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Improved starch recovery from potatoes by enzymes and reduced water holding of the residual fibres

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ABSTRACT

During the industrial extraction of starch from potatoes (*Seresta*), some starch remains within undisrupted potato cells in the fibrous side-stream. The aim of this study was to investigate if enzymatic degradation of cell wall polysaccharides (CWPs) can enhance starch recovery and lower the water holding capacity (WHC) of the "fibre" fraction. The use of a pectinase-rich preparation recovered 58% of the starch present in the "fibre" fraction. Also, the "fibre" fraction retained only 40% of the water present in the non-enzyme treated "fibre". This was caused by the degradation of pectins, in particular arabinogalactan side chains calculated as the sum of galactosyl and arabinosyl residues.

1. Introduction

After the industrial extraction of starch from potatoes, some starch still remains in the fibre-rich by-product. This starch is trapped within more or less intact potato cells (Ramaswamy, Kabel, Schols, & Gruppen, 2013). Albeit representing a low proportion of the starch present in potato tuber, a major challenge is to extract the residual starch. Another challenge is to decrease the high water holding capacity (WHC) of the fibre fraction. This fibre fraction (dry basis), represents 6.4% (w/w) of the total dry matter of potatoes (Grommers & Krogt, 2009, chap 11). The high water holding capacity (WHC) of this fraction negatively affects its processing as an animal feed and increases transport costs.

In the fibre fraction, cell wall polysaccharides (CWPs) constitute 48% (w/w) of the dry matter. They comprise pectins, rich in galactan side chains, cellulose and hemicelluloses, the latter is mainly composed of xyloglucan. Their WHC is affected by interactions between these CWPs, in which water soluble arabinogalactans and xyloglucans are implicated to be important (Ramaswamy et al., 2013, Larsen et al., 2011). Also, it was indicated that long side chains of arabinan and galactan impart a more hydrophillic character to the rhamnogalacturonan RG-I backbone compared to short chains (Larsen et al., 2011). One way to reduce the WHC is by using enzymes that can degrade CWPs. Reduced WHC of pre-treated rape seed meal has been observed upon addition of pectinolytic enzymes (Pustjens et al., 2012). Also, a reduced WHC was found for xylanase-treated wheat cell wall material. However, in that study, it was shown that the WHC of the insoluble residue increased by a factor of 2.5 (Gruppen, Kormelink, & Voragen, 1993). Therefore, it seems that although enzymes can be used to modify CWPs, the type of CWPs degraded determines the extent of WHC of the residual material.

An additional advantage of using enzymes in the starch extraction process is that by degrading fibres these enzymes can loosen the cell wall network in undisrupted cell walls before separating the fibre fraction from the starch fraction. Lab-scale treatment of potatoes with a cellulase improved starch release by 18–28% by hydrolyzing cellulose (Sit, Agrawal, & Deka, 2011). However, at production scale, the actual blending of potatoes and the starch extraction process is different than at lab scale (Grommers and van der Krogt, 2009, chap 11; Wischmann et al., 2007). This is illustrated by main differences observed in the particle size distributions of starch granules between lab scale and industrial scale derived starch (Wischmann et al., 2007). Consequently, only by using a defined *semi-technical* scale set-up, the effects of enzyme addition in the starch recoveries and WHCs are expected to be predictable for industrial scales. Therefore, in this study, potatoes were blended and starch was separated at such a *semi-technical* scale set-up.

Abbreviations: CWPs, cell wall polysaccharides; WHC, water holding capacity.

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Our objective was to test the hypothesis that cell wall degrading enzymes help to release more starch into the starch fraction and lower the WHC of the corresponding fibre fraction at a *semi-technical* scale set-up. The effect of two commercial pectinase-rich enzymes were studied at different concentrations.

2. Materials and methods

2.1. 2.1 Materials

Potatoes of the *Seresta* variety were provided by AVEBE (Veendam, The Netherlands) as soon as they were harvested in August 2012. The potatoes were stored in a dark, dry and ambient (10–15 °C) environment and were used within 7 weeks of harvesting. Enzymes used were Pectinex Ultra-SP (USP), provided by Novozymes (Bagsvaerd, Denmark), and a glucanase rich enzyme preparation (Ebs), provided by Erbsloh (Geisenheim, Germany). The enzyme activities and doses applied are described in Table 1. PNP-β-galactopyranoside, PNP-β-glucopyranoside and PNP-α-arabinofuranoside were obtained from Sigma-Aldrich (USA). PNP-α-xylopyranoside was obtained from Koch-Light Laboratories (Buckinghamshire, UK). To assess the degradation of CWPs by USP and Ebs, defined substrates such as linear arabinan (British Sugar, Peterborough, UK), galactan (Megazyme, Wicklow, Ireland), polygalacturonaic acid (PGA, Fluka Biochemika, Buchs, Switzerland) and tamarind xyloglucan (Dainippon Sumitomo Pharma, Japan) were used. Potato starch was obtained from AVEBE.

