Ethanol-Production Performance of Ozone-Treated Tannin Grain Sorghum Flour[1]

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ABSTRACT

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Ozone has been reported as being able to degrade macromolecules such as cellulose, starch, lignins, and tannins in the textile, pulping, and water-treatment industries. Thus, we hypothesized that ozone treatment may also inactivate tannin activity and increase fermentation efficiency of tannin sorghum lines. The objective of this research was to study the physicochemical properties of ozone-treated whole tannin grain sorghum flour and its fermentation performance in ethanol production. Results showed that the ethanol yields from ozone-treated tannin grain sorghums were significantly higher than yields from the untreated flour. The fermentation efficiency of ozone-treated tannin grain sorghum was approximately 90%, which was 8–14% higher than that of untreated samples at the 36th hr of fermentation. At the end of 72 hr of fermentation, the efficiencies of ozone-treated sorghum flour were 2–5% higher than those of untreated samples. Measured tannin levels of ozone-treated samples decreased significantly from 3.8 to 2.7%. Gel-permeation chromatographic results indicated that both degradation and polymerization processes might have happened to starch molecules during ozone treatment. Rapid Visco Analyzer data showed that the setback of viscosity of ozone-treated flour was lower than that of untreated flours. Distillers dried grains with solubles made from ozone-treated sorghum were low in residual starch (<1%) and high in crude protein (≈35%). Therefore, ozonation could be a novel and useful method to improve ethanol yield and fermentation efficiency of tannin grain sorghum.

The use of ethanol as a gasoline alternative, ethanol production, and ethanol-production capacity in the United States are increasing rapidly because of federal mandates requiring the mixing of ethanol into gasoline. As stipulated by the Energy Independence and Security Act of 2007 and the expanded Renewable Fuels Standard, annual ethanol production will grow to 15 billion gallons by 2012 and to 36 billion gallons by 2022. Currently, ethanol in the United States is produced mainly from crop-based starch-rich grains. Grain sorghum is one of the primary feedstocks for ethanol production. In 2009, more than 30% of the U.S. grain sorghum crop was used for ethanol production. As a major sorghum grower, the state of Kansas used approximately 50% of its 2010 sorghum crop for ethanol production (Agri-Energy Solutions 2009; Jessen 2010).

Grain sorghum is a viable feedstock for ethanol production (D. Wang et al 2008), and the performance of grain sorghum in ethanol production has been studied and evaluated recently (Monk et al 1984; Wu et al 2007, 2010). Ethanol conversion efficiency has been intensively studied because of the importance of, and public concern over, net energy gain in ethanol production. Virtually all of the current commercial sorghum lines in the United States are tannin-free, and most of the previous research studies involving sorghum have focused on these grain sorghum types. Little research has been conducted on ethanol production from tannin sorghum lines. Interest in tannin sorghum utilization has increased as health benefits associated with tannins have been discovered (Awika and Rooney 2004); tannin grain sorghum lines also have been shown to carry agronomic benefits such as resistance to drought, birds, mold, insects, and disease and higher grain yield than nontannin grain sorghum (Reichert et al 1980; Hahn and Rooney 1986). Although growing tannin sorghum has the advantages of lower production and storage costs and higher grain yields, the production of tannin sorghum and its use for ethanol production and animal feed are not desirable, largely because of the adverse effects of tannin on enzyme activity, starch and protein digestion, and ethanol fermentation efficiency. For identity-preserved and specialty markets, however, the use of tannin sorghum is very attractive.

Tannins are a group of highly hydroxylated phenolic compounds that are common in plants. Sorghum tannins have been actively studied for some time now with regard to food or feed uses. Tannins may impact the processing, product quality, and nutritional values of sorghum (Elkin et al 1996; Awika and Rooney 2004; Kobue-Lekalake et al 2009). Sorghum tannins are located in the outer layers of the kernel, beneath the pericarp in the pigmented testa layer of sorghum grain. Tannins protect sorghum grains, but because of their negative effects in animal nutrition, many studies have been conducted to remove or deactivate tannins. Chibber et al (1978) reported that mechanical abrasion or decortication of sorghum coat layers could reduce the tannin content of sorghum flour for food uses by physically removing the tannins. Reichert et al (1980) reported that anaerobic storage of grain sorghum treated with water or HCl solution decreased tannin content in grain sorghum. Daiber and Taylor (1982) steeped sorghum seeds in dilute formaldehyde solution and dilute NaOH to decrease tannin levels. These methods likely degraded or crosslinked the tannins, rendering them inactive. Germination of grain sorghum is another way to reduce tannin content (Reichert et al 1980; Yan et al 2009). Deactivation of tannins during germination was similar to the reduction reported during anaerobic storage of water-treated sorghum. The deactivation mechanism in both processes may be the same, that is, enzymatic degradation (Yan et al 2009).

*The *e*-Xtra logo stands for "electronic extra" and indicates that Figures 3 and 4 appear in color online.

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Mullins and NeSmith (1986) studied ethanol fermentation from bird-resistant (i.e., tannin-containing) and non-bird-resistant (i.e., containing no tannins) grain sorghum and reported that high tannin levels greatly reduced the ethanol-production rate. Wu et al (2007) reported tannin content as a major factor affecting ethanol conversion efficiency of grain sorghum in lines containing tannins. All results showed that ethanol yield and fermentation efficiency increased as tannin content decreased in treated sorghum (Yan et al 2009).

