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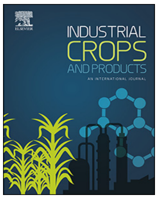

Separate hydrolysis and fermentation and simultaneous saccharification and fermentation methods in bioethanol production and formation of volatile by-products from selected corn cultivars

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ABSTRACT

Corn (*Zea mays* L.) is a widely used raw material in the production of bioethanol. Creating new cultivars and applying appropriate fermentation techniques to increase production efficiency is still important for bioethanol producers. The purpose of the study presented here was to evaluate the correlation between differentiators of corn cultivars and fermentation methods in view of the efficiency of bioethanol production. The five selected cultivars differed by FAO (Food and Agriculture Organization) number, type of hybrid cultivar, and type of grain. The tested samples generally showed significant ($p < 0.05$) differences in starch contents. Ethanol fermentation was investigated using separate hydrolysis and fermentation (SHF) as well as simultaneous saccharification and fermentation (SSF) methods The SSF method proved the most effective for the production of bioethanol, regardless of cultivar. The greatest ethanol yield ($p < 0.05$) was achieved with the semiflint SM Hetman cultivar (81.23% theoretical value) under the SSF process. A negative correlation ($p < 0.05$) between starch content and ethanol yield was demonstrated. Chromatographic analysis of the raw spirits showed that lower levels of fermentation by-products were detected for the SSF method than for SHF.

1. Introduction

Economic development and the limits of conventional energy sources have led to great interest in renewable energy sources. One motive is to prevent the emission of large amounts of carbon dioxide and other toxic compounds from the combustion of fossil fuels, as these are mainly responsible for progressive climate change, and especially the increase in the greenhouse effect. Transport biofuels are of crucial important here, and bioethanol has a significant role among them. Many countries are making the political decision and passing legislation aimed at increasing renewable energy as a fraction of the total energy used (Semenćenko et al., 2011; Semenćenko et al., 2012). As an alternative fuel, bioethanol could potentially replace fossil fuels partially. Increases in the prices of conventional fuels also favor bioethanol. Researchers are currently seeking new materials and technologies that could allow for an increase in production profitability without the use of land devoted to food production or increases in food prices (Sanchez and Cardona, 2008; Vohra et al., 2014; McKechnie et al., 2015).

Traditional bioethanol production technologies from starch raw materials consist of several stages. First, the starch in the raw material is subjected to a gelatinization process which is followed by liquefaction of the starch. This involves the addition of heat and enzymes to accelerate the process. Liquefaction can be carried out under pressurized or nonpressurized conditions. This is followed by the saccharification of starch to fermentable sugars (Wang et al., 2007). An alternative to this method is simultaneous saccharification and fermentation (SSF), in which a liquefaction stage is also present, but hydrolysis to sugars is carried out progressively throughout the process; the released glucose is directly metabolized, protecting the yeast cells against osmotic stress (Öhgren et al., 2007; Prajapati et al., 2015). The enzyme Stargen™ 001 can be used in SSF to hydrolyze granular or uncooked starch, additionally improving the overall ethanol fermentation process (Strąk and Balcerek, 2015; Białas et al., 2010; Nikolić et al., 2012). Furthermore, in the SSF method, the whole process can be carried out in a single reactor, decreasing energy consumption (Shigechi et al., 2004; Prajapati et al., 2015). From the point of view of economics, it is also worth considering how to make use of the by-product of the process, called stillage. This is a rich source of protein, fiber, fat, vitamins, and minerals and can be used wet or dried form as a component of forage for livestock (Cibis et al., 2006; Kim et al., 2008; Mojović et al., 2009).

The main raw materials used for the production of bioethanol are corn (*Zea mays* L.) in the US and sugarcane (*Saccharum officinarum* L.) in

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Brazil (Balat et al., 2008; Borychowski, 2014). Unlike other industrial plants, corn has a wide range of use. This increases its attractiveness to the market, including in Eastern Europe (Belyea et al., 2004; Kwiatkowski et al., 2006). As a starch material, corn grain — which has starch content average 72% d.m. (dry matter) — is very important in the distillation industry (Belyea et al., 2004). Of the cereals, corn is characterized by the highest ethanol performance per unit weight and per unit area of cultivation: 420 L ton^{-1} and 2520 L ha^{-1} (Devantier et al., 2005; Kwiatkowski et al., 2006; Ramchandran et al., 2015). Corn is also distinguished by a high potential yield of up to 15 tons of grain per hectare and by its high starch content (over 60%). In comparison other raw materials that contain starch and sugar (such as wheat (*Triticum* L.), triticale (*Triticosecale*), rye (*Secale* L.), potato (*Solanum tuberosum* L.), sugarbeet (*Beta vulgaris* L.)), corn excels in bioethanol production (Kwiatkowski et al., 2006; Belyea et al., 2004). The possibility of using the corn stillage in animal nutrition shows that the profitability of corn for energy purposes may be even greater.

Over the years, very good progress has been observed in breeding corn, including in terms of the number of cultivars available for sale (Adamczyk et al., 2010). Improving and expanding the existing gene pool will allow more fertile inbred lines. Although corn is a raw material that is widely used, in times of changing climatic conditions, it seems desirable to continually seek new varieties and adapt fermentation methods.

The aim of the study was to screen the distinguishing factors that affect the efficiency of corn fermentation processes and the formation of volatile by-products.

