Fractionation and Characterization of Protein-Rich Material From Barley After Alcohol Distillation

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ABSTRACT

Because of current industrial interests and plans to use barley grain for alcohol production, methods for efficient recovery and utilization of stillage were investigated. Ground barley was fermented to ethanol, the ethanol was distilled, and the residual stillage was separated into distillers' grains, centrifuged solids, and stillage solubles. Distillers' grains and centrifuged solids had protein contents (nitrogen × 5.67, dry basis) of 33 and 61%, respectively, and accounted for 71 and 6% of total barley nitrogen. The protein in distillers' grains was much less soluble than that in barley. Lysine, expressed as grams per 16 g of nitrogen, was 3.9–4.4 for barley and its fractions after fermentation. Eighty-eight percent of the nitrogen in stillage solubles passed through a 10,000 molecular weight cut-off membrane. Permeate from stillage solubles processed by ultrafiltration and reverse osmosis had much lower nitrogen and solids contents than those of stillage solubles; high pressure reverse osmosis was especially effective for reducing nitrogen and solids contents. Thus, ultrafiltration and reverse osmosis can be used to concentrate barley stillage solubles for potential feed utilization while providing a permeate which can be reused for water or safely discarded.

Fermentation of ground cereal grain and subsequent distillation of ethanol leave a protein-rich residue (stillage). Fermentation primarily utilizes starch, whereas other nutrients, such as protein and fat, are concentrated. Corn is most commonly used for commercial ethanol fermentation in the United States, but barley is also beginning to be used for this purpose (Morris 1983). Barley is a major carbohydrate crop in some regions and can be an alternative feedstock for ethanol. Fractionation and characterization of corn stillage (Wu et al 1981, Sweeten et al 1983), corn distillers' dried grains with and without solubles (Wu and Stringfellow 1982), sorghum stillage (Sweeten et al 1983, Wu and Sexson 1984), and wheat stillage (Wu et al 1984) were reported previously. A number of studies on distillers' grains materials leading to food products have been reported. Protein concentrate was made from fermented corn and fermented wheat by alkali extraction (Satterlee et al 1976). Distillers' dried grains flours were used in bread and cookies (Tsen et al 1982, 1983). Brewer's spent grains were incorporated into bread (Finley and Hanamoto 1980), as well as muffins and cookies (Prentice 1978, Prentice et al 1978). Barley distillers' dried grain flour was added to quick breads to build up their fiber and protein content (Eidet and Newman 1984).

This paper reports the separation of barley stillage into various fractions, the use of ultrafiltration and reverse osmosis to process the stillage solubles, and the characterization of these fractions by proximate analysis, molecular size, solubility in various solvents, and amino acid analysis to provide basic information useful in optimizing efficient recovery, optimal utilization, or inexpensive processing of these fractions.

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MATERIALS AND METHODS

Fermentation

Lewis barley, a spring cultivar from Montana, was used in this study. The grain was ground by an Allis roller mill, and material staying on 20-mesh screen was further ground in an Alpine 160 Z pin mill at 9,000 rpm. Ground barley contained 40% each of 12-20 and 20-40 mesh and 20% of through-40 mesh materials. Ground barley (2,332 g, dry basis) was dispersed in 5 L of tap water in a 20-L stainless-steel, temperature-controlled, jacketed fermentor equipped with stirrers. The pH of the slurry was adjusted to 6.2, and 6 ml of Miles Taka-Therm α-amylase (Miles Laboratories, Inc., Elkhart, IN) was added to hydrolyze starch to soluble dextrins. The temperature of the slurry was maintained at 90° C for 1 hr and then 1,337 ml of tap water was added. Miles Diazyme L-100 glucoamylase (18 ml) was added at pH 4.0, 60° C, to convert dextrins to glucose. After 2 hr, the mixture was inoculated with 500 ml of yeast (*Saccharomyces cerevisiae*) at pH 4.5, 30° C. The fermentation was stopped after 66 hr. Two fermentation runs were made. The results of both runs were analyzed, and the average values were used. Additional details of an analogous fermentation procedure were described previously (Wu and Sexson 1984). The yeast and enzymes added together accounted for 2.5% total nitrogen, and no significant effect on the experimental nitrogen determination is likely.

