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Seasonal variability in ethanol concentrations from a dry grind fermentation operation associated with incoming corn variability

Divya Ramchandran[a], David B. Johnston[b], M.E. Tumbleson[a], Kent D. Rausch[a], Vijay Singh[a,*]

[a] Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, 1304 W. Pennsylvania Avenue, Urbana, IL 61801, USA
[b] Eastern Regional Research Center, ARS, USDA, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

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ABSTRACT

Corn from an ethanol plant (commodity corn) and an identity preserved corn hybrid from a seed company (control corn stored at 4 °C) were used to study the effects of incoming corn on dry grind ethanol concentrations. Ethanol concentrations were determined every 2 weeks for 1 year using conventional dry grind procedure. Variations in ethanol concentrations were significant and variability patterns for commodity and control corn followed the same trend. Highest ethanol concentrations were seen in the month of January. Variation with control corn suggested that storage time is a significant factor affecting ethanol concentrations. Effects of different enzyme treatments on mean ethanol concentration over a year were evaluated. Two liquefaction enzymes (optimum pH – 5.8 and 5.1, respectively), two saccharification enzymes (optimum pH – 5.0) and one protease were used in five enzyme treatments (I–V). Final ethanol concentration with enzyme treatment V was (17.5 ± 0.486)%v/v. This was 0.6% higher than enzyme treatment I resulting in an additional ethanol production of 600,000 gallons/year in a 100 million gallon/year ethanol plant. Using more effective enzymes increases overall dry grind ethanol production and ethanol plant profitability.

1. Introduction

In 2012, total production of corn in the US was 10.8 billion bushels, of which 30.8% (3465 million bushels) was used for fuel ethanol production (NCGA, 2013). The corn dry grind industry is the main source of fuel ethanol production in the USA. In the dry grind process, corn starch is converted to glucose using amylases and glucose subsequently is fermented to ethanol by yeast. The primary coproduct produced is distillers dried grains with solubles (DDGS) consisting of nonfermentable components (unconverted starch, fiber, fat, protein and ash) in corn (Khullar et al., 2011). DDGS are marketed as feed for commercial animal production. DDGS are used primarily in animal diets for ruminant and nonruminant animals.

Dry grind ethanol yields depend on the incoming grain quality (Singh, 2012). Ethanol plants encounter seasonal variations in corn quality affecting ethanol yields and DDGS. The observed variability is attributable to three main factors: genetics, growing environment and postharvest practices (Singh, 2012). Singh and Graeber, (2005) studied effects of hybrids and planting locations on ethanol production (with 25% solids content), and reported there was hybrid variability in corn affecting dry grind ethanol yields. Ethanol concentrations varied from 11.2 to 13.8%v/v among 18 different hybrids across 8 growth locations. Variation in ethanol concentration (12.9–14.6%v/v – with 24.5% solids content) was seen due to hybrids with the E-Mill dry grind process (Sharma et al., 2006). Planting environment affected by growth locations, weather conditions and agronomic practices also plays a role in determining ethanol yields (Singh and Graeber, 2005). In the US, corn typically is harvested in September and October, dried to prevent microbial spoilage and stored year round to be used by corn processing plants. Corn wet milling plants have reported processing issues using freshly harvested corn and long term stored corn; freshly harvested corn is more difficult to process than corn stored for 2–3 months and milling quality decreases with storage time (Singh et al., 1998). Variation in extractable starch yields with stored corn was studied (Singh et al., 1998). It was reported that average extractable starch yields across storage time were lower for corn stored at ambient conditions compared to cold room (4 °C). In the dry grind process, not all starch is hydrolyzed to sugars. The left

* Corresponding author. Tel.: +1 217 419 6303/333 9510; fax: +1 217 244 0323.
E-mail address: vsingh@illinois.edu (V. Singh).

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over starch is called unconverted or residual starch and is recovered in the DDGS (Sharma et al., 2010). The amount of residual starch depends on several factors such as the type of raw starch in corn (amylose/amylopectin ratios), processing conditions (temperature, pH, duration of hydrolysis and enzyme activity) and amount of resistant starch (starch resistant toward enzymatic attack) (Sharma et al., 2010). Higher residual starch in DDGS can correspond to lower ethanol production. Plumier et al., (2014) studied effects of storage time and temperature on residual starch contents from the dry grind process and found there was variation in residual starch contents with storage time.

Variability in corn results in substantial losses of quantity and quality of dry grind end products. In a 100 million gallon ethanol plant, an average 3% loss of ethanol due to grain quality is equivalent to 3 million gallons of ethanol per year. The economics of a dry grind facility can be improved by increasing ethanol yields and improving nutritional quality of coproducts. Profitability of a dry grind process can be improved by several factors such as developing modified processing techniques, recovering high value coproducts, using high ethanol yielding hybrids, using lower cost better enzymes, etc. (Singh and Graeber, 2005). Ethanol yields can be increased by using more appropriate enzyme combinations of amylases and proteases. The objectives of this study were to monitor biweekly variation in dry grind ethanol concentrations for corn obtained from an ethanol plant (commodity corn) and identity preserved corn hybrid (control corn) and determine effects of storage time and enzyme treatments on dry grind ethanol production for commodity corn.

