

Effects of Amylose, Corn Protein, and Corn Fiber Contents on Production of Ethanol from Starch-Rich Media¹

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ABSTRACT

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The effects of amylose, protein, and fiber contents on ethanol yields were evaluated using artificially formulated media made from commercial corn starches with different contents of amylose, corn protein, and corn fiber, as well as media made from different cereal sources including corn, sorghum, and wheat with different amylose contents. Second-order response-surface regression models were used to study the effects and interactions of amylose, protein, and fiber contents on ethanol yield and conversion efficiency. The results showed that the amylose content of starches had a significant ($P < 0.001$) effect on ethanol conversion efficiency. No significant effect of protein content on ethanol production was observed. Fiber did not show a significant effect on ethanol fermentation

either. Conversion efficiencies increased as the amylose content decreased, especially when the amylose content was $>35\%$. The reduced quadratic model fits the conversion efficiency data better than the full quadratic model does. Fermentation tests on mashes made from corn, sorghum, and wheat samples with different amylose contents confirmed the adverse effect of amylose content on fermentation efficiency. High-temperature cooking with agitation significantly increased the conversion efficiencies on mashes made from high-amylose (35–70%) ground corn and starches. A cooking temperature of $\geq 160^\circ\text{C}$ was needed on high-amylose corn and starches to obtain a conversion efficiency equal to that of normal corn and starch.

A great amount of research recently has been conducted to increase ethanol yield and conversion efficiency from starch-rich sources. For example, plant breeders have made a great effort to develop new and improved corn hybrids with higher starch content to increase ethanol yields (Bothast and Schlicher 2005). Wang et al (1997, 1998) studied the saccharification and fermentation characteristics of rye and triticale for ethanol production. The saccharification and fermentation efficiencies of oats, barley, wheat, and pearl millet have also been investigated (Thomas and Ingledew 1990, 1995; Thomas et al 1995; Sosulski et al 1997; Wu et al 2006). These authors reported conversion efficiencies of starch to ethanol in the above-mentioned cereal grains were $\approx 90\%$. The effects of other factors such as fermentation temperatures, free amino nitrogen, nitrogen sources, bacterial contamination, and preprocessing of feedstock on ethanol fermentation have also been investigated (Thomas and Ingledew 1990; O'Connor-Cox et al 1991; Jones and Ingledew 1994a,b; Sosulski et al 1997; Narendranath et al 2000). But the relationships among the chemical composition of grains and ethanol production have not sufficiently been studied.

The major components of cereal grains are starch, protein, fiber, and lipids. The bioavailability of starch may differ among grain cultivars and may affect the conversion rate and final yield of ethanol (Moorthy 2002). Starch is a polymer of glucose, composed of various genetically determined ratios of amylose and amylopectin. Amylose is basically a linear polymer with ≈ 200 to 6,000 glucose units (MW 10^5 – 10^6) linked mainly by α -1,4 bonds ($\approx 99\%$) and few α -1,6 bonds ($<1\%$). Amylopectin, on the other hand, is a

much larger and highly branched polysaccharide with up to 3×10^6 glucose units and a MW of $\approx 5 \times 10^8$ and linked by $\approx 95\%$ α -1,4, and 5% α -1,6 bonds. In general, normal cereal starches contain 20–30% amylose and 70–80% amylopectin. Starches with $<5\%$ and $>35\%$ amylose are defined as waxy and high-amylose starch, respectively (Tester et al 2004b). Cereal cultivars with various amylose contents have been developed in corn, rice, wheat, barley, and sorghum (Jacobs and Delcour 1998; Tester et al 2004a,b; Goesaert et al 2005).

Many researchers have studied the structure and physical properties of high-amylose starches. High-amylose starches had higher gelatinization temperatures (Shi et al 1998) and formed stronger gels (Case et al 1998). Starch gels with different amylose contents had different continuous matrix structure (Leloup et al 1991). Higher cooking temperatures and branched starch molecules could retard the reassociation of starch molecules, phase separation, and network development processes during cooling (Case et al 1998; Klucinec and Thompson 1999). The resistance of high-amylose starches to α -amylase was also investigated (Sievrt and Pomeranz 1989, 1990; Richardson et al 2000; Brumovsky and Thompson 2001; Evans and Thompson 2004). They reported that the residual resistant starches found after amylolytic hydrolysis of gelatinized starches consisted mainly of retrograded amylose. Reid et al (1998) reported that the amylose-to-amylopectin ratio of starches significantly affected its fermentation to fatty acid by *Clostridium butyricum*, especially after pancreatin digestion and retrogradation. But there is no information about the effects of amylose content in starches and grains on the production of ethanol and other bioproducts. The objective of this study was to determine the effects of amylose contents of starches, protein, and fiber contents, as well as their interactions, on yeast fermentation of starchy materials to ethanol.

