According to a review by Horton (1969), alkali treatment of *N*-acetylhexosamines leads to the production of three furan compounds which subsequently can react with *p*-DMAB to yield the characteristic spectrum with two distinct maxima. Our observation that the spectra obtained from heated milk and from *N*-acetylhexosamines, heated in a simulated milk salt system, are similar to those obtained by alkali pretreatment suggests that the chromogens formed in milk are the same as those identified in the Morgan–Elson reaction, namely mono- and dianhydro derivatives of *N*-acetylhexosamines containing one and two double bonds, respectively. The relative amounts converted are not known, but may represent only a small portion of the original concentration present in milk (Horton, 1969).

The heat-induced *p*-DMAB reactivity of milk represents additional evidence for the effect of elevated temperatures on the milk system. However, the study has shown that the reaction is independent of the parallel reaction leading to the accumulation of reductones

Reference has been made to a secondary color formation by unheated or heated milk when kept in contact with the *p*-DMAB reaction mixture over a prolonged period (several hours). This reaction is not heat induced and does not appear to involve amino sugars and may have a bearing on the relatively high blank values obtained for unheated milk. This background color appeared to be a contribution mainly by an as yet unidentified compound found in α_s-casein but not in the other milk proteins.

Two direct practical applications of the developed *p*-DMAB reactivity tests are apparent. Since the change in the *p*-DMAB reactivity of milk is directly proportional to the heat treatment of milk, the test may serve as a heat index for high heat-treated milk products and in this respect would complement existing methods that demonstrate heat-induced alterations in milk; and since the solvent mixture used in the test produces a clear supernatant from milk, it may be used for determining *N*-acetylhexosamines in biological fluids containing relatively high concentrations of proteins and lipids. A method for the determination of *N*-acetylhexosamines based upon the formic acid–chloroform solvent mixture has already been reported (Kumar and Hansen, 1972).

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Effect of Southern Corn Leaf Blight on Composition and Selected Physical Characteristics of Corn

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The 1970 corn crop was significantly affected by southern corn leaf blight. We have analyzed heavily damaged, moderately damaged, and undamaged kernels from blight-damaged ears of corn. The kernels were generally smaller in size and lower in weight, and the grain was lower in test weight as blight damage increased. Protein, ash, and fiber content increased with increased blight damage, while oil, starch, and pentosan content decreased. In general, amino acid data agreed favorably with those found for normal corn from years when no blight was present.

Helminthosporium maydis, Nisik et Miyake, Race T, the causative agent of southern corn leaf blight, markedly decreased the yield of corn grown during the 1970 crop year. This disease has been a problem in other years but to a lesser extent. The effect of blight on composition and physical characteristics is important to the farmer, processor, and consumer. The farmer suffers a discount for blighted grain that is downgraded because of kernel damage and low test weight, while dry- and wet-millers encounter higher cleaning losses and lower yields of their principal products (Anderson *et al.*, 1972; Brekke *et al.*, 1972). Several investigators have shown that blighted corn presents no problem in feeding

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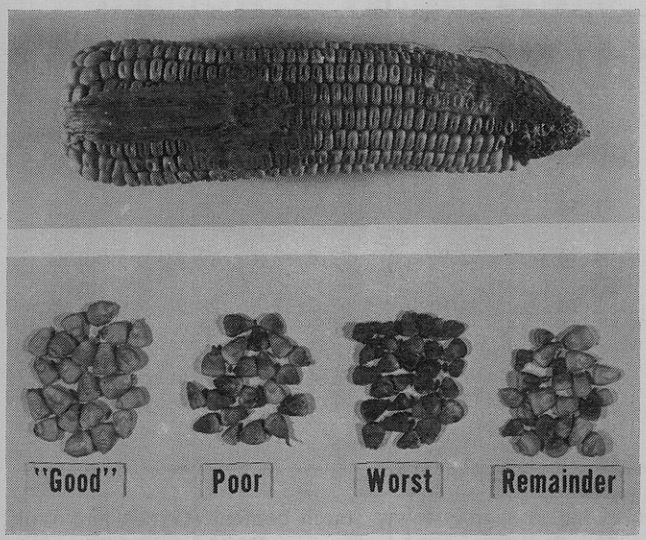

Figure 1. Top, corn severely damaged by southern leaf blight. Bottom, kernels removed from damaged ears by selective hand shelling

Table I. Physical Analyses of Southern Leaf Blight-Damaged Corn[a]

