

COMPARISON OF SACCHARIFICATION METHODS FOR BIOTECHNOLOGICAL UTILIZATION OF WHEAT BRAN

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Abstract

This study compares various methods used for the hydrolysis of lignocellulosic waste materials in terms of sugar yield and the content of inhibiting substances. Wheat bran was used as the lignocellulosic material, and the following hydrolysis methods were investigated: one-step alkaline hydrolysis, repeated alkaline hydrolysis, acid hydrolysis, and a combination of these methods with enzymatic hydrolysis. The combination of acid and enzymatic hydrolysis yielded the highest sugar yield, with a concentration of sugars in the hydrolyzate reaching up to 60 g/L and a yield of around 90%. The total concentration of phenolic substances, which can act as inhibitors during further biotechnological processing of the hydrolyzate, was approximately 270 mg/L GAE when using acid hydrolysis combined with enzymatic hydrolysis.

Keywords: *wheat bran, lignocellulose, saccharification, hydrolysis, biorefinery, circular economy*

1. INTRODUCTION

Earth receives more than 3.7 million EJ/year of solar energy. The amount of energy stored in plants through the process of photosynthesis corresponds to approximately 10 times the global energy demand [1]. Approximately 50% of the plant material produced through photosynthesis is composed of lignocellulose. Lignocellulose is a complex structural material composed of three main components: cellulose, hemicellulose, and lignin. Cellulose, which constitutes about 40-50% of the material, is the primary component of lignocellulose. It is a long-chain polymer composed of glucose units. Cellulose provides structural strength to plant cells and is organized into fibrils that form a rigid network. Hemicellulose is a heterogeneous polymer consisting of various sugars, including xylose, mannose, glucose, and galactose. It makes up approximately 20-35% of lignocellulose. Hemicellulose has a more amorphous structure compared to cellulose and helps bind cellulose fibrils together. Lignin, which accounts for approximately 20-30% of lignocellulose, is a complex aromatic polymer. It provides rigidity and hydrophobicity to plant cell walls. Lignin is composed of various phenolic monomers and is responsible for the woody characteristics of lignocellulosic materials. The presence of lignin confers resistance to degradation in lignocellulosic biomass [2].

Lignocellulosic biomass can be derived from various sources, including agricultural residues (e.g., corn stalks, wheat straw, sugarcane bagasse), forestry residues (e.g., wood chips, sawdust, bark), dedicated energy crops (e.g., switchgrass, miscanthus), and certain non-food crops (e.g., hemp, jute). The annual production of waste materials based on lignocellulose from agricultural raw materials amounts to around 10^8 tons [3]. The extensive production of these materials presents a significant waste management challenge. A significant portion of the produced waste lignocellulosic biomass is commonly disposed of through the simplest method: waste incineration. However, globally, the widespread incineration of lignocellulosic waste material is increasingly contributing to an ecological disaster, primarily due to the substantial release of carbon dioxide into the atmosphere. Meanwhile, lignocellulosic material can be processed using alternative methods instead of simple incineration. Chemical or enzymatic processing of lignocellulosic biomass allows for the creation of numerous value-added products, including biofuels, bio-based chemicals, materials, and bio-plastics [4,5]. To facilitate this, an increasing number of industrial plants, known as biorefineries, are being constructed. This progress in lignocellulosic biomass processing is also motivated by many EU states promoting the circular economy principle as a key concept in sustainable development. The circular economy aims to maximize the value of products, materials, and resources by keeping them in the economic cycle for as long as possible and returning them to the production cycle at the end of their life, while minimizing waste generation. However, it is

important to emphasize that the efficient conversion of lignocellulosic biomass poses some challenges due to its complex structure and resistance to breakdown. Pre-treatment techniques are used to disrupt the lignocellulosic matrix and enhance the accessibility of cellulose and hemicellulose for subsequent conversion processes. One of these crucial processes is saccharification [6]. Saccharification involves the breakdown of polysaccharides into simple sugars, specifically glucose or other monosaccharides. Solutions with high concentrations of these monosaccharides can be subsequently utilized by microorganisms for the production of various essential substances [7].

