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Chemical Properties of *Aspergillus flavus*-Infected Soybean Seeds Exposed to γ-Irradiation during Storage

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ABSTRACT

The aim of the present study was to examine the chemical properties of *Aspergillus flavus*-infected soybean seeds exposed to different levels of γ-irradiation; 0, 1, 3 and 5 kGy, during storage. The results revealed that there was no effect of irradiation at different dose levels on moisture, protein, total lipids and amino acids content of the seeds for overall 60 days of storage under ambient temperature. At zero time, irradiation of *A. flavus*-infected soybean seeds at 5.0 kGy caused a slight increase in peroxide value, no change in acid value, a slight decrease in saponification and iodine values in the crude oil extracted from the seeds. An increase in saturated fatty acids associated with a decrease in un-saturated fatty acids was also observed in the oil extracted from the seeds. Furthermore, at dose level 5 kGy the fungus growth was completely inhibited and there was no detection of aflatoxin B_1 after 60 days of storage. It is concluded that γ-irradiation of *A. flavus*-infected soybean seeds at dose level 5 kGy is sufficient to inhibit fungus growth and aflatoxin production over a storage period of 60 days without changes in major chemical properties of the seeds and the oil extracted from seeds.

Key Words: Chemical properties; soybean; Seed; Storage

INTRODUCTION

Soybean is one of important crops in Egypt. Due to its high nutritive value and protein content it is widely used in animal feed, human food and industrial applications. Soybean oil has commercial value in the field of vegetable oil and is used for consumption as a source of edible oils and protein.

Mycotoxins are toxic substances produced by filamentous fungi that grow on agricultural products either in the field before harvest or during storage (Aziz *et al*., 2002). Aflatoxins and other toxic metabolites have been found in edible tissues, milk and eggs for human use after ingestion of contaminated feed by farm animals (Youssef *et al.*, 1999). *Aspergillus flavus* and *Aspergillus parasiticus* are the primary fungal species that produce aflatoxins in food and feed commodities (Gowrama & Bullerman, 1995).

Various methods of preservation, such as fumigation and heat treatment have been applied to arrest moulds in foods, but none of these methods offers complete control of toxigenic moulds. The objective of the present study was to evaluate the effect of different doses of γ-irradiation on aflatoxin B_1 production and chemical composition of *A. flavus* infected soybean seeds during storage.

MATERIALS AND METHODS

Radiation facWPI_21206ility. Irradiation was performed with a gamma cell (^{60}Co) at National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. The dose rate of the radiation source was 4.95 kGy h^{-1} at the time of experiment.

Conidial suspension preparation. An aflatoxigenic strain of *Aspergillus flavus* locally isolated from wheat seeds at Microbiology Department, National Center for Radiation Research and Technology, Cairo, Egypt was used in this study. Stock cultures were maintained on sabouraud dextrose agar (SDA, pH 6.5). Slants were incubated at 28°C until sporulation was evident and then stored at 4°C. Spore suspensions were prepared from 7-days cultures according Appiah *et al*. (1980) the suspension had a final concentration of 10^6 conidia mL^{-1}.

Irradiation and storage process. Samples (300 g) of soybean (*Glycine max* L. var. Clark) seeds were sealed in polyethylene bags and sterilized by irradiation. After irradiation, all samples were stored at 30°C in a humidified incubator for 0, 30 and 60 days. At the end of each storage period the number of fungal colony-forming units (CFU), aflatoxin B_1 content and chemical analysis were determined.

Samples (50 g) of sterilized soybean seeds in conical flasks were inoculated with spore suspension of *A. flavus* (10^6 conidia g^{-1}) then the moisture content of the seeds was adjusted to 20% using standard methods (Brook & Foster, 1981). Un-inoculated seeds with natural moisture content were served as control. The samples were stored overnight at 4°C and then irradiated with 0, 1, 3 and 5 kGy.

Quantitative estimation of aflatoxin. A 50 g of soybean seeds in conical flasks were extracted with 12.5 mL distilled water plus 125 mL chloroform in warring blender. The chloroform layer was separated in a separating funnel over anhydrous sodium sulphate and transferred into 400 mL beaker. Purification and detection of aflatoxins were carried

out using thin layer chromatography according to Eppley (1968). The plates were developed in chloroform acetone (9:1). The UV fluorescent bands containing aflatoxins with R_f value identical to that of standards were scraped off, eluted with cold methanol (10 mL), filtrated and estimated by spectrophotometry. Aflatoxin B_1 was estimated at wavelength 360 nm. The concentration of aflatoxin B_1 of each flask was determined using the molar extension coefficient recommended by Nabney and Nesbitt (1965). Each treatment in any experiment was done in three replicates.

Chemical analysis. Moisture content, total protein content, total lipids content of the seeds and chemical properties of the oil extracted from the seeds (acid, iodine, proxide & saponifaction values) were determined according to the method described in A.O.A.C. (1980).

Fatty acids identification. For determination fatty acids, the extracted lipids were saponified by boiling under reflux with an excess of dilute aqueous ethanolic alkali. The ether containing the water-soluble hydrolysis products (mainly soap solution & glycerol) was acidified by sulphuric acid to liberate the free fatty acids. The free fatty acids were then extracted with diethylether recovered, dried over anhydrous sodium sulphate and transformed to their methyl esters for GC-MS analysis (Varso, 1972). Fatty acids profile was determined quantitatively using a Gas chromatograph-mass selective detector instrument "GC-MS" type HP 6890 series at National Center of Radiation Research and Technology (NCRRT), Nasr City, Cairo.

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Condition of Analysis. Capillary Hp-Innowax column; 30 m length; 250 µ; 0.25 µ film thickness was used. The oven was programmable with initial temperature of 150°C for one minute, which was then raised in three ramps.

