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CHEMICAL CHANGES IN SOME LEGUME SEEDS INFECTED WITH *Aspergillus flavus* PRODUCING AFLATOXIN

Eisa, E. S.
Agricultural Biochemistry Dept., Fac. of Agric., Mansoura Univ., Egypt.

ABSTRACT

Three legume seeds, namely, winged bean, kidney bean and lentil, were contaminated with *A. flavus*. The infected seeds were incubated at 28°C for 10 days, with moisture content of 20-30%, where suitable amount of aflatoxin was elaborated. The samples were subjected to chemical analysis before and after contamination. Proximate analysis of infected samples showed that crude protein and fibers were increased, while crude lipids, ash and total carbohydrates were decreased comparing with healthy samples. Percentage values of protein fractions were increased in infected samples, whereas water soluble protein showed high increase, salt soluble protein showed moderate increase, while non soluble protein gave slight increase. The values of amino acids in infected seeds were increased in some amino acids and decreased in another acids. Cystine, arginine, glycine, threonine, alanine and phenylalanine were increased in infected seeds. However, aspartic, serine, methaionine, valine, leucine and isolucine were decrease. Arginine showed the highest increase in infected winged bean. It increased from 2582.10 to 3950.70 mg/100 gm sample. Methionine showed the highest decrease in infected lentil. It decreased from 1600.8 to 1144.0 mg/100 gm sample. Fatty acids analysis of legume lipids revealed that oleic, linoleic and linolenic fatty acids were more affected by fungal infection. Oleic and linoleic were increased in infected samples, however linolenic was decreased. The fate of aflatoxins during soaking and boiling infected seeds was investigated. The aflatoxines were fractionated and estimated before and after treatments. Fractionation of aflatoxins on TLC showed that winged bean and kidney bean contained two aflatoxins compounds in untreated samples, one of them was B_1. After soaking of these samples the same two compounds were also found. Aflatoxins of winged bean, which was boiled, contained one aflatoxin while the other compound was disappeared. However, aflatoxins of untreated lentil showed one aflatoxin (B_1), this compound disappeared after treatment this sample by boiling. The results revealed also that aflatoxins of winged bean, with initial value of 31.66 μg/100 gm sample, was reduced to 36.83% by soaking and 82.06% by boiling. Aflatoxin of kidney bean, with initial value of 23.99 μg/ 100 gm sample, was lossed to 39.03% by soaking and 59.08% by boiling. However, aflatoxins of lentil, with initial value of 19.33 μg/100 gm sample, was reduced to 91.0% by soaking and removed completely by boiling.

INTRODUCTION

Fungi are useful for human being life. They participate in production of cheese, bread, antibiotics, vitamins, enzymes, fat and livestock feeds from fermentation of by-products. However, some fungi are harmful to life because of their ability to cause plant diseases, food spoilage and mycotoxins production. Aflatoxins are known as one of the dangerous mycotoxins. They are direct hazard to human health (Butler, 1974 and Krogh, 1989). The aflatoxins are highly toxic fungal metabolites and may

cause a variety of adverse health effects, *e.g*., liver cancer in animal and man, (Mercado *et al*., 1991 and Higuera-Clapara *et al*., 1995). Aflatoxins, especially aflatoxin B_1 is considered the most potent carcinogenic in all naturally produced toxins. Aflatoxin B_1 causes liver cancer, respiratory system cancer and cytochrome P-450 monooxygenase disorder (Hall and Wild, 1994). The contamination of foods and feedstuffs with aflatoxins is serious problem to human and livestock health and consequently, to agricultural economics (Maeba *et al*., 1988). When Egyptian goats had been given feedstuff, contaminated with aflatoxins, they showed decrease in body weight, milk yield and protein content (Selim *et al*., 1996). Contamination of foods with aflatoxins occurs as a result of *A. flavus* growth on wide variety of crops in the fields and during storage, even in the grocery store (Christensen, 1974; Mahoney and Molyneux, 1998; Abdelhamid, 1998). Therefore, many experiments had been performed to reduce the levels of aflatoxins in contaminated crops. Physical sorting, solvent extraction, heating, irradiation, adsorption, biological treatments and the chemical inactivation are presently used to reduce aflatoxins in many commodities (Doyle *et al*., 1982; Hao and Brackett, 1988; Yousef and Marth, 1986; West and Bullerman, 1991 and Aziz, 1998).

