FUNGUS DETERIORATION OF GRAIN: EFFECT OF FUNGUS INFECTION ON AMINO ACIDS AND VITAMINS IN WHOLE WHEAT

by 632

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B. S., National Taiwan University (Formosa), 1967

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE
Food Science

Department of Grain
Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1970

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TABLE OF CONTENTS

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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INTRODUCTION

Some fungi produce mycotoxins. Mycotoxins are metabolites of molds which can produce pathological or undesirable physiological responses in man and animals. These metabolites are occasionally ingested as contaminants in foods which have previously been attacked by molds and may cause outbreaks of disease.

Mycotoxicoses are diseases of animals and humans caused by toxins produced by fungi that have been grown on feeds or foods. Little attention has been focused on mycotoxicoses until recent years. Forgacs (1) in a review article accurately described mycotoxicoses as the neglected diseases.

According to Mirocha *et al.* (2) about 50% of the isolates of genera *Aspergillus*, *Penicillium*, and *Fusarium* can produce mycotoxins. Some of these toxins are lethal to cattle, swine, sheep, and poultry. These molds are often found in feeds and stored grains. Every year the loss from this "neglected disease" is appreciable. In 1934 veterinarians in Illinois estimated that, in the central part of that state alone, in the winter of 1933-34, 5000 horses died of "moldy corn disease" (3). Sippel *et al.* (4) reported the poisoning of 1000 swine by eating molded corn in the southwestern United States in 1952.

The recent discovery of an extremely potent carcinogen, aflatoxin, produced by *Aspergillus flavus* has dramatically changed the status of mycotoxin research. The recognition of aflatoxin

poisoning of turkeys in 1960 (5), the successful isolation of the toxin from cultures of A. flavus (6), the characterization of the aflatoxins B_1, B_2, G_1, and G_2 (7, 8, 9), and studies on the biological effects of these poisons (10, 11, 12, 13, 14, 15, 16) have been well developed.

Hundreds of papers published have discussed the detection, isolation, and description of the mycotoxin produced in foodstuffs. Very few have discussed the nutritional status of the food after mold damage. The object of this research was to investigate the nutritional changes of wheat that had been damaged by the mold Aspergillus flavus. The nutrients studied were amino acids and B-group vitamins. The distribution of aflatoxin in milling products of moldy wheat and moldy corn was also studied.

LITERATURE REVIEW

I. Aflatoxin:

A new disease, called "turkey X disease" was discovered following the widespread outbreaks of deaths of turkey poults in England in 1960 (5). The disease was characterized by acute hepatic necrosis associated with generalized bile duct proliferation.

It was also found that the high prevalence of trout hepatic carcinoma occurred with the wide-scale use of some dry-pelleted commercial feeds in the United States (17). The disease appears in both the nodular and the massive forms and metastases have been observed in hemapoietic tissue in the kidney and in the spleen.

At the same time as the outbreak of turkey X disease in England, a similar disease of ducklings was noted in Kenya and reported to the Central Veterinary Laboratory in Weybridge, England. The ducklings' feed ration contained a peanut meal made from peanuts grown and processed in East Africa. When the meal was withdrawn, animal death ceased. When it was discovered that the peanut meal was contaminated by mold, an important clue was provided in defining the etiology of the toxin. This became the first indication that the problem was not confined solely to Brazilian peanut meal (18). By growing some of the fungal species, Sargeant et al. (6) identified the toxin-producing fungus as Aspergillus flavus Link ex Fries. The British therefore proposed the name "aflatoxin" for A. flavus toxin.

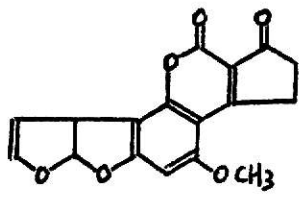
Aflatoxin is also produced by other Aspergilli and some Penicillia, Kulik and Holaday (19) found that extracts of many fungi, isolated from corn grain, contained aflatoxin. A. flavus, A. niger, A. parasiticus, A. ruber, A. wentii, P. citrinum, and P. variable produced measurable amounts of aflatoxin, while cultures of P. frequentans and P. puberulum contained traces. Over half of the A. flavus isolates produced aflatoxin, but only two out of 24 isolates of P. puberulum were toxin producers. Diener and Davis (20) found that 80% of A. flavus isolates produced aflatoxin, mainly B_1.

Chromatographic purification techniques, such as column chromatography and countercurrent distribution, have been used to separate crude extracts of aflatoxin-contaminated nuts into a number of fluorescent compounds. (21, 22). Of these fluorescent components, four have been definitely correlated with toxic activity (7, 23). The generally accepted nomenclature follows the pattern of thin-layer chromatographic separation and the particular fluorescence of each compound when viewed under long-wave-length UV light. Thus the four components are known as aflatoxin B_1 and B_2 showing blue fluorescence and aflatoxin G_1 and G_2 showing green fluorescence. The numbers following the capital letters are in decreasing order of mobility in thin-layer chromatography. The Rf's of B_1 and B_2 are 0.56 and 0.53, and of G_1 and G_2 are 0.48 and 0.46 when aflatoxins are spotted on a thin-layer plate and developed by chloroform : methanol (98.5 : 1.5). In experiments to determine whether a toxic agent may be present in the milk or bovine tissue of cows ingesting rations containing

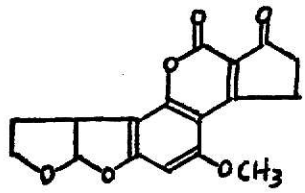
aflatoxin, Allcroft and Carnaghan (18, 24) found the cows excreted a toxic factor in the milk. This toxin has a biological effect in ducklings similar to that caused by aflatoxin. De Iongh et al. (25) showed by thin-layer chromatography on silicic acid (Kieselgel G) that the toxic factor, designated "milk toxin," is a blue-violet fluorescent substance with Rf value much lower than that of aflatoxin B_1. This toxin was named aflatoxin M_1. Originally, the letter M was intended to indicate a toxic metabolite isolated from the milk of cows fed toxic peanut meals and also found in the milk and liver of rats given pure aflatoxin B_1 (18, 25, 26). Aflatoxin M_1 is thus a metabolite of aflatoxin B_1, and the retention of the designation, M, is doubly justified. Aflatoxin M_1 is also present in crude aflatoxin mixtures, in which it can sometimes represent significant proportions of the toxic constituents. Indeed, a batch of contaminated peanuts contained sufficient quantities of aflatoxin M for its isolation and resolution into two components, M_1 and M_2 (27). This has been achieved by using chromatography on paper impregnated with formamide : water (85 : 15), and the solvent system ethyl acetate : benzene (9 : 1), on which M_1 showed a blue-violet fluorescent spot, Rf 0.34 and M_2 showed a violet fluorescent spot, Rf 0.23. The fluorescent intensities of the aflatoxins M_1 and M_2 have been reported to be three times stronger than those of the respective aflatoxins B. The presence of the hydroxyl in aflatoxin M_1 and M_2 has been deduced from the infrared absorption peak at 3425 cm^{-1}(Nujol) and its position from the nuclear magnetic resonance spectrum. Aflatoxin M_2 has been identified as a dihydroderivative of M_1, from which it has

been obtained by catalytic hydrogenation (Pd on carbon in acetic acid).

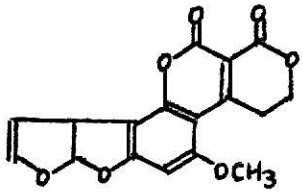
Isolation of two additional aflatoxins, one a fluorescent blue and the other a fluorescent green from cultures of A. flavus was reported by Dutton and Heathcote (28). These two aflatoxins were named B_{2a} and G_{2a} respectively, because they are said to represent hydroxylated B_2 and G_2 aflatoxins in which the hydroxyl groups are situated at the position 2 of the terminal tetrahydrofurano moiety (and not at the position 4 of the same ring as reported for M_1 and M_2) (29). In support of the proposed structure, aflatoxin B_{2a} has been acetylated and proved to give a compound identical with the known acetoxydihydro derivative of aflatoxin B_1. The structures of all the aflatoxins discovered are shown in Fig. 1.



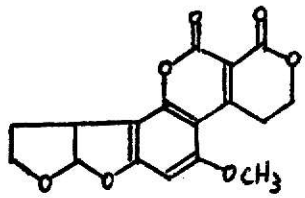
B_1



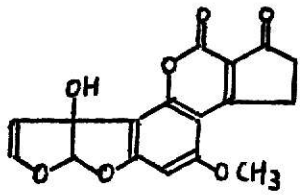
B_2



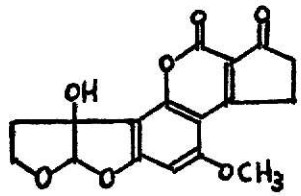
G_1



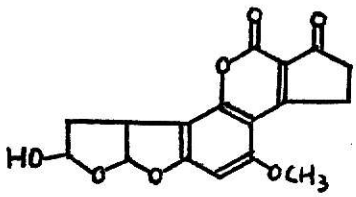
G_2



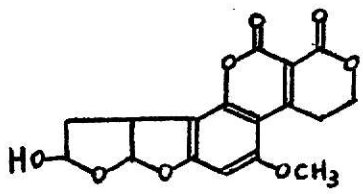
M_1



M_2



B_{2a}



G_{2a}

Fig. 1. Structures of Aflatoxins.

Physical data of aflatoxins are shown in Table I (8, 27, 28, 30, 31, 33, 35, 36, 37, 38, 39).

Table I. Physical Data of Aflatoxins.

Aflatoxin	Molecular formula	Molecular weight	Melting point*	UV absorption(e) 265 mμ	362mμ	Fluorescence(mμ)	Approx Rf**
B_1	$C_{17}H_{12}O_6$	312	268-269	13400	21800	425	0.56
B_2	$C_{17}H_{14}O_6$	314	286-289	11000	20800	425	0.53
G_1	$C_{17}H_{12}O_7$	328	244-246	10000	16100	450	0.48
G_2	$C_{17}H_{14}O_7$	330	237-240	11200	19300	450	0.46
M_1	$C_{17}H_{12}O_7$	328	299	11600	19000***	425	0.40
M_2	$C_{17}H_{14}O_7$	330	293	11900	21000***	-	-
B_{2a}	$C_{17}H_{14}O_7$	330	-	-	-	-	-
G_{2a}	$C_{17}H_{14}O_8$	346	-	-	-	-	-

*Decomposed

**Silica gel G. Thin-layer, $CHCl_3$: CH_3OH (97 : 3)

***At 357mμ.

Aflatoxin is an extremely potent hepatotoxic and hepatocarcinogenic agent. The disease resulting from the ingestion of aflatoxins by turkeys and trout has been described at the beginning of this review. In fact almost all kinds of animals tested are susceptible to this toxic effect.

In a study where a number of chickens fed aflatoxin contaminant died in the first three weeks, histological findings showed there was increased fat deposition in the livers. The livers had a pale color and firm texture. The kidneys also were

enlarged (40).

Newberne (41) studied the effects of feeding two samples of commercial dog food containing 0.5 and 1.75 ppm aflatoxin B_1 and also partially and highly purified preparations of aflatoxin B_1. In all cases the only clinical signs of toxicity were anorexia, weight loss, and icterus. The animals frequently appeared normal in the evening but were found dead the following morning. Histologically, the most prominent alterations were fatty change and bile duct hyperplasia. The results showed that the lethal dose for a dog fell within the same range as that of other very susceptible species such as ducks, guinea pigs, and rabbits.

Sheep are quite resistant to aflatoxin. When 3-month-old lambs are fed rations containing 20% strongly toxic peanut meal for 3 years they fail to show any obvious clinical effects other than a slight growth retardation. No liver damage was observed after 2 years on this ration (24). Allcroft et al. (42) investigated the metabolism of aflatoxin in sheep. Toxic materials similar to aflatoxin M_1, M_2 were found in sheep liver, kidney, and urine.

The most susceptible large farm animals appear to be pigs from 3 to 4 weeks of age. Of mature swine, pregnant sows are the most commonly affected. Pigs are the only species to show generalized jaundice. The liver is discolored from white to bright yellow, and subcutaneous hemorrhages occur (43).

Adult cattle are much more resistant than calves, but field observations have indicated that lactating cows often show a

significant reduction in milk yield within a few days of eating rations containing toxic peanut meal. This effect on milk yield was confirmed experimentally by Allcroft and Lewis (44). Experimental and field observations have shown that calves and yearlings are most susceptible. Microscopic liver lesions show four main features: a) proliferation of bile duct epithelium; b) chronic obliterating endophlebitis of centrilobular and hepatic veins; c) considerable variations in size and shape of parenchymal cells many of which contain abnormally large nuclei, and d) diffuse fibrosis which disrupts lobular structure (45).

Lesions of the liver similar to those generated in ducklings have been produced in monkeys (46). Two animals were given a dose of 500 µg mixed crystalline aflatoxins daily for 18 days, followed by doses of 1 mg/day. Deaths occurred on the thirty-second and thirty-fourth days. Histological examination showed essentially the same changes of fatty infiltration, biliary proliferation and portal fibrosis. Biochemical findings prior to death suggested an impaired liver function (46, 47). The effect of dietary protein level also was investigated (48), and it was shown that a reduced protein intake increased the susceptibility to aflatoxin. At a dose level of 100 µg/day the animals on a low protein diet died while those on a high protein survived. In these experiments, changes in the renal tubules were reported as well as gastrointestinal hemorrhage and ascites. Cuthbertson et al. (49) studied the effects of peanut meal on monkeys and demonstrated that a diet containing 50% toxic meal

(5 ppm aflatoxin in diet) caused rapid development of liver cell damage that was accompanied by biliary proliferation and early death. When the dose was reduce to 18% of the diet, some animals survived for 3 years but the livers showed irregularity in nuclear size, and in one case a coarse cirrhosis.

