Protein-Rich Residue from Ethanolic Fermentation of High-Lysine, Dent, Waxy, and White Corn Varieties

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ABSTRACT

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Corn is the predominant biomass used to make alcohol by fermentation in the United States. Dent corn has been used exclusively for fermentation so far; no information is available for other types of corn. Ground high-lysine (containing the opaque-2 gene), dent, waxy, and white corns were fermented to make alcohol. The stillage residue was then fractionated into distillers' grains, centrifuged solids, and stillage solubles. The highest yield of alcohol and lowest yield of fermentation residue were obtained from dent corn. The centrifuged solids had the highest protein content (37–49%), followed by distillers' grains (31–35% protein). Amino acid analyses of the stillage fractions from high-lysine corns contained higher lysine contents than corresponding fractions from other types of corns. Corn distillers' grains with solubles had a higher ash content than the corn varieties themselves; however, mineral composition of corn types or among corn distillers' grains with solubles was not significantly different. Distillers' grains from high-lysine corn have potential for use in human food and nonruminant feed because of higher predicted protein quality than those from dent corn.

Fermentation of cereal grains to produce ethanol yields a protein-rich stillage material after the alcohol is recovered. After distillation, the stillage is screened or centrifuged to remove suspended and insoluble solids. The remaining soluble fraction usually is evaporated to a syrup, which is marketed as-is or dried in conjunction with the solids from screening or centrifugation to yield distillers' dried grains with solubles (DGS). When the solids from screening or centrifugation are dried alone, the product is called distillers' dried grains (DG).

Corn (Wu et al 1981), wheat (Wu et al 1984), sorghum (Wu and Sexson 1984), and barley (Wu 1986), have been studied as feedstocks for ethanol fermentation. Chemical composition of DGS (Rasco et al 1987), nutritional quality of DGS (Dong et al 1987, Ranhotra et al 1982), and the mineral composition of stillage (Sebree et al 1983) were reported. Corn is most commonly used for commercial ethanol production in the United States (Morris 1983). Among corn types, only dent corn has been used, and little information is available for other types. High-lysine corn, containing the opaque-2 gene, has higher protein quality for humans and nonruminants than does yellow dent corn. White corn has lighter color than yellow corn, and may yield fermentation products that are lighter in color and maybe more acceptable for food use. Waxy corn starch contains amylopectin but no amylose. This paper compares ethanol yield, proximate composition, minerals, and amino acid profiles for fermentation products.

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MATERIALS AND METHODS

Fermentation and Fractionation of Stillage

SL 75, a high-lysine hybrid containing the opaque-2 gene, was from Crow's Hybrid Corn Company (Milford, IL), and waxy corn was a mixture of varieties from National Starch and Chemical Corporation (Watseka, IL). Dent and white corns, flame-dried corn distillers' grains (CDG), and steam-dried corn distillers' grains with solubles (CDGS) came from Agmart Inc. (Monmouth, IL).

Details of fermentation and stillage fractionation procedures were described (Wall et al 1983), and a summary will follow. Ground high-lysine, waxy, and dent corns (2,356 g) were each fermented in duplicate in 20-L stainless steel, temperature-controlled, jacketed fermentors equipped with stirrers. Taka-Therm α-amylase (Miles Laboratories, Elkhart, IN) converted starch to soluble dextrins at 90°C, pH 6.2; Miles Diazyme L-100 glucoamylase hydrolyzed dextrins to glucose at 60°C, pH 4.0; and 9 g of Fermivin yeast (*Saccharomyes cerevisiae*) (G. B. Industries, Des Plaines, IL) converted glucose to alcohol at 30°C for 66 hr, pH 4.5. Steam was used to distill alcohol from the fermented mash by external jacket. Filtration through cheesecloth under suction separated stillage into distillers' grains (solids on cheesecloth) and thin stillage. Centrifugation (model T-1 Sharples, 45,200 × *g*) separated the thin stillage into a solid fraction (centrifuged solids) and stillage solubles (Wu et al 1984).

Analytical Tests

Nitrogen (in quadruplicate), fat (in duplicate), crude fiber, and ash (in duplicate) composition were determined by AACC methods 08-03, 30-26, 32-15, and 46-13 (1983), and protein was calculated from Kjeldahl nitrogen × 6.25 (Watt and Merrill 1963).

