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Investigations on a wheat bran biorefinery involving organosolv fractionation and enzymatic treatment

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HIGHLIGHTS

- Ethanol organosolv fractionation of wheat bran was investigated in detail.
- Enzymatic hydrolysis of the obtained fractions gave a glucose yield of 75%.
- Proteins could be extracted almost quantitatively.
- During the lignin precipitation step, proteins partially coprecipitated.

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ABSTRACT

The present study elucidates the organosolv treatment of wheat bran, the major by-product of the milling industry. The influence of temperature (160–200 °C) and ethanol concentration (30–60% (w/w)) at a given process time of 30 min was investigated. Enzymatic treatments of the organosolv extracts including solid residues led to an overall glucose yield of 75%. The conversion of hemicelluloses into xylose and arabinose was approximately 60% and 45%, respectively. Proteins could be almost completely dissolved, however, practically no free amino acids were obtained. Surprisingly, only around 30% of lignin and 65% of minerals were dissolved. Severe treatment conditions induced the disintegration of fat into glycerol and fatty acids as well as the formation of sugar degradation products. During the lignin precipitation step, proteins partially coprecipitated.

1. Introduction

As the world faces the progressive depletion of fossil resources, the conversion of biomass within biorefineries is seen as a possibility to essentially contribute in particular to the continuous supply of materials in the future. In order to avoid the competition with food production, the utilization of raw materials of high lignocellulosic content is an interesting option. Besides wood and non-food crops, agricultural residues like straw and corn stover as well as other by-products of various origins are of high interest as feedstocks. Wheat bran represents such a by-product, which accrues in enormous quantities during the production of white wheat flour. It is estimated that 150 million tons are produced per year worldwide (Prückler et al., 2014). Currently wheat bran is mainly used as a low value ingredient in animal feed.

As biomass generally shows a complex composition, a separation into certain fractions can be advantageous for subsequent processing (Kamm and Kamm, 2004). In the case of wheat bran, its composition (Table 1) with relatively high levels of protein and starch as well as smaller contents of lignocellulose differs from that of typical lignocellulosic raw materials (Yu et al., 2008). While the glucose extraction from the starch of wheat bran by means of commercially available amylolytic enzymes is performed rather uncomplicated, the recalcitrance of the dense lignocellulosic fraction hinders its easy conversion into free sugars. In order to increase the accessibility of the cellulose for hydrolytic enzymes, a pretreatment seems to be inevitable. In this context, hydrothermal and acidic pretreatments of wheat bran were already investigated in a number of studies (Aguedo et al., 2013; Choteborska et al., 2004; Favaro et al., 2012; Kabel et al., 2002; Kataoka et al., 2008; Palmarola-Adrados et al., 2005; Reisinger et al., 2013; Rose and Inglett, 2010; Tirpanalan et al., 2014; van den Borne et al., 2012). The present work deals with the so-called ethanol organosolv treatment of wheat bran. This process offers the opportunity to separate the biomass into the fractions cellulose, hemicellulose

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Table 1
Chemical composition of the untreated wheat bran.

Analyte	g per 100 g dry mass	Amino acid	g per 100 g dry mass
Total carbohydrates	56.9	Aspartic acid	1.84
Starch	9.1	Threonine	0.64
Glucose (total)	25.4	Serine	0.78
Xylose (total)	20.3	Glutamic acid	3.62
Arabinose (total)	9.3	Proline	1.05
Galactose (total)	1.9	Glycine	1.08
Crude protein	13.2	Alanine	0.96
Klason lignin	8.0	Valine	0.80
Ash	7.0	Isoleucine	0.52
Crude fat	4.3	Leucine	1.10
		Tyrosine	0.52
		Phenylalanine	0.67
		Histidine	0.51
		Lysine	0.78
		Arginine	1.52

and lignin due to differences in their solubility in a mixture of water and an organic solvent, commonly ethanol (Zhao et al., 2009). In the case of wheat bran, this technique could also lead to an extraction of ethanol soluble proteins, in particular prolamin (Idris et al., 2003). By reducing the ethanol concentration after the pretreatment, lignin can be precipitated, which represents an additional advantage of this process. Based on a detailed characterization of the obtained fractions, in the end, the present study enables a comparison between the hydrothermal (Reisinger et al., 2013) and the organosolv treatment of wheat bran.

2. Methods

2.1. Raw material

Wheat bran was kindly provided by the VonWiller mill (Schwechat, Austria) and directly used without further comminution.

