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A comparison between corn and grain sorghum fermentation rates, Distillers Dried Grains with Solubles composition, and lipid profiles ☆

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HIGHLIGHTS

- Grain sorghum and corn fermentations are evaluated and compared.
- Protease addition increases ethanol yields 1–2% for conventional fermentations.
- Lipid profiles in corn and sorghum DDGS are studied.
- Free fatty acids were significantly higher in sorghum DDGS relative to corn.

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ABSTRACT

The aim of this study was to determine if the compositional difference between grain sorghum and corn impact ethanol yields and coproduct value when grain sorghum is incorporated into existing corn ethanol facilities. Fermentation properties of corn and grain sorghum were compared utilizing two fermentation systems (conventional thermal starch liquefaction and native starch hydrolysis). Fermentation results indicated that protease addition influenced the fermentation rate and yield for grain sorghum, improving yields by 1–2% over non-protease treated fermentations. Distillers Dried Grains with Solubles produced from sorghum had a statistically significant higher yields and significantly higher protein content relative to corn. Lipid analysis of the Distillers Dried Grains with Solubles showed statistically significant differences between corn and sorghum in triacylglycerol, diacylglycerol and free fatty acid levels.

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1. Introduction

Fuel ethanol production in the US has steadily grown since 2000 when about 7.6 million m^3 (2 billion gallons) was being produced annually. Since 2010 the US has been producing over 49 million m^3 (13 billion gallons) a year and is currently near 57 million m^3 (15 billion gallons) (RFA, 2016). Essentially all of this production has been using corn as the feedstock. Interest in diversifying feedstocks is increasing and grain sorghum is one of the potential alternative feedstock being considered. Compositionally grain sorghum is very similar to corn (Wang et al., 2008). It is similar in starch and lipid content to corn but has a higher protein content. Sorghum also has improved drought tolerance relative to corn and can be grown with reduced input (water, fertilizer, etc.) requirements (Nghiem et al., 2016).

Investigations into grain sorghum utilization identified a number of potential issues that could impact the mixing of sorghum with corn or using sorghum alone in existing corn ethanol processing facilities (Nghiem et al., 2016). Other researchers have shown the impact of sorghum variety on processing and ethanol yields (Yan et al., 2011; Wu et al., 2007). High tannin varieties were found to have significantly elevated viscosities during liquefaction relative to non-tannin varieties but could be reduced significantly by increasing the amount of alpha amylase (Zhao et al., 2008). Perez-Carrillo et al. (2012) investigated fermentations of corn, sorghum, and decorticated sorghum with and without a protease pretreatment process and found that adding protease could increase ethanol yields.

One of the specific issues identified as a potential problem is the impact on the composition of the distiller's dried grains with solubles (DDGS) and in particular the lipid composition. If the addition of sorghum negatively impacts the value of the DDGS revenue stream, it would have a significant impact on facilities' profitability. Additionally, post fermentation corn oil (also called distillers corn oil) recovery has become common in the majority of ethanol facilities and is also an important revenue stream for

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the facilities. Understanding how the addition of sorghum impacts the quality of the oil (commonly called distillers corn oil), will be important for ethanol producers to know. Composition for sorghum DDGS are reported in the literature for Waxy and non-waxy sorghum varieties as well as for corn, sorghum and corn-sorghum mixture (Yan et al., 2011; Urriola et al., 2009). These results unfortunately do not give detailed lipid information and the samples were not all fermented under identical conditions so no rate information is available.

Moreau et al. (2016) found indications that grain sorghum may contain an endogenous lipase that could impact overall lipid quality. In order to evaluate if grain sorghum would impact DDGS quality or the oil quality, sorghum fermentation were investigated utilizing two fermentation processes with and with protease treatments. These processes allowed fermentation of the grain with and without a thermal treatment prior to fermentation. The thermal treatment would presumably inactivate the endogenous enzyme activity. The overall objective of the research is to begin to identify processing issues that may impact the utilization of grain sorghum in existing corn ethanol facilities and to establish compositional changes in the DDGS product that may impact their value or utilization.

2. Methods

2.1. Materials and enzymes

Corn kernels (yellow dent #2), were purchased from a commercial storage facility and were grown and harvested during the 2014 season. Corn was cleaned and stored at 4 °C until use. Grain sorghum, (var. Macia) was provided by Scott Bean at the USDA-ARS Center for Grain & Animal Health Research in Manhattan, KS. The enzymes used in fermentations were provided by DuPont Industrial Biosciences, Palo Alto, CA. STARGEN™ 002, SPEZYME® RSL, OPTIDEX™ L400 and FERMGEN™. Yeast (Ethanol Red) was provided by Lesaffre. All other chemicals were reagent grade or higher purity.