MATERIALS AND METHODS

Starch and Cereal Samples

The starch samples used in this study were Amioca (essentially pure amylopectin), Melojel ($\approx 28\%$ amylose), Hylon-V ($\approx 50\%$ amylose), and Hylon-VII ($\approx 70\%$ amylose), which were of corn origin. They were kindly provided by the National Starch and Chemical Co. (Bridgewater, NJ). High-amylose (corn-70, corn-55, and corn-35), normal, and waxy corn samples were obtained from Mark Campbell's 2004 summer breeding nursery at the Truman State University Agricultural Research Farm at Kirksville, MO. Corn-70 represents an S5 inbred line derived from the

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cross GUAT209:S13 × (OH43ae × H99ae) possessing ≈70% starch amylose and developed cooperatively between Truman State University and the Germplasm Enhancement of Maize program. Corn-55 and corn-35 were developed from an open-pollinated synthetic cultivar Hsyn-99 that was backcross-converted to possess the recessive starch-altering alleles amylose extender (*ae*) and dull (*du*) sugary-2 (*su2*), respectively. Both corn-55 and corn-35 were developed by David Glover at Purdue University. Normal and waxy sorghum samples were obtained from the Department of Agronomy, Kansas State University (Manhattan, KS). Normal and waxy wheat samples were from the USDA-ARS (Lincoln, NE). The grain samples were ground to a fine meal (≈99% passed a 1.19-mm sieve) using a Magic Mill III Plus grain mill (Magic Mill Products & Appliances, Monsey, NY). The chemical composition of the corn, wheat, and sorghum samples are listed in Table I.

Central-Composite Design

The central-composite design approach was used to study the effects and interactions of corn amylose, protein, and fiber contents on ethanol yield and conversion efficiency. The central-composite design is a type of response-surface methodology (RSM) that is focused on characteristics of the fit response function where optimum estimated response values occur. Five concentrations of amylose (5.56–64.4%), corn gluten (8.5–15.5%) (Sigma, St. Louis, MO), and corn fiber (8.5–15.5%) were used in the formulated fermentation media. The actual contents and arrangements of these factors are detailed in Table II. The software Design-Expert 5 (Stat-Ease Corporation, Minneapolis, MN) was used for

model development and model analysis. Starches and cereal samples with different amylose contents were used to verify the results from the response-surface design tests. All experiments were conducted in triplicate. Results were presented as averages of replicates.

Preparation of Fermentation Media from Starches and Grain Samples

The liquefaction and saccharification processes were the same as those described by Wu et al (2006). The components of the formulated media, containing 20.0 g of starch or 30.0 g of cereal samples, were mixed with 100 mL of distilled water in 250-mL Erlenmeyer flasks. The mixed slurries were digested in a water bath shaker at 95°C for 45 min after the addition of 10 µL of Liquezyme SC DS (240 KNU/g, 1.25 g/mL; Novozyme, Franklinton, NC), an enzyme preparation containing a thermostable α-amylase as major component. During the early stage of digestion, flasks were shaken manually to prevent the formation of a gel. The digested mashes were taken out of the water bath after 45 min and cooled to 80°C, and a second dose of 10 µL of Liquezyme was added to each flask. The liquefaction step was continued in the water bath shaker at 120 rpm for an additional 30 min at 80°C. Then saccharification was conducted in a 60°C water bath shaker at 120 rpm for 30 min after 100 µL of Spirizyme (750 AGU/g, 1.15 g/mL; Novozyme, Franklinton, NC), an enzyme preparation containing a glucoamylase as a major component, was added to each flask. The definitions of KNU and AGU are the same as described by Wu et al (2006). The starch