Except for tissue culture (50, 51), there is no direct evidence as to the degree of susceptibility of man to aflatoxins. Though the majority of the animal species tested, including primates (46, 47), were susceptible, the mouse and sheep showed great resistance to aflatoxins. The factors responsible for this difference are not known. It has been suggested that there is some correlation between the susceptibility to aflatoxins of cells grown in tissue culture and susceptibility of the animals from which the cells derive (52). On this basis man would be expected to be susceptible (52, 53, 54). The aflatoxins should be handled with great care.

Aflatoxin has been detected in infested peanuts, soybean, corn, wheat, milk etc. (55). In the Orient people eat a lot of fermented foods such as soysauce, miso, bean cheese, sake, and koji. Although it has been suspected that such fermented foods are associated with the high rate of liver cancer, (in a case where 25 people became ill and 3 children died from eating moldy rice,) there was no direct evidence that the fermented foods contained detectable amounts of aflatoxin (56). Murakami et al. (57) examined a total of 172 cultures of industrial strains of Aspergilli for the production of aflatoxins in rice koji. None of the fluorescent materials produced had aflatoxin absorption

spectrum. Yokotsuka found that in 64 strains of molds being used in Japan for the production of shoyu, miso and sake, 14 strains produced fluorescent substances resembling those of aflatoxin B group, and 8 strains resembling those of aflatoxin G group. But the melting points of these fluorescent materials were much lower than those of aflatoxins. In fact these fermented foods were free from aflatoxin (58). Wang et al. (59) established a short method convenient for the rapid determination of aflatoxin in alcoholic products. This method was applied to determine the aflatoxin in all the products of Taiwan Tobacco and Wine Monopoly Bureau. The analytical results showed that there was practically no aflatoxin in the alcoholic products.

II. Fungi and Stored Grains:

Not only bacterial flora but also fungal flora infest cereal grains. Christensen and Gordon (50) examined a series of American wheat and corn samples for mold; most of those collected from commercial lots bore molds able to grow at relatively low moisture content. Invasion of the germs of the seeds by molds such as Aspergillus restrictus, A. repens, A. candidus, and A. flavus, always weakens the ability to germinate, thus producing "sick" wheat (61). What is known as "sick" wheat is unable to germinate, dull in appearance and exhibits darkened embryos.

Christensen and Gordon (60) also determined mold populations in "sick" wheat, which were found to be about 10,000 to 2,000,000 per gram. A predominant mold on sound wheat is Alternaria (62).

Alternaria tenuis was found on 64% of the samples examined. Alternaria spp. are common on barley. The degree of infection is independent of the variety but is correlated with the climatic conditions prevailing during growth.

Twelve varieties of rice seed tested by del Prado (63) showed mold populations ranging from 400 to 66,000 per gram. Half of the samples harvested in the Southern area of the U.S.A. were internally infested by molds. Kurada et al. (64) however reported that milled rice produced commercially in the Southern area of the U.S.A. in 1954 was virtually free from internal infection by fungus (64).

The relative humidity of the interseed air and hence the moisture content of the grain influences the composition of the fungal flora of a stored cereal; as the moisture content rises the Aspergilli are replaced by Penicillia which in turn gives way to Mucor. Temperature also influences the composition of the fungal flora. Bottomley et al. (65) found that yellow corn in equilibrium with a relative humidity of 80% supported predominantly Penicillium spp. at 25°C., A. flavus at 30°C., A. glaucus at 35°C., and Mucor spp. at 45°C. Fungi isolated from spoiling grain include Fusarium, Rhizopus, Monilia, Helminthosporium, Curvularia, Hormodendron, Streptomyces, and Cephalosporium.

Tuite and Christensen (66) found the fungi Alternaria, Cladosporium, and Fusarium were common and sometimes abundant in barley seeds before the seeds matured, but no significant numbers or amounts of Aspergilli or Penicillia were found in

seeds at harvest time. In seeds stored at moisture contents of 10-13%, Aspergilli and Penicillia remained static. At seeds moisture contents of 13.8-14.2%, A. restrictus, a slow-growing member of the A. glaucus group, gradually invaded the germs. At moisture contents of 15-17%, A. repens., A. amstelodami, and A. ruber became the dominate flora.

Christensen and Cohen (67) found that washing can reduce the fungal population of wheat by 75-90%. It is impossible to remove the mold spores entirely, consequently mold spores will be found in flour. These workers found, however, that the fungal counts of some flours were higher than those of the washed wheats from which they were milled, and this suggests that mold contamination can occur during the milling process. They also found that the flours from some mills had consistently higher mold counts than those from other mills and concluded that the mold count of flour was determined more by the conditions prevailing within the milling machinery than by the wheat.

Barton-Wright (68) found that the mold count of flour increased during storage if the moisture content was above 16%. At moisture contents of 18% or more the rise in fungal population was much more rapid than in the bacterial population, because the bacteria die off even more rapidly at elevated moisture contents, than the molds do. Over 90% of the fungi found by Barton-Wright in normal flour belonged to the genus Penicillium, but species of the genera Botrytis, Aspergillus and Cladosporium were also present together with some unidentified species. Christensen and Cohen (67) found the chief source of mold contamination of

flour appeared to be molds growing and sporulating in the milling system itself. The predominant molds in most of the flours were Aspergillus glaucus and A. candidus. Unidentified species of Penicillium made up a major portion of the mold flora in only a few samples of commercial flour. Several other genera were found in most samples, but only in small numbers.

Grain in bulk may undergo spontaneous heating. If the moisture is low, around 10 to 12%, the heating is usually due to the presence of insects, for example weevils. This is known as "dry heating." However if the moisture is higher than 14.5%, the "damp grain heating" may take place, owing to the higher-than-normal rate of respiration at these moistures. All cereals respire and generate heat, and the higher the temperature and the moisture, the higher the rate of respiration. The high rate of respiration in damp grain is now thought to be mainly due to the presence of micro-organisms; mold for example, has a high rate of respiration. It is thought that, in the early stages of heating of damp grain, the sub-epidermal mycelium of the grain rather than the external mold is responsible. If the heat generated is not removed, the temperature of the grain rises and spontaneous heating takes place. Evidence indicates that the heating of moist grain is more associated with the presence of molds than with the respiration of the cereal itself.

Wheat infested with molds, if stored while moisture content is high, loses its viability, cannot germinate, and shows a rise in fat acidity. When such wheat is subsequently stored with 13-14% moisture content, the germ damage and fat acidity usually

continue to increase although the mold count decreases. Also heavy mold infestation is found associated with poor baking strength and loss of baking quality during storage (69, 70).

III. Nutritive Value of Cereals:

Cereals are composed of carbohydrates (mainly starch), proteins, lipid, minerals, vitamins, cellulose, hemicelluloses, and a number of other minor constituents. Cereal grains are regarded chiefly as an enery source. Their value as a source of protein, vitamins, and minerals is often overlooked. Kent-Jones (71) showed that in England bread alone, leaving aside household flour, cakes, pastries, biscuits etc., provided in the diet 16.8% of total energy, 19.4% of protein, 15.7% of calcium, 20.4% of iron, 22.1% of vitamin B_1 and 18.7% of niacin requirements. The nutritional attributes of cereals have been studied by many workers. Toepfer (72) collected samples of wheat, flour, and baking products from all over the United States. Analytical determinations of total solid, protein, ash, 6 macro-minerals, 18 amino acids, thiamine, riboflavin, niacin, reducing and non-reducing sugars, starch, lactose, pentosans, fatty acids, the three forms of vitamin B_6, trace minerals and the individual tocopherols of vitamin E have been undertaken in many laboratories. Results will be reported in subsequent articles. Polansky (73) found that durum wheat contained more vitamin B_6 than hard or soft wheats. In milling the hard and soft wheats to flour, generally 10-25% of the B_6 was retained, but in milling durum wheat to semolina about 28% was retained, A large number

of wheats and wheat products has been analyzed for the various forms of vitamin E (74, 75). Although all wheats have similar amounts of the same tocopherols, durum wheats are slightly lower in α- and β-tocopherols and higher in α- and β-tocotrienol. Processing white flour removes the major part of all forms of vitamin E. Desikachar (76) determined the correlation between the degree of polishing rice and the amount of thiamine and phosphorus retained. Kik and Van Landingham (77) pointed out that about 50% of the riboflavin in rice was lost during the milling process. Tkachuk (78) reported on amino acid patterns of six flours milled from four major types of Canadian wheats. All of the amino acid patterns were found to be similar. The results were obtained by automatic ion-exchange chromatographic analysis of 6 N HCl and 6 N Ba $(OH)_2$ hydrolyzates. To provide the quantitative data on the fatty acids in wheat and wheat products Inkpen et al. (79) used chloroform : ethanol : water (200 : 95 : 5) to separate the "extractable" lipid and used the hot 6N HCl to extract the "bound" lipid. The extractable lipids were found to contain more stearate and linoleate but less palmitate than those in bound lipid. Vitamin B in wheat was first studied by Osborne and Mendel (80). They pointed out that most of vitamin B was contained in bran and millings.

In the studies of the distribution of vitamin B_1 in the wheat kernel, it was found that germ made up 2% of whole wheat by weight and contained 16.7% of the vitamin B_1 in whole wheat (81). Vitamin B_2 was less concentrated in the germ than was vitamin B_1

(82, 83, 84, 85). Schultz (86) proposed that thiamine (vitamin B_1) in wheat could be determined by the fermentation method. By using the fermentation method, he found that whole wheat contained 3.6-7.8% µg/g thiamine. He also found the 2% yeast in bread restored practically all of the thiamine removed in the milling process. Calhoun et al. (87) worked out the conditions for liberating vitamins from wheat and showed some of the previous methods to be inadequate. He assayed 9 vitamins in four wheat blends, their flours, and breads, and found that levels of thiamine, riboflavin, niacin, pantothenic acid, and p-amino-benzoic acid were directly related to the protein level of the wheats. This relationship did not exist for biotin, choline, folic acid, and inositol. The spring wheat blends were higher in vitamins (and protein) than were winter wheats. Hashitani and Sako (88) noted that white bread was deficient in nutritive properties particularly in vitamin B_1, this deficiency might be compensated for by use of brewers' yeast. Although riboflavin (vitamin B_2) is not decomposed in the baking of bread, large losses are found when slices of bread are unduly exposed to light (89).

MATERIALS and METHODS

I. Materials:

S_1: Selkirk (Hard Red Spring) harvested in North Dakota in 1968. Kernels red, midlong, hard, ovate; germ midsized to large; crease midwide, middeep; cheeks angular; brush midsized, midlong to long. About 10 lbs. of wheat was obtained from the pilot plant of the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas. This sample was milled to pass through a 30-mesh screen and the product was stored in the refrigerator.

T_1: Triumph (Hard Red Winter) harvested in Kansas in 1968. Kernels red, short to midlong, hard, ovate; germ small; crease midwide, shallow; cheeks rounded; brush midsized, midlong. About 10 lbs. of wheat was obtained from the pilot plant of the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas. This sample was milled to pass through a 30-mesh screen and the meal was stored in the refrigerator.

S_2: Mold damaged Selkirk was prepared by increasing the moisture content of the wheat to 20%, inoculating with Aspergillus flavus spores and incubating at 30°C for 7 months. This wheat was dried under hot air, milled to pass through a 30-mesh screen and the meal was stored in the refrigerator.

T_2: Mold damaged Triumph was prepared by increasing moisture

content of the wheat to 20%, inoculating with Aspergillus flavus spores and then incubating at 30°C. for 7 months. After incubating this wheat was milled to pass through a 30-mesh screen and the meal was stored in the refrigerator.

Moldy Corn: only for aflatoxin determination.

Preliminary analysis of samples is shown in Table 2.

Table 2. Analysis of Samples

	S_1	S_2	T_1	T_2
Test Weight	60.50	-	62.00	-
Pearling (g)	65.70	-	67.00	-
1000 Kernel wt. (g)	33.80	-	29.30	-
Yield (%)	76.40	-	75.37	-
Moisture (%)	11.40	10.90	10.20	13.93
Solids (%)	88.60	89.10	89.80	86.07
Crude Protein (%)	16.45	16.08	16.89	16.08
Ash (%)	1.60	2.61	1.73	2.15

II. Methods:

(1). Ion-exchange chromatography for amino acids.

Each wheat sample was hydrolyzed with 6 N HCl at 110°C. for 22 hr. The hydrolyzate was filtered to remove insoluble materials. The filtrate was then evaporated to dryness and re-dissolved in pH 2.2 citrate buffer solution. An aliquot of this solution was placed in Beckman Model 120 C ion-exchange amino acid analyzer (90). Amino acids were eluted and recorded by automatic machine.

(2). Fluorometry for thiamine and riboflavin.

This method is based on the fact that thiamine is oxidized in alkaline solution to thiochrome, which has an intense blue fluorescence, and riboflavin emits yellowish-green fluorescence. Both of the fluorescences can be measured with a fluorometer. Procedures for analyzing these two vitamins are described below:

(a). Thiamine (B_1).

A 5-g sample was autoclaved with 75 ml 0.1 N H_2SO_4 at 15 lb. pressure for 30 min. (91). The solution was cooled to room temperature and 5 ml 2.5 M NaAc solution was added to neutralize the H_2SO_4. Takadiastase was added to the solution and it was incubated at 45°C for 3 hrs. The enzyme hydrolyzate was diluted to 100 ml and filtered through S & S 597 filter paper.

Five milliliters of the filtrate was pipetted to pass through a Decalso column. The column was washed

with 15 ml distilled water, then the absorbed thiamine was eluted with 25% acidic KCl solution; 25 ml of the eluate was collected. Two 10-ml portions of the eluate were dispensed into two test tubes, 6 ml alkaline $K_3Fe(CN)_6$ solution was added to one of the two tubes, and 6 ml 15% NaOH solution was added to the other tube. Both tubes were extracted with 15 ml iso-BuOH. The fluorescence of the iso-BuOH extracts was determined with an electronic photofluorometer. (Coleman Instruments Inc. Maywood Ill.)