Ozone is a strong oxidant with oxidation potential of 2.07 eV. Ozone has the power to degrade vital components in living cells quickly and to kill microorganisms. It works at low dosage levels and leaves no residue in the treated product. Because of these advantages, ozone has been used in water treatment, food processing (Kim et al 1999), and corn and wheat steeping prior to milling (Ruan et al 2004; Dhillon et al 2009). Previous studies have shown that ozone degrades macromolecules such as lignin (Sugimoto et al 2009), protein, and carbohydrates (Yosef et al 1994; Wang et al 1999, 2008). Seo et al (2007) reported that chitosan could be depolymerized by ozone treatment. Thus, the hypothesis of the current project was that ozone treatment may degrade or inactivate sorghum tannin and reduce its adverse activity during fermentation, thereby increasing the fermentation efficiency of tannin sorghum. A process for deactivating sorghum tannins prior to ethanol fermentation would enable additional uses for tannin-containing sorghum lines grown for special purposes such as nutraceuticals or would prove valuable in areas of the world where tannin-containing sorghums are widely grown.

MATERIALS AND METHODS

Grain Sorghum

Tannin grain sorghum used in this study was cleaned manually and ground into flour with a Magic Mill III Plus grain mill (Magic Mill Products and Appliances, Monsey, NY) set at level 5 for fermentation use. Samples for chemical composition analysis were ground with a UDY cyclone sample mill (Fort Collins, CO) with a 1.0 mm screen.

Experimental Design and Ozonation Treatment

A factorial design was used in this study. Flow rate and treatment time were investigated to determine the main effect and the interaction between the two factors during ozonation. Each factor was run at two levels at room temperature. Ozonation was conducted following a randomized design, and each treatment was run in duplicate. Flour samples (500 g for each treatment) were tumbled in a metal drum (≈10L, Miag, Braunschweig, Germany) equipped with motor rotation. Ozone gas was generated by a pilot-scale ozone generator (Clear Water Tech, San Luis Obispo, CA; donated by Dr. Joseph Montecalvo, California Polytechnic State University) using oxygen from a SeQual oxygen concentrator (SeQual Technologies, San Diego, CA) at the set flow rates (0.7 and 2.0 L/min), which corresponded to ozone flow rates of 0.02 and 0.06 L/min. The ozone concentration in the exiting gas from the ozone generator was measured following an iodometric method (Rakness et al 1996; Chittrakorn 2008). The durations of ozone treatment were 15 and 30 min. The residual ozone exiting the rotary metal drum was entrapped in 2% potassium iodide solution with starch as an indicator. The treatment was conducted in a chemical hood.

Starch Isolation from High-Tannin Grain Sorghum Flour

Twenty-five grams of whole tannin grain sorghum flour (ozone-treated and untreated) was dispersed in 200 mL of distilled water in a flask. The pH was adjusted to 4.0–4.2 with hydrochloric acid, and 0.4% (v/v) protease GC106 (Genencor International, Rochester, NY) was added to hydrolyze protein and facilitate starch extraction. To prevent microbial contamination, 100 µL of 10% NaN_3 was added to each flask. The flasks with mixed flour suspensions were incubated in a 45°C water-bath shaker for 72 hr with constant agitation (150 rpm). The content from the flask was sieved through a no. 200 wire sieve (opening 75 µm), and the retained overs were washed twice with 200 mL (2 × 200 mL) of distilled water to recover starch. The washed overs were discarded, and the throughs were collected and passed through a no. 200 wire sieve (opening 75 µm). Again, the overs were discarded and the throughs were centrifuged at 3,000 × *g* for 30 min. After each centrifugation, the supernatant and tailings were removed and discarded. The prime starch was washed with distilled water and centrifuged at 3,000 × *g* for 30 min. A total of 10 washing and centrifuge cycles were conducted to obtain clean prime starch. The prime starch was freeze-dried for gel-permeation chromatography.

Gel-Permeation Chromatography

Four milligrams of purified starch from whole-grain sorghum flour was mixed with 4 mL of dimethyl sulfoxide (DMSO) and stirred in a boiling water bath for 24 hr. The sample was filtered through a 2 µm filter, and then 200 µL was injected into a gel-permeation chromatography (GPC) instrument (PL-GPC 220, Polymer Laboratories, Amherst, MA) equipped with three different pore sizes of Phenogel columns (100Å, 10^3Å, and 10^5Å, Phenogel GPC, 10 µm, 300 × 7.8 mm), a guard column (Phenomenex, Torrance, CA), and a differential refractive index detector. The eluent system was DMSO containing 5.0 m*M* $NaNO_3$ at a flow rate of 0.8 mL/min. The column oven temperature was controlled at 80°C. Standard dextrans (American Polymer Standards, Mentor, OH) with different molecular weights (MWs) were used for MW calibration.

pH Value Measurement

The pH of whole-grain sorghum flour samples was measured following AACC International Approved Method 02-52.01 (AACCI 2010). Ten grams of flour was added to 100 mL of distilled water. The flour suspension was stirred on a stirring plate for 15 min. Flour samples were allowed to stand for 10 min after removal from the stirring plate, and then the supernatant was decanted and used for pH measurement.

