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**FRACTIONATION OF CORN FIBER, STUDY OF THE STRUCTURES OF
THE OBTAINED FRACTIONS AND THEIR POSSIBLE APPLICATIONS**

PH.D. THESIS

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1. INTRODUCTION

The application of plant biomass as a source of energy, fuel or green chemicals is becoming more important. Biomass can be directly grown for these purposes (e.g.: wheat, corn, rape, willow, etc.), however, the utilization of certain agricultural or industrial by-products is more advantageous. Bioethanol is the most widely used biofuel in the world. It can be used as a substitute of petroleum based fuels or mixed with petrol. Bioethanol can be produced from plants which have a high sugar- or starch content or from cellulose containing plant parts with the the so-called second generation biofuel processes. The second generation procedure is not commercialised yet, application of this technique is more probable in the near future. The renewable raw materials cannot only be utilized as energy carriers, but also as industrial raw materials, for example in the production of biodegradable plastics. The feedstock of bioplastics can be renewable biomass materials of plant and animal origin, for example starch, which I have also used in my work.

In my PhD work I have studied corn fiber, which is the residue of starch production in the corn wet-milling process, i.e. an agroindustrial by-product. In addition, I have used corn starch for the preparation of biologically degradable plastics.

2. OBJECTIVES

The main objective of my work was to develop a fractionation procedure with which the components of the polysaccharide fraction of corn fiber can be efficiently separated, and the obtained polysaccharide fractions can be further utilized for the production of various bioproducts (enzymes, ethanol).

A great emphasis was put on the utilization of the alkali solution obtained in the fractionation procedure for the production of valuable polymeric hemicellulose with high yield. Prior to this, however, it was necessary to find the optimum parameters for the pre-treatments (type of alkali, time of pre-treatment, presence of oxidative agent).

In addition I have studied the use of polymers obtained from corn in thermoplastic starch composites to improve their physical and chemical characteristics and to influence the biodegradation time of the TPS products.

3. LITERATURE BACKGROUND

Lignocellulosic plant tissues are made up of three main components: cellulose, hemicellulose and lignin. These constituents do not occur in nature as independent polymers, they are linked by different bonds and constitute the so called lignocellulose, which makes up the plant cell wall. **Cellulose** is a linear polymer made up of D-glucose units. The monomers are linked by β -(1 \rightarrow 4) bonds, hence the monomers are rotated by 180 degrees compared to one another. Cellulose is the main component of plant cell wall, the most important structural unit of higher plants. The **hemicellulose** component of the plant tissues is a smaller, branched polysaccharide with amorphous structure, which can easily be hydrolyzed. There are several types of hemicelluloses including xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan, etc. **Lignin** is a macromolecule with a very difficult structure. It is made up of differently substituted phenylpropane units, and is responsible for assuring mechanical strength of the cell wall. **Starch** is the most widespread energy storage polysaccharide in plants. It is deposited in the plant cells in the form of variously shaped granules. Starch can be hydrolysed by acids or enzymes α -D-glucose is the product of the complete hydrolysis.

Cellulose and hemicellulose become accessible only with the disruption of the lignocellulosic structure. Physical, chemical, physicochemical and biological pre-treatments can be used. The characteristics of the applied chemicals determine the composition and physical properties of the residual fibers and the soluble fractions. A mild acid hydrolysis saccharifies the hemicellulose content of the fiber, and the sugar monomers get into the liquid fraction. The obtained solution has a high xylose content. Hence, on the one hand it is suitable for ethanol production, on the other hand xylitol, which is a well-known sweetener, can be obtained from it with catalytic or microbial reduction. Cellulose and lignin are retained in the fibrous residue in this case. In the case of alkali pre-treatment the hemicellulose fraction is dissolved in the alkali solution in polymeric form (the structure of lignin is changed, and lignin is partially hydrolysed.). The alkali pre-treatment of corn fiber yields a special compound, the so called corn fiber gum (CFG), which become more and more important for the food industry. Cellulose is mostly used in polymeric form (paper production), pilot studies are performed for its utilization after hydrolysis. In contrast, both starch and its decomposition products are widely used for the production of dehydrated stock, sauces, candies and pills with starch coatings, ethanol and isosugar. A rather novel field of application, the production of bioplastics is at present unfortunately limited.