Amino acids determination. Amino acid determination was performed according to the method of Winder and Eggum (1966). Oxidation with performic acid, to protect methionine and cysteine from destruction, followed by acid hydrolysis was carried out in a closed conical flask for determining all amino acids other than tryptophan. Sample of 20 - 30 mg of dried and defatted samples were weighted in scrow-tubes and 5 mL of performic acid were added. The tubes were closed and placed in ice water bath for 16 h sodium metabisulfate and HCL (6 N) were added to the oxidized mixture. The tubes were placed in an oven at 110°C for 24 h then opened and the content evaporated for dryness in a rotary evaporator. A suitable volume of sodium citrate buffer (pH 2.2) was added to the dried film of the hydrolyzed sample. After all soluble materials were completely dissolved; the sample was ready for analysis. The system used for the analysis was High Performance Amino Acid Analyzer, Biochrom 20 Pharmacia Biotech at National Center of Radiation Research and Technology (NCRRT), Nasr City, Cairo.

Rate °C/min	Final temp °C	Final time/min
17	235	2
1	242	1
8	255	6

Statistical analysis. Analysis of variance (ANOVA) for means was done according to Steel and Torrie (1980). Differences among various treatments were tested using Duncans multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of γ-irradiation and storage on the survival of *A. flavus* in soybean seeds. The sensitivity of fungi to ionizing radiation (Aziz *et al.*, 2005) as well as the efficacy of γ-irradiation for the decontamination of food commodities has been reported (Aziz & Moussa, 2002). In the present study, the results obtained revealed that artificial inoculation of sterilized soybean seeds with *A. flavus* spores (2.4×10^7 CFU g^{-1}), then irradiation at different dose levels, results in a significant decrease of the counts ($p < 0.05$), reaching 1.2×10^3 CFU g^{-1} at irradiation dose level of 3 kGy and there was no growth detected at dose level 5 kGy. Also, the counts of *A. flavus* for un-irradiated soybean seeds increased to 2.5×10^8 and 3.7×10^8 CFU g^{-1} after 30 and 60 days of storage ($p < 0.05$), respectively, whereas the counts of *A. flavus* for 1 and 3 kGy-irradiated soybean seeds increased slightly to 2.1×10^5 and 1.6×10^3 CFU g^{-1}, respectively after 60 days of storage ($p < 0.05$). On the other hand, soybean seeds exposed to 5 kGy exhibited no *A. flavus* growth over storage period, which extended to 60 days (Table I). The results are consistent with Aziz *et al.* (2004), who found that irradiation of maize, chickpeas and groundnut seeds at a dose 4.0 kGy reduced mould growth compared to un-irradiated control and there was no growth at a dose 5.0 kGy.

Effect of γ-radiation and storage on aflatoxin B_1 production in inoculated soybean seeds. There is increasing interest in the use of ionizing radiation for killing the fungal flora and reducing the production of mycotoxins in stored grains. Furthermore, it was reported that fungal strain, condition of storage, humidity, inoculums size and irradiation dose affect mould growth and toxin production (Aziz *et al.*, 2002). In the present study, the results in Table I showed that no aflatoxin B_1 was detected in un-irradiated inoculated seeds at zero time, while after 30 days of storage the amount of aflatoxin B_1 in these seeds was 5.39 µg kg^{-1} seeds and reached 7.82 µg kg^{-1} seeds after 60 days of storage ($p < 0.05$). At dose 1 and 3 kGy of γ-radiation there was less afflatoxin B_1 production (5.33 & 2.51 µg kg^{-1}, respectively) after 60 days ($p < 0.05$) than in the un-irradiated control (7.82 µg kg^{-1}) and aflatoxin B_1 was not detected at 5.0 kGy of gamma radiation over all storage periods. Aziz and Mahrous (2004) and Aziz *et al.* (2005) reported that the total viable population of the mycotoxigenic moulds and mycotoxin production decreased

Table I. Influence of γ-irradiation and storage on the survival of *A. flavus* and aflatoxin production (µg kg^{-1} seeds) of inoculated soybean seeds

Dose in kGy	Un-irradiated		1 kGy		3 kGy		5 kGy	
Storage periods	*A. flavus* CFU/g	aflatoxin B_1 µg kg^{-1}	*A. flavus* CFU/g	aflatoxin B_1 µg kg^{-1}	*A. flavus* CFU g^{-1}	aflatoxin B_1 µg kg^{-1}	*A. flavus* CFUg^{-1}	aflatoxin B_1 µg kg^{-1}
Zero time	$2.4x10^7$	Not detected	$1.6x10^5$	Not detected	$1.2x10^3$	Not detected	No growth	Not detected
30 days	$2.5x10^8$	5.39	$1.9x10^5$	5.11	$1.4x10^3$	2.87	No growth	Not detected
60 days	$3.7x10^8$	7.82	$2.1x10^5$	5.33	$1.6x10^3$	2.51	No growth	Not detected

Table II. Effect of γ-irradiation and storage on moisture, total protein and total lipids contents in soybean seeds (var Clark)

Parameters	Un-inoculated soybean seed Irradiation dose in kGy				*A. flavus* inoculated soybean seeds Irradiation dose in kGy			
	0	1	3	5	0	1	3	5
					Zero time			
Moisture	5.99	5.99	5.82	5.64	20.69	20.38	20.19	20.45
Protein	39.59	39.68	39.60	39.71	39.65	39.95	39.90	40.00
Lipids	25.36	25.30	25.08	25.28	25.19	25.39	25.45	25.40
				30 days				
Moisture	5.70	5.84	5.55	5.55	20.70	20.13	20.24	20.31
Protein	39.73	39.58	39.62	40.05	39.79	40.11	39.99	31.89
Lipids	25.42	25.25	25.48	25.40	25.89	24.56	24.15	25.40
				60 days				
Moisture	5.42	5.18	5.33	5.40	19.99	19.16	19.44	19.08
Protein	40.25	40.01	39.98	40.18	40.40	40.25	40.30	40.12
Lipids	25.23	25.01	25.15	25.62	22.50	25.01	24.75	25.27

