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Effect of Resistant Starch on Hydrolysis and Fermentation of Corn Starch for Ethanol

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Abstract Starch samples with 0% or 30% amylose were subjected to four different liquefaction enzyme treatments (at various temperature and pH conditions) followed by simultaneous saccharification and fermentation (SSF). Resistant starch (RS) measurements were conducted for the initial starch sample, after liquefaction and after SSF. Initial RS was higher for 30% amylose starch samples (16.53 g/100 g sample) compared with 0% amylose (0.76 g/100 g sample). Higher initial RS resulted in lower conversion of starch into sugars and lower final ethanol yields. The four enzymes hydrolyzed RS, but in varying amounts. Higher temperature liquefaction hydrolyzed a larger portion of RS, resulting in higher ethanol concentrations and lower final residual solids (non-fermentables), whereas lower temperature liquefaction hydrolyzed a smaller portion of RS and resulted in lower ethanol concentrations and higher final residual solids. Decreases in resistant starch after high temperature liquefaction were 55% to 74%, whereas low temperature liquefaction decreases were 11% to 43%. For all enzyme treatments, RS content of starch samples decreased further after SSF.

Keywords Amylose · Dry grind corn process · Enzymes · Ethanol · Fermentation · Liquefaction · Resistant starch

Introduction

The U.S. ethanol industry produced 10.25 billion gallons of ethanol in 2008 which was five times of that produced in 2002 [1]. More than 4 billion bushels of corn, representing 33%

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of total U.S. corn production, was utilized for ethanol production. The dry grind corn industry accounted for 82% of current U.S. ethanol production. High cost of raw material (60% of operating cost, [2]) makes it important to have efficient corn starch conversion to maximize ethanol production. During enzymatic starch hydrolysis in the dry grind corn process, not all starch is converted into sugars. Unconverted (residual) starch is recovered in distillers dried grain with solubles (DDGS). The amount of residual starch in DDGS may be dependent on the type of raw starch in corn (native starch available in untreated corn) and process parameters (e.g., temperature, pH, enzymes, and duration of hydrolysis). A part of raw starch is enzyme resistant and has characteristics of crude fiber, remaining unavailable to the enzymes [3], thereby reducing ethanol yield. This portion of raw starch is known as resistant starch (RS).

Resistance of RS to hydrolysis can result from many factors, categorized into four major groups: RS1, physically inaccessible to digestion by entrapment in a non-digestible matrix; RS2, ungelatinized starch; RS3, retrograded starch; and RS4, chemically modified starch [4]. Due to the combination of these factors, RS is thermally stable and does not break down in normal cooking operations and temperatures [3, 4].

Berry [5] showed amylose levels in corn starch correlated with RS levels; Evans and Thompson [6] reported that waxy starch had less RS compared to high amylose starch. Due to its linear chain structure, after gelatinization and during retrogradation, amylose forms a thermally stable RS3 resistant to enzyme [7]. Regular dent and waxy corn, commonly used in the dry grind corn process, have 30% and 0% amylose content, respectively. Corn starch is hydrolyzed and fermented through liquefaction and simultaneous saccharification and fermentation (SSF) steps converting raw starch to ethanol. Conventionally, high temperature (90 °C) and high pH (5.5 to 6.0) have been used during liquefaction. Novel liquefaction enzymes that can work at varying temperature and pH have been introduced. Processing conditions are known to affect RS content in starch [3]. Liquefaction at different temperatures and pH may affect RS in different amounts, therefore, influencing final ethanol yield.

The objective was to study the effect of RS on ethanol concentration in four starch hydrolysis and fermentation processes with varying liquefaction conditions. Starch samples representing regular dent and waxy corn with 30% or 0% amylose were used. To study the fate of RS at each step, measurements of RS, total starch, and solubilized starch were conducted in initial samples, after liquefaction and after SSF.

Materials and Methods

Materials

Three conventional liquefaction α-amylase enzymes and one granular starch hydrolyzing enzyme (GSHE) were used to represent high and low temperature pH combinations (Table 1). Temperature and pH conditions used for these enzymes were according to manufacturer's recommendations. These enzymes differed in original bacterial or fungal culture with optimum activities at different pH and temperatures and mineral requirements for stability. Conventional α-amylase Liquozyme SC was derived from *Bacillus stearothermophilus* and had an activity of 930 KNU/g (KNU=kilo novo units). Spezyme Xtra is α-amylase derived from a genetically modified strain of *Bacillus stearothermophilus* with activity of 14,000 AAU/g (AAU=α-amylase units, one AAU activity is enzyme required to hydrolyze 10 mg starch/min). In laboratory experiments, Spezyme Xtra has

Table 1 Enzymes used in different liquefaction treatments.

