Corey, R. A. Laboratory Test with Bidrin Insecticide. *J. Econ. Entomol.* **1965**, *58*, 658.
Hall, W. E.; Sun, Y. P. Mechanism of Detoxication and Synergism of Bidrin Insecticide in House Flies and Soil. *J. Econ. Entomol.* **1965**, *58*, 845.
Helling, C. S. Pesticide Mobility in Soil, I. Parameters of Thin-Layer Chromatography. *Soil Sci. Soc. Am. Proc.* 1971, *35*, 737.
Menzer, R. E.; Casida, J. E. Nature of Toxic Metabolites Formed in Mammals, Insects, and Plants from 3-(Dimethoxyphosphinyloxy)-*N*,*N*-dimethyl-cis-crotonamide and Its *N*-Methyl Analog. *J. Agric. Food Chem.* **1965**, *13*, 102.

Received for review August 26, 1988. Revised manuscript received February 21, 1989. Accepted March 21, 1989.

Recovery of Protein-Rich Byproducts from Sugar Beet Stillage after Alcohol Distillation

Y. Victor Wu,* Harald C. Nielsen, and Marvin O. Bagby

Ground sugar beets and sugar beet juice were fermented to ethanol. After ethanol was distilled, residual stillage was separated by screening and centrifugation into filter cake, centrifuged solids, and stillage solubles. Pectinase was partially effective in reducing the viscosity of sugar beet slurries before fermentation. Centrifuged solids and stillage solubles had crude protein contents (nitrogen × 6.25, dry basis) of 47 and 24%, respectively, and contained 20 and 79% of the total nitrogen of sugar beet juice. Of the nitrogen in sugar beet juice stillage solubles, 91% passed through a 10-kDa molecular weight cutoff membrane. Permeate from sugar beet juice stillage solubles processed by combined ultrafiltration and reverse osmosis had much lower nitrogen, solids, and ash contents than those of stillage solubles. This practical method to ferment sugar beet juice for ethanol and to recover valuable protein-rich byproducts may have commercial potential.

Although most fuel ethanol produced by fermentation in the United States comes from corn, other fermentable substrates are of interest. Parrish et al. (1985) examined production of readily fermentable carbohydrates and biomass from grain sorghum, Jerusalem artichoke, corn, sugar beet, sweet potato, and sweet sorghum at three temperate locations and concluded that sugar beets are superior to corn in producing fermentable substrates.

Gibbons et al. (1984) reported a continuous, farm-scale, solid-phase fermentation process to produce fuel ethanol and protein feed from fodder beets, which could compete with corn as an ethanol feedstock. Boudarel and Ramirez (1984) studied fermentation of beet juice to ethanol; productivity of an industrial reactor was 30% higher than on a laboratory scale. Larsen et al. (1981) investigated various methods of processing sugar beets for fermentation in relation to alcohol yield. Little is known, however, of yield and composition of fermentation residues from sugar beets or sugar beet juice after ethanol distillation.

Optimum use and efficient processing of fermentation residues are important for commercial success of all ethanol processes. This paper reports on fermentation of ground sugar beets and sugar beet juice to ethanol, effects of commercial pectinases on viscosities of sugar beet slurries before fermentation, yield and composition of stillage fractions, and use of ultrafiltration (UF) and high-pressure reverse osmosis (RO) to concentrate sugar beet juice stillage solubles and produce a permeate suitable for reuse or safe disposal.

MATERIALS AND METHODS

Sugar Beets. Z8 sugar beets (1984 crop) were obtained from Michigan Sugar Co., Carrollton, MI. Z8 is a high-yielding breeding line; its sugar content ranges from 15 to 17% at harvest. Beets were stored at 5 °C for 9 months before arrival and then at 1 °C for 2 weeks before use.

B1230 sugar beets were obtained from D. Cole, U.S. Department of Agriculture, Agricultural Research Service, Fargo, ND. These beets contained 14.8% sugar upon harvest in 1983 and had been stored at 5 °C for 4 months before arrival; beets were then stored at 1 °C for 16 months before use.

Sugar beets were ground in a Cuisinart food processor, and juice was obtained by pressing ground beets at 520 lb/in.^{2} (3540 kPa) in a Model B Carver Laboratory Press (Summit, NJ).