TABLE I
Moisture Content and Chemical Composition (% , db) of Cereal Samples^a

Samples	Moisture (%)	Starch	Protein	Crude Fat	Crude Fiber	Ash
Corn-70	12.1 ± 0.03	62.8 ± 0.10	11.3 ± 0.01	4.7 ± 0.10	2.1 ± 0.03	1.8 ± 0.03
Corn-55	11.1 ± 0.12	61.8 ± 0.10	11.0 ± 0.04	6.3 ± 0.10	1.8 ± 0.03	1.6 ± 0.02
Corn-35	11.8 ± 0.02	65.3 ± 0.06	8.0 ± 0.02	5.7 ± 0.03	2.0 ± 0.08	1.4 ± 0.01
Normal corn	13.0 ± 0.00	72.6 ± 0.15	8.4 ± 0.0	4.0 ± 0.01	2.0 ± 0.05	1.5 ± 0.03
Waxy corn	11.3–12.3	65.3–72.9	8.8–13.7	4.5–6.3	1.1–1.9	1.4–1.8
Normal sorghum	11.6–12.1	71.2–77.9	10.6–14.8	3.2–3.6	1.2–1.6	1.5–1.9
Waxy sorghum	9.6–12.7	68.4–72.4	9.7–12.2	3.3–6.8	1.0–1.7	1.3–1.7
Normal wheat	10.7–14.3	63.4–70.5	13.6–17.8	1.6–3.2	1.5–2.2	1.6–2.1
Waxy wheat	10.2–13.0	59.9–62.8	8.4–10.5	2.6–3.1	1.7–2.0	1.6–1.7

^a Data are either mean ± SD for the same sample or ranges of the same type samples.

TABLE II
Central Composite Design Arrangement of Parameters and Response

Tests	Coded Variables			Actual Variables (%) ^a			Conversion Efficiency (%)
	Amylose	Protein	Fiber	Amylose	Protein	Fiber	
1	-1	-1	-1	18.0	10.0	10.0	86.82
2	1	-1	-1	52.0	10.0	10.0	80.61
3	-1	1	-1	18.0	14.0	10.0	86.98
4	1	1	-1	52.0	14.0	10.0	80.62
5	-1	-1	1	18.0	10.0	14.0	88.25
6	1	-1	1	52.0	10.0	14.0	83.44
7	-1	1	1	18.0	14.0	14.0	86.69
8	1	1	1	52.0	14.0	14.0	80.27
9	-1.732	0	0	5.6	12.0	12.0	89.57
10	1.732	0	0	64.4	12.0	12.0	74.34
11	0	-1.732	0	35.0	8.5	12.0	83.91
12	0	1.732	0	35.0	15.5	12.0	85.04
13	0	0	-1.732	35.0	12.0	8.5	84.10
14	0	0	1.732	35.0	12.0	15.5	84.75
15	0	0	0	35.0	12.0	12.0	84.38
16	0	0	0	35.0	12.0	12.0	84.58
17	0	0	0	35.0	12.0	12.0	86.25
18	0	0	0	35.0	12.0	12.0	84.65
19	0	0	0	35.0	12.0	12.0	84.08
20	0	0	0	35.0	12.0	12.0	84.24

^a Percentage of amylose was based on total starch, and percentages of protein and fiber were based on total dry mass.

hydrolysates (100 mL/flask) were supplemented with 0.5 g of yeast extract, 0.1 g of K₂HPO₄, and 20 ppm of CaCl₂, whereas the cereal hydrolysates (100 mL/flask) were supplemented with 0.3 g of yeast extract and 0.1 g of K₂HPO₄.

High-temperature cooking was conducted in a reactor (Parr Instrument Co., Moline, IL) at 120, 140, and 160°C for high-amylose starch and grain samples. Samples (90.0 g of grain or 60.0 g of starch) were first digested with Liquozyme (≈0.3 KNU/g of starch) at 95°C for 30 min in the reactor with the mixer stirring at ≈200 rpm. Samples were then heated to the designated temperatures (120, 140, or 160°C) for 10 min. After the temperature was cooled to 80°C, a second dose of Liquozyme (≈0.3 KNU/g of starch) was added, and the liquefaction continued for an additional 30 min. The liquefied samples were cooled to ≈60°C and divided into flasks (30 g of dry mass of grains or 20 g of dry mass of starch/flask) for saccharification. Saccharification was conducted at 60°C for 30 min in a water bath shaker at 120 rpm with 100 μL of Spirizyme (≈85 AGU) added into each flask. The supplements were similar to those during the 95°C cooking described earlier.

Insoluble particles in mashes made from high-amylose starches and corn samples were separated by centrifugation at 4,500 rpm for 10 min and were washed twice with 75 mL of distilled water each time.

