Recovery of Protein-Rich By-Products from Sweet Sorghum Grain Stillage after Alcohol Distillation

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ABSTRACT

Because of current interest in sweet sorghum for ethanol production, methods for efficient recovery and utilization of sweet sorghum grain stillage were investigated. Ground sweet sorghum grain was fermented to ethanol, ethanol was distilled, and residual stillage was separated into distillers' grains, centrifuged solids, and stillage solubles. Distillers' grains and centrifuged solids had protein contents (N × 6.25, dry basis) of 42 and 46%, respectively, and accounted for 73 and 8% of total grain nitrogen. Distillers' grains protein was much less soluble than that of unfermented sweet sorghum grain. Seventy-two percent of the nitrogen in stillage solubles passed through a 10,000 molecular weight cut-off membrane. Combined ultrafiltration and high-pressure reverse osmosis effectively processed stillage solubles into a small volume of concentrate with potential feed use and a large volume of permeate, which can be reused for water or safely discarded.

Corn is used almost exclusively for fuel ethanol production in the United States. Parrish et al (1985) examined production of readily fermentable carbohydrates and biomass by grain sorghum, Jerusalem artichoke, corn, sugarbeet, sweet potato, and sweet sorghum at three temperate locations and concluded that sugarbeet and sweet sorghum are superior to corn in productivity of fermentable substrates when sweet sorghum is harvested for both grain and stalk.

Relative amounts of fermentables from sweet sorghum stalk and grain depend on both variety and location. Research over four years showed that sweet sorghum grain production at latitudes 40° N and above failed to produce significant grain, whereas southern latitudes such as in Mississippi and Hawaii yielded much more grain (G. A. Smith 1986, personal communication). Clegg et al (1986) found that grain from Wray sweet sorghum accounted for about 20% of the total alcohol potential as compared to 55% for grain from a relatively sweet hybrid sorghum.

Fermentation of sweet sorghum stalks has been reported (Kargi et al 1985, de Mancilha et al 1984). However, whether fermentation of sweet sorghum grain for ethanol with good yield is feasible is not known. This paper reports fermentation of sweet sorghum grain to ethanol, yield and composition of stillage fractions, and use of ultrafiltration and high-pressure reverse osmosis to concentrate sorghum stillage solubles and produce a permeate suitable for reuse or safe disposal.

MATERIALS AND METHODS

Fermentation

Wray sweet sorghum grain was purchased from Jack Holcomb, Progresso, TX. Grain was ground in a Fitzpatrick Homoloid model JT mill until all passed through a 20-mesh screen (0.85 mm aperture). Ground sorghum grain (2,124 g, db) was dispersed in 5 L of tap water in a 20-L stainless steel, temperature-controlled, jacketed fermentor equipped with stirrers. The slurry was adjusted to pH 6.2, and 6 ml of Miles Taka-therm α-amylase (Miles Laboratories, Inc., Elkhart, IN) was added. The slurry was maintained at 90° C for 1 hr, and then 1,335 ml of tap water was added. The slurry was cooled to 60° C, pH adjusted to 4.0, and 18 ml of Miles Diazyme L-100 glucoamylase was added. After 2 hr the mixture was cooled to 30° C, the pH adjusted to 4.5, and 500 ml of yeast (*Saccharomyces cerevisiae*) culture containing 5 million cells per milliliter was added. Two fermentation runs of 66 hr were made. More details were reported previously (Wu and Sexson 1984).

Fractionation of Stillage

Alcohol was distilled from the fermentor by jacketed steam, and the fermentation residue (stillage) was filtered through cheesecloth under suction. The liquid that passed through the cheesecloth was processed in a continuous centrifuge. The solution that passed through the centrifuge was termed stillage solubles, whereas solids remaining in the centrifuge bowl were designated centrifuged solids. Materials that remained on the cheesecloth were termed distillers' grains. Wet solids were dried at 85° C overnight in a forced-air oven.

Molecular Weight Range of Stillage Solubles

An Amicon model 52 ultrafiltration cell (Amicon Corp., Lexington, MA) with 43-mm diameter membranes was used under 340 kPa (50 lb/in.^{2}) nitrogen pressure. Nominal molecular weight cutoffs for UM05 and PM10 membranes were 500 and 10,000, respectively. Twenty milliliters of stillage solubles were pipetted above each membrane, and 48–58 ml of permeate (solution that passed through the membrane) were collected by adding distilled water above the membrane.