Enzyme name	Type	Temperature (°C)	pH	Manufacturer/supplier
Stargen 001	Granular starch hydrolyzing	30 to 48 (low)	4.0 to 4.2 (low)	Genencor International, Palo Alto, CA, USA
Spezyme Xtra	Liquefaction	60 (low)	5.5 to 6.0 (high)	Genencor International, Palo Alto, CA, USA
Ultra Thin	Liquefaction	90 (high)	4.5 (low)	Valley Research, South Bend, IN, USA
Liquozyme SC	Liquefaction	90 (high)	5.5 to 6.0 (high)	Novozymes, Franklinton, NC, USA

shown optimum liquefying activity at 60 °C. Ultra Thin, a low pH thermostable liquefaction α-amylase derived from *Pseudomonas fluorescens*, had an activity of 120,000 MWU/g (MWU=one modified Wohlgemuth Unit will dextrinize 1 mg of soluble starch to a defined blue value in 30 min). GSHE (Stargen 001) contained α-amylase from *Aspergillus kawachi* and a glucoamylase from *Aspergillus niger* with an activity of ≥456 GSHU/g (GSHU=granular starch hydrolyzing units). Conventional amyloglucosidase (AMG 300L, Novozymes, Franklinton, NC, USA) was obtained from Sigma-Aldrich (St. Louis, MO, USA) which was isolated from *A. niger* and had an activity of ≥300 NU/mL (NU=novo units).

Saccharomyces cerevisiae yeast culture was prepared by dispersing 11 g of active dry yeast (Fleischmann's Yeast, Fenton, MO, USA) and 1 g of yeast malt broth (Sigma, St. Louis, MO, USA) in 89 mL of distilled water and agitated at 50 rpm and 30 °C for 20 min (C24 Incubator Shaker, New Brunswick, NJ, USA). *S. cerevisiae* yeast culture had a viable cell count of 1.8×10^8 cells/mL using Petrifilm plates (3M, St. Paul, MN, USA). Yeast malt broth (BP1422-500) and urea (99.6% ACS grade) were from Fisher Scientific (Pittsburgh, PA, USA).

For pure starch, different amylose contents were prepared from commercially available starches, Hylon VII (70% amylose and 30% amylopectin) and Amioca (~100% amylopectin), obtained from National Starch and Chemical Company (Bridgewater, NJ, USA) and stored at 4 °C. Hylon VII and Amioca were mixed to achieve 30% (db) amylose contents and Amioca was used alone for 0% (db) amylose content in the starch treatments.

Resistant Starch Measurement

A RS measurement kit (KRSTAR, Megazyme, Wicklow, Ireland) based on the method developed by McCleary and Managhan [8] and approved by Association of Official Analytical Chemists (AOAC) and American Association of Cereal Chemists (AACC) was used. The RS measurement method of McCleary and Managhan [8] allowed measurement of RS, solubilized starch, and total starch contents. Slurry samples (50 g) for RS measurement were acquired at the end of liquefaction and at the end of SSF (Figs. 1 and 2). Samples were dried at 49 °C for 24 h. After drying, half (by weight) of the dried sample was stored in Corning culture tubes (Fisher Scientific) at 4 °C for RS assays and remaining half was dried further at 135 °C for 2 h for moisture determination.

Sample material (100±5 mg) was weighed directly into Corning culture tubes (Fisher Scientific). Sodium maleate buffer (pH 6.0) containing pancreatic amylase and amyloglucosidase (3 U/mL, Megazyme) was added (4 mL) to the sample and mixed vigorously

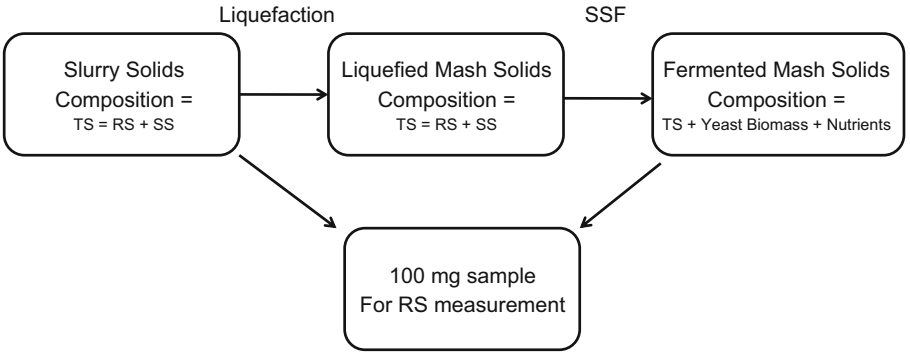

Fig. 1 Composition and sampling of slurry after liquefaction and SSF. *TS* total starch, *RS* resistant starch, *SS* soluble starch

using a vortex mixture (VortexGenie 2, Scientific Industries, Bohemia, NY, USA). Tubes were incubated for 16 h at 37 °C with agitation in a shaking water bath (MaxQ 4000, Barnstead/Labline, Melrose Park, IL, USA). Tubes were treated with 99% ethanol and centrifuged at 1,000×*g* for 10 min (model 5415D, Eppendorf, Westbury, NY, USA). Supernatants were decanted; pellets were resuspended in 50% ethanol. The ethanol centrifugation process was repeated twice. Decanted supernatant from centrifugation were made up to volume in 100-mL volumetric flasks with water. Aliquots (1 mL) were treated with 3 mL glucose oxidase-peroxidase (GOPOD) reagent (Megazyme) and incubated for 20 min at 50 °C. Absorbance was measured at 510 nm against a blank (reagent). Centrifugation pellets were treated with 2 mL of KOH (2 M) in an ice bath with stirring for 20 min. Sodium acetate buffer (pH 3.8) (8 mL) and amyloglucosidase (3,300 U/mL, Megazyme) (0.1 mL) were added; tubes were incubated for 30 min at 50 °C. Aliquots were