Fermentation Processes

The prepared mashes made from 20 g dry mass of starches or 30 g dry mass of ground cereals were adjusted to a value of pH 4.2–4.3 with 2N HCl and inoculated with 5 mL of yeast preculture (strain *Saccharomyces cerevisiae* ATCC 24860). The yeast preculture was prepared as described by Suresh et al (1999) and Zhan et al (2003). The cell concentration of the yeast preculture was checked by its A₆₀₀ value on a BioRite spectrophotometer and by using a counting chamber (Fisher Scientific, Fairlawn, NJ). The A₆₀₀ values of the 48 hr precultures were ≈2.4 for cell concentrations between 2 and 2.8 × 10⁸ cells/mL, which ensured that inoculated mashes had a cell concentration of ≈1.5 × 10⁷ cells/mL.

The ethanol fermentation was performed in an incubator shaker (model I2400, New Brunswick Scientific, Edison, NJ) at 30°C for 72 hr at 150 rpm. Because ethanol fermentation is an anaerobic process, the fermentation flasks were sealed with S-bubblers filled with ≈2 mL of mineral oil. The ethanol fermentation process was monitored by measuring the weights of the fermentation flasks with S-bubblers because the weight loss by CO₂ evolution is proportional to the amount of ethanol produced during ethanol fermentation (Joekes et al 1998). The final ethanol concentration in the fermented beer was determined by the HPLC method after distillation as described in AOAC method 942.06 (AOAC International 1999).

Analytical Methods

Crude fat, moisture, protein, and ash contents were determined by following AOAC official methods 920.39, 925.10, 990.03, and 942.05 (AOAC International 1999). The total starch contents and

amylose and amylopectin contents were determined by using the Megazyme total starch and amylose/amylopectin kits (Bray, Ireland) (Approved Method 76-13, AACC International 2000; Method 996.11, AOAC International 1999) (available at Megazyme at <http://secure.megazyme.com/downloads/en/data/K-AMYL.pdf>). Crude fiber was analyzed by the ANKOM A200 filter bag technique (ANKOM Technology) (available at http://www.ankom.com/09_procedures/procedures3.shtml).

The ethanol concentration was determined by HPLC equipped with a Rezex RCM column (Phenomenex, Torrance, CA) and a Shimadzu RID-10A detector (Columbia, MD). The temperatures of the column and detection cell were set at 80 and 40°C, respectively. The flow rate of the mobile phase (water) was 0.6 mL/min. The retention time of ethanol was ≈16.70 min. Conversion efficiencies were calculated as a ratio of the experimentally determined ethanol yield to the theoretical ethanol yield. The total starch contents in the samples were used to calculate the theoretical ethanol yields, assuming 1.0 g of starch converts to 1.11 g of glucose and that 1.0 g of glucose should generate 0.511 g of ethanol (Thomas et al 1996).

RESULTS AND DISCUSSION

Conversion Efficiencies of Formulated Mashes

Quadratic models are often used in describing the effects of multiparameters on responses and the fitness of a model is assessed by analysis of variance. The conversion-efficiency data from our central-composite design tests were fitted into both quadratic and reduced quadratic models, and the analyses of variance results of both models are shown in Table III. According to Joglekar and May (1987), a good model should have a significance level of $P < 0.05$, an R^2 value >0.800 , and a coefficient of variance (CV) value $<10\%$. On the basis of these criteria, both the full quadratic model and the reduced quadratic model are adequate models for predicting the fermentation efficiency. The reduced quadratic model fits the obtained data better than the full quadratic model, however, because the P value in the lack-of-fit test (0.186) and the adjusted R^2 value (0.893) for the reduced quadratic model are greater than those of the full quadratic model (0.091 and 0.869, respectively). The P values of all the coefficients in the full quadratic model are between 0.18 and 0.94, except those for amylose and amylose squared (<0.0001 and 0.0247), which suggests the necessity of reducing the full quadratic model. The reduced quadratic model is: Efficiency (%) = 88.70 – 0.01513 × A – 0.0028 × A², where A represents the percentage of amylose in starch.

The P values for the coefficients (amylose and amylose squared) in the reduced quadratic model are both <0.0001 , which means that amylose content is a significant predictor of conversion efficiency in ethanol fermentation with media made from starchy materials. Therefore, the reduced quadratic model is an adequate model in predicting conversion efficiency on media made from starches with different amylose contents when cooking at 95°C.