Sample	Proportion,[b] %	Test weight, lb/bu	Weight, g/100 K	Size,[c] %	Germination, %
Moldy ears					
Good kernels	27	51.2	17.0	14	40
Moderately damaged kernels	26	47.3	13.6	10	10
Heavily damaged kernels	18	33.2	8.4	5	0
Remainder of kernels	29	47.8	13.0	11	25
Nonmoldy ears					
Tip kernels		52.8	15.0	10	79
Butt kernels		53.9	19.6	36	81

[a] 10 to 15% moisture content. [b] As percentage by weight of all kernels on the moldy ears. [c] As percentage retained on a $^{21}/_{64}$-in. round-hole perforated sieve.

(Brown 1970), but none have clearly and adequately shown what compositional changes may occur. While the possibility of blighted corn being produced in the United States is greatly lessened because resistant varieties are available, this situation may not hold for other countries; therefore, data on composition and physical characteristics of such corn should be available.

SAMPLES

Samples of yellow corn were obtained from moldy ears collected from a field, located in central Illinois, severely damaged by southern corn leaf blight. The field had been planted with seed having Texas male sterile cytoplasm, and the crop yield was estimated at 8 bu/acre. Ears were hand-picked at about 18% moisture content and dried at room temperature to below 14% in a simple drier. By selective hand shelling, four samples were obtained from the moldy ears. On heavily damaged kernels, the surface of each was dark gray or almost black in color, and these kernels came from the center of moldy areas which were often found on the tip end of the ear. Moderately damaged kernels were taken from the area immediately surrounding the heavily damaged kernels and usually only a portion of each kernel was gray or black. "Good" kernels were pale yellow in color, came predominantly from the butt end of the ear, and were visibly void of blight. The remainder was a mixture of good and damaged kernels remaining on the ear after the first three samples had been removed. These four samples, along with a heavily damaged ear, are pictured in Figure 1.

For comparative purposes and to learn if location on the ear made any noticeable difference, tip and butt kernels were collected separately from the comparatively few nonmoldy ears in the lot.

Samples for the chemical analyses were ground in a cyclone hammer (Udy) mill and passed successively through 0.050-in. round-hole perforated (rhp) and 0.012 × 0.5 in. slotted screens.

PHYSICAL CHARACTERISTICS

Some physical characteristics of the six samples described are reported in Table I. Test weight decreased from 51.2 to 33.2 lb/bu in going from the good to heavily damaged kernels removed from the moldy ears. Based upon test weight the best of any of the first four samples would have graded was U.S. No. 4. Tip and butt kernels from the nonmoldy kernels weighed 53–54 lb/bu, which would correspond to U.S. grade No. 3. Kernel weight also decreased along with test weight. However, all kernel weights were considerably under the range generally typical of good quality normal yellow dent corn; *i.e.*, 25–30 g per 100 kernels. Kernel size as determined by percentage of the sample retained on a $^{21}/_{64}$-in. round-hole perforated sieve decreased with blight damage, and all values, except for the butt-kernel sample, were abnormally low. Low values for kernel size and weight undoubtedly were due in part to the blight damage occurring most often on the tip end of ears, where the kernels naturally are smallest in size and lowest in weight. Good (*i.e.*, predominantly butt) kernels from the moldy ears were not much larger than tip kernels from the nonmoldy ears. This characteristic illustrates one effect that blight had on this lot of corn.

Internal examination of heavily damaged corn kernels revealed little or no vitreous endosperm, along with considerable damage to the germ portion of the kernel. The germ damage is reflected in the germination data (Table I). Heavily damaged kernels did not germinate and good kernels from moldy ears had a considerably lower germination than kernels from nonmoldy ears. Even the latter did not germinate as well as one might expect based upon the mild drying conditions used.

While a precise separation of the damaged kernels present on the moldy ears was difficult to make during hand shelling, the proportions given in Table I are reasonably indicative of the amounts actually present.

During hand shelling, care was necessary not to crush the heavily damaged kernels. With mechanical shelling, most of these and some of the moderately damaged kernels would have been reduced to fines and lost by sieving and aspiration during normal cleaning.

