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# Effect of gamma radiation on aflatoxin and physiochemical properties of *Arachis hypogea* L.

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## ABSTRACT

Aflatoxins are cosmopolitan in distribution and incur several health related problem such as liver cancer, neurological disorders and teratogenic effect in human as well as animals. Several researcher suggested different method of aflatoxin detoxification such as chemical and physical method. In present study effect of different gamma radiation dose ranging from 0.1-15kGy was investigated against total aflatoxin and physiochemical properties of peanut. It was found that gamma radiation are effective in aflatoxin reduction as well as at certain dose radiation does not affect fatty acid profile, oil percentage, Acid value and saponification value. Maximum reduction in total aflatoxin was observed at 10 kGy radiation.

**Keywords** : gamma radiation, *Arachis hypogea*, Aflatoxin

*Arachis hypogea* L. (Groundnuts or peanuts) provide an excellent source of protein and are a valuable cash crop for millions of small-scale farmers in the semi-arid tropics. As a groundnut-based fodder they are consumed by animals in India. Contamination of the groundnut by aflatoxin (complex chemical that can cause cancer) poses a serious threat to human and animal health not only from eating the diseased groundnuts themselves but also indirectly from drinking milk from cows that have eaten infected material and also from edible oil extracted from contaminated seeds. Aflatoxin is produced by the fungus *Aspergillus* which enters the plant, usually through the wounds left by an insect pest. *Aspergillus* get access to plant either during preharvest condition or post harvest condition i.e. storage. The fungi *Aspergillus flavus* Link ex Fries and *Aspergillus parasiticus* Speare have been found to be causal agent for producing aflatoxin in grain when stored. The most important group of toxigenic Aspergilli are the Aflatoxigenic molds, *A. flavus*, *A. parasiticus* and the recently described but much less common species *Aspergillus nomius* all of which are classified in Aspergillus section Flavi (Gams *et al*. 1985). *A. flavus* is widely distributed in nature but *A. parasiticus* is less wide spread, the actual extent of its occurrence being complicated by the tendency for both species to be reported indiscriminately as *A. flavus*. In the last decade, aflatoxin levels were found to exceed an acceptable level limit of 20 ppb (part per billion) stipulated in most export specifications. Aflatoxin contamination has affected maize, peanuts and cottonseeds in Thailand, India, and coconut in the Philippines, Sri Lanka and other Pacific countries. Aflatoxicosis both in humans and animals has been more prevalent in areas where maize and groundnut constitutes a major part of the diet. Bhat *et al.* (1997) reported that about 26% maize and 21% of groundnut sample collected from 11 states exceed the permissible limit of 30 ppb aflatoxin per kg of material. Prevention of Food adulteration act (1954), amended in 1986, U.S. Food and drug Administration (FDA) and the Codex Alimentarius Commission (Anonymous 1989) have recommended a permissible limit of aflatoxin of 30 ppb/kg, 20 ppb/kg and 5 ppb/kg respectively. Few studies have been undertaken in past sampling the food stuffs, available in market for consumption, for presence of fungi. In a study conducted in Andhra Pradesh in 1965, aflatoxin levels were in the range of 1,000 and 5,000 ppb

in 12% of 743 in shell and 141 groundnut cake samples. Aflatoxin levels were highest in samples of the rainy season crop. In 1976, 50% of the groundnut cake samples collected mostly in Madhya Pradesh region was positive for aflatoxin (Reddy *et al*. 1986). Someshekar *et al.* (2004) studied 54 market samples comprising nine different food commodities from Mysore city in order to isolate aflatoxigenic fungi as well as aflatoxin in commodities. 32 samples were contaminated with aflatoxigenic fungi and total mycoflora and aflatoxigenic fungi in different food and feed commodities were in the range of 0.2 to 260 and 0 – 100 CFU x $10^3$/gm respectively. In total, 136 fungi were isolated of which 32 were *A. flavus* strains and 16 of which produce aflatoxin.