Protein and lipids contents are determined on dry weight basis

Table III. Effect of γ-irradiation and storage on peroxide value (mg eq kg^{-1}) of crude oil extracted from un-inoculated and *A. flavus*-inoculated soybean seeds (Var. Clark)

Storage periods	Un-inoculated soybean seeds Irradiation dose in kGy				*A. flavus* inoculated seeds Irradiation dose in kGy			
	0 kGy	1 kGy	3 kGy	5 kGy	0 kGy	1 kGy	3 kGy	5 kGy
Zero time	2.50	3.12	3.59	3.96	2.76	3.38	3.71	4.03
30 days	2.79	3.50	3.99	5.02	3.82	3.92	3.95	5.15
60 days	3.20	4.36	4.50	5.66	5.14	4.95	4.56	5.63

Table IV. Effect of γ-irradiation and storage on acid value (% as oleic acid) of crude oil extracted from un-inoculated and *A. flavus*-inoculated soybean seeds (Var. Clark)

Storage periods	Un-inoculated soybean seeds Irradiation dose in kGy				*A. flavus* inoculated seeds Irradiation dose in kGy			
	0 kGy	1 kGy	3 kGy	5 kGy	0 kGy	1 kGy	3 kGy	5 kGy
Zero time	3.12	3.22	3.38	3.38	3.42	3.38	3.38	3.40
30 days	3.30	3.41	3.52	3.55	5.52	3.78	3.24	3.29
60 days	3.50	3.50	3.62	3.71	6.40	4.41	3.65	3.55

significantly by increasing γ-irradiation doses and no growth or mycotoxin production occurred at 4.0 to 6.0 kGy.

Influence of γ-irradiation and storage periods on moisture, protein and total lipids content in un-inoculated and inoculated soybean seeds. In the present study, the results in Table II showed that the initial moisture content of un-inoculated soybean seeds was 5.99%, while in artificially inoculated soybean seeds with *A. flavus* moisture content raised up to 20%. The results showed also that at zero time the irradiation at different dose levels didn't affect the moisture content of un-inoculated and inoculated soybean seeds ($p < 0.05$). On the other hand storage of the seeds for 60 days slightly decreased the moisture content in un-irradiated and irradiated seeds and for all un-inoculated and inoculated seeds.

Also, from Table II it is clear that the total protein content of un-inoculated seeds was 39.59%, while that in *A. flavus* inoculated seeds was 39.65% at zero time. Irradiation almost had no significant effect ($p < 0.05$) on the total protein content of all soybean seeds (inoculated or not). During storage, the total protein content in all cases was almost constant over 60 days of storage.

In addition Table II revealed that the total lipid content of the un-inoculated and *A. flavus* inoculated soybean seeds

Table V. Effect of γ-irradiation and storage on saponification value (mg KOH/g) of crude oil extracted from un-ioculated and *A. flavus*- inoculated soybean seeds (Var. Clark)

Storage periods	Un-inoculated soybean seeds Irradiation dose in kGy				*A. flavus* inoculated seeds Irradiation dose in kGy			
	0 kGy	1 kGy	3 kGy	5 kGy	0 kGy	1 kGy	3 kGy	5 kGy
Zero time	194.92	194.09	193.93	193.53	194.82	195.32	194.92	193.02
30 days	195.46	195.02	195.98	195.52	208.73	201.43	198.51	194.08
60 days	196.12	196.12	196.39	196.02	226.80	219.99	202.24	195.51

Table VI. Effect of γ-irradiation and storage on iodine value (mg/100g) of crude oil extracted from un-inoculated and *A. flavus* inoculated soybean seeds (Var. Clark)

Storage periods	Un-inoculated soybean seeds Irradiation dose in kGy				*A. flavus* inoculated seeds Irradiation dose in kGy			
	0 kGy	1 kGy	3 kGy	5 kGy	0 kGy	1 kGy	3 kGy	5 kGy
Zero time	131.20	128.00	125.45	121.11	130.90	126.86	123.11	121.02
30 days	127.13	125.90	122.18	118.62	127.63	123.95	120.09	118.97
60 days	123.90	125.11	119.63	116.93	124.20	122.00	118.75	117.75

Table VII. Effect of γ-irradiation and storage on fatty acids content of crude oil extracted from un-inoculated soybean seeds (Var. Clark)

Storage period in days Fatty acids	Carbon No.	Zero time Dose in kGy			30 days Dose in kGy			60 days Dose in kGy		
		0	3	5	0	3	5	0	3	5
Lauric acid	12.:0	1.69	1.79	2.15	2.70	1.96	2.60	3.70	2.52	2.92
Myristic acid	14:0	0.53	0.60	0.90	0.62	0.67	0.94	0.70	0.83	0.96
Palmitic acid	16:0	14.52	14.89	15.83	17.35	15.18	16.51	20.61	16.09	16.98
Stearic acid	18:0	4.49	6.58	7.69	4.76	6.92	8.11	5.00	7.35	8.94
Oleic acid	18:1	20.43	20.18	20.04	20.02	20.10	19.78	19.81	19.86	19.54
Linoleic acid	18:2	50.99	49.06	46.74	47.92	48.48	45.16	45.76	46.87	44.39
Linolenic acid	18:3	4.84	4.21	4.01	3.78	4.18	3.99	2.00	4.09	3.78
Unkmown	20:1	2.61	2.20	2.21	2.40	2.14	2.10	2.20	2.00	1.97
Total saturated (Ts)		21.23	32.86	26.57	25.43	24.73	28.16	30.01	26.79	29.80
Total unsaturated (Tu)		78.87	75.65	73.00	74.12	74.90	71.03	69.77	72.82	69.68
Total		100.10	99.51	99.57	99.55	99.63	99.19	99.78	99.61	99.48
Tu / Ts		3.72	3.17	2.75	2.91	3.03	2.52	2.32	2.72	2.34