2.2. Semi-technical scale separation of "fibre" fraction and "starch" fraction from potatoes

2.2.1. Semi-technical scale set-up

In Fig. 1, a scheme of the *semi-technical* scale set-up is shown. This set-up is known to mimic the commercial starch extraction process well (AVEBE). In all trials, 4 kg of potatoes (fresh weight) were washed extensively until traces of soil were also removed. The potatoes were mixed with $NaHSO_3$ (0.07 w/w % of dry weight of potatoes) and rasped to obtain potato pulp. Rasping was performed with a Nivoba Ultra Rasp RU 40-260 (Nivoba Engineering, Veendam, The Netherlands). Some potato pulp remained in the rasp and was washed out with water. This wash water was combined with potato pulp. Additionally, water was added to the pulp till a total volume of 10 kg was reached. The pulp was weighed and sieved via a centrifugal sieve GL200 (G. Larssons, Bromolla, Sweden) of pore size 125 μm. After sieving once, the pulp was separated into a fibre-rich fraction and a starch-rich fraction. The fibre-rich fraction was again sieved three times through the same sieve and each time a starch-rich fraction was obtained. Therefore, in the end, a total of four starch-rich fractions were obtained and denoted as "starch 1", "starch 2", "starch 3" and "starch 4". One fibre-rich fraction was obtained, which was denoted as "fibre" (Fig. 1). "Starch1-4" and "fibre" fractions were weighed. For enzyme treatments, the pulp was incubated with enzymes at 40 °C for 2 h before sieving. For all trials, the absolute amount of dry matter present in the pulp was 0.9–1.0 kg. All incubations were performed in stirred tank kettles of a capacity of 12 L (model 88.460, Terlet, Zutphen, The Netherlands) and were stirred at a speed of 24 rotations per minute. The trial in which pulp was incubated without enzymes was denoted as "blank trial". The trial in which the pulp was not incubated and sieved directly after rasping was denoted as "standard trial". Three trials were performed with increasing doses of the USP enzyme and denoted as USP1, USP2 and USP3. Two trials were performed with increasing doses of the Ebs enzyme and denoted as Ebs1 and Ebs2. Table 1 shows the properties of enzymes used, as well as the doses applied. In addition, it was assured that the enzymes were unable to degrade starch. For this, the enzymes were incubated at a dose of 1% (w/w) of enzyme liquid on dry matter of starch (AVEBE starch in laboratory of food chemistry stock; 6% (w/w) in milliQ water) at 40 °C for 24 h with head over tail mixing. Samples were centrifuged (30 min, 18,000 × g, 20 °C) before analyses by HPSEC. No differences in the HPSEC profiles were seen upon incubating starch with the enzymes.

All trials were performed before November 2012. Fractions were stored at −20 °C until further analysis. For analysing dry matter contents, the potato pulp, "fibre" and the four "starch" fractions were immediately homogenized and sampled. For other analyses, amounts from 100 to 500 g of each of "starch1-4" fractions were homogenized and pooled in the ratios at which they were obtained. The pooled sample was denoted as "starch". In all experiments, samples of "fibre" and "starch" fractions were shortly inactivated by heating at 100 °C for 5 min before freeze drying prior to analyses. For particle size measurements, samples of "fibre" fractions were immediately frozen. Losses of solids in our *semi-technical* scale experiments were unavoidable and measured. These losses were lower than 2% of the dry matter of potatoes and not included in the mass balance.

2.2.2. Separation of the "starch" fraction into soluble and insoluble parts

Soluble and insoluble parts of the "starch" fraction were prepared by washing the "starch" sample (about 50–75 mg) with sufficient water (2 mL). After incubating these head over tail for 2 h at room temperature, the samples were centrifuged (21,320 g, 30 min, 20 °C) and the supernatant was collected. The washing was performed thrice and all supernatants were pooled together and denoted as "soluble starch". Samples of the residue ("insoluble starch") and the supernatant ("soluble starch") were freeze dried for analysis of carbohydrate and protein contents. About 10–16% (w/w) of the "starch" originally present was recovered as "soluble starch".

2.3. Analysis of enzyme activities:

2.3.1. Characterization of exo-enzyme activities

To measure exo-activities of glycosidases in the two enzyme preparations, synthetic p-nitrophenyl (PNP) glycosides were used. About 25 μL of USP or Ebs or diluted solutions thereof was mixed with 25 μL of 0.1 w/w % of the PNP-glycoside solution. The mixture was incubated with 75 μL of 0.05 M NaOAc buffer, pH 5.0 for one hour at 30 °C. After this, 125 μL of 0.5 M glycine NaOH buffer, pH 9.0 containing 0.002 M EDTA was added and the absorbance of the PNP released was measured at 405 nm. The activity of the enzymes was expressed as units (U) of PNP released per mL of enzyme solution, where 1U corresponds to 1 μmol of PNP released per min.

2.3.2. Enzyme incubations on defined substrates for endo-activities

The ability of enzymes, USP and Ebs, to degrade CWPs was tested by applying them on defined substrates under the same conditions (40 °C, 2 h, presence of $NaHSO_3$ (0.07 w/w % of dry potato), same substrate dilution and enzyme to substrate ratio) that were used in the *semi-technical* scale treatments. The enzymes were diluted and applied such that 6 μg protein of USP and 70 μg protein of Ebs were dosed per 1 mg of soluble PGA, 2 mg of soluble arabinogalactan, 0.5 mg of soluble arabinan and 0.6 mg of tamarind xyloglucan in a final volume of 1 mL. Soluble fractions of arabinan, arabinogalactan and polygalacturonic acid were prepared by stirring (30 min, 40 °C; head over tail) and centrifuging a suspension (2 mg/mL; 21,320 g × 30 min, 20 °C) after which the supernatant was freeze dried. Incubations were performed head over tail in

Table 1
Enzymes characteristics and dose applied for all trials.

	USP	Ebs
Full name	Pectinex Ultra-SP	Acetylesterase comprising β-glucanase complex
Supplier	Novozymes	Erbsloh Geisenheim
Source	*Aspergillus aculeatus*	*Penicillium funicolusum*
Protein content (w/w %)[a]	4 ± 0.01	13 ± 0.4
Activity [b]	**(U/mL)**	
α-1,5 arabinofuranosidase	17	2
β-Galactosidase	67	40
β-Glucosidase	135	432
α-xylosidase	0.15	0.006
Doses applied	**g protein per kg potatoes (dry matter), code**	
	0.01, USP1	0.43, Ebs1
	0.03, USP2	0.86, Ebs2
	0.08, USP3	USP + Ebs = 0.01 USP1 + 0.43 Ebs1

[a] Gram of protein (N%*6.25) per 100 g of enzyme liquid.
[b] Enzyme activity determined by the PNP assays per mL of enzyme liquid.