Model results showed that amylose content significantly affects the conversion efficiency; that is, the greater the amylose content,

TABLE III
Analyses of Variance for Quadratic and Reduced Quadratic Models

Model	Source of Variance	DF	Sum of Squares	Mean of Squares	F	Prob > F
Quadratic model	Regression	9	196.2	21.80	15.02	0.0001
	Residual	10	14.51	1.451		
	Pure error	5	3.118	0.624		
	Lack-of-fit	5	11.39	2.278		
$R^2 = 0.931, R_{adj}^2 = 0.869, CV = 1.43\%, PRESS = 80.0$						
Reduced quadratic model	Regression	2	190.4	95.21	79.96	<0.0001
	Residual	17	20.24	1.191		
	Pure error	5	3.118	0.624		
	Lack-of-fit	12	17.12	1.427		
$R^2 = 0.904, R_{adj}^2 = 0.893, CV = 1.30\%, PRESS = 45.7$						

the lower the conversion efficiency. The other two parameters, corn protein and corn fiber contents, did not have significant effects on the conversion efficiency, which is also clearly demonstrated in Fig. 1. Even though the protein or fiber contents changed from 10 to 14%, the fermentation efficiencies remained essentially the same when the amylose contents were fixed. The fermentation efficiencies decreased remarkably as the amylose contents increased from 18 to 52% at any fixed fiber level (Fig. 1A and B) or fixed protein level (Fig. 1C and D).

Saccharomyces cerevisiae can not utilize protein as its nitrogen source when proteins are present as large polymers; therefore, ammonium, urea, or amino acids have to be added to support the growth of yeast cells (Rose and Harrison 1987). The amount and kinds of nitrogen source not only affect the growth of the yeast cells but also influence the amounts of by-products and ethanol yield. Yeast usually produce more ethanol and less glycerol and other by-products when amino acids are used as a supplemental nitrogen source (Reed and Nagodawithana 1991). O'Connor-Cox et al (1991) reported that a high concentration of free-amino nitrogen not only facilitated the fermentation rate but also increased the ethanol yield. In this study, no effect of corn protein content on fermentation rate or efficiency was observed. This is probably so because yeast extract (0.5 g) containing more than 50% free-amino nitrogen was added to the fermentation medium (100 mL). The supplemented yeast extract evidently provided enough free amino acids to meet the growth and fermentation requirements of the yeast. Little, if any, free-amino nitrogen existed in the corn gluten meal added to the artificial media. *S. cerevisiae* could not hydrolyze cellulose and use it as its sole carbon source. In addition, the nonsignificant effects of fiber contents on fermentation efficiency suggested that there are no fiber-degrading enzymes

present in the Liquozyme SC DS and Spirizyme enzyme preparations.

Fermentation Results from Grains with Different Amylose Contents

To confirm the results from the formulated corn media with different amylose contents in the central-composite design tests, other starchy substrates including corn, corn starch, sorghum, and wheat samples with various amylose contents were used to produce ethanol. Results from fermentation of grains and starches also indicated that amylose content had a significant effect on ethanol fermentation efficiency (Table IV). Again, the conversion efficiency decreased as the amylose content increased. This result is also in agreement with the findings in starch-digestibility studies by Okuda et al (2005). They reported that the digestibility of rice starch was negatively correlated with the amylose content of the starch. Tester et al (2004b) obtained similar results when they studied the amylolytic hydrolysis of waxy, normal, and high-amylose starches. Conversion efficiency to produce ethanol by fermentation is usually 90–95%. The reasons for the imperfect conversion efficiency may include incomplete hydrolyses of starches, glucose consumption (due to the growth of yeast during fermentation), or the inevitable production of by-products during ethanol fermentation (Rose and Harrison 1987; Kosaric and Vardar-Sukan 2001). Starch granules consist of amylose and amylopectin that form a semicrystalline structure. These starch granules are resistant to the digestion of α -amylase and amyloglucosidase. For amylolytic enzymes to readily hydrolyze starch, starch molecules (amylose and amylopectin) have to be changed into amorphous form and become easily accessible to the hydrolyzing enzymes by a process known as gelatinization (Jacobs and Delcour 1998;