This work deals with the processing of wheat bran. Wheat bran, besides other valuable compounds such as wheat germ and parts of the endosperm, remains a major by-product during the milling process. Wheat processing mills can produce up to 50 tons of bran per day and currently there is an interest in processing this material by other methods than, for example, anaerobic digestion. Within the concept of the intended biorefinery processing wheat bran, we focused on the efficiency of saccharification of this lignocellulosic material using alkaline, acid and enzymatic hydrolysis.

2. MATERIALS AND METHODS

2.1. Materials

The wheat bran was provided by Mlýny Voženílek from Předměřice nad Labem (Czech Republic). Chemicals used in this study included sulphuric acid (Analytika, Czech Republic) and sodium hydroxide (Lachner, Czech Republic). Cellulases were used as enzymes (Cellulase, enzyme blend Cellic CTec2 and Amyloglucosidase from *Aspergillus niger*, Sigma-Aldrich, Germany). Acetonitrile, methanol and formic acid (HPLC grade) were purchased from Sigma-Aldrich, Germany. Ferulic acid, sinapic acid, coumaric acid, caffeic acid, gallic acid, dinitrosalicylic acid, glucose, xylose and arabinose (with purity >99%) were purchased from Sigma-Aldrich, Germany. Folin-Ciocalteu reagent was purchased from P-Lab, Czech Republic. Water used in this study was ultra-pure water with $>18.2 \text{ M}\Omega \text{ cm}^{-1}$ resistance (Elga, Veolia, France). The samples were filtered through a syringe nylon filters (0.45 μm pores, Chromspec, Czech Republic).

2.2. Methods

Alkaline hydrolysis was performed with 0.5 M NaOH for 4h at 50°C and stirring at 170 rpm. Acid hydrolysis was performed with 1% H₂SO₄ in an autoclave at 130°C for 40 min. The amount of 100g of wheat bran per 1 liter of solution was always used for hydrolysis. Enzymatic hydrolysis took place for 24 hours on a laboratory shaker at a temperature of 50°C and stirring at an intensity of 170 rpm. Before enzymatic hydrolysis, the pH of the hydrolysates after alkaline or acid hydrolysis was adjusted to a value of 5, which was optimal for the selected mixture of enzymes. Individual sugars were analyzed using high performance liquid chromatography (Agilent 1260, Agilent, USA) with Evaporative Light Scattering Detector (Agilent, USA). Waters Carbohydrate NH₂ column (Waters, USA), 300 mm long with particle size of 3.9 μm was used as a stationary phase. As a mobile phase, mixture of acetonitrile and water (volume ratio 80:20) was used. Sample injection volume was 5 μL and the column temperature was maintained at 30°C using a column thermostat. Individual phenolic compound were analyzed using high performance liquid chromatography (Agilent 1260, Agilent, USA) with Diode array detector (Agilent, USA). Kinetex EVO C18 column (Phenomenex, USA), 250 mm long with particle size of 5 μm was used as a stationary phase. As a mobile phase, mixture of acetonitrile and water (volume ratio 20:80) was used. Sample injection volume was 5 μL and the column temperature was maintained at 35°C using a column thermostat. Total carbohydrates were determined by dinitrosalicylic method [8]. Total phenolic compounds content was determined according to Folin-Ciocalteu method using gallic acid as the standard [9]. Spectrophotometric measurement was performed on Specord Plus UV-VIS spectrophotometer (Analytic Jena, Germany)

3. RESULTS AND DISCUSSION

The composition of wheat bran can vary slightly depending on factors such as the variety of wheat and processing methods. In Table 1 there is a general overview of the composition of wheat bran. The amount of total carbohydrates in wheat bran is reported to be between 600-750 mg/g.