100 mM NaOAc buffer, pH 5.8 (same pH as that of potato juice) and separately in 40 (v/v) % potato juice as a medium. This concentration was used to mimic the concentration of potato juice used in the *semi-technical* scale set-up, which was 4 kg of potato pulp diluted with water to a total weight of 10 kg. Potato juice was collected by blending potatoes through a Sanamat juice extractor (RSA, 240 V, 2.6 A, Uetendorf, Switzerland) and filtering the juice through a 0.2 μm cut off filter (FP30/0.2 CA-S, Whatman, GE Healthcare, Dassel, Germany). The samples were centrifuged (21,320 g × 20 min, 20 °C) and the supernatant was collected for further analyses.

2.4. Analytical methods

All analyses, except for particle size, were performed in duplicate. Particle size measurements were done in quintuplicate.

2.4.1. Dry matter content

Samples were weighed in aluminium cups and dried at 105 °C in a pre-heated oven overnight (16 h) or to more than a day (26–40 h) for some samples until the weight was constant.

2.4.2. Starch content

The total starch and bound starch content (including resistant starch) was determined enzymatically using the Megazyme total starch kit (Megazyme, Wicklow, Ireland). For determining the bound starch content of the “fibre” fraction, 1 g of dried “fibre” was washed with 10 L of water over a sieve of 106 μm (Retsch, Haan, Germany). The residual “fibre” fraction on the sieve was dried and starch content was determined, which was considered to be bound starch.

2.4.3. Constituent monosaccharide composition and content

Constituent monosaccharide composition and content were analysed as described previously (Ramaswamy et al., 2013). From the content of Glc analysed by gas chromatography, the content of starch glucose (Glc) was subtracted to obtain the content of cell wall Glc.

2.4.4. Protein content

The total protein content (N% × 6.25) of the “starch” fractions was determined on a Thermo Quest NA 2100 Nitrogen analyzer (Interscience, Breda, The Netherlands). D-Methionine (Acros organics) was used as an external standard. The protein content of the “soluble starch” fractions was determined after the fractions were re-dissolved in water (1 mg/mL). About 20 μL of the sample or diluted sample was mixed with 200 μL of Bradford reagent (Sigma-Aldrich). A calibration curve of bovine serum albumin (Sigma-Aldrich) was used in the concentration range of 0.25–1.4 mg/mL. After 15 min of incubation at room temperature, the absorbance was measured at 595 nm.

2.4.5. High performance size exclusion chromatography (HPSEC)

An Ultimate 3000 HPLC (Thermo Scientific, CA, USA) was used with three TosoHaas TSK-gel columns in series (4000, 3000 and 2500 SuperAW; each 150 × 6 mm; TosoHaas, Tokyo, Japan) preceded by a TSK Super AW-L guard column (3.5 cm × 4.6 mm; TosoHaas). 20 μL of sample (0.5 mg/mL) was injected and eluted

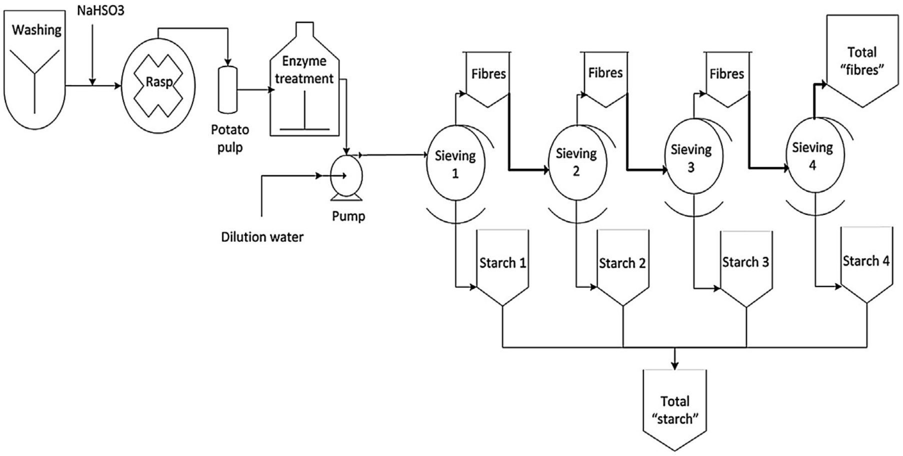

Fig. 1. Flow scheme of the *semi-technical* scale starch separation process.

with 0.2 M sodium nitrate at a flow rate of 0.6 mL/min at 55 °C. Detection was performed with a refractive index detector Shodex R101 (Showa Denko, Kawasaki, Japan). The system was controlled using Chromeleon (version 7) software (Dionex, now ThermoScientific). For calibration, pullulan standards (Sigma; mass range of 180 Da–790 kDa) were used.

2.4.6. *Particle size*

The particle size distribution was determined by laser diffraction using a Malvern Mastersizer 2000 (Malvern, UK). Samples were homogenized and particle size was measured in five replicates. The mean particle size of the volume-weighted ($d_{4,3}$) distributions was calculated as $d_{4,3}$. For the % volume distribution, the size range measured was from 10 to 2000 μm. For confirming whether particle sizes exceeding 2000 μm exist, a number of samples of the freeze dried "fibre" fraction were sieved over a 710 μm sieve and the weight % of particles over the sieve was calculated after freeze drying. Small amounts (about 1 mg) of these particles were wetted with water and analysed by light microscopy using an Axio Scope A1 polarised light microscope (Carl Zeiss; Gottingen, Germany) at a total magnification of 100. Differences in size ranges of cell wall thickness were observed using the Image J software (version 1.44n, National Institutes of Health, Bethesda, MD, USA) after calibration with the slide micrometer.

