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Composition of corncobs as a substrate for fermentation of biofuels

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Abstract. Lignocellulosic residues as for instance corncobs can be used as substrates for the production of biofuels. The corncobs are usually pre-treated in a thermal-physical step (steam explosion) before enzymatic hydrolysis. The pre-treatment process increases the accessibility of the raw material for the hydrolysis. The products of the hydrolyses are used as substrates for the fermentation of bio-ethanol. As an alternative, the hydrolysates could be used for the fermentation of oleaginous yeasts. These yeasts produce and accumulate lipids from glucose during growth. For the energetic use in the form of bio-diesel, the lipids can be converted into fatty acid methyl esters (FAME) in the same way as vegetable oils.
The fibre composition of the raw material was determined in regard to the proportions of cellulose, hemicellulose und lignin. It was investigated whether different corn varieties show varying compositions. The fibre compositions before the pre-treatment of ten different varieties of corncobs (from locations in Upper Austria) were determined. No significant differences were observed (cellulose: 38.8% ± 2.5%, hemicellulose: 44.4% ± 5.2%, lignin: 11.9% ± 2.3% in the dry matter).

Key words: corncob, fibre composition, steam explosion, biofuels, oleaginous yeasts.

INTRODUCTION

Corncobs are an agricultural by-product which is currently used as substrate for combustion. Due to high contents of chlorine, the combustion has to take place in continuously monitored industrial power plants (Gebeshuber, 2011).

In 2012, a total of 219,702 ha (+1.2% compared to 2011) of grain maize were cultivated in Austria. The harvest was about 2,351,370 tonnes. The average yields were 10.7 t per ha. The area used for the cultivation of silage maize was 82,261 ha. Silage maize is shreddered shortly after harvesting, used for silage and can therefore not be used as a substrate for biofuels (Federal Ministry of Agriculture & Environment and Water Management (Lebensministerium), 2013).

As a renewable raw material, the corncobs from grain maize are a potential feedstock for the production of biogas, biodiesel and bioethanol to fulfil the increasing demand for biofuels (Ioannidou et al., 2009). Before its use as a substrate for fermentation processes, the raw material has to be pre-treated to increase the enzymatic accessibility; for example, with the established method of steam explosion (Zimbardi et al., 2002). The lignocellulosic structure is destroyed by treatment with high temperature and saturated steam in a reactor followed by a sudden pressure decrease

(Menon & Rao, 2012), (Eisenhuber et al., 2013). Per ton of corncobs, 700 kg of mono- and oligosaccharides can be obtained by enzymatic hydrolysis. The hydrolysates of corncobs are therefore perfectly suited for biodiesel production with oleaginous yeasts. These species of oil-producing yeasts accumulate up to 50% of fat in their dry mass (Kitcha & Cheirsilp, 2011).

Corncobs are a lignocellulosic material composed of cellulose, hemicellulose and lignin. These polymeric fibres consist of monomeric molecules. Cellulose is built of C6 sugars; hemicellulose mainly of the C5 sugars xylose and arabinose. Lignin consists of phenolic macromolecules.

Cellulose, hemicellulose and lignin are embedded in a complex matrix which is very resistant to enzymatic degradation (Mosier et al., 2005; Menon & Rao, 2012). The aim of this paper was to characterize the composition of ten varieties of corncobs as raw material for the fermentative production of bio-fuels. It should be investigated whether there are significant differences, especially in regard to the proportions of cellulose, hemicellulose and lignin.

MATERIAL AND METHODS

Raw material

Corncobs of ten different varieties of corn (*Zea mays*) were obtained from the Austrian Agency for Health and Food Safety (AGES) (from variety of trials in Upper Austria), partially air dried at 20 ± 2°C and stored in closed plastic boxes until usage. The varieties investigated for this research are not commercially available and are only grown in experimental field trials in Upper Austria. The cobs were chopped up by a garden shredder (Viking GE 260, Kufstein, Austria) to a particle size of 10 mm. The proportions of fat, protein, starch, ashes, cellulose, hemicellulose, and lignin were determined.

Dry matter and ash content

4 g of the air dried samples were dried until constant weight at 105°C. To examine the ash contents ,the crucibles were heated at 550°C for 24 h. The masses were determined using an analytical scale (Kern 770, Balingen-Frommern, accuracy ± 0.00001 g).