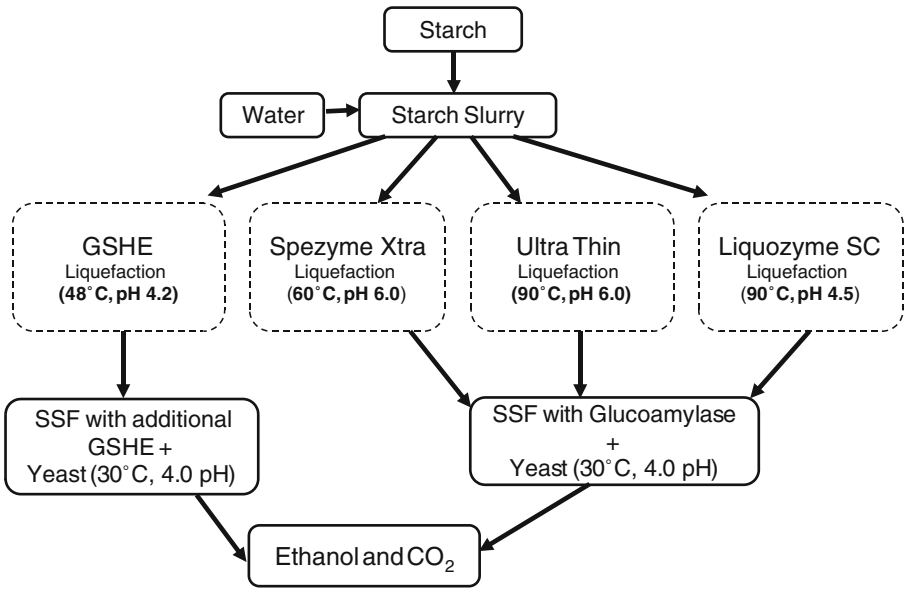

Fig. 2 Procedure flow chart for the different liquefaction enzyme treatments followed by simultaneous saccharification and fermentation (SSF) for the starch samples

removed and treated with GOPOD reagent (Megazyme) and incubated at 50 °C for 20 min. Absorbance was measured at 510 nm against a reagent blank. Glucose solution (1 mg/mL) (Megazyme) was used as a standard. Absorbance data were converted by a reference formula (as given by manufacturer to g RS/100 g db) [9, 10].

RS value for Hylon VII was reported to be 46.25% (of total starch) by McCleary and Managhan [8]. A randomized block design (RBD) was used to determine repeatability and precision of the RS lab procedure by measurement of nine samples of Hylon VII. The coefficient of variation for the RBD experiment was ±4.6% and standard deviation was 2.11 g/100 g sample. To maintain accuracy of the procedure for each RS measurement set, an RS standard (52.5 g/100 g sample) provided with the RS kit and Hylon VII was run; values were compared to actual values. The assay was applicable for samples with RS >2% *w*/*w*; higher errors were obtained for samples with RS contents <2% *w*/*w*.

Procedure for Starch Hydrolysis and Fermentation

Stargen 001 GSHE Procedure

To obtain slurries having 15% solids, starch samples (100 g) were mixed with tap water at 35 °C in a 1,000-mL Erlenmeyer flask (Fig. 2). Slurry temperature was increased to 48 °C for the incubation. Using 10 N sulfuric acid solution, slurry pH was adjusted to 4.2 and GSHE (140 μL) was added. The slurry was maintained at 48 °C for 2 h at 120 rpm in water bath. Incubation resulted in reduced viscosity leading to improved mixing during fermentation. After 2-h incubation, the slurry was cooled to 30 °C and pH was adjusted to 4.0 with 10 N sulfuric acid solution. Simultaneous saccharification and fermentation (SSF) of samples was conducted by adding GSHE (140 μL) and yeast (0.01 g/g starch) to the slurry. Yeast malt broth (2 g) and urea (0.1% of slurry) were added as yeast supplements. Slurry was maintained at 30 °C for 72 h with constant agitation at 100 rpm. All runs were carried out in 1,000-mL flasks in a shaking water bath (model DHOD-182, Bellco Glass, Vineland, NJ, USA). To determine concentrations of ethanol, glucose, fructose, maltose, maltotriose, DP4+, glycerol, and lactic, succinic, and acetic acids, 3 mL sample was drawn from each slurry at 0, 2, 4, 6, 8, 10, 12, 24, 48, and 72 h and analyzed using HPLC.

