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SHORT COMMUNICATION

Valorisation of Potato (*Solanum tuberosum*) Peel Waste: Extraction of Fibre, Monosaccharides and Uronic Acids

Regiane Scharf[1,4] · Rongchun Wang[1,2] · Joanne Maycock[1] · Peter Ho[1] · Shiguo Chen[3] · Caroline Orfila[1]

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Abstract

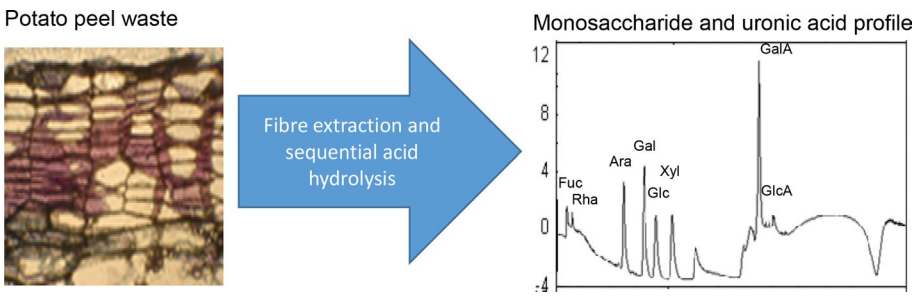
Purpose The food and starch industries generate large quantities of potato peel waste (PPW) that can be exploited for a range of biotechnological and biofuel applications. The purpose of this study was to characterise the ultrastructure and monosaccharide composition of PPW.

Methods The ultrastructure of PPW was observed using light and immunofluorescence microscopy. Fibre was prepared from PPW using mild detergent and subjected to sequential acid hydrolysis followed by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

Results Potato peels are composed of small, stacked cells which generally lack starch granules. These cells are surrounded by thick cell walls rich in pectic polysaccharides. Following sequential extraction, seven different sugars and two uronic acids were identified including (in order of abundance) mannose, galacturonic acid, xylose, glucose, fucose, glucuronic acid, galactose, rhamnose and arabinose. The monosaccharides and uronic acid products showed good stability in the acidic conditions during storage, which would facilitate their downstream purification and eventual commercialisation.

Conclusion PPW is a readily available source of fibre, monosaccharides and uronic acids.

Graphical Abstract



Keywords Potato peel waste · *Solanum tuberosum* · Fibre · Cell wall monosaccharide · Uronic acid · Sequential acid hydrolysis

Statement of Novelty

The monosaccharide and uronic acid composition of potato peel waste, an abundant by-product of the agri-food industry, has been characterised for the first time.

✉ Caroline Orfila
c.orfila@leeds.ac.uk

Extended author information available on the last page of the article

Introduction

Worldwide potato tuber production has been increasing steadily due to rising demand for human nutrition and bio-processing in China, Asia and Africa. It is now the fourth most produced edible crop after rice, maize and wheat, with an estimated annual yield of 370 thousand tonnes in 2013, of which between 20–30% by weight can be discarded during post-harvest processing. The potato processing industry

generates large quantities of unavoidable potato peel waste (PPW) which can pose an environmental burden on both land and water resources due to rapid microbial degradation. Several technologies have been applied to recycle and valorise PPW [1–3]. These include ultrasound, high-pressure processing, sub-critical water extraction, acidic and enzymatic hydrolysis. These have been applied to obtain valuable components from PPW including biopolymers, polyphenols, glycoalkaloids and reducing sugars. The sugars are often used as feedstock for microbial fermentation in bioethanol production [4, 5]. However, the cell wall monosaccharide composition of the extracts has not been previously investigated. It is likely that PPW is a source of a variety of monosaccharides and uronic acids which are themselves valuable products for commercialisation or further processing.

Therefore, the aims of this study were to (1) characterise the structure of PPW and (2) prepare a fibre extract from PPW and to sequentially extract sugars and uronic acids. The monosaccharides and uronic acids were characterised by high performance anion exchange chromatography with pulsed aerometric detection (HPAEC-PAD) which can identify and quantify all cell wall sugars in one chromatographic run. The storage stability the hydrolysis products was evaluated and the potential of PPW as a source of valuable fibre and sugars is discussed.

Materials and Methods

Sampling of PPW

Potato plants (*Solanum tuberosum*) from different cultivars Maris Piper, Russet Burbank and Lady Rosetta were used. Tubers were harvested and prepared the next day. The potatoes were peeled using a domestic OXO Good Grips Swivel Peeler; the thickness of the slices was measured using a Mercer England Thickness gage. The peels referred to here as PPW were immediately frozen and lyophilised.

