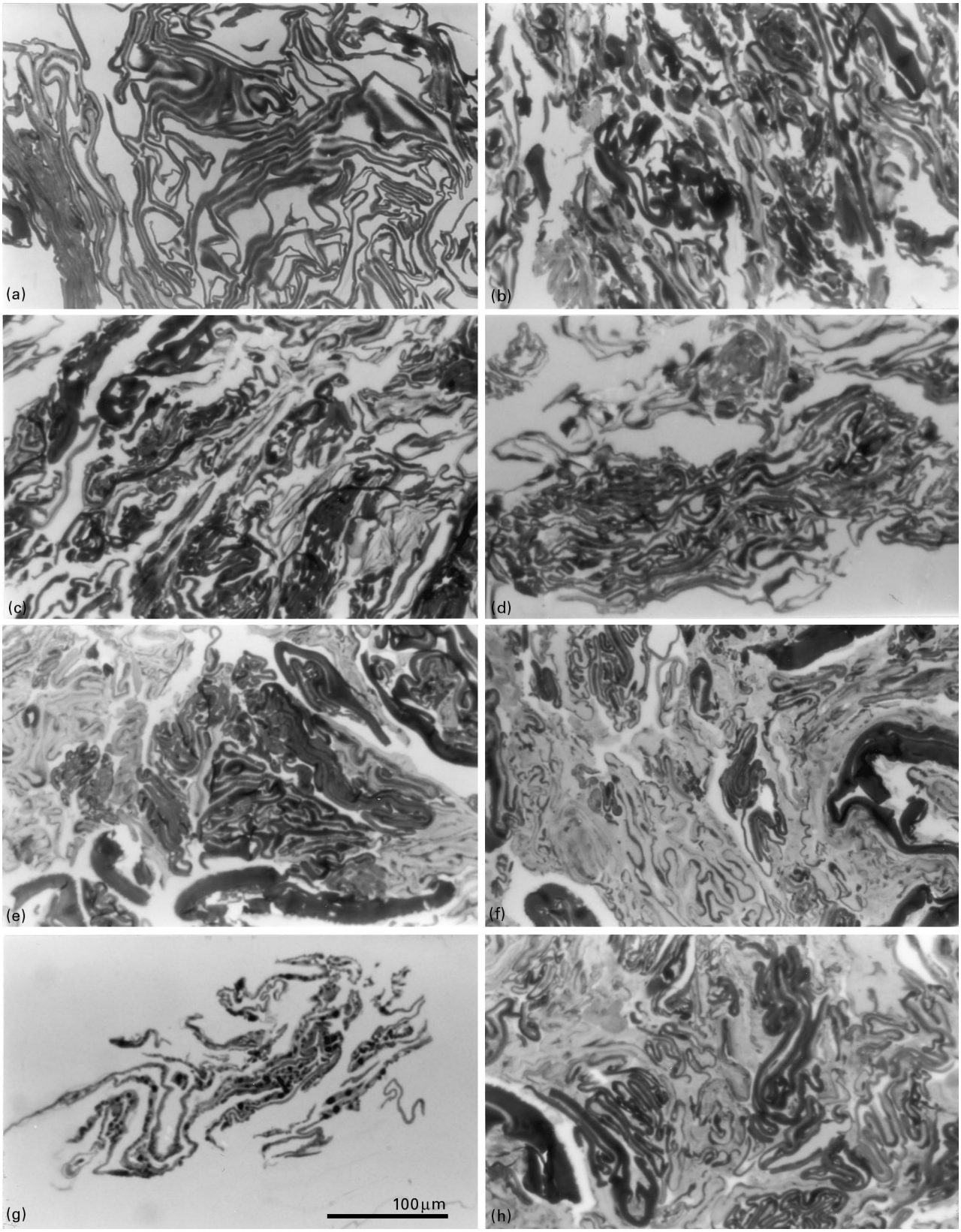

Fig. 1. Sections of onion cell-wall material: (a) before extrusion; (b) sample I, 40% moisture (wb); (c) sample II, 60% moisture (wb); (d) sample III, 80% moisture (wb); (e) sample IV, barrel temperature 120°C; (f) sample V, barrel temperature 140°C; (g) sample VI, barrel temperature 160°C; (h) sample VII, screw speed 250 rpm. Extrusion regimes: increase in moisture content (sample I → II → III), increase in barrel temperature (sample II → IV → V → VI), increase in screw speed (sample V → VII).

Table 3
Water-solubility index (WSI) and water-absorption index (WAI) of onion waste

Sample	WSI (%)	WAI
Control	0.0	12.8
I	0.8	11.9
II	0.5	12.3
III	0.1	12.7
IV	3.3	9.5
V	4.0	8.7
VI	4.4	8.4
VII	4.1	8.7

3.5. Carbohydrate composition of CWM

The chemical changes that occurred in onion CWM following extrusion-cooking were assessed by comparing their carbohydrate compositions.

The methods for CWM preparation were designed to produce large quantities quickly and economically whilst also reducing the onion flavour. The CWMs were analysed for their carbohydrate composition after hydrolysis in 72% (w/w) sulphuric acid (Selvendran & O'Neil, 1987); the absence of starch in the CWM was confirmed by the absence of staining with iodine in potassium iodide.