The present investigation was carried to confirm two purposes. The first aim to estimate effect of the contamination with *A. flavus* on some nutrient components of three legume seeds, *i.e*., winged bean, kidney bean and lentil. The second aim to evaluate the effect of using both soaking and boiling on removing of aflatoxins from these contaminated legume seeds.

MATERIALS AND METHODS

Standard:

Aflatoxins B_1, B_2, G_1 and G_2 standards were obtained from Sigma Chem. Co., USA. Each of them was dissolved in benzene-acetonitrile (98:2) to give a solution of final concentration containing 0.1 μg/ μl

Fungus:

The aflatoxin-producing strain of *A. flavus*, was obtained kindly from Microbiol. Dept., Fac. of Agric., Mansoura Univ., Mansoura, Egypt. The fungus was maintained on slopes of Potato Dextrose Agar (PDA) at 4°C.

Samples:

Mature healthy seeds of winged bean, kidney bean and lentil, free from any infection, were purchased from local market in Mansoura City. Each sample was surface sterilized and divided into two parts. The first part was ground to pass through a 1 mm screen and it was used in chemical analysis as raw sample (control). The second part was used without ground and infected with *A. flavus*. After infection, the samples were dried, ground and subjected for analysis.

Formation of aflatoxins in seeds:

Sterilized legume seeds with 20-30% moisture content were inoculated with *A. flavus* according to the method described by Mercado *et al*. (1991). The *A. flavus* inoculum was prepared by incubating the fungus on acidified PDA plates for 10 days at 28°C. The agar with profuse mycelial and spore growth was cut into 1 cm diameter disks. About 500 gm of seeds sample was inoculated with 10 agar disks of *A. flavus*. The disks were rubbed through the inner surface of the seeds and incubated at 28°C for 10 days (best conditions for aflatoxin production), Davis *et al*. (1966) and Ibrahim *et al*. (1990). Then, the samples were steamed for 10 min to prevent further growth of the mold. The aflatoxins-containing seeds were further air dried to get ride of excess moisture. The infected seed samples were divided into two parts. The first part was ground and subjected for chemical analysis. The second part was used for soaking and boiling treatments.

Removal of aflatoxins by soaking and boiling:

About 100 g of infected legume seeds sample was soaked in a beaker contained about 200 ml water for 24 hours. Another 100 g of the same infected seeds was boiled in a beaker contained also 200 ml water for 20 minutes. The obtained legume seeds samples were dried, ground and subjected for aflatoxins analysis.

Estimation of Aflatoxin:

The seeds sample (20 gm) was blended for 4 min with 2 gm citric acid, 8.4 ml saturated NaCl and 60 ml acetone. The mixture was filtered and the filter cake (residue) was reblended with 50 ml general solution which composed from 196 ml water, 101 ml saturated NaCL, 725 ml acetone and 4.85 gm citric acid. The mixture was filtered into initial filtrate. The filtrate was agitated 1 min with petroleum ether. The lower layer (aqueous layer) was collected and agitated with 40 ml chloroform. The chloroform layer was drained into flask. The aqueous layer was reextracted with 40 ml chloroform : acetone (1 :1) and chloroform layer was collected into same chloroform flask. The chloroform fraction was concentrated and clean up using silicic acid column chromatography. Aflatoxin B_1 was isolated by thin layer chromatography technique using developing solvent system toluene : ethylacetate : chloroform : formic acid (7 : 5 : 5 : 2) according to El-Bazza (1983). Aflatoxin B_1 in the samples was quantified using spectrofluorometer at wavelength 365 nm (Hao and Brackett, 1988).

Proximate analysis:

Moisture, ash, crude fat, crude protein, crude fiber and total carbohydrates were determined by the procedures described in AOAC (1990).

Fatty acids analysis:

Fatty acids were prepared from the extracted lipid seeds and methylated according to Kates (1980). GLC analysis of fatty acid methyl esters was prepared using a Pye Unicam PRO-GC apparatus.

