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STORAGE EFFECTS IN WHEAT

Changes in Lipid Composition in Wheat during Storage Deterioration

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Titratable acidity was about 7% higher in benzene extracts than in petroleum ether extracts of wheat. Benzene extracts of moistened wheat contained more free fatty acids than did extracts of wheat redried to 11 to 12% moisture. Changes in lipid composition during grain deterioration were followed by qualitative and quantitative thin-layer chromatography (TLC) and fractionation on silicic acid columns. Deterioration of wheat was accompanied by formation of at least four unidentified compounds that showed autofluorescence under ultraviolet light. Grain deterioration was accompanied by lowering of polar lipids and rapid disappearance of at least five ninhydrin- or Dragendorff-reagent positive polar lipids. The breakdown of polar lipids was more rapid and more intensive than formation of free fatty acids or disappearance of triglycerides.

DETERIORATION of grain and of milled products in storage is accompanied by increased acidity. As early as 1914, Besley and Boston (*6*) suggested acidity as a factor in determining soundness of corn. The acids formed include free fatty acids, acid phosphates, and amino acids; but at early stages of deterioration, fat acidity increases at a much greater rate than either of the other two types or all types of acidity combined (*24*). Swanson (*22*), Zeleny and Coleman (*24*), and Bolling (*8*) suggested determining fat acidity as one of the best measures of grain damage.

Zeleny (*23*), Geddes (*11*), Milner and Geddes (*19*), Hutchinson (*15*), and James (*16*) have summarized changes occurring in wheat stored under adverse conditions. While a large volume of information is available on the effects of storage conditions on changes in lipid content and free fatty acid levels, relatively little is known about transformations occurring in the wheat lipids during storage. Free fatty acids may result from the action of seed lipases; but it seems that in deteriorating stored wheat the acids result primarily from fungal lipase activity (*3, 4, 12, 20, 21*).

The purpose of this research was to determine changes in composition during breakdown of lipids by molds.

Experimental

Wheat Samples. Sound, hard red winter wheat of the Comanche variety and soft red winter wheat of the Seneca variety stored for about 6 months after harvest at +4° C. were used. The original moisture of the wheat samples was 12.8 and 13.4%, respectively, and each was moistened to 18 or 22%. The wheat samples were placed in narrow-mouthed glass containers, water was added, the contents were mixed thoroughly, the containers were loosely plugged with cotton, and the wheat was allowed to attain desired moisture levels during storage for 48 hours at +4° C. The conditions of storage would seem to allow for relatively easy gas exchange. However, the possibility of limited carbon dioxide accumulation, especially at advanced stages of deterioration, cannot be excluded. The samples were stored at 49° C. and shaken daily, and subsamples were removed for analyses.

Fat Acidity. Fat acidity was determined by two procedures (*2*), with benzene and petroleum ether as extractants. Fat acidity determinations were made on both moistened samples and samples dried to 11 to 12% moisture in a forced draft oven below 60° C. Additionally, free fatty acids were determined in 35 samples of sound wheat harvested in 1963 and stored up to 6 months at 4° C.

Mold Counts. Mold counts were made by the procedure of Christensen (*9*).

Column Chromatography. Lipids from original wheat, and 22% moisture wheat stored at 49° C., were separated into nonpolar and polar fractions. Wheat was ground to pass a 20-mesh sieve on a micro-Wiley mill, and 15-gram samples were extracted in a Stein mill with 100, 50, and 50 ml. of water-saturated 1-butanol, for 4, 2, and 2 minutes, respectively. The combined extracts were decanted, filtered, and evaporated almost to dryness under vacuum in a glass apparatus at about