Lignocelluloses often occur in the processing of plant materials as wastes or by-products. A good example is corn fiber, which I have studied in my work. Corn fiber, which makes up about 10% of

the corn grain, is the outer husk of the grain. It is produced as a by-product in the wet-milling procedure of the corn grain. It consists mostly of carbohydrates, which amount to more than 70% of the corn fiber. The most easily accessible carbohydrate component is starch (about 20%), the amount of which depends on the corn species and the applied procedure. The relative amounts of the other components are constant in a given corn species. The residual 50% polysaccharide consists of hemicellulose (~35%) and cellulose (~15%), which together with the aromatic lignin (~2-4%) form the lignocellulosic structure, which is rather difficult to decompose. It is noteworthy that corn fiber contains valuable oils and proteins, as well (~4%, ~10%, respectively).

4. EXPERIMENTAL METHODS

Elimination of the starch fraction

The dry matter content of the slurry was 10%, the pH was adjusted to 4.5 with 0.05 M acetate buffer α -amylase Termamyl Supra enzyme was used (15 IU/g fiber). A two-step hydrolysis was performed in a pressure cooker (1 hour at 120°C followed by 1 hour at 90°C).

Pre-treatments

The destarched corn fiber was submitted to different alkali pre-treatments, in which the applied alkali solution (1 and 2% KOH or NaOH) and the time of pre-treatment (30, 60, 90 minutes) were optimized in the presence of 0.5% H₂O₂ and without the peroxide agent. The polymeric hemicellulose-B fraction (CFG) was precipitated from the alkali solutions with EtOH. The structure and composition of the CFG samples were determined with classical analytical methods (determination of ash content, protein content by Dumas-method, lignin and polysaccharide content with Hågglund-method) and hyphenated thermoanalytical techniques (thermogravimetry/mass spectrometry and pyrolysis-gas chromatography/mass spectrometry).

Cellulose hydrolysis and fermentation

The enzymatic hydrolysis of cellulose was performed at a pH of 4.8 at 50°C and 250 rpm. 15 FPU/g cellulose Celluclast 1.5 L and 7.5 IU/g cellulose Novozyme 188 enzymes were used. The 1 and 2% KOH and NaOH pre-treated corn fiber samples were used in the ethanol fermentation experiments (SSF). Solka Floc and destarched corn fiber samples were used as reference materials. The experiments were performed in acetate buffer with 25 FPU/g cellulose Celluclast 1.5L and 25 IU/g cellulose Novozyme 188 enzymes at 37°C for 48 hours. The slurry with 5% dry matter content was stirred at 100 rpm. Corn fiber samples with a polysaccharide concentration equal to 5 g/L glucose were used in the fermentation experiments with *Trichoderma reesei* enzyme. The fermentation was

performed at a pH of 5.5 for 5 days at 30°C with continuous stirring at 350 rpm. Solka Floc was used as reference carbon source.