Table VIII. Effect of γ-irradiation and storage on fatty acids contents of crude oil extracted from *A. flavus* inoculated soybean seeds (Var. Clark)

Storage periods Fatty acids	Carbon No.	Zero time Dose in kGy			30 days Dose in kGy			60 days Dose in kGy		
		0	3	5	0	3	5	0	3	5
Lauric acid	12.:0	1.71	1.90	2.22	3.27	2.59	2.60	5.04	3.43	2.98
Myristic acid	14:0	0.58	0.62	0.92	1.04	1.00	1.03	1.51	1.30	1.08
Palmitic acid	16:0	14.70	15.10	15.87	18.88	15.49	16.25	23.10	15.88	16.93
Stearic acid	18:0	4.57	6.71	7.71	5.31	6.20	7.87	5.87	7.02	8.66
Oleic acid	18:1	20.38	20.20	19.95	19.97	19.97	19.88	19.19	19.68	19.62
Linoleic acid	18:2	50.88	49.01	46.70	46.24	48.39	46.26	42.50	46.96	45.36
Linolenic acid	18:3	4.81	4.20	3.90	3.30	4.07	3.76	1.39	3.99	3.80
Unknown	20:1	2.58	2.44	2.11	2.09	2.33	1.96	1.60	1.99	1.79
Total saturated (Ts)		21.56	24.33	26.72	28.50	25.28	27.75	35.52	27.63	29.65
Total unsaturated (Tu).		77.65	75.85	72.66	71.60	74.76	71.85	64.68	72.62	70.57
Total		100.21	100.18	99.38	100.10	100.04	99.61	100.20	100.25	100.22
Tu /Ts		3.60	3.12	2.72	2.51	2.96	2.59	1.82	2.63	2.38

was 25.36 and 25.19%, respectively. Neither irradiation nor artificial inoculation with *A. flavus* had any signaficant effect ($p < 0.05$) on the total lipid content of soybean seeds under investigation at zero time. During storage, the total lipid content of all irradiated (inoculated or not) soybean seeds at different treatments was almost stable up to 60 days of storage. After 60 days of storage the lipid content of un-irradiated inoculated seeds decreased reaching to 22.50%, this decrease might be due to the utilization of lipids by the growing fungus.

In a previous study, Zhou *et al.* (1992) found that the moisture content of soybean seeds ranged from 5.8 to 10.2%. Mohsen (1996) revealed that the moisture content of soybean seeds was 6.35 and that gamma radiation (2.5 – 7.5

kGy) had no effect on the moisture content of soybean seeds and during storage for 12 months the moisture content gradually decreased in both un-irradiated and irradiated seeds this might be due to evaporation upon storage. Seong *et al.* (1994) and Mohsen (1996) found that the protein content of soybean seeds ranged from 33.51 to 53.19%. Farag *et al.* (1985) and Barakat (1988), found a slight increase in the protein content of soybean seeds inoculated with *A. flavus* or *Fusarium solani* and stored at 28°C for one month only, they also reported that the oil content of inoculated soybean seeds with *A. flavus* or *F. moniliforme* decreased in parallel with the increase of moisture content, temperature and storage period and this decrease in oil content was sharp in seeds, which were stored at the optimum temperature of the fungal growth (35°C for *A. flavus* & 25°C for *F. moniliforme*) especially with high moisture content and this means that the fungi utilized the lipids as a source of energy beside the carbohydrates. Recently, Aziz and Mahrous (2004) revealed that *A. flavus* infected seeds behaved differently according to their principle constituents. The authors reported that *A. flavus* caused an increase in protein and decrease in lipids and carbohydrate contents of wheat, soybean and fababean seeds. Also, the authors recorded that there were no changes in chemical constituents of seeds irradiated up to 5 kGy, such as protein, lipids and carbohydrates.

Effect of γ-radiation and storage on the chemical properties of oil extracted from un-inoculated and inoculated soybean seeds. Exposure to γ-irradiation has been reported to increase peroxide value in different oil seeds such as cotton seed, sesame seeds and soybean seeds (Nassar, 1992; Shahin, 1993; Hammad *et al.*, 1994; Mohsen, 1996). In the present study, the results presented in Table III showed that at zero time the peroxide value of the crude oil extracted from un-inoculated un-irradiated soybean seeds was 2.50 mg eq kg^{-1}. From these data, it is clear that irradiation caused an increase in the peroxide value of soybean seeds oil and this increase was proportional to irradiation dose. During storage the peroxide values of soybean seed oil of un-irradiated and 5 kGy irradiated seeds were increased up to 3.20 and 5.66 mg eq kg^{-1}, respectively. The results obtained in Table III also revealed that irradiation caused an increase in peroxide value of crude soybean oil extracted from *A. flavus* infected seeds and this increase was proportional to irradiation dose (2.76 mg eq kg^{-1} at zero kGy & 4.03 mg eq kg^{-1} at 5.0 kGy). Furthermore, the data showed that, during storage the peroxide value of oil extracted from soybean seeds was gradually increased reaching to 5.14 and 5.63 mg eq kg^{-1}) for un-irradiated and 5.0 kGy irradiated *A. flavus* infected seeds after 60 days. The increase in peroxide value could be attributed to the liberation of free radicals, which greatly enhance oil oxidation in different food items containing fat and these free radicals formed by irradiation affect the un-saturated fatty acids of the oil and results in the formation of peroxides and hydroperoxides Fullerton *et al.* (1982), Hanis and Mnukova (1985), reported that peroxide values (Hanis *et al.*, 1988). Fullerton *et al.* (1982) and Hanis and Mnukova (1985), reported that peroxide values increased in direct relation to the radiation dose absorbed by the grains and this increase in peroxide value was not accompanied by off-odors, which are usually observed at peroxide values over 100 μ eq g^{-1} fat.