Conventional Spezyme Xtra Procedure

For conventional hydrolysis and fermentation (Fig. 2) using Liquozyme SC liquefaction enzyme, a 100 g laboratory dry grind procedure was used. Slurry with 15% solids was adjusted to pH of 5.5 to 6.0 using 10 N sulfuric acid solution. Samples were liquefied by increasing slurry temperature to 60 °C and adding 140 μL α-amylase Spezyme Xtra. The slurry was maintained at 60 °C for 2 h with continuous agitation at 120 rpm. After 2 h, slurry temperature was decreased to 30 °C and pH was adjusted to 4.0 with 10 N sulfuric acid. SSF of samples was performed as in the GSHE process except that glucoamylase (140 μL) was added with yeast and yeast supplements (yeast malt and urea) to the slurry instead of GSHE. Slurry was maintained at 30 °C for 72 h with constant agitation at 100 rpm.

Ultra Thin and Liquozymes SC Processes

Liquefaction and SSF procedures used for Spezyme Xtra were similar to those used for Ultra Thin and Liquozyme SC, except the liquefaction temperature was 90 °C and pH was 4.5 for Ultra Thin and pH was 5.5 to 6.0 for Liquozyme SC (Fig. 2).

HPLC Analysis

Samples were centrifuged (model 5415 D, Eppendorf) for 2.5 min at 16,110×*g* (13,000 rpm) to obtain supernatant liquid which was filtered through a 0.2 μm filter and injected into an ion exclusion column (Aminex HPX-87H, Bio-Rad, Hercules, CA, USA) maintained at 50 °C. Sugars (glucose, fructose, maltose, maltotriose), organic acids (lactic, succinic, acetic), and alcohols (ethanol, methanol, glycerol) were eluted from the column with HPLC-grade water containing 5 mM sulfuric acid. Separated components were detected with a refractive index detector (model 2414, Waters Corporation, Milford, MA, USA). Elution rate was 0.6 mL/min; a calibration standard was used prior to each batch run. Data were processed using HPLC software (Breeze™ software, Waters Corporation).

Statistical Design and Analysis

Each hydrolysis and fermentation treatment was performed with three replications and each RS determination was performed with eight determinations. Experiments and assays were arranged in a randomized complete block design. Analysis of variance and Tukey's test (SAS Institute, Cary, NC) were used to compare mean ethanol concentrations and RS values among treatments. Statistical significance level was 5% ($p<0.05$).

Results and Discussion

SSF profiles for glucose and ethanol concentrations for the four liquefaction enzyme treatments on starch samples with two amylose contents (0% and 30% amylose) are shown in Figs. 3, 4, 5, and 6. Initial glucose concentrations (0 to 6 h) were higher for 0% compared to 30% amylose starch showing faster rate of hydrolysis during SSF following liquefaction (Figs. 3 and 4). Similarly, 0% amylose starch had higher ethanol production rates compared to 30% amylose starch (Figs. 5 and 6). Sharma et al. [11] found that starch with 0%

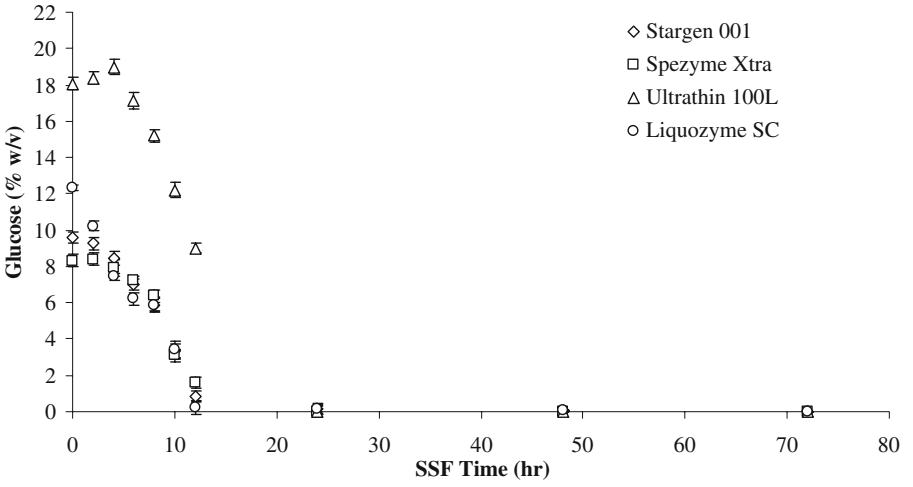

Fig. 3 Glucose concentrations during SSF for 30% amylose starch at 15% solids content. *Error bars* are ±1 standard deviation

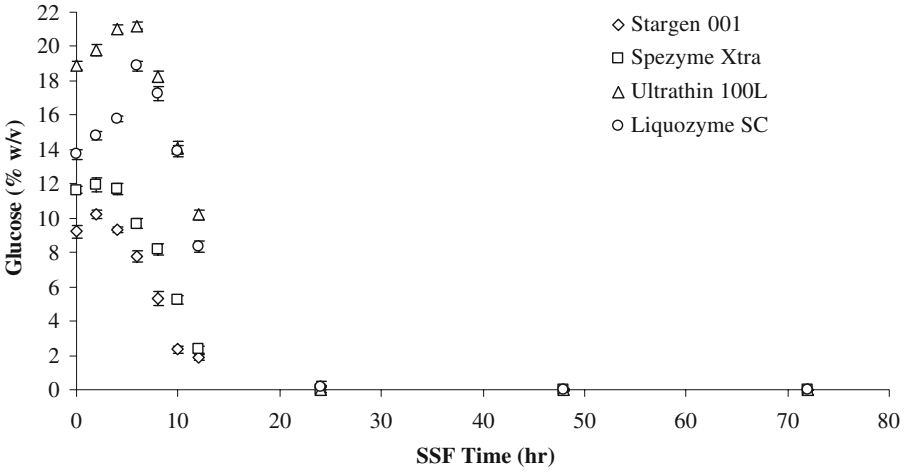

Fig. 4 Glucose concentrations during SSF for four enzyme treatments for 0% amylose starch at 15% solids content. *Error bars* are ±1 standard deviation

