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Recovery of Protein-Rich Byproducts from Oat Stillage after Alcohol Distillation

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Ground oats, ground groats, and oat flour were fermented to ethanol. After ethanol was distilled, residual stillage was separated by screening and centrifugation into distillers' grains, centrifuged solids, and stillage solubles. Oat distillers' grains and centrifuged solids had crude protein contents (nitrogen × 5.83, dry basis) of 19 and 44%, respectively, and contained 67 and 5% of the total nitrogen of oats. Oat flour distillers' grains and centrifuged solids had 43 and 48% protein, respectively, and accounted for 13 and 58% of the total nitrogen of oat flour. Of the nitrogen in oat stillage solubles, 54% passed through a 10K molecular weight cut-off membrane. Permeate from oat stillage solubles processed by combined ultrafiltration and reverse osmosis had much lower nitrogen, solids, and ash contents than did stillage solubles. This practical method to ferment ground oats and oat flour for ethanol and to recover valuable protein-rich byproducts may have commercial potential.

Corn is the most common biomass for commercial ethanol production in the United States (Morris, 1983). After ethanol is distilled, a protein-rich residue (stillage) that contains 5–10% solids remains. This stillage is screened or centrifuged to yield an insoluble solid, corn distillers' dried grains. The remaining soluble fraction with 2–4% solids content is usually evaporated to a syrup and dried with the solids from screening or centrifugation. Drying the soluble fraction requires considerable energy, but discarding this fraction would result in serious pollution.

Ultrafiltration is an efficient process for selectively separating solutions by convective solvent flow through a membrane at moderate pressure. Solutes or particles larger than the specified membrane "cut-off" are quantitatively retained, but solutes smaller than the membrane pores pass through with the solvent. Reverse osmosis separates water from a solution by a membrane that is more permeable to water than to ions and other dissolved matter (Gregor and Gregor, 1978). The solution is pumped at high pressure across the membrane to overcome the osmotic pressure that resists the flow of water. Since ultrafiltration and reverse osmosis involve no evaporation of water, energy consumption is much lower than in concentration by heating.

Fermentation of cereal grains and sugar crops for ethanol and use of reverse osmosis and ultrafiltration to process stillage solubles have been described (Wu, 1986, 1987; Wu and Sexson, 1984; Wu et al., 1981, 1984; Wu and Bagby, 1987). Fermentation of oats for ethanol and characterization of the protein-rich oat fermentation residue have not been described, however. This paper reports fermentation of ground whole oats, ground oat groats, and oat flour to ethanol, fractionation and characterization of the stillage, and use of reverse osmosis (RO) and ultrafiltra-

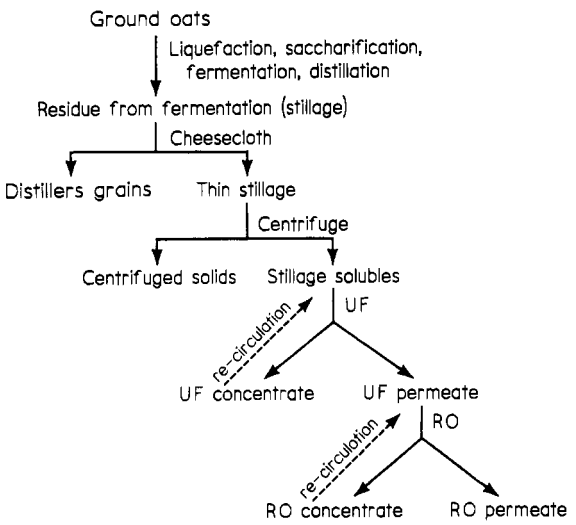

Figure 1. Schematic diagram of oat fermentation, fractionation of stillage, and UF and RO processing.

tion (UF) to process oat stillage solubles.

