Structural Characterization and Enzymatic Modification of Soybean Polysaccharides

The work in this thesis explores the structure of soybean polysaccharides, and examines approaches for the chemical and enzymatic degradation and solubilization of this material. Soybean polysaccharides are produced in large quantities globally as a by-product of various soy production processes. The work presented in this thesis focuses on the insoluble cell wall polysaccharides produced during the manu-facture of soy protein isolate. Soybean polysaccharides are water insoluble and feature an approximate carbohydrate composition (by weight) of 35% galactose, 20% glucose, 20% arabinose, 10% galacturonic acid, 8% xylose, 3% rhamnose, and 3% fucose. Currently, the majority of this material is disposed of as waste, increasing production costs. Opportunities exist for the development of novel functional ingredients from this abundant and underutilized material; however, efforts in this area are currently limited by the material’s insulubility. A central hypothesis of this work was that by obtaining a more complete understanding of the structure of this material, chemical and enzymatic approaches could be developed to modify the polysaccharides, creating soluble polysaccharide fractions that could provide improved functionality in industrial applications.

To address this hypothesis, structural information was obtained through HPAEC compositional analysis and GC-MS linkage analysis. This work was conducted on the whole soybean polysaccharide fraction, instead of only chemically extracted portions of this material like those analyzed in previous studies. Using this linkage data, the polysaccharide classes in soybean were quantified for the first time, with the results (by weight) identifying the primary constituents as: type I arabinogalactan (27.8%), cellulose (23.5%), (glucurono)arabinoxylan (14.4%), arabinan (8.1%), rhamnogalacturonan I/II (6.2%), xyloglucan (2.7%), type II arabinogalactan (2.0%), and homogalacturonan (1.6%). Using this compositional data, a novel chemical solubilization process was developed utilizing hydrogen peroxide at elevated temperatures. This treatment resulted in the release of more than 70% of the original insoluble material as high molar mass, water-soluble polysaccharides. This solubilized fraction is significantly enriched in the non-cellulosic polysaccharides of soy-bean such as arabinogalactan, homogalacturonan, rhamnogalacturonan, arabinan, xyloglucan, and (glucurono)arabinoxylan. These results demonstrate that it is possible to solubilize significant portions of the soybean poly-saccharide using a one-step chemical treatment, which opens new possibilities for the expanded utilization of this material going forward.

The results from this work also highlight the recalcitrance of soybean cellulose and the significant role that this polysaccharide class plays in the overall insolubility of the material. In an effort to address this, lytic polysaccharide monoxygenases (LPMOs) were evaluated for their ability to oxidatively degrade soybean cellulose. The initial investigations utilized TrCel61A, an AA9 LPMO from Trichoderma reesei. This enzyme showed no oxidative activity on native soybean polysaccharides; however, significant oxidative degradation was observed on NaOH pretreated soybean polysaccharides. The oxidation products were evaluated using HPAEC and MS, with the results showing oxidation at both the C1 and C4 positions of cellulose. In addition, a synergistic effect between TrCel61A and a GH5 endo-β-1,4-glucanase was discovered, boosting the glucose release from NaOH pretreated soybean polysaccharides.

Building upon these observations, twenty-three additional LPMOs from seven fungal sources were evaluated (using TrCel61A as a benchmark), with none showing oxidative activity on native soybean polysaccharides. However, NaOH pretreatment of the raw material was shown to improve the enzymatic accessibility of the soybean cellulose through the removal of non-cellulosic polysaccharides. Following this pretreatment, seven LPMOs (including TrCel61A) showed activity on the pretreated soybean polysaccharides. These seven enzymes were subsequently evaluated for their ability to increase the glucose release from this material through hydrolytic boosting of endo-β-1,4-glucanase and beta-glucosidase activities. Significant boosting effects were observed for TrCel61A and one of the newly evaluated LPMOs (Aspte6), resulting in the release of over 36% substrate glucose when compared to only 20% in the absence of the LPMO. Evaluation of the oxidation products from these LPMO treatments with HPAEC and MS showed similar C4 oxidation patterns for all soybean polysaccharide-active LPMOs. In addition, the vast majority of soybean polysaccharide-active LPMOs were also found to have oxidative activity on microcrystalline cellulose. These results demonstrate the ability of enzymatic treatments to solubilize and modify soybean polysaccharides. They also suggest new opportunities to improve upon the enzymatic digestion of this substrate in the future.

Overall, the research conducted in this project has demonstrated the utility of structure-based modification approaches and suggests that the insolubility of soybean polysaccharides is primarily conferred by the cellulosic components. In addition, the results obtained suggest several new opportunities for direct chemical or enzymatic solubilization and degradation of insoluble soybean polysaccharides, paving the way for the improved utilization of this material in the future.

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