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Properties of field-sprouted sorghum and its performance in ethanol production☆,☆☆

Shuping Yan [a,b], Xiaorong Wu [a], Jeff Dahlberg [c], Scott R. Bean [d], Finlay MacRitchie [b], Jeff D. Wilson [d], Donghai Wang [a,*]

[a] Department of Biological and Agricultural Engineering, Kansas State University, Seaton Hall 129, Manhattan, KS 66506, USA
[b] Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506, USA
[c] U.S. National Sorghum Producers, Lubbock, TX 79403, USA
[d] USDA-ARS Grain Marketing and Production Research Center, Manhattan, KS 66502, USA

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ABSTRACT

The objective of this research was to investigate physicochemical and biochemical characteristics of field-sprouted grain sorghum and its fermentation performance in ethanol production. Five field-sprouted grain sorghum varieties, which received abnormally high rainfall during harvest, were used in this study. Enzyme activities, microstructure, flour pasting properties, kernel hardness, kernel weight, kernel size, flour size and particle distribution of field-sprouted grain sorghum were analyzed. The effect of germination (i.e., sprouting) on conversion of grain sorghum to ethanol was determined by using a laboratory dry-grind ethanol fermentation procedure. Sprouted sorghum had increased α-amylase activity; degraded starch granules and endosperm cell walls; decreased kernel hardness, kernel weight, kernel size, and particle size; and decreased pasting temperature and peak and final viscosities compared with non-sprouted grain sorghum. The major finding is that the time required for sprouted sorghum to complete fermentation was only about half that of non-sprouted sorghum. Also, ethanol yield from sprouted sorghum was higher (416–423 L/ton) than that from non-sprouted sorghum (409 L/ton) on a 14% moisture basis.

1. Introduction

The U.S. demand for ethanol has increased sharply in recent years. Currently, feedstock for fuel ethanol production is ~95% corn grain and ~4% sorghum grain (RFA, 2007). Researchers and ethanol producers consider grain sorghum a viable and renewable feedstock (i.e., technically acceptable, fits the infrastructure, and can be economically viable) for ethanol, and sorghum could make a larger contribution to the nation's fuel ethanol requirements (Farrell et al., 2006; Rooney et al., 2007; Wu et al., 2006, 2007).

Both ethanol yield and fermentation efficiency have been used to evaluate the performance of feedstocks in ethanol production. Recent research shows that key factors affecting ethanol yield and ethanol fermentation efficiency of sorghum include starch content, starch digestibility, level of extractable proteins, protein and starch interaction, mash viscosity, amount of phenolic compounds, ratio of amylose to amylopectin, and formation of amylose–lipid complexes in the mash (Wang et al., 2008; Wu et al., 2007; Zhao et al., 2008). In addition to chemical and physical properties of grain sorghum, Yan et al. (2009) studied the effect of germinated sorghum on ethanol fermentation and fermentation efficiency. Results from laboratory-germinated, tannin-containing grain sorghum (i.e., sorghum with a pigmented testa) showed that germination not only decreased tannin content and improved sorghum fermentation performance, but also activated intrinsic enzymes and shortened fermentation time. To a certain degree, germination of feedstocks may not be negative for ethanol fermentation.

Germination, or sprouting, is a common problem for grain when weather is moist during harvest or the environment is humid during storage. When kernels absorb moisture from their surroundings to a sufficient level, the embryo and endosperm are

Abbreviations: FAN, free amino nitrogen; HI, hardness index; HPLC, high-performance liquid chromatography; VAG, Visco-Amylo-graph; SEM, scanning electron microscope; SKCS, single kernel characterization system.

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☆☆ Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

* Corresponding author. Tel.: +1 785 532 2919; fax: +1 785 532 5825.
E-mail address: dwang@ksu.edu (D. Wang).

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hydrated. Hydration switches on the metabolism of the embryo, which sends hormonal signals to the aleurone layer, triggering the synthesis of enzymes responsible for digesting components of the starchy endosperm. Germination promotes the development of cytolytic, proteolytic, and amylolytic enzymes that are not active in dry kernels (Bamforth, 2006; Dewar et al., 1997; Klose et al., 2009) and could cause significant changes in kernel composition and physical properties (Agu and Palmer, 1996; Bamforth, 2006; Beta et al., 2000; Dewar et al., 1997; Elmaki et al., 1999; Iwuoha and Aina, 1995; Lasekan et al., 1995; Muria and Bechtel, 1998; Murty et al., 1984; Osuntogum et al., 1989; Palmer, 1991; Singh and Bains, 1984; Swanston et al., 1994). Germination not only causes compositional changes in the sorghum grain but also initiates a series of biochemical and physiological changes. Intrinsic enzymes such as amylases, proteases, lipases, fiber-degrading enzymes, and phytases are activated; this disrupts protein bodies and degrades proteins, carbohydrates, and lipids to simpler molecules, which increases digestibility of proteins and carbohydrates in the kernel and makes nutrients available and accessible for enzymes (Chavan and Kadam, 1989; Dicko et al., 2006; Subramanian et al., 1992; Ratnavathi and Ravi, 1991; Taylor, 1983; Yan et al., 2009). Balogun et al. (2006) reported that *in vitro* fermentability of sorghum grain was significantly higher when grain was germinated. Research on baby food also showed that germination can activate enzymes, decrease the level of antinutritional factors (tannins, phytic acid), and increase digestibility of macronutrients, bioavailability of minerals, and content of essential amino acids (Correia et al., 2008; Dicko et al., 2006; Subramanian et al., 1992; Taylor, 1983; Taylor et al., 1985).