(b). Riboflavin (B_2).

A 5-g sample was autoclaved with 75 ml 0.1 N H_2SO_4 at 15 lb. pressure for half hour. It was cooled to room temperature, 5 ml 2.5 M NaAc was added to the mixture and let stand 1 hr. This solution was diluted to 100 ml and filtered through Whatman No. 2 filter paper. The filtrate was wheat extract. In order to remove the fluorescence of interfering material the following scheme of reaction was used (92).

	Low Blank Material		High Blank Material	
	Tube A	Tube B	Tube A	Tube B
Wheat Extract (ml)	10	10	10	10
Standard B_2 Solution (ml)	1	-	1	-
H_2O (ml)	1	2	-	1
$KMnO_4$ (4%) (ml)	0.5	0.5	1	1
Time Lapse (min.)	2	2	4	4
H_2O_2 (ml)	0.5	0.5	1	1

The fluorescence of tubes A and B was measured by an electronic photofluorometer (Coleman Instruments Inc., Maywood Ill.), then 20 mg $Na_2S_2O_4$ was added to quench the fluorescence of riboflavin and the blank reading C was taken. If the blank reading was more than 20% of the sample reading by the low-blank procedure, the high-blank procedure had to be used. The riboflavin content of the sample was calculated as below:

$$\text{Riboflavin}\mu g/g = \frac{B-C}{A-B} \times \frac{R}{S} \times \frac{V}{V_1}$$

Where

A = fluorometer reading of sample plus riboflavin standard.

B = fluorometer reading of sample plus distilled water.

C = fluorometer reading of tube B after addition of $Na_2S_2O_4$.

V_1 = mls. of sample solution taken for measurement.

R = μg of riboflavin added to V_1

S = sample weight in g.

(3). Microbiological assay for vitamins.

These techniques have enjoyed wide popularity. For some vitamins such as folic acid and vitamin B_{12}, they are the only method currently available for assay of natural materials (93). Micro-biological methods of vitamin determination are based on the observation that some well-defined micro-organisms can multiply and

produce certain metabolic products only in the presence of some B-group vitamins. If a micro-organism is at first cultured in an optimum nutrient medium, then is transferred to assay medium which contains all nutrients necessary for the optimum growth of this micro-organism except the specific vitamin to be determined, the growth of the micro-organism is inhibited. When the vitamin-containing substrate under investigation is added to the initially clear test medium, multiplication of the micro-organism causes a turbidity that can be measured photometrically, or produces metabolic products that can be determined quantitatively; for instance, the mixture of acids produced by the micro-organism from the glucose in the medium can be titrated. Photometric evaluation of the growth reactions is simpler and can be carried out more rapidly (94). Therefore it was used throughout this experiment.

(a). Vitamin B_6.

There are three forms of vitamin B_6 existing in natural materials, namely pyridoxine, pyridoxal, and pyridoxamine. They differ only by the substituents in the 4-position. Some micro-organisms have a specific response toward each form. In this research, however, only the yeast Saccharomyces carlsbergenesis 4228 (ATCC 9080) was used to determine the total vitamin B_6 (95, 96).

The extraction of vitamin B_6 was carried out by autoclaving 1 g of wheat with 80 ml 0.5 N H_2SO_4 at 120°C. for 1 hr. After it was cooled to room temperature, the solution was adjusted to pH 5.0 with NaOH solution. This solution was diluted to 100 ml and filtered through Whatman No. 2 filter paper. The filtrate was used as the vitamin extract.

The assay medium was obtained in dehydrated form from Difco Laboratories (Detroit, Michigan) (97). Thirty-three and two-tenths gram of the medium was dissolved in hot water and increased to 1 l. Five milliliters of this basal assay medium was dispensed into each of two series of tubes. To one series of tubes containing the assay medium was added graduated quantities of standard known vitamin. To the second series was added varying amounts of the material under assay. Included in each series of tubes was one containing only distilled water and the basal assay medium. This tube was a blank control. The contents of the tubes of each series were then diluted to 10 ml each with distilled water, sterilized, cooled, inoculated, incubated and observed. The growth response was determined turbidimetrically with a Spectronic 20 Spectrophotometer (Bausch & Lomb Optical Company, Rochester, New York).

(b). Vitamin B_{12}.

Procedures for analyzing vitamin B_{12} are essentially the same as for vitamin B_6. The test micro-organism

used was Lactobacillus leichmanii. However, the cleaning of all glasswares and extraction of vitamin from the sample need to be stressed.

In the assay of vitamin B_{12}, as small a quantity as 0.01 ng (1 ng = 10^{-9}g) will give rise to a definite growth response. The importance of clean glassware in the assay of this vitamin was stressed by the U.S. Pharmacopeia Vitamin B_{12} Study Panel, in which they observed that glassware for this purpose required special handling. As many as 12 rinses were necessary to remove interferring substances and give satisfactory results (97).

Extraction of vitamin B_{12} from wheat samples was carried out by dispersing a 1-g sample in 80 ml acetate buffer (pH 4.62). Forty milligrams papain, and 40 mg diastase were added to the suspension, which was kept at 37°C for 24 hours, and then heated at 100°C for 30 min. It was cooled, adjusted to pH 6.6 with NaOH solution, and made up to 100 ml with acetate buffer. The suspension was filtered or centrifuged; the clear solution was diluted to the optimum concentration for assay.

(c). Niacin.

Niacin, or nicotinic acid, a member of the vitamin B complex, has many important functions in human and animal nutrition. Niacin is found in tissues largely in the form of nicotinamide as a constituent of two important

coenzymes, nicotinamide-adenine dinucleotide (NAD) and nicotinamide-adenine dinucleotide phosphate (NADP).

Extraction of niacin was carried out by acid hydrolysis. A 1-g sample was suspended in 50 ml 0.5 N HCl. The suspension was autoclaved at 120° for 1 hr. The mixture was cooled, adjusted to pH 4.5 by addition of 2.5 M NaAc solution, diluted to 100 ml and filtered. The filtrate was adjusted to pH 6.8 and diluted to the optimum concentration for assay. The test organism used in this experiment was Lactobacillus plantarum 17-5 (ATCC 8014).

(d). Pantothenic Acid.

Pantothenic acid is widely distributed in combined form (e.g. in coenzyme A) throughout the vegetable and animal kingdoms. However, the concentrations in which this vitamin is encountered in nature are so low that chemical assay is very difficult. Although a chemical method for the assay of pantothenic acid in wheat has been suggested by Refai and Miller (98), microbiological methods are to be preferred for this type of investigation because of their greater specificity.

In a natural product this vitamin must be liberated from the combined form by enzymatic hydrolysis. A 1-g sample was ground and homogenized with 80 ml standard acetate buffer (pH 4.62); 40 mg papain and 40 mg diastase were added, and the suspension was kept at 37°C for 24 hr. The substrate was then heated to 100°C

for 30 min., allowed to cool, adjusted to pH 6.8 with NaOH solution, diluted to 100 ml with standard acetate buffer solution, and filtered. The filtrate was diluted to the optimum concentration for the test as described above. The test micro-organism was Saccharomyces carlsbergenesis (ATCC 9080).

(4). Thin-layer Chromatography for Aflatoxin.

Aflatoxins were extracted from moldy wheat samples by the procedure, slightly modified, recommended for determining aflatoxins in peanuts and cotton seeds (99). Fifty grams of the wheat sample was suspended in 250 ml of $(CH_3)_2CO$: H_2O (70 : 30) in a 500 ml Erlenmeyer flask. The flask was stoppered and shaken vigorously for 30 min. This solution was filtered. One hundred fifty milliliters filtrate was collected. Sixty milliliters H_2O and 20 ml 20% $Pb(CH_3COO)_2$ solution were added to the filtrate. The mixture was reduced to about 150 ml by boiling, cooled to 20°C in an ice-bath, centrifuged to remove the precipitate, and the supernatant transferred to a separatory funnel, and extracted twice with 50 ml $CHCl_3$. The $CHCl_3$ extracts were combined and evaporated until the volume was about 2-3 ml. This solution was then transferred to a silica-gel chromatographic column quantitatively. One hundred milliliters ethyl ether was percolated through the column. Then 150 ml $CHCl_3$: CH_3OH (97 : 3) was added to elute the aflatoxins. This eluate was evaporated to about 2 ml

and transferred quantitatively to a small vial and evaporated to dryness. Exactly 500 µl $CHCl_3$ was added to the vial to dissolve the aflatoxins.

Chromatoplates (20 cm x 20 cm) were prepared for thin-layer chromatography by mixing 30 g of silica gel G-HR with 60 ml distilled water and spreading a layer of 0.25 mm thick on the plates. Plates were dried at 105°C for 2 hr. and stored in a desiccator. Sample extracts were spotted together with standard aflatoxin solution, according to the figures shown in later pages. The solvent systems used to develop the chromatograms were $CHCl_3$: CH_3OH (97 : 3), and $CHCl_3$: CH_3OH : HOAc (94.5 : 5 : 0.5).

RESULTS AND DISCUSSION

I. Effect of Mold Damage on the Composition of Amino Acids of Wheat Samples.

In Table 3 and Table 4 below, it is shown that after mold deterioration, most of the amino acids in Selkirk wheat were decreased; especially arginine and cysteine were decreased 50.2% and 74.5% respectively. Only alanine and methionine were increased. They were increased 5% and 51% respectively. The increase of methionine was probably derived from cysteine. The ratio of total amino acids to Kjeldahl protein in Selkirk wheat was only 78.86%. That means some amino acids were used to synthesize nitrogenous compounds which still possesed detectable Kjeldahl nitrogen. The large amount of NH_3 in moldy wheat obviously was due to the metabolism of amino acid by the mold.

In Table 5 and Table 6 it is shown that Triumph wheat lost large amounts of lysine (45.3%), histidine (50.1%), and arginine (50.0%). However, methionine, leucine, and phenylalanine increased by 34.5%, 1.5%, and 4.7% respectively. The ratio of total amino acids to Kjeldahl protein was 90.63%. This could mean that Triumph wheat was less damaged by mold than was Selkirk wheat.

Data in Table 3 and Table 5 show that both wheats have very similar amino acid composition. However, after deterioration by mold (Table 4 and Table 6), aspartic acid, threonine, serine, glutamic acid, proline, cysteine, and tyrosine in Selkirk were much lower than those in Triumph, while NH_3 was much higher.

Table 3. Amino Acid Composition of Selkirk Wheat.

Amino Acids	% sample as is moisture	% sample moisture free	g/100 g Kjeldahl Protein	g/100 g Protein Corrected to 100% Recovery Kjeld. Prot.
Lys.	0.389	0.439	2.366	2.301
His.	0.338	0.381	2.052	1.995
NH_3	0.574	0.648	3.489	3.393
Arg.	0.670	0.756	4.074	3.962
Asp.	0.816	0.921	4.963	4.826
Thr.	0.448	0.505	2.722	2.647
Ser.	0.758	0.855	4.607	4.480
Glu.	5.586	6.305	33.958	33.022
Pro.	1.775	2.003	10.788	10.490
Gly.	0.617	0.696	3.751	3.647
Ala.	0.531	0.600	3.203	3.141
Cys.	0.588	0.663	3.573	3.474
Val.	0.681	0.768	4.138	4.024
Met.	0.161	0.182	0.981	0.954
Ileu.	0.569	0.642	3.457	3.362
Leu.	1.125	1.269	6.836	6.648
Tyr.	0.487	0.549	2.958	2.876
Phe.	0.805	0.909	4.894	4.759

Cystine and Methionine by oxidation.

Kjeldahl protein = 16.45%, Protein on moist.-free basis = 18.57%

Moisture content = 11.40%

Total amino acids/ Kjeldahl protein = 102.84%

Total nitrogen recovered/ Kjeldahl nitrogen = 93.34%

Table 4. Amino Acid Composition of Moldy Selkirk Wheat.

Amino Acids	% Sample as is moisture	% Sample Moisture free	g/100 g Kjelkahl Protein	g/100 g Protein Corrected to 100% Recovery Kjeld. Prot.
Lys.	0.295	0.331	1.836	2.329
His.	0.181	0.203	1.125	1.426
NH_3	0.905	1.016	5.627	7.135
Arg.	0.302	0.339	1.879	2.383
Asp.	0.574	0.645	3.573	4.530
Thr.	0.369	0.415	2.297	2.913
Ser.	0.571	0.641	3.553	4.505
Glu.	3.646	4.092	22.677	28.755
Pro.	1.198	1.345	7.451	9.448
Gly.	0.572	0.642	3.557	4.510
Ala.	0.562	0.631	3.494	4.430
Cys.	0.150	0.169	0.935	1.186
Val.	0.660	0.741	4.106	5.209
Met.	0.245	0.275	1.525	1.934
Ileu.	0.484	0.543	3.008	3.814
Leu.	0.979	1.099	6.088	7.728
Tyr.	0.376	0.421	2.335	2.961
Phe.	0.610	0.685	3.796	4.814

Cystine and Methionine by oxidation.

Kjeldahl protein = 16.08%, Protein on moist.-free basis = 18.05%.

Moisture content = 10.90%

Total amino acids / Kjeldahl protein = 78.86%

Total nitrogen recovered / Kjeldahl nitrogen = 84.07%

Table 5. Amino Acid Composition of Triumph Wheat.