The acid value of the oil is a measure of the free fatty acid present, there in. It is normally referred to the degree of hydrolysis of the free acidity of the oils. Results in Table IV show the effect of gamma radiation and storage periods on the acid value of the crude oil extracted from healthy and *A. flavus* infected soybean seeds. It is obvious that soybean seed oil extracted from un-ioculated and *A. flavus* inoculated seeds had acid value of 3.12 and 3.42% at zero time, respectively. During storage, there was a progressive increase in acid values of oils extracted from *A. flavus* infected seeds that reached to 6.40% after 60 days of storage as compared with healthy seeds (3.50%). Irradiation up to 5.0 kGy had no effect on the acid values of oil extracted from un-inoculated and inoculated soybean seeds during storage up to 60 days.

In a previous studies Hafiz (1984) and Afifi (1985) found that acid value of soybean seed oil ranged from 0.53 to 2.8%. On the other hand, Hanis *et al.* (1988) reported that irradiation did not increase greatly the acid value of cereal fat.

Table V show that the saponification value of crude oil extracted from un-irradiated un-ioculated seeds was 194.92 mg KOH g^{-1} and it was observed that irradiation of seeds at dose levels of1, 3 and 5 kGy caused a slight decrease in saponification values for both un-inoculated and *A. flavus* inoculated seeds. From this table, it is clear that during storage, the saponification value of oil extracted from healthy irradiated seeds increased to about 196 mg KOH g^{-1} after 60 days of storage, whereas for oil extracted from *A. flavus* infected seeds it reached 226.80 mg KOH g^{-1}. When *A. flavus* infected soybean seeds were irradiated at dose levels 1, 3 and 5 kGy, the saponification values reached to 219.99, 202.24 and 195.51 mg KOH g^{-1}, respectively after 60 days of storage. This decrease may be attributed to decomposition of some fatty acids in oil due to irradiation treatment. In the present investigation the increase in the saponification value during storage may be due to an increase in low molecular weight fatty acids, which increased during storage. The slight increase in saponifaction value upon storage of some oil seeds had ben shown by several investigators (Shahin, 1993; Hammza, 1994; Mohsen, 1996).

Data presented in Table VI illustrated that the iodine value of crude oil extracted from healthy un-irradiated soybean seeds at zero time of storage was 131.20 mg 100 g^{-1}, whereas for *A. flavus* infected seeds was 130.9 mg 100 g^{-1}. Irradiation of un-inoculated and *A. flavus* inoculated seeds with dose levels 1, 3 and 5 kGy γ-irradiation decreased the iodine value of the crude oil at zero time. The iodine value

was 121.11 mg 100 g^{-1} at irradiation dose level 5 kGy as compared with control. The decrease in iodine value upon irradiation could be attributed to some loss of un-saturated fatty acids of soybean oil by radiation and formation of peroxide compounds, as shown in the increasing of peroxide values of these seeds due to radiation treatment (Table III). In the present study (Table VI) it is clear that, during storage, there was a marked decrease in iodine values of all oils seeds either irradiated or not up to 60 days. These results are in agreement with the results obtained by several investigators (Afifi in soybean oil, 1985; Shahin in sesame oil, 1993; Hamza in corn oil, 1994; Mohsen in soybean & cotton seed oils, 1996), who reported that during storage these was a marked decrease in iodine value of all types of seed oil either-irradiated or not, also, they reported that the same trend of changes in iodine value was observed by irradiation, where irradiation also decrease the iodine value and the decrease was proportionally to the irradiation doses.

Effect of γ-irradiation and storage on fatty acids composition of crude oil extracted from soybean seeds. Table VII indicates that crude oil extracted from un-inoculated soybean seeds contained 21.22% saturated fatty acids. Saturated fatty acids consist of palmetic (14.52%) and stearic acid (4.49%), whereas lauric and myristic were found at low concentrations in both un-irradiated and irradiated soybean oil. On the other hand, the crude oil of soybean seeds contained 78.81% un-saturated fatty acid, diene (linoleic acid) was the most predominant un-saturated fatty acid representing 50.99% of the total fatty acids, monoene (oleic acid) represents considerable amount (20.43%). Triunusaturated fatty acid (linolenic) was present in minor concentration (4.84%). The results are in agreement with those of Afifi (1985) and Basyony *et al.* (1989), who found that linoleic acid was present as a major fatty acid in soybean oil, as it amounted to 57.46% followed by oleic acid 17.60%, palmitic (15.52%), linolenic acid (5.38%) and stearic acid (3.30%). It is evident from Table VII that exposure of soybean seeds to γ-irradiation at 3 and 5 kGy changed the relative percentage of some fatty acids of crude soybean oil, from this table it is clear that the total saturated fatty acids increased to 23.86 and 26.57% and total un-saturated fatty acids decreased to 75.65% and 73.00%, respectively due to irradiation process. These results are in agreement with results obtained by Rady (1981), who found that application of different doses of gamma radiation (1.0, 2.5, 5.0 & 7.5 kGy) to crude rice bran oil and soybean oil induced a remarkable increase in saturated fatty acids and a decrease in un-saturated fatty acids, also the relative percentages of some acids showed pronounced changes due to γ-irradiation.

Results obtained in Table VII revealed that storage induced remarkable changes in fatty acids composition of crude soybean oil either irradiated or not. Storage period for 60 days led to a noticeable decrease in total un-saturated fatty acids for un-irradiated (69.77%) and 3 and 5 kGy irradiated seeds (72.82 & 69.68%), respectively.