Sorghum has been malted and used for production of traditional alcoholic and nonalcoholic beverages for centuries (Dufour et al., 1992). Malting conditions must be controlled to achieve uniform, high-quality sorghum malts and ensure quality required for food products (Dewar et al., 1997). Biofuel ethanol production does not have the same requirements. The most important issues in industrial ethanol production are yield, efficiency, and energy consumption. Our laboratory results in terms of ethanol yield and ethanol fermentation efficiency from artificially germinated high-tannin sorghum suggest that huge potential energy savings exist in production of ethanol from germinated sorghum grain. Using germination-damaged sorghum for industrial ethanol production might benefit the producer and end user by expanding market uses of what has been historically considered a low-value commodity (Suresh et al., 1999; Yan et al., 2009).

The objective of this research was to investigate physicochemical and biochemical characteristics of field-sprouted grain sorghum and its fermentation performance in ethanol production.

2. Materials and methods

2.1. Grain sorghum

Five field-sprouted sorghum varieties (DK5400, DK5311, Asgrow567, Pio8313, and Pio82G10) from south central Texas, which received abnormally high rainfall during harvest, were used in this study. The received dry samples had visible shoots but no visible mold-contamination. Non-sprouted DK5400 was used as a control. Samples were carefully cleaned, and foreign materials were removed manually. Samples were ground to flour with a Magic Mill III plus grain mill (Magic Mill Products & Appliances, Monsey, NY.) set at the level IV for fermentation. Samples for chemical composition analysis were ground with an Udy cyclone sample mill (Udy, Fort Collins, CO) with a 0.5-mm screen.

2.2. Particle size analysis

Size distributions of sorghum flour were measured with an LS 13 320 single wavelength laser diffraction particle size analyzer using the Tornado dry powder system (Beckman Coulter Inc., Miami, FL). Samples were run in duplicate.

2.3. Morphological structure of field-sprouted grain sorghum

Microstructures of field-sprouted sorghum kernels and control grain sorghum were examined with a scanning electron microscope (SEM) with an accelerating voltage of 5.0 kV (Hitachi S-3500N, Hitachi Science Systems, Ltd., Japan). Samples were vacuum coated with a mixture of 60% gold and 40% palladium particles by using a Sputter Coater-Desk II SPUTTER/ETCH UNIT (Denton Vacuum, LLC, NJ).

2.4. Measurement by the single kernel characterization system

Kernel hardness, kernel weight, and kernel size of field-sprouted sorghum samples and the control were measured with a single kernel characterization system 4100 (SKCS; Perten Instruments, Springfield, IL) controlled by Microsoft Windows Software SK4100 as optimized for sorghum (Bean et al., 2006). Data presented are the mean values of 300 kernels.

2.5. Analysis of enzyme activity and flour pasting properties

A Megazyme alpha-amylase assay kit was used to measure α-amylase activity (CU/g). Flour pasting properties were determined with a Brabender Visco-Amylo-graph (VAG, C. W. Brabender Instruments Inc., NJ). For VAG sample preparation, 14 g of sorghum flour with 14% moisture content and distilled water (100 mL) was added to the amylograph bowl at room temperature. A 20-min measurement profile with a heat/cool rate of 7.5 °C/min was used as follows: increase the slurry temperature from room temperature to 95 °C in the first 6 min, hold at 95 °C for 5 min, decrease from 95 to 50 °C in 5 min, and hold at 50 °C for 2 min. Each sample was analyzed in duplicate.

2.6. Microorganism, preparation of mashes and inoculation

Dry alcohol yeast (*Saccharomyces cerevisiae*, Red Star Ethanol Red) provided by Fermentis (Lesaffre Yeast Corp., Milwaukee, WI), was used for simultaneous saccharification and fermentation. Before inoculation, dry yeast was activated by adding 1.0 g of cells into 19 mL of preculture broth (containing 20 g glucose, 5.0 g peptone, 3.0 g yeast extracts, 1.0g KH_2PO_4, and 0.5 g $MgSO_4 \cdot H_2O$ per liter) and incubated at 38 °C for 30 min in an incubator operating at 200 rpm. The activated yeast culture had a cell concentration of roughly 1×10^9 cells/mL.