Microscopy of Peel Samples

Fresh peel specimens (0.5 cm^3) were fixed in 4% formaldehyde in PEM buffer (50 mM Pipes, 5 mM $MgSO_4$ and 10 mM EGTA, pH 6.9). Fixative was removed with PEM and the samples were washed with phosphate buffered saline (PBS), dehydrated in an ethanol series (30–70%) and embedded in Steadman wax (9:1 polyethylene glycol 400 distearate and 1-hexadecanol). Wax embedded samples were sectioned using a microtome with metal blade at 11° and 50 µm thickness. Sections were placed onto polysine-coated glass slides, followed by dewaxing with ethanol series (97–50%). Sections were then stained with 0.1% Toluidine Blue for 10 min, washed for 10 min in PBS, mounted with anti-fading glycerol phosphate buffered solution (Citifluor AF1, Agar Scientific, UK) and covered with a glass cover slip. Observations were made with a BH2 Olympus microscope. For immunofluorescence labelling of pectin, sections were incubated with 150 µL of 3% (w/v) milk protein in PBS for 1 h to reduce nonspecific binding. Monoclonal antibodies JIM5 and JIM7 which recognize different patterns of methyl esterification on homogalacturonan were kindly provided by Professor Paul Knox (Centre for Plant Sciences, University of Leeds, UK). The sections were incubated overnight at 4 °C in primary antibody (either JIM5 or JIM7), diluted 1:5 in PBS with milk. Control sections were incubated in PBS alone. Samples were washed twice with 0.1% v/v Tween 20 in PBS for 10 min. All sections were incubated at room temperature for 1 h in secondary antibody (anti-rat goat IgG conjugated to FITC, Sigma), diluted 1:100 in PBS with milk. Samples were washed 10 min with 0.1% v/v Tween 20 in PBS plus 10 min in PBS. Samples were then stained with 0.1% Toluidine Blue for 10 min to reduce autofluorescence interference, washed for 10 min in PBS, mounted with antifading glycerol phosphate buffered solution (Citifluor AF1, Agar Scientific, UK) and covered with a glass cover slip. Observations were made with a Confocal Zeiss Axioplan Imaging LSM 510 Meta.

Preparation of Fibre from PPW

Lyophilised PPW (10 g) was homogenised using a homogeniser (Ultra Turrax, IKA, Staufen, Germany) at 22,700 g with 50 mL of cold mixed-cation buffer (MCB) (10 mM NaOAC, 3 mM KCl, 2 mM $MgCl_2$ and 1 mM $CaCl_2$, pH 6.5) containing Triton X-100 (2 mg/ml) for 5 min at 4 °C. The detergent suspension was removed by washing through a metal sieve (45 µm) with 100 mL of chilled MCB without Triton X-100. This step removed intact starch granules that could recovered by sedimentation. The pellet was washed with 2 L of water and finally with 10 mL of 50% acetone to remove moisture. After washing, the fibre material was dried overnight in an oven at 35 °C.

Sequential Hydrolysis of PPW Fibre

Fibre hydrolysis was performed in duplicate in three sequential steps: firstly, 2 mg of PPW fibre were hydrolysed with 1 mL of 0.1M trifluoroacetic acid (TFA) for 1 h at 100 °C. Samples were centrifuged at 2800 g at 4 °C for 10 min and the supernatant was collected for analysis. Secondly, the solid residue was hydrolysed with 2 M TFA for 1 h. Tubes were centrifuged at 2800 g at 4 °C for 10 min and the supernatant was collected for analysis. Finally, the residue was hydrolysed with 1 M H_2SO_4 for 1 h. TFA was removed using a centrifugal evaporator (Genevak, Surrey, UK). Dried samples were resuspended with 1 mL of milli-Q purified water

and filtered using a 0.45 µm nylon filter prior to monosaccharide and uronic analysis. H_2SO_4 was neutralised using NaOH, and the sample diluted with milli-Q purified water prior to chromatographic analysis. Samples were kept chilled at all times and not exposed to bright light.