Pasting Properties of Sorghum Flour by Rapid Visco Analyzer

A Rapid Visco Analyzer (RVA) (RVA-3D, Newport Scientific, Warriewood, Australia) was used to test pasting properties of the sorghum flours. Sorghum flour (4.0 g of 14% moisture content standard flour, i.e., 3.44 g dry mass) and water (25 mL, including water from the sample flour) were mixed at 50°C; the slurry was held at 50°C for 1 min and then heated from 50 to 95°C. The hot paste was held at 95°C for 2.5 min, cooled to 50°C, and held at 50°C for 2 min. The total process took 13 min.

Microorganism, Preparation of Mashes, and Inoculation

The dry yeast (*Saccharomyces cerevisiae*, Red Star Ethanol Red) was provided by Fermentis (Milwaukee, WI) and was used for simultaneous saccharification and fermentation (SSF). Before inoculation, dry yeast was activated by adding 1.0 g of cells to 19 mL of preculture broth (containing 20 g of glucose, 5.0 g of peptone, 3.0 g of yeast extracts, 1.0 g of KH_2PO_4, and 0.5 g of $MgSO_4 \cdot H_2O$ 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 approximately 1×10^9 cells/mL.

Liquozyme SC DC (Novozymes, Franklinton, NC), a heat-stable α-amylase from *Bacillus licheniformis,* was used for liquefaction. Enzyme activity of the Liquozyme SC DC was 240 KNU/g (one kilo Novo unit, KNU, is the amount of enzyme that breaks down 5.26 g of starch per hr at Novozymes' standard method for determination of α-amylase). Spirizyme Fuel (Novozymes, Franklinton, NC), an amyloglucosidase from *Aspergillus niger*, was used for saccharification. Enzyme activity of the Spirizyme Fuel

was 750 AGU/g (one AGU is the amount of enzyme that hydrolyzes 1 μmol of maltose per min under specified conditions).

Whole-grain sorghum flour (34 g, as is) was dispersed in a 250 mL Erlenmeyer flask with 100 mL of fermentation broth containing 0.1 g of KH_2PO_4 and 20 μL of Liquozyme (240 KNU/g). The flasks were transferred to a 70°C water-bath shaker operating at 170 rpm. The water-bath temperature gradually increased from 70 to 95°C over a period of 30 min and kept at 86°C for 60 min. After 90 min of liquefaction, flasks were removed from the water-bath shaker and cooled to room temperature. Materials sticking to the inner surface of the flasks were scraped back into the mash with a spatula, and the inner surface was rinsed with 2–3 mL of distilled water using a fine-tipped polyethylene transfer pipette. One hundred microliters of amyloglucosidase, 0.3 g of yeast extract, and 1 mL of activated yeast culture (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 SSF ethanol fermentation. Each sample was run in duplicate.

Fermentation and Distillation

Ethanol fermentation was conducted at 30°C in an incubator shaker (I2400, New Brunswick Scientific, Edison, NJ) operating at 150 rpm for 72 hr. The fermentation process was monitored by measuring the weight loss of each flask from 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 distilled on a distillation unit. Distillates were collected in a 100 mL volumetric flask immersed in ice water. When distillates in the volumetric flask approaching the 100 mL mark (≈0.5 mL below the mark), the distillation process was stopped. Distillates in the volumetric flask were equilibrated in a 25°C water bath for at least 2 hr before adjusting the total volume to 100 mL with distilled water. Distillates were analyzed for ethanol by a Shimadzu HPLC (Columbia, MD) with a Rezex RCM Monosaccharide Ca+2 (8%) column (Phenomenex, Torrance, CA) and refractive index detector (Wu et al 2006). The mobile phase was double deionized water at 0.60 mL/min, and the oven temperature was 80°C.

Tannin Measurement

Tannin contents in the control whole-grain sorghum flour and ozone-treated whole-grain sorghum flours were determined by following the modified vanillin assay procedures for measurement of condensed tannin (Price et al 1978). Pure catechin (Sigma, St. Louis, MO) was used as a standard for calibration. The whole-grain sorghum flours for tannin testing were freshly ground on the day of assay with a UDY cyclone sample mill with a 0.5 mm screen.

Color Measurement

The *L*, a*, b** color spaces system was developed in 1976 and adopted by the International Commission on Illumination (CIE), which became a joint ISO and CIE standard (ISO 11664-4:2008(E)/CIE S 014-4/E:2007 and ISO 11664-5:2009(E)/CIE S 014-5/E:2009). *L** is a measure of the lightness, with values of 0 for black and 100 for white; *a** describes red-green color, with positive *a** values indicating redness and negative *a** values indicating greenness; and *b** describes yellow-blue color, with positive *b** values indicating yellowness and negative *b** values indicating blueness. A Minolta Chroma Meter (CR-210, EnTest, Carrollton, TX) was used for color determination. The instrument was calibrated with a white calibration tile. The colorimeter was set to an illuminant condition C and a 2° standard observer. Each sample was placed in the standard sample holder for color measurement. The test was done in duplicate. In this study, the effects of ozonation on sorghum color were measured against the untreated control sample.

Analytical Methods

Moisture content was determined following AACCI Approved Method 44-15.02A (2010). Total starch content was measured with Megazyme total starch test kits and the DMSO procedures according to AACCI Approved Method 76-13.01 (2010). AOAC official methods were used to analyze sorghum flour samples for crude protein (990.03), ash (942.05), crude fiber (962.09), and crude fat (920.39) (AOAC International 2000).