Free sugars

20 g of the air-dried corncobs were grinded with a commercial coffee mill (Moulinex, Coffee Grinder, AR 100; Alençon, France). 5 g of the substance were transferred into a 25 mL reaction tube and filled with HPLC grade water to 25 g. For the extraction of soluble substances, the tubes were put into a laboratory shaker at 200 rpm for one hour. Saccharides, organic acids, ethanol, and furans were quantified using a high performance liquid chromatography (HPLC). The HPLC system Agilent Technologies, Santa Clara, 1200 Series with a Varian Metacarb 87 H column (300 * 7.8 mm) at 65°C, H_sSO_4 (c = 5 mmol L^{-1}) eluent and an isocratic flow rate of 0.8 mL min^{-1} was used.

Starch content

The starch content of the chopped corncobs was detected with the enzymatic test kit 'Total Starch Assay Kit; α-Amylase / Amyloglucosidase Method' (Megayzyme, Ireland), according to the AOAC Method 996.11 and AACC Method 76.13 (and improvements).

Protein content

The protein content was quantified with the nitrogen determination system Dumatherm (Gerhardt, Dumas analytical system, DTM) correspondent to the ASBC-AOAC Method 997.09. Protein is detected indirectly by measurement of the total nitrogen content by combustion. The nitrogen of the sample is burned and reacts to NO_3^- and is further reduced by elementary copper to form N_2. The N_2 is measured by conductivity.

Fat content

The fat content was determined quantitatively according to the method AOAC 983.23 (Chloroform-Methanol Extraction Method, gravimetric detection).

Approximately 10 g of chopped and air dried corncobs were grinded with a coffee mill. 5 g of the grinded substrate was weighed into a round-bottomed flask. Extraction with chloroform-methanol was done in accordance with AOAC 983.23. The fat content was measured via gravimetric analysis after the extraction.

Fibre analysis

The contents of cellulose, hemicellulose and lignin were detected gravimetrically according to the modified neutral (Neutral Detergent Fibre = NDF) and acid detergent method (Acid Detergent Fibre = ADF), adapted from the method described by Van Soest et al. (1991).

The principle of the detection of cellulose, hemicellulose and lignin is gravimetric analysis by hot filtration, extraction with organic solvents, and drying, followed by determination of the ash contents of the samples.

Quantification of Acid Detergent Lignin (ADL) was replaced by direct determination of cellulose and was conducted by hot liquid extraction with acetic acid, nitric acid, and organic solvents followed by filtration according to the method of Horwitz & Latimer (2005). For this purpose, hot liquid extraction with an acid detergent solution according to the method described by Horwitz & Latimer (2005), Neumann & Basler (1976) was performed, followed by filtration with an organic solvent.

The Hemicellulose content was calculated as the difference between NDF (cellulose, hemicellulose and lignin) and ADF. Lignin was detected indirectly by determination ADF (lignin and cellulose) and defined as the difference between ADF and cellulose.

RESULTS AND DISCUSSION

Fibre analysis

The contents of cellulose, hemicellulose and lignin of ten different varieties of corncobs were determined. For each of them, three repetitions of analyses were carried out. The following proportions of cellulose, hemicellulose and lignin were detected: cellulose: 38.8% ± 2.5%; hemicellulose: 44.4% ± 5.2% and lignin: 11.9% ± 2.3%).

Fig. 1 shows the percentage shares of the polymeric fibres cellulose, hemicellulose and lignin of the different varieties. The individual varieties listed on the x-axis are labelled with numbers and letters. The nomenclation of the corn varieties (origin: experimental field trials) is in accordance with the Austrian Agency for Health and Food Safety (AGES).