Fractionation of Stillage

After the alcohol was distilled from the fermentor by circulating steam through the outer jacket, the residue (stillage) was filtered through cheesecloth. The materials that remained on the cheesecloth were labeled as "distiller's grains." The thin stillage that passed through the cheesecloth was centrifuged to give "centrifuged solids" and "stillage solubles" (Wu and Sexson 1984).

Fractionation of Stillage Solubles

An Amicon model 52 ultrafiltration cell with 43-mm diameter membranes under 50 lb/in.^{2} (340 kPa) of nitrogen pressure was used. The nominal molecular weight cutoffs (MWCO) for UM05 and PM10 membranes were 500 and 10,000, respectively (Amicon 1972). Fifteen milliliters of stillage solubles were introduced above each membrane, and 52–55 ml of permeate (solution below the membrane) was collected by adding distilled water above the membrane (Wu and Sexson 1984).

Protein Extraction

Ground barley (10 g) was put in a stainless-steel cup with 100 ml of solvent and blended for 5 min in a Waring Blendor. The slurry was then centrifuged at 10,400 × *g* for 10 min, the supernatant decanted, and the residue extracted with the next solvent. Solvents used sequentially were water, 0.5*M* sodium chloride, 70% ethanol, 70% ethanol + 0.1% dithiothreitol (DTT), and borate + 0.1% DTT + 0.5% sodium dodecyl sulfate (SDS) at pH 10.4. 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 any other pH adjustment. For barley distillers' grains, 4 g of sample and 100 ml of solvent were used. Each supernatant and the final residue were analyzed for nitrogen, and the amount and percent of nitrogen were calculated for each fraction.

Reverse Osmosis

An OSMO Econo Pure reverse-osmosis (RO) unit (Osmonics, Inc., Minnetonka, MN) equipped with OSMO-112 Sepralators (1.0 m^2 membrane, hold-up volume about 600 ml) was used for RO at 200 lb/in.^{2} (1,360 kPa) and ultrafiltration (UF) at 100 lb/in.^{2} (680 kPa). For RO, a SEPA-97 membrane with a MWCO of 200 for organics was used. For UF, a SEPA-0 cellulose acetate membrane with a MWCO of 1,000 for organics was used. The solution that passed through the membrane was termed permeate, and the solution retained by the membrane is called concentrate. The concentrate stream was recirculated back to the initial solution. Samples of permeate and concentrate plus initial solution (termed concentrate subsequently) were taken for analyses. The flow rate of permeate was 15 L/hr for UF and 3.6 L/hr for RO. More details on RO and UF were reported previously by Wu et al (1983).

A model UHPROLA-100 RO system (Village Marine Tec, Gardena, CA) equipped with a SW30-2521 module with 1.1 m^2 polyamide membrane (Filmtec Corp., Minneapolis, MN) was used for RO at 800 lb/in.^{2} (5,440 kPa) at 23° C. The flow rate of permeate was 21 L/hr. The hold-up volume of the membrane module is 605 ml.

Analyses

Protein, fat, fiber, and ash contents were determined by AACC approved methods (1976), and protein was calculated from Kjeldahl N × 5.67. Moisture was determined by heating samples at 100° C to constant weight, and starch was determined by a polarimetric method (Garcia and Wolf 1972). Analyses for glucose, glycerol, and ethanol were made by a Waters ALC 200 high-performance liquid chromatograph equipped with refractive index detector (Waters Associates, Milford, MA) on a Bio-Rad HPX-42C (300 × 7.8 mm, o.d.) column (Richmond, CA) with water eluant. Nitrogen determinations were made in quadruplicate, and solids, ash, and moisture contents were determined in duplicate.

