Engineering aspects of enzymatic fiber solubilization from potato pulp

Potato pulp is a low-value by-product of the potato starch production. However, it contains valuable fibers consisting of rhamnogalacturonan I (RGI) with large galactan and arabinan side chains that can be extracted using a combination of polygalacturonase (PG) and pectin lyase (PL) which degrades the surrounding homogalacturonan (HG). The RGI backbone is linked by glycosidic bonds to HG and by degradation of HG the target carbohydrate is solubilized and thereby recoverable. Due to the complex structure of RGI with large galactan and arabinan side chains – also known as hairy-RGI – the fiber has the potential to act as a prebiotic. Prebiotics are especially valuable to the swine industry where post weaning diarrhea is problematic. When piglets are separated from the sow they are subjected to a critical transition from milk onto a diet based on plant polysaccharides causing imbalance in the intestinal system. Prebiotics can counteract this imbalance by maintaining a healthy microbiota in weaning piglets.

Fast enzymatic release of the potential prebiotic hairy-RGI fiber from potato pulp demanded excessive availability of HG. However, at pH 6.0 the availability of HG was strongly affected by the presence of calcium as calcium forms gels by interacting with protonated low-methoxylated (LM) HG hindering enzyme attacks and degradation of the HG. Initially, two types of potato pulp FiberBind, a dried commercial potato pulp product containing ~7000 ppm of calcium, and PUF, a dried calcium reduced product containing ≤200 ppm of calcium, were tested for precipitable dry matter after a 1 min. reaction at 60°C and pH 6.0 using 1% substrate and with or without enzymes. The enzymes were PL from Erwinia nidulans and PG from Aspergillus aculeatus each dosed at 1.0% (w/w) enzyme/substrate (E/S). The study was carried out at various concentrations of ethylenediaminetetraacetic acid (EDTA) or phosphate. EDTA is a known calcium chelating agent.

Assessment of the dosage-response effects of phosphate and EDTA on the two potato pulp substrates indicated a need for a significant molar surplus of the chelating agent for maximal effect on polysaccharide release induced by enzymatic cleavage of HG. To get a more general understanding of the calcium gel formation four potential chelating agents were tested for their effect on inducing high yields of precipitable oligo- and polysaccharides from sugar beet pulp, citrus peel, FiberBind and PUF. The results revealed a correlation between degree of protonation at pH 6.0 and yield of fibers.

Maximum polysaccharide yield was obtained for FiberBind where the enzymatic treatment in presence of citric acid yielded 22.5% of initial dry matter. Besides the effect from the applied chelating agent other factors such as production date of potato pulp also had an effect on the yields of fibers from potato pulp. Seven samples drawn within the potato starch campaign of 2011 were characterized according to monosaccharide compositions, degree of methylation (DM) and acetylation (DAc), and content of the glycoalcaloids α-solaline and α-chaconine. The monosaccharide composition, DM, and content of α-solaline remained rather unchanged, whereas DAc and content of α-chaconine decreased significantly during the campaign.

The seven samples were subjected to the same enzymatic treatment as described above and the precipitated polysaccharides were characterized based on their monosaccharide compositions and molecular size distribution. The yields of enzymatically solubilized potato polysaccharides and the solubilized galactan proportion increased during the potato starch campaign. The data suggested that potato pulp produced late in the campaign would be preferable to upgrade, due to the higher yield and lower risk of toxicity from glycoalcaloids. The outcome may be the result of an inherent effect of the higher ripeness of the potatoes late in the campaign.

Producing fibers from potato pulp, i.e. FiberBind, is more profitably done at dry matter contents of more than 1% (w/w). However, increased dry matter can lead to highly viscous suspensions and inefficient mixing. To investigate practical processing of FiberBind suspensions viscosity studies were performed in a Rapid-ViscoAnalyzer (RVA, Newport Scientific, NSW, Australia) examining the viscosity reducing effect of enzyme activities, which included PL and PG from E. nidulans both dosed at 1% (w/w) (E/S) and tested at 60°C, as well as two commercial α-amylases Termamyl® SC and Fungamyl® 800L dosed at 0.2% (w/w) (E/S) tested at 60°C and 70°C. Especially, PL was efficient for viscosity reduction possibly due to the high DM (~70%) of FiberBind hindering PG attacks on the HG backbone. Starch degradation at 60°C and 70°C did not cause viscosity reduction to the same extent as pectinases, suggesting, if necessary, starch removal contemporary with release of polysaccharides by PL and PG. Scaling up the enzymatic extraction of polysaccharides using 3% (w/w) dry matter to 4L gave 29.7% (of initial dry matter) which was comparable to lab. scale enzymatic extraction using 1% (w/w) dry matter.

The enzymatically extracted polysaccharides produced in 4L as well as polysaccharides produced in pilot plant scale were structurally characterized through linkage analysis producing partially methylated alditol acetates (PMAA) analyzed via gas chromatography (GC) mass spectrosocopy (MS). The two products showed almost identical linkage patterns. As expected, large galactan and arabinan chains made up most of the polysaccharides. ~50% of the glycosidic linkages in the extracted products were (1→4) galactopyranosyl (Galp) linkages with a low degree of branching: a branch point was observed for every 30 (1→4) Galp residue. High contents (~23%) of (1→4) arabinofuranosyl (Araf) residues was detected as well, but no branch points were observed. Lower contents of rhamopyranosyl (Rhap) and galactopyranosyl uronic acid (Gal pA) were observed as well the structure to be hairy-RGI.

The prebiotic effect of the fiber was tested in weaning piglets a minimal basal medium containing 5 g/L of the polysaccharides and inoculated with content from the terminal ileum of piglets. Hereafter the suspension was incubated anaerobically for 24 hours at 37.5°C. The microbiota composition was analyzed by extracting DNA, amplifying 16S rRNA variable regions by PCR, and taxonomically classification against the Ribosomal Database Project II. After 24 hours incubation the pH had dropped and the concentration of short chain fatty acids (SCFA) had increased. The microbiota was dominated by the phylum Firmicutes. In particular, the proportion of Clostridia, decreased significantly, whereas the proportion of Lactobacillus was increased significantly. In piglets Lactobacillus is associated with a healthy microbiota suggesting a prebiotic effect of the enzymatically extracted polysaccharides.