Amino acids analysis:

Dried sample (0.2 gm) was hydrolyzed with 10 ml HCl 6 N for 24 hours at 110°C. The HCl was evaporated and the residue was dissolved in 10% isopropanol and diluted to known volume. The obtained amino acids were determined quantitatively using the paper chromatographic technique according to Block *et al.* (1958). The amino acids hydrolyzates and the references expected for them were spotted in Watman No 1 filter paper sheets. The spotted sheets were developed separately using two solvent systems, *i.e*., butanol : acetic acid : water, 4:1:5, (upper layer) and butanol : acetic acid : water, 4:1:1. The amino acids were visualized using ninhydrin reagent as described by Helimann *et al*. (1957). The spots of amino acids were cut into separately pieces, extracted with methanol and the optical density was measured at 500 nm.

Protein fractionation:

Water-soluble protein (WSP), salt soluble protein (SSP) and non-soluble protein (NSP) fractions were separated according to Reddy and Srikar (1991). 5 gm of each of legume seeds samples were homogenized with distilled water and centrifuged at 5000 rpm for 15 min to separate WSP fraction. The residue was treated with 5% NaCl solution buffered with 0.02% M $NaHCO_3$ to pH 7.2 and then centrifuged to separate SSP fraction. The residue contains NSP fraction which was calculated by difference between total protein and WSP + SSP.

RESULTS AND DISCUSSION

Proximate analysis:

Legume seeds, *i.e.*, winged bean, kidney bean and lentil were inoculated with *A. flavus* and incubated at 28°C for 10 days until enough aflatoxins were produced. The proximate analysis of these seeds before and after infection is shown in Table 1. The obtained data revealed that crude protein and fibers were higher in infected samples than those of raw samples. Crude protein values of winged bean, kidney bean and lentil increased from 30.54 to 35.05%; 28.40 to 32.59% and 27.31 to 31.62% in infected samples, respectively. Fibers content increased in infected samples from 8.40 to 10.35% in winged bean, 7.95 to 8.13% in kidney bean and 3.26 to 5.43% in lentil. These increases may be due to mycelia formation in seeds and to the metabolic role of fungus in conversion carbohydrates to protein. On contrary, crude lipid, ash and total carbohydrates contents were lower in infected samples than those of raw samples. Ash content showed the highest decrease in infected lentil sample. It decreased from 3.28 to 1.11%. However, total carbohydrates revealed moderately decrease in infected seeds. It decrease from 54.16 to 51.98% in winged bean, 58.81 to 56.68% in kidney bean and 64.10 to 61.49% in lentil. Lipid content showed the highest

decrease in infected samples. It decreased from 2.66 to 0.49% in winged bean, 1.59 to 0.40% in kidney bean and 2.05 to 0.35% in lentil. This decrease is probably because these components are metabolized by *A. flavus* (Diener and Davis, 1969 and Davranov *et al*., 1996)). El-Sawah (1997) found that 30% of total carbohydrate content of soybean was consumed for the fungal growth, and at the same time, the protein content in the material was increased by about 13.15%.

Table 1: A proximate analysis of legume seeds samples (gm/100 gm dry sample).

Components (%)	Samples					
	Before infection			After infection		
	Winged bean	Kidney bean	Lentil	Winged bean	Kidney bean	Lentil
Crude protein	30.54	28.40	27.31	35.05	32.59	31.62
Crude lipid	2.66	1.59	2.05	0.49	0.40	0.35
Ash	4.24	3.25	3.28	2.13	2.20	1.11
Fibers	8.40	7.95	3.26	10.35	8.13	5.43
Total Carbohydrates	54.16	58.81	64.10	51.98	56.68	61.49

Protein fractions:

Percentage value of protein fractions of legume seed samples before and after infection is shown in Table (2). The results indicated that all protein fractions were higher in infected legume samples than those of uninfected samples. Water soluble protein fraction showed the highest increase levels in infected legume seeds. The percentage values of this fraction increased from 9.92 to 12.70% in winged bean, 10.15 to 13.03% in kidney bean and 10.25 to 13.02% in lentil. Salt soluble protein fraction showed a moderate increase in infected legume samples. This fraction increased from 4.48 to 5.62% in winged bean, 5.02 to 5.97% in kidney bean and 6.46 to 7.35% in lentil. However, a slight increase in non-soluble protein fraction was noticed in infected legume samples. These results may be attributed to destruction or hydrolysis of high protein molecules of samples to low molecules by proteolytic enzymes of fungus as well as the producing mycelia and spores during growth of fungus (Diener and Davis, 1969).