MATERIALS AND METHODS

Materials. Ogle oats came from Illinois Foundation Seed, Champaign, IL. Whole oat groats (dehulled oats) and oat flour of unknown cultivar were from the Quaker Oats Co., Barrington, IL. Ogle oats and whole groats were ground in an Alpine Model 160Z pin mill at 9000 rpm twice and once, respectively. A Ro-Tap testing sieve shaker (W. S. Tyler Co., Cleveland, OH) equipped with 25-, 35-, 45-, 60-, and 100-mesh screens (707-, 500-, 354-, 250-, and 149-μm openings, respectively) in series gave particle size distribution of oat products. Average particle sizes for ground oats, ground groats, and oat flour were 304, 361, and 174 μm, respectively, based on weight and average particle size of each screened fraction.

Fermentation. Ground oats (3172 g dry basis) were put in a 20-L stainless steel, temperature-controlled, jacketed fermentor equipped with stirrers. Five liters of water was added, and the pH was adjusted to 6.2. Six milliliters of Taka-therm α-amylase (Miles Laboratories, Elkhart, IN) was added, and the mixture was maintained at 90 °C for 1 h with stirring. The slurry was cooled to 60 °C by adding 1.21 L water and by activating the cooling jacket. The pH of the mixture was then adjusted to 4.0, and 18 mL of Diazyme L-100 glucoamylase (Miles Laboratories) was added. The slurry was kept at 60 °C for 2 h with agitation and then cooled to 30 °C. The mixture was adjusted to pH 4.5, and 500 mL of yeast (*Saccharomyces cerevisiae*) containing 500 million cells/mL was added. Samples were withdrawn at 0, 24, 48, and 66 h when fermentation was stopped. Figure 1 is a schematic diagram of oat fermentation, fractionation of stillage, and UF and RO recovery.

Ground oat groats and oat flour (2531 g dry basis each) were also fermented. The procedure was identical with that for ground oats.

Fractionation of Stillage and Molecular Weight of Stillage Solubles. Wu (1987) described these procedures in detail.

UF and RO. UF with cellulose acetate membrane at 100 lb/in.^{2} and RO with polyamide membrane at 1000 lb/in.^{2} (6800 kPa) were described earlier (Wu, 1987). Flow rates were 8.6 L/m^2 per h for UF permeate and 19 L/m^2 per h for RO permeate. All nitrogen, solids, and ash were recovered from UF and RO permeate, concentrate, and hold-up fractions.

Analyses. Nitrogen, fat, crude fiber, and ash contents were determined by AACC approved methods (1983). Crude protein value is from Kjeldahl N × 5.83 (Jones, 1931). Moisture value is from heating samples at 100 °C to constant weight. Starch content comes from a polarimetric method (Garcia and Wolf, 1972) and from a glucoamylase (Dintzis and Harris, 1981) method with gas–liquid chromatography of alditol acetates (Blakeney et al., 1983). Neutral detergent fiber content (the sum of cellulose, lignin, and water-insoluble hemicellulose) is from the method of McQueen and Nicholson (1979). Total dietary fiber was determined by the method of Prosky et al. (1988). High-performance liquid chromatography yielded glucose, ethanol, and glycerol contents (Wu and Bagby, 1987).

Amino acid analyses are from a Beckman Model 334 gradient liquid chromatograph system (Beckman Instruments, San Ramon, CA). A rotary evaporator was used to dry samples hydrolyzed in refluxing 6 N hydrochloric acid (109 °C) after 24 h. Citrate buffer (pH 2.2) dissolved the residues. Sulfur amino acid values were from performic acid oxidation of the sample (Moore, 1963). Amino acid data were calculated by the method of Cavins and Friedman (1968).

Conductivity values of stillage fractions are from a Radiometer CDM 2e conductivity meter with a CDC 104 NS cell at 27 °C.

Table I. Effect of Fermentation Time on Oat Slurry Composition

		g/100 mL		
substrate	h	glucose	ethanol	glycerol
ground oats	0	15.3	0	0
	24	1.8	6.2	0.50
	48	0.03	7.0	0.51
	66	0	7.2	0.21
ground groats	0	16.8	0	0
	24	0.38	8.0	0.62
	48	0.22	8.7	0.66
	66	0.18	8.6	0.65
oat flour	0	20.7	0	0
	24	0.15	8.0	0.67
	48	0.11	7.7	0.64
	66	0.06	7.1	0.64