TABLE I
Effect of Fermentation Time on Corn Slurry Compositions[a]

Corn/ Fermentation Time (hr)	g/100 g Slurry		
	Glucose	Glycerol	Ethanol
High-lysine			
0	15.3 (0.2)	0 (0)	0 (0)
24	0.88 (0.81)	0.68 (0.05)	8.2 (0.3)
48	0.06 (0.02)	0.73 (0.12)	8.7 (0.05)
66	0.03 (0.02)	0.71 (0.09)	8.6 (0.1)
Dent			
0	15.2 (0.05)	0 (0)	0 (0)
24	2.6 (0.5)	0.56 (0.00)	6.8 (0.1)
48	0.05 (0.01)	0.64 (0.01)	8.6 (0.2)
66	0 (0)	0.64 (0.02)	8.9 (0.2)
Waxy			
0	14.1 (0.5)	0.03 (0.03)	0 (0)
24	0.84 (0.56)	0.66 (0.02)	7.2 (0.1)
48	0.05 (0.01)	0.69 (0.02)	8.0 (0.4)
66	0 (0)	0.72 (0.02)	8.2 (0.3)
White			
0	16.0	0	0
24	2.4	0.60	6.3
48	0.05	0.61	7.6
66	0.06	0.63	7.4

[a]Value in parentheses is standard deviation.

Phosphorus was measured by the AOAC method 2.019 (1984). Starch was determined by a polarimetric method (Garcia and Wolf 1972), and moisture (in duplicate) was measured by heating samples in an air-oven at 100°C to constant weight. Moisture in CDG and CDGS came out slowly upon heating, therefore, a constant weight method rather than a fixed heating time gave better results. Minerals were determined by atomic absorption (Garcia et al 1974). Neutral detergent fiber (in duplicate) (McQueen and Nicholson 1979) was measured without sodium sulfite as an estimate of total dietary fiber. Glucose, glycerol, and ethanol contents were determined by high-performance liquid chromatography (Wu et al 1984).

For amino acid analysis, each sample was hydrolyzed for 24 hr by refluxing in 6*N* hydrochloric acid. The sulfur amino acids were determined after oxidation of the sample with performic acid (Moore 1963). Tryptophan analysis was conducted after samples were hydrolyzed for 18 hr in 6*N* sodium hydroxide. Hydrolyzed samples were evaporated to dryness, and residues were dissolved and analyzed in a Beckman model 334 amino acid analyzer system (Beckman Instruments, San Ramon, CA). Available lysine was determined by the method of Rao et al (1963). Data were analyzed by the method of Cavins and Friedman (1968).

RESULTS AND DISCUSSION

Yields and Chemical Composition of Fermentation Products

Fermentation of each corn was monitored at 0, 24, 48, and 66 hr by analyzing samples for glucose, glycerol, and ethanol. At 0 hr, glucose concentration was 14.1–16.0 g/100 ml slurry, and no glycerol or ethanol was detected (Table I). Glucose concentration decreased greatly by 24 hr and almost disappeared at 48 or 66 hr. Maximum ethanol concentrations (7.6–8.9 g/100 ml slurry) occurred at 48 hr for high-lysine and white corns and at 66 hr for dent and waxy corns. Glycerol was produced through a side reaction, and its concentration was between one-tenth and one-fourteenth of that of ethanol.

Means from overall analysis of variance of four corn slurry compositions from Table I as determined by least significant

TABLE II
Yield and Composition of Fermentation Products from Corn (dry basis)

