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# Time of harvest affects the yield of soluble polysaccharides extracted enzymatically from potato pulp

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A B S T R A C T

Potato pulp is a co-processing product from potato starch production. The pulp mainly consists of the tuber cell walls, which are rich in pectin and cellulose. The potato pulp pectin is dominated by galactan branched rhamnogalacturonan 1 which after enzymatic solubilization has shown promising properties as bifidogenic prebiotic fibers. The potato starch processing campaign is based on processing of fresh potatoes (in Denmark the campaign lasts from September to December). This study examines the effect of time of harvest and processing during the campaign on the yield of enzymatically solubilized potato polysaccharides applying a recently developed enzymatic process using 1.0% (w/w) [enzyme/substrate (E/S)] pectin lyase from *Aspergillus nidulans* and 1.0% (w/w) [E/S] polygalacturonase from *A. aculeatus* at 60 °C, 100 mM citric acid, pH 6.0 for 1 min. Seven samples drawn within the potato starch campaign of 2011 were characterized: the yields of enzymatically solubilized potato polysaccharides and the solubilized galactan proportion increased during the potato starch campaign. The data thus suggest that potato pulp produced late in the campaign would be preferable for upgrading to the bifidogenic fibers; this outcome may be the result of an inherent effect of the higher maturity of the potatoes late in the campaign.



*Keywords:* Potato pulp; Fiber; Enzymatic extraction; Potato starch campaign; Rhamnogalacturonan I; Galactan

## 1. Introduction

Potato pulp is a high-volume, low value by-product resulting from industrial production of potato starch. The pulp is mainly made up of the potato tuber cell walls, and typically also contains some residual starch. A key feature of the potato pulp cell wall polysaccharides is their high content of galactose and galacturonic acid (Serena and Knudsen, 2007; Meyer et al., 2009; Thomassen and Meyer, 2010; Ramaswamy et al., 2013). Accordingly, in agreement with the presence of highly complex arabinogalactan structures in raw potatoes (Øbro et al., 2004), the main type of cell wall polysaccharides in potato pulp is pectin in the form of homogalacturonan (HG) and notably rhamnogalacturonan I (RG I), of which the latter is predominantly branched with high molecular weight, linear (β1,4-)galactan and arabinogalactan side chains (Byg et al., 2012), including some (β1,3-)galactan stretches (Khodaei and Karboune, 2013). In addition to pectin, the pulp cell wall material also contains some cellulose, typically constituting ~10–22% by weight of the dry matter (w/w DM) (Meyer et al., 2009; Thomassen and Meyer, 2010; Gao et al., 2012), as well as low levels of hemicellulose mainly as xyloglucan (Ramaswamy et al., 2013). Hence, despite vast geographical differences in the origin of the reports and hence the potato raw materials used, the compositional data for the potato pulp cell wall composition are very consistent. Significant variation is found in the residual starch content, however, probably as a result of differences in starch extraction efficiencies among different factories; hence, reported starch levels in freshly processed potato pulp range from 20 to 30% by weight of the dry matter (Meyer et al., 2009; Thomassen and Meyer, 2010; Ramaswamy et al., 2013), but a content of up to 35% by weight was recently

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reported in industrial potato pulp from China (Gao et al., 2012). Most of the protein is washed out during the potato starch production process, which is a "washing-shredding-washing-separation" process, so the protein level in potato pulp is only 4–6% (w/w DM) (Serena and Knudsen, 2007; Gao et al., 2012; Ramaswamy et al., 2013), and lignin and lipid contents are also low, typically ~2–4% and <1% (w/w DM), respectively (Serena and Knudsen, 2007; Gao et al., 2012).

Annual production levels of potato pulp as a side-product from potato starch production are in the order of millions of tons with $\sim 1 \times 10^6$ tons produced annually in Europe (Mayer and Hillebrandt, 1997), $1 \times 10^5$ tons in Northern Japan (Yunoki et al., 2004), and nearly $5 \times 10^6$ tons produced in China each year (Gao et al., 2012). In Denmark alone, the annual production of wet potato pulp is ~75 000 tons. Currently, this pulp is not upgraded to any significant degree, and the enormous amounts available create a large incentive for developing effective processes for its utilization and valorization.

It has thus recently been shown that the starch and presumably some of the galactan in potato pulp may be converted to ethanol by consolidated processing using the saccharolytic/cellulolytic fungus *Acremonium cellulolyticus* (Gao et al., 2012). Our own work has mainly focused on the prospects of extracting the high-molecular weight RG I galactan polysaccharides that may make up a high-value functional food ingredient product, due to their documented effects as fermentable dietary fibers in humans (Olesen et al., 1998); weight gain retarding effects in rats (Lærke et al., 2007), and prebiotic bifidogenic potential (Thomassen et al., 2011; Michalak et al., 2012). The solubilized high molecular weight potato pulp RG I galactans moreover exhibit desirable techno-functional effects (rheological, color, and other effects) when incorporated into food products such as bread and liver pâté (Kaack et al., 2006a,b).