RESULTS AND DISCUSSION

Yield and Composition of Oat Fermentation Products. Table I shows the effect of fermentation time on oat slurry composition. Fermentation volumes were 11.4, 8.8, and 10.4 L, respectively, for ground oats, ground groats, and oat flour. At zero time only glucose was present. The glucose value at zero time for oat flour is in error, based on amounts of starch in oat flour. Oat flour (2531 g, dry basis) (Materials and Methods) had 1579 g of starch (oat flour had 62.4% starch from Table II) and can yield 1754 g of glucose (162 g of starch yields 180 g of glucose). If oat flour slurry had 20.7% glucose (Table I), the weight of glucose would be 2153 g in a fermentation volume of 10.4 L, much higher than 1754 g glucose that can be obtained from starch of oat flour. Repeat analysis gave the same glucose value for oat flour slurry at zero time. An unknown compound may have been released with glucose from oat flour, but not from oat groats or whole oats. Ethanol concentration reached a maximum after 24 h for oat flour, 48 h for ground groats, and 66 h for ground oats. Glucose concentration was lowest for oat flour and highest for ground oats after 24-h fermentation. Glycerol was also present from 24 to 66 h in all oat fermentations as a byproduct. Ethanol yield from ground oats, oat flour, and ground groats was 95, 93, and 87% of theoretical value based on starch, respectively. Fermentation of oat flour appears advantageous to that of ground oats and groats, because only 24 h was necessary to reach maximum ethanol concentration.

Table II gives yield of distillers' grains, centrifuged solids, stillage solubles and ethanol, and compositions of each starting material and fermentation fraction on a dry basis. Oat flour (174 μm) stillage and ground oats (304 μm) stillage filtered well. Ground groats (361 μm) stillage did not filter properly on cheesecloth, so it was centrifuged to yield a solid fraction containing both distillers' grains and centrifuged solids, plus a liquid stillage soluble fraction. It is not apparent why filtering ground

Table II. Yield and Composition (%) of Oat Fermentation Products (Dry Basis)

oat product	% of starting weight	protein[a]	fat	ash	starch	crude fiber	NDF	TDF
ground whole oats	–	11.3	4.4	2.9	48.2	13.2	26.3	33.8
DG	40.4	18.8	9.7	3.9	1.0	25.4	56.8	63.5
CS	1.4	43.6	14.8	2.1	1.0	5.2	20.3	24.3
SS	9.2	26.3	1.4	13.2	–	–	–	7.2
ethanol	26.0	–	–	–	–	–	–	–
ground groats	–	16.9	6.3	2.1	61.8	2.0	7.1	12.5
DG + CS	27.8	43.8	21.2	3.3	5.0	9.1	22.6	20.6
SS	9.4	21.0	2.1	11.3	–	–	–	7.5
ethanol	30.5	–	–	–	–	–	–	–
flour	–	14.0	6.3	1.5	62.4	1.2	4.4	8.0
DG	4.1	43.1	27.1	2.5	1.4	7.7	17.9	21.6
CS	17.1	47.5	24.9	1.9	1.0	5.1	17.9	27.3
SS	12.7	23.9	7.9	11.0	–	–	–	5.0
ethanol	32.9	–	–	–	–	–	–	–

[a] N × 5.83. Key: DG = distillers' grains, CS = centrifuged solids, SS = stillage solubles, NDF = neutral detergent fiber, TDF = total dietary fiber, – = not analyzed.

Table III. Nitrogen Distribution and Content of Whole Oat Stillage Solubles

membrane	approx MW	fraction	% of total N	N content, % dry basis
YM2	<1000	permeate	39	3.19
	>1000	concentrate	61	12.10
PM10	<10000	permeate	54	3.31
	>10000	concentrate	46	8.92

groats stillage was difficult. This may be due to larger particle size of ground groats compared with oat flour, whereas hull in ground oats may act as a filtering aid.