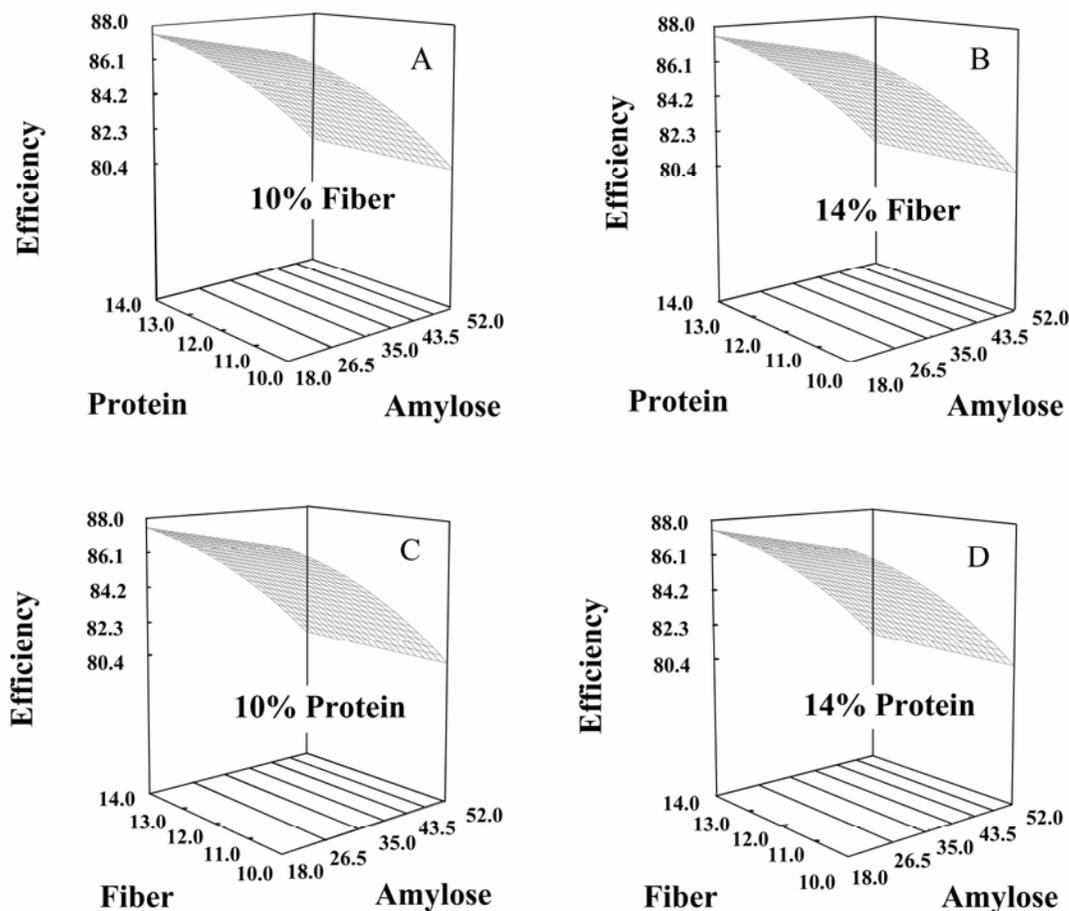


Fig. 1. Effects of amylose, fiber, and protein contents on fermentation efficiency.

Tester et al 2004b). Starches from different botanical sources or from the same source but with different amylose contents may have very different gelatinization temperatures and ranges, which can be determined by differential scanning calorimetry (DSC) thermal analysis (Shi et al 1998; Klucinec and Thompson 1999; Liu et al 2005). The gelatinization temperatures of starches from different sources can be as low as 60°C (Ji et al 2004) or as high as 144–166°C for Hylon-V and 154–171°C for Hylon-VII (National Starch Food Innovation 2005). Because of the existence of starch granules with high gelatinizing temperatures, and the formation of amylose-lipid complex and reassociation of amylose molecules during gelatinization (Boltz and Thompson 1999) and enzymatic hydrolysis, there is always some starch that escapes hydrolysis by amylolytic enzymes. This was observed in an earlier study by Hebeda and Leach (1974), in which ≈2% of the starch in industrial processes for dextrose production remained undigested as insoluble particles in the hydrolysates. Evans and Thompson (2004) reported no clear relationship between the amylose content and enzyme-resistant starch contents. But, in most instances, resistant-starch contents increase as the amylose content in starch increases (Berry 1986; Sievert and Pomeranz 1989). For example, the amylose levels in starches from waxy corn, potato, wheat, normal corn, pea, amylomaize V, and amylomaize VII are <1.0, 20, 25, 26, 33, 53, and 70%, respectively, whereas their enzyme-resistant starch contents are 2.5, 4.4, 7.8, 7.0, 10.5, 17.8, and 21.3%, respectively (Sievert and Pomeranz 1989, 1990). Results from our study showed a similar trend; the insoluble particles from corn starch with ≈6, 18, 52, and 64% amylose were 0.5, 7.6, 13.5, and 29.4%, respectively, after cooking at 95°C and liquefied with α-amylase and saccharified with glucoamylase.

