Protein-Rich Residue from Wheat Alcohol Distillation: Fractionation and Characterization

Y. V. WU, K. R. SEXSON, and A. A. LAGODA, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture,[1] Peoria, IL 61604

ABSTRACT

Cereal Chem. 61(5): 423–427

Whole wheat and flour were fermented to make alcohol. The residue, after alcohol was distilled, was fractionated into distillers' grains, centrifuged solids, and distillers' solubles. Distillers' grains and centrifuged solids had protein contents of 29 and 57%, respectively, and accounted for 36 and 21% of total hard wheat nitrogen. Half of the nitrogen in distillers' solubles of flour passed through a 10,000 molecular weight membrane. Lysine, expressed in grams per 16 g of N, was considerably higher in distillers' grains and centrifuged solids than wheat.

Fermentation of cereal grains to make alcohol produces a protein-rich material (stillage) after the alcohol is distilled. The fermentation process predominantly consumes the starch in cereal grains, and other nutrients such as protein are thereby concentrated. Alcohol fermentation of corn is done commercially, and a small amount of wheat is also used for this purpose. Fractionation and characterization of corn stillage and of corn distillers' dried grains with and without solubles were reported previously (Wu et al 1981, Wu and Stringfellow 1982). Satterlee et al (1976) prepared protein concentrate from fermented corn and fermented wheat by extraction with alkali. Tsen et al (1982, 1983) incorporated distillers' dried grain flours in bread and cookies. Prentice (1978) and Prentice et al (1978) blended brewers' spent grain with flour for muffin and cookie formulations. Finley and Hanamoto (1980) incorporated various fractions of brewers' spent grains into bread. Some composition data on wheat distillers' grains and wheat distillers' grains with solubles are available (National Research Council 1956). Since corn is not available for alcohol fermentation in some parts of the United States and various regions of the world, wheat is often used as the substrate for yeast. Wheat and wheat flour differ appreciably from corn and degermed corn flour, respectively, in composition and properties, and the by-products of wheat fermentation may have better characteristics for food than does corn. The purpose of this study was to fractionate and characterize the protein-rich distillation residues from fermented hard and soft whole wheats and their flours.

[1]Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over others firms or similar products not mentioned.

MATERIALS AND METHODS

Wheat

Newton wheat, a typical hard red winter wheat in Kansas, was grown in 1981. Daws wheat, a soft white winter wheat, was from Washington. The wheat was milled in a laboratory model Allis rolls until all passed through No. 12 screen (whole grain), milled in the Allis rolls until 83% of the wheat passed through No. 12 screen (83% extraction flour), or in a Buhler mill with 70% of the wheat collected in the flour bins (70% extraction flour).

Fermentation

Ground wheat or wheat flour was dispersed in 5 L of tap water in a 20-L stainless steel, temperature-controlled, jacketed fermentor equipped with stirrers. The dry basis weights used were 2,350, 2,065, 1,838, 1,950, 1,927, and 1,811 g for Newton wheat, Newton flour (83% extraction), Newton flour (70% extraction), Daws wheat, Daws flour (85% extraction), and Daws flour (70% extraction), respectively. The pH of each slurry was adjusted to 6.2, and 6 ml of Miles Taka-therm α-amylase (170,000 Modified Wohlgemuth Units/g) was added. One Modified Wohlgemuth Unit is that amount of enzyme which will dextrinize one milligram of soluble starch to a dextrin of a definite size in 30 min under the conditions of the assay. The temperature of the fermentor was maintained at 90°C for 1 hr to gelatinize and degrade starch to soluble dextrins. Then tap water was added to the fermentor. The volumes of tap water added at this stage were 1,328, 1,307, 1,315, 1,316, 1,309, and 1,337 ml for Newton wheat, Newton flour (83% extraction), Newton flour (70% extraction), Daws wheat, Daws flour (85% extraction), and Daws flour (70% extraction), respectively. The fermentor was cooled to 60°C, the pH was adjusted to 4.0, and to hydrolyze dextins 18 ml of Miles Diazyme L-100 glucoamylase (100 Diazyme units/ml) was added. The suspension was incubated for 2 hr. The mixture was cooled to 30°C and adjusted to pH 4.5, and 500 ml of yeast (*Saccharomyces cerevisiae*) in the logarithmic growth phase containing an average of 5 million cells per milliliter was added. The yeast inoculum was made from 9 g of Fermivin dry yeast (G. B. Fermentation Industries, Des Plaines, IL), 0.3% yeast extract, 0.5% peptone, and 1.0% glucose in 500 ml of tap water. Each fermentation was stopped after 66 hr.