PROXIMATE ANALYSIS

If blight affects corn composition, the effect should be revealed in proximate analysis of the grain (Table II). Fat acidity (AOAC, 1960 standard procedure) values increased from 29 to 100 as blight damage increased. These values are below the range of 112 to 284 reported by Baker *et al.* (1959) for samples incurring 100% blue eye mold damage and also below the 224–282 range for samples having 100% cob rot damage. However, only kernels from the nonmoldy ears had fat acidity values below 22, the level reported by Baker *et al.*

Table II. Proximate Chemical Analysis of Blight-Damaged Corn[a]

Type of kernels	Fat acidity, mg KOH/10 g of dry grain	Crude protein, %[b]	Crude fat, %	Ash, %	Crude fiber, %	Starch, %	Amylose, % of starch	Pentosans, %
Moldy ears								
Good kernels	29	10.2	3.7	1.54	2.5	73.0	29.7	7.23
Moderately damaged kernels	81	10.9	3.2	1.74	2.9	72.0	29.3	6.64
Heavily damaged kernels	100	13.0	2.0	2.14	4.9	68.2	28.9	6.38
Remainder of kernels	45	10.9	3.5	1.63	2.7	71.8	28.7	7.20
Nonmoldy ears								
Tip kernels	16	9.9	3.9	1.50	2.3	71.4	29.5	8.01
Butt kernels	12	10.6	3.9	1.48	2.5	71.6	29.7	7.57

[a] Analyses reported on dry solids basis. [b] Percent nitrogen × 6.25.

Table III. Effect Upon Composition of Partial Loss of Fat and Starch in Good Kernels as Compared to Heavily Damaged Kernels

Constituent	Good kernel composition, g	Component loss, %	Good kernel composition after loss, g	Good kernel composition after loss, %
Fat	4	63.0	1.5	1.9
Protein	10	0.0	10.0	13.0
Starch	73	30.0	52.6	68.2
Other	13	0.0	13.0	16.9
Total	100		77.1	

(1957) as the approximately upper limit for freshly harvested corn. Deyoe *et al.* (1968) found that an increase in protein content accompanied grain sorghums of low test weight. In our study, as the degree of blight damage increased, small-to-moderate increases occurred in protein, ash, and fiber (AOAC 1965, standard procedure) contents (Table II). Crude fat (AOAC, 1960, standard procedure) content decreased as blight damage increased, and heavily damaged kernels contained approximately 50% less oil than the good kernels. The decreased oil content was expected in view of the visible damage to the germ portion of the kernel. Heavily damaged kernels also had a slightly lower starch content (Garcia and Wolf, 1972); however, amylose content (Wolf *et al.*, 1970) of the starch fraction remained constant. The pentosan content (AACC Approved Method, 1962) decreased with increasing blight damage.

The variation in chemical composition is in general agreement with changes observed by Bressani and Conde (1961) during maturation of corn from 23 to 58 days after flowering. The results suggest that the blight prevented the corn kernels from reaching their normal state of maturity.

Helminthosporium maydis is said to attack the starch and fat in a corn kernel (Hesseltine *et al.*, 1971). Assuming partial destruction of only these two components, appreciable quantities could be lost from a kernel without making large changes in the proximate analysis when the latter is made on a percentage basis. Differences between the heavily damaged and good kernels recorded in Table II could result from loss of more than one-half the fat and about one-fourth the starch normally present, as is shown by compositional data given in Table III.

AMINO ACID ANALYSIS

When protein content of a grain changes, the possibility of an alteration in amino acid pattern must always be considered. To answer this question, all six samples were analyzed in dup-

Table IV. Amino Acid Content of Blight-Damaged Corn

Amino acid	Good kernels	Moldy ears: Moderately damaged kernels	Moldy ears: Heavily damaged kernels	Moldy ears: Remainder of kernels	Nonmoldy ears: Tip kernels	Nonmoldy ears: Butt kernels	LSD[a]
			g/16 g of Nitrogen				
Lysine	3.40	3.48	3.34	3.42	3.29	3.23	0.56
Histidine	3.04	2.86	2.25	2.93	3.01	3.05	0.30
Arginine	5.50	5.17	4.70[b]	5.47	5.40	5.25	0.55
Aspartic acid	7.33	7.08	7.69	7.42	7.10	7.16	0.64
Threonine	3.96	3.96	4.39	4.06	3.92	3.87	0.46
Serine	4.97	4.99	5.22	5.07	4.96	5.00	0.34
Glutamic acid	20.24	19.53	20.14	19.90	20.67	20.86	1.00
Proline	9.25	10.41	10.63	9.56	9.23	9.68	1.43
Glycine	4.47	4.30	4.58[b]	4.45	4.39	4.19[b]	0.16
Alanine	7.76	7.80	7.60	7.73	7.77	7.82	0.45
Cystine	1.44	1.33	1.45	1.53	1.43	1.65	0.37
Valine	5.05	5.73	5.33	5.03	5.18	4.94	1.25
Isoleucine	3.61	3.69	3.93	3.57	3.59	3.53	0.41
Leucine	13.09	12.51	13.41	12.51	13.16	13.32	2.29
Tyrosine	4.71	4.63	4.57	4.57	4.77	4.40	0.55
Phenylalanine	5.25	5.04	5.60	5.07	5.32	4.88	0.57
Methionine	3.12	2.99	3.05	2.93	3.17	3.08	0.23