Since the desirable potato cell wall polysaccharides consist of a backbone of RG I with large side chains of galactan/arabino galactan they can be extracted using pectin modifying enzyme activities that catalyze the cleavage of HG stretches linked by glycosidic bonds to the RG I backbone. In order to particularly target the solubilization of the high-molecular weight galactan fibers of the RG I region of the potato pectin, we recently developed a minimal enzymatic treatment procedure based on catalyzing the breakdown of the HG surrounding the RG I regions by use of a combination of mono-component pectin lyase (EC 4.2.2.10) attacking mainly the α1,4 bonds of methoxylated galacturonic acids and polygalacturonase (EC 3.2.1.15) attacking mainly the bonds between non-methoxylated galacturonic acid residues in HG (Thomassen et al., 2011). The use of monocomponent enzymes appears favorable, because undesired enzyme catalyzed degradation of the released fibers is avoided. Until now, studies on enzymatic dietary fiber extraction from potato pulp have mainly been carried out only on selected single batches of the pulp (Meyer et al., 2009; Thomassen et al., 2011; Byg et al., 2012; Khodaei and Karboune, 2013). However, during the potato starch campaign, which e.g. in Denmark usually sets off in early September and terminates in the middle of December, and is based exclusively on processing of freshly harvested potatoes, potatoes of slightly different ripeness or maturity are processed (in Denmark, the varieties processed are mainly *Solanum tuberosum* L. cv. Oleva, Kardel, and Kuras, respectively).

It has previously been shown that when potato pulp was fermented with *Rhizopus oryzae* the date of the starch extraction affected the fermentation, and the changes were assumed to be a result of variations in the pectin quality during the starch processing campaign (Saito et al., 2006). The accessibility of the pectin for enzymes may also be affected by maturation. When the homogalacturonan is synthesized, the polymer is normally methyl-esterified, which prevents it from forming calcium pectate also known as egg-box structures. After homogalacturonan is placed in the cell wall pectin methyl esterase may de-esterify homogalacturonan allowing the formation of calcium gels and increase cell wall rigidity (Sabba and Lulai, 2005). The action of pectin methyl esterase generates regions in the cell wall matrix that can be cleaved by the action of other cell wall-based enzymes such as polygalacturonases and pectate lyases, thus loosening the pectic network. As the pectin becomes more soluble its "grip" on the surrounding cell wall is loosened (Kashyap et al., 2001; Verhertbruggen and Knox, 2006). Hence, the optimal time point for obtaining maximal yields of enzymatically solubilized potato polysaccharides within the potato starch campaign may be hypothesized to be dependent on a possible inherent effect of the potato maturity and may also depend on the potato pulp composition. The objective of this study was to test this hypothesis by examining if the yields of enzymatically solubilized potato polysaccharides correlated with the pulp composition, and whether an optimal time, i.e. early or late in the campaign, could be identified for this enzymatic upgrading of the pulp.

## 2. Materials and methods

### 2.1. Substrates and enzymes

Potato pulp was sampled on the following days from the industrial production of potato starch at KMC Andelskartoffelmelsfabrikken, Brande, Denmark: 7th of September 2011, 21st September 2011, 4th of October 2011, 18th of October 2011, 1st of November 2011, 15th of November 2011, and 6th of December 2011. The samples were frozen immediately and stored at −21 °C until characterization.

Monocomponent endo-polygalacturonase from *Aspergillus aculeatus* was a gift from Novozymes A/S (Bagsværd, Denmark) (see also Thomassen et al., 2011). The *Pichia pastoris* pectin lyase clone AN2569.2 from *Aspergillus nidulans* (now *Emericella nidulans*) was obtained from the Fungal Genetic Stock Center, Carnegie Institution, Stanford, CA. *P. pastoris* fermentation was principally done according to Stratton et al. (1999) in 5 L fermentation as described in detail previously (Silva et al., 2011). Dosing of the pectin lyase was done on the basis of protein content (22.0 g/L), whereas dosing of the monocomponent endo-polygalacturonase, presented as a lyophilized powder, was done directly on dry weight basis.

### 2.2. Chemicals

Trifluoroacetic acid (TFA), D-(+)fucose, α-L-rhamnose, L-(+)arabinose, D-(+)galactose, D-(+)xylose, D-(+)mannose, D-glucorunic acid, D-(+)galacturonic acid, citric acid anhydrous ≥99.5%, sulfuric acid 72% (w/v), 2-propanol anhydrous 99.5%, sodium acetate, sodium hydroxide 50% (w/v), sodium azide, and pullulans with the following average molecular masses 1.3, 12, 110, and 400 kDa were purchased from Sigma–Aldrich (Steinheim, Germany). D-(+)Glucose was purchased from Merck (Darmstadt, Germany).

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