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Ethanol yields and elevated amino acids in distillers dried grains with solubles from maize grain with higher concentrations of essential amino acids

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ABSTRACT

Concentrations of the essential amino acids, lysine and tryptophan, present in corn distillers dried grains with solubles (DDGS) are insufficient to fully meet the nutritional requirements in non-ruminant feeds. Mutations known to increase lysine and tryptophan concentrations were combined by breeding into two maize hybrid backgrounds that differ for grain protein concentration (FR1064 × Mo17 normal protein, FR1064 × IRHP1 high protein). These mutant hybrids were compared to their non-mutant control isolines for field performance and ethanol and DDGS processing using laboratory scale procedures: conventional dry grind process and modified dry grind process using granular starch hydrolyzing enzyme (GSHE).

The mutant hybrid grain contained higher concentrations of a number of amino acids, with notable increases to at least 0.55% w/w lysine and 0.18% w/w tryptophan. Due to lower grain yield and starch concentrations, final ethanol yields of FR1064 × Mo17: *o2; asaC28* hybrid were reduced compared to control for both the conventional and modified dry grind processes. However, with the modified process, ethanol yield for FR1064 × IRHP1: *o2; asa2-C28* hybrid was similar to its control isoline. DDGS yields increased for both mutant hybrids compared to controls. Although amino acid profiles were similar for DDGS recovered from both the conventional and modified processes, DDGS from the mutant hybrids contained higher concentrations of lysine (1.48–1.70% w/w) and tryptophan (0.32–0.39% w/w) compared to controls (1.09% w/w lysine, 0.22% w/w tryptophan). Using these mutant corn hybrids at an ethanol plant resulted in lower ethanol yields; however, this loss can be recovered with the higher DDGS yield and increased nutritional value of the DDGS.

1. Introduction

In 2015, total ethanol and animal feed production were 14.7 billion gallons and 40 million metric tons, respectively (RFA, 2016). Majority of the DDGS produced in the US is used as an ingredient in diets pertaining to dairy and beef cattle (76%) owing to its high fiber content; whereas, only a small percentage is used in poultry (8%) and swine (15%) diets (RFA, 2016).

Improvements in the fuel ethanol production processes are adapted by ethanol plants to expand the existing markets for DDGS (Rausch and Belyea, 2006). For example, fractionation technologies, elusieve process, enzymatic milling (E-mill), quick germ quick fiber (QGQF) processes have been developed to recover additional co-products and increase the nutritional value of DDGS (Singh et al., 1999 and Radhakrishnan et al., 2005). Martinez-Amezcua et al. (2007) compared nutritional value of DDGS recovered from conventional dry grind process to modified processes such as elusieve and QGQF processes. Crude protein content of DDGS samples from modified processes were higher compared to conventional DDGS. However, amino acid contents (lysine, threonine and tryptophan) for all the DDGS samples were similar. DDGS properties from a modified process (E-mill process) were evaluated and compared to conventional DDGS (Kim et al., 2010). It was reported that E-mill DDGS had higher amino acid contents, amino acid digestibilities and true metabolizable energy (TME) compared to the conventional DDGS (Kim et al., 2010). Jacela et al. (2014) investigated the nutritional value of high protein DDGS (recovered from dry fractionation process) for swine diet formulations. It was reported that both amino acid concentrations and amino acid digest-

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ibility were higher for high protein DDGS compared to traditional DDGS. Low oil DDGS produced after extracting oil from DDGS is another valuable co-product with higher crude protein and lower oil contents (Saunders and Rosentrater, 2009).

Using specialty corn hybrids in the dry grind process is another approach to increase the profitability of ethanol plants. Sharma et al. (2007) investigated the effects of using different corn types such as waxy corn, high amylose corn and normal dent corn in the dry grind process. Waxy corn resulted in highest ethanol yields and fastest fermentation rates; whereas, high amylose corn gave lowest ethanol yields. This was attributable to the high amount of resistant starch due to the high percentage of amylose in high amylose corn (Sharma et al., 2007, 2010). Another example of a specialty corn hybrid is the Quality Protein Maize (QPM, reviewed in Vasal, 2000) or high protein quality corn (HPQC) which was developed to improve the protein quality of corn. Lysine and tryptophan are the two most limiting essential amino acids in corn grain. These amino acids are added as supplements to animal feeds (Lysine in broiler diets and both lysine and tryptophan in swine diets) and are produced using bacterial fermentations which is expensive (Prasanna et al., 2001). There are few reported studies on the effect of using such corn hybrids in the dry grind process. Wu (1986) compared the fermentation properties of using a high lysine corn hybrid (SL 75) to normal dent corn in the dry grind process. Compared to high lysine corn, dent corn resulted in higher ethanol yields. High lysine fermentation products (distillers grains; distillers grains with solubles) contained higher lysine and tryptophan contents compared to the corresponding dent corn fermentation products.

The main goal of this study was to evaluate the potential of using certain mutant corn hybrids with high lysine and tryptophan contents in the dry grind process. The hybrids selected were double mutants containing the *opaque-2* mutation (*o2*) and a feedback-insensitive form of the alpha subunit of anthranilate synthase (*asa2-C28*). The *o2* mutation was initially described by Mertz et al. (1964) as reducing accumulation of zein proteins, leading to higher amounts of available lysine and tryptophan, and is the main genetic factor contributing to Quality Protein Maize and other HPQC genotypes. The *asa2-C28* mutation was first identified in a screen for maize tissue cultures capable of growing in the presence of the toxic tryptophan analog, 5-methyltryptophan. These tissue cultures were subsequently regenerated into plants with elevated levels of free tryptophan in seeds and reduced feedback inhibition of anthranilate synthase by tryptophan (Hibberd et al., 1986). Later work identified a single nucleotide change in the ASA2 gene encoding the alpha subunit of anthranilate synthase that conditions the same methionine to lysine mutation associated with loss of feedback inhibition of anthranilate synthase in a number of organisms (Anderson et al., 2000).