Amino Acids	% Sample as is Moisture	% Sample moisture-free	g/100 g Kjeldahl Protein	g/100 g Protein Corrected to 100% Recovery Kjeld. Prot.
Lys.	0.438	0.488	2.595	2.517
His.	0.356	0.397	2.109	2.046
NH_3	0.668	0.744	3.954	3.835
Arg.	0.825	0.919	4.888	4.740
Asp.	0.892	0.993	5.281	5.122
Thr.	0.463	0.516	2.743	2.660
Ser.	0.777	0.866	4.602	4.464
Glu.	5.601	6.238	33.164	32.165
Pro.	1.874	2.087	11.095	10.761
Gly.	0.671	0.747	3.973	3.853
Ala.	0.581	0.647	3.438	3.334
Cys.	0.482	0.537	2.853	2.767
Val.	0.737	0.821	4.365	4.234
Met.	0.169	0.188	0.998	0.968
Ileu.	0.564	0.628	3.338	3.237
Leu.	1.120	1.247	6.630	6.431
Tyr.	0.498	0.554	2.947	2.859
Phe.	0.698	0.777	4.133	4.009

Cystine and Methionine by oxidation.

Kjeldahl protein = 16.89%, Protein on moist.-free basis = 18.81%

Moisture content = 10.20%

Total amino acids / Kjeldahl protein = 103.11%

Total nitrogen recovered / Kjeldahl nitrogen = 97.47%

Table 6. Amino Acid Composition of Moldy Triumph Wheat.

Amino Acids	% Sample as is Moisture	% Sample moisture-free	g/100 g Kjeldahl Protein	g/100 g Protein Corrected to 100% Recovery Kjeld. Prot.
Lys.	0.230	0.267	1.431	1.579
His.	0.171	0.199	1.066	1.176
NH_3	0.553	0.643	3.441	3.796
Arg.	0.391	0.454	2.430	2.681
Asp.	0.740	0.860	4.602	5.078
Thr.	0.444	0.516	2.760	3.045
Ser.	0.724	0.841	4.503	4.969
Glu.	4.635	5.385	28.822	31.801
Pro.	1.401	1.627	8.710	9.611
Gly.	0.617	0.717	3.838	4.234
Ala.	0.550	0.639	3.422	3.775
Cys.	0.447	0.519	2.780	3.068
Val.	0.659	0.766	4.100	4.523
Met.	0.218	0.253	1.353	1.493
Ileu.	0.533	0.620	3.317	3.660
Leu.	1.087	1.263	6.762	7.461
Tyr.	0.473	0.549	2.939	3.243
Phe.	0.700	0.814	4.356	4.806

Cystine and Methionine by oxidation.

Kjeldahl protein = 16.08%, Protein on moist.-free basis = 18.68%

Moisture content = 13.93%

Total amino acids / Kjeldahl protein = 90.63%

Total nitrogen recovered / Kjeldahl nitrogen = 81.82%

II. Effect of Mold Damage on the Vitamin Content of Wheat Samples.

The content of thiamine in Selkirk and Triumph wheats after mold damage is shown in Table 7. It can be seen that more than 40% of the thiamine originally present in the wheat was lost. Recovery test was carried out by adding 4μg thiamine·HCl to 1 g of the sample and following the same procedure of thiamine analysis as described under Methods. The results of the recovery test are shown in Table 8. The low recovery (79.2%) from moldy Selkirk wheat may have been due to the presence of some quenching material produced by the mold.

The riboflavin content of wheat was largely increased after the wheat had been infected by mold. The increase of riboflavin in Selkirk wheat was almost 300% and in Triumph about 180% as shown in Table 9. Part of the high analytical values was probably the result of the fluorescent materials produced by mold. Recovery test was carried out by adding 1μg riboflavin to 1 g of the sample. Results are shown in Table 10. The percentages of recovery fall in the range of 83.0 to 96.0%.

Microbiological techniques have been proved to be satisfactory for the analysis of B-group vitamins. The results of pyridoxine analysis are shown in Table 11 and Fig. 2. The pyridoxine contents of the two wheats were very close (4.18μg/g and 4.35μg/g). Mold tends to synthesize pyridoxine in wheat. However, the increase in Selkirk (46.2%) was more than in Triumph (18%). Recovery of this vitamin was about 87%. Vitamin B_{12} content in wheat (Table 12) is very low, being in the region of 10^{-9} g. There were many

discrepancies in assay values reported. This could result from the failure to extract the total cyanocobalamin present in the sample. The extraction method used in this experiment was proposed by Strohecker (100). Vitamin B_{12} content in Selkirk was about 0.076 μg/g, and in Triumph was about 0.061 μg/g. After the wheat was infected by mold the amount of vitamin B_{12} in Selkirk and in Triumph was increased to 0.283 μg/g and 0.085 μg/g respectively. It is obvious that Selkirk wheat was an excellent substrate for vitamin B_{12} synthesis by mold. The net increase in vitamin B_{12} was about 276%. The sigmoid-shaped calibration curve as shown in Fig. 3 suggests that maybe this vitamin is associated with some enzyme reaction (101). The recovery of this vitamin was about 93%.

The niacin content of wheat is relatively high; as shown in Table 13, it was about 67 μg/g in Selkirk wheat and about 87 μg/g in Triumph. It seems that mold was able to synthesize a great deal of niacin in Selkirk wheat but decomposed it in Triumph wheat. The calibration curve in Fig. 4 shows that a very narrow range of niacin concentrations can be used for assay. It is because definite growth of the organism was always observed in the blank control. The recovery of this vitamin was in the range of 83.2% to 87.0%.

The pantothenic acid content in Selkirk was 11.8 μg/g while in Triumph it was 10.5 μg/g. Mold infection has little effect (only 12.3% increase) on Triumph wheat, but increased a tremendous amount (463%) of this vitamin in Selkirk. The calibration curve (Fig. 5) is sigmoid-shaped. Recovery was almost 100% (95.2-101.3%).

From the above discussion it is seen that the vitamin contents in wheats studied tended to increase after mold infection, although thiamine in both wheats, and niacin in Triumph decreased a bit. This was because the mold could utilize the substrates to synthesize vitamins. Also, the spring wheat (Selkirk) was found to be a much better substrate than winter wheat (Triumph). The reason that mold can synthesize tremendous amounts of vitamins B_2, B_{12}, and pantothenic acid in spring wheat but not in winter wheat is unknown. If the wheat is completely free from toxic material, the use of this moldy wheat as a vitamin supplement in feed is worth further study.

Table 7. Effect of Mold Damage on the Thiamine Content of Wheat.

Sample	Thiamine (μg/g)			Average Content in Wheat (μg/g)	Moisture Free Basis	% Change
S_1	5.65	5.29	5.40	5.45	6.20	-
S_2	3.34	2.95	3.14	3.14	3.52	-43.3
T_1	5.05	4.87	4.80	4.91	5.54	-
T_2	2.72	2.50	2.62	2.61	2.84	-48.7

Table 8. Recovery Test of Thiamine*

Sample	Thiamine (μg/g)			Average	% Recovery
S_1	9.25	8.98	8.53	8.92	86.7
S_2	6.69	6.00	6.25	6.31	79.2
T_1	8.70	8.66	7.20	8.19	82.0
T_2	6.44	6.65	6.69	6.59	99.5

* 4 μg of thiamine·HCl was added to 1 g sample

Table 9. Effect of Mold Damage on the Riboflavin Content of Wheat.*

Sample	Riboflavin (μg/g)			Average Content in Wheat (μg/g)	Moisture Free Basis	% Change
S_1	1.08	1.10	1.08	1.05	1.19	-
S_2	4.21	4.14	4.20	4.18	4.70	+295
T_1	1.14	1.07	1.10	1.10	1.24	-
T_2	3.28	3.07	3.15	3.17	3.46	+179

* Low blank method was used to analyze S_1 and T_1, high blank method was used to analyze S_2 and T_2.

Table 10. Recovery Test of Riboflavin**

Sample	Riboflavin (μg/g)			Average	% Recovery
S_1	2.00	2.00	1.93	1.98	93.0
S_2	4.92	5.12	5.00	5.01	83.0
T_1	1.97	1.85	2.00	1.94	84.0
T_2	4.15	4.15	4.10	4.13	96.0

** 1 μg of riboflavin was added to 1 g sample.

Table 11. Effect of Mold Damage on the Vitamin B_6 Content of Wheat.

Standard (ng/tube)	Turbidity (100-T*)			Average
0	00.0	00.0	00.0	00.0
2	20.5	19.8	20.0	20.1
4	31.0	32.5	31.0	31.5
6	44.0	42.0	43.0	43.2
8	49.2	49.0	49.3	49.2
10	56.5	56.5	55.3	56.1
12	60.8	61.0	60.6	60.8
14	63.5	64.5	62.8	63.6
16	67.0	67.2	67.4	67.2
18	69.5	69.0	69.0	69.2
20	73.0	69.5	71.4	71.3

Sample**	1 ml	3 ml	5 ml	Sample+Pyridoxin (1 ml + 2.5 ng)	Average % Content in Wheat (µg/g)	% Change	% Recovery
S_1	25.5	51.3	62.8	37.5	4.18	-	88.5
S_2	33.7	62.0	69.0	42.7	6.12	+46.2	88.5
T_1	26.0	51.7	64.5	38.2	4.35	-	89.0
T_2	30.5	55.2	68.0	40.5	5.13	+18.0	86.4

* T = Transmittance

** Sample 1 g was made up to 1500 ml

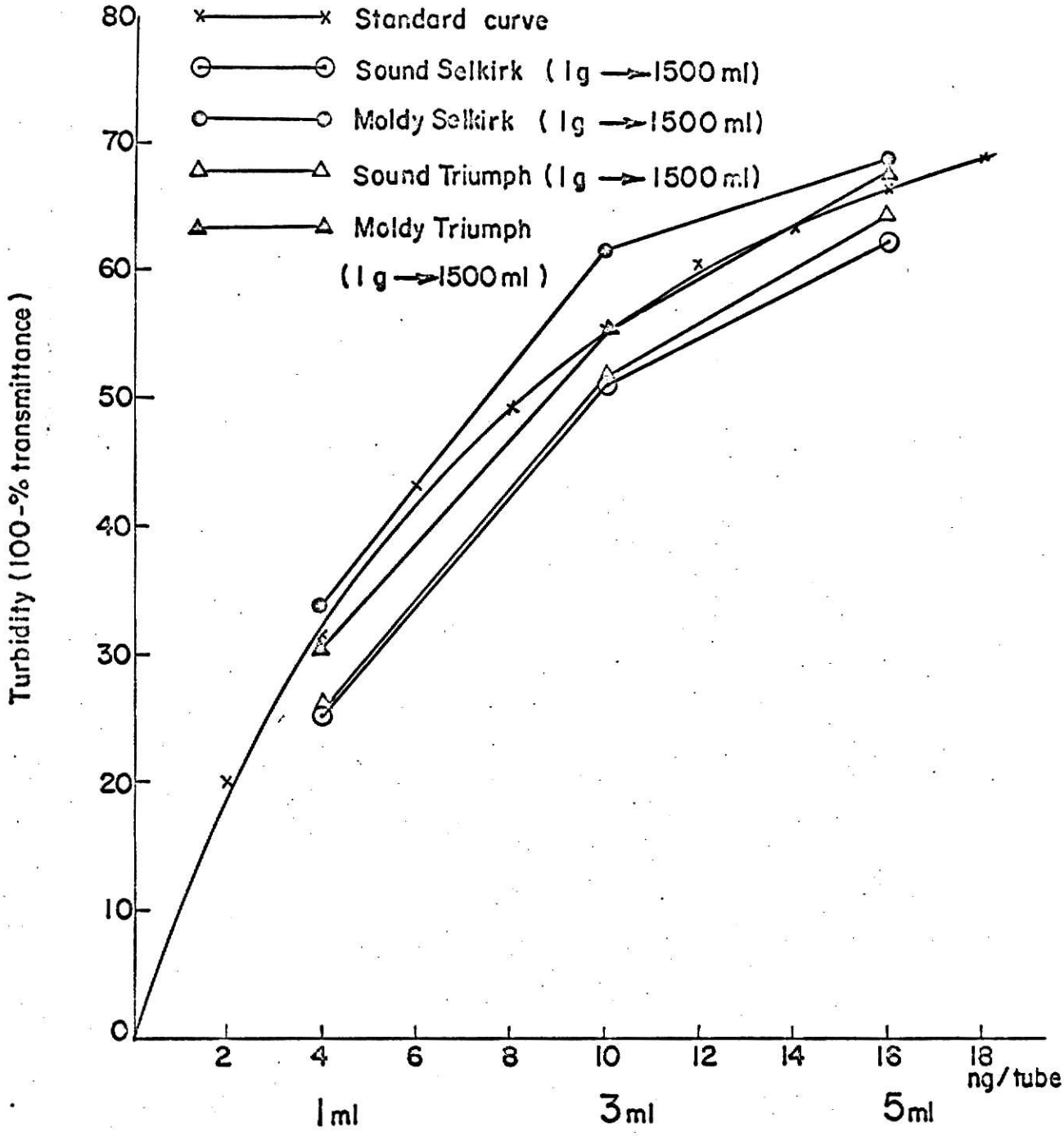


Fig.2. Effect of mold damage on the vitamin B_6 content of wheat.

Table 12. Effect of Mold Damage on the Vitamin B_{12} Content of Wheat.