2.4.7. *Water holding capacity*

WHC measurements were performed using the Baumann's apparatus as described previously (Ramaswamy et al., 2013). The WHC was expressed as the mL of water held per g of dry matter of "fibre" fraction. In addition, the term "retained WHC" was used to relate the amount of water held by the enzyme treated "fibre" fraction $\left(\left(\frac{mL}{g}\right)^E\right)$ to the amount of water held by the blank "fibre" fraction $\left(\left(\frac{mL}{g}\right)^B\right)$ and denoted as WHC^R. $\therefore WHC^R = \left(\frac{\left(\frac{mL}{g}\right)^E \times (g)^E}{\left(\frac{mL}{g}\right)^B \times (g)^B}\right) \times 100$, where $(g)^E$ and $(g)^B$ represent the amount of enzyme treated "fibre" and blank "fibre" recovered from potato (on dry matter), respectively.

3. Results and discussion

3.1.1. *Recoveries of total solids, starch and CWPs in "starch" and "fibre" fraction*

The potatoes used contained 28.5% (w/w) dry matter. Based on dry matter, the starch and cell wall polysaccharide (CWP) contents were 70 and 9% (w/w), respectively (Table 2A). These values are common for potatoes.

Recovery of solids (dry matter), starch and CWPs in the "fibre" and "starch" fraction is shown in Fig. 2. The recovery of solids from potato ranged from 87 to 100% (w/w) for all the trials (Fig. 2). The recoveries of CWPs ranged between 96 and 100% (Fig. 2), which indicated that microbial contamination was minimal in all trials. In addition, the total starch recovered in the "standard trial" (see 2.2.1) was 96% (w/w) on dry matter, of which 94% was recovered in the "starch" fraction and 2% in the "fibre" fraction. These results are in close agreement with the starch recovery on commercial scale (Grommers and van der Krogt, 2009, chap 11).

For the "standard" and "blank" trials, already only one-third of all CWPs were recovered in the "fibre" fraction and the rest of CWPs were present in the "starch" fraction. Findings on such distributions of CWPs over "starch" and "fibre" fractions for the industrial scale process have not been reported. The amount of CWPs in the "fibre" fractions decreased at higher enzyme doses applied and the decrease was more obvious for USP than for Ebs.

Starch in the "fibre" fraction can be distinguished as free starch, which is easily removable from partly intact cells, and as bound starch, which is enclosed within intact or partly intact cells (Leszczynski, 1989). Since the amount of starch recovered in the "fibre" fraction decreased upon enzyme treatments (USP2, USP3 and Ebs2, Fig. 2), we determined whether the decrease of starch was due to removal of bound starch. Our results showed no difference between the contents of total and bound starch in the "fibre" fraction. This implied that all free starch was already washed out during the sieving process and recovered in the "starch" fraction (data not shown). The recoveries of starch from potatoes ranged between 87 and 104%.

Furthermore, the "fibre" fraction recovered from the blank trial (non-enzyme incubation) showed similar recoveries of starch and CWPs (Fig. 2) and protein contents (Table 2A) compared to the standard trial (no incubation), again excluding the possibility of microbial contamination. Overall, considering the scale of experimental set-up, it was concluded that the mass balances of starch, CWPs and dry matter of these *semi-technical* scale trials were good to enable investigation of the effect of enzymes.

3.1.2. *Effect of enzymatic degradation of cell wall polysaccharides, starch removal from "fibre" and resulting water holding capacity*

The proportion of CWPs and starch removed from "fibre" fractions and their effect on the WHC is presented in Fig. 3. In this figure, the removal of CWPs and starch is related to that of the blank "fibre" fraction (treatment without enzymes). For both USP and Ebs, the release of starch was linearly correlated to the removal of CWPs ($r^2 = 0.98$ for USP and 1.00 for Ebs) The removal of CWPs and starch by the combined action of USP and Ebs was almost equivalent to the sum of the individual treatments showing an additive effect. These results indicate that USP and Ebs degraded cell walls in potato pulp, which increased the starch recovery in the "starch" fraction. At laboratory scale, such a relation has been reported previously for starch extraction from potatoes assisted by cellulases (Sit et al., 2011). Our results showed that also at the larger *semi-technical* scale set-up, enzymes assisted considerable release of starch from the "fibre" fraction.

In addition, the WHC of the "fibre" fractions was investigated in relation with the removal of CWPs from these "fibres". Fig. 3 shows WHC^R, which presented the percentage of water held by the total amount of "fibres" related to the blank "fibres" (see 2.4.8). The USP3 (0.08 g of USP) treated "fibres" showed the lowest amount of water held, which was only 40% of the water held by the blank "fibre" fraction. The WHC^R reduced more by the action of USP than by Ebs, mainly because USP released more CWPs than Ebs. Since starch is not expected to contribute substantially to the WHC (Pinnavaia & Pizzirani, 1998), it was concluded that an increased removal of CWPs decreased the WHC^R of "fibre" fractions. Enzyme aided reduction in WHC has been reported also for wheat bran and rye bran (Petersson, Nordlund, Tornberg, Eliasson, & Buchert, 2013). To understand the effect of the type of CWPs present on the amount of water held, the CWPs in the various fractions are described in more in detail.