45° C. The extracts were kept under vacuum in a desiccator 40 hours over P_2O_5 at 4° C., and dissolved three times with Skellysolve B, and the combined upper layers were evaporated under vacuum after centrifugation. The lipids were dissolved in 80 ml. of a chloroform-methanol mixture (2 to 1) and washed with 17.5 ml. of 0.04% calcium chloride, followed by two washings with 10 ml. each of aqueous 0.02% calcium chloride solutions, and the volume of the washed, lipid-containing layer was made to 50 ml. with chloroform. Total lipids were determined by drying of two 5-ml. fractions to constant weight, and the remaining 40 ml. were concentrated under vacuum to about 2 ml. for separation on silicic acid columns, 15 cm. long and 2 cm. in diameter. Twenty-gram lots of silicic acid (Mallinckrodt, Chemical Works) for chromatography of lipids were washed with distilled water and dried at 120° C. for 4 hours. Up to 500 mg. of lipids could be separated on the column. The silicic acid was washed twice with 60 ml. of a 7 to 1 and once with 60 ml. of a 15 to 1 chloroform-methanol mixture, and finally with 80 ml. of chloroform. The slurry was transferred to columns, and the concentrated lipid extract applied on top of the column. The neutral lipids were eluted with 120 to 150 ml. of chloroform and the polar lipids with 120 to 150 ml. of methanol. The completion of elution was monitored by thin-layer chromatography (TLC). Each of the two fractions was concentrated to 100 ml. under vacuum, and two 10-ml. portions were drawn from each for determination of neutral and polar lipid content, respectively. The remaining fractions were freed of solvent under reduced pressure, dissolved in a 2 to 1 mixture of chloroform-methanol, and used for TLC.

Preparation of Lipid Fractions for Quantitative TLC. For calibration purposes the lipid extract from Kaw, a variety of hard red winter wheat, prepared as described under column chromatography, was separated into 10 fractions by elution from silicic acid (from Bio-Rad Laboratories, Richmond, Calif.), employing the apparatus described by Hirsch and Ahrens (*14*). The neutral lipids were eluted by the solvents given by Barron and Hanahan (*5*), followed by elution of polar lipids, according to Hanahan and coworkers (*13*). The ten fractions obtained contained as main components: hydrocarbons; sterol-esters and esters of high alcohols; triglycerides; sterols and higher alcohols; diglycerides; monoglycerides; polyglycerol phosphatides and glycolipids; phosphatidyl ethanolamine and phosphatidyl serine; inositol phosphatide, lecithin, and lysophosphatidyl ethanolamine; and lysolecithin. The fractions were evaporated to almost dryness under reduced pressure, and dissolved in a 2 to 1 mixture of chloroform-methanol.

Thin-Layer Chromatography (TLC). Glass plates (20 × 20 cm.) were coated with a 250-micron layer of silica gel G (E. Merck, A. G., Darmstadt, Germany) in a conventional manner, using a commercial spreader (C. A. Brinkmann and Co., Great Neck, N. Y.). The plates were dried for 3 hours at 130° C. The most useful solvents for ascending development of lipid spots (20 to 40 μg.) were chloroform, a mixture of petroleum ether–ether–acetic acid (80:20:1) (ether mixture), or a mixture of chloroform–methanol–water (65:25:4) (chloroform mixture). All solvents were of analytical grade, redistilled from glass; ether was redistilled from above metallic sodium. The spots were located and visualized by exposure to iodine vapor; by spraying the plates, after they dried, with sulfuric acid and heating for 15 minutes at 130° C.; with 0.2% solution of ninhydrin in butanol and 1% pyridine (*17*) (as a spray specific for free amino acids); with a modified Dragendorff's reagent (*18*) for choline phosphatides and galactolipids; or with a molybdenum spray (*10*) for the detection of polar lipids. Lipids separated by thin-layer chromatography were tentatively identified by comparison of R_f values with literature data (*7*, *17*, *18*), use of specific sprays, and comparison of R_f values with those of pure compounds. Among the nonpolar lipids, lauric acid, myristic acid, 1-monopalmitin, 2-monopalmitin, 1,2-dipalmitin, 1,3-dipalmitin, tripalmitin, and tristearin (H. L. Mitchell, Biochemistry Department, Kansas State University) were used. Among the polar lipids (phosphoglyceride standards from Applied Science Laboratories, Inc., State College, Pa.), plant lecithin, phosphatidyl ethanolamine, and phosphatidyl serine were used. Plates were viewed under incandescent light. Additionally, plates were observed under ultraviolet light (long wave, 3570 A.) prior to and after being sprayed with sulfuric acid.