Liquozyme SC DC (Novozyme, Franklinton, NC), a heat-stable α-amylase from *Bacillus licheniforms* was used for liquefaction. Enzyme activity was 240 KNU/g (One Kilo Novo Unit, KNU, is the amount of enzyme that breaks down 5.26 g of starch per hour for Novozyme's standard method for determination of α-amylase). Spirizyme Fuel (Novozyme, Franklinton, NC), an amyloglucosidase from *Aspergillus niger*, was used for saccharification. Enzyme activity was 750 AGU/g (One AGU is the amount of enzyme that hydrolyzes 1 μmol of maltose per minute under specified conditions).

Whole sorghum flour (30 g, db) was dispersed in a 250 mL Erlenmeyer flask with 100 mL of fermentation broth containing 0.1 g KH_2PO_4 (preheated to about 60 °C), and 20 μL Liquozyme (240 KNU/g) added to the flask. The flasks were transferred to a 70 °C water bath shaker operating at 170 rpm. The water bath

temperature was gradually increased from 70 °C to 85 °C over about 30 min. After 60 min at 85 °C, flasks were removed from the water bath shaker and cooled to room temperature. Materials sticking on the inner surface of the flasks were scraped back into the mash with a spatula, and then the inner surface was rinsed with 2–3 mL of distilled water by using a fine-tipped polyethylene transfer pipette. The pH of the mashes was adjusted to around 4.2–4.3 with 2N HCl. After the pH of each mash was adjusted, 100 μL amyloglucosidase, 0.3 g yeast extract, and 1 mL activated yeast broth (1×10^9 cells/mL) were added to each flask. Inoculated flasks were sealed with S-bubblers/airlocks and transferred to an incubator shaker for ethanol fermentation. Each sample was run in duplicate.

2.7. Fermentation and distillation

Ethanol fermentation was conducted at 30 °C in an incubator shaker (Model I2400, New Brunswick Scientific, Edison, NJ) operating at 150 rpm for 72 h. The fermentation process was monitored by measuring the weight loss of evolved carbon dioxide (CO_2) during fermentation.

At the end of fermentation, all fermented mash in each 250 mL flask was transferred to a 500 mL distillation flask. Each Erlenmeyer flask was washed with distilled water four times (4 × 25 mL). The washing water was pooled in the distillation flask, and then the distillation flask was distilled on a distillation unit. Distillates were collected in a 100 mL volumetric flask immersed in ice water. When distillates in the volumetric flask approached the 100 mL mark, the distillation process was stopped. Distillates in the volumetric flask were equilibrated to 25 °C and adjusted to 100 mL with distilled water if necessary. Distillates were analyzed for ethanol by a Shimadzu HPLC with a Rezex RCM column (Phenomenex, Torrance, CA) and refractive index detector (Wu et al., 2006).

2.8. Analytical methods

Methods for the analyses of dry matter/moisture, starch, crude protein, ash, crude fiber, and crude fat of samples were AOAC Official Methods 930.15, 996.11, 990.03, 942.05, 962.09, and 920.39 (AOAC International, 2000), respectively. Free amino nitrogen (FAN) was analyzed by the European Brewery Convention method (EBC, 1987) with modification. Grain sorghum flour (150 mg) was mixed with 1.5 mL deionized distilled water in a 2.5 mL microcentrifuge tube, vortexed five times within 10 min, then centrifuged at 12 000 rpm for 20 min. At this point, the supernatant was ready for FAN analysis. Glucose, glycerol, and ethanol in the finished beers were determined by HPLC (Shimadzu Scientific Instruments, Columbia, MD) according to the method described by McGinley and Mott (2008). The column used was a Rezex ROA column (Phenomenex, Torrance, CA), and the detector for HPLC was a refractive index detector (model RID-10A, Shimadzu) with the detection unit maintained at 40 °C. The mobile phase was 5 mM sulfuric acid at a flow rate of 0.6 mL/min and the oven temperature was 65 °C. HPLC data were analyzed with Shimadzu EZStart 7.4 software. Fermentation efficiency was calculated as a ratio of actual ethanol yield (determined by HPLC) to theoretical ethanol yield (calculated from the total starch content in the sample).

2.9. Statistical analyses

All experiments were performed at least in duplicate. Tabular results presented are mean values of repeated experimental data. An ANOVA was conducted to determine the significant differences at a 5% significance level ($P < 0.05$).