3.1.3. *Effect of cell wall polysaccharide distribution, composition and water holding capacity of the "fibre" fractions*

3.1.3.1. *Cell wall polysaccharide yield and composition of "fibre" fractions*

The most abundant carbohydrate residues in potato were cell wall glucosyl (Glc (48 mol%)), galactosyl (Gal (19 mol%)) and uronyl (UA (15 mol%))(Table 2A) residues indicating that CWPs mainly

Table 2
Composition of CWPs and WHC of the "fibre" fractions (A), yields of carbohydrate residues in the "fibre" fractions and WHC^R of the "fibre" fractions (B).

(A)

Trial [a]	WHC of "fibre" fraction (mL/g)	w/w%			CWPs in "fibre" fraction: Molar composition (mol %)[d]							
		Protein	Starch	CWP	Rha	Fuc	Ara	Xyl	Man	Gal	Glc[e]	UA
Potatoes		9	70	9	7	1	6	2	2	19	48	15
Standard[b]	14.2	6	28	50	3	<1	8	4	2	35	25	23
Blank[c]	15.5	6	27	50	2	<1	8	4	2	36	27	22
USP1	16.8	8	25	48	3	<1	8	4	2	32	31	21
USP2	13.9	9	27	48	2	<1	8	5	3	23	39	21
USP3	13.3	10	23	44	2	<1	8	6	4	15	44	21
Ebs1	16.3	8	25	48	2	<1	8	4	2	30	32	21
Ebs2	16.3	8	25	51	2	<1	8	4	2	27	33	23
USP + Ebs	15.5	10	22	49	2	<1	8	4	3	26	35	23

(B)

Trial [a]	Yield in "fibre" fraction (w/w% based on dry matter of potatoes)										
	Dry matter	CWPs	Rha[d]	Fuc[d]	Ara[d]	Xyl[d]	Man[d]	Gal[d]	Glc[d,e]	UA[d]	WHC^R (%)
Potatoes	100	100	100	100	100	100	100	100	100	100	
Standard[b]	5.4	31	11	15	36	45	29	52	20	44	
Blank[c]	6.5	37	10	8	41	48	25	61	27	49	100
USP1	5.3	30	10	10	33	42	25	44	24	37	88
USP2	4.4	24	7	6	24	39	26	25	24	29	60
USP3	3.1	16	4	2	15	30	22	10	18	18	40
Ebs1	5.8	32	8	11	33	42	26	43	27	42	93
Ebs2	5.0	29	8	8	31	41	21	36	25	39	85
USP + Ebs	4.4	24	7	7	25	35	25	29	23	32	67

[a] Refer to Table 1 for details of trials.
[b] Trial in which potato pulp is not incubated.
[c] Trial in which potato pulp is incubated without enzymes.
[d] Rha, rhamnose; Fuc, fucose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acid.
[e] Non-starch glucose.

comprised cellulose, arabinogalactan (galactans with or without Ara substitutions) and homogalacturonan (HG) (Table 2A). The presence of rhamnosyl (Rha (7 mol%)) residues pointed out that pectins were constituted of rhamnogalacturonan-I (RG-1) and homogalacturonan (HG) (Meyer, Dam, & Lærke, 2009).The 6 mol % of arabinosyl (Ara) residues indicated that the side chains on RG-I contained Ara residues.

In the "fibre" fraction from the standard trial, 31% of CWPs were recovered (Table 2B), of which Gal, Glc and UA were the most abundant constituent residues (Table 2A). This showed that the

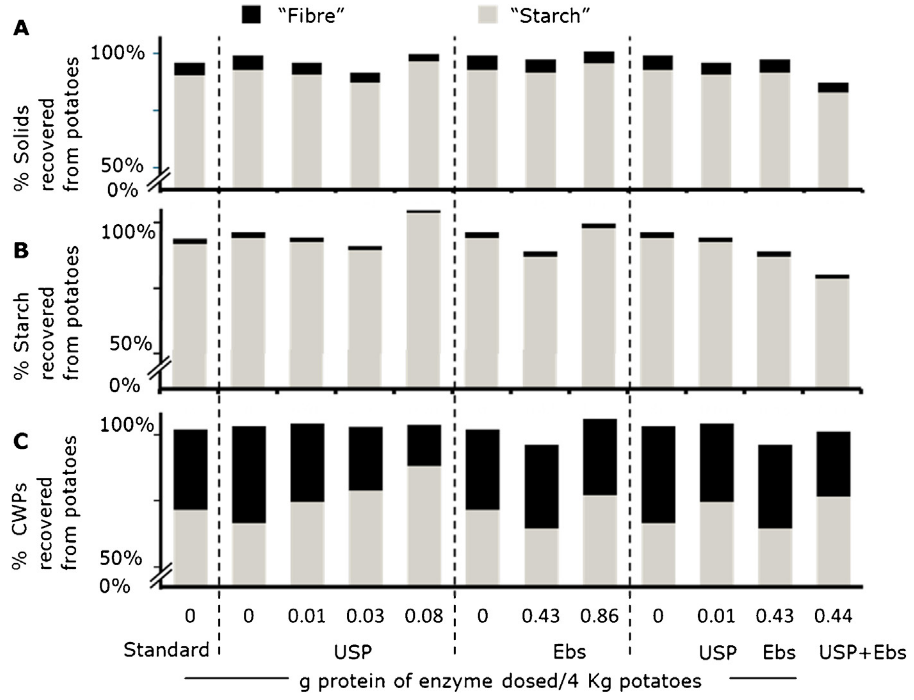

Fig. 2. Recovery and distribution of total solids (A), starch (B) and CWPs (C) from potatoes over "starch" and "fibre" fractions. The standard trials were performed twice. Standard deviations of the recoveries for this trial were between 0.06 and 0.16%. The starch and CWP contents were analysed in duplicate for all trials and average values were used to calculate the recoveries. Standard deviations were between 0.0008 and 5.3%.

