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Extracted Sweet Sorghum Substrates as a Source of Fermentable Sugars

Scientific Note

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ABSTRACT

Simple sugars of variously prepared sweet sorghum culms were extracted to different extents depending upon water temperature, extraction time, and size of the culm substrate. Sonication of succulent culms (≤15 mm split sections) suspended in water followed by centrifugation extracted 88% of the available sugars. However, hot water extraction of freeze-dried and milled sorghum quantitatively extracted the simple sugars, which were 2/3 sucrose and 1/3 glucose and fructose. Following the sugar removal, the residue was extracted with a 12% NaOH solution to leave a cellulosic residue that was nearly quantitatively converted (98%) to glucose by enzymatic hydrolysis. Ninety percent of the pentosans in the culms were extracted by the alkali treatment, and then 91% of these pentosans were precipitated from the alkaline extract with acidified ethanol.

Index Entries: Sweet sorghum; extractions; sugars; pentosans; sonication.

INTRODUCTION

Because of its genetic diversity, sweet sorghum (*Sorghum bicolor* (L.) Moench) is especially adaptable to geographic and climatic conditions, soil types, fertilizer, and overall agronomic practices (*1–4*). Furusaki et al.

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(5) state that sweet sorghum is potentially exploitable as a biomass energy source. Due to economic considerations, approaches for developing new sorghum varieties must consider grain and biomass yields as well as sugar yields. McBee et al. (6) have discussed adapting cultivars for dual purposes, such as for grain and for biomass, that can be converted to usable energy forms. The first step in using sweet sorghum as a chemical feedstock might involve expressing the sugar-rich juice from the culms. However, this technique is both capital- and energy-intensive, and alternative methods need to be found. Bryan et al. (7) stated that the problems of extracting sugar efficiently from sweet sorghum without complex processing methods have not been solved. The sugars in fresh sorghum present a problem in milling because of their stickiness. Water extraction of wet, chopped sorghum culms would alleviate this problem. However, the desired concentration of sugars in solution for fermentation requires that excessive dilution be avoided. Previously, we demonstrated that hemicelluloses (pentosans) can be effectively extracted from milled wheat straw by a 12% NaOH solution (8). Good yields of these extracted pentosans were recovered from the filtrates by acidified ethanol precipitation. If chemical feedstocks are to be commercially produced from sweet sorghum, water extraction to remove sugars would logically precede the extraction of pentosans and lignin by alkali. Techniques to fractionate the three major components (sugars, pentosans, and cellulose) of sweet sorghum by using these two stages are discussed in this paper.

MATERIALS AND METHODS

Raw Material

Sweet sorghum, *Sorghum bicolor* (L.) Moench, variety Wray was grown in central Illinois on reclaimed stripmine land. For an optimal yield of sugar, the stalks were harvested on d 140 and stripped of leaves. Most of the culms were sectioned and frozen, although some were dejuiced by pressing.

Dejuicing

Short sections (50–75 mm) of culms were pressed in a Carver mold (57 mm diam.) with a hydraulic press to 15,000 psig (104×10^6Pa). Pressure was released to 10,000 psig (69×10^6Pa), and the process was repeated 4–6 times. Then, the bagasse was freeze-dried and milled in a Wiley-type mill equipped with a screen containing 1-mm-diam. openings.

Water Extraction

Sorghum culms were chopped at high moisture (75% moisture, 25 mm) or dried (freeze-dried except for second stage of extraction when samples were air-dried) and then milled (4–10% moisture, < 1 mm).

The sorghum culms (10 g, moisture-free basis) were mixed with 100 mL (or equivalent to 1:10 ratio) of distilled water and allowed to steep for 1 or 3 h at 97–100°C or 24 h at room temperature. Samples were filtered and washed with 300 mL of hot water. Fibrous residues were freeze-dried and milled (< 1 mm) for chemical analyses. Filtrates were analyzed for sucrose, glucose, and fructose by high-performance liquid chromatography (HPLC) with a Bio-Rad (Richmond, CA) HPX-87H size exclusion column and water as the mobile phase.

