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OCCURRENCE OF AFLATOXIN AND AFLATOXIGENIC MOULDS IN COFFEE BEANS AND DECONTAMINATION BY GAMMA-IRRADIATION

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SUMMARY: The survey of 25 commercial samples of coffee beans for aflatoxin has been conducted in 1989. Moulds were counted in all samples to be 102 to 104 per gram and were identified as *Aspergillus flavus, A. ochraceus, A. restrictus, A. fumigatus, A. wentii, A. glaucus, A. niger and Penicillum* species, Aflatoxin B_1 was detected in 7 samples at a level of 8-40 mg/kg and aflatoxin B_2 was detected in 3 samples at a level of 6-32 mg/kg. Three from nine isolates of *A. flavus* produced high levels of aflatoxin B_1 on coffee beans (35-260 mg/kg) and on MYG Liquid medium (16-87 mg/kl). A study on the inactivation of moulds in coffee beans showed that gamma irradiation doses of 4-6 KGY were required to decrease the mould counts below 102 per gram. Toxigenic moulds were eliminated with 5 KGY irradiation. In the storage study at humidity higher tha. 90% at 30°C in polyethylene pouches during 1 to 2 months, the mould counts increased more than 106/g, while samples subjected to 4KGY irradiation were free from moulds and aflatoxins.

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INTRODUCTION

One measure of the quality of coffee beans is the number of mciroorganisms present at any given time during storage. Deterioration as a result of mould growth, particularly mycotoxigenic moulds on agricultural commodities represents a public health hazard (Beuchat,1984). Aflatoxins are considered at present to be one of the most dangerous contaminants in food and feed products (Refai, 1988).

The presence of toxigenic fungal propagules in raw food material is expected and populations and types of fungi vary considerably with the nature of the product (Beuchat, 1979). Several reports have been published delineating environmental conditions required for metabolic procces of moulds in stored foods (Corry, 1978).

The use of ionizing radiation in the preservation of food products has proved to be suitable method for the elimination of pathognic microorganisms (Aziz, 1982). The evaluation of organoleptic and antimicrobial effecets gamma radiation on coffee beans has been limited.

This study presents data on the occurrence of aflatoxins and aflatoxigenic moulds in coffee beans and the efficacy of gamma irradiation for decontamination of coffee beans immediatly and during different storage periods.

MATERIAL AND METHODS

Material:

Twenty five samples of coffee beans obtained from the local market were used in this investigation.

Mycological studies:

Five grams of each sample were ground in blender under sterile conditions then mixed with 0.01 tween 80 sterile water and homogenized for one minute. Each suspension was diluted 102 to 104 times with the same sterile water and then 0.2 ml aliquots were spread on the surface of MYG chloramphenicol agar containig malt extract 10g, yeast extract 4g. glucose 4g, agar 20g and chloramphenicol 20 mg per litre pH (6.0). Moulds were counted and later isolated and identified.

Estimation of aflatoxin producing ability:

The qualitative and quantitative test for aflatoxin producing strains was carried out by inoculating triplicate flasks of aflatoxin free coffee beans and MYG liquid medium with 1.0 ml spore suspension of *A. flavus*.Spore suspension was prepared by adding 5 ml of distilled water to a 7 day old malt exract agar slant of *A. flavus* isolate and agitated. The spore suspension was collected in conical flasks and approximayaly 1 ml equavilant to 107 conidia.

Aflatoxins in each coffee beans and liquid culture media as well in the original sample (three analyses/ 25 samples, 25g each) was determined by TLC (Aziz,1987).

Gamma-irradiation:

Twenty five grams in polyethylen pouches of the coffee beans and one milliletre spore suspension (107 spore/ 1 ml) + 9 ml sterile saline solution in test tubes were irradiated at an increasing doses of 1 up to 8 KGY. Cobalt 60 Egypt's Indutrial Meaga Gamma I Irradiation NCRRT City, Cairo, Egypt was used for the irradiation procedure. After each treatment, suitable serial dilutions were made and the surviving counts were recorded on MYG agar meduim. Also, irradiated samples and isolates were analyzed for detecting the presence of aflatoxin.