3. Results and discussion

3.1. Chemical composition of field-sprouted grain sorghum and control grain sorghum

Table 1 shows chemical composition of the five field-sprouted samples and the control (non-sprouted, DK5400C). The FAN in the non-sprouted sample was lower than that in the sprouted samples even though the non-sprouted sorghum sample had the highest protein content. Enzymatic degradation of protein by activated intrinsic proteases during sprouting resulted in an increase in FAN contents and short peptides, which accounted for the significant increase in FAN levels of field-sprouted sorghum samples (Agu and Palmer, 1996; Evans and Taylor, 1990; Ogbonna et al., 2003; Taylor, 1983). These activated intrinsic proteases have optimal temperatures of around 50 °C and retain much activity at 70 °C for some time (Ogbonna et al., 2004). FAN contents in the mashes of sprouted sorghum samples will further increase during the slurry and liquefaction process. Also, α-amylase activity in the non-sprouted control was lower than that in sprouted grain sorghum, which agrees with results reported by Murty et al. (1984). The diverse values of FAN and α-amylase activity also revealed that samples had experienced different degrees or durations of field sprouting. All field-sprouted samples had high starch content (>66% wb).

3.2. Results from SKCS and particle size analyzer

The SKCS originally was designed to analyze wheat kernels but has been modified to measure grain hardness, kernel size, and kernel weight for sorghum (Bean et al., 2006). The SKCS can provide rapid measurements of sorghum grain information based on the variability present in the samples. Non-sprouted sorghum had higher values for kernel hardness, kernel weight, and kernel diameter than field-sprouted sorghum (Table 1). Hardness is one of the most important traits for grain milling; it affects grain milling

Table 1
Chemical composition, kernel hardness and kernel size of grain sorghum samples.

Sorghum samples	Chemical composition (% wb)						FAN (mg/L)	α-amylase activity (CU/g)	Hardness index	Kernel weight (mg)	Kernel diameter (mm)
	MC	Ash	Protein	Fiber	Lipids	Starch					
DK5400C	10.38^{f}	1.62^{a}	11.59^{a}	1.15^{e}	3.22^{a}	64.50^{d}	162.0^{e}	5.60^{c}	78.97^{a}	30.00^{a}	1.98^{a}
DK5400	12.28^{d}	1.18^{c}	6.66^{e}	2.12^{ab}	2.27^{c}	67.30^{c}	221.9^{c}	12.03^{b}	49.12^{e}	24.76^{c}	1.68^{c}
DK5311	12.97^{a}	1.19^{c}	7.02^{d}	2.24^{a}	2.29^{c}	66.78^{c}	234.8^{bc}	12.60^{b}	52.56^{d}	25.42^{bc}	1.73^{bc}
Asg567	11.92^{e}	1.26^{b}	7.60^{b}	2.07^{bc}	2.34^{c}	67.80^{bc}	284.3^{a}	15.79^{a}	56.75^{c}	25.53^{b}	1.76^{b}
Pio82G10	12.71^{b}	1.10^{e}	7.27^{c}	1.95^{cd}	2.40^{bc}	69.28^{ab}	189.5^{d}	13.35^{b}	68.30^{b}	25.98^{b}	2.01^{a}
Pio8313	12.52^{c}	1.15^{d}	6.96^{d}	1.91^{d}	2.49^{b}	69.65^{a}	258.4^{ab}	13.18^{b}	66.19^{b}	26.00^{b}	1.78^{b}

Superscript letter in the same column indicates significant difference ($P < 0.05$).
FAN = Free amino nitrogen.
MC = Moisture content (wb).

quality and parameters such as particle size, damaged starch, and flour water absorption. The hardness index (HI) obtained from the SKCS is inversely related to particle size less than 200 μm. Grains with higher HI values had a lower percentage of small particle size. The field-sprouted Pio sorghum varieties had higher HI than other samples. With the same setting on the mill, the sample with high HI had a larger portion of particles with diameters bigger than 200 μm. In contrast, the portions of smaller particles (<200 μm) in sprouted DK sorghum samples was higher than that in the Pio samples. The non-sprouted DK5400C had the highest HI, highest amount of large particles (>200 μm), and lowest amount of small particles (<200 μm). The particle size distribution curve of the control sample had two pronounced peaks around 18 and 450 μm, whereas the field-sprouted samples had 3 peaks – an extra peak at about 125 ± 5 μm (data not shown). The samples with higher HI also had larger particles, whereas samples with a low HI had smaller particles. These results are in agreement with those reported by Beta et al. (1995) and Lee et al. (2002). In addition, Lasekan et al. (1995) reported that sorghum variety affected germination and sugar production from sorghum malts. Our HPLC data agree with the above trends (data not shown). One purpose of this study was to evaluate fermentation performance of sprouted sorghum for ethanol production. Naidu et al. (2007) reported that particle size significantly affects ethanol yield. Our results showed that ethanol yield was inversely related to kernel HI (a linear regression equation with $R^2 = 0.855$). This is probably because sorghum with higher HI had a higher percentage of large particles. Previous research has shown a negative relationship between particle size and ethanol yield (Kelsall and Lyons, 2003; Naidu et al., 2007).