Ultrasonic Extraction of Sugars

Two-gram samples of chopped (15-mm sections, split lengthwise), succulent sweet sorghum culms in 20 mL of water (1:10) were prepared. One-third of the samples were extracted with ultrasonic energy by using a Model T 32A power suply (Branson Sonic Power Company, Danbury, CT) capable of producing up to 550 W. The sonifier delivered 165 W at 20 kHz to the converter. A booster horn was connected to the converter to increase by 50% the longitudinal mechanical vibrations of the probe. The probe was inserted to one-half (9 mm) of the liquid's height in a 100-mL beaker. Sample slurries were sonicated for 1 min, temperatures recorded, materials stirred, sonicated for an additional 1 min, and temperatures recorded. The slurries were transferred into tubes and centrifuged at 2200 rpm in an International Centrifuge Universal Model UV (International Equipment Company, Boston, MA) for 10 min. Of the remaining two-thirds of the samples, one-half was allowed to steep at room temperature and filtered, and the other half was steeped and centrifuged as described above. Duration time of each experiment was 24 min. All extracts were analyzed for soluble sugars by HPLC as described earlier.

Alkali Extraction

Milled (< 1 mm), water-extracted sorghum culms (8 g, moisture-free basis) were mixed with 80 mL of 12% NaOH solution (w/v) and allowed to steep 1 h at 100°C. Samples were filtered and washed with 400 mL of hot water. Residues were water washed further to remove alkali, filtered, and then freeze-dried. Filtrates were precipitated with 95% ethanol either following adjustment to pH 4.5 with acetic acid or without pH adjustment. The ratio of ethanol:filtrate was 2:1. After standing 24 h, the precipitates were collected on filter paper, washed with absolute ethanol, air-dried, and analyzed.

Cellulase Treatments and Chemical Analyses

Various plant materials were treated with cellulase to determine the availability of cellulose for enzymatic reaction (*9*). Glucose yields were determined by HPLC as described above. Sweet sorghum culms for the cellulase treatment had been freeze-dried. The treated residues from alkali extraction had been frozen but never dried.

Cellulose contents were measured by a monoethanolamine method (*10*) and reported on an ash- and pentosan-free basis. Samples were analyzed for lignin contents by an ultraviolet spectrophotometric method (*11*). Pentosan contents were determined by TAPPI Standard Method T223m, and ash contents measured by ignition at 600 ± 25°C.

RESULTS AND DISCUSSION

At this Center, expressed juice from sweet sorghum grown on sludge-amended stripmine soil was readily fermented to ethanol by *Saccharomyces cerevisiae* in 72% yield (*12*). Commercial utilization of sweet sorghum for chemical feedstock may require methods of sugar extraction other than typical dejuicing processes. Compositions of sorghum fractions from six treatment regimes are given in Table 1, B–G. The "80% ethanol solubles" measures readily solubilized materials, primarily simple sugars. Yields of both residue and 80% ethanol solubles decrease as treatments progress from dejuicing by pressing (B) to aqueous extractions on milled (< 1 mm) material (E, F). Treatment variables are time, temperature, particle size, and multistage extractions. With increasingly rigorous treatment, more sugars and solubles are removed, leaving less residue. These results correlate with increasing amounts of sugars in the extraction liquors, and because cellulose, pentosans, and lignin, generally are not soluble in water, their concentrations increase in the residue with more rigorous aqueous extractions.

With chopped culms (25 mm), results are similar for both long-term ambient and hot water extractions (C, D). However, long-term ambient steeping may be slightly more effective at removing sugars.

Double extraction with hot water (F) is slightly more effective at removing sugars than is double extraction with first ambient and then hot water (E). Treatments E and F started with chopped culms (25 mm) that were extracted once and then air-dried and milled (< 1 mm) before the second extraction. A single hot water extraction of milled (< 1 mm) culms is more effective in removing sugars than are the extractions of the never dried, succulent samples (25 mm) followed by a hot water extraction of the milled material. No treatment was effective in removing more than 78% of the total sugars from chopped culms, but the 3 h hot water steeping of milled material removed 99% of the sugars (G). Treatments C, E, and F indicate sopme losses (8–12%) in material, probably because of deterioration during steeping and/or drying, possibly through fermentation.