Pectinases. Clarex L, Spark-L HPG, Pectinol 80SB, and Klerzyme Liquid 200 pectinases were described by Wu and Bagby (1987). Pectinex 3XL (Novo Laboratories, Wilton, CT) is a purified pectolytic enzyme preparation from a selected strain of *Aspergillus niger*. Ultrazyme 100 is a pectolytic enzyme from Swiss Ferment Co. (Basel, Switzerland). Optimum pH values for Clarex L, Spark-L HPG, Pectinol 80SB, Pectinex 3XL, and Klerzyme Liquid 200 are 3.5, 3.5, 4.5, 3.5, and 3.4 and optimum temperatures are 50, 50, 55, 50, and 60 °C, respectively. Ground sugar beets (100 g) were incubated with 0.3 and 1.5 mL of pectinase at optimal temperatures and pH values, stirred periodically for 6 h with a spatula (since suspensions were too thick to stir magnetically), and centrifuged at 6000*g* for 20 min.

Fermentation. Viscosities of sugar beet slurries without pectinase treatment were too high for proper stirring without significant aqueous dilution. Ground sugar beets (4900 g) were put in a 20-L stainless-steel, temperature-controlled, jacketed fermentor equipped with stirrers. The pH was adjusted to 3.5, and the mixture was maintained at 90 °C for 30 min with stirring. The temperature was then reduced to 50 °C, 74 mL of Pectinex 3XL and 500 mL of hot water were added, and the slurry was maintained at 50 °C for 3 h with agitation. The slurry was then adjusted to pH 4.0, and 18 mL of Diazyme L-100 glucoamylase (Miles Laboratories, Elkhart, IN) was added. The mixture was maintained at 50 °C for 2 h with agitation and cooled to 30 °C. The slurry was then inoculated with 500 mL of yeast (*Saccharomyces cerevisiae*) containing 500 million cells/mL and fermented at pH 4.5. Samples were withdrawn at 0, 24, 48, and 66 h, at which time fermentation was stopped. Figure 1 is a schematic diagram of sugar beet fermentation, fractionation of stillage, and UF and RO recovery.

For fermentation of sugar beet juice (8758 g), pH was adjusted to 6.2, 6 mL of Taka-therm α-amylase (Miles Laboratories) was

Northern Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, 1815 North University Street, Peoria, Illinois 61604.

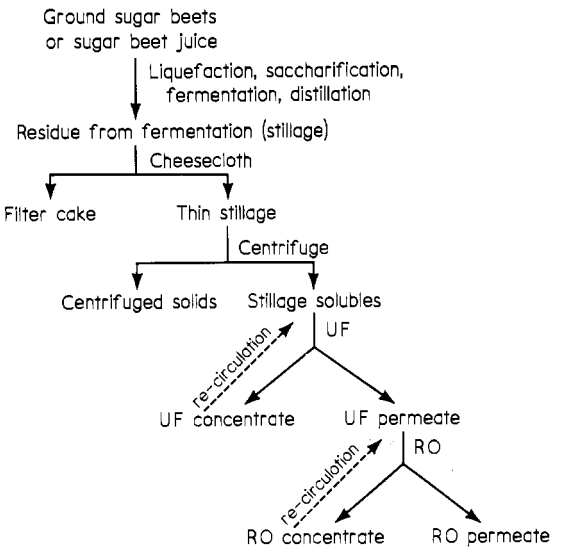

Figure 1. Schematic diagram of sugar beet fermentation, fractionation of stillage, and UF and RO processing.

added, and the mixture was maintained at 90 °C for 1 h with stirring. Subsequently, Diazyme L-100 and yeast were added as described above.

Fractionation of Stillage and Molecular Weight Range of Stillage Solubles. Wu and Bagby (1987) and Wu (1988) reported the procedures and details.

UF and RO. UF at 100 lb/in.^{2} and RO at 1000 lb/in.^{2} were described by Wu (1988). The flow rate was 31 L/m^2 per h for UF permeate. The flow rate of RO permeate was 20 L/m^2 per h. All nitrogen, solids, and ash were recovered in combined fractions from UF and RO.

