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Effect of corn grain variety on the bioethanol production efficiency

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HIGHLIGHTS

- 258 different corn varieties were used in ethanol fermentation.
- The hybrids of corn were created with four male parents.
- Higher starch content caused lower efficiency of starch saccharification.
- Ethanol productivity depended on variety and starch content in corn grains.
- Corn hybrids with S80660A male parents have the biggest impact on ethanol efficiency.

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ABSTRACT

The impact of corn grain variety on the ethanol production effectiveness was determined. The screening of 258 various corn samples were done. The endosperm of recognized 258 types of corn was semi flint as a consequence of crossing between female parent with dent type seeds and male parent with flint type seeds. The tested cultivars fall into two groups, ie. 133 belong to single cross (SC) and 125 belong to three-way cross (TC) hybrids. These hybrids of corn were created with four male parents: 96 with S61328, 61 with S80660A, 20 with S07787A and 8 with S09347. The corn samples were mashed and fermented with distillery yeast *Saccharomyces cerevisiae* which produced ethanol from corn mashes yielding maximum 81.33% of the theoretical value. It was noted that the corn samples with higher starch content had lower efficiency of starch saccharification (-0.35, $p < 0.05$). This fact generates negative correlation between variables ethanol (L/100 kg starch) and starch content (-0.43, $p < 0.05$). The highest level of reducing sugars in grains led to higher productivity of ethanol. The comparisons in groups generated with multivariate exploratory techniques (cluster analysis, *k*-means clustering, PCA) showed that it is possible to distinguish a statistically different cluster with the highest ethanol yield (35.6 L of ethanol per 100 kg grains). Moreover, the PCA analysis showed that hybrids of corn with S80660A male parent have the biggest impact on efficiency of ethanol. The presented results clearly prove that not only starch content in corn grains determine the ethanol productivity.

1. Introduction

From many years, countries around the world are struggling with problems concerning their energy security. Depletion of conventional energy sources, ie. coal, oil and natural gas, the fuel crisis and the general increase in demand for energy, has led to the searching for its new sources [1,2]. Also, an attention has been noticed to the increase in greenhouse gas emissions associated with fossil fuel use. One of the solutions to this problem is to increase the use of biofuels [3–5]. Ethanol is the most widely and most frequently used biofuel. Ethanol may be obtained from the agricultural products, products from food and biomass processing industries, that are divided into three groups: raw sugar (e.g. sugar beet, sugar cane), raw starch (e.g. corn, rye, triticale) and lignocellulosic materials (such as grass, wood) [6,7].

Corn grain, used for ethanol production purposes, is efficient in high crop ($8.0\ t\ ha^{-1}$) and ethanol yield ($417\ L\ t^{-1}$) per hectare. Long-term growth is one of the biggest obstacles to the use of corn as a raw material for ethanol production. However, there is an

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opportunity to overcome this problem, thanks to advances in variety research. Diversity in ethanol yield was already observed when fermenting various corn hybrids from different growth locations [8–11].

The first step toward ethanol production from corn grain via pressureless cooking method is a wet or dry milling. The hydrolyzing process must be preceded by gelatinization of the starch. Pressure cooking is very effective for further fermentation of starchy materials but production costs are high due to the high energy consumption in the cooking process. Alternative to classical (pressure) method of starch liquefaction is non-pressure cooking fermentation system as well as simultaneous saccharification and fermentation (SSF) method or fermentation with stillage recycling [12–17]. Non-pressure method allows obtaining high yields of ethanol, while saving energy and reducing the fermentation time [10,18,19].

It was reported that the amount of native starch hydrolysis by amylases decreases with increased amylose content [20]. This fact can directly affect the performance of ethanol. Rendleman and Jacob [21] subjected to hydrolysis *in vitro* starches from different botanical origins using human salivary α-amylase. The rate of digestion of 100% corn amylose < hybrid high-amylose corn starch (64–66% amylose) < waxy maize starch (99–100% amylopectin) but susceptibility to hydrolysis of cooked waxy maize starch was similar to ordinary corn starch (approximate 25% amylose) [21]. Analogous results were obtained by subjecting to hydrolysis starch granules from rice cultivars [22], and potatoes [23]. The hydrolysis rates of starch granules from low-amylose rice cultivars were higher than those from normal amylose content rice cultivars. The amylose-free sweet potato starch was also much more susceptible to hydrolysis than control native sweet potato starch. Resistance of raw corn starches to α-amylase is affected also by granules size and the susceptibility to hydrolysis of amylo-maize starch decreases in the smallest granules with the greatest amylose content [24].

The aim of this study was screening of 258 various corn samples to evaluate the factors which determine ethanol yield and selecting the most effective varieties of corn for non-pressure method of ethanol production.

2. Materials and methods

2.1. Raw material

258 various corn samples were used in the research (varieties, experimental hybrids), obtained from the Plant Breeding Smolice IHAR Group, Poland.