The yield of CWM from fresh onion waste was 3.5% on a fresh weight basis. The carbohydrate composition of CWM, consisting of rhamnose, fucose, arabinose, xylose, galactose, mannose, glucose and uronic acid, was comparable to that reported by Ng et al. (1998). Generally, there was no significant change in the carbohydrate composition (on a mol % basis) of control and extrudates for different extrusion conditions (Table 4).

3.6. Water extraction of extruded CWM

The dried extrudates were extracted with water (20°C). The extracts were rich in pectic polysaccharides as indicated by the high levels of uronic (galacturonic) acid and galactose, and lower levels of arabinose. Small quantities of xylose are likely to have originated from xyloglucans (Redgwell & Selvendran, 1986). An increase in barrel temperature (sample II, IV, V and VI) resulted in an increase in the water-soluble polymers (WSP) and WSI (Table 3) complementing an earlier study on the effect of pressure-cooking on onions (Lecain, Ng, Parker, Smith & Waldron, 1998). This indicates that extrusion-cooking-induced changes may be due to β-elimination (Sajjaanantakul, Van-Buren & Downing, 1989; Greve, Shackel, Ahmadi, McArdle, Gohlke & Labavitch, 1994).

The effect of extrusion on the levels of total pectic

Table 4
Carbohydrate composition of water-soluble and water-insoluble polymers from control and extruded onion CWM. t: trace

		Recovery (% CWM)	Carbohydrate (mol%)								Total μg/mg	Ratio UA:NS
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA		
Cell-wall material												
	Control	100	1	1	3	4	1	13	32	44	829	3
	I	100	1	1	2	4	1	12	27	51	817	4
	II	100	1	1	3	4	1	14	29	47	832	3
	III	100	1	1	3	4	1	14	30	46	856	3
	IV	100	1	1	3	4	1	12	28	51	821	3
	V	100	1	1	3	4	1	12	28	49	818	3
	VI	100	1	1	3	4	1	13	33	43	869	3
	VII	100	1	1	3	4	1	12	31	45	843	3
Water-soluble polymers												
	Control	2	t	1	3	2	1	40	4	49	598	1
	I	3	2	3	8	9	1	35	11	31	549	1
	II	2	1	3	8	9	2	34	10	34	479	1
	III	2	1	2	6	6	1	29	8	45	506	1
	IV	3	2	2	6	6	1	36	8	38	719	1
	V	6	3	2	7	3	1	31	4	49	775	1
	VI	18	2	2	6	2	t	34	2	51	917	1
	VII	6	2	2	6	3	1	29	6	51	837	1
Water-insoluble polymers												
	Control	98	1	1	3	4	1	12	33	44	832	3
	I	97	1	1	3	4	1	12	28	50	831	3
	II	98	1	1	3	4	1	13	30	47	852	3
	III	98	1	1	3	4	1	13	28	49	834	3
	IV	97	1	1	3	4	1	12	30	48	864	3
	V	94	1	1	2	4	1	11	32	48	842	3
	VI	82	1	1	2	5	1	7	39	44	838	3
	VII	94	1	1	3	4	1	11	35	44	849	3

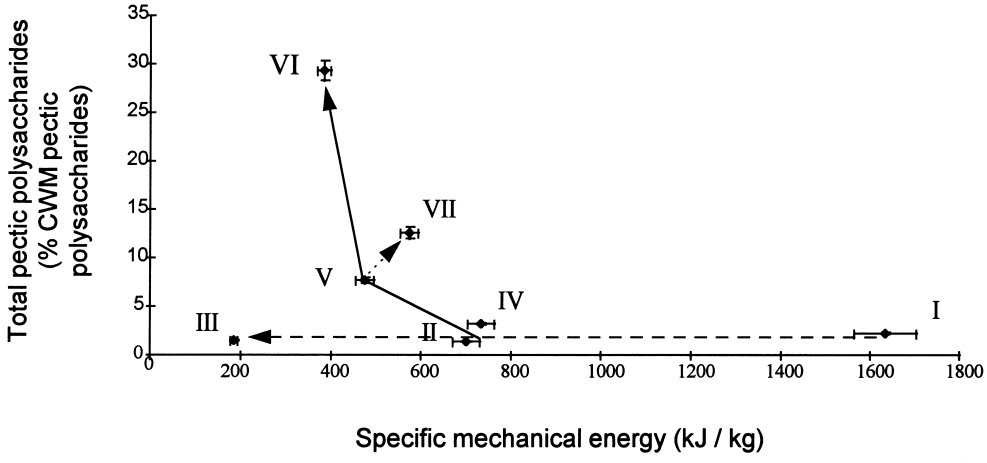

Fig. 2. Changes in total pectic polysaccharides (μg/mg CWM total pectic polysaccharides) of water-soluble polymers in relation to specific mechanical energy (SME). Symbols: — increase in barrel temperature; ···► increase in screw speed; – – –► increase in moisture content.