Analyses. Protein, fat, and ash contents were determined by American Association of Cereal Chemists approved methods (1983); crude protein was calculated from Kjeldahl N × 6.25. Moisture was determined by heating samples at 100 °C to constant weight, and starch was measured 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). Nitrogen determinations were made in quadruplicate, moisture in triplicate, and dietary fiber and ash in duplicate. Analyses for ethanol, sucrose, glucose, fructose, and glycerol were made with a Waters ALC 200 high-performance liquid chromatograph (Waters Associates, Milford, MA) on a Bio-Rad HPX-87H (300 × 7.8 mm (o.d.)) column (Richmond, CA) at room temperature with water as eluant at 36 mL/h. Conductivity of sugar beet juice stillage fractions was measured at 27 °C by a Radiometer CDM 2e conductivity meter with a CDC 104 cell.

Amino acid analyses were performed on a Beckman Model 334 gradient liquid chromatograph system (Beckman Instruments, San Raman, CA). After 24-h reflux in 6 N hydrochloric acid, hydrolyzed samples were dried in a rotary evaporator and residues were dissolved in pH 2.2 citrate buffer. Sulfur amino acids were determined after oxidation of the sample with performic acid (Moore, 1963). Data were calculated automatically (Cavins and Friedman, 1968).

RESULTS AND DISCUSSION

Effect of Pectinase on Slurry Viscosity. The high viscosity of sugar beet slurries caused stirring problems before fermentation and in processing stillage. The volume of supernatant was used as a measure of effectiveness of pectinase to reduce viscosity. This volume ranged from 2 mL for Klerzyme (the least effective pectinase) to 50 mL for Pectinex 3XL (the most effective pectinase); Clarex L (47 mL) was slightly less effective than Pectinex 3 XL. Changing pH from 3.5 or temperature from 50 °C (optimal values of Pectinex 3XL) decreased volume of supernatant. Pectinex 3XL at 1.5 mL/100 g of ground sugar beet was chosen to reduce viscosity of beet slurry at pH 3.5 and 50 °C prior to fermentation. In comparison, 0.3 mL of Clarex L/100 g of sweet potato reduced viscosity of slurry more effectively (Wu and Bagby, 1987) than 1.5 mL of Clarex L/100 g of sugar beet.

Table I. Effect of Fermentation Time on Sugar Beet Slurry and Sugar Beet Juice Composition[a]

substrate	h	g/100 mL sucrose	glucose	fructose	ethanol	glycerol
B1230 sugar beets	0	0	3.5	4.0	0.2	0.1
	24	0.1	0.1	0.1	3.7	0.3
	48	0	0.2	0.2	3.7	0.3
	66	0	0.3	0.3	3.6	0.4
Z8 sugar beet juice	0	8.9	1.3	1.1	0	0
	24	0.2	2.7	4.0	2.1	0.2
	48	0.2	0	0	5.1	0.4
	66	0.1	0	0	4.8	0.4

[a] Pectinex 3XL (74 mL) was added to 4861 g of wet B1230 sugar beets. Final ethanol yields were 96% and 89% of theoretical values for sugar beets and sugar beet juice, respectively.

Effect of Fermentation Time on Composition. Compositions of sugar beets and of sugar beet juice slurries after various fermentation times are shown in Table I. Slurries had been pretreated with Pectinex 3XL pectinase, α-amylase, and glucoamylase. After 24-h fermentation, almost all sugars disappeared from sugar beets, while ethanol reached its maximum concentration. For Z8 juice after 24-h fermentation, sucrose almost disappeared but considerable glucose and fructose remained. Maximum concentration of ethanol was achieved at 48 h for juice, and almost all sugars disappeared. For both sugar beets and juice, glycerol also formed.

Yield and Composition of Sugar Beet Fermentation Products. B1230 sugar beets contained 14.9% dry matter, of which 59.9% was sucrose, fructose, and glucose (Table II); therefore, B1230 sugar beet contained 8.9% sugar before fermentation, compared with 14.8% (Materials and Methods) sugar at harvest. The large loss in sugar during 20 months of storage was due to respiration. A smaller loss in sugar would be expected for a shorter storage time. Fermentation residue accounted for 47% of the dry weight of B1230 sugar beets, of which filter cake was the largest fraction. Filter cake had about twice as much protein and neutral detergent fiber and had a higher fat content than B1230 sugar beets. B1230 stillage solubles had a protein content similar to filter cake but contained more ash.