Table IX. Effect of γ-irradiation and storage on the content of amino acids in un-inoculated soybean seeds (Var. Clark)

Storage periods	Zero time Dose in kGy			30 days Dose in kGy			60 days Dose in kGy		
Amino acids	**0**	**3**	**5**	**0**	**3**	**5**	**0**	**3**	**5**
Aspartic	31.0	31.0	31.2	30.4	30.4	30.0	30.2	30.4	30.8
Threonine	12.6	12.2	12.4	12.6	12.8	12.2	11.8	11.6	11.0
Serine	15.4	15.0	15.6	14.4	14.0	14.6	14.2	14.8	14.4
Glutamic	52.8	52.2	52.4	51.0	51.2	51.2	49.8	49.4	49.4
Proline	11.4	11.8	11.4	11.4	11.2	11.2	11.8	10.0	11.0
Glycine	12.2	12.2	12.4	11.8	11.0	11.2	11.2	11.6	10.6
Alanine	12.0	11.6	11.2	11.2	11.4	11.2	11.2	11.4	11.6
Cystine	3.2	0.8	0.8	2.8	0.8	0.8	0.6	0.6	0.6
Valine	11.2	11.4	11.0	10.6	10.2	10.2	10.4	10.4	10.0
Isoleucine	10.6	10.6	10.2	10.2	10.6	10.2	10.0	10.4	10.2
Leucine	19.2	19.8	18.3	18.6	18.4	18.4	18.4	18.2	18.0
Tyrosine	7.6	7.4	7.6	7.8	7.0	7.2	7.8	7.6	7.2
Phenylalanine	12.4	12.2	12.6	12.6	12.0	12.2	12.0	12.8	12.0
Histidine	7.0	7.2	7.4	7.2	7.6	7.2	6.6	6.4	6.2
Lysine	14.2	14.2	14.6	14.4	14.4	13.4	12.0	12.2	11.4
Arginine	16.8	16.8	16.6	16.4	16.8	16.0	15.2	15.4	14.8
Total A.A. (mg/g)	249.6	246.4	245.7	243.4	239.8	237.2	233.2	233.2	229.2

Table X. Effect of γ-irradiation and storage on the content of amino acids in *A. flavus*-inoculated soybean seeds (Var. Clark)

Storage periods	Zero time Dose in kGy			30 days Dose in kGy			60 days Dose in kGy		
Amino acids	**0**	**3**	**5**	**0**	**3**	**5**	**0**	**3**	**5**
Aspartic	23.4	23.4	23.2	21.2	21.8	21.4	21.6	21.2	21.4
Threonine	10.8	10.8	10.6	9.8	9.2	9.4	9.8	9.0	9.2
Serine	12.6	12.6	12.6	12.4	11.6	11.6	11.8	11.8	11.2
Glutamic	40.6	40.6	39.4	37.6	37.6	37.0	37.6	37.4	36.0
Proline	61.0	61.0	61.4	61.2	61.2	61.0	61.4	61.0	49.6
Glycine	11.8	11.8	10.4	9.4	9.0	9.0	9.6	9.0	9.0
Alanine	11.6	11.6	11.8	11.6	11.0	11.8	11.8	11.8	11.0
Cystine	2.6	2.6	2.4	1.0	1.0	0.4	0.4	0.4	0.4
Valine	11.0	11.0	10.2	9.0	9.4	9.2	9.2	9.6	9.6
Isoleucine	10.8	10.8	9.8	8.2	8.0	8.0	8.2	8.0	8.2
Leucise	18.4	18.4	18.2	16.6	16.0	16.0	16.0	16.4	16.4
Tyrosine	8.2	8.2	7.8	7.2	7.0	7.0	7.0	6.8	6.8
Phenylalanine	13.4	13.4	12.2	10.8	10.2	10.0	10.4	10.8	10.8
Histidine	7.0	7.0	6.6	6.0	6.2	6.2	5.6	5.6	5.2
Lysine	13.2	13.2	13.0	10.4	10.0	10.6	9.4	9.2	9.0
Arginine	17.4	17.4	16.4	13.0	13.6	13.2	11.6	11.4	10.8
Total A.A. (mg/g)	273.8	273.8	266.0	245.4	242.8	241.8	241.4	239.4	224.6

The inoculation of soybean seeds with *A. flavus*, has not induced significant changes in fatty acids composition as compared with the un-inoculated seeds (Table VIII). The crude oil of inoculated seeds contained 77.65 and 21.56% un-saturated and saturated fatty acids. Also, it is clear that the linoleic acid was the predominant un-saturated fatty acid representing 50.88% followed by oleic acid (20.38%) and linolenic acid (4.81%). Saturated fatty acids consisted of palmetic acid (14.70%) and stearic acid (4.57%). In addition, the exposure of inoculated soybean seeds to γ-irradiation at dose levels 3 and 5 kGy changed the composition percentage of some fatty acids for crude

soybean oil (Table VIII). It is clear that the total saturated fatty acids increased to 24.33% and 26.72%, respectively and the total un-saturated fatty acid decreased to 75.85 and 72.66%, respectively. Furthermore, at the storage period up to 60 days the un-saturated fatty acids of the inoculated and non-irradiated seeds decreased to 64.68%, whereas for the irradiated samples at 3 and 5 kGy decreased to 72.62 and 70.57%, respectively. On the other hand, the total saturated fatty acids increased to 35.59, 27.63 and 29.65%, for un-irradiated and 3 and 5 kGy irradiated seeds, respectively.

El-Sayed *et al.* (1979); Rady (1981); Cherry (1983); Nassar (1992) and Mohsen (1996) showed that the percentage of saturated fatty acids of some oil was proportionally increased with the increase in dose level and the percentage of the un-saturated fatty acids were inversely proportional with the increase in the dose level. The authors concluded that this may be due to partial oxidation of un-saturated fatty acids double bonds. The authors also found that storage led to a noticeable decrease in total un-saturated fatty acids for crude oil extracted from both un-irradiated and irradiated oily seeds due to saturation of some of double bonds by autoxidation reaction with the aid of some factors as fungi, enzymes, moisture, air light and temperature.