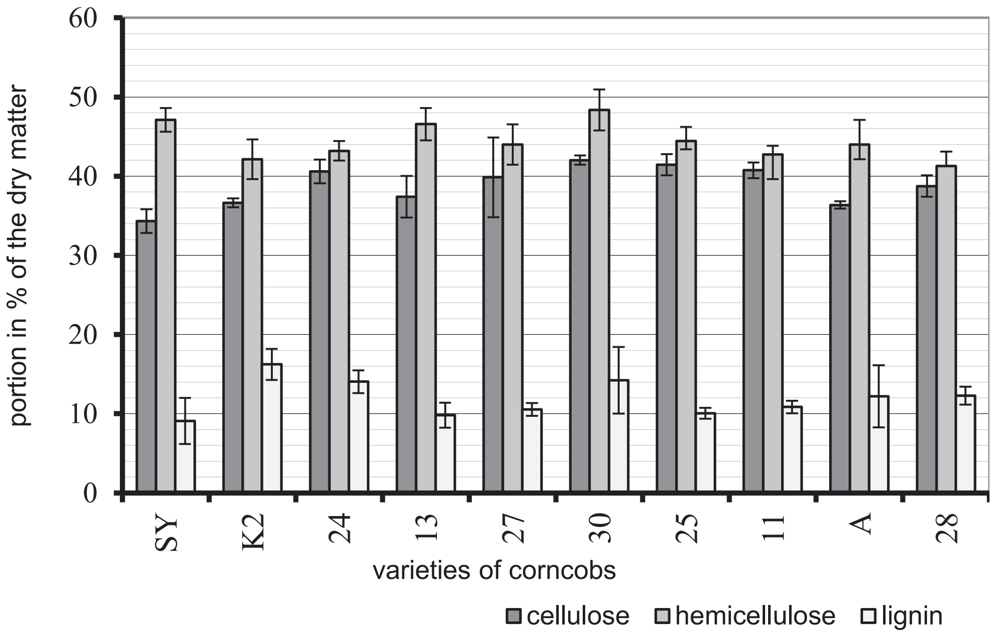

Figure 1. Composition of the fibres in different varieties of fresh corncobs.

In Fig. 2, the fibre contents (cellulose, hemicellulose and lignin) of ten different corncobs are visualized. The fat content was 0.3% ± 0.02 (mixture of all corncobs). The starch value in the raw material was 0.67% (m/m) ± 0.12. The protein content was 4.26% ± 0.96. The ash content was 2.88% ± 0.11 of the dry mass.

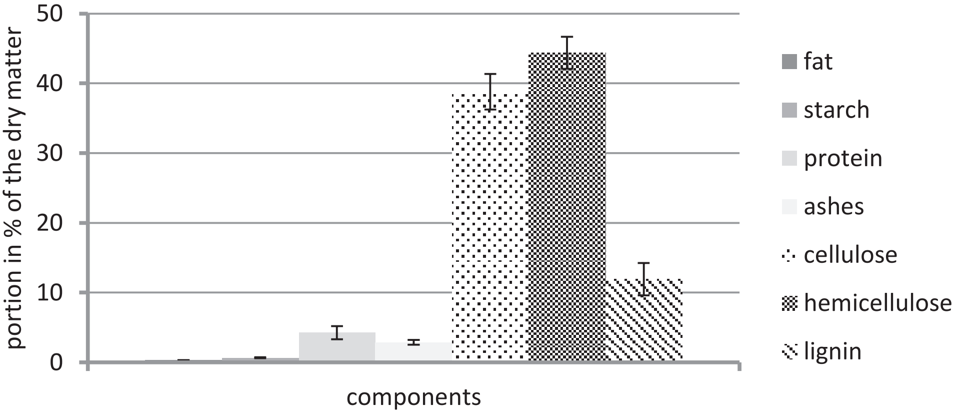

Figure 2. Average shares of fat, starch, protein, ashes, cellulose, hemicellulose, and lignin of the ten different varieties of corncobs tested.

CONCLUSIONS

According to the results of the fibre analyses, there are no significant differences (significance level $\alpha = 0.05$) between the corncobs tested. Consequently, it is not necessary to choose one specific plant variety for using as a renewable energy source, namely as a substrate for the fermentation of bio-ethanol or fat by oleaginous yeasts. The polymeric fibre proportions are in accordance with the literature reviewed. The analyses published from Wang et al. indicated contents of cellulose of 40–44%, of hemicellulose of 31–33%, and of lignin of 16–18% (Wang et al., 2011). Obviously, the hemicellulose content differs from the literature and is significantly higher. For further research, it might be necessary to compare the results with another established method. Consequently, the contents of the ten different varieties of corncobs examined for this paper will be additionally compared with acid hydrolysis.

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