Z8 sugar beets contained 14.2% dry matter, of which 71.7% was sucrose, fructose, and glucose (Table II). This sugar content before fermentation (10.2%) is less than that expected at harvest (15–17%). Z8 sugar beet juice accounted for 79% of the wet weight, 72% of the dry weight, 63% of the total nitrogen, and 85% of the total sugar of Z8 sugar beets. Fermentation products accounted for 15% of the dry weight of Z8 sugar beet juice, stillage solubles being the largest fraction (16 L of stillage solubles was produced/kg of ethanol). All fermentation fractions from Z8 sugar beet juice contained higher concentrations of protein than present in juice. Centrifuged solids also had fat and neutral detergent fiber contents higher than those of juice, while stillage solubles had a higher ash concentration than did juice.

Theoretical ethanol yields for B1230 sugar beets and Z8 sugar beet juice were calculated from the sum of sugar (Table II) and dry-matter contents. Attained ethanol yields for sugar beets and juice were 96 and 89%, respectively, of theoretical values.

Nitrogen Distribution and Content of Sugar Beet Juice Stillage Solubles. Nitrogen distributions and contents of permeates and concentrates are shown in Table

Table II. Yield and Composition of Sugar Beet Fermentation Products[a]

product	% of residue	% dry basis						
		protein	fat	NDF	ash	sucrose	fructose	glucose
B1230 sugar beet		10.8	0.6	20.2		42.6	9.3	8.0
filter cake	71.8	23.1	2.3	42.2	11.5			
centrifuged solids	0.6	50.8			5.6			
stillage solubles	27.6	23.2			22.3			
Z8 sugar beet juice		4.50	0.03	0.2	3.5	81.1	1.4	2.8
filter cake	0.2	38.1						
centrifuged solids	20.4	46.5	1.0	30.3	4.2			
stillage solubles	79.4	24.2			25.6			
Z8 sugar beets		7.1	0.3	13.3	6.3	70.7	1.0	0
Z8 pressed sugar beets residue		7.5	0.6	39.4	5.0	35.4	2.1	0.5

[a] NDF = neutral detergent fiber. Fermentation products accounted for 47% of B1230 sugar beet dry weight and 15% of Z8 sugar beet juice dry weight. Z8 sugar beet juice accounted for 79% of the wet weight, 72% of the dry weight, and 85% of the total sugar of Z8 sugar beets. Dry matters of B1230 sugar beets, Z8 sugar beets, and Z8 sugar beet juice were 14.9%, 14.2%, and 14.3%, respectively.

Table III. Nitrogen Distribution and Content of Z8 Sugar Beet Juice Stillage Solubles

membrane	approx MW	fraction	% of total N	N content, % dry basis
YC05	<500	permeate	56	2.36
	>500	concentrate	44	4.14
PM10	<10000	permeate	91	3.03
	>10000	concentrate	9	11.07

Table IV. Amino Acid Composition of Sugar Beets and Their Fermentation Products[a]

	g/16 g nitrogen recovered					
	beets	beet juice	juice-centrifuged solids	juice-stillage solubles	beet filter cake	beet stillage solubles
aspartic acid[b]	8.5	8.9	11.5	5.7	10.7	9.7
threonine	3.4	2.2	6.4	2.4	5.1	3.3
serine	3.9	2.3	6.3	2.8	5.1	4.2
glutamic acid[c]	25.5	38.1	12.7	40.8	15.6	18.7
proline	3.0	2.4	3.9	6.1	4.7	4.7
glycine	3.3	2.3	5.4	4.5	5.9	4.4
alanine	3.4	3.9	6.4	4.5	7.6	7.9
valine	4.2	2.4	7.3	1.4	6.0	3.5
half-cystine	1.6	1.8	2.1	1.1	1.3	1.8
methionine	1.9	0.4	2.1	2.2	1.9	0.3
isoleucine	2.9	2.1	6.0	1.3	4.5	2.4
leucine	4.0	2.7	9.1	1.7	8.7	2.8
tyrosine	2.8	0.8	4.6	1.1	4.2	1.2
phenyl-alanine	1.9	1.4	5.3	0.9	3.6	1.3
lysine	3.6	1.6	7.9	2.6	4.0	3.2
histidine	2.4	1.1	2.5	0.8	2.7	1.8
arginine	4.5	2.2	6.5	1.8	3.4	0.0