2.2. Organosolv fractionation

The organosolv pretreatment was performed in a 2 L pressure-tight agitator vessel with a double jacket heated by thermal oil (Kiloclave type 3, Büchiglasuster, Switzerland). For each trial, 1160 mL ethanol/water-solvent (30%, 45% and 60% (w/w) ethanol) were added to 290 g wheat bran. The slurries were heated to the target temperatures (160–200 °C) and held for a reaction time of 30 min. During the whole procedure, the slurries were continuously stirred at 400 rpm. The obtained mixtures were centrifuged at 7000 rpm equal to an rcf of 10,722*g* (Sorvall Evolution RC), and the lignin was precipitated from the supernatants by dilution with deionized water to a final ethanol concentration of 10% (w/w). The lignin precipitate was separated by centrifugation under above described conditions.

2.3. Enzymatic hydrolysis

In order to gain monomeric sugars, pretreated lignocellulosic biomasses have to undergo an additional hydrolysis step, in this work by application of amylolytic, cellulolytic and hemicellulolytic enzymes. Cellulase (0.017 units/mg), endo-1,3(4)-ß-glucanase (0.017 units/mg) and endo-1,4-ß-xylanase (1 unit/mg) from *Trichoderma longibrachiatum* were obtained from Sigma Aldrich. The starch degrading commercial enzymes Termamyl 120 L and AMG 300 L were received from Novozymes. An enzyme cocktail displaying concentrations of 0.04 g/mL cellulase, 0.04 g/mL ß-glucanase, 0.01 g/mL xylanase, 5 µL/mL Termamyl and 5 µL/mL AMG, dissolved in sodium citrate buffer (0.1 mol/L, pH 4.8), was used.

Both the solid residue of the organosolv pretreatment and the supernatant of the lignin precipitation step were used for enzymatic hydrolysis. The former was first washed four times with ethanol at concentrations equivalent to those used for the organosolv pretreatment itself and then once with deionized water. To 1 g washed residue, 1 mL of the enzyme cocktail and 9 mL sodium citrate buffer was added. This equals a cellulase activity of around 17 units per g cellulose. To 5 mL supernatant of the lignin precipitation, 1 mL of the enzyme cocktail and 4 mL sodium citrate buffer was added. Supplementation with 10 mg/mL of NaN_3 prevented microbial growth. The mixtures were prepared in duplicate and incubated for 72 h at 40 °C and shaken regularly. Since in the present study emphasis was paid on the organosolv fractionation, the enzymatic process was not further optimized. However, to ensure a high degree of carbohydrate hydrolysis, the enzymes were applied in excess.

2.4. Analytical methods

All measurements were performed with the supernatants of the centrifuged organosolv-slurries before and after the lignin precipitation step as well as with the enzymatically hydrolyzed samples. The mentioned fractions were used for analysis of free and total sugars, degradation- and by-products, free and total amino acids, dry matter and ash content, ion chromatography as well as for nitrogen determination using the Kjeldahl method. The protein content was calculated using a nitrogen-to-protein-factor of 5.26 (Tkachuk, 1969). Dry matter and the ash content were determined according to the Standard Laboratory Analytical Procedures (LAP) for biomass analysis provided by the National Renewable Energy Laboratory (Sluiter and Sluiter, 2011a,b). Unless stated otherwise, all analytical procedures were performed at least in duplicate. The accuracy of the obtained data was ±10%.

2.4.1. Carbohydrate and degradation product analysis

The sugar composition of the original bran, free and total sugar concentrations (glucose, xylose, arabinose and galactose) as well as the levels of degradation products (furfural, hydroxymethylfurfural, glycerol, acetic and levulinic acid) in solution were measured according to the Standard Laboratory Analytical Procedures (LAP) for biomass analysis provided by the National Renewable Energy Laboratory (Sluiter and Sluiter, 2011a,b). The sugars were separated using a Phenomenex Rezex RPM-Monosaccharide column, the degradation products using a Phenomenex Rezex ROA-Organic Acid column, each equipped with the appropriate precolumn. The starch content of the original bran was determined using the Megazyme Total Starch (AA/AMG) enzyme assay kit, according to AOAC 996.11.

2.4.2. Amino acid analysis

The amino acids were analyzed via HPLC using pre-column derivatization with ortho-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) and fluorescence detection (Cigic et al., 2008; Henderson and Brooks, 2010).

The mobile phases were prepared as follows: mobile phase A: 1.4 g anhydrous Na_2HPO_4, 3.8 g $Na_2B_4O_7{\cdot}10H_2O$ and 32 mg NaN_3 were dissolved in 1 L demineralized water, and the pH was adjusted to 8.2 with concentrated HCl; mobile phase B: a mixture of acetonitrile:methanol:water was prepared equal to a volumetric ratio of 45:45:10.

The following reagents were used for the derivatization procedure: 0.1 M borate buffer in water with pH 9.9; OPA-reagent: 10 g/L OPA dissolved in 0.02 M borate buffer (pH 9.9) with 0.8% of 3-mercaptopropionic acid; FMOC-reagent: 5 mg/mL FMOC