Table 2: Protein fractions of legume seed samples (gm/100 gm dry sample).

Protein fraction (%)	Samples					
	Before infection			After infection		
	Winged bean	Kidney bean	Lentil	Winged bean	Kidney bean	Lentil
Water soluble protein	9.92	10.15	10.25	12.70	13.03	13.02
Salt soluble protein	4.48	5.02	6.46	5.62	5.97	7.35
Non soluble protein	16.14	13.23	10.60	16.73	13.59	11.25
Total protein	30.54	28.40	27.31	35.05	32.59	31.62

Amino acids:

The amino acids composition of protein legume seeds is shown in Table (3). The results revealed that the essential amino acids lysine, histidine, arginine, leucine and isolucine and non-essential amino acids, aspartic acid and serine were present in relatively large amounts in all legume samples. Essential amino acids, *i.e*., threonine, alanine, methaionine, valine and phenylalanine, and non essential amino acid glutamic were found in median levels. While, the amino acids cystine, glycine and tyrosine were present in low amounts. The results indicated that the values of amino acids in infected samples were increased in some amino acids and decreased in another acids comparing with the raw samples. The amino acids cystine, arginine, glycine, threonine, alanine and phenylalanine were increased in infected legume samples. On contrary, the amino acids aspartic, serine, methaionine, valine and leucine + isolucine were decreased in infected samples. The amino acid arginine showed the highest increase in infected winged bean sample, it increased from 2582.10 to 3950.70 mg/100 gm sample. The amino acid methaionine showed the highest decrease in infected lentil sample, it decreased from 1600.80 to 1144.00 mg/100 gm sample. The amino acids lysine, histidine and tyrosine did not change in winged bean sample and they gave slight changes in two another samples.

Table (3): Effect of infection with *A. flavus* on amino acids composition of legume samples (mg/100 gm dry sample).

Amino Acid	Samples					
	Before infection			After infection		
	Winged bean	Kidney bean	Lentil	Winged bean	Kidney bean	Lentil
Cystine	527.09	1190.35	932.31	1287.69	1740.03	1280.15
Lysine	2920.13	2980.00	2978.20	2918.20	3016.15	2964.63
Histidine	2750.14	2580.10	2551.40	2751.42	2377.64	1802.30
Arginine	2582.10	1613.04	2700.25	3950.70	2114.00	2766.65
Aspartic	2570.69	1422.58	2850.40	2120.49	1032.11	2788.10
Glutamic	2069.84	1876.88	1130.67	2413.33	1828.37	1629.28
Serine	2890.40	2728.76	1523.52	2517.65	2595.10	1439.38
Glycine	580.65	562.80	678.50	878.62	1141.33	1321.00
Threonine	2057.14	1785.76	628.92	2757.14	1854.28	1235.72
Alanine	1054.50	1651.90	1710.36	1507.28	2109.34	1853.32
Tyrosine	584.82	769.23	342.90	588.10	892.31	353.85
Methaionine	2338.46	1769.22	1600.80	1911.47	1474.00	1144.00
Valine	1505.16	1623.70	1541.18	1449.40	1584.40	1420.80
Phenylalanine	345.85	1330.10	1665.70	1980.14	1896.15	2126.67
Leucine + Isoleucine	2782.05	2405.13	2810.59	2154.75	2396.36	2378.20
Unknown	---	---	---	586.17	1706.66	1566.70
Total	28559.02	26189.55	25145.70	31770.55	29758.23	28070.74

The results show also that, unknown amino acids were appeared in infected legume samples and they did not present in the healthy samples. These unknown amino acids reached to high value in kidney bean sample (1706.66 mg/100 gm sample). The obtained results for increase some amino acids in infected samples may be attributed to the mycelia and spores, which produce in seeds by fungus during growth. However, the decrease of some amino acids may be due to consumption of these amino acids by the fungus during its growth (Diener and Davis, 1969).