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

III. Permeate accounted for 56% of the nitrogen with the YC05 membrane, which has a nominal MWCO of 500. Permeate from the PM10 membrane contained 91% of the nitrogen of stillage solubles. Both concentrate fractions had higher nitrogen contents than the corresponding permeates, indicating that larger molecules are richer in nitrogen. In comparison, 29% of the nitrogen from grain sorghum stillage solubles and 48% of the nitrogen from corn stillage solubles were in compounds having nominal molecular weights less than 500, and 86% of the nitrogen from grain sorghum stillage solubles and 100% of the nitrogen from corn stillage solubles were in compounds having nominal molecular weights less than 10 000 (Wu et al., 1981; Wu and Sexson, 1984).

Amino Acid Composition. Table IV reveals that sugar beets are rich in glutamic acid plus glutamine. Beet juice has higher glutamic acid plus glutamine, but lower threonine, serine, glycine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine than sugar beets. Juice-centrifuged solids have higher aspartic acid plus asparagine, threonine, serine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine but lower glutamic acid plus glutamine than beet juice. Juice stillage solubles have higher proline, glycine, methionine, tyrosine, and lysine but lower aspartic acid plus asparagine, valine, half-cystine, isoleucine, leucine, phenylalanine, and histidine than beet juice. Beet filter cake has higher aspartic acid plus asparagine, threonine, serine, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, and phenylalanine but lower glutamic acid plus glutamine and arginine than sugar beets. Beet stillage solubles have higher proline, glycine, and alanine but lower glutamic acid plus glutamine, methionine, leucine, tyrosine, phenylalanine, histidine, and arginine than sugar beets.

UF and RO of Sugar Beet Juice Stillage Solubles. Table V shows volumes and concentrations of nitrogen, solids, and ash of Z8 sugar beet juice stillage solubles and their UF and RO fractions. Concentrations of nitrogen and solids in UF permeate were approximately three-fourths those of stillage solubles. The nitrogen, solids, and ash concentrations of RO permeate decreased to 0.45, 1.3, and

Table V. Ultrafiltration and Reverse Osmosis of Z8 Sugar Beet Juice Stillage Solubles[a]

	vol, mL	mg/mL		
		nitrogen	solids	ash
stillage solubles	7510	0.644	18.4	5.83
permeate (UF)	7305	0.490	14.0	5.06
concentrate (UF)	156	1.13	30.3	6.50
permeate (RO)	6145	0.0022	0.181	0.068
range, 10 fractions	545–646	0.0011–0.0058	0.105–0.369	0.0276–0.167
concentrate (RO)	938	0.742	21.3	7.64
range, 10 fractions	43–100	0.434–1.48	12.0–42.6	4.39–15.24

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

1.3%, respectively, of the concentrations in UF permeate. Solids and ash concentrations of RO permeate and RO concentrate of sugar beet juice stillage solubles UF permeate were linearly related to conductivity (correlation coefficients 0.987–0.997). Thus, conductivity can rapidly monitor solids and ash concentrations of RO permeate and RO concentrate. Conductivity of RO permeate (0.19 mS/cm) was much lower than that of cold tap water at room temperature (0.72 mS/cm). Therefore, combined RO and UF are very efficient in processing sugar beet juice stillage solubles into a small volume of concentrate and a large volume of permeate suitable for reuse as water.

Nitrogen and solids concentrations of the RO permeate from UF permeate slowly increased in nitrogen and solids concentrations during the first two-thirds of the RO process but then increased more rapidly (Table V). Nitrogen and solids concentrations of RO concentrates increased at a relatively constant rate during RO.

CONCLUSIONS

Fermentation of ground sugar beets to ethanol is hindered by high slurry viscosities. The best tested commercial pectinase only partially reduced this viscosity; in contrast, a lower concentration of pectinase was effective for sweet potatoes (Wu and Bagby, 1987). Addition of water to ground sugar beets can reduce slurry viscosity, but this decreases fermentable sugar concentration and resulting concentration of ethanol, which will be more expensive to recover.