Several researchers reported that a high-temperature treatment (Würsch and Koellreutter 1992; Ezeogu et al 2005) or stirring of the starch slurry during gelatinization (McCleary and Monaghan 2002; Woo and Seib 2002) can significantly decrease the enzyme-

resistant starch content in starch-based samples. These observations and the features of DSC thermograms of high-amylose starches (Shi et al 1998; Klucinec and Thompson 1999; Brumovsky and Thompson 2001) suggest that cooking high-amylose starches at higher temperature, with shearing, may significantly increase the digestibility of the high-amylose starches and therefore improve the conversion efficiency.

Improving Fermentation Efficiency by High-Temperature Cooking and Stirring

High-temperature cooking and stirring in a Parr Reactor at 120, 140, and 160°C for 10 min was used to increase the hydrolyzing and fermentation efficiencies of both high-amylose corn and high-

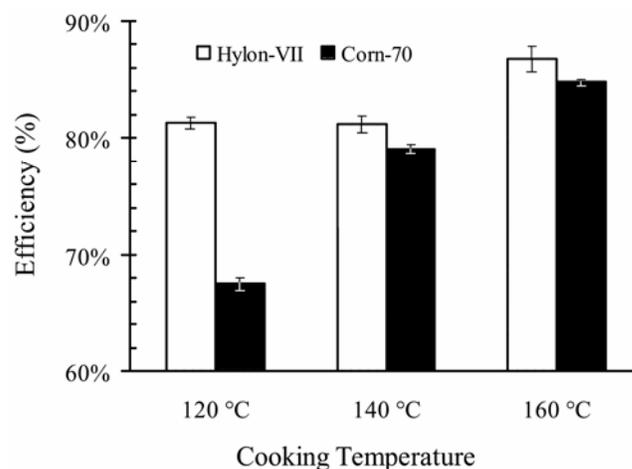


Fig. 2. Effects of cooking temperature on fermentation efficiencies of mashes made from Hylon-VII and Corn-70.

TABLE IV
Effects of Cooking Temperature (95 or 120°C) on Fermentation Efficiency of Starches with Different Amylose Contents^a

Sample	Amylose (% db)	Efficiency ^b (%)		Increase in Efficiency (%)
		95°C	120°C	
Ground corn				
Corn-70	73.9	52.9 ± 2.44	67.5 ± 0.55	27.6
Corn-55	53.6	61.8 ± 0.57	75.2 ± 0.53	21.6
Corn-35	40.3	80.4 ± 0.95	81.7 ± 0.40	1.64
Normal corn	22.8	88.7 ± 1.17	87.8 ± 0.83	-0.96
Waxy corn	7.5	89.6 ± 0.62	88.5 ± 0.46	-1.25
Corn starches				
Hylon-VII	66.2	68.7 ± 0.59	80.9 ± 0.54	18.9
Hylon-V	51.8	81.3 ± 0.15	86.5 ± 0.25	6.73
Melojel	27.5	89.8 ± 0.34	89.2 ± 0.82	-0.73
Amioca	5.9	90.9 ± 0.73	90.4 ± 0.81	-0.56
Ground wheat				
Normal wheat	23.3–25.2	86.6–87.6	–	–
Waxy wheat	1.8–2.1	91.6–91.9	–	–
Ground sorghum				
Normal sorghum	23.2–25.7	85.6–88.9	–	–
Waxy sorghum	3.0–3.2	87.1–89.2	–	–

^a Data are either mean ± SD for the same sample or ranges of the same type samples.

^b Fermentation efficiency (%) = Actual ethanol yield/theoretical ethanol yield × 100.

TABLE V
Insoluble Residues Recovered by Centrifuging Mashes Made from Different Starches (20 g dry mass) Cooked at Different Temperatures^a

Starch	Cooking at 95°C			Cooking at 120°C			Difference (%)
	Residues (g)	% of Original Starch	Starch (%) in Residues	Residues (g)	% of Original Starch	Starch (%) in Residues	
Hylon-VII	5.88	29.4	94.1 ± 0.10	2.64	13.2	90.4 ± 0.20	16.2
Hylon-V	2.71	13.5	89.6 ± 0.38	1.16	5.80	79.2 ± 0.28	7.75

^a Mean ± standard deviation.