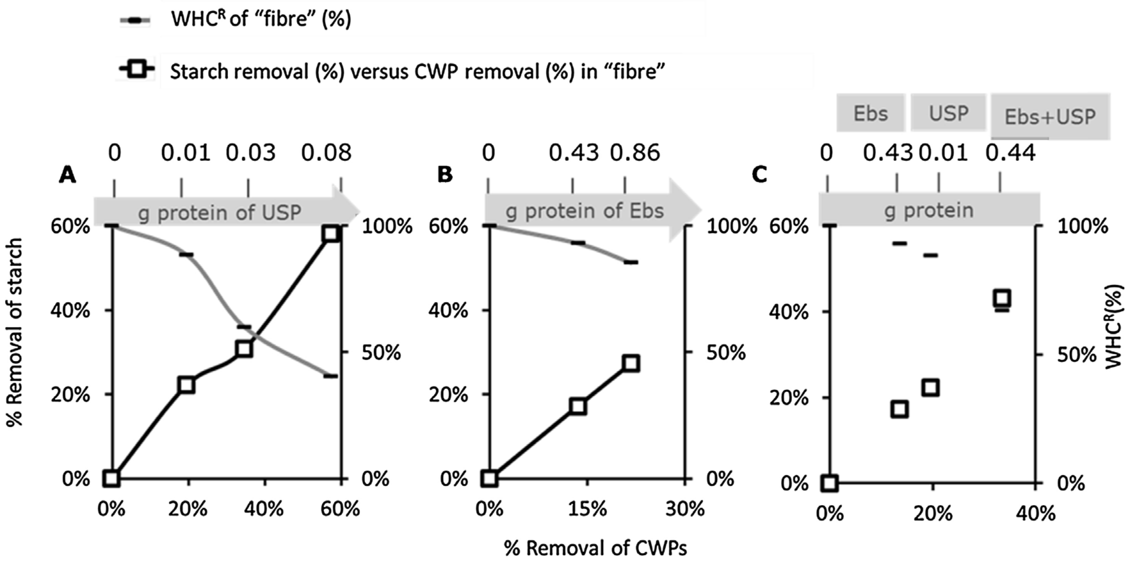

Fig. 3. Removal of starch (% relative to the blank) related to the removal of CWPs (% relative to the blank) from the "fibre" fraction and WHC^R of "fibres" (%) for treatments with USP (A), Ebs (B) and a combination of USP and Ebs (C).

"fibre" fraction was abundant in arabinogalactan, cellulose and HG. This fraction represented about 35% of a total of Rha and UA residues, 49% of a total of Ara and Gal residues, and 21% of other CWPs (total of xylosyl (Xyl), mannosyl (Man) and non-starch glucosyl (Glc)) residues from potato. The galactosyl units analysed belong mostly to polysaccharides originating from cell walls. Small amounts of galactolipids may be present (less than 1.85% (w/w) in the "fibre" fraction from the standard trial (Prescha, Świe drych, Biernat, & Szopa, 2001).

With increasing doses of USP or Ebs, the carbohydrate composition of the resulting "fibre" fractions changed, pointing to changes in the CWP network. Mostly, Gal seemed to decrease in the "fibre" fraction while Glc increased (Table 2A). Modifications in the CWP network influence the WHC of "fibre" (Ramaswamy et al., 2013). Therefore, the amount of CWP residues recovered in the "fibre" fractions were used to understand the effect of the removal of CWPs on the WHC of the "fibre" fraction. The yield of Gal (Table 2B) in "fibre" fractions decreased substantially upon enzyme treatment indicating substantial removal of galactan. In view of the lowered WHC (Table 2A) of the fractions obtained at increasing doses of USP, it was concluded that galactans largely affect the WHC of the "fibre" fraction. The recoveries of Ara decreased from 41% in the blank "fibre" to 15% in USP3 (0.08 g protein) treated "fibre" (Table 2B). Because Ara is present in potatoes both as pectic arabinan and as substitutions on pectic galactan, the decrease in Ara could indicate that arabinans and/or Ara residues substituted on galactan were removed. For Ebs treated "fibres", the decrease in the recovery of Ara was much lower as it was for Gal. It was possible that arabinans also affected the WHC, since they have been shown to hydrate even more than arabinogalactans (Larsen et al., 2011). Also, USP treatments decreased the recoveries of UA and Rha in the "fibre" fractions more than Ebs treatments (Table 2B) pointing at the removal of HG and RG-I. Although it could not be concluded if degrading the pectic backbone also lowered the WHC, previous studies have shown the degradation of HG by polygalacturonase and pectin mehyl esterase lowered the WHC of dehulled lupins (Ali, Williams, Martin, & Sipsas, 2005).

Next to pectic CWPs, a small decrease in the recoveries of Xyl and CW Glc was observed upon addition of USP (Table 2B), indicating the removal of xyloglucans (XGs). Although the role of XGs in the WHC of potato fibre has not been conclusively identified before, XGs have been indicated previously to possess a high WHC (Nishinari, Yamatoya, & Shirakawa, 2000).

The extent of solubilisation of the individual carbohydrate residues was correlated to WHC^R in supplementary Fig. 1. From this figure, it was again clearly shown that the WHC^R decreased in proportion to the removal of CWPs, of which the major carbohydrate residues removed were Gal residues while Xyl residues remained the most in the "fibres". This was also discussed above (Table 2B). The reduction in the WHC^R values were relatively lower for Ebs treated "fibre" fractions despite the higher protein dosage for the Ebs treatments than the corresponding USP treated "fibre" fractions (Table 2B). The fact that the presence of arabinogalactans and arabinans were shown to increase the hydration of RG-I in a previous study (Larsen et al., 2011), and that their higher solubilisation by USP than Ebs correlated to a larger drop in WHC^R of USP treated "fibres" than Ebs treated "fibres", assisted us to conclude that the solubilisation of pectic arabinogalactan helps to lower the WHCs (WHC and WHC^R) of the "fibre" fractions in this study.