Quantitative Determination of Lipids by Thin-Layer Chromatography. Employing the densitometer with scanning stage and Varicord recorder (Photovolt Corp., New York, N. Y.), lipid fractions from silicic acid columns and lipid mixtures separated by TLC were measured quantitatively (*7*). Compounds separated by silicic acid column chromatography were used for preparing calibration curves.

Table I. Fat Acidity in Lipids of 35 Sound Wheat Samples Extracted with Benzene and Petroleum Ether

(Mg. KOH used to neutralize free fatty acids in 100 grams of wheat)

Fat Acidity	Benzene Extract	Petroleum Ether Extract
Maximum	17.6	16.4
Minimum	10.3	10.0
Average	13.1	12.3

Results and Discussion

Fat acidity determined in 35 samples of sound wheat was higher in benzene than in petroleum ether extracts (Table I). TLC has shown that the difference could be attributed, in part at least, to different lipid components in the two extracts.

Table II. Fat Acidity in Benzene Extracts of 18% Moisture Wheat Stored at 49° C.

(Mg. KOH used to neutralize free fatty acids in 100 grams of wheat, dry matter basis)

Length of Storage, Days	Comanche				Seneca			
	Moistened Wheat		Redried Wheat		Moistened Wheat		Redried Wheat	
	Moisture, %	Fat acidity, mg. KOH	Moisture, %	Fat acidity, mg. KOH	Moisture, %	Fat acidity, mg. KOH	Moisture, %	Fat acidity, mg. KOH
8	17.7	21.7	11.7	15.6	17.0	20.9	11.9	15.6
15	17.1	43.1	11.6	34.9	16.4	27.5	11.6	21.3
31	16.7	53.3	11.7	45.5	15.8	38.1	12.0	34.5
42	14.6	47.1	...	...	13.8	41.0	...	...
56	12.0	39.0	...	...	11.8	39.8	...	...
70	9.7	47.1	...	...	9.6	48.7	...	...

Table III. Fat Acidity in Benzene Extracts, and Mold Counts (Colonies per Gram) of 22% Moisture Wheat Stored at 49° C.

(Mg. KOH used to neutralize free fatty acids in 100 grams of wheat on a dry matter basis)

Length of Storage, Days	Comanche			Seneca		
	Moisture, %	Mold count × 10^3/gram	Fat acidity, mg. KOH	Moisture, %	Mold count × 10^3/gram	Fat acidity, mg. KOH
0	12.8	1	12.3	13.4	1	13.1
0	22.3	1	23.8	22.0	1	23.4
7	21.7	...	24.2	21.3	...	23.4
14	20.9	250	27.5	20.5	180	27.1
21	20.8	...	33.2	20.3	...	32.8
28	21.7	925	35.3	20.9	1125	36.9
42	21.6	1875	30.8	20.7	1475	44.3
56	21.9	50	27.1	21.0	2475	43.1
70	22.5	...	23.4	21.4	687	34.9
100	22.4	...	22.6	21.3	...	27.9
132	24.4	...	19.7	22.7	...	24.6