Effect of γ-irradiation and storage on the content of amino acids in soybean seeds. The present results revealed that irradiation of un-inoculated and *A. flavus*-inoculated soybean seeds at dose level 3 and 5 kGy did not cause any measurable decrease in the content of amino acids. Amino acid analysis revealed the occurrence of 16 amino acids in all soybean seeds under investigation. Glutamic acid was the predominant amino acid followed by aspartic acid and leucine (Table IX & X). Taha (1997) reported that glutamic acid, leucine and arginine showed the highest relative contents and accounted over 35% of total amino acid content of soya protein, whereas methionine and cystine exhibited 2.5% of total amino acid content. Mahrous *et al.* (2003) reported that the sulfure containing amino acids are limited in most cereals and legumes.

Furthermore, Table IX shows that by increasing the storage period up to 60 days, there was a significant decrease for total amino acids in un-inoculated seeds to 233.2 mg g^{-1} and for 3 and 5 kGy irradiated seeds to 233.2 and 229.2 mg g^{-1}, respectively. Also the same trend was observed for the content of amino acids in *A. flavus*-inoculated soybean seeds and stored for 60 days (Table X). Total amino acids for un-irradiated seeds decreased to 241.4 mg g^{-1}, whereas for the 3 and 5 kGy irradiated seeds decreased to 239.4 and 224.6 mg g^{-1}, respectively. Hanis *et al.* (1988) reported that irradiation of wheat, corn and oat meals up to 10 kGy did not cause any measurable decrease in the content of amino acids and they noticed that a dose of 25 kGy destroyed about 39% of methionine in wheat meal, 26% in corn meal and 31% in oat meal. In addition the authors noticed also a loss of about 33% of cysteine in corn meal, but in the two other cereals meals cysteine did not show any measurable decrease. Data in the literatures did not indicate much radiation destruction of amino acids in various food and feeds up to dose 70 kGy. Farag and Diaa-El-Din (1998), Seda *et al.* (2002) and Taha (1997) mentioned that the sulfur containing amino acids (cystine & methionine) were much more sensitive to radiation at low dose levels and 36% of cystine and 19% of methionine were lost by irradiation at 10 kGy, whereas lysine, isoleucine, phenylalanine, valine, tyrosine, arginine and histidine were more sensitive to the higher dose levels of 20, 30 and 40 kGy. Mahrous *et al.* (2003) showed that irradiation of wheat, barley, maize and sorghum grains up to a dose of 10 kGy did not cause any measurable decrease in the content of amino acids except methionine, which was destroyed by about 15 - 22% and at 15 kGy, methionine was also destroyed by about 22 - 32%, while there was no significant changes in the other amino acids. According to Urbain (1986) and Diehl (1991) the changes that occur in amino acids are due to deamination, decarboxylation and oxidation of sulfhydryl and aromatic groups.

It is concluded that irradiation at dose level 5 kGy is sufficient to control fungi contaminated soybean seeds and inhibited the growth of *A. flavus* and aflatoxin B_1 production all over the storage period of 60 days without changes in major chemical composition of the seeds, with slight effect on fatty acids composition and chemical properties of oil extracted from seeds. Also, γ-irradiation almost had no effect on the major amino acid content of soybean seeds after 60 days of storage under the ambient temperature.

REFERENCES

A.O.A.C., 1980. *Official Methods of Analysis of the Association of Official Analytical Chemists 15th Ed*, Washington D.C. USA

Afifi El-Sayed, A., 1985. *Effect of Gamma Irradiation on Some Plant oil*. M.Sc. Faculty of Agriculture Moshtohor, Zagazig University Egypt

Appiah, V., G.T. Odamten and D.S. Langerak, 1980. *In vitro Studies of the Combination of Heat and Radition on Conidia of Aspergillus flavus*. IFFIT Report No. 3

Aziz, N.H. and S.R. Mahrous, 2004. Effect of γ-irradiation on aflatoxin B_1 production by *Aspergillus flavus* and chemical composition of three crop seeds. *Nahrung/Food*, 48: 234–8

Aziz, N.H. and L.A.A. Moussa, 2002. Influence of gamma radiation on mycotoxin-producing moulds and mycotoxins in fruits. *Food Control*, 13: 281–8

Aziz, N.H., S.A. El-Zeany and L.A.A. Moussa, 2002. Influence of γ-irradiation and maize lipids on the production of aflatoxin B_1 by *Aspergillus flavus*. *Nahrung/Food*, 46: 327–31

Aziz, N.H., Z.A. Matter and A.M. Shahin, 2005. Detection of fumonisin B_1 produced by *Fusarium moniliforme* and its control by gamma radiation and food preservatives. *New Egypt J. Microbiol*., 10: 96–107

Aziz, N.H., L.A.A. Moussa and F.A.E. El-Far, 2004. Reduction of fungi and mycotoxins formation in seeds by gamma-radiation. *J. Food Safety*, 24: 109–27

Barakat, M.I. El-Khaleely, 1988. Soybean seed deterioration caused by fungi under storage conditions. *Ph.D Thesis*, Faculty of Agriculture Fayoum, Cairo, University

Basyony, A.E., M.I. El-Rafaei, M. Galal and M.I. Barakat, 1989. Effect of gamma irradiation on seed, borne, fungi and soybean seeds components during storage. *Agric. Res. Rev*., 67: 619–28

Brook, R.C. and G.H. Foster, 1981. Drying, cleaning and conditioning. *In*: Finney, E.E. Jr. (ed.), *Handbook of Transportation and Marketing in*