Statistical Analysis

Differences in traits among treatments were determined with the LSD LINE option of PROC GLM at a significant level of 0.05 (SAS 9.1.3 service pack 4 for Windows, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Effect of Ozonation on pH Values of Sorghum Flours

Figure 1 shows pH-value changes of the tannin sorghum flours after ozone treatment. Compared with the pH of the control sorghum flour, pH values of all the ozone-treated sorghum flours

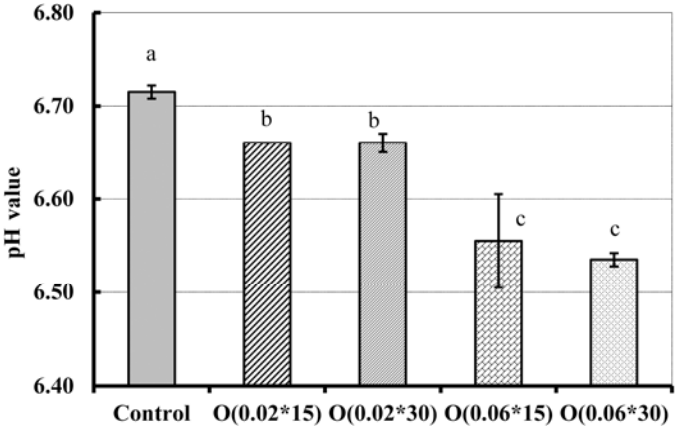

Fig. 1. Effect of ozone treatments on pH value of grain sorghum flour. Numbers in parentheses after the letter O are ozone doses designated by ozone flow rate (L/min) × treatment duration (min). Different letters above the bars indicate values different at $\alpha = 0.05$ level.

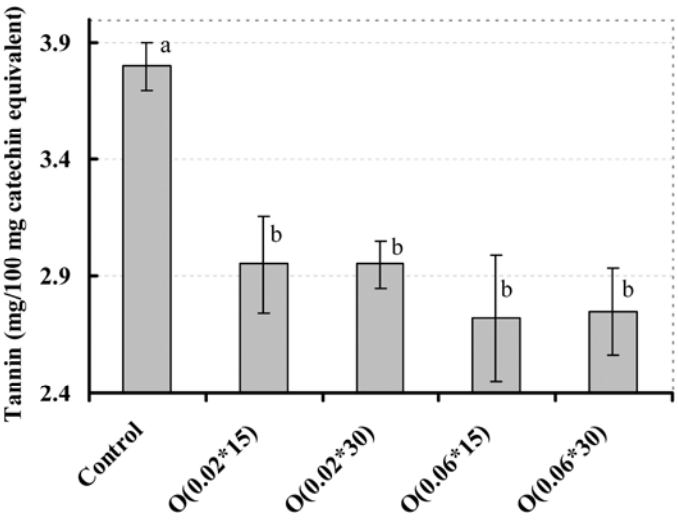

Fig. 2. Effect of ozone treatments on tannin content in grain sorghum flours. Numbers in parentheses after the letter O are ozone doses designated by ozone flow rate (L/min) × treatment duration (min). Different letters above the bars indicate values different at $\alpha = 0.05$ level.

were lower following treatment. The pH value of the same sorghum flour decreased as ozonation doses increased (higher ozone flow rates, longer treatment time, or both). Statistically, the pH values were significantly different among treatments with ozone flow rates at 0, 0.02, and 0.06 L/min ($P < 0.05$). pH values of ozone-treated sorghum flour were significantly different from the control; however, no significant difference was found between pH values of the flours ozonated for 15 min and those ozonated for 30 min ($P > 0.05$).

Decreasing pH values after treatment mean more $[H^+]$ ions in the water slurry systems from treated flours. Increases of carboxyl and carbonyl contents in ozone-treated starches have been reported to be proportional to the doses of ozone (Chan et al 2009). The increase in carboxyl groups in the oxidized starches was the result of oxidative breakdown of starch polymeric molecules. In whole-grain sorghum flour, additional carbohydrate polymers such as hemicelluloses and cellulose contribute to the formation of carboxyl groups during ozone treatment. This formation of carboxyl groups could be one explanation for the behavior of pH decrease in ozonated sorghum flours depicted in Figure 1.

Effect of Ozonation on Sorghum Tannins

Measured tannin levels in ozone-treated sorghum flours decreased by more than 20% compared with the untreated control (Fig. 2). The tannin content decreased as ozone levels increased. At either treatment time (15 or 30 min), the tannin contents in sorghum flours treated at the higher ozone flow rate (0.06 L/min) were lower than those treated at the lower flow rate (0.02 L/min), but no significant difference in tannin content was found among the treated samples. The combined effect of flow and time dose might be the reason for such different results. At either treatment duration, the combined ozone doses tripled when the flow rate was raised from 0.02 to 0.06 L/min, whereas the combined ozone dose only doubled at either flow rate when the treatment time increased from 15 min to 30 min. If triple dosage change is the minimum required to cause significant changes in tannin content, then double combined ozone doses might show a decreasing trend in tannin content but not enough to cause significant changes.

As previously reported, ozone can degrade macromolecules such as lignin and starches. Similar degradation could have happened to tannins in our ozone-treated sorghum flours. Size-exclusion HPLC was used to test the tannins in the treated sorghum flour samples. Unfortunately, the size-exclusion HPLC analysis could not differentiate between tannins in the ozone-treated samples and nontreated control (data not shown). One possible reason could be that the changes in tannin molecules were too small for size-exclusion HPLC to detect. Ozone treatment could change the structures of some functional groups in tannin molecules and therefore affect their enzyme-inhibition and protein-precipitating activity; however, such changes (formation of carboxyl, carbonyl groups, or breaking of a limited number of short branches) were not dramatic enough for size-exclusion HPLC to detect and differentiate.