amylose (100% amylopectin) was hydrolyzed at a higher rate, compared to starch with amylose, producing higher glucose concentrations at fast rates for various temperature conditions.

High temperature liquefaction treatments (Ultra Thin 100L and Liquozyme SC) resulted in high initial glucose concentrations for both 30 (18.99% *w*/*v* at 4 h and 12.33% *w*/*v* at 0 h of SSF, respectively) and 0% amylose starch (21.22% and 18.88% *w*/*v* at 6 h of SSF, respectively) (Figs. 3 and 4; Table 2). For low temperature liquefaction treatments (GSHE and Spezyme Xtra), glucose concentrations remained less than 12% *w*/*v* for both 30% and 0% amylose starch (Figs. 3 and 4). High temperature liquefaction (Liquozyme SC and Ultra Thin 100L) achieved higher ethanol concentrations than low temperature liquefaction

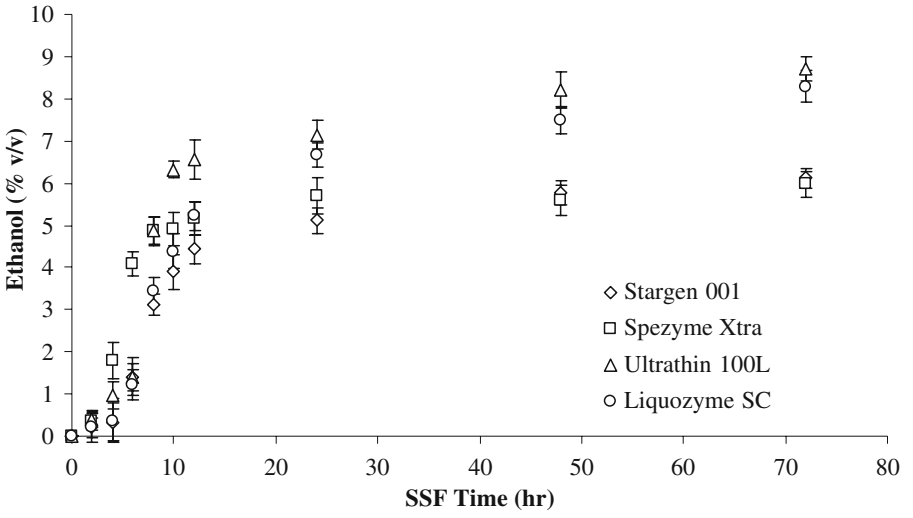

Fig. 5 Ethanol concentrations during SSF for four enzyme treatments for 30% amylose starch at 15% solids content. *Error bars* are ±1 standard deviation

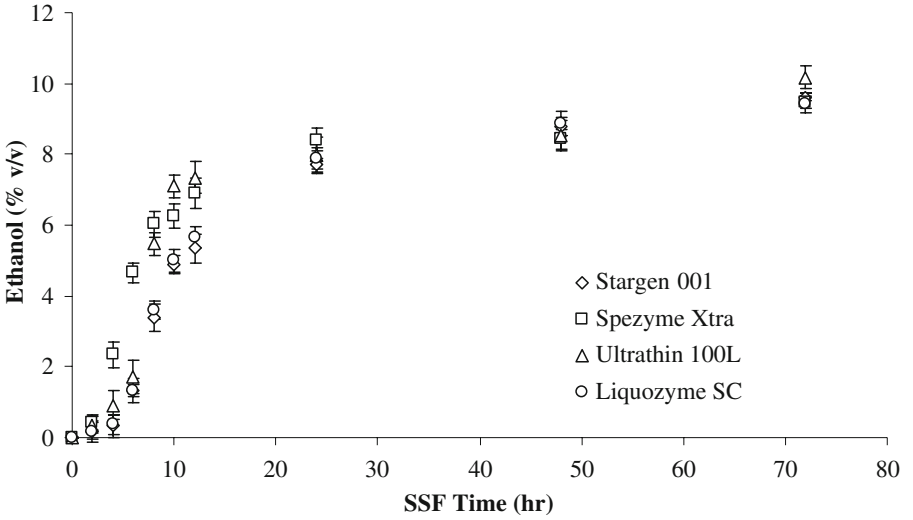

Fig. 6 Ethanol concentrations during SSF for four enzyme treatments for 0% amylose starch at 15% solids content. *Error bars* are ±1 standard deviation

(GSHE and Spezyme Xtra) for 30% amylose starch (Table 2). Mean ethanol concentration, across all liquefaction treatments, was higher for 0% amylose starch (9.68% *v*/*v*) compared to 30% amylose starch (7.28% *v*/*v*) (Table 2). High temperature liquefactions hydrolyzed a larger percentage of starch with higher amylose content compared to low temperature liquefaction (Figs. 7 and 8). Starch with 0% amylose was hydrolyzed equally during liquefaction and SSF by all treatments. For 0% amylose, starch is completely hydrolyzed and the low temperature liquefaction helped maintain low glucose levels during the SSF period. This resulted in a similar ethanol concentration to high temperature liquefaction (Table 2). A low glucose concentration in fermentation mash was desirable because high glucose concentrations are known to cause osmotic stress to yeast [12].