All fermentation fractions had higher protein and fat contents than the corresponding starting materials, except stillage solubles from whole oats and ground groats had lower fat than whole oats and groats, respectively. Centrifuged solids from oat flour gave the highest protein content (47.5%) of all fractions. Distillers' grains from whole oats contained oat hulls with low protein content; therefore, oat flour distillers' grains had much higher protein than oat distillers' grains. Stillage solubles contained highest ash contents among all fractions. Starch practically disappeared for ground oats and oat flour after fermentation as shown by the low starch contents of distillers' grains and centrifuged solids. The higher yield of ethanol for oat flour (100% groats) compared with ground groats was a result of better conversion of oat flour starch to ethanol.

Ground oats contained hull, which had high crude fiber, neutral detergent fiber (NDF), and total dietary fiber (TDF) (Frolich and Nyman, 1988). Oat distillers' grains (Table II) had higher crude fiber, neutral detergent fiber, and total dietary fiber contents than did oat flour distillers' grains because oat distillers' grains contained hull. Prosky et al. (1985) reported total dietary fiber of quick-cooking oats ranged from 10.0 to 13.0%. Frolich and Nyman (1988) found oat kernel (dehulled oats) had 11.5% total dietary fiber. Total dietary fiber of 12.5% for ground groats and 8.0% for oat flour (Table II) appears reasonable considering differences in materials and processing.

Nitrogen Distribution and Content of Oat Stillage Solubles. YM2 (molecular weight cut-off of 1000) and PM10 membranes (molecular weight cut-off 10000) separated whole oat stillage solubles into permeate and concentrate (Table III). YM2 permeate volume was 38.5 mL; each milliliter of permeate contained 0.201 mg of nitrogen and 6.35 mg of solids. YM2 concentrate volume was 15.5 mL; each milliliter of concentrate had 0.778 mg of nitrogen and 6.60 mg of solids. Concentrates (larger molecules) had considerably higher nitrogen contents than permeates (smaller molecules) for both membranes. Most nitrogenous materials in oat stillage solubles were low in molecular weight (e.g., amino acids and peptides); less than half the total nitrogen was from materials higher than 10 000 molecular weight. For comparison, no nitrogen from corn stillage solubles came from material with molecular weight higher than 10 000 (Wu et al., 1981), and 43–45% of the nitrogen from wheat stillage solubles originated from materials with molecular weights higher than 10 000 (Wu et al., 1984).

Table IV. Amino Acid Composition of Whole Oat Fermentation Products[a]

	g/16 g N recovered				amino acid requirement pattern for preschool child, g/16 g N (FAO, 1985)
	whole	oat distillers' grains	centrifuged solids	stillage solubles	
aspartic acid[b]	8.3	8.7	8.9	7.6	
threonine	3.6	3.9	3.9	3.8	3.2
serine	5.1	5.1	5.1	5.7	
glutamic acid[c]	21.3	21.3	23.3	19.5	
proline	5.7	6.0	6.0	5.2	
glycine	5.1	5.2	5.0	6.3	
alanine	4.6	5.5	6.1	4.4	
valine	5.6	6.2	6.3	4.0	3.3
half-cystine	2.6	2.4	2.1	3.7	2.3
methionine	1.9	2.1	2.1	0.8	
isoleucine	3.8	4.5	4.6	3.0	2.6
leucine	7.8	8.6	8.9	5.0	6.2
tyrosine	3.6	3.6	4.3	3.9	5.9
phenylalanine	5.3	5.9	6.3	3.4	
lysine	4.3	4.4	3.7	4.6	5.4
histidine	2.2	2.2	1.7	2.1	1.8
arginine	8.6	7.7	6.7	6.2	

[a] Tryptophan not determined. [b] Includes asparagine. [c] Includes glutamine.

Amino Acid Composition. Table IV reveals that oats are rich in glutamic acid plus glutamine and have a good amino acid composition compared with FAO (1985) amino acid requirement pattern for preschool child, except for lysine. FAO data are adjusted to grams/16 g of nitrogen from grams/100 g of protein in Table IV. Distillers' grains, centrifuged solids, and stillage solubles from whole oats have amino acid compositions similar to that of oats, except stillage solubles have higher glycine and half-cystine but lower valine, methionine, isoleucine, leucine, phenylalanine, and arginine contents. Ground groats and oat flour have amino acid compositions similar to oats. Also, there are no large differences between composi-