Effect of Ozonation on Starch Molecule Distribution

Figure 3 shows the MW distribution of starches from ozone-treated high-tannin sorghum flour and control sorghum flour as determined with GPC. Compared with the MW distribution of starch in the control sorghum flour, the MW distribution of starches in ozone-treated high-tannin sorghum flours were either shifted toward the low-MW side or the proportion of the low-MW fraction increased. Several previous investigations demonstrated that ozone treatment could change structural, physicochemical, and functional properties of starches (Kuakpetoon and Wang 2006; An and King 2009; Chan et al 2009). These studies speculated that the low final viscosity of ozone-treated starch could be attributed to degraded starch molecules and weakened starch granules during ozonation, but no data directly supported their assumptions. Our GPC data revealed two different types of changes in ozone-treated sorghum starches. First, our data confirmed that ozonation can cause different degrees of degradation to starch molecules, which is in accordance with ozone-treated corn, sago,

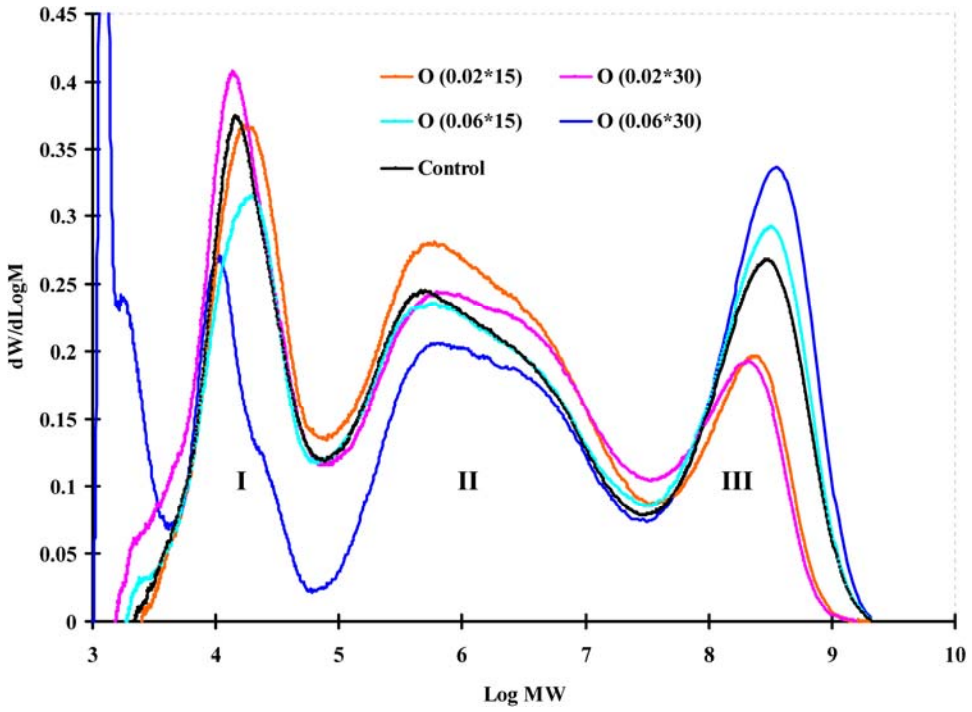

Fig. 3. Effect of ozone treatment on molecular weight distributions of sorghum starches. Gel-permeation chromatography was performed on a PL-GPC 220 with three 300 × 7.8 mm Phenogel columns (100Å, 10^3Å, and 10^5Å) and a differential refractive index detector. The eluent system was dimethyl sulfoxide containing 5.0 m*M* $NaNO_3$ at 0.8 mL/min. The oven temperature was 80°C. Fraction I was low-molecular-weight amylose; fraction II was intermediate components; and fraction III was high-molecular-weight amylopectin. Numbers in parentheses after the letter O are ozone doses designated by ozone flow rate (L/min) × treatment duration (min).

and tapioca starches (Chan et al 2011). The shift of GPC curves of the ozone-treated starches toward the low-molecular-weight side (left) is direct evidence of such degradation (Fig. 3). The portion of the lower-MW amylose peak (fraction I) increased after ozone treatment, especially after high ozone dose treatment, but the shift toward the low-MW direction was not proportional to the ozone dosage. Instead, more low-MW starches were found in samples treated at the lower flow rate than at the higher flow rate. On the other hand, GPC curves showed that some cross-linking among starch molecules might have occurred, because the high-MW portions (fraction III) of the GPC curves were obviously larger than that of the nontreated control. The GPC curves of starches treated at the lower flow rate had larger middle portions (fraction II, LogMW 5.5–7.5) than did the control, whereas the GPC curves of starches from samples treated at the higher ozone flow rate had larger peaks in the high-MW end (fraction III, LogMW 9–10). Our data show that both oxidative degradation and cross-linking could have affected sorghum starches during ozone treatment. One explanation for the decrease in low-MW starch molecules in samples treated with a higher ozone dose could be the formation of larger molecules from cross-linking of oxidative degraded starch molecules (starches with carboxyl and carbonyl groups). Another explanation for the shifting of starch GPC curves of the ozonated samples could be that one part of the starches was degraded under the more intensive ozonation condition to the extent that it was not recovered in the starch-recovery procedures used in this study; therefore, the purified starch samples were enriched with high-MW starch molecules. The result would be GPC curves of starches from more intensively ozonated samples shifted toward the higher-MW side, as shown in Figure 3. At present, no information is available regarding the types of cross-linking reactions and the structural features of cross-linked molecules. Further studies are needed to elucidate the mechanism behind such changes. To obtain a treated product with the appropriate amount of oxidized starch molecules and suitable physical and chemical properties for ethanol production, ozone treatment doses (control of flow rate and treatment time) need to be further optimized.