Field experiments were conducted as an univariate in random blocks completed in accordance with the methodology of the study of the economic value of the corn varieties (VCU) developed by the Research Centre for Cultivar Testing in Słupia Wielka (RCCT, 2009). Corn was forecrop, good wheat soil complex. The three-component fertilizers (NPK) were used as pre-seed fertilization in pure ingredient per 1 ha: 138, 85 and 180 kg and on leaf of 5–6 leaves stage of corn – Multifoliar Ku (Ekoflora, Poland) and magnesium sulfate in an amount of 1.5 l ha^{-1} and 5 kg ha^{-1}, respectively. Chemical protection against weeds – Lumax® herbicide (Syngenta, Poland) at a dose of 4 l ha^{-1}; in phase 2 corn husks. Harvesting plant density – 80,000 ha^{-1}.

2.2. Microorganisms and enzymes

Distillery yeast *Saccharomyces cerevisiae*, Fermiol strain (Lasaffre Company, France) was used for fermentation experiments. Dry yeast was hydrated before use and the slurry was added to fermentation media, corresponding to 0.5 g of dried yeast per L of mash. Enzyme preparations have been used in fermentation processes: Termamyl SCDS (thermostable α-amylase; Novozymes; 0.2 ml kg^{-1} of grain) was used for ground corn liquefaction and San Extra L (glucoamylase; Novozymes; 0.6 ml kg^{-1} of grain) was applied for starch saccharification. Additionally Optimash VR (a complex of xylanase and cellulase; 0.08 ml kg^{-1} of grain) was used to decompose the non-starchy polysaccharides and GC 106 Protease (0.08 ml kg^{-1} of grain) was used to hydrolyze the peptide bonds (Genencor International).

2.3. Fermentation

The corn grain samples were ground on laboratory mill WZ-1 (size of the meal was ⩽0.8 mm) and mixed with water in a proportion of 1:6 and pH of the fermentation media was adjusted to 5.0–5.5. Than non-pressure cooking (100 °C, 1 h) was used for gelatinizing of the starch. Next, liquefaction (80 °C, 20 min) with Termamyl SCDS and saccharification (55–60 °C, 100 min) with San Extra L, Optimash VR and GC 106 Protease, were performed. Fermentation media after hydrolysis were cooled down to 30 °C and inoculated with *S. cerevisiae*, Fermiol strain. The fermentation media, before inoculation with yeast, were supplemented with diammonium phosphate in the amount of 0.4 g L^{-1}. The obtained corn fermentation media were characterized by a density of 143 g grain per kg. The samples were incubated at 30 °C for 72 h.

After fermentation the pH was measured in the mashes and the distillation process was applied to evaluate the ethanol yield.

2.4. Analytical methods

Dry matter of ground corn grain samples was determined directly by drying at 130 °C to constant weight [25]. The starch content was analyzed according to Hölm et al. [26]; the released glucose was estimated by LC-MS method. The content of reducing sugars in grains and stillage was determined by LC-MS method. The ethanol concentration of the raw distillates was checked on DDM 2909 Automatic Density Meter (Rudolph Research Analytical, USA).

LC-MS analysis. The ion-exclusion high-performance liquid chromatography electrospray ionization mass spectrometry (IE-HPLC–ESI-MS) analysis was performed using a Dionex UltiMate 3000 UHPLC (Thermo Fisher Scientific, Sunnyvale, CA, USA) coupled to a Bruker maXis impact ultrahigh resolution orthogonal quadrupole-time-of-flight accelerator (qTOF) equipped with an ESI source and operated in positive-ion mode (Bruker Daltonik, Bremen, Germany). The IE chromatographic separation was achieved with a Rezex™ RCM-Monosaccharide Ca^{2+} (8%), LC Column 300 × 7.8 mm (phenomenex, Torrance, CA, USA). The ESI-MS settings were as follows: capillary voltage 4500 V, nebulizing gas 3.0 bars, and dry gas 10 L/min at 200 °C. The scan range was from mass-to-charge ratio (m/z) 80–1200. The mobile phase was composed of water. The flow rate was 0.4 mL/min with an isocratic elution. The postcolumn continuous infusion (0.1 mL/min) of ammonium hydroxide solution at 0.5% was used. The sample injection volume was 5 μL. The column temperature was set at 80 °C. The ESI-MS system was calibrated using sodium formate clusters introduced by loop-injection at the beginning of the LC-MS run. The LC-MS data were processed using Data Analysis 4.1 software (Bruker Daltonik, Bremen, Germany). Molecular ions $[M + NH_4]^+$ were extracted from full scan chromatograms and peak areas were integrated. The extraction window of individual ion chromatograms was ±0.05 m/z units. The D-glucose-^{13}C was used as an internal standard. It was added in a constant amount to samples, the blank and calibration standards. This substance has been used for calibration by plotting the ratio of the analyte signal to the