Standard (ng/tube)	Turbidity (100-T)			Average
0.00	0.00	0.00	0.00	0.00
0.05	3.0	3.5	3.0	3.2
0.10	7.0	6.0	5.5	6.2
0.15	12.0	11.0	7.0	10.0
0.20	13.0	13.0	16.0	14.0
0.25	21.5	20.5	19.0	20.3
0.30	24.0	24.5	23.5	24.0
0.35	33.5	31.0	33.5	32.7
0.40	41.5	39.0	39.5	40.0
0.45	49.0	49.5	51.5	50.0
0.50	51.5	51.0	52.0	51.5
0.55	56.0	56.5	54.0	55.5
0.60	58.5	59.0	58.5	58.7
0.65	63.0	63.0	63.0	63.0
0.70	65.5	66.5	65.5	65.8
0.75	66.0	67.0	66.0	66.3

Sample	1 ml	3 ml	5 ml	Sample+Cyanocobalamin (1ml+0.15 ng)	Average Content in Wheat (μg/g)	%Change	%Recovery
S_1*	6.3	26.5	51.5	18.8	0.076	-	93.5
S_2**	4.5	16.5	39.0	16.0	0.285	+276	90.0
T_1*	4.5	19.5	41.0	17.0	0.061	-	92.0
T_2*	7.5	30.5	55.5	21.0	0.084	+37	94.8

* Sample 1 g was made up to 750 ml

** Sample 1 g was made up to 3750 ml

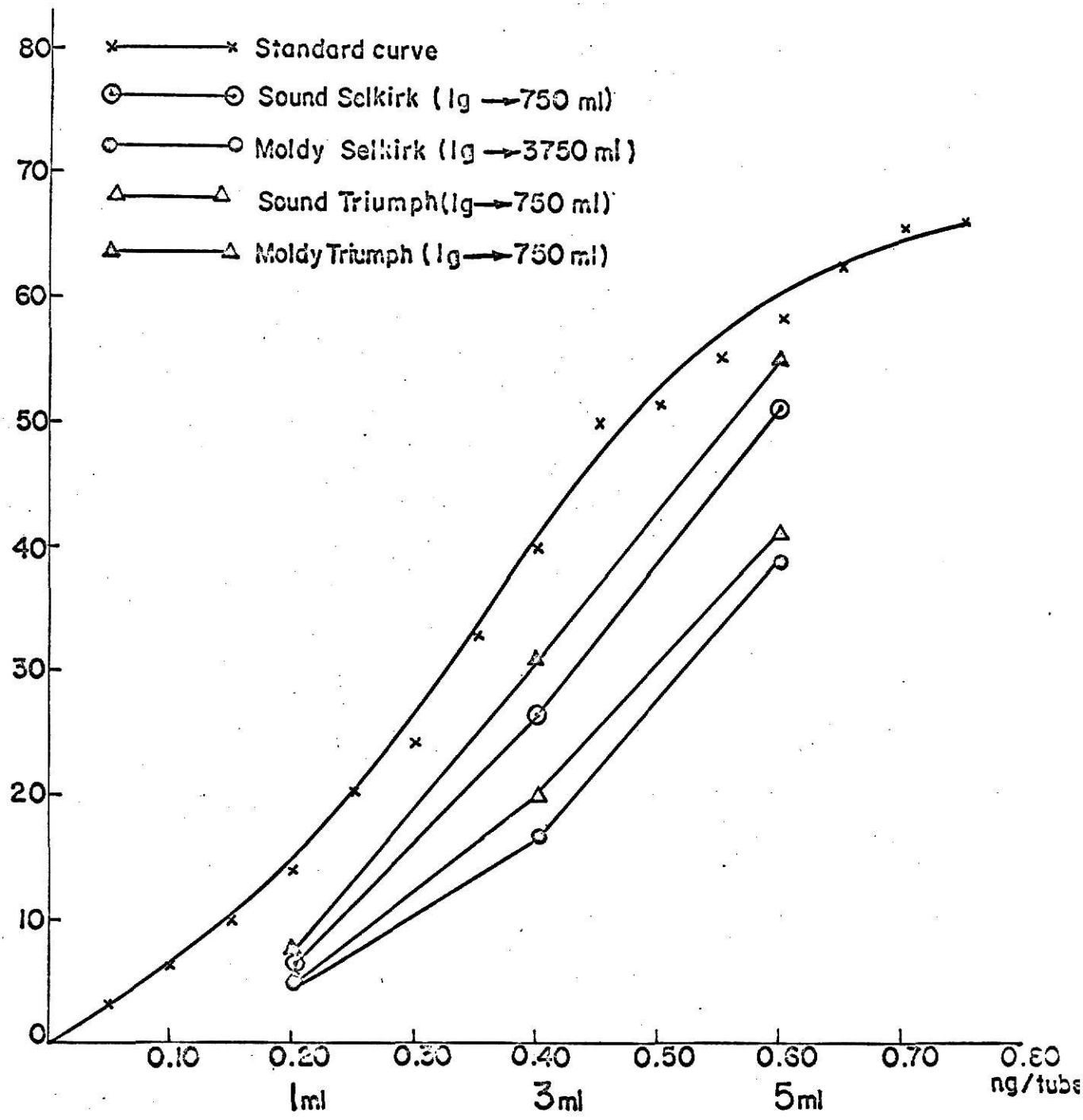


Fig. 3. Effect of mold damage on the vitamin B_{12} content of wheat.

Table 13. Effect of Mold Damage on the Niacin Content of Wheat.

Standard (µg/tube)	Turbidity (100-T)			Average
0.00	00.0	00.0	00.0	00.0
0.05	20.0	23.0	20.0	23.0
0.10	35.4	36.0	34.2	35.2
0.15	38.6	39.0	39.1	38.9
0.20	42.5	41.5	43.5	42.5
0.25	44.0	44.0	45.0	44.3
0.30	43.5	44.4	45.5	44.5
0.35	45.0	43.5	49.5	46.0
0.40	48.5	48.0	47.5	48.0
0.45	47.5	47.5	47.5	47.5
0.50	49.5	48.0	46.5	48.0
0.75	49.0	49.0	49.0	49.0

Sample*	1ml	3ml	5ml	Sample+Niacin (1ml + 0.1µg)	Average Content in Wheat (µg/g)	%Change	%Recovery
S_1	28.5	42.5	46.0	39.2	67.0	-	85.5
S_2	36.0	46.8	48.5	42.2	120.0	+79.1	83.2
T_1	31.5	45.0	47.0	40.5	87.0	-	85.0
T_2	27.5	41.5	45.5	39.0	65.0	-25.3	87.0

* Sample 1 g was made up to 1000 ml

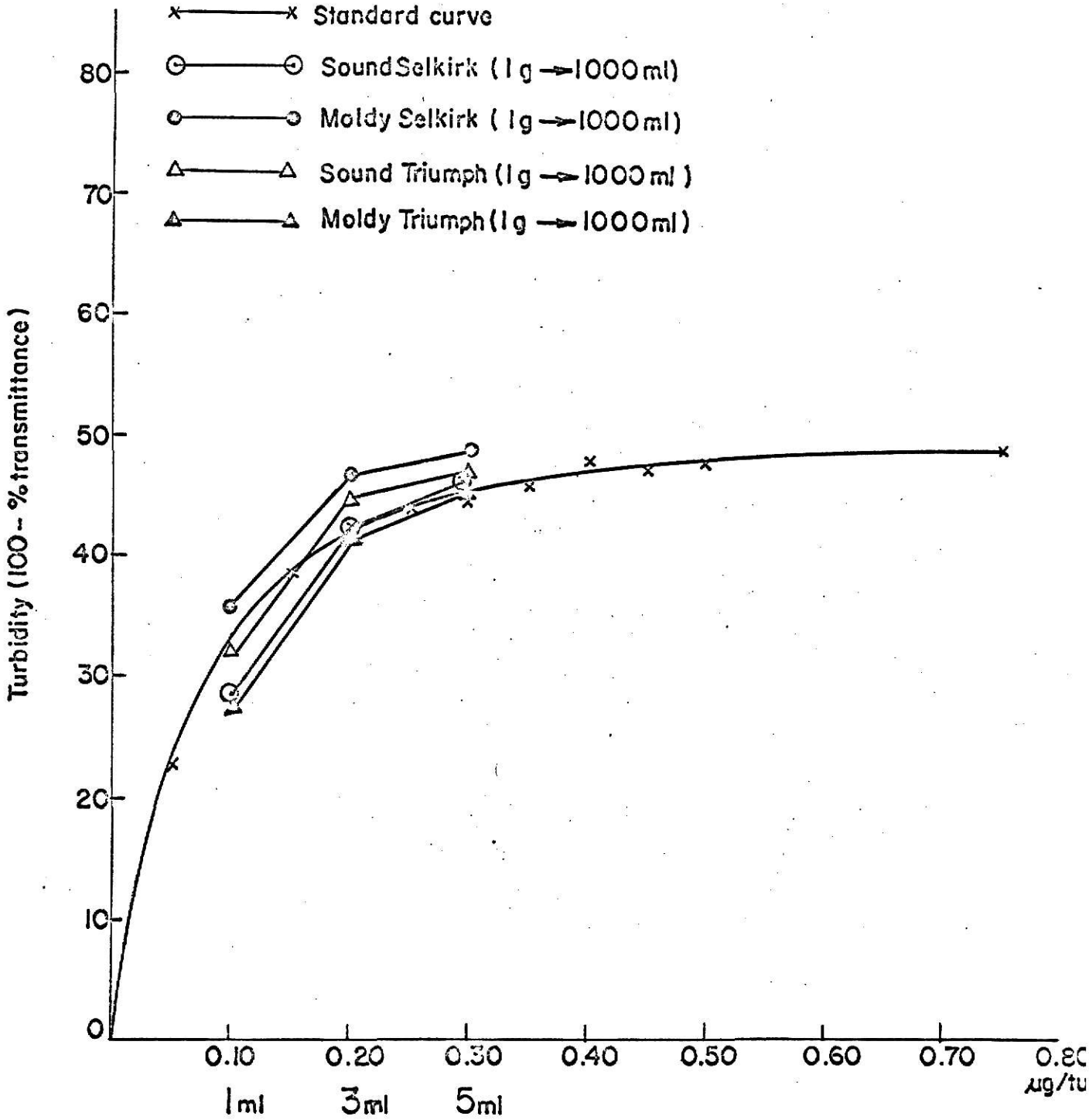


Fig.4. Effect of mold damage on the niacin content of wheat.

Table 14. Effect of Mold Damage on the Pantothenic Acid Content of Wheat.

Standard (µg/tube)	Turbidity (100-T)			Average
0.00	00.0	00.0	00.0	00.0
0.01	2.6	3.0	2.8	2.8
0.02	8.0	7.5	8.5	8.0
0.03	20.0	19.5	19.9	19.8
0.04	35.5	33.5	34.5	34.5
0.05	44.0	45.5	45.5	45.0
0.06	54.0	53.5	56.0	54.5
0.07	61.2	60.0	60.8	60.6
0.08	62.5	63.2	63.3	63.0
0.09	64.8	65.6	66.7	65.7
0.10	65.5	66.5	66.3	66.1
0.15	73.0	71.2	70.9	71.7

Sample	1ml	3ml	5ml	Sample+Ca - Pantothenate (1ml + 0.02µg)	Average Content in Wheat (µg/g)	% Change	%Recovery
S_1*	5.0	44.0	62.5	27.5	11.8	-	98.5
S_2**	6.0	48.0	65.0	28.7	66.5	+463.0	95.2
T_1*	4.0	35.5	59.5	26.0	10.5	-	101.3
T_2**	5.0	44.0	62.5	26.5	11.8	+ 12.3	96.5

* Sample 1 g was made up to 750 ml

** Sample 1 g was made up to 3750 ml

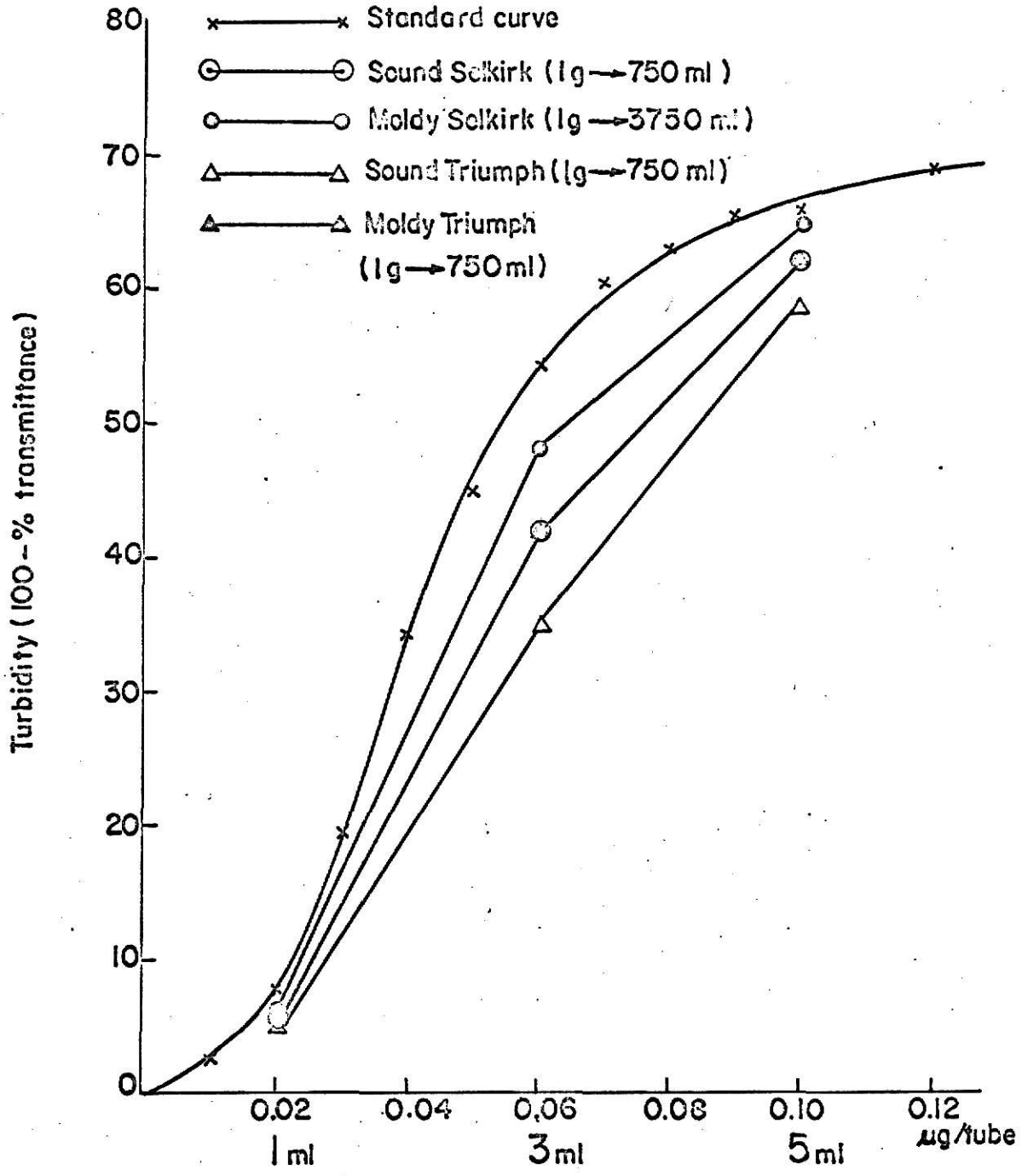


Fig.5. Effect of mold damage on the pantothenic acid content of wheat.

