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SHORT COMMUNICATION

Effect of gamma irradiation on microbial load, aflatoxins and phytochemicals present in *Trigonella foenum-graecum*

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Abstract The effects of gamma irradiation on microbial load, total aflatoxins and phytoconstituents content of *Trigonella foenum-graecum* have been studied. Gamma irradiation at a dose of 2.5 kGy resulted in 2 log reduction of the total aerobic microbial count. A complete sterilization was, however, observed at 10 kGy. The total aflatoxin level decreased gradually with increase in gamma irradiation dose as compared to its un-irradiated counterparts, whereas the high performance liquid chromatography (HPLC) profile showed no change in the levels of phytochemicals up to the gamma irradiation dose of 10 kGy. HPLC profiles, however, differed in peak areas, and retention times of the components. These results suggest that gamma irradiation at a dose of 5.0 kGy was very effective for microbial decontamination because it did not adversely affect the active components of *T. foenum-graecum.*

Keywords Gamma irradiation · Microbial load · Aflatoxins · *Trigonella foenum-graecum*

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Introduction

Microbiological contamination of spices, particularly by pathogenic non-spore forming bacteria, is one of the most significant public health problems and an important cause of human suffering all over the world. These microorganisms are present on the surface of the plants and originate from the plant environment, namely soil, water and air. Some pathogenic microorganisms grow inside the plant's tissue resulting in rapid spoilage of the medicinal properties of the spices and cause food-borne illness (Chmielewski and Migdal 2005). The need for decontamination of spices was realized principally from the fact that these materials are added directly to food. According to the World Health organization (WHO), in 1992 infectious and parasitic diseases represented the most frequent cause of death (35%) worldwide, the majority of which occurred in developing countries (Loaharanu 1994). Insect infestation is also a major problem of stored spices in the domestic market. The inhibition of microbial growth and disinfestations are important for the acceptance of spices. Good quality of the spices may be achieved by decontamination, which should be fast and effective against all microorganisms (Migdal et al. 1998). The conventional method of decontamination was based on fumigation with ethylene oxide gas, which is now prohibited in most countries due to health safety reasons (Uijl 1992). Irradiation is a well known decontamination process used to reduce spoilage and eradicate pathogenic microorganisms (Sokhey and Hanna 1991). Ionizing irradiation has become one of the most promising methods for food sanitization, because it causes very effective disruption of DNA molecules in the nuclei of cells (Diehl 1995).

Sterilization of spices by ionizing radiation has increasingly gained commercial interest, since, spices are

spoiled by microorganisms during handling, transportation and storage. In the present study we have determined the effects of different doses of gamma irradiation on the microbial population and the aflatoxins and phytochemicals present in *Trigonella foenum-graecum* (fenugreek).

Materials and methods

Sample

Fresh unwashed *T. foenum-graecum* seeds were purchased from the local market, and packed in polythene bags. Gamma irradiation was conducted at Shriram Applied Radiation Centre (SARC), Shriram Institute for Industrial Research, New Delhi, India. The source for gamma rays was cobalt-60. Samples were irradiated in air-packed cardboard boxes without opening the boxes and the dosimeters were placed at different positions in the boxes to ascertain uniform doses of gamma radiation. Different doses of gamma irradiation (2.5, 3.0, 4.0, 5.0 and 10.0 kGy) were applied. Un-irradiated samples were also prepared and used as a control.

Microbiological analysis

Total aerobic microbial count and sterility testing were done following the method described by the Indian Pharmacopoeia (Anonymous 2007).

Total aflatoxins

Total aflatoxins were determined by using Ridascreen® enzyme immunoassay and its level was quantified spectrophotometrically at λ 450 nm.

Extraction of phytochemicals

The un-irradiated and irradiated *T. foenum-graecum* seeds were ground to powder. The powdered plant material (10 g) was extracted with 150 ml of ethanol (HPLC grade) for 6 h by using Soxhlet equipment (Khan et al. 1988). The extract was filtered using Whatman filter paper no. 1 and stored in labeled volumetric flasks in a refrigerator until further use.