Preparation and testing of bioplastics

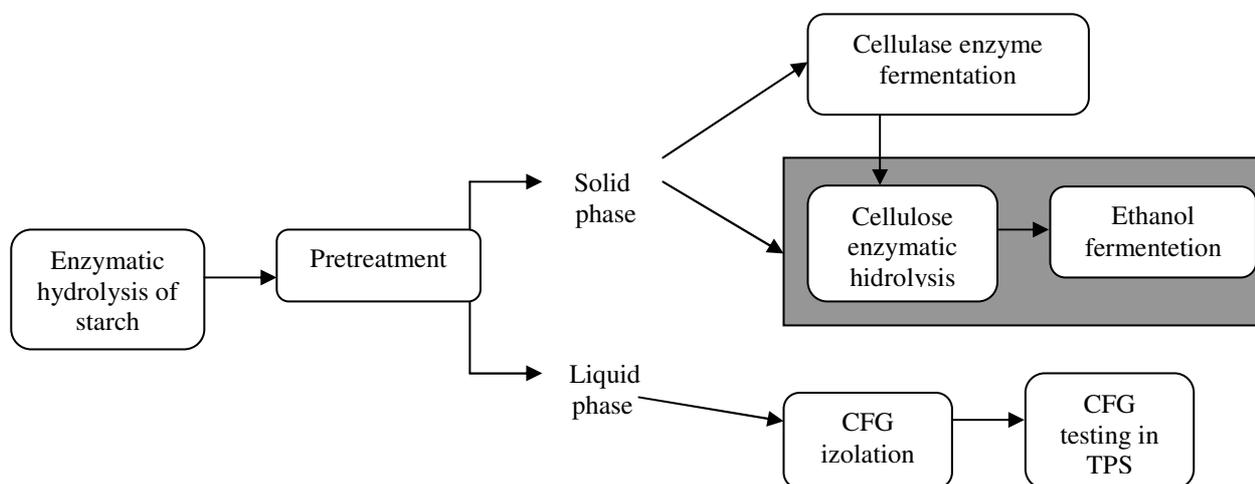
Mixtures consisting of 70 g dry corn starch, 30 g glycerol and 10 g additive were pressed into plates under pressure at 160°C. Dumbbell specimen were cut from the pressed plates, and were used for mechanical testing. The dumbbell specimen were used for mechanical testing, whereas the pieces remained after cutting were used for studying enzymatic degradability and water absorption.

For the evaluation of the results *Statistica v8* was used.

5. RESULTS

5.1. Pre-treatment of corn fiber

I managed to eliminate 99.8% of the starch fraction of corn fiber, which is the first step of process shown in the figure. As a result of the hydrolyzation of starch, the glucan content of corn fiber decreased from 41.8% to 20.4% (the latter value is due to the presence of cellulose in DCF). As no other components of the corn fiber were eliminated in the first step, their relative amounts increased in the DCF sample.



The destarched corn fiber was pre-treated with alkali solutions under different circumstances. First, the effect of 0.5% hydrogen peroxide on the yield and purity of the solid products of 2.5% NaOH pre-treatment was studied. In addition, the pre-treatment time needed at 120°C was also optimized. The highest hemicellulose yield, which was 83.5%, was obtained in the pre-treatment in the presence of peroxide at 120°C for 120 min. The experiments performed at 120°C for 120 min with NaOH solution and at 120°C for 90 min with NaOH solution in the presence of peroxide were

equally successful. 51.6% of the hemicellulose content of the alkali solution used for the pre-treatment was precipitated with ethanol in the form of solid CFG. The statistical evaluation of the experiments performed in the presence of peroxide indicates that the time of pre-treatment influences the efficiency in solving the hemicellulose and the isolation of CFG. On the other hand, the peroxide applied in the pre-treatments does not have a significant role on hemicellulose solubilization and CFG precipitation.

Py-GC/MS and TG/MS were used to study the structure of CFGs obtained from the solutions after alkali and alkaline peroxide pre-treatment. It was established that the main pyrolysis products were released in the thermal decomposition of polysaccharides, and most of these carbohydrate peaks corresponded to hemicellulose decomposition compounds. Both samples contained smaller amounts of lignin, fatty acid and phytosterol pyrolysis products. In spite of the similar pyrograms, the amounts of the polysaccharide decomposition products were different in the case of the two samples. In addition, the amount of impurities (fatty acids, lignin derivatives and aromatic compounds) in the precipitated hemicellulose samples was smaller if peroxide was present in the pre-treatment step. These results suggest that the presence of peroxide has a significant effect on the structure and purity of the isolated polymeric hemicellulose.

The composition of the residual solid fibers obtained after the pre-treatments was also determined. The cellulose content of these samples has subsequently been hydrolysed with cellulase enzymes. According to the results, 91% of the cellulose content of the alkali and alkaline peroxide pre-treated fibers was saccharified. The accessibility of cellulose improved to a great extent compared to the original and destarched samples. It has been established that the addition of peroxide in the pre-treatment step does not significantly influence the enzymatic degradability of the pre-treated fibers.