Analysis of Monosaccharide Composition Using High Performance Anion Exchange Chromatography Amperometric Detection (HPAEC-PAD)

The monosaccharide composition was determined by HPAEC-PAD using a method adapted from [6]. The column used was PA20 (Dionex, Thermo Scientific). Column temperature was 30 °C, flow rate of 0.30 mL/min and injection volume was 10 µL. The 65 min elution program consisted of linear gradient from 10 µM to 5 µM NaOH from 0 to 1.5 min, followed by isocratic elution with 5 µM NaOH from 1.5 to 30 min, linear gradient up to 1 M NaOH from 30 to 40 min, column washing with 1M from 40 to 45 min, linear gradient to 10 mM from 45 to 55 min following equilibration of the column with 10 mM NaOH from 55 to 65 min. Monosaccharides were detected using a pulsed amperometric detector with gold working electrode and silver reference electrode. Monosaccharide standards were L-fucose, L-rhamnose, L-arabinose, D-galactose, D-glucose, D-xylose, D-mannose, D-galacturonic acid and D-glucuronic acid. D-Fructose was used as an internal standard. A standard mixture run was performed before sample analysis to determine response factor. The external standard method of calibration was used, with each curve prepared from seven different concentrations of standard solutions. The range of standards concentration used was from 0.30 to 100 µg/mL. The external standard (fructose) concentration used was 200 µg/mL.

Method validation for analysis of sugars was performed according to ICH recommendations and the European Commission Directive for the performance of analytical methods including linearity, precision and accuracy, which are principal components of quantification. Linearity was investigated by analysis of peak area response versus concentration over a range of 11 ng/mL to 150 µg/mL. For calibration curves, the peak areas of the Dionex chromatogram were plotted against on-column amount and analysis was performed on two separate occasions with triplicate injections of each concentration. Precision and accuracy were evaluated for galactose at three quality control (QC) concentration (25, 50 and 100 µg/mL). The lower QC is representative of the lower concentration of hydrolysed sugars from CW samples, and the high QC level is near the upper boundary of the standard curve. Intra-day precision and accuracy was calculated from triplicate injection of the 3 concentrations on the same day. Inter-day precision and accuracy was determined by analysis of triplicate injections of the 3 concentrations on the 3 separate days. Values for precision are expressed as relative standard deviation (R.S.D) and relative error (R.E.) for accuracy.

To assess storage stability, hydrolysates and standards were stored for 3 days at 4 °C and re-analysed.

Results and Discussion

Potato Peel Structure Investigated Using Microscopy

Thin sections (50 µm) were stained with toluidine blue to visualise the cellular organisation of potato skin, the main constituent of PPW (Fig. 1 a–c). The micrographs show that for all varieties of potato, the skin consists of stacks of between 8 and 15 oblong cells measuring 40 µm in length on the axis parallel to skin surface, by around 10 µm in width on the axis perpendicular to skin surface. A clear layer of suberin is observed on the most external surface. There does not appear to be any starch granules within the skin cells, however are abundant in larger and rounder periderm cells underneath the skin (indicated with single arrow head on Fig. 1a). This is likely to be the main source of starch in PPW. Labelling of the same sections with the anti-pectin antibodies JIM5 and JIM7 showed abundance of pectin. Both methyl esterified (Fig. 1f) and non-methyl esterified (Fig. 1e) homogalacturonan were localised to cell walls of all cells in the skin and periderm. Mild autofluorescence (Fig. 1d) suggested the presence of cell wall phenolics. These micrographs suggest that potato peels are a rich source of cell wall material and pectin in particular.

Preparation of Fibre from PPW

The tightly packed cells of peel tissue make extraction of fibre difficult. To break open the cells and remove the lipids and proteins from cell membranes, as well as the suberin layer, PPW was homogenised in a cold buffer containing triton X-100, a non-ionic surfactant. This step allowed recovery of some intact starch granules. Even through starch is not visible in skin cells, it is our experience that periderm cells are often present in PPW. The yield of fibre was around 20–24% (dry weight) of PPW.

Chromatographic Separation of Cell Wall Monosaccharides and Uronic Acids

We used HPAEC-PAD to separate eight sugars and two uronic acids in one chromatographic run (Fig. 2a; Table 1). Peak area varied linearly with on-column amount over the ranges used ($R > 0.98$). Intra-day and inter-day precision was calculated for galactose as R.S.D. $< 4.6\%$, $< 1.7\%$ and $< 6.4\%$ for 25 µg/µL, 50 µg/mL and 100 µg/mL concentrations

