

**FINAL REPORT OF THE NKFIH PROJECT 121024 ENTITLED
„STUDY OF THE PREBIOTIC EFFECT OF
CORN-FIBRE-DERIVED SACCHARIDES”**

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Specific and thorough literature review was carried out, and two Hungarian review papers were published. The first review focuses on the application of prebiotic oligosaccharides obtained from agricultural by-products. These could serve as functional components of foods by preserving health through the effects on gut microbiota. The influence of the bacteria residing in the colon on the organism is highly complex. The most important effects are achieved by the production of short chain fatty acids and lactate. In recent years several studies have been published comparing the fermentation properties of carbohydrates derived from potential sources that point out the significance of the structural differences of xylan from various raw materials. The development of novel prebiotic oligosaccharides has gained much attention in the past decades and is still an ongoing process.

In the second review the possible ways of utilisation of agricultural by-products and the development and production of novel prebiotic carbohydrates are discussed. The xylooligosaccharides (XOS) are molecules obtained by the hydrolysis of xylan, which have numerous positive impacts on health when applied as functional components of foods. This review addresses the methods of the production of XOS from lignocelluloses focusing on two intensively researched emerging technologies: the enzymatic treatment and the autohydrolysis. We also present our research corresponding to this topic, in which the production of XOS using commercial enzyme preparations and applying mild sulphuric acid treatment is investigated. For industrial application the presented technologies must be cost-efficient, therefore the careful optimization of the process parameters is of utmost importance.

Preliminary experiments were performed regarding the adaptation of the methods not used in our laboratory before this project, i.e. the hydrophobicity and co-aggregation ability measurements. According to the literature the epithelial adhesion can be assessed by co-aggregating the *Lactobacillus* strain with *Saccharomyces cerevisiae*. Both the hydrophobicity and co-aggregation ability measurements resulted in high standard deviations, which implies that under the applied conditions these methods cannot be used for the assessment of the epithelial adhesion.

Three strains were selected to study the prebiotic effect of corn fibre saccharides: *Lactobacillus acidophilus* LA5, CHR HANSEN (Denmark), *Lactobacillus acidophilus* 150, Exquim (Spain) (La150), *Lactobacillus casei* 01, CHR HANSEN (Denmark) (*L. casei*). Fermentation assays were carried out for each strain in media containing the liquid fraction obtained by mild acid hydrolysis of corn fibre as carbon source. Control experiments were also planned, in which commercial fructo-oligosaccharide preparation was used instead of the corn-fibre-derived saccharides. In the fermentation assays the optical density, the pH, the viable cell count, the concentrations of monomer and oligomer sugars, the concentrations of lactic and acetic acids

were monitored. Applying inulin as fructo-oligosaccharide carbon source significant differences were observed in cell growth: La150 showed the highest growth rate, LA5 could grow moderately, while *L. casei* was not able to grow. All the three strains could grow well on the hydrolysate of 0.75% sulphuric acid, since the hydrolysate contained monomer sugars, which could be metabolized by all the three strains. Applying the hydrolysate of 0.15% sulphuric acid differences were observed in cell growth: La150 showed the highest growth rate, LA5 could grow moderately, while *L. casei* showed the lowest growth rate. Therefore, these observations imply that the xylo-oligosaccharides derived from corn fibre can stimulate all the three selected *Lactobacillus* strain, and they have the most expressed prebiotic effect for La150. Preliminary experiments for the assessment of *Lactobacillus* tolerance against different stress factors were carried out, however, the standard deviations were high, and significant differences compared to the control without stress factor were not obtained.

Beside corn fibre other biomass materials were also investigated, and various chemical and biochemical conversion routes were studied to establish the complete utilisation of the biomass materials. Hence, the biomass materials were fractionated and converted into various sugar fractions, which could be fermented into alcohols, sugar alcohols and organic acids. The concept of biorefining was developed in the 21st century, and it is defined as the sustainable processing of biomass into a wide spectrum of products like chemicals, materials and energy. Lignocelluloses are promising raw materials for biorefining. Lignocellulosic materials represent an abundant and inexpensive source of sugars that can be microbiologically converted into industrial products. Xylitol is a natural polyol, which is used in foods as a sweetener. Traditional chemical conversion of xylose into xylitol is difficult, requires harsh conditions and results in high price of xylitol. The microbial conversion of xylose into xylitol could be an attractive alternative. In our study wheat straw was used as raw material, and it was treated with diluted sulphuric acid to hydrolyse the hemicellulose. The xylose-rich hydrolysate was fermented with *Hansenula anomala*, which produced xylitol. We found that using activated carbon as detoxification was not necessary, since it did not have positive effect on xylitol yield, however it caused significant loss of initial xylose and glucose. The most important problem was to produce a more concentrated xylose solution, which might be achieved by recycling the hydrolysate.

Xylitol can also be produced by heterogeneous catalytic hydrogenation of xylose over Raney®-nickel. Hydrogenation is followed by several purification steps, which makes the chemical production relatively complex and expensive. Our goal was to investigate the treatments (activated carbon, bio-purification) of corn stover hydrolysates and to hydrogenate xylose to xylitol. An activated carbon treatment was used for eliminating inhibitor compounds and increasing the efficiency of the bio-purification step. It was found that glucose can completely be eliminated from the hydrolysate. Hydrogenations of corn stover hydrolysate showed that high reaction temperature resulted in high sugar alcohol yields and selectivity. The flow rate, at a given temperature, had no significant effect on xylitol yield.