amylose starch samples (Table IV and Fig. 2). When cooking at 120°C, the fermentation efficiencies increased 27.6% for corn-70, 21.6% for corn-55, 18.9% for Hylon-VII, and 6.73% for Hylon-V. However, because of considerable amounts of starch in the mash that escaped the amylolytic digestion, their efficiencies were still significantly ($P < 0.01$) below those of samples with normal or low amylose contents. The insoluble particles separated from the hydrolysates by centrifugation accounted for 13.5 and 29.4% of the original high-amylose starch when cooked at 95°C, and 5.8 and 13.2% when cooked at 120°C (Table V). The conversion efficiency increased as cooking temperature increased. The highest conversion efficiency was obtained with cooking temperature at 160°C. Results indicated that 120 and 140°C temperatures were not high enough to gelatinize and disrupt the starch granules in Hylon-VII and corn-70, even with mechanical stirring (Fig. 2). At 160°C, both the Hylon-VII and Corn-70 samples had conversion efficiencies similar to those of normal and waxy starch and cereal samples. This was in good agreement with the results described by Case et al (1998), who reported that higher cooking temperature (>160°C) not only retarded the gelation process of the cooked starch but also significantly reduced the strength of the starch gels. The unorganized starch molecules produced by high-temperature cooking are more accessible to amylolytic enzymes, which results in high conversion efficiencies.

Although the conversion efficiencies for both high-amylose corn and high-amylose starch samples were improved significantly ($P < 0.01$) after high temperature cooking at 120, 140, and 160°C, the efficiencies for high-amylose ground corn samples were still significantly ($P < 0.01$) lower than those for high-amylose starch samples (Table IV and Fig. 2), when compared at the same cooking temperature. Two factors may contribute to the lower conversion efficiency for high-amylose corn samples. First, the formation of lipid-amylose complexes may have contributed to the efficiencies for high-amylose corn being lower than those for high-amylose starches. The lipid-complexed amylose in cereal starch could range from a few percentages of the total starch (Morrison 1995) to >55% (Tester et al 2004a). The lipid contents in high-amylose corn samples were ≈4.5%, some of which are monoacyl lipids (Morrison et al 1993). The amylose complexes with monoacyl lipids, not triglycerides. Monoacyl lipid in corn starch increases as amylose increases, so there might be more amylose-lipid complexes in high-amylose starch. Therefore, the amount of amylose-lipid complexes is likely larger in the ground corn mashes than in the starch mashes, which contain <1% lipids. Because amylose-lipid complexes are resistant to enzymatic hydrolysis, the percentage of hydrolyzable starches in the high-amylose corn samples will be less than that in the high-amylose starches. Second, particle sizes of the samples may play an important role in the digestibility of starch. Marshall (1992) found that the gelatinization temperature increased by ≈10°C as the particle sizes of milled rice increased from 50 μm to ≈1,000 μm. The particle sizes of corn starch granules are mostly <30 μm (Tester et al 2004a,b), whereas the particle sizes of the ground cereal are mostly in the range of 400–900 μm (Garber et al 1997). Therefore, the percentage of gelatinized and disrupted starch granules in the mashes from high-amylose ground corn (coarser samples) will be less than the percentage from the high-amylose corn starch samples, which will inevitably lead to a lesser degree of hydrolysis of starch and conversion efficiency for the high-amylose corn samples.

Results in Table IV also show that the efficiencies of samples with <35% amylose decreased slightly after high-temperature cooking. The slight decrease (0.56 to 1.25%) in efficiencies could be because Maillard reactions between amino groups and free glucose had consumed more reducing sugars and free amino acids during high temperature cooking than during the 95°C cooking process (Colonna et al 1992). The darkened color of hydrolysates from high-temperature cooking indicated more Maillard reaction products than did hydrolysates from 95°C cooking.

CONCLUSIONS

Fermentation of mashes to ethanol in shaking-flask tests using the central-composite design showed that amylose in starches adversely influences the ethanol conversion efficiency when starchy substrates were cooked at atmospheric pressure. Fermentation on mashes made from corn, sorghum, and wheat samples with different amylose contents confirmed the adverse effects of amylose on conversion efficiency. When the amylose content in the starches of cereals is <30% of starch, the effects of amylose contents on ethanol fermentation efficiency are not significant. Corn protein and corn fiber contents in the fermentation mashes do not have significant effects on the conversion efficiency. High-temperature cooking, especially at 160°C, can significantly increase the conversion efficiency of high-amylose starch and corn samples.

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