Table V. Ultrafiltration and Reverse Osmosis of Oat Stillage Solubles[a]

	vol, mL	mg per mL		
		N	solids	ash
stillage solubles	5200	2.25	50.2	6.93
permeate (UF)	4895	0.756	22.3	5.38
concentrate (UF)	214	6.88	121	8.26
permeate (RO)	3567	0.0022	0.049	0.0052
range, 10 fractions	346–432	0.0014–0.0032	0.026–0.076	0–0.032
concentrate (RO)	996	0.902	27.5	5.96
range, 10 fractions	96–100	0.548–1.50	15.5–46.4	3.79–10.2

[a] In addition to permeate and concentrate, hold-up and wash fractions were also collected for UF and RO. UF and RO permeate, concentrate, and hold-up fractions contained all nitrogen, solids, and ash. Permeate from UF (4765 mL) was used as feed solution for RO. The lower number in each range of nitrogen, solids, or ash concentration was the value for the first RO fraction, and the higher number was that for the last fraction.

tions of fermented fractions from ground groats and oat flour (not shown in Table IV).

UF and RO of Oat Stillage Solubles. Concentrations of nitrogen and solids in UF permeate were 34 and 44% of those of stillage solubles (Table V), respectively. The nitrogen, solids, and ash concentrations of RO permeate decreased to 0.29, 0.22, and 0.10%, respectively, of concentrations in UF permeate. Nitrogen and solids concentrations of the RO permeate from the UF permeate increased slowly during the first four-fifths of RO, but then increased more rapidly. Nitrogen and solids concentrations of RO concentrate, however, increased at a relatively constant rate during the entire RO process. The RO permeate contained 75% of the total volume (4765 mL of UF permeate used for RO), 0.22% of total nitrogen, 0.16% total solids, and 0.072% of total ash of the UF permeate from oat stillage solubles. Alternatively, the RO permeate contained 71% of the total volume, 0.069% of total nitrogen, 0.068% of total solids, and 0.053% of total ash of oat stillage solubles.

Conductivity of RO Permeate and Concentrate. Solids and ash concentrations of RO concentrate and solids concentration of RO permeate are linearly related to conductivity for oat stillage solubles (not shown). The first eight of ten RO permeate fractions contained no ash. Correlation coefficients of conductivity versus milligrams of ash per milliliter of RO concentrate and conductivity versus milligrams of solids per milliliter of RO concentrate were 0.992 and 0.999, respectively. The correlation coefficient of conductivity versus milligrams of solids per milliliter of RO permeate was 0.796, which was significant at the 0.006 level. Conductivity measurements, which are more rapid than solids and ash determinations, can thus monitor solids concentration of RO permeate and solids and ash concentrations of RO concentrate. Conductivities for RO permeates (0.028–0.142 mS/cm at 27 °C) are lower than that of tap water (0.88 mS/cm).

CONCLUSIONS

Optimum use of fermentation residue, after ethanol is distilled, is important for commercial success of an overall ethanol process. Since a higher percentage of ground oats than corn became fermentation residue, economics of the oat process depend even more on having suitable processes for economically recovering valuable byproducts. The nutritional value of oats, based on its amino acid composition, is better than that of corn. Oat fermentation products also appear to have better amino acid compositions than do corresponding products from corn.

For each kilogram of ethanol produced, 7 L of oat stillage solubles or 5.4 L of oat flour stillage solubles is also present. Gregor and Jeffries (1979) reported that the total cost for equipment, power, and labor for combined UF and RO was $0.93/1000 L of stillage treated, versus $2.20/1000 L for fuel alone by the evaporative method. UF combined with RO appears to be a practical and economical method to process stillage solubles from oats or oat flour. A large volume of dilute solution can yield a small volume of concentrate and a large volume of permeate that can be safely disposed or reused as water.

Recovery of nitrogen and solids from oat stillage solubles by combined UF and RO was more than 99.9% (include hold-up fraction) assuming that only RO permeate is discarded. Oat distillers' grains have moderate protein content and are rich in total dietary fiber. Oat flour distillers' grains and centrifuged solids are rich in protein and have moderate total dietary fiber contents. These fermented oat products may thus provide valuable food-grade products.

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