Fractionation of Stillage

Alcohol was distilled from the fermentor by steam, and the residue (stillage) was filtered through cheesecloth under suction (Fig. 1). The thin stillage that passed through the cheesecloth was centrifuged at 45,000 rpm in a model T-1 Sharples continuous centrifuge with a bowl having a 4.5-cm inside diameter. The solution that passed through the continuous centrifuge was designated distillers' solubles. The solids that remained in the bowl of the centrifuge were termed centrifuged solids. The materials that remained on the cheesecloth were the distillers' grains. The wet distillers' grains and wet centrifuged solids were dried overnight in a forced-air oven at 90°C or were freeze-dried.

Ultrafiltration

Two kinds of ultrafiltration apparatuses with different membranes were used. Amicon ultrafiltration cell model 52 (Amicon Corp., Lexington, MA) with two membranes 43 mm in diameter was used for most experiments. Each membrane is characterized by its nominal molecular weight cutoff; for example, 500 for UM05 and 10,000 for PM10; above these levels most species are retained by the membrane. Distillers' solubles (10 ml) was pipetted into the cell above each membrane, and distilled water was added under 50 lb/in.^{2} of nitrogen, so that the volume of solution above the membrane remained approximately constant. About five volumes of solution below the membrane was collected. The volume of solution above and below the membrane was measured, the nitrogen content of each solution was determined, and the percentage of total nitrogen in each fraction was calculated.

A Millipore immersible CX molecular separator (Millipore Corp., Bedford, MA), consisting of a Pellicon molecular filtration membrane cast on a cylindrical porous plastic core, was also used in some experiments as an additional check. The CX separator was immersed in 3 ml of distillers' solubles in a small test tube, vacuum was applied to the separator, and solution was sucked through the membrane. This membrane has a nominal molecular weight cutoff of 10,000. The volume and nitrogen content of each fraction were determined.

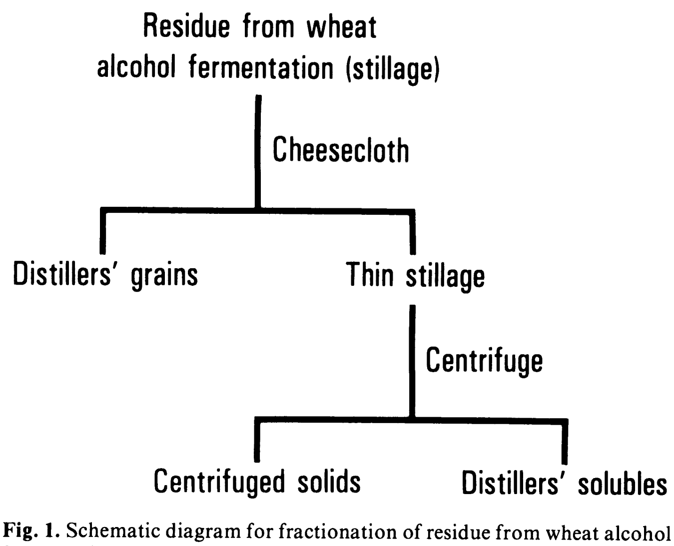

Fig. 1. Schematic diagram for fractionation of residue from wheat alcohol fermentation.

Protein Extraction

Ground Newton wheat (16.7 g) was put in a stainless steel cup with 100 ml of solvent and blended for 5 min in a Waring Blendor. The sample after blending was centrifuged at 10,400 × *g* for 10 min, the supernatant was decanted, and the residue was extracted with the next solvent. Solvents used sequentially were 1% sodium chloride, water, 0.01*N* acetic acid (twice), and 0.03*N* sodium hydroxide. Newton flour was extracted the same as Newton wheat. For wheat flour centrifuged solids, one more 0.03*N* sodium hydroxide extraction was added, and the solvent-to-solid ratio was increased from 6:1 to 12.5:1. For wheat distillers' grains and wheat centrifuged solids, one more 0.03*N* sodium hydroxide extraction and two more 0.1*N* sodium hydroxide extractions were performed, and the solvent-to-solid ratios were increased to 20:1. A higher solvent-to-solid ratio was needed for centrifuged solids and distillers' grains compared with wheat or wheat flour, because the former absorbed more solvents.