Table 1. Chemical composition of wheat bran [10-23]

Component	Content [%]	Component	Content [%]
ash	2.4–8.1	glucose	21.4–39.4
protein	13.5–17.7	xylose	10.5–18.9
lignin	3.0–10.2	arabinose	7–12
starch	8.8–34.2	galactose	1.2–1.5
cellulose	11	mannose	0.7
β-glucans	2.2–2.8	ferulic acid	0.5–1.5
fructans	3.4–4.0		

Bataillon et al. [20] stated in their work that the yield of arabinose and xylose from bran after alkaline hydrolysis is 40%. Considering the content of xylose and arabinose in wheat bran, it should be realistic to obtain a combined total of 65-122 mg of arabinose and xylose from 1 gram of bran through alkaline hydrolysis. The investigation of wheat bran provided by mills revealed that alkaline hydrolysis yielded 61 mg/g of arabinose and 12 mg/g of xylose (Figure 1), consistent with literature findings. Assuming a similar yield of glucose from alkaline hydrolysis at 40%, it should be realistic to obtain 51-112 mg of glucose from 1 gram of wheat bran. The yield of glucose from alkaline hydrolysis of bran was 72 mg/g (Figure 1), corresponding to the estimated theoretical yield. The total concentration of sugars in the solution after alkaline hydrolysis was 5.9 g/L. To determine the possibility of obtaining a higher proportion of sugars through repeated alkaline hydrolysis, the wheat bran residue underwent a second alkaline hydrolysis. It was found that by repeating the alkaline hydrolysis, it is possible to double the yield of carbohydrates (Figure 2). The total concentration of sugars in the solution after alkaline hydrolysis was 15.2 g/L.