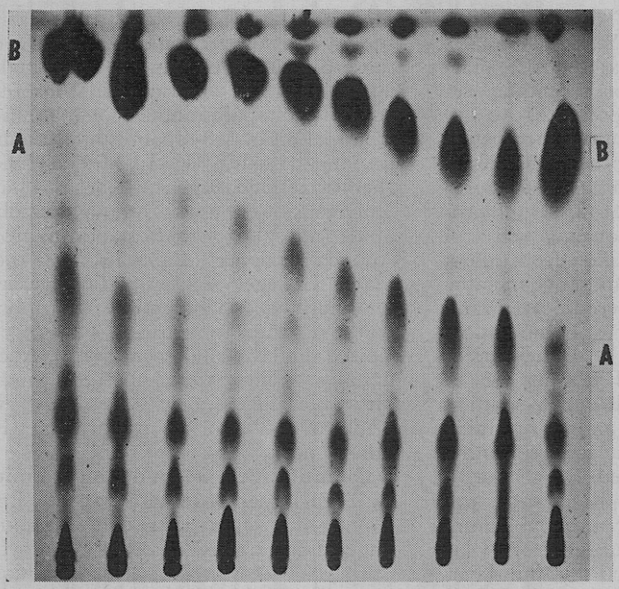

Figure 1. Thin-layer chromatography of benzene extracts of 22% moisture hard wheat stored for 0, 0, 14, 28, 42, 56, 70, 100, 132, and 0 days at 49° C.

Developed with chloroform; spots visualized by spraying with sulfuric acid; picture taken in UV. *A.* Free fatty acids. *B.* Triglycerides. First sample original wheat, other samples moistened to 22%

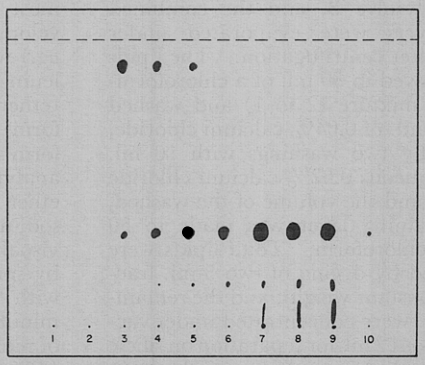

Figure 3. Thin-layer chromatography of benzene extracts of hard wheat stored for 0, 0, 14, 28, 42, 56, 70, 100, 132, and 0 days at 49°C.

Developed with ether mixture; spots observed under UV light. First sample original wheat, other samples moistened to 22%

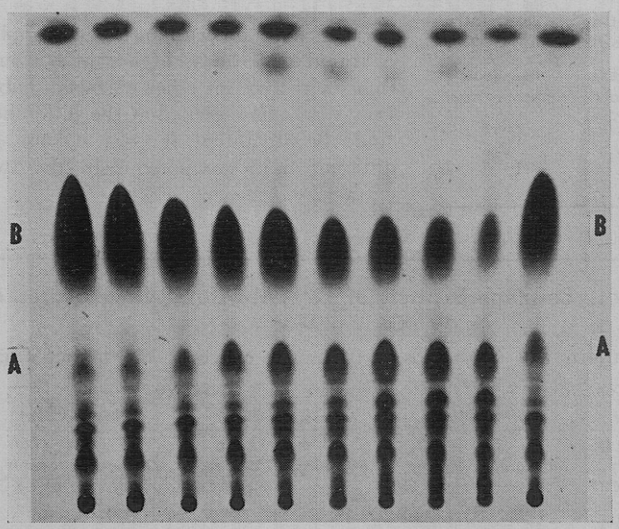

Figure 2. Thin-layer chromatography of benzene extracts of hard wheat stored for 0, 0, 14, 28, 42, 56, 70, 100, 132, and 0 days at 49° C.