Composition

Protein, fat, fiber, and ash were determined by AACC approved methods (1976). Starch was measured by a polarimetric method (Garcia and Wolf 1972). Protein was calculated from N × 5.7 and included free amino acids, peptides, and other nitrogen compounds. Moisture was determined by heating samples to constant weight at 100°C. Analyses for glucose, glycerol, and ethanol were made 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. Fat, ash, and moisture determinations were in duplicate and four values were averaged for protein.

For amino acid analysis, 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. A portion of the acid hydrolysate was analyzed in a Dionex D 300 amino acid analyzer (Dionex Corp., Sunnyvale, CA), and the data were computed automatically by the method of Cavins and Friedman (1968).

Functional Properties

Gluten ball-forming ability. A gluten ball was formed by mixing 2 g of vital gluten, devitalized gluten, and wheat flour distillers' grains with 3 ml of 0.1% sodium chloride at room temperature.

Mixograms. A 10.0-g Swanson-Working Mixograph (National Mfg. Co., Lincoln, NE) described by Finney and Shogren (1972) was used. For Newton flour and Newton flour centrifuged solids, enough water and wheat starch were added to give water absorption of 61 and 11% protein. For Daws flour and Daws flour centrifuged solids, water absorption of 58 and 8% protein were used. Mixograms were stopped after 7–10 min of mixing.

RESULTS AND DISCUSSION

Composition and Yield of Fermentation Products

Since we are interested primarily in the protein-rich residue from wheat alcohol distillation, only general statements are made for alcohol, glucose, and glycerol. No glucose was left after 66 hr of fermentation, and the amount of glycerol formed was around 10% of the alcohol. The yield of alcohol was higher for soft wheat and soft wheat flours compared with the hard wheat counterparts, both on an absolute basis and as a percent of theoretical. The attained ethanol yield (expressed as percent of theory) of wheat and wheat flour in general did not differ greatly from that of corn as reported by Wall et al (1983).

Table I lists the protein, fiber, fat, starch, and ash contents of wheat, wheat flour, and their fermentation products. The yield of each fermentation product is calculated as percent of grain or flour as well as percent of fermentation residue. For hard wheat grain, 35% of the original weight was recovered as fermentation residue, of which distillers' grains, centrifuged solids, and distillers' solubles accounted for 47, 15, and 38%, respectively. The protein content of fermentation products from hard wheat ranged from 29 to 57%, about two to four times that of the starting wheat. The fat content of distillers' grains was several times that of the starting wheat, but the fat content of centrifuged solids was not much higher than the wheat. The distillers' grains and centrifuged solids had higher ash content than wheat, but the distillers' soluble had the highest ash content by far. Part of the ash from the distillers' solubles was derived from the salt formed as a result of neutralization of acid and base in pH adjustments. Distillers' grains and centrifuged solids both have considerably higher fiber than hard wheat, but distillers' grains had the highest value by far. The absence of starch and glucose at the end of fermentation suggested that all starch in wheat was converted to glucose and all glucose was converted by yeast to alcohol and a small amount of glycerol. Protein, fat, fiber, and ash contents of wheat distillers' grains and wheat distillers' solubles in Table I are between the maximum and minimum values reported by the National Academy of Sciences (National Research Council 1956). However, our fractionation procedure included a centrifuged solids fraction that was not reported by the National Academy of Sciences (National Research Council 1956). Hard wheat distillers' grains had similar protein and fat, higher fiber, and lower ash contents than brewers' spent grains (Finley and Hanamoto 1980).

Hard wheat flour (70% extraction) had lower protein, fat, fiber, and ash contents than whole hard wheat. The lower yield of wheat flour fermentation residue compared with whole wheat was due to the higher starch content of wheat flour. The smaller particle size of wheat flour resulted in smaller particle size of fermentation residue, which passed through cheesecloth completely. Only centrifuged solids and distillers' solubles were obtained after fermentation of wheat flour. About two-thirds of the fermentation residue from wheat flour was accounted for by distillers' solubles, in contrast to about one-third for wheat. Both centrifuged solids and distillers' solubles had about 50% protein content, about four times that of the flour. The fat and ash contents of centrifuged solids and distillers' solubles were higher than that of the flour. The compositions and yields of 83% extraction flour fermentation products were close to the figures from the corresponding materials from hard wheat grain (not shown in Table I).