Table 2 Ethanol and glucose concentrations for starch samples with 0% or 30% amylose for various liquefaction enzyme treatments.

Liquefaction treatments	Ethanol (% *v*/*v*) at 72 h of SSF		Mean	Glucose (% *w*/*v*) at 0 h of SSF		Mean
	0% Amylose	30% Amylose		0% Amylose	30% Amylose	
Stargen 001	9.62±0.11[a]	6.12±0.23	7.87a[b]	9.21±0.37	9.61±0.31	9.41a
Spezyme Xtra	9.49±0.17	5.97±0.32	7.73a	11.65±0.22	8.32±0.34	9.99a
Ultra Thin 100L	10.16±0.32	8.72±0.29	9.44c	18.92±0.26	18.05±0.34	18.48c
Liquozyme SC	9.44±0.27	8.29±0.37	8.87b	13.71±0.28	12.33±0.17	13.02b
Mean (across liquefaction treatments)	9.68y[c]	7.28x		13.37y	12.08x	

[a] Data points±standard deviation

[b] Means followed by same letters in same column are not different ($p<0.05$)

[c] Means followed by same letters in the last row for ethanol or glucose are not different ($p<0.05$)

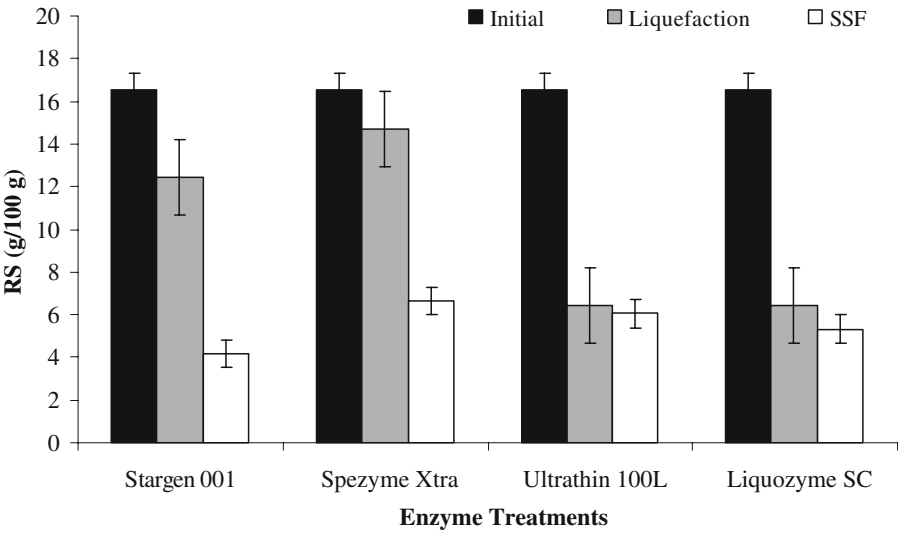

Fig. 7 Resistant starch content for four enzyme treatments, initial (0 h), after liquefaction (2 h), and after SSF (72 h) with 30% amylose starch. *Error bars* are ±1 standard deviation

RS for Starch Sample with 30% Amylose Content

Corn starch contains two fractions: (1) solubilizable starch, which can be hydrolyzed by enzymes to sugars and utilized by yeast to make ethanol and (2) RS, which is difficult to hydrolyze. Initial RS content for 30% amylose content was 16.53 g/100 g of total raw starch (Fig. 7). RS content decreased after liquefaction and after SSF, for all four enzyme