Storage studies:

Polyethylene pouches each containing 25 grams of coffee bean samples were stored after irradiation at 30 °C and relative humidity 70-90%. Mould counts and aflatoxin analysis were carried out every two weeks over three months.

RESULTS AND DISCUSSION

Through this study it was found that the species of aspergilli and penicilli represented the most common fungal populations isolated and identified from the different coffee bean samples. *Aspergillus flavus* (68%) *A. ochraceus* (33%)*A. restrictus* (16%)*A. fumigatus*(16%) *A. wentii* (8%), *A. glaucus* (8%), *A. niger* (35%) *Penicillium cyclopium* (44%) *P. chrsogenum* (8%) and *P. variable* (8%) were widespread in all samples. These observation agree with data recorded by Beuchat (1984). The data recorded in (Table -1) revealed that of the samples of coffee beans, ten samples were contaminated with aflatoxins, seven with aflatoxin B1 (8 - 40 Mg/Kg) and three with aflatoxin B2 (6 - 32 Mg/Kg). Alfatoxins are naturally occurring compounds and were reported to easily contaminate corn, peanut, cotton seed, rice, milk, certain meat products and poultry diets (Bean and fernando, 1985) Generally, aflatoxin B_1 was investigated as the most-potent carcinogenic metabolite produced by fungi in different food products, (Hsieh, 1983). During this investigation, from the nine isolates of *A. flavus*,Three produced aflatoxin B_1 om coffee beans (35-260 Mg/g) and on MYG-Liquid medium (6-87 Mg/ml) (Table-1). *Aspergillus flavus* was observed as the main popular fungal species capable of producing aflatoxins in food and feed commodities (Wei et al., 1985).

As a result of mould growth and mycotoxin production in different food products, many counstries used both fumigation with ethylen oxid and heat for sterilizing these products. However, these methods have several disadvantages for application such as toxic residues

TABLE (1) DETECTION OF AFLATOXINS IN COFFEE BEAN SAMPLES AND ASPERGILLUS FLAVUS ISOLATES:

NO. OF TESTED COFFEE BEANS SAMPLES	25
NO. OF POSITIVE SAMPLES	10
AMOUNT OF AFLATOXIN B1 (Mg/Kg IN 7 SAMPLES	8 - 40
AMOUNT OF AFLATOXIN B2 (Mg/Kg IN 3 SAMPLES	6 - 32
NO. OF ISOLATES TESTED	9
NO. OF TOXIGENIC ISOLATES	3
AMOUNT OF AFLATOXIN B1	
ON EXPTLY CONTONINATED COFFEE BEAN (Mg/Kg	35 - 260
ON MYG MEDIUM (Mg/ ML	16 - 87

Table (2) Effect of different doses of gamma-irradiation on the number of moulds (gram/sample) and afltoxin concentratios in coffee bean (Three analysis/sample).

RADIATION DOSES IN KGY	COUNT OF MOULD PER GRAM	AFLATOXIN CONCENTRATION	
		AFLATOXIN B MG/Kg	AFATOXIN B MG/Kg
0	9.70 X 10	40	32
1	1.50 X 10	37	32
2	8.20 X 10	22	21
3	1.80 X 10	13	16
4	3.5 X 10	5	7
5	0.00	TRACES	TRACES
6	0.00	TRACES	TRACES
7	0.00	ND	ND
8	0.00	ND	ND

TRACES: LESS THAN 1 MG/K
ND: NOT DETECTED

are left and organoleptic properties are changed (Guri et al., 1986).