3.3. Morphological structure of field-sprouted grain sorghum

Fig. 1 shows SEM images of germ, endosperm, and whole kernels of field-sprouted and non-sprouted sorghum. Starch granules in germ (Fig. 1A) had many more holes than those in endosperm (Fig. 1B). These holes indicate that starch granules were degraded or attacked by activated enzymes during field sprouting. Grain contains abundant enzymes in the germ. While grain kernels are dry, enzymes are inactive (because of enzyme inhibitors) and will remain so until moisture content of the kernels is high enough to trigger germination. The new shoot and root will emerge from the kernel when the embryo begins to germinate. As the intrinsic enzymes (e.g., proteases, amylases, and lipases) in sorghum kernels are activated (Correia et al., 2008), the reservoir chemical constituents (e.g., proteins, starch, and lipids) are degraded by these enzymes into simple compounds that are used to make new compounds (i.e., shoot and root). Because of water intake rate and germination, macromolecules in the germ are broken down by enzymes more rapidly than those in the endosperm (Fig. 1A, B, and 1C). Moss (1977) studied the rate of moisture movement into the kernel using autoradiography. There was an initial rapid movement of water into the germ and along the edge of the endosperm region. Because of rapid movement, the effect of germination/sprouting is more pronounced in the germ than in the endosperm. Enzymes are working not only on starch granules but also on protein and cell walls (Correia et al., 2008; Glennie et al., 1983). Fig. 1 shows starch granules and cell walls of sprouted sorghum kernels (Fig. 1A and B); relative position of endosperm, germ and root of sprouted kernels (Fig. 1C); and starch granules and cell walls of non-sprouted grain