III. Effect of Mold Damage in Aflatoxin Content of Wheat and Corn.

Thin-layer chromatography was used to study aflatoxin content and distribution in milled products of wheat and corn.* The results are shown in Figs. 6 to 9. Aspergillus flavus produced 9.15 ppb aflatoxin B_1 and 12.5 PPb aflatoxin G_1 in moldy spring wheat (Selkirk), while produced 16.6 ppb aflatoxin B_1 and trace aflatoxin G_1 in moldy corn. But there was no detectable amount aflatoxin in moldy winter wheat (Triumph). In Figs. 6 and 7 are shown the identities of aflatoxins on thin-layer plates developed by different solvent systems. In Fig. 8, it is shown that most aflatoxins remained in the bran when the wheat was milled. High-ash flour and low-ash flour had only traces. In Fig. 9, it is seen that the concentration of aflatoxin B_1 in the germ of corn was about seven times that of feed or grits, and was about five times that of the meal. The percentages of distribution of aflatoxin B_1 in the milled products of corn were: feed 12.8%, germ 27.6%, grits 5%, and meal 54.6%.

*Moldy wheats were milled to bran (39.4%), high-ash flour (flour 1) (15.3%), and low-ash flour (flour 2) (45.3%). Moldy corn was milled to feed (22%), germ (6.4%), grits (7.6%), and meal (64%).

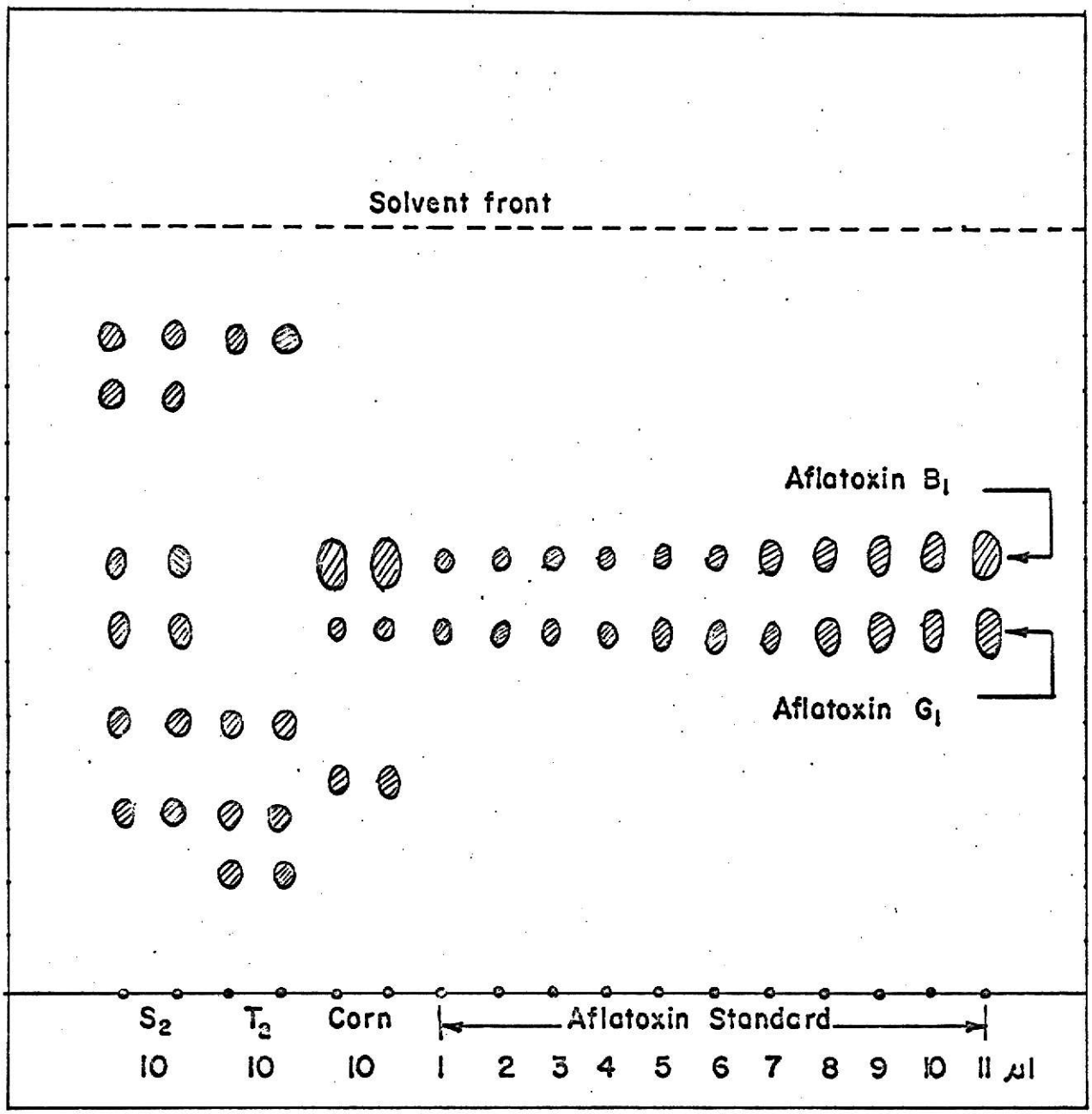


Fig. 6. Thin-layer chromatogram of moldy Selkirk wheat, moldy Triumph wheat, and moldy corn.
Developing solvent: CH_3OH: $CHCl_3$ (3:97, v/v).

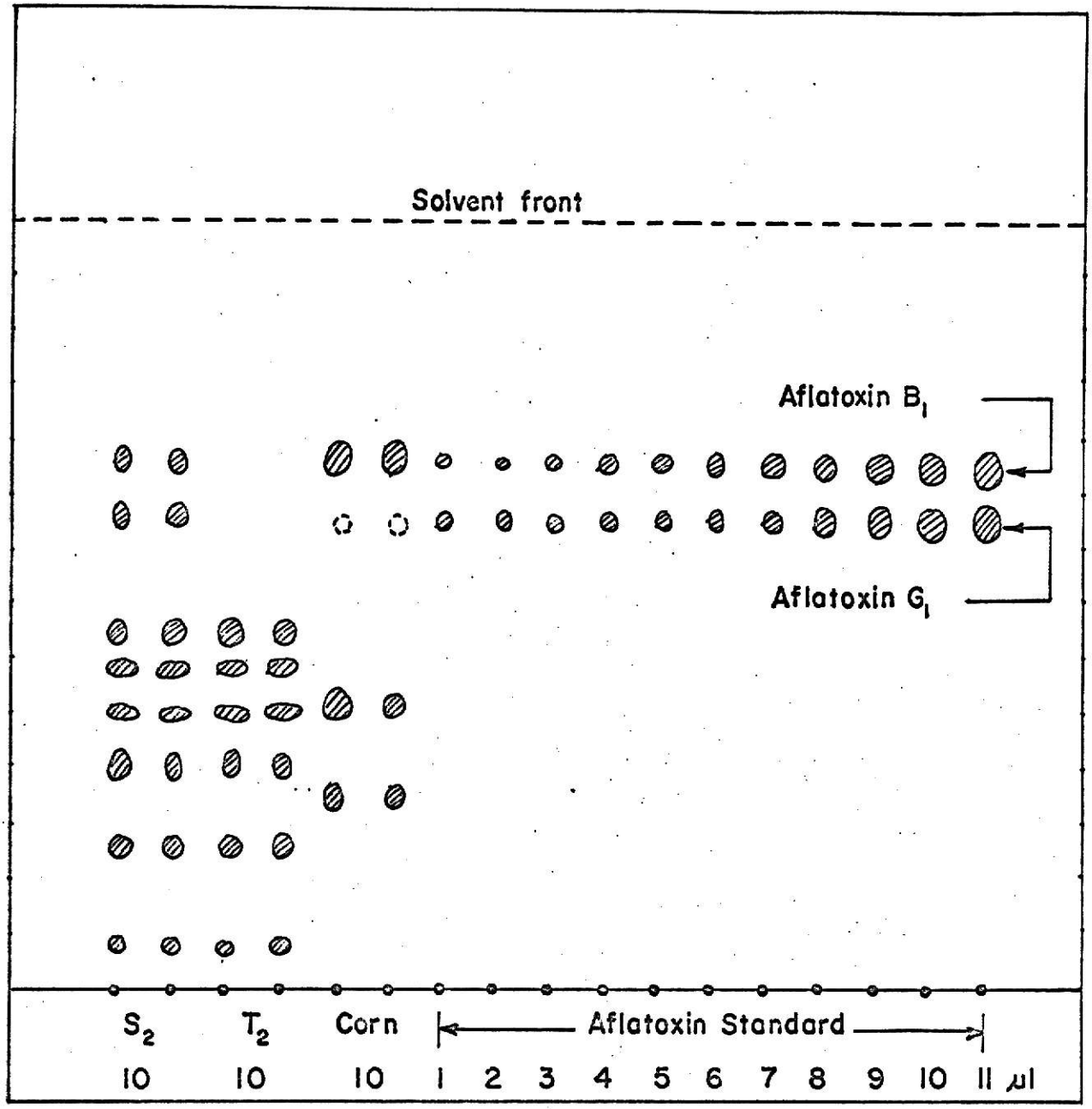


Fig.7. Thin-layer chromatogram of moldy Selkirk wheat, moldy Triumph wheat and moldy corn.

Developing solvent: $CH_3OH : CHCl_3 : HOAc$ (5:94.5:0.5, v/v).

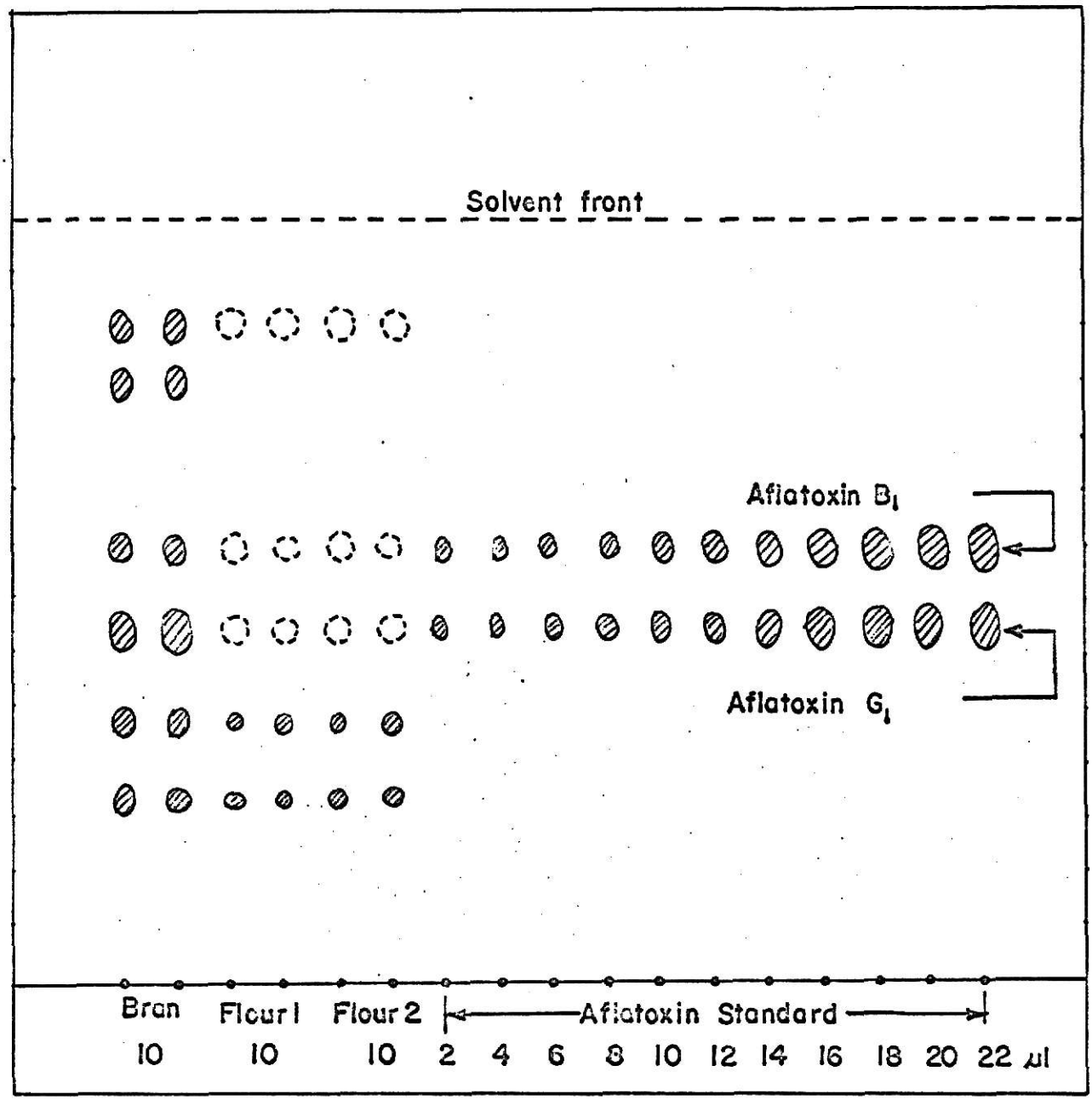


Fig. 8. Thin-layer chromatogram of milling products of moldy Selkirk wheat.

