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Effect of Storage Period and Irradiation Doses on Red Chillies

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Abstract: The samples of red chillies were packed in High Density Polyethylene (HDPE) bags and irradiated using 2, 4 and 6 kGy gamma radiation. The irradiated samples were stored at room temperature and relative humidity along with control (0 kGy) for 90 days. The samples were evaluated for proximate composition, total phenolics and aflatoxin. Irradiation and storage showed non-significant effect on proximate composition and total phenolics whereas irradiation showed significant effect on aflatoxins as compared to control. There was gradual decrease in aflatoxin contents with the increase level of gamma rays. It was concluded that red chillies for better quality retention can be safely stored in polyethylene bags. The use of radiation can be helpful for the preservation of chillies with respect to the production of aflatoxin during storage.

Key words: Red chillies, irradiation, storage studies, aflatoxin, polyethylene bag

INTRODUCTION

According to American Spice Trade Association (ASTA) “a spice is any dried plant product used primarily for seasoning purposes.” This includes sherbs, spice seeds, roots, spice blends and other plant based materials. The spices are often produced in countries possessing tropical and sub-tropical climate conditions, which experiencing extreme ranges of rainfall, temperature and humidity (Martins *et al*., 2001). In recent years, the natural occurrence of aflatoxins in spices has been reported and studied by several researchers (Martins *et al*., 2001; Fazekas *et al*., 2005; Zinedine *et al*., 2006; Romagnoli *et al*., 2007; Aydin *et al*., 2007).

Aflatoxin is a type of mycotoxin, produced by species of *Aspergillus*, especially *Aspergillus flavus* and *Aspergillus parasiticus*. These are found every where in air and soil (Rustom, 1997). These posses a significant threat to human as well as animal health, due to inherited side effects like carcinogenic, mutagenic and teratogenic (Blesa *et al*., 2004). The International Agency of Research on Cancer has classified aflatoxins as Group 1 carcinogens (IARC, 2002).

The contamination of aflatoxin can significantly be reduced at preharvest by adopting good crop and cultural practices. Aflatoxins are generally removed from the food stuff through physical, chemical and biological means. However, due to recently emerged food safety concerns, use of irradiation is considered as an effective, safe, widely applicable food processing method. This is used to reduce food poisoning, food spoilage and extension of shelf life with out any detrimental effects on food quality. Gamma radiation is being used for more than 60 food products in about 50 countries.

Within UK seven categories of foods have been approved for irradiation. The regulations across the world make provision for labeling to ensure that consumers are fully informed whether foods or ingredients have been irradiated.

In developing countries like Pakistan, the problem of aflatoxin persists especially after harvesting during storage due to poor processing conditions. Red chillies are usually sun-dried in open fields and then stored under poor conditions favoring aflatoxin production. Pakistan is sixth largest exporter of chillies in the world. However, the export has declined after detection of aflatoxin by the European Union Food Authorities since 2006. Russell (2007) found samples of red chilli pods and red chilli powder from different areas of Pakistan contaminated with aflatoxins and noted few samples contained higher level of aflatoxins than limit prescribed by the EEC (European Commission) regulations.

The export of any food products which contain aflatoxins higher than that of levels prescribed by World Trade Organization is impossible to import or export in the WTO regime. The present project was designed to assess the affects of gamma radiation on aflatoxin contaminated red chillies.

MATERIALS AND METHODS

Procurement and storage of red chillies: The samples of red chillies were procured from the local market of Faisalabad. The samples were homogenized by grinding red chillies into fine powder and mixing thoroughly. Then samples were analyzed for aflatoxin. After aflatoxin determination powder red chillies were packed in HDPE bags.

Irradiation of red chillies: The red chillies samples (1 Kg each) contaminated with Aflatoxin were packed separately in HDPE bags and were irradiated by applying 2, 4 and 6 kGy dose levels, in a cobalt-60 gamma irradiator (Issle Dovatel GIK-7-2, Russia), at a dose rate 0.4461 kGy/h at Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan. The irradiated samples were stored at room temperatures (24-28°C) and relative humidity (45-60%) in paper box along with control (0 kGy) for 90 days.