3.1.3.2. Insight on starch quality: Cell wall polysaccharides and proteins in the "starch" fractions

In the starch separation process, contamination of the insoluble "starch" rich fraction with water insoluble CWPs and proteins should be avoided, since that may necessitate additional cleaning steps (Grommers and van der Krogt, 2009, chap 11). The recovery of CWPs and proteins in the "insoluble starch" fraction was calculated from the difference in the content of CWPs and proteins in the "starch" fraction and the "soluble starch" fraction. The "insoluble starch" fraction was not directly analysed because it was complicated to obtain accurate values for CWP and protein content. The content of CWPs in the "soluble starch" fraction increased with higher enzyme doses, from 10 w/w % for the blank "starch" fraction to 22 w/w % for USP3 (0.08 g protein) and 16 w/w % for Ebs2 (0.86 g protein). Corresponding to this increase, the recovery of CWPs in the "insoluble starch" fraction decreased with higher enzyme doses, which was more obvious for USP than Ebs treatments (Fig. 4). It is beneficial that these CWPs to accumulate as soluble solids in the commercial process, because these solubles (in potato juice) are separated from "starch" before the "starch" fraction is purified (). The content of proteins in the "soluble starch" fraction also increased slightly, from 16 ± 2.5 for the blank "starch" fraction to 24 ± 3.6 for USP3 (0.08 g protein). This was reflected by negligible differences in the recoveries of proteins in the "insoluble

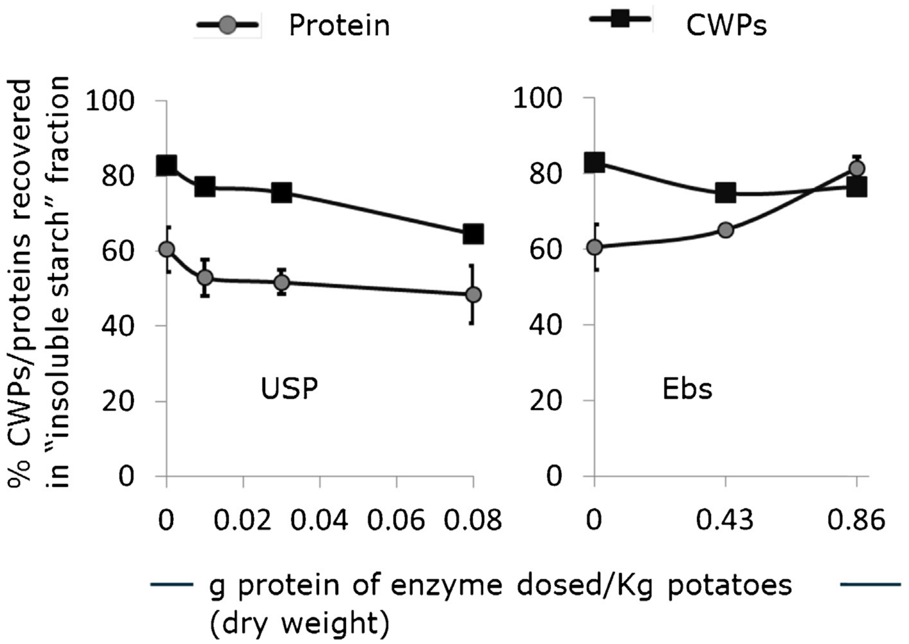

Fig. 4. Percentage (w/w) of CWPs and proteins recovered in "insoluble starch" fraction from total CWPs and total proteins originally present in the "starch" fraction.

starch" fractions (Fig. 4), implying that the "insoluble starch" was free from any additional build-up of proteins upon treatment with USP. Only for Ebs2 (0.86 g protein), the amount of protein recovered in the "insoluble starch" increased by 35% compared to the blank "insoluble starch" fraction. Therefore, the results indicated that for treatments with USP, the insoluble "starch" fraction was by large not contaminated with CWPs and proteins.

3.1.4. 3.1.4 Particle size

The particle size of the "fibre" fraction was studied to determine the effect of the enzyme treatments on the size distribution of all particles in the "fibre" fractions. Fig. 5A showed that the % of the volume diameter of most particles measured fell between the size range from 100 to 2000 μm and the maximum volume % of particles fell in the size range of about 600–850 μm.

After treatment with USP, the volume % of particles decreased only slightly compared to the blank and hardly any effect was observed for Ebs treatments (data not shown). However, since calculations on volume distribution are not absolute and could also include agglomerated particles, the weight distribution based on actual recoveries of fibre particles larger than 710 μm was determined. Only 5% of the total dry matter of the blank "fibre" was larger than 710 μm and was comparable to the 3% of the USP3 treated "fibre". These results confirmed that most particles were less than 710 μm and that enzyme addition did not cause substantial differences in the particle weight distribution. The cell wall width of these particles larger than 710 μm was determined microscopically (supplementary figure) to observe if morphological differences were substantial before and after treatment with USP3. The thickness of the cell wall varied from about 3–30 μm (supplementary figure 2) and not much difference was observed between treatments. This pointed out that the removal of about 58% of CWPs compared to the blank did not modify the morphology of the cell walls substantially, concluded by the microscopic analyses performed.