Developing solvent: CH_3OH: $CHCl_3$ (3:97, v/v).

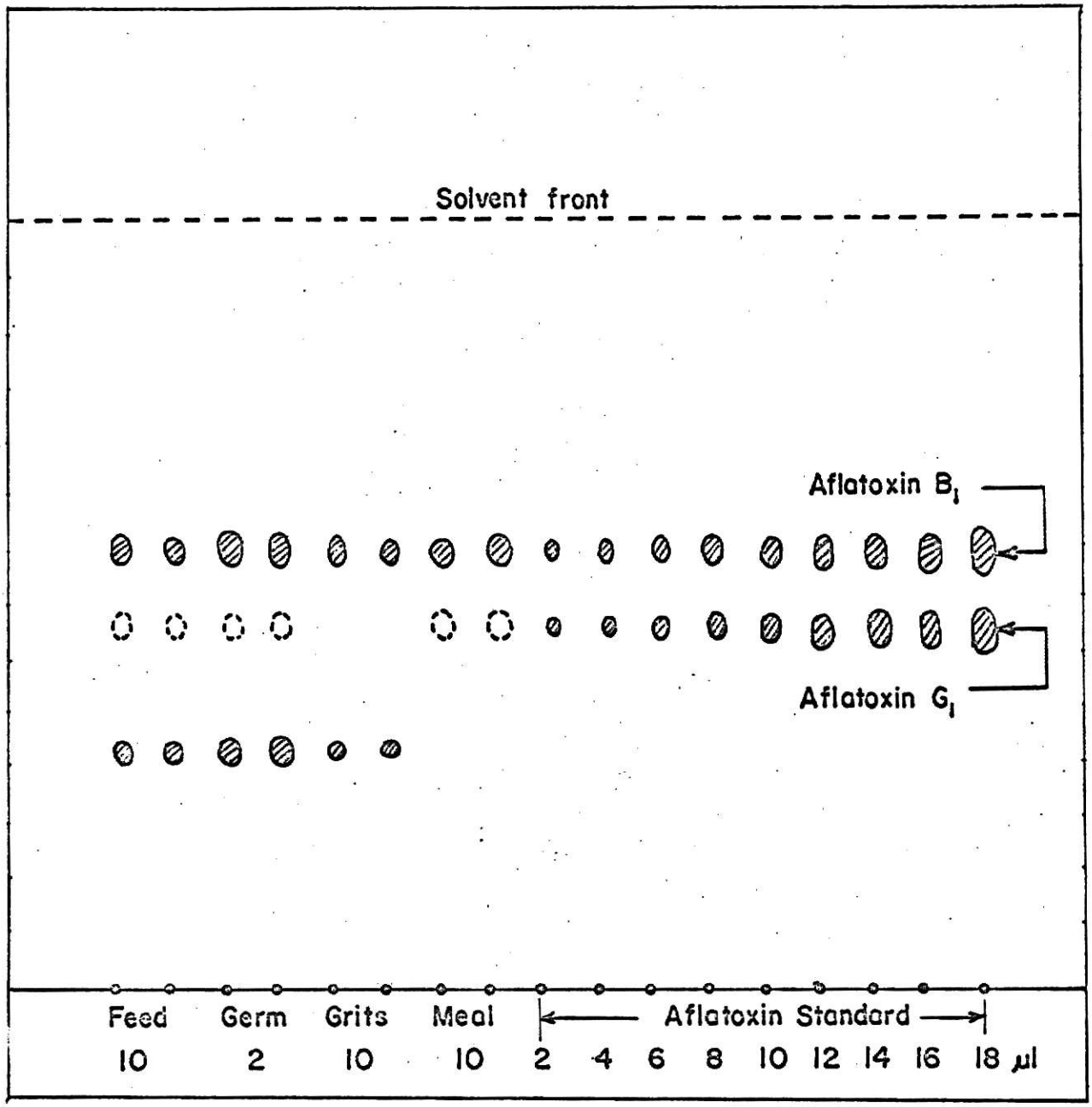


Fig. 9. Thin-layer chromatogram of milling products of moldy corn.

Developing solvent: $CH_3OH:CHCl_3$ (3:97, v/v).

SUMMARY AND CONCLUSION

This study was conducted to investigate amino acids, B-group vitamins, and aflatoxin in Hard Red Spring (Selkirk) and Hard Red Winter (Triumph) wheats which had been deteriorated by Aspergillus flavus.

The amino acid compositions of both wheats before mold deterioration were quite similar, but after mold deterioration, arginine and cysteine in Selkirk wheat were decreased 50.2% and 74.5% respectively. However, methionine was increased 51%. The increase of methionine was possibly derived from cysteine. In Triumph wheat, lysine, histidine, and arginine were decreased by 45%, 50.1% 50.0% respectively, however, methionine was increased by 34.5%. The ratio of total amino acids to Kjeldahl protein in Selkirk wheat and Triumph wheat was 78.86% and 90.63% respectively. This means Selkirk wheat was more damaged by mold than was Triumph wheat because more amino acids in the protein of Selkirk wheat were metabolized to form other nitrogenous compounds which still possessed detectable Kjeldahl nitrogen.

In spring wheat large amounts of B-group vitamins were synthesized: the net increase of riboflavin was 295%, vitamin B_6 46%, vitamin B_{12} 276%, niacin 79.1%, and pantothenic acid 463%. But in winter wheat much lower amounts of B-group vitamins were synthesized: riboflavin 179%, vitamin B_6 18%, vitamin B_{12} 37%, and pantothenic acid 12.3%.

Some vitamins were lost in wheat after mold deterioration. Thiamine decreased by 43.3% in Selkirk wheat and 48.7% in Triumph

wheat. Niacin decreased by 25.3% in Triumph wheat.

Distribution of aflatoxin in milling products of moldy wheats and moldy corn was shown by thin-layer chromatogram. Spring wheat contained 9.15 ppb of aflatoxin B_1 and 12.5 ppb of aflatoxin G_1, while winter wheat contained no significant amount of aflatoxin. Almost all aflatoxins in wheat were present in bran, only a trace amount was detected in either high-ash or low-ash flour. Corn was milled to feed (22%), germ (6.4%), grits (7.6%), and meal (64%). Aflatoxin distribution in these milling products was: feed 12.8%, germ 27.6%, grits 5%, and meal 54.6%. The concentration of aflatoxin in germ was at least five times that in meal.

Moldy wheat contained a much higher amount of B-group vitamins than unmolded wheat. If it is completely free from toxic material, the use of moldy wheat as a vitamin supplement in feed is worthy of further study.

ACKNOWLEDGMENT

The author wishes to express his appreciation of his major adviser, Professor Robert J. Robinson, who provided the guidance and research facilities for the project.

He also owes much gratitude to Professor Gerald D. Miller and to Professor Raymond K. Burkhard for their encouragement and advice.

He acknowledges his debt to Professor Charles W. Deyoe and Mrs. S. Geng for their help in analyzing amino acids of the wheat samples.

Finally, the author thanks Miss Grace E. Givin for reading the manuscript.

LITERATURE CITED

1. FORGACS, J. Mycotoxicoses, the neglected diseases. Feedstuffs 34, 124-134 (1967).
2. MIROCHA, C.J., CHRISTENSEN, C.M., and NELSON, G.H. Toxic metabolites produced by fungi implicated in mycotoxicoses. Biotechnol. Bioeng. 10, 469 (1968).
3. GRAHAM, R. Cornstalk disease investigations. Vet. Medicine 31, 46-50 (1936).
4. SIPPEL, W.L., BURNSIDE, J.E., and ATWOOD, B.F. A disease of swine and cattle by eating molded corn. Proc. Am. Vet. Med. Assoc., 90th Ann. Meeting, Toronto pp. 174-181 (1954).
5. BLOUNT, W.P. Turkey X disease. Turkeys 9, 52 (1961).
6. SARGEANT, K., SHERIDAN, A., O'KELLY, J., and CARNAGHAN, R.B.A. Toxicity associated with certain samples of groundnuts. Nature 192, 1096-1097 (1961).
7. DE IONGH, H., BEERTHUIS, R.K., VLES, R.O., BARRETT, C.B., and ORD, W.O., Investigation of the factor in groundnut meal responsible for "turkey X disease". Biochem. Biophys. Acta. 65, 548-551 (1962).
8. VAN DER MERWE, K.J., FOURIE, L., and SCOTT, DE B. The structure of aflatoxins. Chem. Ind. (London) PP. 1660-1661 (1963).
9. WICK, E.L., and WOGAN, G.N. Aflatoxin B_2: chemical identity and biological activity. Science 142, 1191-1192 (1963).
10. ARMBRECHT, B.H., BIERBOWER, G.W., and UNDERWOOD, P.C. Experimental aflatoxicoses in Peking white ducklings and Coturnix quail. Toxicol. Appl. Pharmacol. 7, 478 (1965).

11. BARNES, J.M., and BUTLER, W.H., Carcinogenic Activity of aflatoxin to rats. Nature 202, 1016 (1964).

12. BUTLER, W.H. Early hepatic parenchymal changes induced in the rat by aflatoxin B_1. Am. J. Pathol., 49, 113-128 (1966).

13. BUTLER W.H. Acute toxicity of aflatoxin B_1 in guinea-pigs. J. Pathol. Bacteriol. 91, 277-280 (1966).

14. DETROY, R.W., and CIEGLER, A. Inhibition of protein and aflatoxin B_1 synthesis by D-ethionine in Aspergillus parasiticus. Bacteriological Processings (1970) (Am. Soc. for Microbiol.).

15. LANCASTER, M.C., JENKINS, F.P., and PHILIP, J. McL. Toxicity associated with certain samples of groundnuts. Nature 192, 1095-1096 (1961).

16. SPENSLEY, P.C. Aflatoxin, the active principle in turkey X disease. Endeavour, 22, 75-79 (1963).

17. WOOD, E.M., and LARSON, C.P. Hepatic carcinoma in rainbow trout. Arch. pathol. 71, 471-479 (1961).

18. ALLCROFT, R., and CARNAGHAN, R.B.A. Groundnut toxicity-Aspergillus flavus toxin in animal products. Vet. Record 74, 863 (1962).

19. KULIK, M.M., and HOLADAY, C.E. Aflatoxin: A metabolic product of several fungi. Mycopathol. Mycol. Appl. 30, 137-140 (1966).

20. DIENER, U.L., and DAVIS, N.D. Aflatoxin production by isolates of Aspergillus flavus. Phytopathology 56, 1390-1393 (1966).

21. BROADBENT, J.H., CORNELIUS, J.A., and SHONE, G. The detection and estimation of aflatoxin in groundnuts and groundnut materials. Analyst 88, 214-216 (1963).

22. VELASCO, J. Determination of aflatoxin in cotton seed by ferric hydroxide gel cleanup. J. Asso. Offic. Anal. Chemist 53, 611-616 (1970).

23. NESBITT, B.F., O'KELLY, J., SARGEANT, K., and SHERIDAN, A. Toxic Matabolites of Aspergillus flavus. Nature 195, 1062-1063 (1962).

24. ALLCROFT, R., and CARNAGHAN, R.B.A. Groundnut toxicity- An examination for toxin in human food products from animals fed toxic groundnut meal. Vet. Record 75, 259-263 (1963).

25. DE IONGH, H., VLES, R.O., and VAN PELT, J.G. Milk of mammals fed by an aflatoxin-containing diet. Nature 202, 466-467 (1964).

26. BUTLER, W.H., and CLIFFORD, J.I., Extraction of aflatoxin from rat liver. Nature 206, 1045-1046 (1965).

27. HOLZAPFEL, C.W., STEYN, P.S., and PURCHASE, I.F.H. Isolation and structure of aflatoxins M_1 and M_2. Tetrahedron Letters 25, 2799-2803 (1966).

28. DUTTON, M.F., and HEATHCOTE, J.G. Two new hydroxy-aflatoxins. Biochem. J. 101, 21-22 (1966).

29. LILLEHOJ E.B., and CIEGLER, A. Biological activity of aflatoxin B_{2a}. Appl. Microbiol. 17, 516-19 (1969).

30. ASAO, T., BUCHI, G., ABDEL-KADER, M.M., WICK, E.L., and WOGAN, G.N. Aflatoxin B and G. J. Am. Chem. Soc. 85, 1706-1707 (1963).

31. ASAO, T., BUCHI, G., ABDEL-KADER, M.M., CHANG, S.S., WICK, E.L., and WOGAN, G.N. The structures of aflatoxins B_1 and G_1. J. Am. Chem. Soc. 87, 882-886 (1965).

32. BUCHI, G., FOULKES, D.M., KURONO, M., and MITCHELL, G.F. The total systhesis of racemic aflatoxin B_1. J. Am. Chem. Soc. 88,

4534-4536 (1966).

33. VAN SOEST, T.C., and PEERDEMAN, A.F. An X-ray study of dihydroaflatoxin B_1. Koninkl, Ned. Akad. Wetenschap. Pro. Ser. B67, 469-472 (1964).

34. HARTLEY, R.D., NESBITT, B.F. and O'KELLY, J. Toxic metabolites of Aspergillus flavus, Nature 198, 1056-1058 (1963).

35. CHEUNG, K.K., and SIM, G.A. Aflatoxin G_1: Direct determination of the structure by method of isomorphous replacement. Nature 201, 1185-1188 (1964).

36. CHANG, S.B., ABDEL-KADER, M.M., WICK, E.L., and WOGAN, G.N. Aflatoxin B_2: Chemical identity and biological activity, Science 142, 1191 (1963).