One of the concerns with using conventional dry grind process was the high temperature liquefaction treatment (>82 °C) which might result in destruction of lysine. Therefore, the hybrids were also processed using a modified dry grind process using granular starch hydrolyzing enzyme (GSHE). This is a one-step fermentation process where GSHE is added along with the yeast. GSHE converts starch to dextrins at low temperatures ≤ 48 °C and hydrolyzes dextrins to sugars to be converted into ethanol. Specific objectives of this study were to evaluate the fermentation properties (rates and ethanol yields) of these mutant corn hybrids using conventional and modified dry grind processes and to evaluate the nutritional value of DDGS recovered from both the processes and compare them with their control isolines.

2. Materials and methods

2.1. Genetic materials and grain yield measurements

The *o2-R* mutant allele (Schmidt et al., 1987) was introgressed via backcrossing into the B73 and Mo17 inbred lines followed by selfing to homozygosity by Robert J. Lambert at the University of Illinois. The B73: *o2* inbred line was used as the donor for introgression into the FR1064 inbred line (Illinois Foundation Seeds) by backcrossing six times followed by selfing to homozygosity of *o2*, where the presence of *o2* allele was tracked by observation of the mutant phenotype among kernels of self-pollinated ears from the same plants used as male parents in each backcross generation. Similarly, the Mo17: *o2* inbred line was used as the donor for introgression into the Illinois Reverse High Protein1 (IRHP1) inbred line described in Uribelarrea et al. (2004). The *asa2-C28* mutation was obtained from seed initially deposited with In Vitro International (subsequently transferred to ATCC but no longer maintained) and introgressed into each of the FR1064, IRHP1, and Mo17 inbred lines via at least six backcrosses followed by selfing to homozygosity. The *asa2-C28* allele was tracked during the breeding program using the molecular marker assay described in Anderson et al. (2000). To generate double mutants for *o2* and *asa2-C28*, the converted inbred lines for each individual mutation were crossed to each other, self-pollinated, and progeny plants homozygous for both mutations identified using visual selection for *o2* and the molecular marker assay for *asa2-C28*. The double mutant inbred lines were then crossed to each other to generate hybrid seeds for field evaluation.

A set of four hybrids were evaluated in this study: FR1064 × Mo17: *o2; asa2-C28* (FM*o2C28*) and its control non-mutant FR1064 × Mo17 isoline (FM), as well as FR1064 × IRHP1: *o2; asa2-C28* (FRH*o2C28*) and its control isoline (FRH). The four hybrids were grown in each of three replicated field plots during 2012 at the Crop Sciences Research and Education Center located near the University of Illinois campus in Urbana, IL. Each plot was 5.3 m long by 3 m wide, in four rows with 0.76 m spacing, and received pre-plant applications of 200 kg N fertilizer per hectare (as 28% urea ammonia nitrate) and GuardsmanMax herbicide (dimethenamid and atrazine, BASF, Florham Park, NJ). Plots were planted on May 5 at 77,800 plants per hectare with in-furrow tefluthrin soil insectide (ForceG, Syngenta, Greensboro, NC). They received 2.5 cm of overhead irrigation just prior to flowering, and five ears were harvested by hand from the middle portion of the center two rows of each plot on October 5 (14% grain moisture). Bulked shelled grain from each of the four hybrids were hand cleaned using a 12/64" (4.8 mm) sieve by removing the broken corn and foreign material (BCFM). Cleaned corn samples were ground using a hammer mill (1100W, model MHM4, Glen Mills, Clifton, NJ) equipped with 0.5 mm sieve. Moisture content of ground corn samples were determined using a standard two stage oven method (Approved Method 44-19, AACC International, 2000). Corn samples were processed using two laboratory scale processes: conventional dry grind procedure and modified dry grind procedure using granular starch hydrolyzing enzyme.

2.2. Enzymes and other materials

All the enzymes used in this study were obtained from Dupont Industrial Biosciences (Palo Alto, CA, U.S.A). In the conventional procedure, Spezyme CL, Distillase and Fermgen enzymes were used as alpha amylase, glucoamylase and protease enzymes, respectively. Spezyme CL consists of a thermostable alpha amylase and was obtained from a genetically modified strain of *Bacillus licheniformis*. It had an activity of 15,225 AAU/g (AAU = Alpha Amylase Units), a specific gravity (sg) of 1.17g/mL and an optimum pH range of 5.5–6.5. Distillase consists of amylase (1,4-α-D-105 glucan glucanohydrolase – EC 3.2.1.1), glucoamylase (1,4-α-D-glucan hydrolase – E.C. 3.2.1.3) and Aspergillopepsin I (EC 3.4.23.18). These enzymes were obtained from genetically modified strains of *Trichoderma reesei*. It had an activity of 380 GAU/g (GAU = Glucomylase Units), a sg of 1.10–1.14 g/mL and an optimum pH range of 4.0–4.5. Fermgen (sg = 1.12 to 1.20 g/mL) was an acid protease enzyme obtained from *Trichoderma reesei* with a declared activity of 1000 SAPU/g (SAPU = Spectrophotometric Acid Protease Units). Optimum pH range was 4.0–5.0. In the modified process, GSHE Stargen 002 along with protease enzyme, Fermgen,