Fatty acids:

Fatty acids composition of legume seeds lipids is listed in Table (4). It could be noticed that unsaturated fatty acids were the main constituent in legume seeds lipids. Linolenic fatty acid was the major essential fatty acid in both winged bean and kidney bean lipids, it represented 35.04% and 21.44% of the total fatty acids in these two samples, respectively. Linoleic was the major essential fatty acid in lentil lipids, it represented 33.07% of the total fatty acids in this sample. Oleic fatty acid was found in moderate amounts in lipids of winged bean and lentil, but it was found in small amount in kidney bean lipids. Its values were 14.90%, 20.54% and 3.99% in lipids of winged bean, lentil and kidney bean, respectively. Saturated fatty acids showed the lowest content in lipid legume samples. Palmitic acid was the major fatty acid, its values were 14.75%, 11.08% and 10.62% of total fatty acids in lipids of winged bean, kidney bean and lentil, respectively. The results indicated that the fatty acids oleic, linoleic and linolenic were more affected by fungus infection of seeds. Oleic acid and linoleic fatty acids were increased in all infected seeds lipids. However, linolenic fatty acid was decreased. Oleic acid in infected seeds increased from 14.90 to 22.94% in winged bean, 3.99 to 12.09% in kidney bean and 20.54 to 32.29% in lentil. Linoleic acid increased from 21.91 to 23.74% in winged bean, 18.51 to 28.82% in kidney bean and 33.07 to 35.27 in lentil. While, linolenic acid was decreased in infected seeds from 35.04 to 18.78% in winged bean, 21.44 to 5.66% in kidney bean and 9.04 to 6.29 in lentil. The results revealed also that, there was a slight change in values of some other fatty acids in present samples, whereas, some of these acids were decreased and some increased. These obtained results may be attributed to the action of lipase synthesized by the fungus in seeds (Khor *et al.,* 1986, Ku and Hang, 1995 and Hauka *et al.*, 1999).

Table (4): Effect of infection with *A. flavus* on fatty acids composition of legumes oil samples.

Fatty acid %	RT	Samples					
		Before infection			After infection		
		Winged bean	Kidney bean	Lentil	Winged bean	Kidney bean	Lentil
Caproic (6)	2.40	1.19	1.55	1.19	0.70	0.06	0.48
Caprilic (8)	4.65	0.51	1.34	0.17	0.51	0.39	0.24
Cparic (10)	7.24	0.17	3.27	0.56	1.01	2.42	0.59
Lauric (12)	9.73	0.34	4.35	1.64	1.78	4.96	1.65
Myristic (14)	12.03	0.40	3.33	1.34	1.77	3.55	0.80
Palmitic (16)	14.28	14.75	11.08	10.62	9.39	18.28	14.55
Stearic (18)	16.63	---	2.32	---	---	1.02	---
Oleic (18:1)	17.10	14.90	3.99	20.54	22.94	12.09	32.29
Linoleic (18:2)	18.10	21.91	18.51	33.07	23.74	28.82	35.27
Linolenic (18:3)	19.30	35.04	21.44	9.04	18.78	5.66	6.29
Arachidonic (20)	20.41	---	1.45	0.37	---	1.74	---
Behenic (22)	27.55	0.66	2.20	0.27	1.10	0.89	---
Unknowns		10.13	25.17	21.19	18.28	20.12	7.84