The stability of aflatoxin with respect to different physical and chemical agents is well known (Annonymous 1979). Autoclaving at 120°C for up to 1 h does not destroy this mycotoxin, and even after sterilization in an acid or alkaline medium, slight mutagenic activity is still detectable (Van Duck & Voorde, 1980). In vivo, the activity of aflatoxin B1 is markedly reduced by hydroxylation at the 9a position. The stability of this mycotoxin is, however, limited in highly polar solvents. Visible light and UV light are also able to stop biological activity (Anonymous 1979), but these sources of radiation have a low penetration capacity in solids and liquids. Therefore, the action of gamma rays on aflatoxin B1 stability was tested.

## MATERIALS & METHODS

**Sample collection—**Peanut (*Arachis hypogea*) variety G-10 was purchased from Market of Gujarat (India). Immediately the samples are stored at 4°C.

**Gamma Irradiation—**The source of the gamma irradiation was "Shri Ram Applied Radiation Centre" (SARC), of the Shri Ram Institute for Industrial Research, Delhi. SARC- fully automatic and computerized plant with a capacity of 500 kilocuries Cobalt-60 can process 10,000 $m^3$ of medical products (0.1 g/cc) annually. Gamma radiation has been carried out on the peanuts of Gujrat-10 variety. The peanuts were packed in polythene bags of 1 kg weight. A dose of 0.1 kGy to 15 kGy was used for the study.

**Quantification of Total Aflatoxin by ELISA—**5g fine ground powder of peanut seeds or irradiated seeds drawn in triplicate from each peanut seed lot were extracted with 25mL of 70% aqueous methanol using a laboratory homogenizer and filtered through Whatman no. 1 filter paper. 100µL of each filtrate were diluted with 600µL of dilution buffer and 50µL of diluted sample employed to immunoaffinity column for cleanup the samples. Aflatoxin content was finally eluted with 0.5mL of HPLC grade methanol and total aflatoxin content were determined following aflatoxin detection kit obtained from R- Biopharm AG, Darmstadt, Germany.

50µL of standard solution of aflatoxin and cleaned eluted sample in duplicate added to the wells of microtiter plate. After that 50 µL of peroxidase enzyme conjugate and 50µLof mouse monoclonal anti-aflatoxin antibodies were added to each well and incubated at room temperature in the dark for 30 minutes. After washing thoroughly with 250 µL distilled water three times, 50µL of urea peroxidase (substrate) and 50µL of tetramethylbenzidine (chromogen) were added to each well, mixed thoroughly and incubated for 30 minute at room temperature in the dark. Reaction was stopped by adding 100µL 1M Sulphuric acid (stop reagent) and the absorbance was measured at 450nm using Bio-Rad ELISA microplate reader Model 680.

**Determination of fatty acids—**Fatty acid analysis was done according to the method described in IS : 548 (Part 3) - 1976. A Hewlett-Packard 5840A Gas chromatography (Palo Alto. CA) with thermal conductivity detector and microprocessor was used. Working conditions of Gas Chromatography :

Column 1 – 10% DEGA on chromosorb WAW (80-100 mesh), Injector and detector temperature 300 $^0$C each; oven temperature 180, 190, 200 and 210 $^0$C.

Column 2- 10% SE-30 0n chromosorb WAW (80-100 mesh); Injector and detector temperatures 300 $^0$C, column temperatures 240,250,260 and 270 $^0$C.

Hydrogen gas was used as carrier gas at a flow rate of 40ml /min. and the oven temperature was maintained with an accuracy of 0.3 $^0$C, the sample (5 l.) was injected and the retention time of each sample was noted.

A standard fatty acid methyl ester mixture (Sigma Chemical Co.) was used to identify sample peaks. Commercial mixtures of fatty acid methyl esters were used as reference data for the relative retention times. Quantitative analysis of the fatty acids was performed using the heptadecanoic acid methyl ester as internal standard. The results are reported by mean values of two replicates.

**Chemical properties of peanut**

**Oil percentage**—Oil percentage was determined according to the method described in IS:7874 (Part 1)-1975 in which 2.5g of the prepared sample dried at 135°C for 2hr in oven and extracted with Petroleum Ether in soxhlet apparatus. Petroleum ether fraction was evaporated on steam bath, cooled and weighed.