Products[a]	Yield, % of Corn	Composition[b](%)					
		Protein	Fat	Crude Fiber	Neutral Detergent Fiber	Ash	Starch
High-lysine corn	···	8.8 (0.3)	4.2 (0.0)	2.7	10.2 (0.2)	1.2 (0.1)	70.3
DG	21.9 (0.4)	31.0 (1.0)	17.1 (0.1)	16.0 (0.3)	41.4 (0.4)	2.0 (0.0)	5.5 (1.5)
CS	0.9 (0.3)	48.7 (0.4)	4.6 (0.1)	8.6 (0.7)	36.6 (2.5)	2.5 (0.0)	7.6 (0.2)
SS	6.9 (0.2)	28.3 (0.4)	nd[c]	nd	nd	16.3 (0.1)	nd
DGS	29.7 (0.4)	32.4 (0.7)	12.7 (0.4)	12.4 (1.7)	38.3 (1.2)	5.0	7.3 (0.2)
Ethanol	37.1 (0.1)	···	···	···	···	···	···
Dent corn	···	9.1 (0.2)	3.1 (0.1)	2.7	10.9 (0.2)	1.2 (0.1)	73.7
DG	19.7 (0.8)	35.1 (0.8)	12.1 (0.1)	13.7 (0.9)	41.1 (1.0)	2.0 (0.0)	10.1 (0.7)
CS	1.9 (0.2)	41.0 (1.1)	2.0 (0.2)	7.1 (1.7)	38.7 (2.8)	2.4 (0.0)	14.0 (2.0)
SS	7.0 (0.3)	19.1 (0.2)	nd	nd	nd	19.0 (0.2)	nd
DGS	28.6 (0.3)	33.4 (1.1)	9.9 (0.0)	9.6 (0.2)	30.4 (0.7)	5.2 (0.1)	12.1 (1.1)
Ethanol	40.7 (1.1)	···	···	···	···	···	···
Waxy corn	···	10.3 (0.2)	4.4 (0.1)	3.4	12.3 (0.5)	1.5 (0.1)	70.4
DG	26.3 (1.7)	33.2 (0.6)	11.8 (0.3)	12.1 (1.1)	37.4 (0.3)	2.3 (0.1)	10.0 (2.0)
CS	1.3 (0.4)	46.2 (0.7)	4.4 (0.0)	12.5 (0.3)	34.5 (2.6)	2.5 (0.0)	7.3 (0.7)
SS	7.4 (0.4)	15.6 (0.2)	nd	nd	nd	13.2 (0.0)	nd
DGS	35.0 (0.9)	31.3 (1.6)	10.6 (0.3)	10.9 (1.1)	31.7 (0.1)	4.2 (0.0)	11.1
Ethanol	39.0 (0.5)	···	···	···	···	···	···
Whate corn	···	9.1 (0.1)	5.1 (0.1)	1.6	7.9 (0.0)	1.0 (0.1)	70.9
DG	20.2	35.3 (0.6)	13.5 (0.1)	11.5	33.5 (0.1)	1.2 (0.1)	12.8
CS	3.0	36.8 (0.3)	7.2 (0.1)	7.0	37.9 (0.3)	1.9 (0.0)	12.8
SS	7.7	16.3 (0.2)	nd	nd	nd	15.4 (0.1)	nd
DGS	30.9	29.1 (0.1)	12.3 (0.0)	8.8	26.1 (0.6)	4.3 (0.0)	16.2
Ethanol	35.0	···	···	···	···	···	···

[a]DG = distillers' grains, CS = centrifuged solids, SS = stillage solubles, DGS = distillers' grains with solubles. Carbon dioxide was vented during fermentation and accounted for the rest of the material balance for corn.
[b]Value in parentheses is standard deviation.
[c]nd = Not determined.

differences ($P < 0.05$) showed that there was no significant difference in glucose and ethanol concentrations at 48 and 66 hr. There were significant differences in glucose and ethanol concentrations at 24 and 0 hr, however. There were significant differences in glycerol concentration between 0 and 24 hr, but no difference between 24, 48, or 66 hr.

Dent corn yielded the most ethanol and least DGS; waxy corn yielded most DGS (Table II). Centrifuged solids had the highest protein content of all fractions. Protein, fat, crude fiber, and neutral detergent fiber contents of DG are several times those of the starting corns. Stillage solubles had the highest ash but lowest protein content of all fermentation by-products. White corn contained the least crude fiber and neutral detergent fiber of all corns studied. White corn fermentation products had lighter color than the corresponding dent corn product. This may indicate potential for white corn fermentation products in foods such as bread or baked goods, where light color is desirable.

The data from Table II were analyzed by protected ($P < 0.05$) least significant differences. Centrifuged solids from high-lysine corn had higher protein content than centrifuged solids from dent and waxy corns. Stillage solubles from high-lysine corn had higher protein content than stillage solubles from dent, white, and waxy corns. Distillers' grains from high-lysine corn had lower starch content than distillers' grains from dent and white corns.