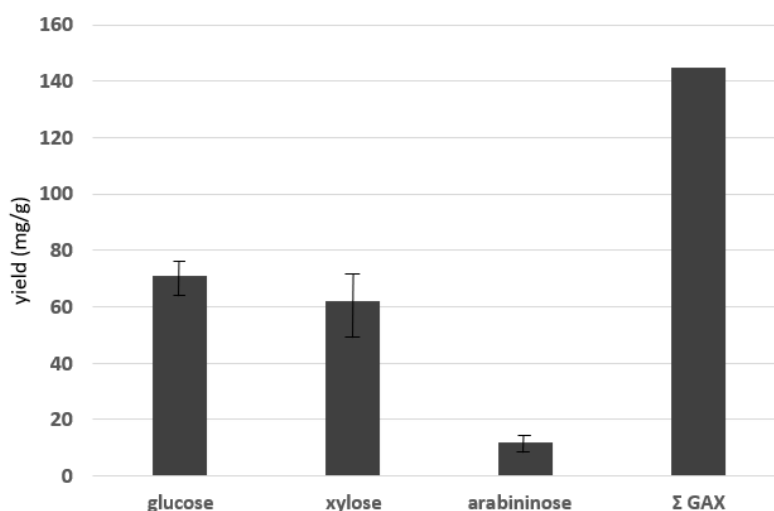


Figure 1. Yields of sugars from alkaline hydrolysis

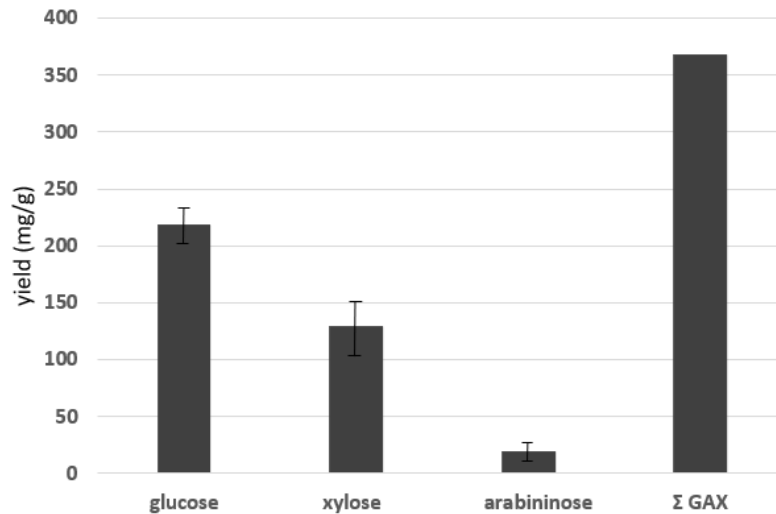


Figure 2. Yields of sugars from repeated alkaline hydrolysis

As can be seen in Figure 3, acid hydrolysis resulted in slightly increased sugar release in comparison with alkaline hydrolysis. Demirel et al. [24] and Zhao et al. [10] reports up to 60% hydrolysis efficiency using dilute sulfuric acid, which is consistent with the results achieved. By acid hydrolysis it was possible to obtain a solution with a concentration of total sugars of 20.7 g/L.

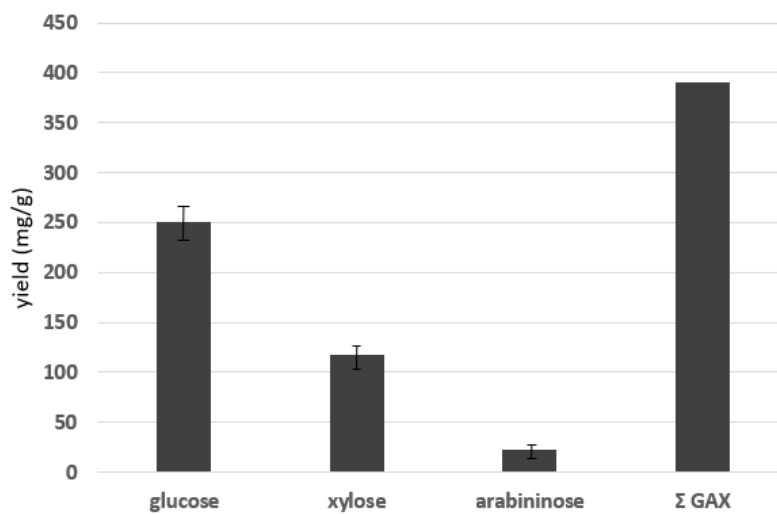


Figure 3. Yields of sugars from acid hydrolysis

Integrating alkaline hydrolysis with enzymatic hydrolysis improved the sugar yield and concentration of total sugars in final hydrolysate (Figure 4). The whole process was more efficient as repeated alkaline hydrolysis, with the concentration of sugars in the hydrolyzate reaching 39.8 g/L. The increase in saccharification efficiency is caused by the removal of part of the lignin from the structure of wheat bran after alkaline hydrolysis and the subsequent increase in the porosity of the material. The increase of porosity subsequently enables more efficient enzymatic hydrolysis.