2. Materials and methods

Composite corn was obtained biweekly from a Midwestern ethanol plant from October, 2012 to September, 2013. Corn was purchased from a 50 mile radius around the plant. A composite container was filled with corn samples collected directly from the pickup trucks before they were transferred to the silos for storage on a daily basis. The sample was thoroughly mixed, and around 2 kg corn was collected from the composite container on a biweekly basis for this study. An identity preserved yellow dent corn hybrid obtained from a seed company was used as control corn. Control corn was stored at 4 °C for 1 year. Subsamples (around 600 g) were used every 2 weeks for analysis. Corn was cleaned using a 12/64″ (4.8 mm) sieve by removing the broken corn and foreign material (BCFM). Cleaned corn was stored at 4 °C prior to use. Cleaned corn was ground using a 0.5 mm sieve in a laboratory hammer mill (1100W, model MHM4, Glen Mills, Clifton, NJ). Moisture content of ground corn was determined using a two stage standard oven method (Approved Method 44–19, AACC International, 2000). Ground corn (100 g dry basis, db) was used for dry grind fermentation. Two replicates were used for each corn sample. Active dry yeast (Ethanol Red Lesaffre Yeast Corp., Milwaukee, WI) was used for fermentation. A urea stock solution (50%w/v from 99.6% ACS grade) was prepared and used as the nitrogen source for yeast obtained from Fisher Scientific, Fair Lawn, NJ. Two liquefaction enzymes (AA-I and AA-II), two saccharification enzymes (GA-I and GA-II) and a protease (P) were provided by a commercial enzyme company. Alpha amylases (AA-I and AA-II) breaks down starch to dextrins during liquefaction and glucoamylase enzymes (GA-I and GA-II), subsequently hydrolyzes dextrins to simple sugars such as glucose, fructose, maltose and maltotriose during saccharification which can be further consumed by yeast to produce ethanol. Protease aids in breaking down the protein matrix and increases the accessibility of starch granules to amylases and also results in the production of free amino nitrogen which can be utilized by yeast during fermentation (Vidal et al., 2011). AA-II and GA-II were newer generation enzymes relative to AA-I and GA-I. A total of five enzyme treatments (I–V) were evaluated (Table 1). Enzyme treatments and dosages were selected based on manufacturer's recommendations (Table 1).

Table 1
Enzyme treatments and dosages[a] used in dry grind process.

	Enzyme treatments				
	I	II	III	IV	V
Liquefaction Enzyme	AA-I (18.8 μL)	AA-I (18.8 μL)	AA-I (18.8 μL)	AA-II (25.7 μL)	AA-II (25.7 μL)
Saccharification Enzyme	GA-I (61.5 μL)	GA-II (56.3 μL)	GA-II (56.3 μL)	GA-II (56.3 μL)	GA-II (56.3 μL)
Protease	–	–	P (2.86 μL)	–	P (2.86 μL)

[a] Enzyme dosages in μL/100 g corn dry basis.

Table 2
Activity measurements of enzymes used.

Enzymes	Alpha amylase units[a]	Glucoamylase units[b]	Protease units[c]	Total protein[d]
AA-I	3090	1721	ND[e]	451
AA-II	6420	7960	4	3010
GA-I	994	7638	ND[e]	5674
GA-II	4626	8951	ND[e]	8107
P	ND[e]	ND[e]	4	508

[a] Alpha amylase units – μmol of maltose released per minute per mL of enzyme.
[b] Gluco amylase units – μmol of glucose released per minute per mL of enzyme.
[c] Protease units – μmol of tyrosine residues released per minute per mL of enzyme.
[d] Total protein expressed in μg of protein.
[e] Not detected.

2.1. Enzyme activity measurements

Enzyme protein contents were measured using Bradford's method (1976). Bovine serum albumin used as the protein standard and Bradford's reagent were purchased from Bio–Rad Hercules, CA. Amylase activities were measured as an increase in reducing sugars and expressed as the amount of reducing sugars released per minute by 1 mL of enzyme (μmol of sugar/min mL). Alpha amylase and glucoamylase activities were determined using DNS assay using pregelatinized corn starch and maltodextrins as substrate, respectively. Standard sugars used for alpha amylase and glucoamylase assays were maltose and glucose, respectively (Fisher Scientific, Fair Lawn, NJ). Alpha amylase and glucoamylase assays were conducted at 85 and 32 °C, respectively, for 5 min. Protease activity was determined at 32 °C (Abe et al., 1977). Bovine hemoglobin was used as the substrate. Protease activity was expressed as the amount of tyrosine liberated by trichloroacetic acid soluble products per minute by 1 mL of enzyme at 280 nm. Amino acid tyrosine obtained from Fisher Scientific (Fair Lawn, NJ) was used as the standard. Each activity was determined in 20 mM sodium phosphate buffer at the optimum pH of the enzyme (Table 2). The optimum pH of AA-I, AA-II, GA-I, GA-II and P enzymes were 5.8, 5.1, 5.0, 5.0 and 5.0, respectively.

2.2. Composition analysis

Chemical composition of the incoming corn (starch, protein and oil content) was analyzed using Near-infrared reflectance (NIR) analyzer (Model Infratec 1229, Whole grain analyzer, FOSS, Eden Prairie, MN) at Illinois Improvement Crop Association, Champaign, IL. Duplicate readings for each sample were taken for analysis. The NIR instrument was calibrated using wet chemistry methods of the Corn Refiners Association (CRA). Protein, oil and starch content was