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Ethanol, feed components and fungal biomass production from field bean (*Vicia faba* var. *equina*) seeds in an integrated process

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HIGHLIGHTS

- Ethanol, feed and fungal biomass production from field beans in an integrated processes.
- Field beans hydrolyzed by enzymes or by a combination of H_3PO_4/$Ca(OH)_2$ and enzymes.
- Fermentation by yeast yielded 38–42 g L^{-1} ethanol with 71–79% efficiency.
- Solid residues contain up to 32% protein and reduced content of antinutritionals.
- *Neurospora intermedia* cultivation on thin stillage increased ethanol and protein.

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ABSTRACT

The use of field beans, a non-food leguminous crop, was studied for ethanol, feed components and fungal biomass production. The seeds were hydrolyzed using enzymes or with combination of acid (H_3PO_4) and alkaline ($Ca(OH)_2$) pretreatment and enzymatic hydrolysis. Fermentation by *Saccharomyces cerevisiae*, with or without removal of suspended solids, yielded 38.3–42.5 g L^{-1} ethanol (71.3–79.2% efficiency). The filtration residues contained ca. 247–326 g kg^{-1} crude protein, 10.6–15.5% acid detergent fiber and 19.9–29.1% neutral detergent fiber. They were enriched in phenolics (by up to 93.4%) and depleted in condensed tannin (by up to 59.3%) in comparison to the raw material. The thin stillages were used for cultivation of edible fungus *Neurospora intermedia* which produced 8.5–15.9 g L^{-1} ethanol and 4.8–16.2 g L^{-1} biomass containing over 62% protein. The mass balances showed that fermentation of unfiltered mashes was more efficient yielding up to 195.9 g kg^{-1} ethanol and 84.4% of protein recovery.

1. Introduction

Dehydrated ethyl alcohol (bioethanol) is a predominant transportation biofuel with global annual production volume of almost 93 million cubic meters in 2014 and a production increase of 53.4% from 2007 (RFA, 2016). Currently the vast majority of bioethanol is produced in moderate climate from food-grade agricultural crops such as grains (wheat, corn, rye), tubers (potatoes, Jerusalem artichokes) and roots (sugar beets) (Sebayang et al., 2016), known as first generation (1G) raw materials. This has caused concerns about ethanol production influencing food prices (Tyner, 2013). Therefore, the use of non-food raw materials for production of biofuels is gaining worldwide importance. In this matter, bioconversion of lignocellulosic residues and industrial wastes to ethanol is in the scope of many research activities. However, most of these materials require different, often harsh and costly, processing treatments compared to 1G feedstocks which make their industrial applications complicated (Chen and Fu, 2016). Therefore, novel, non-food and highly available raw materials for ethanol production are sought to, at least partially, replace 1G ethanol raw materials.

Another important issue is the choice of substrate to increase first generation bioethanol production and the proper tillage of arable land. Intense use of one crop often causes its cultivation in a monoculture which leads to soil nutrients depletion, extensive use of chemical fertilizers and reduction of crop yield (Lal, 2009). Therefore, proper intercropping should be applied to increase production and limit the use of chemical fertilizers. It would be beneficial if the intercrop could also be used for ethanol production in

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similar conditions as the main crop to ensure the continuity of supplies to the ethanol plant. Intercrops of special interest both from the agricultural and ethanol production perspective are the leguminous plants like peas or beans. They fix nitrogen in soil (Neugschwandter et al., 2015) and may be used in rotation with i.e. wheat or corn so their agricultural application is justified (Stolz and Nadeau, 2014; Luce et al., 2015). Moreover legume seeds are rich in starch (20–50%) and crude protein (15–30%) which make them promising material for processing into value added products like biofuels or feed components (Karlsson et al., 2015). Previous studies proved that ethanol production from various beans is possible. Fermentation of field peas (Nichols et al., 2005), common beans (Nichols et al., 2011) or carob pods (Turhan et al., 2010) hydrolyzates or extracts resulted in high ethanol yield. Moreover, the fermentation residuals (stillages) obtained from field peas and common beans are rich in protein and fiber that make them useful as animal feed just like dried distiller's grain with solubles (DDGS) obtained from grains (Nichols et al., 2011).

An interesting leguminous crop with low food value are field beans (*Vicia faba* var. *equina*), a variety of faba beans (*Vicia faba*). The global production quantity of faba beans and field beans exceeded 3.5×10^6 metric tons in 2013 with China as a predominant producer (ca. 1.5×10^6 metric tons) (http://www.fact-fish.com/statistic/broadbeans,horsebeans,production quantity). It is cultivated mostly for the purpose of high protein feed production (Micek et al., 2015). However, its feed quality is limited due to high content of antinutritional compounds like condensed tannins, trypsin inhibitors or α-galactosides which decrease protein digestibility and causes gastric problems in livestock (Gulewicz et al., 2014). Therefore suitable processing of field beans is important to increase its quality as animal feed. In the production of 1G ethanol several processing factors are used to degrade raw material components like high temperature and enzymatic hydrolysis which could degrade antinutritional compounds i.e. phytic acid (Vidal-Valverde et al., 1998) as well. Moreover additional processing like acid/alkali treatment reduces some unwanted compounds in legumes (Medugu et al., 2012).