The rate-enhancing effects of ultrasound on a variety of chemical processes have been demonstrated (Kristol et al. (*13*)). However, as Patel et al. (*14*) point out, ultrasonics in chemical engineering projects is still in the initial developmental phase. Because of the high cost of drying, it is important that an efficient method of extracting sugars from chopped, wet sorghum be developed. As the water extraction (steeping) of

Table 1
Compositional Enhancement of Sweet Sorghum as a Function of Aqueous Extractions
Analyses of Treatment Fractions, % (oven-dry basis)

	Residual solids							Liquor extracts			
Treatment[a]	Yield[d]	80% Ethanol solubles	Cellulose[e]	Pentosans	Lignin	Ash	Cellulose conversion	Total sugars	Sucrose	Glucose	Fructose
A None	—	58.7	16.7	16.2	5 3	2.8	44.6	47.8	33.0	8.7	6.1
B Hydraulic press[f]	65.5	33.1	27.2	23.9	9.1	2.3	—	25.7	17.1	5.2	3.4
C Ambient water (24 h, 25 mm)	62.4	19.9	32.5	27.6	9.0	2.5	—	27.0	17.0	5.4	4.6
D Hot water (1 h, 25 mm)	65 9	28.4	29.5	24.2	9.4	2.4	—	23.4	15.4	5.0	3.0
E Two stage amb. water (24 h, 25 mm)								27.6	18.2	5 1	4.3
hot water (1 h, < 1 mm)	32.8	3.6	40 2	31.9	11.4	1.3	—	6.4	1.5	1.7	3.2
								[34.0]	[19.7]	[6.8]	[7.5]
F Two stage hot water (3 h, 25 mm)								23.0	14.5	5.1	3.4
hot water (1 h, < 1 mm)	35.0	4.1	40.8	31 2	12.8	1.2	—	14 1	9.3	2.9	1.9
								[37.1]	[23.8]	[8.0]	[5.3]
G Two stage hot water (3 h, < 1 mm)								47.4	31.6	9.1	6.7
								Liquor solids[f]			
hot 12% NaOH (1 h, < 1 mm)	20.5	1.0	86.1	7.8	4.0	0.3	98.5	Yield[b] 18.0(19.8)	Pentosans 73.8(64.8)	Lignin 2.6(2.5)	Ash 12.4(24.6)

[a]Water sorghum culms or water-extracted sorghum, 10 1, 12% NaOH solution water-extracted sorghum, 10 1.
[b](Fraction weight − sample weight) × 100
[c]Monoethanolamine method (*10*)
[d]Culms were pressed in a cylindrical mold (57 mm-diam).
[e]Offset values are sums of values in brackets.
[f]Solids precipitated by addition of acetic acid and ethanol to filtrates. Values in parentheses represent solids precipitated by addition of ethanol only to filtrates.

chopped, succulent sweet sorghum was only partially effective in removing sugars (≤ 78%), the use of ultrasonic energy with the water extraction was explored. Even if milling should be required before the pentosan extraction with alkali, the removal of sugars would eliminate the problem of stickiness and help prevent storage-loss of material because of fermentation or spoilage. Figure 1 shows the effect of extracting sugars by either steeping-filtration, steeping-centrifugation, or sonication-centrifugation. The first two treatments were performed at ambient temperatures. Temperatures of 70–74°C were observed during the ultrasonic treatments that should increase the yield of sugar extracted. An analytical extraction method (50% v/v ethanol/water) showed that the sweet sorghum culms contained 47.8% sugars; thus the yield of 42.1% sugars (sucrose, glucose, and fructose) from sonication and centrifugation represents 88% of the original sugars. Simple water steeping and