**Acid value**—Acid value of peanut oil was determined according to the IS 548(Part-1):1964 in which 5 gm of oil taken into 200 ml conical flask and 50 ml of freshly neutralized hot ethyl alcohol with 1 ml of phenolphthalein indicator solution was boiled for 5 minute and titrated against aqueous alkali solution. Acid value was calculated using following formula:

Acid value = 56.1 VN/ W

Where

V = volume in ml of standard potassium hydroxide solution used

N = normality of standard potassium hydroxide solution

W = Weight in gm of the oil taken

**Saponification value**—Saponification value of oil was determined according the IS 548(Part-1):1964 in which 5 gm of oil was refluxed with 25 ml of alcoholic hydroxide. Boiled the sample until sample was saponified indicated by the absence of any oil matter and appearance of clear solution. Titrated against standard hydrochloric acid with phenolphthalein indicator. Saponification value was calculated using following formula :

$$\text{Saponification value} = \frac{56.1\,(B\text{-}S)\,N}{W}$$

Where

B = Volume in ml of standard hydrochloric acid required for the blank

S = Volume in ml standard hydrochloric acid required for the sample

N = Normality of the standard hydrochloric acid

W = Weight in gm of the sample taken

## RESULT & DISCUSSION

Peanut make an important contribution to the diet in many countries. Peanut seeds are a good source of protein, lipid and fatty acids for human nutrition (Grosso & Guzman 1995). Detoxifying method or preservative are said to be ideal if they had least or no effect on nutrition content, oil properties and taste of food commodities.

***Effect of Gamma Radiation on Fatty acid profile***—The peanut oil belongs to the oleo- linoleic acid group of oils. The more important component fatty acids are Palmitic, Oleic, Linoleic, Arachidic, Stearic and Behenic acids. Myristic, Erucic and Seric acids as well as trace amounts of odd carbon fatty acids. The components are: palmitic acid 8.3%; stearic acid 3.1%; arachidic acid 2.4%; behenic acid 3.1%; lignoceric acid 1.1%; oleic acid 56.0% and linoleic acid 26.0% (by weight). The presence of small proportions of behenic, arachidic and lignoceric acids is characteristic of this oil (Anonymous 2001). Fatty acid composition of vegetable oil plays an important role to decide its quality (Jambhulkar & Joshua 1998).

Results of effect of gamma radiation on fatty acid composition are presented in Table 1. It is clear from the data that Palmetic acid, oleic acid and linolic acid are the main constitutes of the peanut oil but the presence of arachidic acid and euric acid differentiate the peanut oil from other edible oil. Myrestic fatty acid is usually found in very small amount in peanut oil or even sometimes it may be absent. Low energy doses upto 3 kGy of gamma irradiation have not shown much affect on the fatty acid composition of peanut oil, while increasing the dose from 5 kGy to 15 kGy also showed little effect on fatty acid profile except for lignoceric acid, which was not detected due to treatment. Yalcin *et al.* (2011) investigated the effect of irradiation (2.5, 4.0, 5.5, and 7.0kGy) on chemical properties and volatile contents of linseed and observed the consistent decrease in both protein and oil content of the irradiated linseed samples with increasing doses. Irradiation causes irregular changes in Palmitic and Stearic acid content. No adverse effects on the nutritional value or the sensory quality of dried fruits and nuts have been reported by Khan (1993). Previous studies showed that gamma rays interact with fat molecules to cause oxidation, decarboxilation, dehydration and polymerization reactions leading to lipid oxidation (Gomes *et al*. 2003). Inayatullah *et al.* (1987) found that the peroxide value of soybean was significantly increased by irradiation with 0.25–5 kGy. Chiou (1994) revealed that the peroxide content of peanut oils extracted from the irradiated peanuts increased with increased irradiation dose (2.5, 5.0, 7.5 and 10 kGy). Kashani & Valadon (1984) in their study on Iranian pistachios concluded there was a slight increase in peroxide values but no off flavoring occurred at 1 kGy

irradiation dose. Diehl (1990) suggested the texture deformation caused by irradiation is less in foods that have low water content.

***Effect of Gamma Radiation on Oil properties of Peanut*** — Table 2 shows the effects of Gamma irradiations on oil of peanut. Lower dose from 0.1 kGy to 1.0 kGy decreasing oil content in the irradiated peanut samples. Upto 3 kGy dose of Gamma irradiation, the oil decreased in very low amount from 48% to 45% but in higher doses like 5 kGy to 15 kGy, it decreased drastically from 48% to 36%. At 3 kGy dose of Gamma irradiation, the oil content maintained the good quality of peanut as it under the PFA limits.