2. Material and methods

2.1. Raw material

Five cultivars of corn, originating from the IHAR Plant Breeding Group in Smolice, were used in the research. The tested cultivars were Tonacja, Dumka, Rosomak, SM Hetman (Smolice Hetman), and SM Hubal (Smolice Hubal); these differed from each other by FAO number (Food and Agriculture Organization of the United Nations; an indicator of cultivars maturity, namely the length of the growing period required for the accumulation of dry matter), type of hybrid cultivar, and type of grain. They were planted in 2016 in class IVa soil (reclaimed land of average quality, improved).

Tonacja is a trilinear (TC) cultivar of the semiflint grain type. Cultivar entered in the register of The Research Centre for Cultivar Testing in 2014. Its FAO number of 220–230 places it in the early cultivar group. The advantages of cultivar include its versatility of use: for grain, silage, grit and bioethanol. Tonacja has a very high resistance to Fusarium cobs (caused by fungi belonging to the genus *Fusarium*) and knobby smut (caused by fungus *Ustilago maydis* (D.C.) Corda). This cultivar tolerates poorer soils, giving a fairly large grain yield compared with other early cultivars.

Dumka is a trilinear cultivar of the semident grain type. Cultivar entered in the register of The Research Centre for Cultivar Testing in 2008. Its FAO number of 230 classifies it as a mid-early cultivar. This cultivar is good to use for grain, silage, and bioethanol. Soil requirements are defined as average. Dumka has a good tolerance for knobby smut corn. This is a cultivar resistant for root and stem lodging.

Rosomak is a single-type (SC) cultivar of semiflint/semident grain type. Cultivar entered in the register of The Research Centre for Cultivar Testing in 2013. Its FAO number of 250–260 classifies it as a medium-late cultivar. This is the first cultivar grown thanks to the doubled haploid lines (DH). According to the manufacturer of the cultivar, the homozygosity of such a line used in the culture process can significantly shorten the time needed to get the cultivar compared with conventional inbreeding. This cultivar tolerates poorer soils and is very tolerant to drought. The resistance to diseases as Fusarium cobs and knobby smut, economic values associated with the yielding and innovation of this cultivar enabled her to gain the recognition of both corn producers and consumers.

Cultivar SM Hetman is a trilinear cultivar of the semiflint grain type. Cultivar entered in the register of The Research Centre for Cultivar Testing in 2016. Its FAO number of 240 classifies it as a mid-early cultivar. The cultivar has excellent seedling vigor, which has a very good effect on yield potential and high tolerance to drought. Cultivar SM Hetman is characterized by very high resistance to Fusarium stalk, especially the cobs. Long greening of plants (characteristic stay green) causes that the cultivar is suitable for cultivation for silage. In addition, the directions of use are the production of grain, grit and bioethanol.

Cultivar SM Hubal is a trilinear cultivar of semiflint grain type. Cultivar entered in the register of The Research Centre for Cultivar Testing in 2016. Its FAO number of 240 classifies it as a mid-early cultivar. In studies SM Hubal performed very good results in both the production of silage (CCM) and grain (101% standard). This cultivar is characterized by high resistant to plant diseases. The agronomic profile can distinguish a very good initial growth, high tolerance to drought and weaker soil position as well as characteristics stay green and dry down at high level. These features confirm its versatility of use both for silage and grain.

2.2. Microorganisms

1.1.2 Two different *Saccharomyces cerevisiae* strains recommended for the selected fermentation methods were used in the study. In the SHF method, *Saccharomyces cerevisiae* distillery yeast in the form of a dried Fermiol formulation (Lesaffre, France) was used. Fermiol is used in traditional fermentations in distillery industry. In the SSF method, the Ethanol Red strain (Lesaffre, France) was used for ethanol production from corn mashes. Ethanol Red was used in SSF process as the manufacturer recommended this strain for higher gravity mashes. The dry yeast was hydrated before use and the slurry (corresponding to 0.5 g of dried yeast L^{-1}) was added to the mash.

2.3. Enzymes

Four enzyme preparations were used in SHF processes: Termamyl SC DS (thermostable α-amylase from *Bacillus licheniformis*; Novozymes, Denmark) was used for ground corn liquefaction, and San Extra L (glucoamylase from *Aspergillus niger*; Novozymes, Denmark) was used for starch saccharification. Additionally, Optimash VR (a complex of xylanase and cellulase from *Penicillum funiculosum*; Genencor International, USA) was used to decompose the nonstarchy polysaccharides, and GC 106 Protease (from *Aspergillus niger*; Genencor International, USA) hydrolyzed the proteins present in the flour.

The SSF process was conducted using bacterial α-amylase Amylex BT2 (Genencor International, USA), produced by fermentation of a genetically unmodified strain of *Bacillus stearothermophilus*, for corn starch liquefaction. Otherwise, Diazyme SSF containing glucoamylase and protease (both derived from *Aspergillus niger*; Genencor International, USA) was used for corn starch hydrolysis.

The enzymes used in SSF for the uncooked unliquefied starch were Stargen™ 001 (granular starch hydrolyzing enzyme containing α-amylase from *Aspergillus kawachi* and glucoamylase from *Aspergillus niger*; Genencor International, USA), Optimash VR, and GC 106 Protease.

2.4. Fermentation processes

The corn grain samples were ground prior to analysis on a WZ-1 laboratory mill (size of the meal was < 0.8 mm). Volume of the fermentation media was 150 ml. After 72 h of fermentation, distillation was performed. The whole fermentation medium has been subjected to distillation.

Separate hydrolysis and fermentation (SHF) method. The ground grain was mixed with water (in a 1:6 ratio), the pH of the fermentation