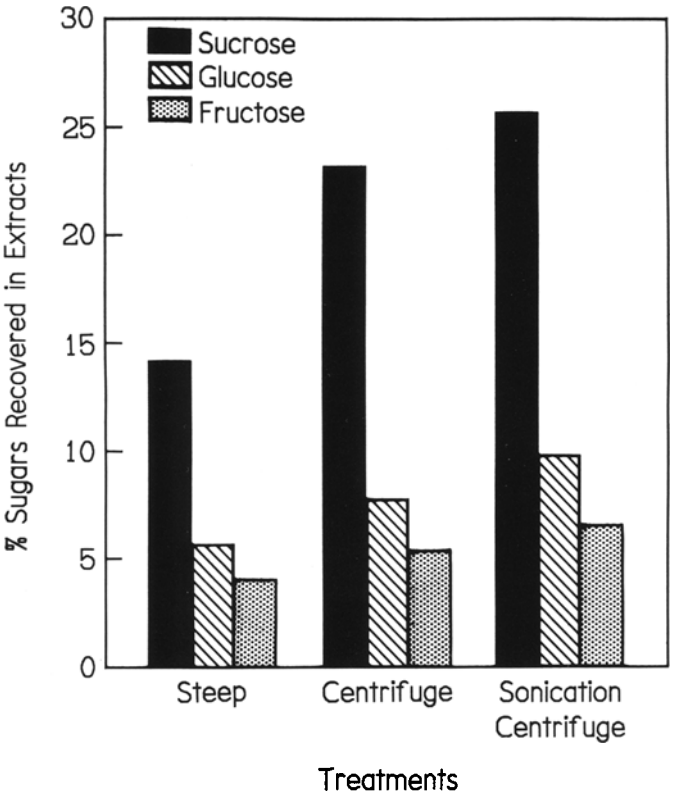

Fig. 1. Effect of steeping-filtration, steeping-centrifugation, and sonication-centrifugation on sucrose, glucose, and fructose extraction from sweet sorghum culms (dry plant basis). Water:sweet sorghum culms, 10:1; ambient temperature; <15-mm split sections; 24 min duration; centrifugation, 2200 rpm for 10 min; sonication, two 1-min treatments (165 W at 20 kHZ), temperature attained 70–74°C.

centrifugation yielded 76% of the available sugars. In these experiments, the sorghum culm sections were relatively small (<15-mm split sections). Even so, the rind may require vigorous physical or mechanical treatment to permit a more effective attack by ultrasonic energy.

When freeze-dried, milled sweet sorghum is treated with water for 3 h at 99°C and washed, a quantitative yield (47%) of sugar is realized (Table 1, G).

The second phase of this treatment was extraction with hot 12% NaOH solution, which solubilized most of the lignin and pentosans and left a residue rich in cellulose (86%), of which 98% is converted to glucose by cellulase. Alkali extraction can result in many changes in the polysaccharide. Most of the pentosans are recoverable from the extraction liquor by acidification followed by precipitation with ethanol. Acidification causes precipitation of the higher molecular weight polysaccharides. These liquor solids contained 74% pentosans or 82% of the initial sorghum culm pentosans. Of the pentosans extracted (90%), 91% were recovered with acidified ethanol precipitation. Eighty-eight percent were precipitated when ethanol was used alone. Similar observations were reported for the alkali extraction of dejuiced sweet sorghum culms (bagasse) (*15*). However, 22% of the extracted lignin from the dejuiced sorghum and only 10% of the extracted lignin from the water-extracted sorghum were recovered by acidified ethanol precipitation. We showed previously that a treatment of milled wheat straw with a 12% NaOH solution for 4 h at 80°C removed 88% of the wheat straw pentosans and 85% of the extracted pentosans were precipitated by acidified ethanol (*8*). Further, the cellulose in the straw residue was essentially all converted (98%) to glucose by enzymatic hydrolysis. Detroy et al. (*16*) have reported on the fermentation of glucose derived from the cellulase hydrolysis of cellulose in modified wheat straw. Ready fermentation of alkali-prepared, acid-hydrolyzed pentosans extracted from wheat straw also has been reported by Detroy et al. (*17*).

CONCLUSIONS

Sonication and centrifugation of succulent, chopped sweet sorghum culms (< 15-mm split sections) suspended in water extracted 88% of the available sugars. All of the sugar (47% yield, based on dry culms) was extracted with hot water from freeze-dried, milled sweet sorghum culms. Alkali extraction of the hot water-extracted, milled sweet sorghum culms removed 90% of the available pentosans, and 91% were recovered by precipitation. These can be acid-hydrolyzed to xylose as the primary sugar. Cellulose in the residue is essentially all convertible to glucose (98%). We believe that three-fourths of the dry weight of sweet sorghum culms is convertible to fermentable sugars by this process.

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