Table 2 shows the effects of different energy doses of gamma irradiation on oil properties of peanut oil. Acid value and saponification value have been determined with oil content itself of the entire irradiated peanut. PFA limits of all properties also shown in the table to clarify the good quality of peanut oil. As oil content decreased with increasing dose of gamma irradiation, the oil properties increased with the increasing irradiation but more than 6 in acid value and more than 3 in free fatty acid makes the oil bitter. In higher doses of gamma i.e. 5 kGy to 15 kGy, all three properties increased and went beyond the PFA limits. Lower doses maintained the properties under the limits.

**Table 1—- Effect of gamma radiation on fatty acid properties of peanut sample.**

| | Irradiation with Gamma | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **STD** | **control** | **0.1kGy** | **0.2kGy** | **0.5kGy** | **1.0kGy** | **3.0kGy** | **5.0kGy** | **10.0kGy** | **15.0kGy** |
| Palmetic | 8.41 | 9.37 | 10.52 | 10.46 | 9.89 | 9.68 | 9.58 | 9.36 | 9.88 | 9.73 |
| Oleic | 58.1 | 56.58 | 56.61 | 56.57 | 56.82 | 56.12 | 56.11 | 57.79 | 58.20 | 58.73 |
| Linolic | 26.6 | 25.33 | 25.64 | 25.41 | 25.46 | 25.38 | 25.14 | 25.54 | 25.12 | 23.45 |
| Lignoceric | 1.30 | 1.16 | 1.10 | 1.12 | 1.12 | 1.16 | 1.12 | ND | ND | ND |
| Arachidic | 2.14 | 1.16 | 1.53 | 1.35 | 1.39 | 1.56 | 1.74 | 1.86 | 1.54 | 1.46 |
| Behemic | 2.21 | 2.56 | 2.49 | 2.62 | 2.71 | 2.74 | 2.68 | 2.74 | 2.46 | 2.39 |
| Euric | 0.40 | 0.45 | 0.49 | 0.48 | 0.42 | 0.41 | 0.41 | 0.40 | 0.231 | 1.58 |

Fatty Acid composition (in %), ND- Not Detected

The results have shown that with increasing radiation dose the oil content decrease from 48.02% to 36.36% (in gamma radiation) at a dose of 15 kGy. Only 3% decrease in oil content has been observed with a dose of 3 kGy. The acid value and free fatty acid content (w.r.t. oleic acid) have changes under the PFA limits only with the dose of 3 kGy. The saponification value of oil increased with increasing radiation dose. An increase in saponification value indicates an increase in quantity of alkali required for saponification, which may be due to breakdown of linkage of the triglycerides and the diglycerides. The saponification value showed the oil contained long chain fatty acids. In lipids particularly unsaturated fatty acids, radiolytic decomposition occurs via a preferential break at the level of the carbonyl function of the double bond. This decomposition induces the formation of some peroxide and volatile compounds responsible for off-odours. The increasing

**Table 2- Effect of gamma radiation on oil properties of peanut.**

| | Irradiation with Gamma | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **STD** | **control** | **0.1kGy** | **0.2kGy** | **0.5kGy** | **1.0kGy** | **3.0kGy** | **5.0kGy** | **10.0kGy** | **15.0kGy** |
| Oil (%) | 42-48 | 48.02 | 47.93 | 47.26 | 46.44 | 46.01 | 45.20 | 43.96 | 40.29 | 36.36 |
| Acid value$^{\$}$ | < 6.0 | 1.39 | 2.01 | 2.43 | 2.58 | 2.89 | 3.0 | 5.44 | 6.14 | 6.96 |
| Sap. Value$^{\#}$ | 188-196 | 182 | 185 | 189 | 190.3 | 191.4 | 192.9 | 195.9 | 197.5 | 198.4 |

# Saponification value, $ Acid value (mg/gm).

trends is not much high, it only goes 192 and 190 in 3 kGy in comparison to 182 of non-irradiated sample. Although the overall saponification value varies from 182 to 198.4. Maximum saponification value has been observed in 15 kGy of gamma radiation. Dose higher than 3.0kGy had some changes oil percentage, acid value and saponification value as compared to control. No significant change due to irradiation in the composition of dried fruits and nuts (Inayatullah *et al.* 1987) has been reported within a range of exposure doses sufficient to kill all infesting insects.