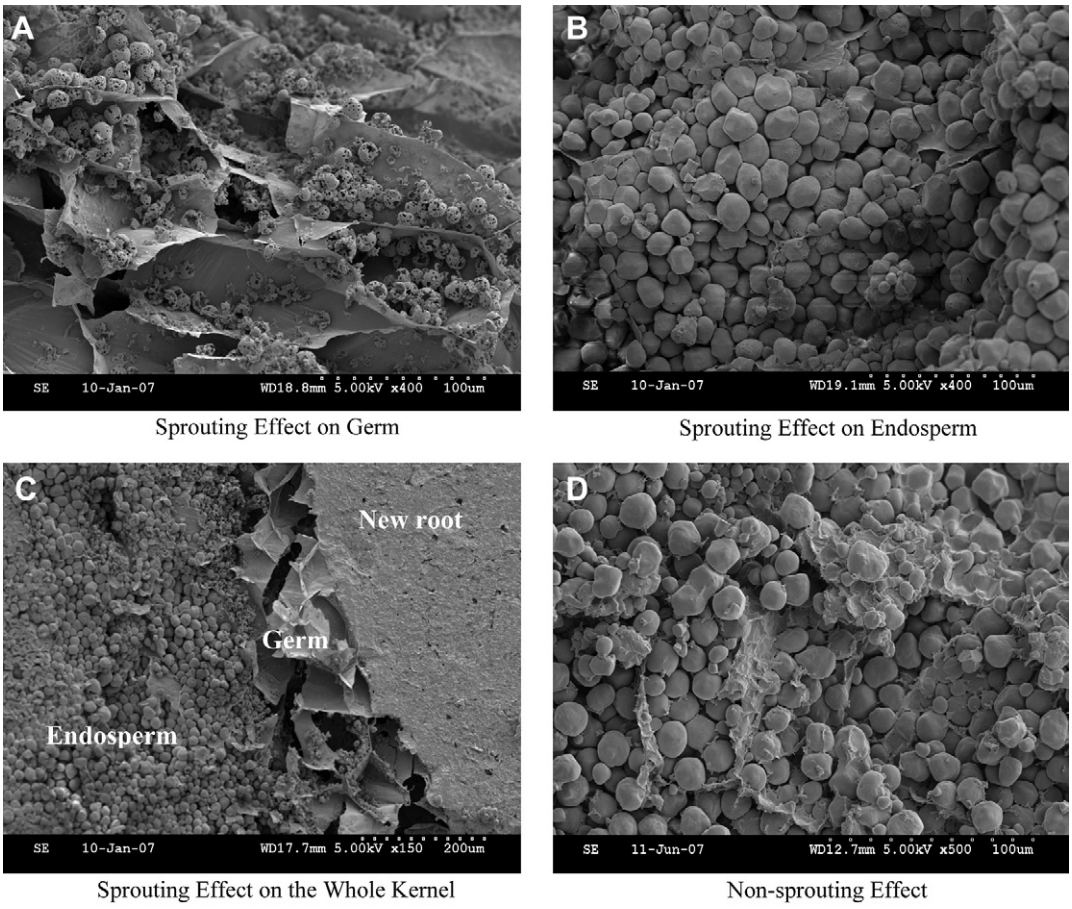

Fig. 1. Scanning electron microscope images of starch granules: A: Germ of field-sprouted sorghum. B: Endosperm of field-sprouted sorghum. C: Cell walls of field-sprouted grain sorghums. D: Non-sprouted sorghum.

sorghum kernels (Fig. 1D). These SEM images clearly indicate that various degrees of damage occurred to starch granules in both the germ and endosperm of sprouted sorghum kernels (Fig. 1A, B and 1C). Cell walls of the sprouted sorghum kernels also were degraded by the activated intrinsic cell-wall-degrading enzymes and apparently were thinner than cell walls of non-sprouted sorghum kernels. These intrinsic enzymes mainly convert part of the insoluble polymers in sorghum kernels into soluble smaller molecules, which makes the sprouted sorghum a better feedstock for ethanol production. After field-sprouted grain sorghum is harvested, shoots and roots of some field-sprouted kernels might not be noticeable if they have shrunk during drying. Therefore, total mass of field-sprouted grain sorghum kernels might not change. In a laboratory germination test, the significant decrease in mass was due to the loss of solubles during rinsing and loss of shoots or roots during drying (Yan et al., 2009). In industrial biofuel production, field-sprouted grain sorghum may be a better feedstock because of its easy digestibility of enzymatically damaged starch granules, thin cell walls, and higher content of readily available sugars.

3.4. Pasting properties of field-sprouted sorghum flour

The effect of sprouting on viscosity was analyzed with a Brabender Visco-Amylo-graph. The Visco-Amylo-graph curves of field-sprouted sorghum were significantly different from those of non-sprouted sorghum in terms of peak viscosity, holding strength, final viscosity, peak temperature, and peak time (Fig. 2). In general, field-sprouted sorghum flour had a short peak time (took less time to reach the peak viscosity), clear holding strength, and low final viscosity (low setback). In addition, field-sprouted sorghum required less time to begin pasting than non-sprouted sorghum, indicating that starch in the sprouted flour swelled easily and consumed less energy during the cooking process. Compared with field-sprouted samples, non-sprouted sorghum had no peak viscosity but a significantly higher final viscosity. This is due to the difference in α-amylase activity and high content of damaged starch granules in the sprouted sorghum compared with the non-sprouted sorghum. Compared with intact starch granules in non-sprouted sorghum, enzyme-damaged starch granules swell readily and easily break down into small fragments, resulting in low peak and final viscosities in the field-sprouted sorghum pasting profile.

On the other hand, differences in peak viscosity, holding strength, and final viscosity also were observed among the field-sprouted sorghum varieties, which could be due to degree of sprouting and differences in kernel hardness that resulted in different particle sizes and degrees of damaged starch. Obviously, HI of the non-sprouted kernels was significantly higher than that of all sprouted samples. There were an inverse correlation between peak viscosity and kernel HI. Among the five sprouted samples, DK5400 had the lowest HI and highest peak viscosity and Pio82G10 had the highest HI and lowest peak viscosity. Among all samples, DK5400C had the highest final viscosity and lowest α-amylase activity, whereas Asgrow567 had the lowest final viscosity and highest α-amylase activity (Table 1). This indicates that sprouted sorghum samples originally had very different hardness and/or were at very different stages of the germination process because differences in time and duration of exposure to high moisture conditions before harvest (e.g., unfavorable weather in the field) would result in sprouted kernels with different enzyme activities and related degraded products (Evans and Taylor, 1990; Ogbonna et al., 2003).

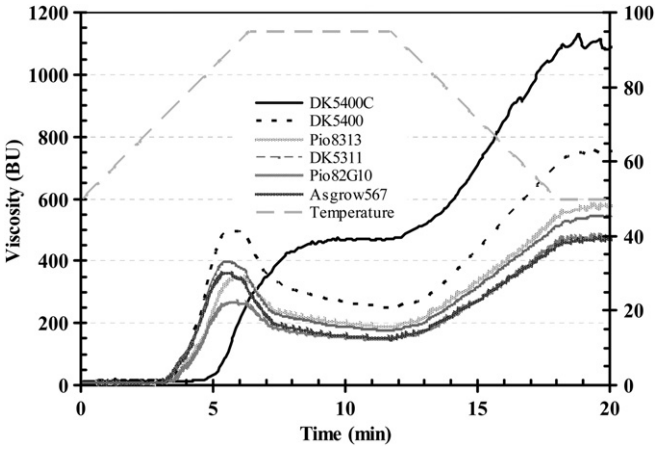

Fig. 2. Pasting properties of flours from five field-sprouted sorghum varieties and non-sprouted control (DK5400).