Developed with ether mixture; spots visualized by spraying with sulfuric acid; picture taken in UV light. *A.* Free fatty acids. *B.* Triglycerides. First sample original wheat, other samples moistened to 22%

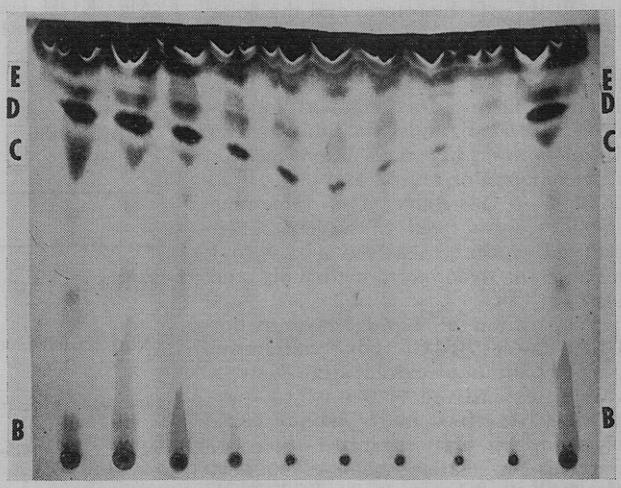

Figure 4. Thin-layer chromatography of polar lipids from benzene extracts of 22% moisture hard wheat stored for 0, 0, 14, 28, 42, 56, 70, 100, 132, and 0 days at 49° C.

Developed with chloroform mixture; spots visualized by spraying with sulfuric acid. Tentatively identified as: *B.* Phosphatidyl serine. *C.* Phosphatidyl choline. *D.* Digalactosyl glyceride. *E.* Monogalactosyl glyceride

Free fatty acids in wheat moistened to about 18% moisture increased with length of storage at 49° C. The benzene extracts contained consistently less titratable acidity, if dried prior to extraction to 11 to 12% moisture, than if titration was performed on extracts of moistened grain (Table II). The moisture decreased as a result of partial loss of water of the wheat stored at 49° C. in loosely plugged containers for relatively long times. Consequently, fatty acids remained at a relatively uniform level after 6 weeks, presumably as a result of checked fungal activity in the partly dehydrated samples. The checking of fungal activity was probably also due in part to accumulation of carbon dioxide in the containers. TLC of benzene extracts from damp wheat showed a number of unidentified compounds that were absent from extracts of the dry wheat.

Chromatograms of benzene extracts of 22% moisture wheat stored for up to 132 days at 49° C. show a consistent increase in fatty acids (*A*) and a decrease in triglycerides (*B*) (Figures 1 and 2). The increase in fat acidity, concomitant with increase in fungal growth, is summarized in Table III. Microbial counts of viable organisms in wheat stored beyond 70 days showed abundant bacterial growth. Consequently, mold counts of wheat stored longer than 70 days are not included in Table III. Fungal attack was accompanied by elaboration of at least four compounds varying in R_f values, which could be seen under ultraviolet light without spraying the plate (Figure 3). The autofluorescing compounds formed seem related to

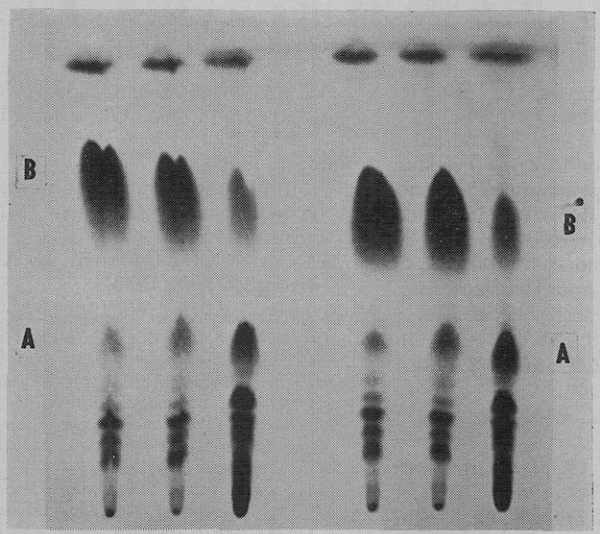

Figure 5. Thin-layer chromatography of neutral lipids from water-saturated butanol extracts of 22% moisture hard wheat

Left to right. Original wheat, wheat stored for 140 days at 4° C., and wheat stored for 140 days at 49° C. Right half is replication of left 3 spots. Developed with ether mixture; spots visualized by spraying with sulfuric acid; picture taken in UV light. *A.* Free fatty acids. *B.* Triglycerides