***Effect of Gamma Radiation on Aflatoxin content*—**Table 3 show the effect of irradiation on TAF (Total Aflatoxin) content. TAF was determined by using ELISA. At 10.0 and 15.0 kGy TAF was not detected in peanut sample. Total aflatoxin reduced 11.6%, 54.7%, 75.0%, 81.5%, 91.5%, and 99.3% at 0.1, 0.2, 0.5, 1.0, 3.0 and 5.0 kGy respectively. Iqbal *et al.* (2012) studied the effect of gamma radiation on moisture content, total mold content, *Aspergillus* count and aflatoxins of pepper and found effective in reducing total mold and *Aspergilllus* counts. Total mold counts in irradiated peppers immediately after treatments were significantly lowered compared with those in non-irradiated samples, achieving 90 and 99% reduction at 2 and 4 kGy doses, respectively. *Aspergillus* counts were significantly reduced, by 93 and 97%, immediately after irradiation at doses of 2 and 4 kGy, respectively. A radiation dose of 6 kGy completely eliminated the population of total molds and *Aspergillus* fungi. Aziz and Moussa (2002) reported that the fungal floras in the different fruit samples are sensitive to gamma-radiation, and were completely inhibited at 5.0 kGy radiation dose. The toxicity of Peanut meal contaminated with $AFB_1$ was reduced by 75% and 100% after irradiation with gamma rays at a dose of 1 and 10 kGy, respectively (Temcharoen & Thilly 1982). El-Bazza-Zernab *et al*. (2001) exposed the *A. flavus* isolate to gamma radiation dose level from 0.0 to 3.0 kGy. The gradual decrease in the growth occurred by increasing the irradiation dose upto 2.5 kGy. Low doses of gamma radiation did not affect its production up to 0.5 kGy and the mycelial weight markedly increased the total production reaching 3000 µg/L. Thereafter a decrease in its production was observed by increasing the dose. Aziz & Youssef (2002) showed that application of radiation at 10 kGy significantly detoxify aflatoxin $B_1$ by 82-88%. Aziz *et al*., (2004) showed that at 4 kGy in maize gamma rays significantly destroyed 60.9%-66.7% of aflatoxin. Kumari et al. (2009), reported the complete inhibition of Aflatoxin B1 and ochratoxin at 5 kGy in triphala.

A flatoxin contamination is unavoidable, numerous strategies for their detoxification have been proposed. These include physical methods of separation, thermal inactivation, irradiation, solvent extraction, adsorption from solution, microbial inactivation, and fermentation. Chemical methods of detoxification are also practiced as a major strategy for effective detoxification. Control of aflatoxins is the need of the hour, since their occurrence in foods and feeds is continuously posing threats to both health and economics all over the world. Besides the postharvest preventive measures, it is imperative that suitable detoxification methods are developed for inactivating or removing aflatoxins from the contaminated commodities, as the toxins are also produced by *A. flavus* and *A. parasiticus* even during pre-harvest stages of crop production. At present there is no effective method for detoxification of aflatoxin. Therefore gamma radiations are more attractive and effective for aflatoxin decontamination in peanut and other food commodities. We recommend 3.0 – 5.0 kGy doses for aflatoxin reduction, at this concentration no significant changes occur in physiochemical properties of peanut; beyond these doses i.e. at 10.0 and 15.0 kGy significant changes has been observed in Peanut sample.

**Table 3—Effect of gamma radiation on total aflatoxin in peanut samples.**

| | Irradiation with Gamma | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **0.1kGy** | **0.2kGy** | **0.5kGy** | **1.0kGy** | **3.0kGy** | **5.0kGy** | **10.0kGy** | **15.0kGy** |
| TAF* | 77.3 | 68.3 | 35 | 19.3 | 14.3 | 6.6 | 0.5 | ND | ND |

TAF- Total Aflatoxin, ND- Not Detected, * Value in ppb (part per billion)

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