[a] Least significant difference (0.05 level) for samples. [b] Significant variation (0.05 level).

licate by the procedure of Benson and Patterson (1965) and the results were statistically evaluated (Table IV). Only arginine and glycine showed statistically significant variation at the 5% level and the variation was associated with the heavily damaged kernels. When a mold is present, the appearance of another protein could be expected; however, none was detected in a quantity sufficient to alter the protein composition. Several unusual amino acid peaks were noted in the heavily damaged kernels as might be expected with mold or bacteria present, but their quantity was small. Only two amino acids were significantly affected, and then only in heavily damaged kernels. Blighted corn had essentially the same protein composition as the nonmoldy corn tested.

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Titrimetric Method for Determination of Medicagenic Acid in Alfalfa (*Medicago sativa*)[1]

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A titrimetric method for the quantitative determination of medicagenic acid (a biologically active sapogenin present in alfalfa saponins) in the presence of other nonactive sapogenins in the acid hydrolysates of alfalfa tops and roots has been elaborated. The amount of medicagenic acid containing saponins can be derived from the content of medicagenic acid by means of a conversion factor. The content of these saponins in alfalfa tops and roots is 0.11 and 0.96%, respectively, thus accounting for the greater toxicity of the latter toward various organisms.

During recent years there has been interest in determining the relationship of saponin content in alfalfa (*Medicago sativa*) to a number of adverse physiological effects. At least ten different saponins are present in alfalfa. Soyasapogenols A, B, C, D, and E and medicagenic acid were identified in the aglycone moiety and glucose, galactose, arabinose, xylose, rhamnose, and glucuronic acid in the carbohydrate moiety (for review see Birk, 1969).

It has been shown recently that several biological activities of alfalfa saponins, such as hemolysis and larval and fungal growth inhibition, are exerted only by those saponins which contain medicagenic acid as their aglycone (Gestetner *et al.*, 1971a,b; Shany *et al.*, 1970a). The need for determination of the amounts of medicagenic acid in the presence of the other aglycones is therefore evident. Since medicagenic acid is a triterpenoid acid with two carboxyl groups Djerassi *et al.*, 1957), which are absent from the other alfalfa sapogenins, a titrimetric method has been elaborated for this purpose.

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[1] This is Part VIII of a series on "Alfalfa Saponins;" Part VII appeared in *Israel J. Chem.* (1972).

MATERIALS AND METHODS

Quantitative Determination of Medicagenic Acid. A mixture of alfalfa sapogenins isolated from purified saponin extracts (SE) prepared from alfalfa (*Medicago sativa*) tops flour, Hairy Peruvian variety, or from its roots, according to the method of Shany *et al.* (1970b), was used. A sample of 30 mg of SE was dissolved in 30 ml of 1 *N* H_2SO_4 in dioxane–water (1 : 3) and boiled under reflux for 12 hr. This period ensures complete hydrolysis of alfalfa saponins (Shany *et al.*, 1970b). The liberated sapogenins were extracted with four portions of ether (50 ml each); the combined extracts were washed with water for removal of residual H_2SO_4 and concentrated to dryness in a rotary vacuum evaporator.

The sapogenin mixture was dissolved in 50 ml of absolute methanol. Aliquots of 1 ml, to which two drops of thymolphthalein has been added, were titrated with 0.1 *N* methanolic KOH using an Agla micrometric syringe. The color transition at pH 10.0 marked completion of the titration of both carboxyl groups. Prior to and during the titration, nitrogen was introduced through the solution in order to remove carbon dioxide and as a means of stirring. The calculation of the amount of medicagenic acid was based on a molecular weight of 502 (Djerassi *et al.*, 1957).