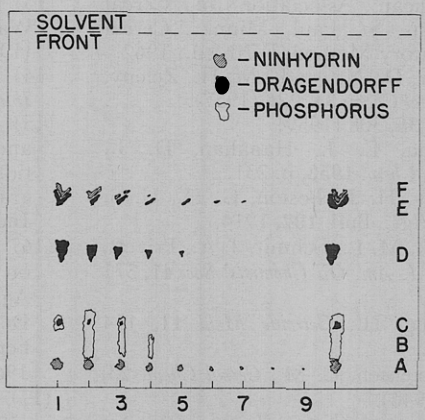

Figure 6. Diagrams of thin-layer chromatography of benzene extracts of 22% moisture soft wheat stored for 0, 0, 14, 28, 42, 56, 70, 100, 132, and 0 days at 49° C.

Developed with chloroform mixture. Combined results from spraying one plate with ninhydrin followed by Dragendorff reagent, and one plate with ninhydrin followed by phosphorus reagent. *First sample original wheat, other* samples moistened to 22%. Identification as in Figure 4

Table IV. Total, Neutral, and Polar Lipids (Dry Matter Basis) of 22% Moisture Wheat Stored at 49° C.

Length of Storage, Days	Comanche				Seneca			
	Total lipids, %	Neutral, %	Polar, %	Recovery, %	Total lipids, %	Neutral, %	Polar, %	Recovery, %
0[a]	1.97	1.26	0.63	96.0	2.27	1.47	0.77	98.7
140[b]	2.00	1.20	0.67	94.0	2.24	1.43	0.76	97.8
140	1.52	0.98	0.38	89.5	1.65	1.22	0.39	97.6
148	1.43	1.06	0.37	100.0	1.60	1.22	0.36	98.9
160	1.17	0.94	0.23	99.8	1.38	1.13	0.25	100.0

[a] In original wheat.
[b] In wheat stored at 4° C.

Table V. Neutral and Polar Lipid Fractions (Dry Matter Basis) of 22% Moisture Soft Wheat Stored at 49° C., as Determined by Quantitative TLC

Length of Storage, Days	Moisture, %	Nonpolar Fractions		Polar Fractions		
		Triglycerides, %	FFA, %	D, %	E, %	F, %
0	13.4	0.669	0.033	0.091	0.018	0.049
0	22.0	0.714	0.040	0.113	0.057	0.094
14	20.5	0.550	0.045	0.098	0.024	0.073
28	20.9	0.479	0.055	0.088	0.009	0.042
42	20.7	0.419	0.073	0.034	0.007	0.011
56	21.0	...	...	0.026	0.007	0.010
70	21.4	0.329	0.125	0.018	...	0.005
100	21.3	0.270	0.160	...	0.004	...
132	22.7	0.258	0.225	0.009	0.003	...
132[a]	21.4	0.528	0.045	0.099	0.027	0.073

[a] At 4° C.

metabolic processes of fungi attacking wheat. The timing and sequence of their formation might be of interest in following wheat deterioration. Preliminary information indicates that the fluorescing compounds are elaborated by some fungi only.

The intensive disappearance of polar lipids in deteriorating grain is illustrated in Table IV and Figure 4. Whereas both neutral and polar lipids decreased during storage (Tables IV and V), the neutral lipids decreased only slowly and actually constituted at advanced stages of deterioration a larger fraction of the residual lipid extract as polar lipids rapidly disappeared. The decrease in triglycerides and increase in fatty acids in the nonpolar fraction of water-saturated butanol extracts are shown in Figure 5.