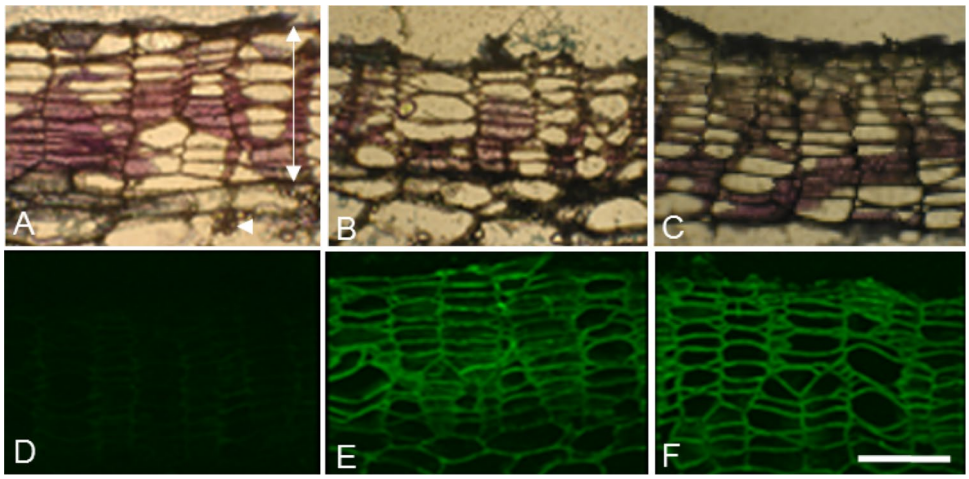


Fig. 1 Light and fluorescence microscopy of wax-embedded sections of potato tuber peel. Top row show toluidine blue stained sections of varieties Lady Rosetta (**a**), Maris Piper (**b**) and Russet Burbank (**c**). Double arrow shows skin tissue composed of stacked cells. Single arrow head shows presence of starch granules in periderm tissue underneath skin. Bottom row shows immunofluorescence of skin (Maris Piper only) with no antibody (**d**, negative control), with JIM5 (**e**) and JIM7 (**f**) antibodies showing abundant and even presence of both methyl-esterified and non-esterified pectin. Scale bar = 50 um

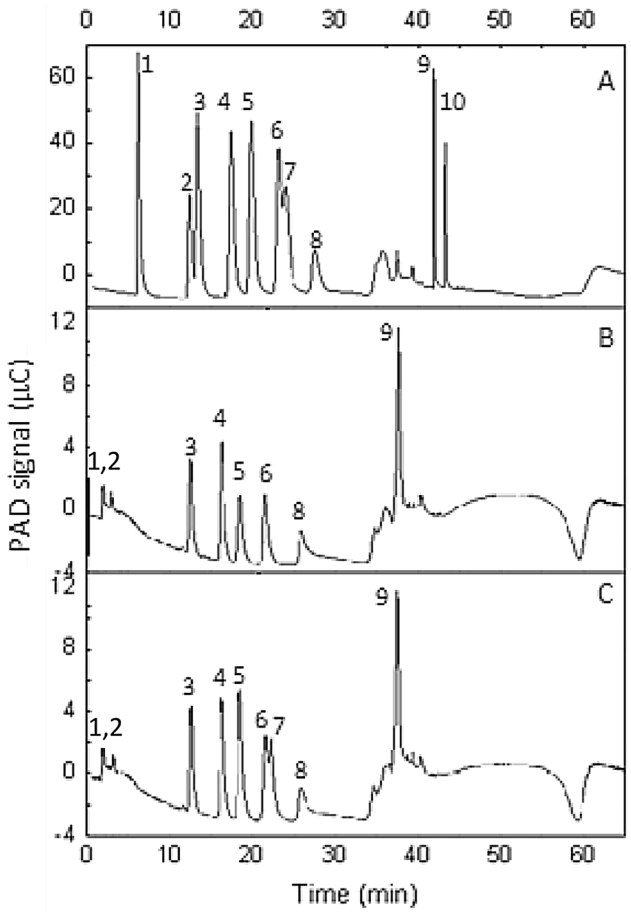


Fig. 2 HPAEC-PAD separation of **a** standards at 25 µg/mL **b** fibre hydrolysate and **c** fibre hydrolysate spiked with mannose. mL (1) fucose, (2) rhamnose, (3) arabinose, (4) galactose, (5) glucose, (6) xylose, (7) mannose, (8) fructose – used as internal standard (9) galacturonic acid and (10) glucuronic acid

Table 1 Chromatographic performance of eight monosaccharides and two uronic acids separated within the same chromatographic run using HPAEC-PAD