Protein Extraction

Ground sweet sorghum grain (10 g) was blended with 100 ml of solvent for 5 min in a Waring Blendor with a stainless steel cup, and then centrifuged at 10,400 × *g* for 10 min; the supernatant was decanted, and residue was extracted with the next solvent. Solvents used sequentially were water (2×), 1% sodium chloride, 60% *tert*-butanol (2×), 60% *tert*-butanol + 0.05% dithiothreitol (DTT) (2×), and borate + 0.5% sodium dodecyl sulfate (SDS) + 0.05% DTT (pH 10.6). The borate solution was made from 500 ml of 0.05*M* sodium tetraborate, 430 ml of 0.2*N* sodium hydroxide, and 49.66 g of sodium chloride, without pH adjustment. For sweet sorghum distillers' grains, 5 g of sample and 100 ml of solvent were used. Each supernatant and the final residue were analyzed for nitrogen.

Reverse Osmosis

An OSMO Econo Pure reverse osmosis (RO) unit (Osmonics, Inc., Minnetonka, MN) equipped with OSMO-112 Sepralator (1.0 m^2 membrane, hold-up volume about 600 ml) was used for ultrafiltration (UF) at 680 kPa (100 lb/in.^{2}). For UF, a SEPA-0 cellulose acetate (CA) membrane and a polysulfone (PS) membrane with a molecular weight cutoff of 1,000 for organics were used. Solutions retained by the membrane were called concentrates, and those that passed through membranes were termed permeates. Concentrate streams were recirculated into the initial solution. Samples of permeate and of concentrate plus initial solution (subsequently called concentrate) were removed for

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analyses. Flow rates were 15 L/hr for CA-UF and 13 L/hr for PS-UF.

A model UHPROLA-100 RO system (Village Marine Tec, Gardena, CA) having a SW30-2521 module with 1.1 m^2 polyamide membrane (Filmtec Corp., Minneapolis, MN) was used for RO at 5,440 kPa (800 lb/$in.^2$) and 6,800 kPa (1,000 lb/$in.^2$) at 25° C. The hold-up volume of the membrane module is 605 ml. The flow rate of permeate was 21 L/hr at 5,440 kPa and 31 L/hr at 6,800 kPa. The UF permeate was used as the feed solution for RO.

Analyses

Protein, fat, crude fiber, and ash contents were determined by AACC approved methods (1983), and protein was calculated from Kjeldahl N × 6.25 (Watt and Merrill 1963). Moisture was determined by heating samples at 100° C to constant weight, and starch was determined by a polarimetric method (Garcia and Wolf 1972). Dietary fiber, the sum of cellulose, lignin, and water-insoluble hemicellulose, was determined by the neutral detergent method (McQueen and Nicholson 1979). Glucose, glycerol, and ethanol were analyzed by high-performance liquid chromatography on a Bio-Rad HPX87H (300 × 7.8 mm) column (Richmond, CA) with 0.01*N* sulfuric acid eluant at 45° C.

Samples containing 1 mg of nitrogen for amino acid analyses were hydrolyzed for 24 hr by refluxing in 6*N* hydrochloric acid. Hydrolyzed samples were evaporated to dryness in a rotary evaporator. Residues were dissolved in pH 2.2 citrate buffer and analyzed with a Dionex D300 amino acid analyzer (Dionex Corp., Sunnyvale, CA). Data were evaluated by computer (Cavins and Friedman 1968).

RESULTS AND DISCUSSION

Yield and Composition of Sweet Sorghum Grain Fermentation Products

To determine the feasibility of fermenting sweet sorghum grain to make ethanol, we examined fermentation products by various methods. Ethanol, glycerol, and glucose concentrations were 8.2, 0.60, and 0.05% by weight, respectively, after 66 hr of fermentation. The ethanol yield averaged 89% of theoretical, based on the sum of starch and sugar contents of sweet sorghum grain. This value was close to those achieved for corn (88%), sorghum (86%), and barley (90%) (Wall et al 1983, Wu and Sexson 1984, Wu 1986).

Table I lists yields and compositions of fermentation products from sweet sorghum grain. Fermentation residue accounted for 36% of sweet sorghum grain compared with 32% of sorghum grain (Wu and Sexson 1984). Distillers' grains were the largest fraction (73%) of fermentation residue. Both distillers' grains and centrifuged solids had higher protein, fat, crude fiber, and neutral detergent fiber than the original sweet sorghum grain. Stillage solubles had the highest ash content of all fractions; this ash value included salt formed during pH adjustments before fermentation. In general, the composition of sweet sorghum grain was not much different from that of grain sorghum. The sugar content of mature sorghum grains ranges from 0.9 to 2.0% in normal varieties (Edwards and Curtis 1943), compared with 1.6% for Wray sweet sorghum grain.