To identify the polar lipids metabolized by the proliferating fungi, lipids separated on TLC by chloroform mixture were sprayed with reagents specific for free amino acids, choline and galactolipids, and phosphorus-containing lipids (Figure 6). These compounds are tentatively identified as phosphatidyl serine (*B*), phosphatidyl choline (*C*), digalactosyl glyceride (*D*), and monogalactosyl glyceride (*E*). Phosphatidyl ethanolamine had a R_f value intermediate between digalactosyl glyceride and monogalactosyl glyceride. Two-dimensional TLC (*1*) indicated that monogalactosyl glyceride, separated by the one-dimensional method, also contained phosphatidic acid. It now seems possible to suggest that early stages of grain deterioration might be detected by following the disappearance of certain polar lipids or by assays of enzymes involved in metabolism of polar lipids, provided the enzymes are elaborated by fungi and absent from sound wheat.

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NUTRITIVE VALUE OF GRAINS

Amino Acids and Proteins in Sorghum Grain

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Determination of the protein content and amino acid composition of sorghum grain indicates variations due to hybrids and location. Protein values, N × 6.25, ranged from 8.65 to 12.50%. Analyses by ion exchange procedures of 30 samples of grain sorghums representing 15 different hybrids grown at two locations show variations in amino acid content. Statistical analysis of the amino acid data, per cent of sample and per cent of amino acid in the protein, indicated significant differences ($P < 0.05$) in amino acid level due to hybrids. A significant ($P < 0.05$) location effect was found for the amino acids as per cent of the sample. Of amino acids important from a nutritive standpoint, methionine ranged from 1.22 to 1.97% and lysine from 1.81 to 2.49% of the protein.

Protein is one of the most important portions of animal diets. Since feed grains make up approximately 50% or more of the diet, protein composition and content of the grains are highly important. The importance of grain sorghums has increased materially in the last ten years, during which standard varieties were largely replaced by hybrids. In 1952 United States production was 90,741,000 bushels; 1962 it was 509,137,000 bushels, or 5.6 times more. Studies have indicated that the feed values of grain sorghums and corn are equal. Oklahoma workers (*20*) show that grain sorghums (sorghum) could effectively replace corn in chick diets. Other work has indicated similar replacement values with swine and sheep (*2, 9, 12*). More recent work with laying hens has indicated that sorghum may not be as well utilized as corn. Malik and Quisenberry (*11*) found combinations of corn and sorghum more effectively utilized than sorghum alone.

Research has indicated that hybridization lowered the protein content of corn (*6*) and that quality of protein, as measured by amino acid analysis, varies. Differences in amino acid composition have been ascribed largely to the increasing percentage of zeins in the protein as total protein in the grain increases (*6–8, 16, 17, 22*). Other workers (*14*) have not detected significant differences in methionine, tryptophan, and lysine levels in corn when protein content ranged from 8.9 to 13%. Doty (*5*) reported that amino acid composition of corn might be genetically controlled. Wolfe and Fowden (*21*) found considerable differences in amino acids present in various corn varieties. Largest differences were for arginine, histidine, lysine, leucine, threonine, and valine, and between varieties least related genetically.

Table I. Effects of Hybridization and Location on Average Protein Content of Hybrid Sorghum Grain

Hybrid	Location: Hiawatha	Manhattan	Newton	Mean
	% Protein[a]			
59CH5	12.4	11.8	12.2	12.1
61MH233	12.0	10.3	11.3	11.2
60MH173	11.1	10.0	12.3	11.1
60MH172	11.1	9.9	12.1	11.0
59CH71	11.4	10.3	11.2	11.0
61MH235	11.3	10.4	11.3	11.0
KS651	11.3	10.3	11.1	10.9
59MH153	10.8	10.1	11.9	10.9
58MH105	11.5	9.9	11.2	10.9
60MH177	10.8	9.6	11.9	10.8
59MH152	10.6	9.3	11.5	10.5
RS610	10.7	10.0	10.6	10.4
KS652	10.6	9.8	10.6	10.3
60MH212	10.5	9.2	10.6	10.1
KS701	10.2	9.0	10.4	9.9
Mean	11.1	10.0	11.3	

[a] N × 6.25.