Despite the similar morphologies observed, in Fig. 5B, it is shown that the volume weighed average size ($d_{4,3}$) of fibres decreased more with USP than with Ebs. Reduced particle size was only observed for USP treated "fibre" fractions, USP2 (0.03 g protein) and USP3 (0.08 g protein). In view with the reduced WHC of USP2 (0.03 g protein) and USP3 (0.08 g protein) "fibre" fractions compared to the blank "fibre", it is possible that a decrease in particle size lowers the WHC of "fibre". The results are supported by observations on sugar beet fibre where a decrease in particle size from 540 to 205 um lowered the WHC from 8.5 to 7.3 (Thibault, Renard, & Guillon, 2001).

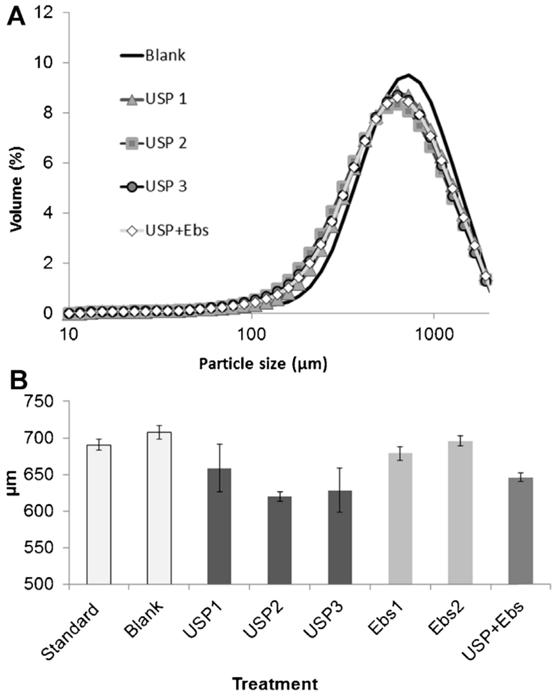

Fig. 5. A. Particle size distribution in volume % of the "fibre" fractions. B. Volume weighed average sizes of the "fibre" fractions. Error bars represent the deviation of 5 particle size measurements per sample.

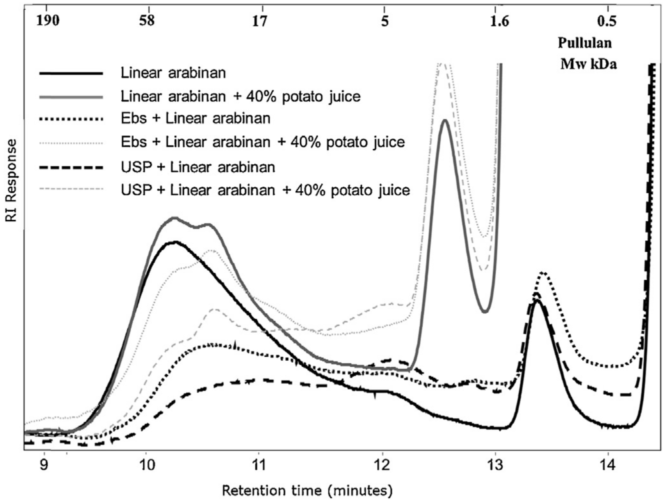

Fig. 6. HPSEC profiles of arabinan treated with Ebs and USP in the presence and absence of potato juice (40%).

3.1.5. Enzyme activities and inhibition by potato juice

The activities of USP and Ebs on model substrates were tested. The exo-activities of the enzymes (Table 1) indicated that USP was more enriched in arabinofuranosidase, xylosidase and slightly more in β-galactosidase compared to Ebs. The hydrolysis of purified CWPs (arabinogalactan, arabinan, polygalacturonic acid and tamarind xyloglucan, see 2.1 and 2.3.2) by USP and Ebs at their highest doses (USP3 and Ebs2) was observed by the degradation of high molecular weight (Mw) material to low Mw (supplementary Fig. 3) in buffer. This indicated the presence of galactanase, arabinanase, polygalacturonase and endo-glucanase activities. These results confirmed the degradation of arabinogalactan, HG and XG by the enzymes. However, in the presence of potato juice (PJ), the profiles were interfered by components in PJ (data not shown).

Only for arabinan degradation, representative HPSEC profiles were obtained (Fig. 6). In buffer, the profiles of both USP and Ebs degraded arabinan were comparable, showing similar abilities to degrade arabinan. However, in 40% potato juice (PJ), substantial high Mw material remained after enzyme digestion. This was especially the case with Ebs than with USP (Fig. 6). This indicated that in PJ, the enzymes showed lower abilities to degrade high Mw arabinan than in buffer (absence of PJ). This was more obvious for Ebs than for USP. Since the pH of potato juice and buffer were the same, these results suggested that both USP and Ebs were suppressed by components in PJ, of which Ebs seemed to be suppressed more than USP. This could be explained either due to the lack of activities to degrade arabinan in the presence of PJ and/or lack of accessibility of the enzymes to arabinan in PJ. Possibilities of inhibition of cell wall degrading enzymes by components in potato juice, such as phenolic acids or proteins have been mentioned in previous studies (Lyon & McGill, 1989; Bulantseva, Tkhang, Buza, Krinitsyna, & Protsenko, 2005).

4. Conclusion

The study showed that starch recovery from "fibre" increased substantially (58%) upon enzyme treatment (0.08 g protein of USP) by solubilising arabinogalactan (A + G, 80% w/w), arabinan and homogalacturonan (HG; 63% w/w each) from "fibre". The absence of these CWPs also caused the "fibre" fraction to retain only 40% of the total amount of water originally present. This pointed at the importance of pectic arabinogalactan side chains in contributing to the WHC of the "fibre" fraction. It can be expected that at industrial scale, USP or enzymes similar to USP might help to release starch from potatoes and lower the WHC of the "fibre" fraction.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.07.019.

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