Mineral Composition of Corn and Corn Distillers' Grains with Solubles

Dent, high-lysine, and waxy corns are rich in magnesium, potassium, and phosphorus (Table III); mineral profiles are similar. Mineral contents of CDGS are considerably higher, due to hard water (high in calcium and magnesium used in processing), neutralization during pH adjustments (forming sodium chloride), and from concentrating minerals during fermentation (when starch was consumed, other components such as minerals were concentrated). Commercial steam-dried CDGS had higher calcium and iron but lower sodium contents than CDGS made in the laboratory; this may reflect differences in the amount or concentration of concentrated solubles added back when the material was co-dried, or different water and chemicals used in processing. Commercial flame-dried CDG generally had lower mineral contents than commercial CDGS because distillers' solubles are richer in minerals than distillers' grains. These analyses all indicate that CDGS is rich in calcium, magnesium, iron, potassium, and phosphorus, making CDGS a potentially valuable source of minerals in food and feed.

Amino Acid Composition of Corn and Fermentation Products

Table IV shows that dent corn is rich in glutamic acid plus glutamine, leucine, aspartic acid plus asparagine, and proline.

TABLE III
Mineral Composition of Corn and Corn Distillers' Grains with Solubles (CDGS)
(ppm, dry basis)

Products[a]	Ca	Na	Mg	K	Fe	Al	Cu	Cr	Se	Mn	Zn	P
HL	0[b]	0[b]	1,300	3,300	19	8.0	3.6	0[b]	45	6.4	21.2	2,200
HL CDGS	690	2,700	4,300	14,000	100	47	16	5.3	40	22	90	7,800
D	0[b]	0	1,500	3,200	19	5.2	2.9	1.4	53	5.0	21	3,000
D CDGS	700	3,000	4,600	14,000	65	19	12	5.2	0[d]	17	70	8,800
Wa	0[b]	6	1,500	3,300	23	22	3.3	0[b]	16	8	28	2,700
Wa CDGS	500	2,200	3,900	11,000	71	17	11	3.5	14	20	60	3,600
D CDG[c]	200	120	1,000	1,000	170	140	8.9	3.9	27	9.2	46	6,200
D CDGS[d]	1,000	370	3,900	11,000	300	56	25	3.2	51	24	70	6,400

[a]HL = high-lysine corn, D = dent corn, Wa = waxy corn.
[b]Not detected. The detection limits by atomic absorption for Ca, Na, Cr, and Se are 0.002, 0.0002, 0.005 and 0.36 ppm, respectively.
[c]Commercial flame-dried corn distillers' grains.
[d]Commercial steam-dried corn distillers' grains with solubles.