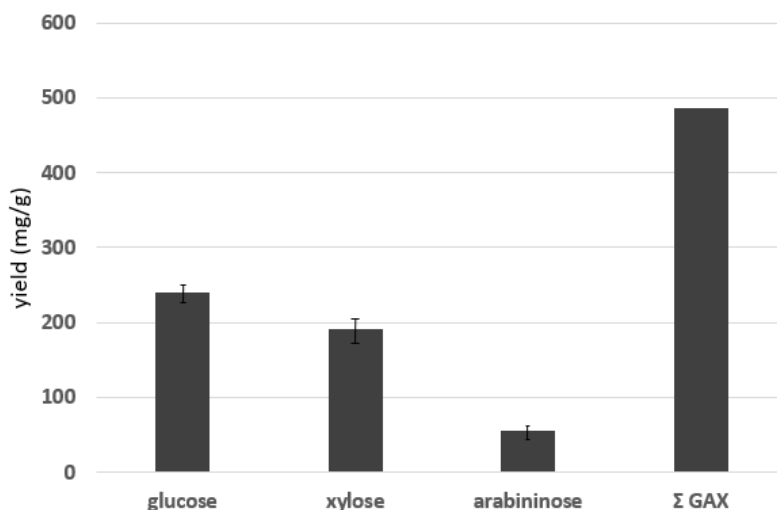


Figure 4. Yields of sugars from combined alkaline and enzymatic hydrolysis

The highest yield of carbohydrates was recorded using acid hydrolysis followed by enzymatic hydrolysis (Figure 5). With this wheat bran hydrolysis procedure, it was possible to obtain a solution with a sugar concentration of up to 60 g/L. Considering the total carbohydrate content of wheat bran of 600 to 750 mg/g, a yield of 80 to 95% was achieved by this hydrolysis method.

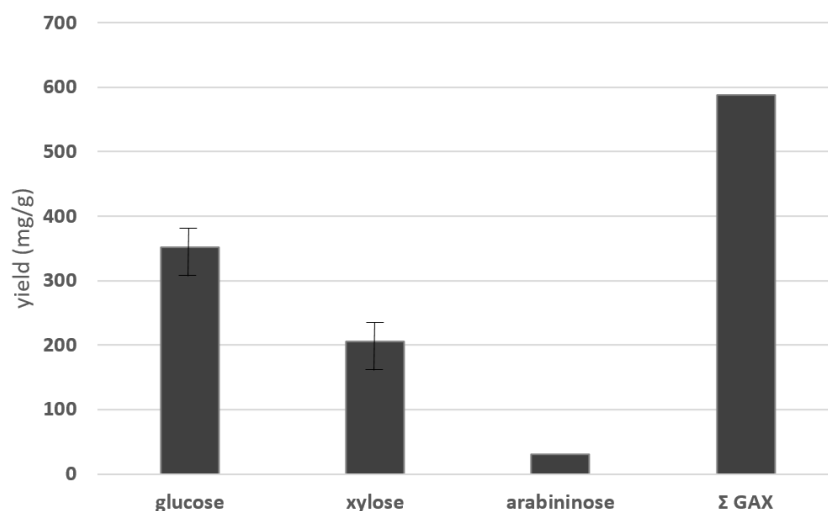


Figure 5. Yields of sugars from combined acid and enzymatic hydrolysis

During the saccharification of lignocellulosic materials, various inhibitors are produced as by-products of the process [25]. These inhibitors, primarily including vanillin, syringaldehyde, and ferulic acid, can hinder the efficient conversion of sugars during the biotechnological production of various chemicals. After saccharification, the following phenolic substances were identified in the solution: ferulic acid, sinapic acid, coumaric acid, and caffeic acid. Ferulic acid reached the highest concentration in the sugar solution, up to 155 mg/L. The concentration of sinapic acid reached up to 22 mg/L. Other phenolic acids were present in amounts less than 5 mg/L. When determining the total concentration of phenolic substances, a significant difference was found between alkaline hydrolysis followed by enzymatic hydrolysis and acid hydrolysis followed by enzymatic hydrolysis. While the concentration of total phenolics after alkaline hydrolysis followed by enzymatic hydrolysis was 458±42 mg/L GAE, in acid

hydrolysis followed by enzymatic hydrolysis, the concentration of total phenolics in solution was 271 ± 35 mg/L GAE. However, the total concentration of phenolics found after alkaline hydrolysis followed by enzymatic hydrolysis may be overestimated by the higher concentration of proteins in the solution after alkaline hydrolysis.

4. CONCLUSIONS

This work demonstrates that the optimal method for hydrolyzing wheat bran to obtain a sugar solution suitable for further biotechnological use is a combination of acid hydrolysis with enzymatic hydrolysis. This method achieved a high sugar yield of around 90% and produced hydrolysates with a sugar concentration of up to 60 g/L. However, the hydrolyzate contains phenolic substances, particularly ferulic acid, which may pose challenges for further use of hydrolyzate. Additionally, other potential inhibitors like furfural and 5-hydroxymethylfurfural may be present in the hydrolyzate, but their concentrations were not monitored in this study.

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