The corn fiber fractions pre-treated with two different solutions for three different time periods (6 different samples) were used as carbon sources in cellulase enzyme fermentation with *Trichoderma reesei* Rut C30 strain. Corn fiber (CF), destarched corn fiber (DCF) and Solka Floc – SF, which is a widely used carbon source in cellulase enzyme fermentation, were used as reference materials. β -glucosidase and filter paper (FPA) activities of the produced enzymes were determined in the supernatant after 5 days of cultivation. No significant differences were observed in the filter paper activities of the enzymes grown on pre-treated fibers. The average enzyme yield was 0.61 FPU/ml, which is 19.7 and 26.2% higher compared to the feedstock corn fiber (0.49 FPU/ml) and destarched corn fiber (0.45 FPU/ml), respectively. The alkali and alkaline peroxide pre-treated samples behaved similarly in cellulase enzyme production, hence the addition of peroxide in the pre-treatment step did not influence enzyme fermentation. On the other hand, the time of pre-treatment

was decisive. The characteristics of the β -glucosidase activities were similar to the filter paper activities. The only difference is that a higher β -glucosidase activity was observed with the CF sample, which can probably be attributed to the fact that starch increases the production of the given enzyme.

Based on the successful results reached in the pretreatments, further optimization experiments were performed in which the type of the alkali was KOH and NaOH without oxidativ agent, and the concentration of the alkali solutions were decreased from 2.5% to 1 and 2%. The temperature and the time of the pretreatment were 120°C and 1 hour. The liquid fractions were separated by centrifugation in contrast to the previous experiments. I managed to dissolve hemicellulose with all used alkali solutions with a high yield. The 2% NaOH solution proved to be the best: 88% of the hemicellulose was dissolved; subsequently, 82.4% of the dissolved hemicellulose could be isolated from the supernatant (based on the hemicellulose content of the fiber). According to the results of the thermoanalytical experiments the type and concentration of the alkali solution influence the solubilization of hemicellulose and the isolation of CFG, however, they do not have a significant effect on the structure of CFG. I have worked out a pre-treatment with which the efficiency of CFG isolation could be increased from 44% (data available in previous research papers) to 82.4%.

The 1 and 2% KOH and NaOH pre-treated corn fibers were studied in simultaneous saccharification and fermentation (SSF). The conversion of the cellulose content of the pre-treated samples to ethanol was 90-97%, which is about four times higher compared to the conversion obtained with the original corn fiber. The ethanol conversion of the destarched fiber was only 24%, since owing to the lignocellulosic structure only a part of the cellulose content was accessible to the cellulase enzyme, presumably only the cellulose chains on the outer surface could be saccharified to glucose. SSF experiments performed with the Solka Floc positive reference material resulted in an ethanol yield of only 54%.

5.2. Improvement of the characteristics of thermoplastic starch

The tensile strength of the unfilled sample was 9.00 MPa, whereas the addition of zein and hemicellulose increased the tensile strength to 11.50 and 10.37 MPa, respectively. With these two additives the tensile strength proved to be better even compared to the positive polycaprolactone containing reference material, which is available in the market (3.70 MPa). The tensile strength of the cellulose filled TPS sample (3.35 MPa) was comparable to that of the polycaprolactone filled one, and was considerably lower compared to the plastic prepared without additive. On the other

hand, the mechanical data of both the additive free and polycaprolactone containing bioplastics are comparable to TPS plastics available in the market. I have been the first who studied TPS-based plastics prepared with zein and hemicellulose additives. It was found that these additives improved the mechanical characteristics of TPS significantly.

The application of the two novel additives - zein and hemicellulose - resulted in 20 - 30% lower water absorption at relative humidities of 44 - 100% compared to the unfilled TPS blend. I have been the first who proved that the use of zein and hemicellulose resulted in lowering the water uptake of the TPS systems to a value comparable to that of the reference polycaprolactone containing TPS plastic.