Degradation of antinutritional compounds from field beans would be a crucial aspect of integrated processing into several value added products like feed components, feed biomass and ethanol. Previous research proved that by-products play an important role in the bioethanol production economics from starch-containing raw materials (Lennartsson et al., 2014). The most important are the dried distillation residues which constitute a highly nutritional animal feed known as DDGS (Kim et al., 2008). Furthermore, the liquid fraction of the stillage (thin stillage) could be used as a medium for production of edible biomass and ethanol by some filamentous fungi like *Rhizopus oligosporus* (Rasmussen et al., 2014) or *Neurospora intermedia* (Ferreira et al., 2014). As reported by Ferreira et al. (2015) *N. intermedia* cultivation on thin stillage, originated from a wheat bioethanol production plant, is a sustainable solution to improve the ethanol production process and a valuable biomass with high protein and lipid content suitable as feed additive. The cultivation of *N. intermedia* on industrial grade thin stillage can lead to production of 10% additional ethanol per year with production of additional high-value biomass (Ferreira et al., 2014) and it is currently evaluated in industrial scale at the largest bioethanol plant in Sweden (Agroetanol, Norrköping) (http://www.nyteknik.se/energi/svamp-ger-mer-etanol-i-norrkoping-6394095 [in Swedish]) what clearly indicates its sustainability. Fungal cultivation on industrial wastewaters and solid wastes is a promising way for process diversification, potentially resulting in production of many value-added products like enzymes, organic acids, alcohols and cell wall-constituents (chitosan) (Ferreira et al., 2013).

In this study, the use of field beans, a non-food, limited feed feed value crop, was for the first time evaluated for production of several value added products (biofuel, feed and edible fungal biomass) in an integrated and waste-free processes. Moreover, several processing treatments were evaluated for improved ethanol production and degradation of antinutritional condensed tannin content, which were also reported here for the first time. Additionally, the liquid distillation wastes- thin stillages were evaluated for innovative process of cultivation of edible, ethanol producing fungus *N. intermedia*. The effect of processing conditions of raw material on the cultivation efficiency is reported here for the first time as well.

This investigation was aimed at developing an integrated processing technology of field bean seeds into several products: ethanol, feed components and fungal biomass. The treatments includes enzymatic hydrolysis and acid/alkali treatments with H_3PO_4/$Ca(OH)_2$ of field bean mashes, separation of mashes into liquid and solid fraction by filtration, ethanol fermentation by distiller's strain of *Saccharomyces cerevisiae*, distillation and cultivation of an edible fungi *N. intermedia* on the liquid fraction of the stillage.

2. Materials and methods

2.1. Raw material

Field bean seeds of high-condensed tannin Granit cultivar were used throughout the research. It was donated by a local farmer outside Wrocław, Poland. The material was ground by a knife mill (Rotary Mill, Brabender, Germany) with an internal sieve of 2 mm. The obtained meal was characterized by the content of dry matter (885.50 ± 0.25 g kg^{-1}), starch (378.10 ± 0.35 g kg^{-1} dry solids), crude protein, ash, total phenolics and condensed tannins (values given in Table 3).

2.2. Enzymes and microorganisms

Commercial carbohydrate hydrolyzing enzyme preparations produced by Novozymes (Denmark) were used throughout the study. Termamyl SC DS (thermostable α-amylase with a declared activity of 240 kilo novo units (KNU) g^{-1}), San Extra L (glucoamylase with a declared activity of 400 amyloglucosidase units (AGU) g^{-1}) and Viscoferm (a blend of endo-1,3(4)-β-glucanase, cellulase and endo-1,4-xylanase of unspecified activities) were used to degrade carbohydrate polymers in the seeds. Active dry yeast *S. cerevisiae* Ethanol Red (Fermentis, France) were used in the ethanol fermentation step. The yeast was rehydrated in sterile distilled water prior to inoculation. *N. intermedia* CBS 131.92 was obtained from Centraalbureau voor Schimmelcultures (The Netherlands). The fungus was maintained on a PDA plates containing 39 g L^{-1} of potato dextrose LAB-AGAR medium (Biocorp, Poland). The spore suspension for inoculation was prepared by flooding the plates with 20 mL of sterile distilled water and releasing the spores with sterile spreader (Ferreira et al., 2014). Spore concentration was counted microscopically using a Burker hemocytometer chamber.

2.3. Pretreatment, hydrolysis and fermentation

Field bean fermentation media was prepared in similar way to the dry-grind ethanol production process with some modifications as depicted in Fig. 1. The meal was weighted into stainless steel mashing cups, water was added to desired weight and the cups were placed in a mashing apparatus water bath (LB-12, Lochner Labor + Technik GmbH, Germany). For acid/alkali pretreated variants phosphoric acid or calcium hydroxide, were added in the