High performance liquid chromatography analysis for phytochemicals

High performance liquid chromatography (HPLC) of ethanol extract (20 µl) was performed on Shimadzu diode array detector under the following conditions: column C-18, 4.6 × 250 mm, 10 µm; mobile phase ethanol: water (80:20); flow rate of 1.0 ml min^{-1}; column temperature 28°C. Detection was done at 450 nm.

Results and discussion

Total aerobic microbial contamination in the un-irradiated sample of *T. foenum-graecum* was found to be about 10^7 cfu g^{-1}. Gamma irradiation at a dose of 2.5 kGy resulted in 2 log reduction in the total viable count (10^5 cfu g^{-1}). No microbial load was, however, recorded in the samples irradiated with 5.0 kGy. Furthermore, the samples treated with 5.0 kGy gamma radiation showed bacterial growth on day 11 after incubation, however, no fungal growth was recorded in sterility tests. Gamma radiation at 10 kGy resulted in complete sterilization of powder and the sterility was maintained for 14 days. Our results are in agreement with those who reported that a minimum dose of 10 kGy was necessary to obtain a product of good microbiological quality (Kim et al. 2000; Van et al. 1988).

The data in Fig. 1 show the total aflatoxin content of *T. foenum-graecum* in response to different doses of gamma radiation. Un-irradiated sample showed maximum (64.3 ppb) level of total aflatoxins. Total aflatoxins levels decreased gradually with increase in gamma radiation dose. These results are in agreement with the findings of Aziz and Moussa (2002) who reported that mycotoxin production decreased with increasing irradiation dose.

The data of Fig. 2 show the HPLC profile for *T. foenum-graecum* treated with different doses of gamma irradiation. No change in the phytochemicals was recorded up to 10.0 kGy gamma irradiation. However, HPLC profiles differed as far as the peak areas and retention times of the

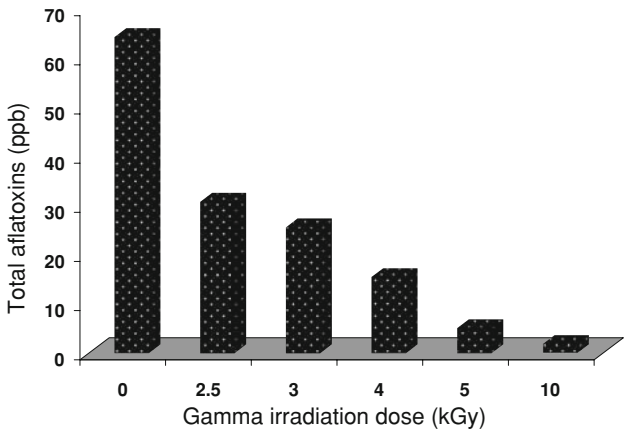

Fig. 1 Total aflatoxins content of *Trigonella foenum-graecum* in response to different doses of gamma irradiation. The data in each column are means of two independent experiments with four replicates each. The maximum variation from mean value was <5%