The protein, fat, fiber, and ash contents of fermentation products from soft wheat were similar to those from hard wheat, except that the protein contents of soft wheat fermentation products were lower than the corresponding products from hard wheat, reflecting the difference in protein content of hard and soft wheats. Likewise, the soft wheat flour fermentation product had lower protein content than the corresponding hard wheat flour product. In addition, the distillers' solubles from soft wheat flour accounted for three-fourths of the fermentation residue, a higher proportion than from hard wheat flour.

The protein contents of soft wheat fermentation products were similar to the corresponding fractions from corn, and the fat contents of wheat distillers' grains and centrifuged solids were lower than those of the corresponding fractions from corn (Wu et al 1981). Also, the wheat distillers' solubles accounted for a higher weight fraction (38 versus 20%) and larger percentage of total nitrogen of the fermentation residue than did the corn distillers' solubles (33 versus 20).

TABLE I
Composition and Yield of Fermentation By-Product from Wheat and Wheat Flour (dry basis)

	Percent of Residue	Percent Content: Protein N × 5.7	Fat	Fiber	Ash	Starch
Hard wheat						
Wheat	···	15.7	1.3	3.4	1.5	64.8
Distillers' grains	47	29.2	6.0	19.9	2.4	0
Centrifuged solids	15	57.2	1.8	6.8	2.2	0
Distillers' solubles	38	37.4	nd[a]	nd[a]	8.8	nd[a]
Residue, percent of grain	35	···	···	···	···	···
Hard wheat flour (70% extraction)						
Wheat flour	···	12.4	0.9	0.3	0.5	79.6
Centrifuged solids	36	49.0	6.1	9.5	1.7	0.7
Distillers' solubles	64	51.0	nd[a]	nd[a]	3.8	nd[a]
Residue, percent of flour	21	···	···	···	···	···
Soft wheat						
Wheat	···	10.5	1.5	2.6	1.6	68.5
Distillers' grains	48	23.0	6.7	18.9	2.2	0
Centrifuged solids	15	46.1	5.4	8.4	2.4	2.8
Distillers' solubles	37	33.4	nd[a]	nd[a]	9.1	nd[a]
Residue, percent of grain	35	···	···	···	···	···
Soft wheat flour (70% extraction)						
Wheat flour	···	8.8	0.9	0.2	0.5	81.5
Centrifuged solids	26	35.3	6.8	14.8	1.7	9.3
Distillers' solubles	74	44.0	nd[a]	nd[a]	4.0	nd[a]
Residue, percent of flour	21	···	···	···	···	···

[a]nd = Not determined.

Nitrogen Distribution and Content of Distillers' Solubles Fractions

Table II shows the nitrogen distribution and content of wheat and wheat flour distillers' solubles fractions separated by ultrafiltration membranes. About 80% of the total nitrogen stayed above the UM05 membrane for distillers' solubles from hard wheat, hard wheat flour, and soft wheat flour; the nitrogen contents of the above UM05 fractions were higher than those that passed through the membrane. These observations indicate that the smaller molecules that went through the membrane had lower nitrogen content than the larger molecules that stayed above the membrane. About 70% of the total nitrogen of soft wheat distillers' solubles stayed above the UM05 membrane, and the nitrogen content of the above UM05 fraction was higher than that of the fraction that passed through the membrane. Between 51 and 59% of the nitrogen

TABLE II
Nitrogen Distribution and Content of Distillers' Solubles Fractions from Wheat and Wheat Flour

Distillers' Solubles	Membrane[a]	Fraction	Percent of Total N	N Content (% db)
Hard wheat	UM05	Above	81	9.28
		Through	19	3.81
	PM10	Above	57	10.63
		Through	43	4.6
Hard wheat flour	UM05	Above	83	11.18
		Through	17	6.62
	PM10	Above	51	11.35
		Through	49	6.98
Soft wheat	UM05	Above	71	7.60
		Through	29	4.71
	PM10	Above	55	10.08
		Through	45	4.98
Soft wheat flour	UM05	Above	82	10.63
		Through	18	4.48
	PM10	Above	59	12.71
		Through	41	5.95

[a]The UM05 and PM10 membranes have nominal molecular weight cutoffs of 500 and 10,000, respectively.

of distillers' solubles from wheat and wheat flour stayed above the PM10 membrane, and the nitrogen contents of the above PM10 fractions were higher than those of the fractions that passed through the membrane. Ultrafiltration by CX separator and by dialysis tube on the two distillers' solubles from wheat flour gave the same nitrogen distribution as those from the PM10 membrane. Substantial percentages of the nitrogenous molecules in wheat and wheat flour distillers' solubles were proteins and large peptides. In contrast, corn distillers' solubles contained smaller nitrogenous molecules, because all nitrogen passed through PM10 membrane and 48% passed through UM05 membrane (Wu et al 1981).