The enzymatic degradability of the starch content of the prepared samples was also studied. The aim was to lower the initial rate of degradation with the help of the selected additives. I managed to hydrolyse 87.7% of the starch content of the unfilled sample during a 72-hours enzymatic hydrolysis. In the case of the use of hemicellulose additive 57.2% of the starch was saccharified, whereas an additional 21 and 31% of the starch was dissolved in the case of the polycaprolactone and protein containing samples, respectively. According to the results, the addition of hemicellulose resulted in lowering the decomposition rate compared to the negative (TPS without additive) and positive (polycaprolactone additive used) reference materials.

As I have been the only one who studied zein and hemicellulose additives in TPS systems, I have performed additional detailed experiments with these additives. At this time, however, the samples were smelted with plasticorder after being mixed by hand. The amount of glycerol was optimized with a constant starch: additive ratio. As reference TPS samples without additives were used. The 32% glycerol containing sample had the highest tensile strength (17.7 MPa) in the reference series. Among the samples containing hemicellulose and zein additives, the TPS systems containing 31% glycerol had the highest tensile strength, 19.5 and 16.9 MPa, respectively. The enzymatic degradation of these samples was further studied. The results show that the rate of degradation decreased to a great extent due to the perfect mixing and smelting. Only 7.65% of the starch content of the unfilled sample was saccharified after 72 hours of hydrolysis, which is only slightly higher compared to the zein containing sample. Only 4.85% of the starch content of the hemicellulose composite could be decomposed, which is 36.6% better compared to the additive free TPS. These results indicate that the application of hemicellulose additive, which I have used first, results in a decrease in the initial rate of degradation.

6. THESES

- I. I have managed to develop a process with which the efficiency of CFG isolation from corn fiber increased from 44% [Doner, L.W. 2001] to 82.4% [Gáspár, M. 2007]. I managed to precipitate 95% of the hemicellulose content of the solution after pre-treating the destarched corn fiber with 2% NaOH solution for 1 hour at 120°C.
- II. I have been the first who combined CFG isolation with the alkali pre-treatment of the corn fiber. As a result, the enzymatic accessibility of the corn fiber increased to >91% (2.5% NaOH solution or 2.5% NaOH solution with 0.5% peroxide, 1 hour pre-treatment at 120°C), the ethanol yield based on the cellulose content was >95% (PCFs prepared at 120°C for 1 hour with 1 and 2% KOH and NaOH solutions). In addition, I have been the first who proved that the residual fiber of the alkali pre-treatments is an excellent carbon source in cellulase enzyme fermentation. (0.61 FPU/ml FPA enzyme and 0.28 IU/ml β -glucosidase enzyme could be produced using corn fibers pre-treated with 2.5% NaOH or 2.5% NaOH solution with 0.5% peroxide for 30, 60 and 120 minutes at 120°C as carbon sources) [Gáspár, M. 2003; Gáspár, M. 2007; Gáspár, M. 2005a].
- III. I have established that the addition of 0.5% hydrogen peroxide in the alkali pre-treatment of corn fiber does not have a significant role in determining the yield of hemicellulose (CFG). Nevertheless, the data of classical analytical methods and thermoanalytical analysis (Py-GC/MS and TG/MS), which I have used first to study the composition and structure of CFG samples, confirm that the purity of CFG increases if hydrogen peroxide is present in the pre-treatment step [Gáspár, M. 2008].
- IV. I have been the first who studied the effect of CFG additives in thermoplastic starch composites. I have found that the addition of 10% CFG increases the tensile strength compared to the TPS blend prepared without additive. In addition, I have succeeded in lowering the initial degradation rate of the starch content, which is advantageous with respect to the biodegradation characteristics of TPS composites [Gáspár, M. 2005b; Gáspár, M. 2009].
- V. I have been the first to study the effect of zein additive in thermoplastic starch composites. I have found that the addition of 10% zein increases the tensile strength and lowers the water absorption compared to the unfilled TPS sample. [Gáspár, M. 2005b; Gáspár, M. 2009].