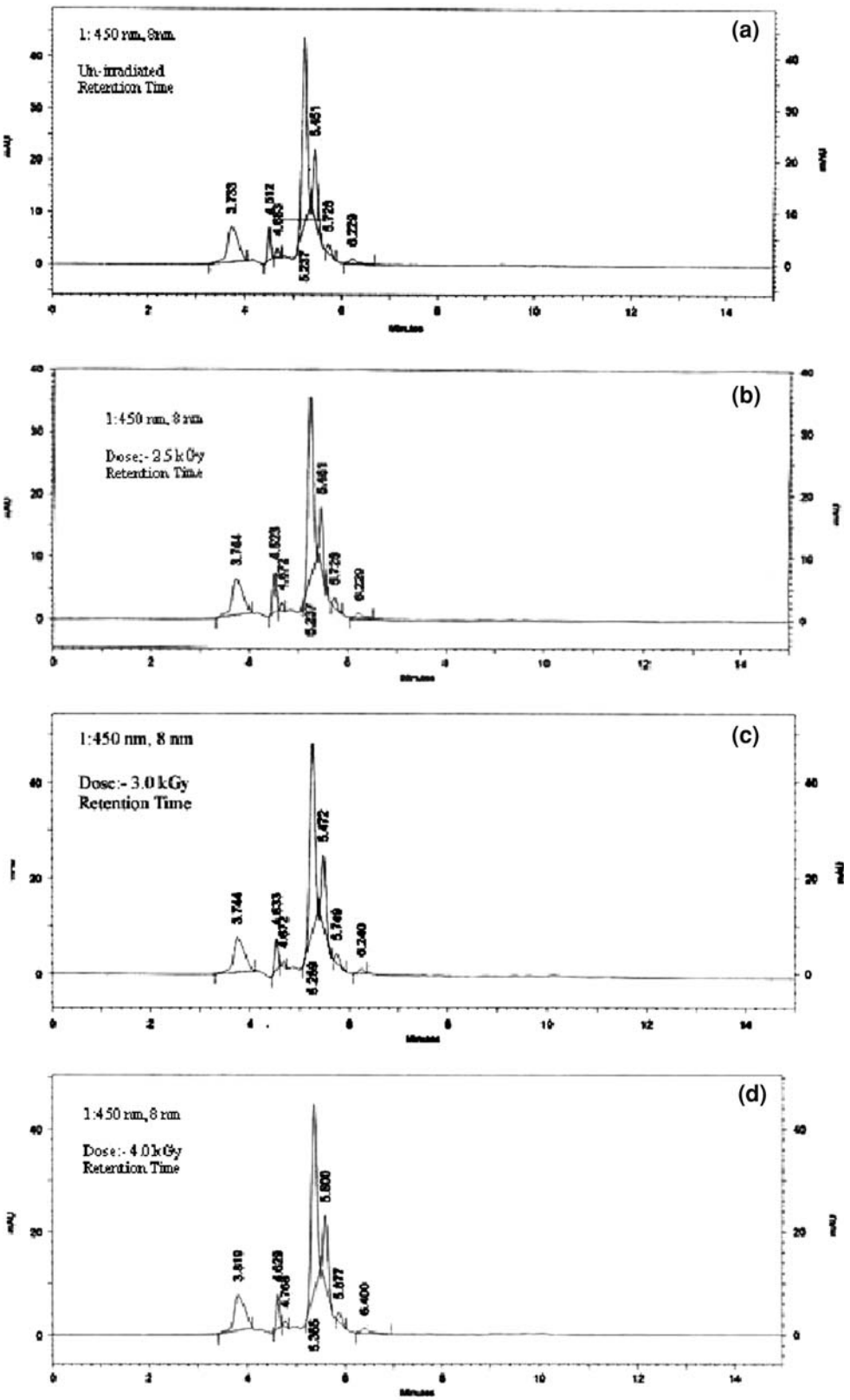

Fig. 2 HPLC profile for *Trigonella foenum- graecum* treated with different doses of gamma irradiation. Un-irradiated (**a**), irradiated with 2.5 (**b**), 3.0 (**c**), 4.0 (**d**), 5.0, (**e**) and 10.0 kGy (**f**)