Amino acid analyses were carried out, in duplicate or triplicate, using a Glenco MM-100 amino acid analyzer (Glenco Scientific Inc., Houston, TX) or a Dionex D 300 amino acid analyzer (Dionex Corp., Sunnyvale, CA). Each sample was hydrolyzed for 24 hr by refluxing in 6*N* hydrochloric acid. The hydrolyzed sample was evaporated to dryness in a rotoevaporator, and the residue was dissolved in pH 2.2 citrate buffer. The data were computed automatically by the method of Cavins and Friedman (1968). The sulfur amino acids were determined after oxidation of the sample with performic acid as described by Moore (1963).

RESULTS AND DISCUSSION

Yield and Composition of Barley Fermentation Products

To determine the feasibility of fermenting barley to make ethanol, we examined the fermentation products by various methods. The average concentrations of ethanol, glycerol, and glucose were 7.9, 0.6, and 0% by weight, respectively, after 48 hr of fermentation. The attained ethanol yield was 90% of theoretical yield and close to that achieved for corn under identical experimental conditions (Wall et al 1983).

Table I lists the yield and composition, on a dry basis, of fermentation products from barley. Fermentation residue accounted for 41% of the barley grain compared with 30% of the corn grain. This higher percentage of residue was a result of the lower starch percentage in barley compared with corn (Wall et al 1983) and indicates that optimal utilization and processing of this residue is even more important than for corn. Distillers' grains were the largest fraction (72%) of fermentation residue. Both distillers' grains and centrifuged solids had higher protein, fat, and fiber than the original barley. Stillage solubles had the highest ash content of all fractions; in part, these ash values include salt formed during pH adjustments before fermentation. Newman and Gras (1983) reported that two barley distillers' dried grains obtained from a small ethanol distillery had 90% dry matter and averaged 27.5% protein, 3.3% ether extract, 4.0% ash, and 16.8% crude fiber. The

TABLE I
Yield and Composition of Fermentation Products from Barley (Dry Basis)[a]

Products	% of Residue	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Starch (%)
Barley		13.4	2.2	3.9	2.6	62.0
Distillers' grains	72	32.6	6.0	16.6	4.4	0
Centrifuged solids	3	60.5	4.3	5.9	2.5	1.3
Stillage solubles	25	16.7	nd[b]	nd	13.1	nd

[a] Residue accounted for 41% of the barley grain.
[b] nd = not determined.

National Research Council (1956) reported barley distillers' grains had 27.7% protein, 11.6% ether extract, 10.1% crude fiber, and 1.8% ash on a dry basis. A direct comparison of these values with those in Table I is difficult, because no fractionation procedures are given; in general, however, compositions agree closely with those of our materials (Table I).

Nitrogen Distribution and Content of Barley Stillage Solubles Fractions

Barley stillage solubles were fractionated by ultrafiltration, using two membranes, according to molecular weight. The nitrogen distributions and contents of the permeates and concentrates are shown in Table II. Permeate accounted for 31% of the nitrogen with the UM05 membrane, which has a nominal MWCO of 500. Concentrate (larger molecular weight material) had about twice the nitrogen content of the permeate. With the PM10 membrane (MWCO of 10,000), only 12% of the nitrogen was in the concentrate. This small percentage, compared with the relatively large (69%) amount of nitrogen in the UM05 concentrate, indicates that most of the nitrogenous materials in stillage solubles are peptides. The PM10 concentrate also had a higher nitrogen content than the permeate, thus indicating that the larger molecules have higher nitrogen. For comparison, 48% of the nitrogen from corn stillage solubles was in forms having molecular weights of 500 or less, and all nitrogenous materials had molecular weights of 10,000 or less (Wu et al 1981).