TABLE I
Yield and Composition of Fermentation Products from Sweet Sorghum Grain (dry basis)[a]

Products	% of Residue	Protein (%)	Fat (%)	Crude Fiber (%)	NDF[b] (%)	Ash (%)	Starch (%)
Sweet sorghum grain	...	13.6	4.8	2.8	12.5	1.7	69.6
Distillers' grains	73	41.6	12.7	8.0	40.6	1.5	5.2
Centrifuged solids	8	46.4	6.6	8.7	34.5	2.1	7.0
Stillage solubles	19	14.2	nd	nd	nd	19.1	nd

[a] Residue accounted for 36% of sweet sorghum grain; nd = not determined; protein = N × 6.25. The sweet sorghum grain also contained 1.4% sucrose and 0.2% glucose.
[b] NDF = Neutral detergent fiber.

Nitrogen Distribution and Content of Sweet Sorghum Grain Stillage Solubles

Sweet sorghum grain stillage solubles were fractionated by two ultrafiltration membranes, according to molecular weight. Nitrogen distributions and contents of permeates and concentrates are shown in Table II. Permeate accounted for 44% of the nitrogen with the UM05 membrane, which has a nominal molecular weight cutoff of 500. This permeate fraction had a much higher nitrogen content than the concentrate, indicating that the permeate was relatively rich in amino acids and small peptides. Permeate from the PM10 membrane accounted for 72% of the nitrogen of stillage solubles, indicating that most nitrogenous compounds in stillage solubles were amino acids and peptides. In comparison, 29% of the nitrogen from grain sorghum stillage solubles and 48% of the

TABLE II
Nitrogen Distribution and Content of Sweet Sorghum Grain Stillage Solubles

Membrane	Approximate Molecular Weight	Fraction	% of Total N	N Content (% dry basis)
UM05	<500	Permeate	44	8.65
	>500	Concentrate	56	1.50
PM10	<10,000	Permeate	72	2.21
	>10,000	Concentrate	28	2.65

TABLE III
Protein Distribution of Sweet Sorghum Grain and its Distllers' Grains

Fraction	% of Total N[a]	
	Sweet Sorghum Grain	Sweet Sorghum Distillers' Grains
Water extract	10	2
1% NaCl extract	2	1
60% *tert*-Butanol extract	28	2
60% *tert*-Butanol + DTT extract[b]	26	0
Borate + SDS + DTT extract, pH 10.6[b]	10	8
Residue	18	79

[a] Total N recovered did not add up to 100% due to loss of materials upon transfer.
[b] DTT = dithiothreitol; SDS = sodium dodecyl sulfate.

TABLE IV
Amino Acid Composition[a] of Sweet Sorghum Grain and Its Fermentation Products

Amino Acid	Sweet Sorghum Grain	Distillers' Grains	Centrifuged Solids	Stillage Solubles
Alanine	9.6	11.3	8.7	7.2
Arginine	4.2	3.8	5.6	7.5
Aspartic acid	6.8	7.2	9.4	9.4
1/2 Cystine	1.2	1.4	1.3	2.0
Glutamic acid	22.0	26.3	19.3	14.2
Glycine	2.8	2.9	4.0	7.6
Histidine	2.4	2.3	2.6	3.7
Isoleucine	3.8	4.5	5.0	2.9
Leucine	14.8	17.6	13.2	5.4
Lysine	2.2	1.9	5.4	7.1
Methionine	2.1	1.8	2.5	1.3
Phenylalanine	5.2	6.3	5.7	2.9
Proline	7.8	9.0	7.1	9.7
Serine	4.3	5.0	5.4	5.4
Threonine	3.1	3.6	4.6	4.6
Tyrosine	4.7	5.3	5.1	3.0
Valine	5.0	5.5	6.5	5.0

[a] Grams of amino acid per 16 g of nitrogen recovered. Tryptophan not determined. Aspartic acid includes both aspartic acid and asparagine, and glutamic acid includes both glutamic acid and glutamine.

TABLE V
Ultrafiltration and Reverse Osmosis of Sweet Sorghum Grain Stillage Solubles

Material[a]	Volume (ml)	Nitrogen (mg/ml)	Solids (mg/ml)	Ash (mg/ml)
Stillage solubles	4,900	0.616	27.5	5.06
Permeate (UF, PS)	4,574	0.287	16.2	3.88
Concentrate (UF, PS)	223	1.56	52.1	5.37
Permeate (RO, 6,800 kPa)	3,467	0.0010	0.0348	0.0034
range, 10 fractions	340–360	0.0007–0.0013	0.021–0.071	0.0004–0.0093
Concentrate (RO, 6,800 kPa)	947	0.347	19.5	4.76
range, 10 fractions	70–100	0.229–0.578	12.4–33.8	3.18–7.82
Stillage Solubles	5,900	0.572	25.0	4.80
Permeate (UF, CA)	5,760	0.210	11.4	3.50
Concentrate (UF, CA)	208	2.10	67.5	7.43
Permeate (RO, 5,440 kPa)	4,417	0.00033	0.0285	0.004
range, 10 fractions	285–470	0.00022–0.00057	0.0116–0.059	0–0.0065
Concentrate (RO, 5440 kPa)	990	0.302	16.3	5.03
range, 10 fractions	90–100	0.17–0.567	9.93–30.7	2.99–9.57