Fig. 2 continued

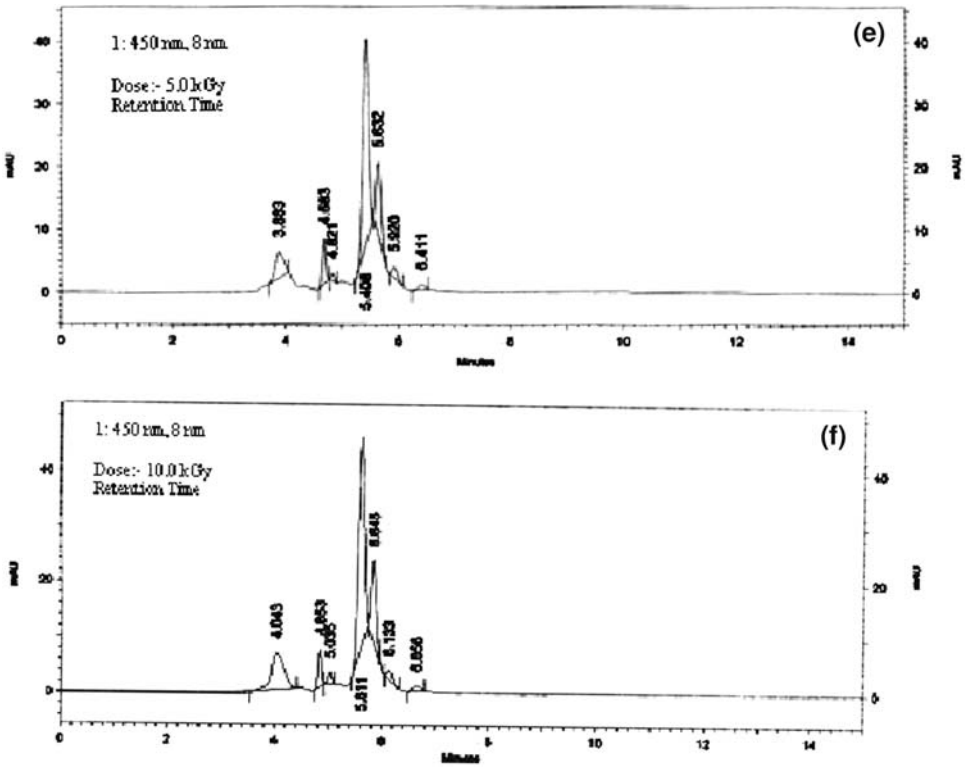

Table 1 HPLC analysis of ethanol extracts of *Trigonella foenum- graecum* treated with various doses of gamma radiation

Un-irradiated		2.5 kGy		3.0 kGy		4.0 kGy		5.0 kGy		10.0 kGy	
Peaks	Area	Peaks	Area	Peaks	Area	Peaks	Area	Peaks	Area	Peaks	Area
Retention time (min)											
3.733	19.65	3.744	19.9	3.744	20.8	3.819	20.6	3.883	21.4	4.043	22.6
4.512	5.1	4.523	5.3	4.533	5.4	4.629	5.5	4.683	5.8	4.853	6.1
4.683	0.9	4.672	0.9	4.672	1.1	4.768	1.2	4.821	1.5	5.035	1.7
5.237	50.4	5.237	50.4	5.259	50.5	5.365	50.8	5.408	51.2	5.611	57.8
5.451	17.2	5.461	17.5	5.472	17.5	5.600	18.0	5.632	18.0	5.845	18.5
5.728	2.1	5.728	2.1	5.749	2.4	5.877	2.4	5.920	2.5	6.133	3.0
6.229	1.4	6.229	1.4	6.240	1.5	6.400	2.0	6.411	2.3	6.656	2.4

The data in each column are means of two independent experiments with four replicates each. The maximum variation from mean value was <5%

components of the ethanol extracts were concerned (Fig. 2). These results are also supported by Koseki et al. (2002).

The data of HPLC analysis of ethanol extracts of *T. foenum-graecum* treated with various doses of gamma radiation are shown in Table 1. Gamma irradiation at 5.0 kGy resulted in a much lower level of microbial contamination and was found to be the only treatment effective enough to meet the standards set by processors operating under Hazard Analysis Critical Control Points (HACCP) or International Standards Organization (ISO). These results are also supported by the earlier workers (Tjaberg et al. 1972; Loaharanu 1994; Thayer et al. 1996; Olson 1998) who stated that the treatment with ionizing energy is more effective against bacteria than thermal treatment, and does not leave chemical residue in food products. Thus, it can be concluded that treatment with gamma radiation is more effective against bacteria without alteration in phytoconstituents present in *T. foenum-graecum*.

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