TABLE II
Nitrogen Distribution and Content of Barley Stillage Solubles Fractions

Membrane	Approximate Molecular Weight	Fraction	% of Total N	N Content (% db)
UM05	<500	Permeate	31	1.99
	>500	Concentrate	69	3.89
PM10	<10,000	Permeate	88	3.06
	>10,000	Concentrate	12	4.81

TABLE III
Protein Fractions of Barley and Barley Distillers' Grains

Fraction[a]	% of Total N[b]	
	Barley	Barley Distillers' Grains
Water extract	9	4
0.5*M* NaCl extract	9	3
70% ethanol extract	30	9
70% ethanol + DTT extract	3	2
Borate + SDS + DTT extract, pH 10.4	21	26
Residue	24	45

[a]DTT = dithiothreitol, SDS = sodium dodecyl sulfate.
[b]Total N recovered did not add up to 100% owing to loss of materials from a number of transfers.

TABLE IV
Amino Acid Composition[a] of Barley and Its Fermentation Products

Amino Acid	Barley	Distillers' Grains	Centrifuged Solids	Stillage Solubles
Aspartic acid	5.6	6.0	5.7	6.7
Threonine	3.3	3.5	3.4	4.8
Serine	4.2	4.2	4.1	5.5
Glutamic	29.1	30.7	35.3	28.9
Proline	9.6	11.1	11.4	12.4
Glycine	3.8	3.7	3.2	7.5
Alanine	4.0	4.3	4.0	4.5
Valine	5.0	5.3	5.1	4.5
Cystine	2.3	1.3	1.3	1.9
Methionine	1.7	1.8	2.1	1.0
Isoleucine	3.4	4.0	4.4	3.0
Leucine	6.6	7.7	8.2	5.6
Tyrosine	3.3	3.5	3.9	3.7
Phenylalanine	5.1	6.1	6.4	3.3
Lysine	4.0	3.9	4.0	4.4
Histidine	2.4	2.3	2.3	2.9
Arginine	5.7	5.5	5.2	4.8

[a]Grams of amino acid per 16 g nitrogen recovered. Tryptophan not determined.

Protein Fractions of Barley and Barley Distillers' Grains

Sequential extraction with a series of solvents was used to fractionate proteins from barley and barley distillers' grains (Table III). Water, 0.5*M* sodium chloride, 70% ethanol, and borate + SDS + DTT extracted albumins, globulins, prolamins, and glutelins, respectively. Prolamin (hordein) was the largest fraction in barley, whereas cross-linked prolamin or ethanol-soluble reduced glutelin extracted by 70% ethanol + DTT was the smallest fraction. Barley distillers' grains had much lower prolamin than barley, and only about half the total nitrogen was extracted by this series of solvents. The low protein solubility of barley distillers' grains indicated that the protein was denatured during fermentation and by heating.

Amino Acid Composition

Amino acid compositions of barley and its fermentation products are listed in Table IV. Lysine, the first limiting amino acid for cereal grains, is relatively high in barley and its fermentation products (3.9–4.4 g/16 g nitrogen) compared with 2.5–3.4 for corresponding products from corn (Wu et al 1981). Glutamic acid is the most abundant amino acid in all fractions. The amino acid composition of distillers' grains is very similar to that of barley, and these are not much different than the composition of centrifuged solids. Stillage solubles had higher aspartic acid, threonine, serine, proline, glycine, and histidine but lower methionine, leucine, phenylalanine, and arginine than barley. The nutritional value of barley and its fermentation products appears better than the corresponding products from corn, wheat, and sorghum (Wu et al 1981, 1984; Wu and Sexson 1984).