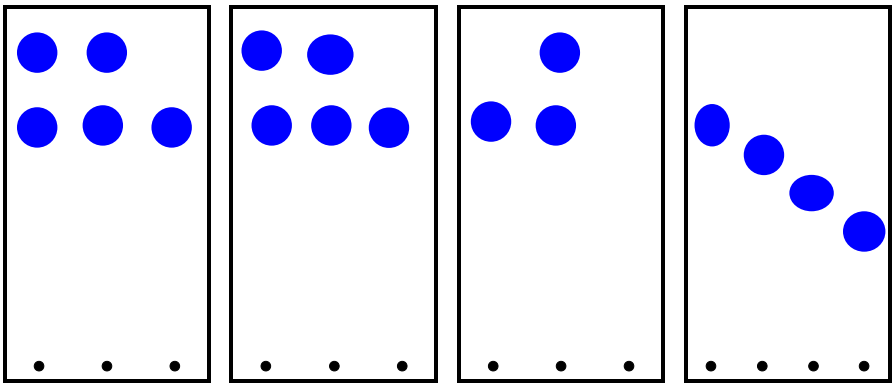

Fig. (1).: TLC chromatogram of aflatoxins of untreated seeds.

Fig. (2).: TLC chromatogram of aflatoxins of soaked seeds.

Fig. (3).: TLC chromatogram of aflatoxins of boiled seeds.

Fig. (4).: TLC chromatogram of standard aflatoxins.

(1): winged bean aflatoxin; (2) kidney bean aflatoxin and (3) lentil aflatoxin.

Removal of aflatoxin content:

The infected legume seeds were subjected to two treatments, *i.e.*, soaking in water for 24 hours and boiling for 20 minutes to reduce the levels of aflatoxins in these seeds. The aflatoxins were fractionated and estimated before and after treatments. The results from Fig. (1) revealed that TLC chromatogram of aflatoxins of winged bean and kidney bean before treatments (raw) contained two aflatoxins. The compound with low R_f gave the same R_f and colour of B_1 aflatoxin. However, aflatoxin of untreated lentil seeds showed one compound, which gave the same R_f of B_1 aflatoxin. The results from Fig. (2) indicated that the two aflatoxins, which found in untreated winged bean and kidney bean samples (Fig. 1), were also found after treatment these seeds by soaking. The results of Fig. 3 showed that TLC chromatogram of aflatoxin of boiled winged bean contained one compound while the other compound disappeared. However, the same chromatogram revealed that aflatoxin of boiled kidney bean contained the same two aflatoxin which was found in untreated seeds. From this result it can said that soaking or boiling of these two seeds samples did not remove aflatoxin completely. On contrary, from results in chromatogram of Figure 3, it could be noticed that the aflatoxin compound, which present in untreated lentil sample was disappeared after boiling of this seeds. Data shown in Table 5 indicated that aflatoxin of winged bean seeds with initial value of 31.66 μg/100 g was reduced to 36.83% by soaking and 82.06% by boiling. Aflatoxin of lentil seeds with initial value of 19.33 μg/kg sample was reduced to 91.0% by

soaking and removed completely by boiling. The removal of aflatoxins by soaking or boiling from kidney bean seeds seemed partially effective. However, 39.03% of aflatoxins in this sample was removed by soaking and 59.08% was removed by boiling (Table 5). From these results it can said that the boiling treatment was more affect on removal of aflatoxins from seeds. On the other hand, treatment such seeds by soaking or boiling did not eliminate completely the hazard of aflatoxins and thus the consumer would be exposed to its adverse effects when ingested. Formation and control of aflatoxin in foods and its fate during processing of foods was investigated by Bullerman *et al*. (1984) and Emara (1996). Boiling and steeping of sorghum, rice and other foods resulted in aflatoxin loss to various levels (Stoloff, 1986).

Table (5): Effect of soaking and boiling on B_1 aflatoxin levels (μg/100 gm dry sample) in infected legume samples.

Samples	Raw		Soaking	Boiling	
	μg/100 gm	μg/100 gm	Loss %	μg/100 gm	Loss %
Winged bean	31.66	20.00	36.83	5.68	82.06
Kidney bean	23.99	14.63	39.03	9.82	59.08
Lentil	19.33	17.40	91.0	0.00	100.0

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التغيرات الكيميائية فى بعض بذور البقوليات المصابة بفطر الأسبرجلس فلافس المنتج للأفلاتوكسين

عيسى سالم عيسى

قسم الكيمياء الزراعية – كلية الزراعة – جامعة المنصورة – المنصورة – مصر .