For these reasons, there have been many reports which indicate that radiation treatment is a suitable method for decontaminating food products and commerical scale use for radiation process has been successful in several countries (Depster, 1985). The data recorded in (Table 2) revealed that the viable count of mould decreased significantly by increasing the radiation doses. It was noticed that 4-6KGY was the lethal dose for the moulds isolated from examined coffee bean samples. As a result of gamma irradiation treatment, the concentrations of aflatoxins in the coffee beans decreased greatly by increasing the irradiation does (AFB_1 from 32 Mg/Kg at OKGY to 5 Mg/KG at 4 KGY) Table-2). Chang and Markakis (1982) reported that, in general, increasing the radiation dose at the range of 0 to 4 KGY resulted in decreasing aflatoxin formation in barley. The effect of gamma irradiation on the viability of *A. flavus* aflatoxin production in synthetic medium and coffee beans is reported in (Table 3)., *Aspergillus flavus* produced much more aflatoxin B_1 on coffee beans as compared to synthetic meduim. Generally by increasing the gamma; irradiation doses the concentration of aflatoxin B_1 decreased and could not detected at dose 6 KGY. El-Hadi (1986) reported that when the two strains of *A. flavus* producing aflatoxins were exposed to increasing doses of gamma-irradiation up to 5 KGY, the aflatoxin quantities decreased and finally it was completely inhibited.

The effect of storage periods and gamma-irradiation on the mould viability and aflatoxin production in coffee beans is represented in (Table -4). Mould growth occurred rapidly in the coffee beans during the first month of storage and reached more than 106 per gram at 30°C and 90% relative humidity. Also aflatoxin B_1 concentrations increased greatly by increasing the storage periods and reached to 415 Mg/g after 4 months. When coffee beans were irradiated with a dose of 4 KGY,

TABLE (3): EFFECT OF GAMMA IRRADIATION ON THE COLONY FORMATION OF A.FLAVUS AND AFLATOXIN B_1 PRODUCTION

IRRADIATION DOSES KGY	TOTAL VIABLE COUNTS PER ML	AFLATOXIN PRODUCTION (A)	
		COFFEE BEAN MG/G	MYG MEDIUM MG/ML
0	250×10^{7}	260	87
1	1.90×10^{5}	185	69
2	5.10×10^{4}	125	42
3	3.30×10^{3}	88	33
4	6.00×10^{2}	60	26
5	3.00×10^{1}	25	8
6	0.00	16	TRACES
7	0.00	TRACES	ND
8	0.00	ND	ND

(A) : INCUBATED FOR 10 DAYS AT 25 C
TRACES: LESS THAN 1 MG / GRAM OR ML
ND : NOT DETECTED .

Table (4) : Effect of storage periods of non-irradiated and irradiated coffee beans on the number of moulds and aflatoxin B_1 production.

Radiation Doses in KGY	Mould counts / gram sample after different storage periods in weeks													
	0		2		4		6		8		10		12	
	N	AFB1	N	AFB1	N	AFB1	N	AFB1	N	AFB1	N	AFB1	N	AFB1
0	8.10×10^{4}	45	3.50×10^{5}	60	6.10×10^{6}	135	8.10×10^{7}	226	3.00×00^{7}	336	2.00×10^{8}	415	2.00×10^{8}	420
1	3.50×10^{4}	32	3×10^{4}	32	4×10^{4}	45	6×10^{5}	90	6×10^{5}	87	8×10^{6}	110	8×10^{6}	122
2	8.20×10^{3}	19	8×10^{3}	19	9×10^{2}	13	3×10^{2}	7	8×10^{1}	5	3×10^{1}	TRACES	0. 00	ND
3	1.80×10^{2}	12	1.8×10^{2}	13	7×10^{1}	8	7×10^{1}	6	5×10^{1}	2	0.00	ND	0.00	ND
4	3.50×10^{1}	3	2×10^{1}	TRACES	0.00	ND	0.00	ND	0.00	ND	0.00	ND	0.00	ND

N : THE NUMBER OF MOULDS/GRAM SAMPLE
TRACES: LESS THAN 1Mg/K
ND : NOT DETECTED
AF1 : AFLATOXIN B1 Mg/K

mould growth as well as aflatoxins was not oberved for more than 2 months of storage. The different effect can be explained by that in coffee beans samples irradiation reduced the rate of increase in a which therfore inhibited the rapid increase of mou ds. On the contrary, the increase of moulds in whole form samples was independent of the dose but it was related to the increase in aw (Juri et al., 1986).

So, in this study irradiation can suppress the growth of moulds in coffee bean samples with a dose of 4-6 KGY. The toxigenic moulds disappeared and the mycoflora became dominated by non toxigenc *Aspergilli* during storage under summer conditions.

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