Effect of Ozonation on Pasting Properties of Sorghum Flour

Pasting properties of ozone-treated tannin grain sorghum flours and control flour analyzed with an RVA are shown in Figure 4. Not all curves showed clear peak viscosity and breakdown viscosity. This could be caused by two factors; one is high tannin content (≈4%) in the flour. Tannins have the ability to bind, coagulate, and precipitate proteins. This conclusion has been reviewed by Butler et al (1984), who summarized that under optimal conditions, sorghum tannin is capable of binding and precipitating at least 12 times its own weight of protein by means of hydrogen bonding, hydrophobic interaction, electrostatic attraction, and covalent bonding associated with oxidation. Because tannins interact with proteins during mashing or during the RVA analysis, the tannin–protein complexes will inhibit the water-absorption rate of starch granules and prevent starch granules from swelling rapidly. Another factor could be the particle size of sorghum flours. Whole sorghum flour samples used in this study were prepared with a Magic Mill III Plus grain mill set at level 5. The particle sizes of such prepared flours were relatively larger than those from a cyclone mill with 1.0 mm screen or industrial flours. Normally, the sizes of cereal starch granules are in the range of 0–50 μm. If a 1.0 mm (1,000 μm) screen was used, some large particles in the ground sorghum flours could contain more than 100 starch granules. As a result, water-absorption rates by starch granules in larger particles will be inhibited. Sudden increases in mash viscosities of tannin sorghum samples during mashing confirmed this assumption.

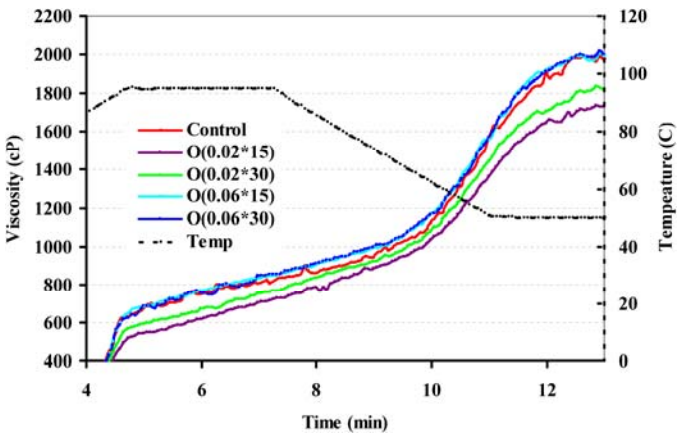

Fig. 4. Pasting properties of ozonated high-tannin sorghum flours and a nontreated control flour measured with a Rapid Visco Analyzer with a standard 13 min procedure. Numbers in parentheses after the letter O are ozone doses designated by ozone flow rate (L/min) × treatment duration (min).

The setback viscosity indicates the degree of retrogradation of starch molecules during cooling. A high setback value indicates a high tendency of starch molecules to retrograde. Pasting curves in Figure 4 show that the setback viscosities of sorghum flours treated with low ozone flow rates were ≈200 cp lower than those of sorghum flours ozonated at the higher flow rates. This result agrees with Figure 3, which shows that amylopectin degraded more in samples treated at the lower flow rate, but some cross-linking occurred in samples treated at the higher flow rate. Usually, the lower setback viscosity is a sign of higher α-amylase activity. Lower setback viscosity is a good trait for ethanol production from grain sorghum, because low setback viscosity means easy disruption of starch granules and less tendency to form retrograded starch molecules, which are more resistant to enzyme hydrolysis, during mashing (Yan et al 2010).

Effect of Ozonation on Ethanol Yield and Fermentation Efficiency

Ethanol yields from ozone-treated whole-grain sorghum flour and control sorghum flour are listed in Table I. Ethanol yields from all the ozone-treated sorghum flours were significantly higher than that from the nontreated control sorghum flour. Ethanol yields from samples treated at the lower flow rate were significantly higher than those from both sorghums treated at the higher ozone flow rate and the nontreated control ($P < 0.05$). This indicates that the ozone flow rate had a significant effect on ethanol yields in the treated flow rate range. The favorable effects of ozonation on ethanol yields were not found to be proportional to ozone doses as measured by flow rates (0.02 versus 0.06 L/min). In fact, some favorable effects might have been offset partially as ozone flow rate increased from 0.02 to 0.06 L/min. On the other hand, when we examined the effect of ozonation time on ethanol yield, no significant difference in ethanol yield was found between 15 and 30 min of ozonation ($P > 0.05$). Nevertheless, ethanol yields from ozone-treated sorghum samples showed decreasing trends as treatment time increased at both flow rates. The explanation for insignificant changes in

TABLE I
Ethanol Yields from Ozonated Grain Sorghum and Control Sorghum[a]

Sample	Ethanol Yield (L/ton)	Ethanol Yield (gal/bu)
Control	356.5 ± 5.76	2.68 ± 0.04
O (0.02 × 15)	375.5 ± 5.40	2.82 ± 0.04
O (0.02 × 30)	373.0 ± 1.44	2.80 ± 0.01
O (0.06 × 15)	364.4 ± 1.80	2.74 ± 0.01
O (0.06 × 30)	363.0 ± 3.96	2.73 ± 0.03

[a] Numbers in parentheses after the letter O are ozone doses designated by ozone flow rate (L/min) × treatment duration (min).

ethanol yields could be that the 30 min duration was not long enough. Interactions between the ozone flow rate and treatment duration on ethanol yield were not significant ($P > 0.05$).