Ultrafiltration and Reverse Osmosis of Barley Stillage Solubles

Table V summarizes the results of UF and RO on barley stillage solubles; UF values are averaged from two experiments. Permeate from UF contained 34% of the nitrogen, 45% of the solids, and 68% of the ash of barley stillage solubles. Concentrate from UF contained 16% of the nitrogen, 12% of the solids, and 7% of the ash of barley stillage solubles. The remaining nitrogen, solids, and ash

TABLE V
Ultrafiltration and Reverse Osmosis of Barley Stillage Solubles

Substance[a]	Volume (ml)	Nitrogen (mg/ml)	Solids (mg/ml)	Ash (% db)
Stillage solubles	4,765	1.52	53.5	13.3
Permeate (UF)	4,648	0.52	24.8	20.0
Concentrate (UF)	256	4.50	118.6	8.2
Permeate (RO, 200 psi)	4,011	0.052	2.09	41.5
range, 10 fractions	395–416	0.014–0.137	0.22–7.14	
Concentrate (RO, 200 psi)	1,255	0.923	42.2	19.4
range, 10 fractions	100–355	0.437–1.14	20.1–72.7	18.4–23.2
Permeate (RO, 800 psi)	3,527	0.0015	0.042	28
range, 10 fractions	312–383	0.00027–0.0025	0.020–0.076	
Concentrate (RO, 800 psi)	450	0.669	32.1	19.3
range, 9 fractions	50	0.435–0.941	21.5–44.8	18.4–20.9

[a]In addition to the permeate and concentrate, hold-up volume in the machine and water loss from evaporation during processing also contributed to the initial volume. The permeate from ultrafiltration (UF) was used as the feed solution for reverse osmosis (RO).

of the stillage solubles were in the hold-up and wash fractions (not shown in Table V). The percent recovery of nitrogen, solids, and ash for each UF and RO run was 99, 94, and 96 or higher, respectively.

The permeate from UF was then used as the feed solution for RO at 200 and 800 psi. The nitrogen and solids contents of permeate and concentrate initially increased slowly as volume of concentrate decreased; however, near the end of RO at 200 psi, rapid increases of nitrogen and solids contents were observed. The total RO permeate at 200 psi had 2.4% nitrogen, 2.5% solids, and 6.1% ash of the UF permeate. However, the RO permeate fractions at 800 psi had 0.23% nitrogen, 0.15% solids, and 0.20% ash of the UF permeate; thus nitrogen, solids, and ash contents of RO permeates decreased by 10–30 times when the pressure increased four times from 200 to 800 psi. An increase in efficiencies of this magnitude has not been reported previously. Clearly, the higher pressure is recommended for RO. UF and RO of barley stillage solubles are more efficient than stillage solubles from dry-milled corn fractions at the same pressures (Wu and Sexson 1985).

CONCLUSION

Corn is by far the major cereal grain used for ethanol production (Morris 1983). Three small ethanol plants used barley as feedstock, but their combined ethanol capacity was less than 1% of the total. Optimum use of the fermentation residue, after ethanol is distilled, plays an important role in the commercial success of the overall ethanol process. Because a higher percentage of the grain became fermentation residue for barley than for corn, the economics of the barley process depend even more on having suitable processes for recovering valuable by-products at a minimal cost. The nutritional value of barley, based on amino acid composition, is superior to that of corn. Also the fermentation products of barley, based on amino acid composition, appear better than the corresponding products from corn, wheat, and sorghum. The U.S. Department of Agriculture (1983) reported the amount of barley grown in this country increased from 1980 to 1982. The total U.S. barley production in 1982 was 522 million bushels, with North Dakota the leading producer followed by Montana and Idaho.

Barley distillers' grains and centrifuged solids may have potential as a food source, because they have high protein contents and an amino acid balance equivalent to that of barley grain. The relatively low molecular weight of barley stillage solubles indicates that proteolysis of some proteins occurs during production of ethanol from barley. Ultrafiltration and reverse osmosis are capable of concentrating barley stillage solubles for potential feed use, while providing a permeate which can be reused or safely disposed of. High-pressure reverse osmosis at 800 psi is remarkably effective, because it produces a permeate from stillage solubles having very low nitrogen, solids, and ash contents.

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