[a] UF = Ultrafiltration; RO = reverse osmosis; PS = polysulfone; CA = cellulose acetate; 6,800 kPa = 1,000 lb/in.^{2}; 5,440 kPa = 800 lb/in.^{2}.

nitrogen from corn stillage solubles was present in compounds having molecular weights less than 500, and 86% of the nitrogen from grain sorghum stillage solubles and 100% of the nitrogen from corn stillage solubles was present in compounds having molecular weights less than 10,000 (Wu et al 1981, Wu and Sexson 1984).

Protein Fractions of Sweet Sorghum Grain and Its Distillers' Grains

Water, 1% sodium chloride, 60% *tert*-butanol, 60% *tert*-butanol + DTT, and borate + SDS + DTT were used to extract albumins, globulins, prolamins, cross-linked prolamins, and glutelins, respectively. Prolamins and cross-linked prolamins are the two largest protein fractions in sweet sorghum grain (Table III). Sweet sorghum distillers' grains had little prolamin and no cross-linked prolamin, and only 13% of the total nitrogen was extracted by this series of solvents. The low protein solubility of sweet sorghum distillers' grains suggests that protein was denatured or aggregated or both during fermentation and by heating.

Amino Acid Composition of Sweet Sorghum Grain and Its Fermentation Products

Table IV shows that glutamic acid (or glutamine) and leucine are the two most abundant amino acids in sweet sorghum grain. The amino acid composition of sweet sorghum distillers' grains is, in general, close to that of sweet sorghum grain because 73% of the protein from sweet sorghum grain is accounted for by the distillers' grains. The centrifuged solids and stillage solubles have much higher lysine contents than sweet sorghum grain. In general, amino acid compositions of sweet sorghum grain and distillers' grains are similar to those of grain sorghum and its distillers' grains (Wu and Sexson 1984).

Ultrafiltration and Reverse Osmosis of Sweet Sorghum Grain Stillage Solubles

UF was used to remove large molecules that may foul RO membranes. The permeate from UF with the PS membrane accounted for 93% of the volume, 43% of the nitrogen, 55% of the solids, and 72% of the ash of stillage solubles (Table V). Permeate from UF with the CA membrane accounted for 98% of the volume, 36% of the nitrogen, 44% of the solids, and 71% of the ash of stillage solubles. The CA membrane exhibited a faster flow rate than the PS membrane, and the CA permeate had smaller amounts of nitrogen and solids. The UF permeate was used as feed solution for RO. For RO at 5,440 kPa (800 lb/in.^{2}), the RO permeate accounted for 78% of the volume, 0.12% of nitrogen, 0.20% of solids, and 0.09% of the ash of the UF permeate. For RO at 6,800 kPa (1,000 lb/in.^{2}), the RO permeate accounted for 78% of volume, 0.27% of nitrogen, 0.17% of the solids, and 0.07% of the ash of the UF permeate. In comparison, the RO permeate obtained at 1,360 kPa (200 lb/in.^{2}) accounted for 92% of the volume, 9% of the nitrogen, and 16% of the solids of UF permeate from sorghum stillage solubles (Wu and Sexson 1984). High-pressure RO at 5,440 or 6,800 kPa is therefore very efficient for processing large volumes of sweet sorghum grain stillage solubles into small volumes of concentrate and large volumes of permeates suitable for reuse or disposal.

CONCLUSIONS

Parrish et al (1985) showed that sweet sorghum, when harvested for grain also, is superior to corn in productivity of fermentable substrates. We have now demonstrated that sweet sorghum grain can be fermented to ethanol with good yield, and that stillage solubles can be efficiently processed by ultrafiltration combined with high-pressure reverse osmosis. The grain of sweet sorghum contributes significantly to the total alcohol potential of the sweet sorghum plant, making possible successful commercial production of alcohol from both stalk and grain.

The high protein contents of sweet sorghum distillers' grains and centrifuged solids indicate potential of these fractions in foods. Protein solubility of sweet sorghum distillers' grains is even lower than that of grain sorghum and corn distillers' grains (Wu and Sexson 1984, Wu et al 1981). Proteins from corn distillers' grains and sorghum distillers' grains were used more efficiently by calves and lambs than was soybean meal protein; more protein from distillers' grains escaped degradation by rumen microorganisms, and was digested and absorbed from the lower gastrointestinal tract, resulting in more growth (Klopfenstein et al 1978). Sweet sorghum distillers' grains may likewise be utilized more efficiently by calves and lambs than soybean meal protein.

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