3.5. Ethanol production from field-sprouted grain sorghum

Fig. 3A shows ethanol yield of five field-sprouted sorghum varieties and the non-sprouted control. The ethanol fermentation process essentially was completed within 36 h for the sprouted sorghum, and ethanol yield did not increase significantly after the 36th hour, indicating the fermentation process using field-sprouted sorghum could be stopped at the 36th hour after yeast inoculation in the beginning of fermentation. This result agrees with results from a study on laboratory-germinated high-tannin sorghum (Yan et al., 2009) and further confirms that using sprouted grain sorghum for ethanol production could shorten the fermentation

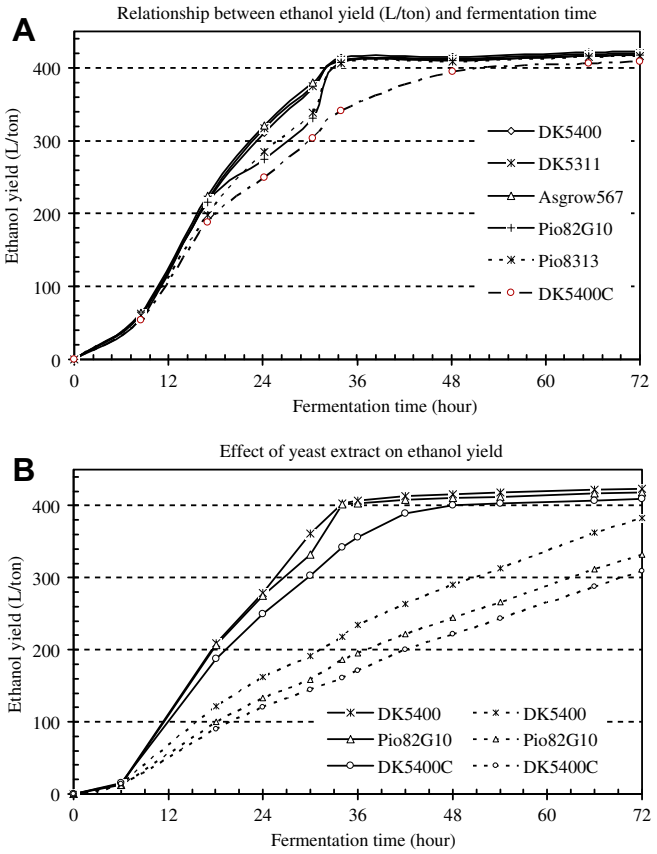

Fig. 3. A: Ethanol yields of five field-sprouted sorghum varieties and a non-sprouted (DK5400C). B: Effect of yeast extract on ethanol yield (solid lines: with yeast extract; dashed lines: without yeast extract).

time without significantly decreasing ethanol yield. Grain damaged by sprouting may lose value for food applications but may not affect ethanol production and final ethanol yield. In this study, ethanol yield from field-sprouted sorghum actually was slightly higher than that from the non-sprouted control sorghum (Fig. 3A). The actions of cell-wall-degrading enzymes in the field-sprouted sorghum might have contributed to this high yield. The fermentation process for sprouted grain could be much shorter than that required for normal grain (Wu, 1989; Yan et al., 2009).

Fermentation course varied among sorghum varieties in the 18th–30th hour (Fig. 3A). The two Pio varieties had lower ethanol yield than the other three varieties within the same fermentation period (at 24 h). This might be due to kernel hardness, particle size, and availability of nutrients for yeast in the mash. The harder sorghum had larger particles, which might prevent nutrients from being released rapidly to the mash during this period. However, as the fermentation process proceeds in the mash and water continues penetrating into larger particles, the structures of larger particles eventually would be disrupted and nutrients would be released into the mash. On the other hand, availability of FAN for yeast might affect fermentation course and rate. Pio82G10 had the lowest FAN, highest HI, and lowest ethanol yield during the 18th–30th hour (Table 1 and Fig. 3A).