2.2. Fermentation

Conventional fermentations were conducted by finely grinding grain using a Bunn (Springfield, Ill) (model G2) burr mill and the moisture content determined using AOAC Official Method 930.15 (AOAC International, 2012). The grain was then mixed with water in a tared beaker at higher than the final solids content (30%), to allow for rinsing. The particle size of the grain slurry was further reduced using an IKA (Wilmington, NC) T25 Disperser with an 18G dispersing element at 16,000 rpm for 3–5 min until slurry was uniform. The slurry was then adjusted to pH 5.8 using 1 N HCl and a mechanical mixer. Alpha amylase (SPEZYME® RSL) was added at 0.5 mL/kg mash and the slurry heated to 95 °C for 60 min. After cooling, supplemental N (400 ppm) was added as urea and the pH adjusted to 4.5 with 1 N HCl. Glucoamylase (OPTIDEX® L-400) was added at a dosage of 0.4 mL/kg of mash and yeast (Ethanol Red) was added (1.1 g/kg of mash) to start the fermentation. The total slurry weight was then readjusted to reach the final 30% solids content. The mash was divided (800g) into tarred 1 L flasks and if required, protease (FERMGEN®) was added at 0.9 mL/kg grain. The flasks were stoppered and a 21-gauge needle inserted to vent CO_2 produced.

Native starch fermentations were conducted by finely grinding the grain and preparing the slurry as described above. Following the particle size reduction, the pH was adjusted to 4.5 using 1 N HCl. A native starch hydrolyzing enzyme mixture (STARGEN™ 002) was then added at 3 mL/kg of grain. Supplemental N (400 ppm) was added as urea and yeast added (1.1 g/kg of mash) to start the fermentation (Ethanol Red). The total slurry weight was then readjusted to reach the final 30% solids content. The mash was divided (800 g) into tarred 1 L flasks and if required, protease (FERMGEN®) was added at 0.9 mL/kg grain. The flasks were stoppered and a 21-gauge needle inserted to vent CO_2 produced during fermentation.

Conventional and native starch fermentation flasks were incubated in a New Brunswick Scientific Innova® 44 incubator at 30 °C with shaking at 200 rpm. The flasks were incubated for up to 96 h and periodically weighed. The difference between the flask starting weight and the flask weight during fermentation is due to the release of CO_2 through the inserted needle. This allows rate determinations to be followed without disturbing the anaerobic fermentation and indirectly becomes a measure of ethanol production. A 1 mL sample was taken at the end of fermentation for HPLC determination of maltodextrins (DP4+), maltotriose (DP3), maltose, glucose, fructose, succinic acid, lactic acid, acetic acid, glycerol, methanol and ethanol as described in Johnston and McAloon (2014).

2.3. Distillers Dried Grains with Solubles production and analysis

After fermentation, the flasks were heated to 85 °C to remove the ethanol produced so the remaining material could be safely dried. The remaining slurry was transferred to a tared drying tray and dried in a forced air oven at 55 °C overnight. The dried material (now DDGS) was weighed to determine the initial yield and then ground in a blade grinder (Krups, model 203) to prepare a uniform material for further analysis. Protein was determined using AOAC method 930.05. Fiber contents (acid detergent, neutral detergent) were determined using the ANKOM Tech methods (ANKOM Tech Methods, 1995). Crude fiber was determined using AOAC method Ba 6a-05 (AOCS, 1998). Ash was determined using AOAC 942.05 and ICP using AOAC 985.01 (AOAC, 2012). Starch was determined using AACC 76-13.01, (AACC International, 2000). Calculated values for Total digestible nutrients, Net energy (lactation), Net energy (maintenance), Net energy (gain), Digestible energy, and Metabolizable energy were done according to National Research Council, 2001.

2.4. Extraction

Sorghum and corn kernels were ground to 20 mesh (87 μm) with a Wiley Mill (Thomas Scientific, Philadelphia, PA) immediately before use. All extractions were performed in a Dionex Accelerated Solvent Extractor (ASE) Model 200 (Dionex, Sunnyvale, CA) as previously described (Johnston et al., 2005). Samples (1 g of ground corn or sorghum DDGS) were placed in an 11 mL extraction vessel. Extractions were conducted at 50 °C and 1000 psi with methylene chloride. The extractor was programmed to extract each sample with three × 7.5 ml portions of solvent, for 10 min each. The entire extract (21.5 ml) from each sample was pooled, the solvent was evaporated under a stream of N_2, and the mass of the lipid residue was measured with an analytical balance. The lipid residue was then dissolved in hexane/isopropanol, 97/3, v/v, and HPLC analyses were conducted.

2.5. Nonpolar lipid

Triacylglycerols, diacylglycerols, free Fatty acids, free sterols, phytosterol esters, and ferulate phytosterol esters were quantitatively analyzed by an updated version of a normal-phase HPLC method with ELSD (Moreau and Hicks, 2005). These nonpolar lipid components were identified by comparison with the retention time of commercial standards. Quantitative analysis of each component was achieved by injecting multiple samples of each