Fermentation efficiency is an important parameter for evaluating the performance of a material for ethanol production. Ethanol fermentation efficiencies from ozone-treated whole-grain sorghum flour and control sorghum flour are shown in Figure 5. By the end of the 72 hr fermentation process, the efficiencies of ozone-treated grain sorghum flours were 2–5% higher than that of the control flour. The efficiencies of the samples treated at the lower flow rate were ≈3% higher than those of the samples treated at the higher flow rate. When we examined the fermentation efficiencies at the 36th hr, the efficiencies of ozone-treated sorghum samples ranged from 86 to 92%, whereas the efficiency of the nontreated control sorghum was 78%. Efficiencies of samples treated at the lower ozone flow rate (0.02 L/min) were 12.9–13.8% higher than that of the nontreated control, whereas the fermentation efficiencies of samples treated at the higher ozone flow rate were 8–10% higher than that of the nontreated control. Figure 5 shows that the fermentation efficiency at the end of the fermentation process (72 hr) did not increase much after the 36th hr except for the control. Therefore, fermentation time could be shortened to 36 hr to save energy without obvious loss in ethanol yield if ozone-treated grain sorghum flour is used for ethanol production. This indicates that ozone treatment could be a novel way to shorten fermentation time and increase the production capacity of ethanol plants.

Effect of Ozonation on Sorghum Flour Color

Figure 6 shows results from color measurement with a colorimeter. Compared with the nontreated control grain sorghum flour, ozone-treated tannin sorghum flours had higher L^* values, indicating that they became lighter colored. As ozonation time and ozone flow rate increased, the whiteness of sorghum flour increased, whereas a^* values (redness) showed a declining trend as ozone dosage increased. Yellowness (b^* values) varied among the treatments. Xiang (2009) reported that sorghum colors are determined and affected by many factors such as pericarp and the presence of pigment testa layer.

Besides tannins, many other naturally occurring compounds such as lignin, carotenoids, and anthocyanins may give plant-originated materials dark colors. Degradation of these compounds usually leads to a lighter-colored material (Miki et al 1994; Henry et al 2000; Tiwari et al 2009). As a powerful oxidant, ozone definitely has the potential to degrade such pigment compounds, including tannins, and turn sorghum flour color lighter, which could have contributed to the color changes (lighter and brighter, reduction in redness, and variable in yellowness) of ozone-treated sorghum.

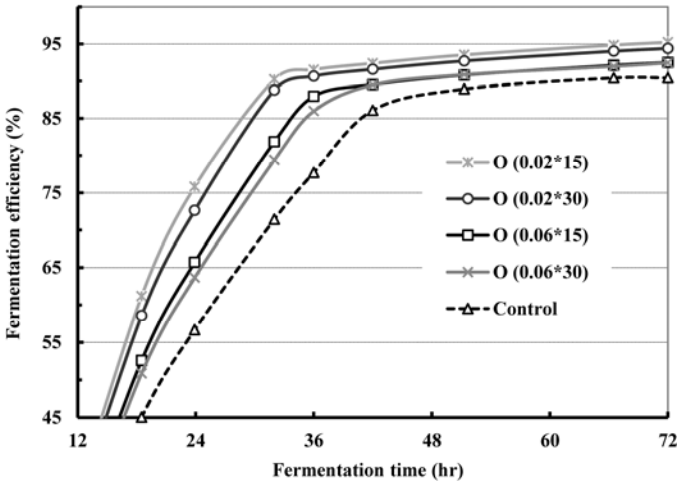

Fig. 5. Fermentation efficiencies of ozonated high-tannin sorghum and a nontreated control using a laboratory dry-grind process. Numbers in parentheses after the letter O are ozone doses designated by ozone flow rate (L/min) × treatment duration (min).

Effect of Ozonation on Distillers Dried Grains with Solubles

Distillers dried grains with solubles (DDGS) is a by-product from ethanol production. Its composition and quality are critical for its market value as animal feed, which accounts for a major portion of revenues in ethanol plants. Protein and fat contents are the two major variables that affect the nutritional values, and thus market prices, of DDGS. Table II shows the chemical composition of DDGS from ozone-treated and nontreated samples. Normal DDGS has crude protein content of 25–29% and crude fat content of 7–11% (Saunders and Rosentrater 2009). Data in Table