TABLE III
Percent Nitrogen Distribution of Soluble Fractions of Hard Wheat and Its Fermentation Products

Fraction	Hard Wheat	Distillers' Grains Dried at 90°C	Distillers' Grains Freeze Dried	Centrifuged Solids Dried at 90°C	Centrifuged Solids Freeze Dried
1% NaCl Extract	18	15	24	7	8
Water wash	5	6	7	6	7
1st 0.01*N* HOAc Extract	12	3	5	3	6
2nd 0.01*N* HOAc Extract	27	2	3	3	2
1st 0.03*N* NaOH Extract	21	8	11	12	17
2nd 0.03*N* NaOH Extract	...	11	10	13	29
1st 0.1*N* NaOH Extract	...	11	7	21	8
2nd 0.1*N* NaOH Extract	...	8	3	9	2
Residue	18	39	21	21	12

TABLE IV
Percent Nitrogen Distribution of Soluble Fractions of Hard Wheat Flour and Its Fermentation Products

Fraction	Hard Wheat Flour	Centrifuged Solids Dried at 90°C	Centrifuged Solids Freeze Dried
1% NaCl Extract	15	13	12
Water wash	4	7	12
1st 0.01*N* HOAc Extract	62	5	10
2nd 0.01*N* HOAc Extract	7	2	9
1st 0.03*N* NaOH Extract	9	9	19
2nd 0.03*N* NaOH Extract	...	19	8
Residue	6	39	25

Protein Fractions of Wheat and Wheat Flour Fermentation Products

The solvents used for Table III were designed to compare the solubility of protein from wheat and its fermentation products. Sodium chloride solution will extract albumin and globulin. Water wash is needed to remove most of the salt for subsequent extraction of gluten by acetic acid, because the solubility of gluten in acetic acid is greatly reduced by the presence of salt. The residual salt resulted in a smaller amount of wheat nitrogen in the first acetic acid extract than that in the second extract. Acetic acid extracted only 5 to 8% nitrogen from distillers' grains and centrifuged solids, compared with 39% from wheat. Freeze-dried distillers' grains and centrifuged solids had more soluble protein than the corresponding materials dried at 90° C. The decrease in protein solubility in wheat fermentation products resulted from the drying at 90° C as well as from the fermentation process itself and the distillation of alcohol at the end of fermentation.

The nitrogen distribution of soluble fractions of wheat flour and its fermentation products is listed in Table IV. The solvents used were identical to those in Table III, except that the last two sodium hydroxide extractions were not performed. For wheat flour, 69% of the nitrogen was removed by acetic acid, compared with 7 and 19% for centrifuged solids dried at 90° C and freeze-dried, respectively. The protein in freeze-dried centrifuged solids was more soluble than that in materials dried at 90° C. The decrease in protein solubility of wheat flour fermentation products was again due to drying at 90° C, as well as to the fermentation process and the distillation of alcohol after fermentation.

Amino Acid Composition

The amino acid compositions of wheat and its fermentation products showed high levels of glutamic acid and proline (Table V). Wheat is deficient in lysine and low in isoleucine and threonine compared to the amino acid pattern for high-quality protein for human consumption (National Academy of Sciences 1980). Substantial increases in lysine, threonine, and isoleucine were observed for distillers' grains and centrifuged solids compared with wheat. Therefore, distillers' grains and centrifuged solids are expected to be nutritionally better than wheat. Distillers' solubles, however, had lower lysine and isoleucine than wheat and are probably nutritionally inferior to wheat. Soft wheat had higher lysine content than hard wheat; the distillers' grains and centrifuged solids from soft wheat also had higher lysine content than the corresponding material from hard wheat. The amino acid compositions of hard wheat 83% extraction flour and its

TABLE V
Amino Acid Composition of Wheat, Distillers' Grains, Centrifuged Solids, and Distillers' Solubles[a]