Pressing sugar beets yielded juice that contained 79% of the total weight and 85% of the total sugar. This juice had low viscosity, and a higher concentration of ethanol resulted from fermentation. The sugars in this juice were converted to ethanol in high yield. This represented a significant cost saving over methods based on fermentation of crude sugar or diffusion juice. Thus, fermentation of sugar beet juice may be more feasible and desirable than that of ground sugar beets.

Fermentation of sugar beet juice also produced 16 L of stillage solubles/kg of ethanol. 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, compared with \$2.20/1000 L for fuel alone by the evaporative route. UF combined with RO thus appears to be a practical and economical method to process sugar beet juice stillage solubles. A large volume of dilute solution can be separated into a small volume of concentrate and a large volume of permeate that can be reused as water or safely discarded.

Recovery of nitrogen and solids from sugar beet juice stillage solubles in concentrates from combined UF and RO is more than 99%. Sugar beet juice centrifuged solids had high lysine content and may provide valuable food-grade or feed products.

ACKNOWLEDGMENT

We gratefully acknowledge technical assistance of N. H. Harrison, J. P. Anderson, and A. A. Lagoda. We thank J. F. Cavins for amino acid analyses.

Registry No. Ethanol, 64-17-5.

LITERATURE CITED

American Association of Cereal Chemists. *Approved Methods*, 8th ed.; AACC: St. Paul, MN, 1983.

Amicon Corporation. *Amicon ultrafiltration applications manual*; Publication No. 427. Amicon: Lexington, MA, 1972.

Boudarel, M. J.; Ramirez, A. Improvement of conditions of continuous fermentation with recycle for the production of ethanol from beet juice with *Saccharomyces cerevisiae*. *Ind. Aliment. Agric.* **1984**, *101*, 1151–1158.

Cavins, J. F.; Friedman, M. Automatic integration and computation of amino acid analyses. *Cereal Chem.* **1968**, *45*, 172–176.

Garcia, W. J.; Wolf, M. J. Polarimetric determination of starch in corn with dimethyl sulfoxide as a solvent. *Cereal Chem.* **1972**, *49*, 298–306.

Gibbons, W. R.; Westby, C. A.; Dobbs, T. L. A continuous, farm-scale, solid-phase fermentation process for fuel ethanol and protein feed production from fodder beets. *Biotech. Bioeng.* **1984**, *26*, 1098–1107.

Gregor, H. P.; Jeffries, T. W. Ethanolic fuels from renewable resources in the solar age. *Ann. N.Y. Acad. Sci.* **1979**, *326*, 273–287.

Larsen, D. H.; Doney, D. L.; Orien, H. A. Production of ethyl alcohol from sugar beets. *Dev. Ind. Microbiol.* **1981**, *22*, 719–724.

McQueen, R. E.; Nicholson, J. W. G. Modification of the neutral detergent fiber procedure for cereals and vegetables by using alpha amylase. *J. Assoc. Off. Anal. Chem.* **1979**, *62*, 676–680.

Moore, S. On the determination of cystine as cysteic acid. *J. Biol. Chem.* **1963**, *238*, 235–237.

Parrish, D. J.; Gammon, T. C.; Graves, B. Production of fermentables and biomass by six temperate fuel crops. *Energy Agric.* **1985**, *4*, 319–330.

Wu, Y. V. Characterization of sweet potato stillage and recovery of stillage solubles by ultrafiltration and reverse osmosis. *J. Agric. Food Chem.* **1988**, *36*, 252–256.

Wu, Y. V.; Sexson, K. R. Fractionation and characterization of protein-rich material from sorghum alcohol distillation. *Cereal Chem.* **1984**, *61*, 388–391.

Wu, Y. V.; Bagby, M. O. Recovery of protein-rich byproducts from sweet potato stillage following alcohol distillation. *J. Agric. Food Chem.* **1987**, *35*, 321–325.

Wu, Y. V.; Sexson, K. R.; Wall, J. S. Protein-rich residue from corn alcohol distillation: fractionation and characterization. *Cereal Chem.* **1981**, *58*, 343–347.

Received for review August 31, 1988. Accepted January 9, 1989. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.