Proximate analysis of red chillies: The samples of non-radiated and irradiated red chillies were analyzed for the moisture content, crude protein, crude fat, crude fiber, ash and Nitrogen Free Extract (NFE) according to their respective methods described in AACC (2000).

Total phenolics: The total phenolics in red chillies were determined according to the method described by Singleton *et al.* (1999) by using folin ciocalteu reagent. The gallic acid was used as standard. The sample infusion (50 µL) was taken in a glass stoppered test tube and added 0.5 mL of folin ciocalteu reagent. The 10 mL volume was adjusted and incubated at ambient temperature for 15min. In each sample 1.5 mL sodium carbonate solution (20%) was added and incubated in water bath at 37°C for 20 min. The absorbance was measured in spectrophotometer (Cecil CE-7200, Sweden) at 755 nm.

Aflatoxin in red chillies

Sample extraction and clean-up: For analysis of aflatoxin in red chillies, sample extraction and clean up was done as described by Richard (2000). A 25 g finely ground homogeneous sample was taken into 250 mL conical flask and 100 mL solvent (acetonitrile: water, 84:16) was added. The flasks were placed in a horizontal shaker for 1hour. Romer MycoSep® column 228 (Romer Laboratory Inc., USA) was used for clean up and residue was evaporated by nitrogen gas. After drying, samples were re-dissolved in 300 µL mobile phase (acetonitrile: methanol: water; 1:1:2) for High Performance Liquid Chromatography (Alberts *et al.*, 2006). The concentration of aflatoxins in each sample was expressed as µg kg^{-1}.

High Pressure Liquid Chromatographic (HPLC) analysis: The HPLC system (Perkin Elmer 200 Series, USA) was used for analysis of aflatoxin in red chillies. The specifications of the HPLC were: the Series 200 UV-Vis detector with wavelength fixed at 365 nm; Total Chrom, Chromatography Data System (CDS) software. The analysis was carried out by following the conditions: column temperature 30°C, flow rate of mobile phase; 1 $mLmin^{-1}$. All analyses were performed isocratically using degassed HPLC grade acetonitrile: methanol: water (1:1:2, v/v/v) (Merck, Germany) as a mobile phase. The reverse-phase chromatographic column (Discovery C_{18} (250 x 4.6 mm, 5 µm), Supelco, Bellefonte, PA, USA) was used for the detection of aflatoxins (B_1, B_2, G_1 and G_2). During analysis of samples through HPLC, a standard solution (Biopure, Mix 1 supplied by Romer lab, USA) was injected after every 10 samples in order to assess the retention time verification and instrument calibration.

Quantification of aflatoxins: A 20 µL aliquot was used for each HPLC injection. Aflatoxins were identified with reference to retention time of standards and by spiking the samples with standards. The standard curves were developed using concentration versus peak area for quantification of aflatoxins.

Statistical analysis: The data obtained for each parameter was subjected to statistical analysis using Co-STAT-2003 software to determine the level of significance. The significant differences between means were further compared using Duncan Multiple Range test (DMRt) according to the methods described by Steel *et al.* (1997).

RESULTS AND DISCUSSION

Gamma irradiation is a phytosanitary treatment applied to food and herbal materials that improves the hygienic quality and reduces the losses due to microbial contamination and insect damage (IAEA, 1992; Farkas, 1998).