Peak	Compound	Rt (min) Mean ± SD[a]	RSD (%)[b]	LOQ (ng/mL)	R^2
1	Fucose	6.15 ± 0.18	2.88	47	0.9999
2	Rhamnose	12.09 ± 0.46	3.79	47	0.9996
3	Arabinose	13.10 ± 0.45	3.46	47	0.9999
4	Galactose	16.81 ± 0.63	3.77	47	0.9997
5	Glucose	19.09 ± 0.72	3.79	93	0.9993
6	Xylose	22.26 ± 0.81	3.65	93	0.9998
7	Mannose	23.08 ± 0.83	3.62	47	0.9999
8	Fructose	26.62 ± 1.06	4.00	47	0.9999
9	Galacturonic acid	41.80 ± 0.05	0.13	93	0.9823
10	Glucuronic acid	43.10 ± 0.03	0.69	93	0.9999

n = 2, 3 repetitions each

Rt retention time, *SD* standard deviation, *RSD* relative standard deviation, *LOQ* limit of quantification

respectively. Good intra-day and inter-day accuracy was demonstrated across the concentration range with relative error < − 7%. The precision and accuracy meet performance criteria for analytical methods, with precision (R.S.D.) and accuracy (R.E) within 15% (Table 1). All sugars and uronic acids were stable upon storage in acidic conditions for 3 days at 4 °C, stability ranged between 95–100% (data not shown).. Injection of hydrolysates shifted peaks to earlier retention times compared to standards in water (Fig. 2b), but all compounds were confirmed by spiking. Separation of xylose and

Table 2 Yield percentage of monosaccharide and uronic acids released from PPW fibre after sequential hydrolysis

	Yield percentage[a]			
	0.1 M TFA	2 M TFA	1 M H_2SO_4	Total extractable
Extract yield	15	27	28	70
Monos				
Fuc	0.65	1.72	0.90	3.27
Rha	1.49	0.98	0.00	2.47
Ara	2.12	0.14	0.08	2.34
Gal	1.49	0.95	0.12	2.56
Glu	0.52	1.24	1.80	3.56
Xyl	0.32	1.98	1.37	3.67
Man	0.00	0.00	7.84	7.84
GalA	1.15	0.55	2.31	4.01
GlcA	1.78	0.14	1.25	3.18
Total monos	9.50	7.72	15.68	32.89

mannose was problematic but spiking of samples confirmed their separation even in fibre hydrolysates (Fig. 2c).

Monosaccharide and Uronic Acid Composition of PPW Fibre Hydrolysates

Sequential acid hydrolysis using increasingly stronger acid hydrolysed the fibre and extracted mixtures of sugars and uronic acids with different compositions. Table 2 shows the yield of the extracts and sugars extracted at each step. The analysis indicated that PPW contains polymers made of a variety of monosaccharides, including neutral sugars and uronic acids. A large proportion of arabinose, galactose, rhamnose and uronic acids was extracted with the mild TFA conditions (0.1 M), indicating presence of branched rhamnogalacturonan I (RG I) that can be easily extracted and is acid labile. Meanwhile stronger acidic conditions extracted glucose, xylose, galactose and fucose, indicative of xyloglucans, as well as uronic acid and rhamnose, but little arabinose, indicating presence of galactan rich rhamnogalacturonan I. Finally, the strongest acid tested in this study (1 M H_2SO_4) extracted mannose, glucose, xylose and uronic acids, indicating presence of xyloglucans, glucomannans and pectin. The composition of the residue was not determined. This composition analysis shows that PPW contains mixture of biopolymers including pectins and hemicelluloses that can be easily hydrolysed using relatively mild acid conditions. The high arabinose content suggests that potato RGI may be higher in arabinan than previously reported [6].

Conclusion

This study demonstrated the potential of potato peel waste as a useful source of fibre, a variety of monosaccharide and uronic acid which could be further purified and commercialised.

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Affiliations

Regiane Scharf[1,4] · Rongchun Wang[1,2] · Joanne Maycock[1] · Peter Ho[1] · Shiguo Chen[3] · Caroline Orfila[1]

[1] School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK

[2] School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin 150001, China

[3] Zhejiang Key Laboratory for Agro-Food Processing, Department of Food Science and Nutrition, Fuli Institute of Food Science, Zhejiang University, Hangzhou 310058, China

[4] Present Address: Nestlé Product Technology Centre (PTC), York YO31 8FY, UK