37. DUTTON, M.F., and HEATHCOTE, J.G. The structure, biochemical properties and origin of the aflatoxins B_{2a} and G_{2a}. Chem. Ind. (London) pp. 418-421 (1968).

38. LIJINSKY, W., and BUTLER, W.H. Purification and toxicity of aflatoxin G_1, Pro. Soc. Exptl. Biol. Med. 123, 151-154 (1966).

39. MASRI, M.S., LUNDIN, R.E., PAGE, J.R. and GARCIA, V.C. Crystalline aflatoxin M_1 from urine and milk. Nature 215, 753-755 (1967).

40. ASPLIN, F.D. and CARNAGHAN, R.B.A. The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens. Vet. Record 73, 1215-1219 (1961).

41. NEWBERNE, P.M., RUSSO, R., and WOGAN, G.N. Acute toxicity of aflatoxin B_1 in the dog. Pathol. Vet. 3, 331-340 (1966).

42. ALLCROFT, R., ROGERS, H. LEWIS, G., NABNEY, J. and BEST, P.F. Metabotism of aflatoxin in sheep: excretion of the "milk toxin". Nature 209, 154-155 (1966).

43. HARDING, J.D.J., DONE, J.T., LEWIS, G., and ALLCROFT, R. Experimental groundnut poisoning in pigs, Res. Vet.Sci. 4, 217-229 (1963).

44. ALLCROFT, R., and LEWIS, G. Groundnut toxicity in cattle: Experimental poisoning of calves and a report on clinical effects in older cattle. Vet. Record 75, 487-493 (1963).

45. LOOSMORE, R.M., and MARKSON, L.M. Poisoning of cattle by Brazilian groundnut meal. Vet. Record 73, 813-814. (1961).

46. TULPULE, P.G., MADHAVAN, T.V., and GOPALAN, C. Effect of feeding aflatoxin in young monkeys. Lancet 1, 962 (1964).

47. MADHAVAN, T.V., GOPALAN, C., and TULPULE, P.G. Aflatoxin-induced Hepatic fibrosis in Rhesus monkeys. Arch. Pathol., 79, 466-69 (1965).

48. MADHAVAN, T.V. and GOPALAN, C. Effect of dietary protein on aflatoxin liver injury in weanling rats. Arch. Pathol., 80, 123-126 (1965).

49. CUTHBERTSON, W.F.T., LAURSEN, A.C., and PRATT, D.A.H. Effect of groundnut meal containing aflatoxin on cynomologus monkeys. Brit. J. Nutr. 21, 893 (1967).

50. LEGATOR, M.S., and WITHROW, A. Aflatoxin: effect on mitotic division in cultured embryonic lung cells. J. A. O. A. C. 47, 1007-1009 (1964).

51. LEGATOR, M.S., ZUFFANTE, S.M., and HARP, A.R. Aflatoxin: effect on cultured heteroploid human embryonic lung cells. Nature 208, 345-348 (1965).

52. GABLIKS, J., SCHAEFFER, W., FRIEDMAN, L., and WOGAN, G., Effect of aflatoxin B_1 on cell cultures. J. Bacteriology, 90, 720-23 (1965).

53. LEGATOR, M.S. Biological effects of aflatoxin in cell culture. Bacteriological Rev., 30. 471-77 (1966).

54. ZUCKERMAN, A.J., TSIQUAYE, K.N., and FULTON, F. Tissue culture of human embryo liver cells and the cytotoxicity of aflatoxin B_1. Brit. J. Exptl. Pathol. 48, 20-27 (1966).

55. AYRES, J.C., LILLARD, H.S., and LILLARD, D.A. Mycotoxins: Methods for detection in foods. Food Technology 24, 161 (1970).

56. TUNG, T.C., and LIN, K.H. Aflatoxin in foods in Taiwan. J. Vitaminol. 14, 48-52 (1968).

57. MURAKAMI, H., TAKASE, S., KUWABARA, K. Nonproductivity of aflatoxin by Japanese industrial strains of Aspergillus. II. Production of fluorescent substances in rice koji, and their identification by absorption spectrums. J. Gen. Appl. Microbiol. 14, 97 (1968).

58. YOKOTSUKA, T., SASAKI, M., KIKUCHI, T., ASAO, Y., and NOBUHARA, A. Compounds produced by molds I. Fluorescent compounds produced by Japanese industrial molds. Nippon Nogeikagaku Kaishi 41, 32 (1967).

59. WANG, H.H., SHIH, T.H., CHIOU, C.M., FAN, T.I., CHUANG, H.L., and TAN, C.L. A short method for the determination of aflatoxin in wine products. J. of the Chinese Agr. Chem. Soc. 6, 69 (1968).

60. CHRISTENSEN, C.M., and GORDON, D.R. The mold flora of stored wheat and corn and its relation to heating of moist grain. Cereal Chem. 25, 40 (1948).

61. CHRISTENSEN, C.M. Grain Storage Studies. XXI. viability and moldiness of commercial wheat in relation to the incidence of germ damage. Cereal Chem. 32, 507 (1955).

62. MILNER, M., CHRISTENSEN, C.M., and GEDDES, W.F. Grain storage studies v. chemical and microbiological studies on "sick" wheat. Cereal Chem. 24, 23 (1947).

63. DEL PRADO, F.A., and CHRISTENSEN, C.M. Grain storage studies. XII. The fungus flora of stored rice seed. Cereal Chem. 29, 456 (1952).

64. KURADA, H., OGASAWARA, K., and FRAMPTON, V.L. Microflora of milled rice. Cereal Chem. 34, 47 (1957).

65. BOTTOMLEY, R.A., CHRISTENSEN, C.M., and GEDDES, W.F. The influence of various temperatures, humidities and oxygen concentrations on mold growth and biochemical changes in stored yellow corn. Cereal Chem. 27, 271 (1950).

66. TUITE, J.F. and CHRISTENSEN, C.M. Grain Storage studies XVI. influence of storage conditions upon the fungus flora of barley seed. Cereal Chem. 32, 1 (1955).

67. CHRISTENSEN, C.M., and COHEN, M. Numbers, kinds, and source of molds in flour. Cereal Chem. 27, 178 (1950).

68. BARTON-WRIGHT, E.C. Studies on the storage of wheaten flour: III. Changes in the flora and the fats and the influence of these changes on gluten character. Cereal Chem. 15, 521 (1938).

69. DIRK, B.M., BOYER, P.D., and GEDDES, W.F. Some properties of fungal lipases and their significance in stored grain. Cereal Chem. 32, 356 (1955).

70. SORGER-COMENIGG, H., CUENDET, L.S., CHRISTENSEN, C.M. and GEDDES, W.F. Effect of mold growth during temporary exposure of wheat to high moisture contents upon the development of germ damage and other indices of deterioration during subsequent storage. Cereal Chem. 32, 270 (1955).

71. KENT-JONES, D.W. Presidential Address Royal Institute of Chemistry. J. Royal Institute of Chemistry. May 1956.

72. TOEPFER, E.W. and HEWSTON, E.M. Nutrient composition of selected wheats and wheat products , description of samples. Cereal Chem. 46, 560 (1969).

73. POLANSKY, M.M., and TOEPFER, E.W. Nutrient composition of selected wheats and wheat products IV vitamin B-6 components. Cereal Chem. 46, 664 (1969).

74. HALL, G.S., LAIDMAN, D.L. The determination of tocopherols and isoprenoid quinones in the grain and seedlings of wheat. Biochem. J. 108, 465 (1968).

75. SLOVER, H.T., LEHMANN, J. and VALIS, R.J. Nutrient composition of selected wheats and wheat products. Cereal Chem. 46, 635 (1969).

76. DESIKACHAR, H.S.R. Determination of the degree of polishing in rice II. Determination of thiamine and phosphorous for processing control. Cereal Chem. 32, 78 (1955).

77. KIK, M.C., and VAN LANDINGHAM, F.B. Riboflavin and thiamine in rice. Cereal Chem. 20 563 (1943).

78. TKACHUK, R. Amino acid composition of wheat flours. Cereal Chem. 43, 207 (1966).

79. INKPEN, J.A., QUACKENBUSH, F.W. "Extractable" and "Bound" Fatty acids in wheat and wheat products. Cereal Chem. 46, 580-588 (1969).

80. OSBORNE, T.B., and MENDEL, L.B. The nutritive value of the wheat kernel and its milling products. J. Biol. Chem. 37, 557-601 (1919).

81. BELL, M., and MENDEL, L.B. The distribution of vitamin B in the wheat kernel. Am. J. Physiol. 62, 145 (1922).

82. CONNER, R.T., STRAUB, G.J. Thiamine and riboflavin in wheat and corn. Cereal Chem. 18, 671 (1941).

83. MORGAN, A.F., and HUNT, M. J. Vitamin content of wheat products. Cereal Chem. 12, 411 (1935).

84. MUNSELL, H.E. and DEVANEY, G.M. The vitamin B and G content of wheat germ. Cereal Chem. 10 287 (1933)

85. SOMERS, G.F., COOLIDGE, M.H., and HAMNER, K.C. The distribution of thiamin and riboflavin in wheat grains. Cereal Chem. 22, 333 (1945).

86. SCHULTZ, A.S., ATKIN, L., and FREY, C.N. The vitamin B_1 content on wheat, flour, and bread. Cereal Chem. 16, 643 (1939).

87. CALHOUN, W.K., BECHTEL, W.G., and BRADLEY, W.B. The vitamin content of wheat, flour, and bread. Cereal Chem. 35, 350 (1958).

88. HASHITANI, Y., and SAKO, T. Vitamin content of bread. Cereal Chem. 9, 107, (1932).

89. ANDREWS, J.S., BOYD, H.M., and TERRY, D.E. The riboflavin content of cereal grains and bread and its distribution in

products of wheat milling. Cereal Chem. 19, 55 (1942).

90. SPACKMAN, D.H., STEIN, W.H., and MOORE, S. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30, 1190 (1958).

91. BECHTEL. W.G., and HOLLENBECK, C.M. A revised thiochrome procedure for the determination of thiamine in cereal products. Cereal Chem. 35, 1 (1958).

92. BECHTEL, W.G., and HILL, G. Fluorometric procedure for riboflavin in cereals and cereal products. Cereal Science Today 7, 198 (1962).

93. PEARSON, W.N. Riboflavin p. 99-136, In The Vitamins, vol. VII ed. by Gyorgy, P.N., and Pearson, W.N., Academic Press, New York, and London (1967).

94. LOY, H.W. JR., PARRISH, W.P., SCHIAFFINO, S.S. The use of turbidity measurements in microbiological methods for vitamins. J. A.O.A.C. 39, 172 (1956).

95. ATKIN, L., SCHULTZ, A.S., WILLIAMS, W.L., and FREY, C.N. Yeast Microbiological methods for determination of vitamins. Ind. & Eng. Chem. Anal. Ed. 15, 141 (1943).

96. PARRISH, W.P., LOY, H.W., JR., and KLINE, O.L. Further studies on the yeast method for vitamin B_6. J.A.O.A.C. 39, 157 (1956).

97. DIFCO LABORATORIES, Microbiological assay of vitamins and amino acids. p. 1-2. Detroit, Michigan (1964).

98. REFAI, F.Y., and MILLER, B.S. The determination of Pantothenic acid in wheat. Cereal Chem. 29, 469 (1952).

99. PONS, W.A., JR., CUCULLU, A.F., LEE, L.S. ROBERTSON, T.A., FRANZ, A.O., and GOLDBLATT, L.A. Determination of aflatoxin

in agricultural products: use of aqueous acetone for extraction. J.A.O.A.C. 49, 554-562 (1966).

100. STOHECKER, R., and HENNING, H.M. Vitamin B_{12} p.167, In Vitamin Assay, translated by Libman, D.D. Verlag Chemie, GMBH. Weinheim/Bergstr (1966).

101. WEISS, W., EDELSON, H., and LOY, H.W. The nature of the dose-response curve and its effect upon the error of the assay of vitamin B_{12}. J.A.O.A.C. 39, 167 (1956).

FUNGUS DETERIORATION OF GRAIN: EFFECT OF FUNGUS INFECTION ON AMINO ACIDS AND VITAMINS IN WHOLE WHEAT

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE
Food Science

Department of Grain

Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1970

ABSTRACT

The amino acid compositions of both spring wheat and winter wheat were quite similar. But after deteriorated by mold, arginine and cysteine in spring wheat were decreased 50.2% and 74.5% respectively. However, methionine was increased 51%. In winter wheat, lysine, histidine, and arginine were decreased 45%, 50.1%, and 50.0% respectively. However, methionine was increased 34.5%. The ratio of total amino acids to Kjeldahl protein in spring wheat and winter wheat were 78.86% and 90.63% respectively. This means the spring wheat was more damaged by mold than was the winter wheat because more amino acids in the protein of spring wheat were metabolized to form other compounds which still possessed detectable Kjeldahl nitrogen.

In spring wheat large amounts of B-group vitamins were synthesized, thus: riboflavin 295%, vitamin B_6 46%, vitamin B_{12} 276%, niacin 79.1%, and pantothenic acid 463%. But in winter wheat much less amounts were synthesized, thus: riboflavin 179%, vitamin B_6 18%, vitamin B_{12} 37%, and pantothenic acid 12.3%.

Some vitamins were lost in wheat after mold deterioration. Thiamine was lost 43.3% in spring wheat and 48.7% in winter wheat. Niacin was lost 25.3% in winter wheat.

The moldy spring wheat contained 9.15 ppb of aflatoxin B_1 and 12.5 ppb of aflatoxin G_1, while winter wheat contained no significant amount of aflatoxin. Almost all aflatoxins in wheat were present in bran, only trace amount was detected in both high ash and low ash flours. In the milling products of corn, germ contained

highest concentration of aflatoxin, meal contained much less, feed and grits contained least.