Amino Acids	Hard Wheat Grain	Hard Wheat Dist. Grain	Hard Wheat Cent. Solids	Hard Wheat Dist. Soluble	Soft Wheat Grain	Soft Wheat Dist. Grain	Soft Wheat Cent. Solids	Soft Wheat Dist. Soluble
Aspartic	5.7	7.1	6.5	4.5	6.0	7.8	7.6	4.9
Threonine	3.2	4.3	4.2	3.2	3.3	4.5	4.5	5.5
Serine	5.0	5.7	5.4	5.4	5.1	5.4	5.7	5.5
Glutamic	33.2	26.2	29.4	38.0	31.0	22.9	27.2	35.1
Proline	11.2	9.8	10.9	13.5	10.9	8.5	9.3	13.9
Glycine	4.4	5.4	4.4	5.0	4.8	5.8	4.8	5.3
Alanine	3.8	5.2	4.5	3.4	4.3	5.7	5.2	3.8
Valine	4.8	5.4	6.1	3.5	4.5	6.0	5.9	3.6
Methionine	1.2	1.5	1.8	0.9	1.4	1.6	1.6	0.9
Isoleucine	3.4	3.8	4.8	2.8	3.7	4.0	5.0	2.8
Leucine	7.1	8.0	8.5	6.0	7.4	8.1	8.9	6.0
Tyrosine	3.5	3.7	4.0	3.3	3.7	3.7	4.2	3.3
Phenylalanine	4.7	5.1	5.4	4.9	4.9	4.9	5.5	5.0
Lysine	2.8	3.9	3.7	2.6	3.2	4.4	4.1	2.8
Histidine	2.4	2.7	2.4	2.8	2.5	2.9	2.5	2.5
Arginine	5.7	7.6	5.8	4.6	6.0	7.8	6.0	4.6

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

fermentation products were close to the figures from the corresponding materials from hard wheat grain (not shown in Table V).

Functional Properties

Because gluten accounts for most of the wheat protein and wheat gluten is frequently used in baked goods, we evaluated wheat flour distillers' grains for that purpose. The inability to form a gluten ball is a good indication of denaturation. A vital wheat gluten, a devitalized wheat gluten, and the two wheat flour centrifuged solids (freeze-dried and dried at 90° C) were compared in their abilities to form gluten balls. The vital wheat gluten formed a cohesive and elastic gluten ball. The devitalized wheat gluten could not form a gluten ball. The hard and soft wheat flour centrifuged solids that were dried at 90° C formed weak gluten ball with little cohesiveness. The same centrifuged solids that were freeze-dried formed moderately cohesive gluten balls.

Mixograms of hard wheat flour centrifuged solids (freeze-dried and dried at 90° C) were compared with hard wheat flour. The centrifuged solids dried at 90° C had few dough-forming properties, but the freeze-dried centrifuged solids showed some dough-forming properties. When the soft wheat flour centrifuged solids (freeze-dried and dried at 90° C) were compared with soft wheat flour, the same trend as the hard wheat flour and centrifuged solids was observed. It appears from gluten ball-forming property and mixogram studies that the wheat flour-centrifuged solids dried at 90° C were denatured as far as baking bread is concerned. However, the freeze-dried centrifuged solids were only partially denatured and retained some functional properties for breadmaking.

CONCLUSION

The protein contents of hard wheat fermentation products were higher than the corresponding fractions from corn, and the fat contents of the wheat fermentation products were lower than those of the corresponding fractions from corn (Wu et al 1981). The lower fat contents of the wheat fermentation products may result in a better taste and better storage stability of the wheat products compared with those from corn. The lysine contents of wheat distillers' grains and centrifuged solids were higher than that of whole wheat and higher than those from corn. The soft wheat and its fermentation products have better essential amino acid composition contents than those from hard wheat. Since the proximate composition and amino acid composition of 83–85% extraction flour fermentation products were close to those of wheat grain, there appeared to be no advantage to ferment the 83–85% extraction flour to alcohol. The fiber content of centrifuged solids from 70% extraction flour was considerably lower than hard wheat distillers' grains, and the use of hard wheat flour centrifuged solids instead of distillers' grains may be desirable where fiber content of a food product such as baby food may be a limiting factor. The yield of soft wheat per acre is higher than hard wheat, and soft wheat also has higher starch contents; therefore, soft wheat will produce more alcohol per acre. Although the price of wheat is usually higher than corn, fermentation of wheat to make alcohol may be justified under certain conditions, such as in areas where corn is not grown or when a better-quality fermentation by-product is needed.

ACKNOWLEDGMENT

We thank N. E Harrison for technical assistance.

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[Received November 16, 1983. Accepted May 9, 1984]