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

	High-Lysine Corn[b]				Dent Corn				Waxy Corn				White Corn			
Amino Acids	Grain	DG	CS	DGS	Grain	DG	CS	DGS	Grain	DG	CS	DGS	Grain	DG	CS	DGS
Aspartic acid[c]	8.9	8.6	9.4	7.7	6.2	7.0 (0.1)	9.0	7.1	7.2	6.7	9.2	6.9	6.9	7.6	9.2	7.3
Threonine	3.9	4.6	5.1	4.4	3.5	4.0 (0.0)	4.9	4.1	4.0	3.9	5.1	4.1	3.6	4.2	5.0	4.5
Serine	4.7	5.3	5.2	4.9	4.6	5.5 (0.1)	5.3	5.0	5.3	5.3	5.6	5.2	4.8	5.5	5.5	5.6
Glutamic acid[d]	16.9	16.9	13.9	16.7	18.0	20.2 (0.5)	14.3	17.6	20.1	19.5	15.2	19.1	19.6	22.5	15.9	20.0
Proline	9.0	8.5	6.4	9.5	8.8	9.5 (0.1)	6.1	8.8	9.0	8.8	6.2	9.2	8.4	9.6	6.8	10.4
Glycine	5.2	5.0	5.0	5.5	3.7	3.9 (0.1)	4.5	4.1	4.2	3.7	4.7	4.1	4.0	3.7	4.7	4.1
Alanine	6.4	7.1	6.4	7.0	6.9	7.7 (0.3)	6.6	7.4	8.0	7.9	7.1	8.0	7.2	8.1	7.4	8.2
Valine	5.6	5.8	6.6	5.8	4.8	5.3 (0.2)	6.3	5.3	5.2	5.0	6.4	5.0	5.1	5.6	6.5	5.4
Half-cystine	2.3	2.3	1.6	2.2	2.1	1.8	1.3	2.2	1.8	2.4	1.7	1.4	2.0	1.7	1.3	1.8
Methionine	1.6	···	2.0	1.5	1.3	1.9 (0.1)	1.8	1.8	2.1	2.4	1.9	2.3	1.5	···	1.9	1.4
Isoleucine	3.3	4.0	4.6	3.8	3.2	3.9 (0.1)	4.5	3.8	3.6	3.7	4.6	3.8	3.4	3.9	4.6	3.8
Leucine	9.1	10.7	9.6	9.9	11.9	14.5 (0.3)	10.4	12.6	13.2	13.9	10.8	13.4	12.1	15.4	11.5	13.8
Tyrosine	3.8	4.0	4.2	3.9	3.9	4.4 (0.1)	4.2	4.1	4.5	4.4	4.6	4.6	4.0	4.6	4.5	4.7
Phenylalanine	4.2	5.1	5.3	4.7	4.7	5.9 (0.1)	5.2	5.2	5.2	5.5	5.5	5.6	4.9	6.1	5.4	5.3
Lysine	4.4	4.2	6.1	4.4	3.1	2.9 (0.3)	5.5	3.1	2.8	2.7	6.4	3.0	3.2	2.2	5.7	3.0
Histidine	3.5	3.4	2.8	3.6	2.9	2.9 (0.1)	2.5	2.8	2.9	2.6	2.6	2.8	2.9	2.7	2.7	2.9
Arginine	7.1	6.7	7.1	6.5	5.8	5.7 (0.1)	5.9	5.5	6.1	5.1	6.4	4.8	5.9	4.9	6.1	5.1
% Available lysine	3.4	3.8	nd[e]	3.3	2.7	2.5	nd	2.8	nd	nd	nd	nd	nd	nd	nd	nd
Tryptophan	1.4	1.3	1.8	1.1	0.9	1.0	1.8	0.8	nd	nd	nd	nd	nd	nd	nd	nd

[a]Grams of amino acid per 16 g of nitrogen recovered. Value in parenthesis is standard deviation. Average % nitrogen recovery was 97.6.
[b]DG = distillers' grains, CS = centrifuged solids, DGS = distillers' grains with solubles.
[c]Aspartic acid includes asparagine.
[d]Glutamic acid includes glutamine.
[e]nd = Not determined.

Among the essential amino acids, dent corn is low in lysine, tryptophan, and isoleucine (National Academy of Sciences 1980). There were no significant differences in amino acid composition between any pair of fractions from dent corn, because correlations were 0.90 or greater.

High-lysine corn contains more lysine than dent corn (4.4 vs. 3.1 g/16 g N); all high-lysine corn fermentation products also contained more lysine than the corresponding dent corn products (Table IV). High-lysine corn also contained more tryptophan than dent corn. High-lysine corn and its fermentation products also contained more available lysine than dent corn and its corresponding fermentation products. Correlations of amino acid compositions between any pair of fractions from high-lysine corn were 0.90 or greater indicating no significant differences in composition.

The amino acid compositions of waxy corn, white corn, and their fermentation products (Table IV) were not significantly different from those of dent corn and its products. (Correlations of amino acid compositions between any pair of fractions within or across corn type were all 0.90 or greater.) Since lysine is the first limiting amino acid in dent corn, the higher lysine content of centrifuged solids and of all high-lysine fermentation products suggests better protein quality for these products.

CONCLUSIONS

Among dent, waxy, high-lysine, and white corns, dent corn yields the most alcohol and least fermentation residue. Waxy corn yielded the most fermentation residue of all corns. Centrifuged solids had the highest protein and lysine contents of residue fractions.

Fermentation products from high-lysine corn have potential for human food and for nonruminant feeds because of their higher lysine content than fractions from dent corn. White corn fermentation products may also find applications in baked goods, where lighter color is desirable. CDGS are rich in mineral nutrients.

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