Effect of irradiation doses and storage on proximate composition of red chillies: Mean square for proximate composition of red chillies revealed non-significant differences in moisture, ash, crude protein, crude fat, crude fiber and Nitrogen Free Extract (NFE) during 90 days storage in HDPE. It is evident from the results that chemical composition of red chillies remained intact up to 6 kGy irradiation doses. The interaction of these parameters also showed non-significant effect (Table 1). The moisture, ash, crude protein, crude fat, crude fiber and NFE contents varied non-significantly from 11.33-11.41, 5.62-5.68, 9.13-9.20, 15.02-15.12, 26.07-26.18 and 32.49-32.75%, respectively due to irradiation of red chillies up to 6 kGy (Table 2). The present findings are in close agreement with the results of El-Niely (2007) who found no change in proximate composition of peas, cowpeas, lentils, kidney beans and chickpeas treated with different radiation doses (5-10 kGy). During storage there was a non-significant increase in moisture content (11.26-11.49%) and NFE (31.87-33.68%) and decrease in protein (9.34-8.94%), fat (15.34-14.76%), ash (5.75-5.55%) and fiber contents (26.44-25.59%) with the increase of storage intervals (Table 3).

The results showed a slight change in protein content of red chillies due to the application of radiations during storage. This increase was might be due to the presence of some enzymes that may cause autolysis during the storage of foods that are seldom inactivated at high radiation doses (6 kGy) (Eggum, 1979). Irradiation does not alter the elemental composition of

Table 1: Mean squares of proximate composition of red chillies at different irradiation doses and storage periods

SOV	df	Moisture	Ash	Protein	Fat	Fiber	NFE
Irradiation (I)	3	0.015^{NS}	0.008^{NS}	0.012^{NS}	0.022^{NS}	0.026^{NS}	0.153^{NS}
Storage (S)	3	0.122^{NS}	0.095^{NS}	0.349^{NS}	0.781^{NS}	1.766^{NS}	7.171^{NS}
I x S	9	0.003^{NS}	0.001^{NS}	0.001^{NS}	0.003^{NS}	0.004^{NS}	0.019^{NS}
Error	32	0.294	0.063	0.170	0.428	1.669	2.304
Total	47						

** Highly significant at $p\leq0.01$; NS = Non-Significant

Table 2: Effect of irradiation doses on proximate composition of red chillies

Irradiation dosage (kGy)	Proximate composition (%) Moisture	Ash	Protein	Fat	Fiber	NFE
0	11.41	5.62	9.13	15.02	26.07	32.75
2	11.37	5.64	9.15	15.05	26.11	32.69
4	11.36	5.66	9.17	15.08	26.15	32.60
6	11.33	5.68	9.20	15.12	26.18	32.49

Table 3: Effect of storage intervals on proximate composition of red chillies

Storage (Days)	Proximate composition (%) Moisture	Ash	Protein	Fat	Fiber	NFE
0	11.26	5.75	9.34	15.34	26.44	31.87
30	11.32	5.69	9.24	15.19	26.35	32.22
60	11.39	5.61	9.13	14.98	26.13	32.76
90	11.49	5.55	8.94	14.76	25.59	33.68

foods (Stewart, 2001). A little and non-significant variation has been observed in fat content of red chillies during storage due to the application of different irradiation doses might be due to auto-oxidation and dint of action of high energy radiation on lipid molecules (Nawar, 1986). Slightly and non significant change in NFE content evident in the present study because the oligosaccharides were broken down to a considerable extent when the samples were irradiated (0-6 kGy) (Siddhuraju *et al.*, 2002)

Effect of irradiation doses and storage on total phenolics in red chillies: Mean squares revealed non-significant variations in total phenolics of red chillies due to irradiation doses and storage intervals. The interaction between irradiation doses and storage intervals (IxS) also showed non-significant effect on total phenolics (Table 4).

Mean for effect of irradiation doses on red chillies are given in Table 5. Total phenolics in all samples were ranged from 43.15-43.36 (mg/100g). Mean for total phenolics remained 42.31-44.00 (mg/100g) during 90 days storage (Table 6). In the present study, non-significant increase in the phenolic contents of irradiated red chillies might be due to the release of phenolic compounds from glycosidic compounds and the degradation of larger phenolic compounds into smaller ones by gamma radiation (Harrison and Were, 2007).