Agriculture, Vol. II, Pp: 63–110. CRC Press, Boca Raton, FL

Cherry, J.P., 1983. Cotton seed oil. *J. AOCS*, 60: 312A

Diehl, J.F., 1991. Nutritional effect of combining irradiation and other treatment. *Food Control*, 2: 20–5

Duncan, D.B., 1955. Multiple Range test and multiple. *F-test Biometrics*, 11: 1–12

Elppey, R.M., 1968. Screening method for zearalenone, aflatoxin and ochratoxin. *A.J. AOAC*, 51: 74–8

El-Sayed, S.A., M.S. Raouf, M.M. Mourad and A.H. Rady, 1979. Effects of gamma irradiation on fatty acids of tomato seeds oil. *Arab. J. Neuc. Sci. Applic*., 61: 2–12

Farag, M. and H. Diaa El-Din, 1998. The nutritive value for chicks fed full-fat soybean irradiated at up to 60 kGy. *Anim. Feed Sci. Tech*., 73: 319–27

Farag, R.S., F.A. Khalil, S.M. Mohsen and A.E. Basyouny, 1985. Effect of cirtain fungi on the lipids of wheat kernels, sesame and soybean seeds. *Grasosy Aceities*, 36: 362–7

Fullerton, F.R., D.L. Greenman and D.C. Kendal, 1982. Effects of storage conditions on nutritional qualities of pemipurified (AIN-76) and natural ingredient (NIH-07) diets. *J. Nutr*., 112: 570–6

Gowrama, J. and L.B. Bullerman, 1995. *Aspergillus flavous* and *A. parasiticus* aflatoxigenic fungi of concern in foods and feeds. *J. Food. Prot*. 58: 1395–404

Hafiz, S.A., 1984. Biochemical studies on some souybean varieties. *M.Sc. Thesis*, Faculty of Agriculture, Cairo, University Egypt

Hammad, A.H.I., M.M. Ragab, A. Effat and A.A. Shahin, 1994. Effect of irradiation and fungal infection on the chemical composition of oil properties of sesame seed. *Seventh Conference of Phytopathology April, 19-21, 1994, Cairo, Egypt*, Pp: 155–63

Hamza, R.G., 1994. Biochemical studies on lipids as affected by gamma irradiation. *M.Sc. Thesis,* Faculty of Agriculture, Ain Shams University, Cairo, Egypt

Hanis, T. and J. Mnukova, 1985. Radiation and heat induced changes in contents of vitamin A and acid peroxide values in cereal-based mixtures during storage, 424. *In*: *Proc. International Conference of Food Science and Technology*. Manila, Philippines

Hanis, T., J. Mnukova, P. Jelen, P. Klir, B. Perez and M. Pesek, 1988. Effect of gamma irradiation on survival of natural microflora and some nutrients in cereal meals. *Cereal Chem*., 65: 381–3

Mahrous, S.E., N.H. Aziz and A.A. Shahin, 2003. Influence of gamma irradiation on the occurrence of pathogenic microorganisms and nutritive value of some cereal grains. *Isotope Rad. Res*., 35: 551–86

Mohsen, G.I.M., 1996. Effect of gamma radiation on microbial contamination and poisoning substances in some oil seeds and their products. *Ph. D. Thesis*, Department of Food Science and Technology, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt

Nabney, J. and B.F. Nesbitt, 1965. Aspectrophotometric method for determining the aflatozins. *Analytis*, 90: 155–66

Nassar, O.M., 1992. Biochemical studies on irradiated fats and oils. *Ph. D. Thesis*, Faculty of Science, Ain Shams University, Cairo, Egypt

Rady, A.H., 1981. Effect of gamma-irradiation and estrification on crude rice bran oil properties. *Ph. D. Thesis*, Faculty of Agriculture Cairo, Egypt

Seda, Hanen, A., A.A. Mahmoud, A.A. Ibrahim and H.F.G. El-Niely, 2002. Effect of radiation processing on sensory and chemical characteristics of (Giza-2) (Vicia faba). *Egypt J. Rad. Sci. Applic*., 15: 119–39

Seong, R.C., S.J. Park, H.S. Chung and Y.B. Kim, 1994. Differences in chemical composition of soybean seeds with different usages. *Korean J. Crop Sci*., 39: 382–8

Shahin, Azza, A.M., 1993. Control of fungus diseases during storage of certain economic crops using gamma radiation. *Ph. D Theis*, Faculty of Agriculture Cairo University, Cairo, Egypt

Steel, R.G.D. and J.H. Torrie, 1980. *Principals and Procedures of Statistics: A Biometrical Approach*, 2nd ed. International Student Edition, Mc GRAW-Hill. International Book Company, London

Taha, S.A., 1997. Influence of ionizing radiation upon the amino acid and fatty acid composition of soybean. *Zagazig J. Agric. Res*., 24: 273–82

Urbain, W.M., 1986. Radiation chemistry of food components. *In*: Schweigert, B.S. (ed.), *Food Irradiation and Food Science and Technology*, P: 37. Academic Press, New York

Varso, H.H., 1972. A procedure for isolation and quantitative determination of volatile fatty acids from meat products. *J. Food Sci*., 37: 136–9

Winder, K. and O.B. Eggum, 1966. Protein hydrolysis. A description of method used. Department of animal physiology in Copenhagen. *Acta Agric. Scandinavia*, 16: 115

Youssef, M.B., S.R. Mahrous and N.H. Aziz, 1999. Effect of gamma-irradiation on aflatoxin B_1 produced by *Aspergillus flavous* in ground beef stored at 5^{o}C. *J. Food Safety*, 19: 231–9

Zhou, X.N., J.Y. Gai and R.H. Ma, 1992. Studies of the components of seed storage protein and their correlations in soybeans. *Soyabean Sci*., 11: 191–7

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