polysaccharides of the WSP extracts (summation of uronic acid, galactose and arabinose) as a function of the total pectic polysaccharides in the CWM is shown in Fig. 2. where the extrusion data are shown at their respective specific mechanical energy values. Increasing barrel temperature (samples II and IV to VI) resulted in an increase in solubility of pectic polysaccharides from 1.5 to 29.2% (Fig. 2). In contrast, changes in water content of the feed onion material (samples I, II and III) and the screw speed (samples V and VII) had relatively little effect on pectic solubility (Fig. 2). In all water-soluble polysaccharides, the uronic acid: pectic neutral sugar (arabinose + galactose) (UA:NS) ratio was lower than that of the parent CWM. Interestingly, extrusion-cooking seems to degrade relatively more arabinose side-chains than galactose side-chains, unlike pressure-cooking which had a significantly more degradative effect on the galactose side-chains (Lecain et al., 1998). However, the degree of degradation of cell-wall polymers of onions during pressure-cooking has been shown to be affected by the variety used; cell walls from the variety Delta were much more strongly degraded than those from the variety Hysam. In addition, the increase in xylose and glucose may reflect the extrusion-related increase in the solubilisation of hemicellulose (Table 4). The extrusion-induced increase in soluble pectic polymers may have a consequent effect on the functional and nutritional values (Gourgue et al., 1994).

3.7. Molecular weight profiles

Water-soluble polysaccharides (WSP) of CWM and extrudates were investigated for molecular weight (MW) profiles by chromatography on Sepharose CL-4B. The MW profile of CWM-derived WSP contained one peak with a maximum of approximately 40 000 Da (Fig. 3). WSPs of extrudates resolved one broad peak with a maximum at MW 40 000 Da which revealed an increase in the total water-soluble carbohydrate. This bears similarity to the changes which occur in WSP following pressure-cooking (Lecain et al., 1998) where a slight decrease in the peak molecular weight of water-soluble polysaccharides was also observed. It is likely that extrusion-cooking treatments of high energy input lead to some depolymerisation. Studies of starch extrusion have associated a decreased expansion of extrudate with increased molecular degradation (Kirby et al., 1988; Tang & Ding, 1994) which corresponds to increasing SME dissipation (Ollett, Parker, Smith, Miles & Morris, 1990).

4. Conclusion

The above work has demonstrated that:

1. Extrusion-cooking offers a way of modifying onion cell-walls which contain a significant amount of dietary fibre especially rich in pectic polymers, with little starch. This could be a useful component of fibre-enriched foodstuffs.
2. In common with the extrusion of other materials, increasing the barrel temperature (from 100 to 160°C) resulted in a decrease in die pressure and is associated with a reduction in specific mechanical energy.
3. Extrusion-cooking at barrel temperatures of 100, 120 and 140°C resulted in a small, but significant, increase in the soluble fraction of the extruded material. A barrel temperature of 160°C caused a considerable increase in the soluble components which were rich in soluble pectic polymers and some hemicelluloses. This is probably due to an increase in β-eliminative degradation and consequent depolymerisation.
4. Increasing the barrel temperatures from 100 to 140°C resulted in swelling of the cell walls. At a barrel temperature of 160°C, swelling of cell walls was not observed, probably due to the solubilisation of pectic polymers during fixation, and loss from the cell-wall material.

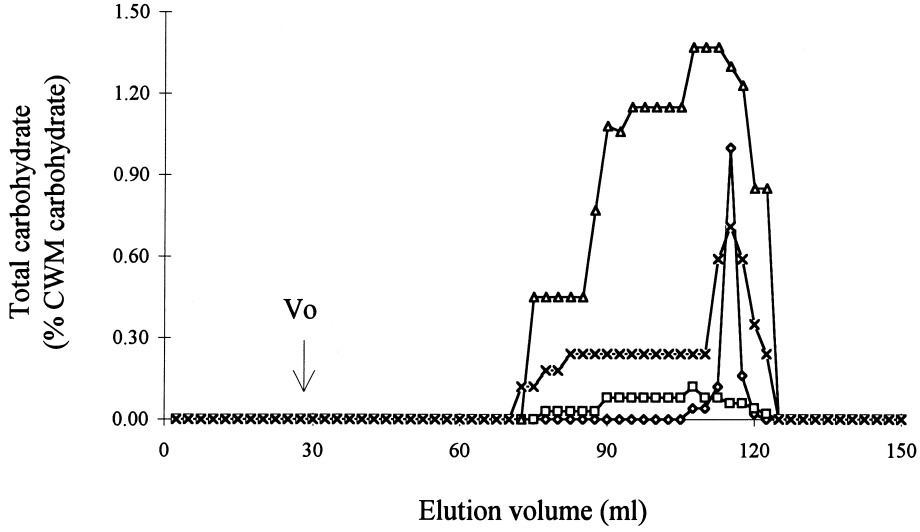

Fig. 3. Molecular weight profiles of water-soluble polysaccharides of CWM and extruded material. Symbols: –◇– control CWM; –□– Sample III: extruded, 80% wb moisture content; –△– Sample VI: 160°C temperature; – × – Sample VII: 250 rpm screw speed.

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