فى هذا البحث أختير ثلاث أنواع من بذور البقوليات وهى الفاصوليا واللوبيا والعدس ، وتم إصابتها بفطر الأسبرجلس فلافس وحضنت على درجة حرارة 28°م لمدة 10 أيام ومحتوى رطوبة 20 – 30% حتى أنتجت كمية مناسبة من الأفلاتوكسينات ، وأجرى للعينات تحليل كيميائى ، وأظهرت نتائج التحليل الأولى إحتواء العينات المصابة بالفطر على نسبة عالية من البروتين والألياف ونسبة منخفضة من الدهن والرماد والمواد الكربوهيدراتية . ولقد تميزت العينات المصابة بإرتفاع مكونات البروتين لها ، فقد أظهرت البروتينات الذائبة فى الماء زيادة عالية والبروتينات الذائبة فى الملح زيادة متوسطة بينما أظهرت البروتينات الغير ذائبة زيادة طفيفة . ودلت نتائج تحليل الأحماض الأمينية للعينات المصابة بإرتفاع قيم بعضها وإنخفاض قيم البعض الآخر ، فلقد إزدادت قيم الأحماض الأمينية سستين ، أرجنين ، جليسين ، ثريونين ، ألانين ، فنيل ألانين بينما إنخفضت قيم الأحماض الأمينية أسبارتيك ، سيرين ، مثيونين ، فالين ، ليوسين والأيزوليوسين . وأظهر الحامض الأمينى أرجنين أعلى زيادة فى عينة بذور الفاصوليا المصابة حيث إرتفعت من 2582.1 إلى 3950.7 ملليجرام/100 جرام عينة جافة بينما أظهر الحامض الأمينى ميثيونين أعلى إنخفاض فى عينة العدس المصاب حيث إنخفضت من 1600.8 إلى 1144 ملليجرام / 100 جم عينة جافة . وبينت نتائج تحليل الأحماض الدهنية لدهن العينات بأن الأحماض الدهنية أوليك ، لينوليك ، لينولينيك كانت الأكبر تأثراً بالإصابة الفطرية حيث تميز دهن العينات المصابة بإرتفاع نسبة الحامض الدهنى أوليك والحامض الدهنى لينوليك بينما إنخفضت نسبة الحامض الدهنى لينولينيك .

عوملت العينات المصابة بالنقع والغليان بغرض إزالة الأفلاتوكسينات منها وأجرى فصل وتقدير وصفى وكمى للأفلاتوكسينات قبل وبعد المعاملة . وأظهر الفصل الكروماتوجرافى على الطبقات الرقيقة لأفلاتوكسينات بذور الفاصوليا واللوبيا بإحتوائها على مركبين من الأفلاتوكسينات ، أحدهما B_1 وبعد معاملة هاتين العينتين بالنقع وجد أنهما أحتويا أيضاً على نفس المركبين من الأفلاتوكسينات بينما أظهرت أفلاتوكسينات بذور الفاصوليا المعاملة بالغليان إحتوائها على مركب واحد أفلاتوكسين بينما إختفى المركب الآخر . ولقد أوضح كروماتوجرام أفلاتوكسينات عينة العدس بإحتوائه على مركب واحد وهو أفلاتوكسين B_1 وعند معاملة العينة بالغليان إختفى هذا المركب من العينة . وبالتقدير الكمى للأفلاتوكسينات بعد معاملة العينات أظهرت النتائج أن عينة الفاصوليا المحتوية على أفلاتوكسينات قدرها 31.66 ميكروجرام/100 جرام عينة جافة قد إنخفضت إلى 36.83% بالنقع و 82.06% بالغليان ، وإنخفضت الأفلاتوكسينات فى عينة اللوبيا والتى نسبتها 23.99 ميكروجرام / 100 جرام عينة بواسطة النقع إلى 39.03% وبالغليان إلى 59.08% ، بينما أظهرت عينة العدس المحتوية على أفلاتوكسينات قدرها 19.33 ميكروجرام / 100 جرام عينة بإنخفاض حاد فى محتوى الأفلاتوكسينات حيث إنخفض بنسبة 91.00% بالنقع بينما إختفت الأفلاتوكسينات تماماً بالغليان .