One of the most important physicochemical changes that occurs during germination is degradation of the proteinaceous matrix that holds starch granules within the cells in the endosperm and conversion of these substances into soluble peptides and amino acids, which contribute to the increased FAN and provides nutrients for yeast growth. The effect of FAN on the fermentation process was further confirmed by the presence of yeast extracts during fermentation (Fig. 3B). Sorghum mashes with added yeast extract (solid lines in Fig. 3B) had a much faster fermentation rate and took less time (36 h vs. 72 h) to complete fermentation than sorghum mashes without yeast extract (dashed lines in Fig. 3B). These results support previous findings that FAN is a positive factor for the fermentation process (Pérez-Carrillo and Serna-Saldívar, 2007; Pierce, 1987, 1982). *S. cerevisiae* can assimilate amino acids and low-molecular-weight peptides but not proteins. The non-sprouted control sample, DK5400C, had the lowest FAN among the samples (Table 1) and the lowest ethanol yield at the end of the 72 h fermentation both with and without added yeast extract. Without the addition of yeast extract, fermentation rates depended on the availability of FAN in the mashes. Sprouted DK5400 had the highest FAN content among three samples and the fastest fermentation rate and highest ethanol yield both with and without added yeast extract. This further supports the idea that FAN is important for yeast growth and fermentation rate, especially for yeast proliferation. Sprouted sorghum with high FAN content benefits ethanol fermentation efficiency and reduces fermentation time.

The HPLC analysis of finished beer showed a significant amount of sugar left in the finished beer (72 h fermentation) when fermentation was conducted using sorghum mashes without added yeast extract (Fig. 4). There was little sugar left in the finished beer when yeast extract was added to the sorghum mashes. In addition, the amount of sugar remaining varied among the three samples (peak area is proportional to the sugar concentration). Among three samples without added yeast extract, the non-sprouted control sample (DK5400C) had the most amount of residual sugar left and the field-sprouted sample of the same sorghum variety (DK5400) had the least residual sugar. The three samples appear in the same order when ranked in terms of FAN content and ethanol level: DK5400 > Pio82G10 > DK5400C (Table 1 and Fig. 3). This finding is in agreement with previous observations (Pierce, 1982, 1987).

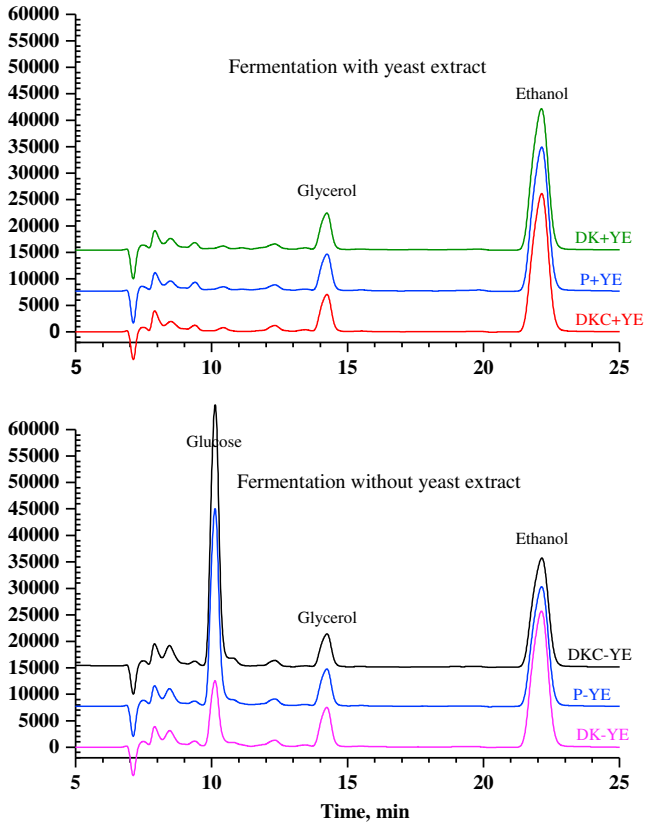

Fig. 4. Ethanol yield and residual glucose contents in the finished beers analyzed using HPLC with Phenomenex ROA column (DKC: non-sprouted DK5400C; P: field-sprouted Pio82G10; DK: field-sprouted DK5400; +YE: with yeast extract; −YE: without yeast extract).

4. Conclusion

Field sprouting damaged starch granules, protein matrices, and cell walls in sorghum kernels, consequently decreasing kernel hardness, kernel weight, and kernel size. Field sprouting also changed the chemical composition and pasting properties of field-sprouted grain sorghum, which could shorten fermentation time without decreasing ethanol yield. Field-sprouted grain sorghum had relatively high FAN content. The FAN provided efficient buffering capacity and optimal yeast performance, and field-sprouted sorghum had a more rapid fermentation rate than non-sprouted sorghum. FAN played a key role in increasing conversion efficiency for ethanol production. Using weathered and/or sprouted sorghum from regions affected by unusually high moisture events during grain fill and harvest may provide an opportunity for ethanol producers to maintain ethanol production efficiency, while shortening processing time. This could offer sorghum producers an opportunity to receive a premium, or at least a fair market value, for sorghum when such environmental events occur.

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