7. POSSIBLE FIELDS OF APPLICATION

The alkali fractionation that I have studied in my work offers an excellent possibility for the utilization of corn fiber, which is a by-product of corn grain wet milling process. The elimination of the starch content followed by alkali pre-treatment renders the separation of cellulose and hemicellulose polysaccharides possible. The technology is easy to use, and can be well inserted into the industrial processing of corn grain. 3000 tons of corn fiber are produced daily at Hungrana Starch and Isosugar Manufacturing and Trading Co. Ltd. (Szabadegyháza, Hungary), and a small amount of which is used currently as mixing component of animal feed product. The price of 1 ton of corn fiber to be used as forage is about 18000 Ft at present.

If 1 ton of corn fiber was processed according to the technology, which I have outlined in my work, the income would work out as follows. The 200 kg starch content of the corn fiber could be used for the production of about 102 kg of ethanol (taking into consideration that the factory produces ethanol from starch with about 90% efficiency at present). The 150 kg cellulose content could be used for the production of about 81 kg of ethanol following alkali fractionation. The wholesale price of bioethanol produced from glucose is about 200 Ft/l, which means that the 183 kg ethanol, which could be produced from the corn fiber would mean an income of 46300 Ft. The price of CFG varies from 376 Ft/kg (US data) to tenths of thousands of forints depending on the purity and size of the polymer. About 287 kg of CFG could be isolated from 1 ton of corn fiber, which would mean an additional income of 108000 Ft/day (the calculation is based on the 376 Ft/kg price). This simplified calculation, taking into consideration that the applied procedures require a very small amount of chemicals (the 48% NaOH has a wholesale price of 120 Ft/kg at BorsodChem at present), implies that the procedure can be very advantageous economically. The procedure could be rendered more economic if a part of the ethanol prepared from the pre-treated fibers was used for CFG isolation, and after the precipitation of the polymeric hemicellulose the ethanol was recovered by distillation or membrane technology. A further incentive for the on-spot processing of corn fiber is that the factory produces enzymes that are required for starch saccharification and *Saccharomyces cerevisiae*, which is needed for ethanol fermentation.

8. PUBLICATIONS

This thesis is based on the following publications:

- I. M. Gáspár, T. Juhász, K. Réczey, Fractionation and utilization of corn fibre carbohydrates, *Process Biochemistry*, 2005a, 40/3-4, pp. 1183-1188. IF: 2,336
- II. M. Gáspár, G. Kálmán, K. Réczey, Corn fiber as a raw material for hemicellulose and ethanol production, *Process Biochemistry*, 2007, 42/7, pp. 1135-1139. IF: 2,336
- III. E. Mészáros, M. Gáspár, K. Réczey, E. Jakab, G. Várhegyi, Thermal behavior of corn fibers and corn fiber gums prepared in fiber processing to ethanol, *Journal of Analytical and Applied Pyrolysis*, 2009a, 85, pp. 11-18. IF: 2,120
- IV. M. Gáspár, K. Réczey, Kukorica rost poliszacharidok hasznosítása frakcionálással, *IX. Nemzetközi Vegyészkonferencia proceeding*, Kolozsvár, Románia, 2003. pp. 76-80.
- V. M. Gáspár, Zs. Benkő, G. Dogossy, K. Réczey, T. Czigány, Reducing water absorption in compostable starch-based plastics, *Polymer Degradation and Stability*, 2005b, 90, pp. 563-569. IF: 2,073
- VI. M. Gáspár, D. Dienes, K. Réczey, P. Müller, B. Pukánszky, Influencing the properties of TPS by natural additive polymers derived from corn fiber, *Cost 868 meeting on Biotechnology and Biopolymers in Textile, Packaging, Cosmetics and Medical Applications*, Poszter, Isztambul, Törökország, 2009b. február 19-20.

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M. Gáspár, Á. Sárdi, K. Réczey, Hemicellulose-B izolálása kukorica rostból, *Műszaki Kémiai Napok'03 Proceeding*, Veszprém, 2003., pp. 380-385.

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E. Mészáros, M. Gáspár, K. Réczey, E. Jakab, G Várhegyi, Thermal analysis and pyrolysis techniques for studying the composition of differently pretreated corn fibers in fiber processing to ethanol, *Centenáriumi Vegyészkonferencia*, Sopron, 2007. május 29. - június 1.

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