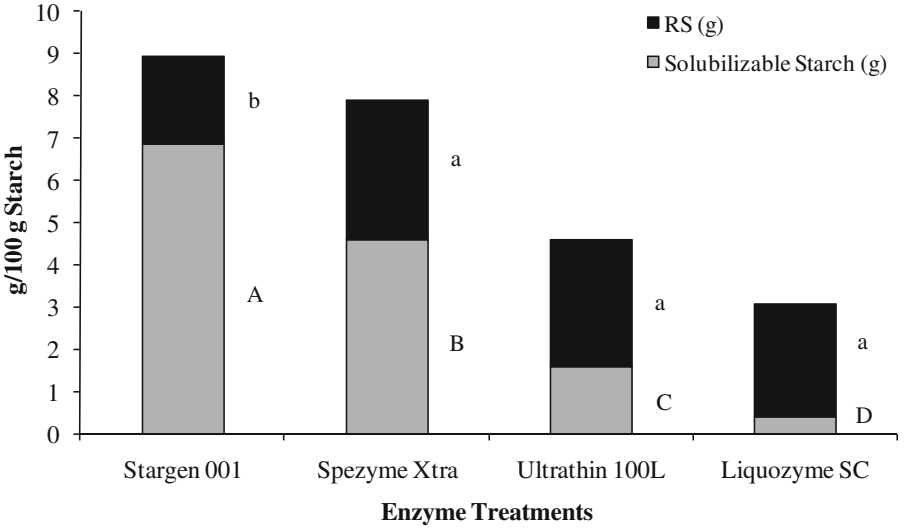

Fig. 8 Total, solubilizable, and resistant starch fractions in residual fermented mash after 72 h of SSF for four enzyme treatments, for 30% amylose starch. RS and solubilizable starch followed by same letters are not different ($p<0.05$). *RS* resistant starch

treatments (Fig. 7). RS reduction, relative to initial RS content, after liquefaction, was highest (60%) for high temperature liquefaction treatments, i.e., Ultra Thin (16.53 to 6.43 g/100 g starch) and Liquozyme SC process (16.53 to 6.42 g/100 g starch). For low temperature liquefaction, decreases in RS contents after liquefaction were low, 24.78% and 11.18% for GSHE and Spezyme Xtra, respectively, relative to initial RS content.

For the starch samples treated with high temperature liquefaction, a larger portion of RS was hydrolyzed and lower RS content was present at the start of SSF, leading to higher starch to glucose conversion. Correspondingly, higher final ethanol concentrations were observed for starch samples liquefied at high temperature (8.72% and 8.30% *v*/*v* for Ultra Thin 100L and Liquozyme SC, respectively, Table 2) compared to samples liquefied at low temperature (6.12% and 5.97% *v*/*v* for GSHE and Spezyme Xtra, respectively). Enzymes need a certain chain length of starch polymer to attach and cleave the bonds to release sugars. The necessary chain length may not be available in the case of RS as it may have a structure or rigid arrangements (RS1 or RS3) not allowing enzymes to attach and hydrolyze. High temperature liquefaction is able to break part of RS structure and make it available to enzymes to be hydrolyzed into sugars. Hence, higher ethanol concentrations were observed for high temperature liquefaction. Low temperature liquefaction treatment may not be able to break the complex RS structure and a higher percentage of initial starch (mostly RS) can be diverted into the DDGS stream.

After SSF, fermented mash had residual solids contents of 21.28, 24.98, 15.67, and 13.22 g (db) for GSHE, Spezyme Xtra, Ultra Thin, and Liquozyme SC processes, respectively. Solids remaining after starch hydrolysis and fermentation were mixtures of yeast biomass, unutilized nutrients, and residual starch (Fig. 1). Higher residual solids content indicated higher residual starch and lower starch to glucose or ethanol conversion. Residual starch (in residual solids after SSF) consisted of two fractions: solubilizable starch and RS. The total residual starch content, solubilizable starch fraction and RS fraction in these residual solids for four enzyme treatments are presented in Fig. 8. Raw starch (prior to liquefaction and SSF) contains RS and enzymes may or may not hydrolyze all or part of RS. After SSF, high RS in residual starch would be indicative that RS was unavailable to enzymes and was not hydrolyzed to sugars. If residual starch was primarily solubilizable starch, it indicated inefficiency/lower activity of the enzyme or that more enzyme was needed.

After SSF, the GSHE process had the highest residual starch (Fig. 8). GSHE had lower percentage (23.25%) of this residual starch as RS and remaining percentage was the solubilizable starch. Larger portion of residual starch as solubilizable starch fraction was indicative of lower activity of GSHE; therefore, it can be stipulated that higher dosage of GSHE may have been required. The other three enzymes had smaller solubilizable starch fraction in residual starch (Fig. 8). RS fractions in residual starch for Spezyme Xtra, Ultra Thin, and Liquozyme SC processes were 41.94%, 65.64%, and 87.02%, respectively (Fig. 8). Compared to the other three enzymes, Liquozyme SC hydrolyzed the highest portion of the available starch. RS contents were similar for Spezyme Xtra, Ultra Thin, and Liquozyme SC processes but the solubilizable starch fractions were different (Fig. 8).

RS for Starch Sample with 0% Amylose Content

Results for 0% amylose starch were similar to that from liquefaction and SSF of 30% amylose starch samples. RS content for 0% amylose starch (0.76 g/100 g) was low compared to 30% amylose starch (16.53 g/100 g) (Figs. 7 and 9) indicating higher amylose results in higher RS in corn starch. As 0% amylose starch resulted in <2% *w*/*w* of RS, all