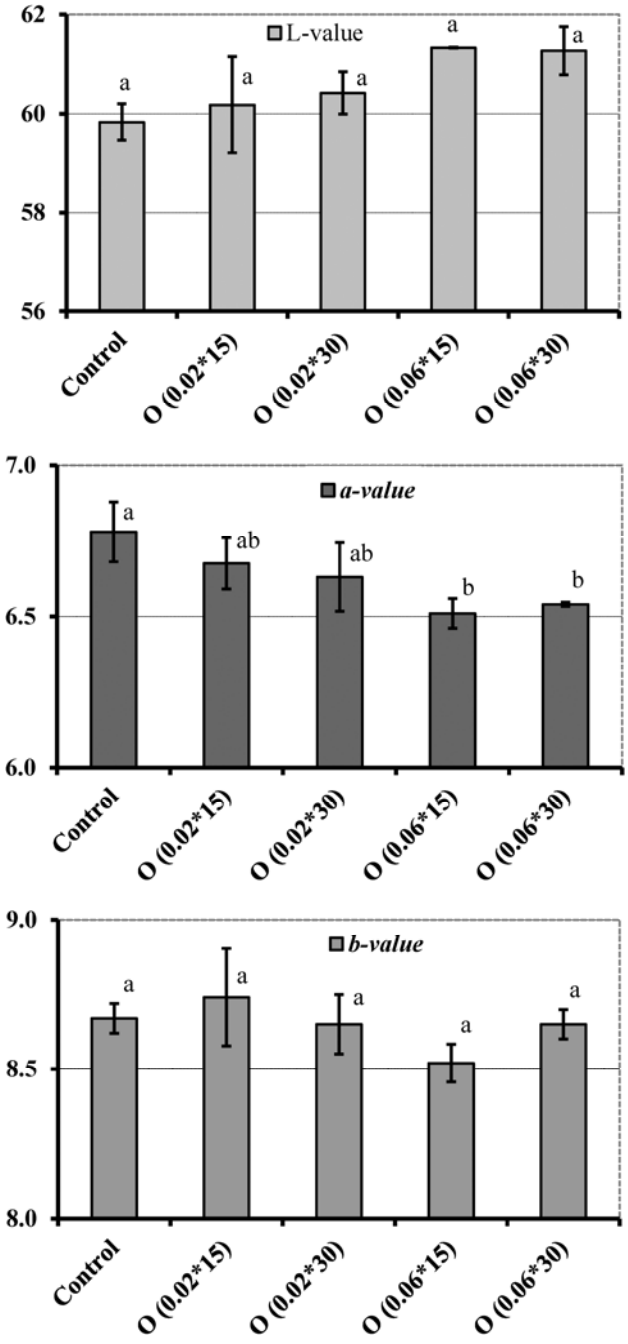

Fig. 6. Effect of ozone treatment on sorghum flour color measured in L^*, a^*, b^* color space. Numbers in parentheses after the letter O are ozone doses designated by ozone flow rate (L/min) × treatment duration (min). Different letters above the bars in the same frame indicate values different at $\alpha = 0.05$ level.

TABLE II
Proximate Analysis Results on Major Components of Distillers Dried Grains with Solubles (%, db) from Ozonated Sorghum Samples and Nontreated Control[a]

Sample	Starch	Crude Protein	Crude Fats	Crude Fiber	Ash
Control	1.69 ± 0.01	33.8 ± 0.01	8.56 ± 0.01	4.99 ± 0.01	5.66 ± 0.01
O (0.02 × 15)	0.95 ± 0.01	35.3 ± 0.01	8.14 ± 0.02	4.74 ± 0.03	5.87 ± 0.11
O (0.02 × 30)	0.94 ± 0.00	35.1 ± 0.88	8.63 ± 0.03	4.99 ± 0.15	5.76 ± 0.00
O (0.06 × 15)	1.03 ± 0.15	35.1 ± 1.22	8.51 ± 0.02	4.86 ± 0.05	5.84 ± 0.35
O (0.06 × 30)	0.99 ± 0.08	34.8 ± 0.26	8.48 ± 0.16	4.88 ± 0.48	5.74 ± 0.16

[a] Numbers in parentheses after the letter O are ozone doses designated by ozone flow rate (L/min) × treatment duration (min).

II show that DDGS from sorghum has much higher crude protein content than and a comparable crude fat content to normal ethanol-industry DDGS, which gives sorghum DDGS a label of better quality, at least in terms of protein content. Comparing DDGS from different treatments within this study, protein content was higher in DDGS from ozone-treated samples than from the nontreated control. Residual starch content in normal industrial DDGS is around 5% (Belyea et al 2004); starch residues in DDGS from ozone-treated samples were less than 1%, lower than residues in DDGS from the nontreated control (1.69%), which is reasonable given the higher fermentation efficiencies and higher ethanol yields of the ozone-treated sorghum flours. Although the treated sorghum flours still showed high levels of tannins, the fast ethanol-fermentation rates and high fermentation efficiency of ozonated sorghum samples indicated that tannins' adverse activities had been greatly removed, if not totally eliminated, at least toward the hydrolysis enzymes and yeast activity. Overall, ozone treatment not only enhanced fermentation efficiencies and ethanol yields but also generated a DDGS by-product with higher protein content.

CONCLUSIONS

Ozonation not only decreased tannin content and pH value of high-tannin sorghum but also affected sorghum flour color, properties of starch granules, and starch molecular distribution. Fermentation efficiency is an important parameter in evaluating the performance of a material for ethanol production, and ethanol fermentation efficiency from ozone-treated sorghum increased over 10% compared with the control. This indicates that ozonation has significant impact on ethanol yield and fermentation efficiency and is an effective way to increase ethanol yield and shorten the fermentation process without decreasing ethanol yield.

Safety Warning. Ozone is a strong oxidizing agent and can be harmful to the upper respiratory tract and the lungs. Caution should be taken to avoid exposure to the ozone environment (<8 hours at 0.1 ppm, or <15 min at 0.3 ppm atmosphere concentration).

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