Similarly increase in phenolic acids in irradiated cloves and nutmeg (Variyar *et al.*, 1998) and almond skin extract has been reported (Harrison and Were, 2007). Contrary, a decrease in the amount of total phenolics has been observed in dehydrated rosemary treated with 10-30 kGy (Koseki *et al.*, 2002).

The slight increase in total phenolics in red chillies was might be due to the low irradiation doses as well as the difference in the nature of samples tested by the different researchers.

Table 4: Mean squares of total phenolics on red chillies at different irradiation doses and storage periods

SOV	df	Total Phenolics
Irradiation (I)	3	0.097^{NS}
Storage (S)	3	6.085^{NS}
I x S	9	0.031^{NS}
Error	32	4.598
Total	47	

**Highly significant at $p\leq0.01$; NS = Non-Significant

Table 5: Effect of irradiation doses on total phenolics on red chillies

Irradiation dosage (kGy)	Total Phenolics (mg/100g)
0	43.15
2	43.25
4	43.31
6	43.36

Table 6: Effect of storage intervals on total phenolics on red chillies

Storage (Days)	Total Phenolics (mg/100g)
0	44.00
30	43.53
60	43.22
90	42.31

Effect of irradiation doses and storage on aflatoxin in red chillies: Mean square of irradiation doses showed significant effect on aflatoxin while storage as well as interaction of irradiation doses and storage showed non-significant effect on aflatoxin (Table 7). The results in the present study showed that total aflatoxin decreased (11%) due to increase in irradiation doses (0-6 kGy) while increased during 90 days storage (Table 8).

Table 7: Mean squares for effect of radiation and storage on aflatoxin decontamination in red chillies

SOV	df	Total aflatoxin
Irradiation (I)	3	0.235**
Storage (S)	3	0.002^{NS}
I x S	9	0.001^{NS}
Error	32	0.001
Total	47	

Table 8: Effect of radiation and storage on aflatoxin decontamination in red chillies (µg/kg)

	Irradiation dosage (kGy)				
Days	0	2	4	6	Mean
0	3.35	3.26	3.14	3.00	3.18
30	3.38	3.28	3.15	3.01	3.20
60	3.40	3.32	3.17	3.00	3.22
90	3.39	3.32	3.17	3.02	3.23
Mean	3.38^{a}	3.29^{b}	3.15^{c}	3.00^{d}	

The findings of previous scientists and research workers are still cotroversary. Aflatoxin stability was investigated in corn and corn-based products by Christensen *et al.* (1977) who found that conventional processing for instance cooking, roasting, frying and baking were not able to eliminate aflatoxins completely since they are relatively heat stable.

Failure of irradiation to destroy aflatoxins in foods, feeds and fungal cultures has also been confirmed by Frank and Grunewald (1970) and Frank *et al.* (1971). Kume *et al.* (1987) found that the dose required for pure aflatoxin destruction is greater than 50 kGy.

While Fresh maize, chick peas and groundnut seeds irradiated up to 6 kGy resulted in detoxification of aflatoxin B_1 (Aziz *et al.*, 2006). In the present study, irradiation doses up to 6 kGy were applied keeping in view the minimum harm to composition and nutrition attributes. Hence, aflatoxins in red chillies were not completely destroyed. However, higher dose level (maximum dose level 10 kGy) allowed by WHO (1994) should also be tested.

Conclusion: The use of radiation (6 kGy) can be helpful for minimizing aflatoxin in red chillies. There were no detrimental changes in chemical composition of red chillies radiated up to 6 kGy and stored in High Density Polyethylene (HDPE) bags up to 90 days. The total phenolics contents also remained non-significant during storage after irradiation. The chances of recontamination also reduced, as gamma radiation was applied after packing red chillies in HDPE bags. Red chillies can be safely stored in HDPE bags for their better quality retention.

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