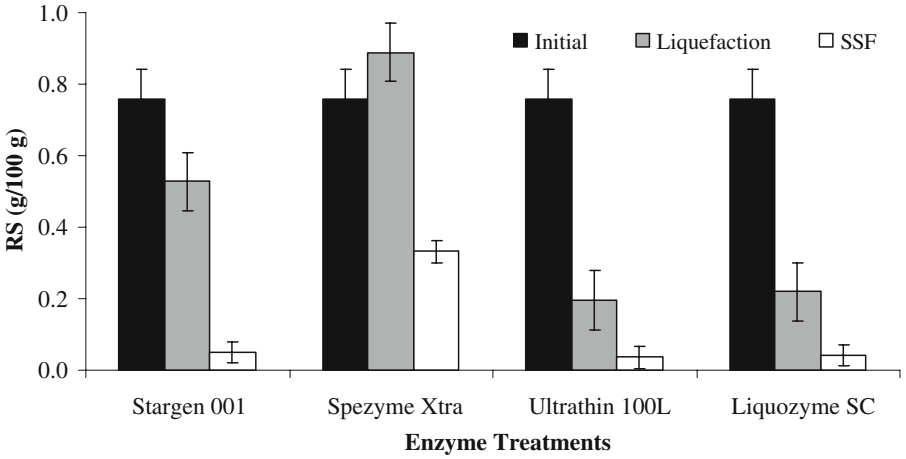

Fig. 9 Resistant starch contents for four enzyme treatment processes, initial (at 0 h), after liquefaction (at 2 h), and after SSF (at 72 h) for 0% amylose starch. *Error bars* are ±1 standard deviation

RS measurements were performed with eight replications for better estimation of error. Final ethanol concentrations were similar for all four enzyme treatments (9.62%, 9.49%, 10.16%, and 9.45% *v*/*v* for GSHE, Spezyme Xtra, Ultra Thin, and Liquozyme SC, respectively) (Fig. 6).

For all four enzyme treatments, RS content decreased or remained similar after liquefaction and decreased further after SSF (Fig. 9). RS decrease after liquefaction were highest for high temperature liquefaction treatments, 57.2% and 55.9% (decrease from initial) for Ultra Thin (0.76 to 0.2 g/100 g starch) and Liquozyme SC (0.76 to 0.22 g/100 g starch), respectively (Fig. 9). This was consistent with 30% amylose starch indicating an ability of high temperature liquefaction to break higher amounts of RS.

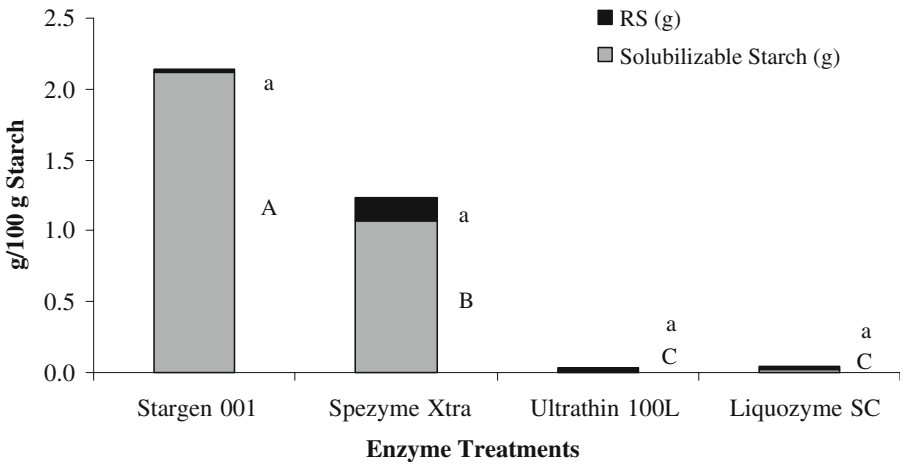

Fig 10 Total, solubilizable, and resistant starch fractions in residual fermented mash after 72 h of SSF for four enzyme treatments, for waxy corn with 0% amylose starch. RS and solubilizable starch followed by same letters are not different ($p<0.05$). *RS* resistant starch

After SSF, total residual solids were calculated to be 7.27, 8.62, 8.02, and 8.11 g (db) for GSHE, Spezyme Xtra, Ultra Thin, and Liquozyme SC processes, respectively. Residual solids for all four enzyme treatments were similar showing similar starch-to-glucose or ethanol conversions. GSHE and Spezyme Xtra liquefaction treatments had lower (1.15% and 13.41%, respectively) percentage of residual starch as RS and the remaining as solubilizable starch (Fig. 10), indicating higher dose of enzymes was required. For high temperature enzymes Ultra Thin 100L and Liquozyme SC, low residual starches were observed (Fig. 10) which was indicative of higher starch conversion to ethanol.

Conclusions

Initial resistant starch RS content was higher for 30% amylose starch compared to 0% amylose starch. This was consistent with previously published data that higher amylose content results in higher RS in starch. Higher initial RS in starch resulted in lower conversion of starch into sugars and hence to lower final ethanol yield. During higher temperature liquefaction, a larger portion of resistant starch was hydrolyzed, resulting in higher ethanol yields and lower final residual solids. During lower temperature liquefaction, a smaller portion of resistant starch was hydrolyzed; this resulted in lower ethanol yields and higher final residual solids. Starch sample with lower initial RS contents (0% amylose content samples) resulted in higher ethanol yields and similar final dry solids, irrespective of liquefaction enzyme treatment; therefore, it was indicative of similar starch conversions.

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