Spice Extracts as Dose-Modifying Factors in Radiation Inactivation of Bacteria

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Three spices, chili, black pepper, and turmeric, were tested for the effect of their aqueous extracts on the sensitivity of three bacteria, *Escherichia coli*, *Bacillus megaterium*, and *Bacillus pumilus* spores, to γ-radiation. It was found that the extracts of the three spices offered protection to these organisms against inactivation by γ-radiation. These spice extracts were also tested for their protection of naked plasmid DNA. Radiation-induced degradation of plasmid pUC18 DNA was reduced in the presence of the spice extracts. The maximum protection was offered by the chili extract followed by that of black pepper and turmeric. The two known antioxidants, curcumin and piperine from turmeric and black pepper, respectively, were shown to protect the plasmid DNA from the degradation by γ-radiation. Experiments with the plasmid pUC18 DNA indicated that the spice extracts probably protected microorganisms by protecting their DNA. These studies indicated the importance of spices among ingredients in food as dose-modifying factors during radiation processing.

Keywords: *Spice extracts; dose modification; γ-radiation; food processing*

INTRODUCTION

Radiation processing is being increasingly accepted as an important method for the preservation and hygienization of foods (Josephson and Peterson, 1983; Nair and Sharma, 1994; Loaharanu, 1998). One of the applications of this technology is the microbial decontamination of prepared foods for shelf-life extension under refrigeration and preparation of shelf-stable sterile products for storage at ambient temperature. These foods include ready-to-eat intermediate-moisture foods, hospital diets, and rations for astronauts, adventure groups, and the military. It is well-known that food constituents offer protection to microbes during irradiation (Josephson and Peterson, 1983). Most prepared foods contain spices as common ingredients that are added for the enhancement of aroma, flavor, and palatability (Giese, 1994). All major and many minor spices have been studied for hygienization using γ-radiation (Farkas, 1988; Sharma et al., 1984, 1989). However, the effect of spices as food components on the radiosensitivity of food microflora has not been tested. In this paper we report the result of a study carried out to assess the effect of aqueous extracts of three major spices on the sensitivity of two vegetative cells and spores of bacteria and a plasmid DNA to γ-radiation.

MATERIALS AND METHODS

Spices and Chemicals. The powders of spices red chili (*Capsicum annum*), turmeric (*Curcuma longa*), and black pepper (*Piper nigrum*) were obtained from a local market. Curcumin and piperine were obtained from Sigma Chemical Co. All other reagents were of analytical grade and obtained from standard sources.

Microorganisms. Locally isolated strains of *Escherichia coli* and *Bacillus megaterium* were used as test organisms in these studies. A loopful from the stock culture was inoculated in a Luria−Bertani (LB) broth medium and allowed to grow overnight. The overnight-grown culture was pelleted by centrifugation at 5000*g* for 10 min, and the pellet was resuspended in sterile saline (0.85%). The procedure was repeated twice to wash the bacterial pellet of the medium constituents. The suspension of bacterial cells was diluted in sterile saline to obtain cell densities of $\sim 10^7$ colony-forming units (cfu)/mL.

For studying the radiosensitivity of spores a standard spore-forming bacterium, *Bacillus pumilus* (ATCC-14884), was used. To prepare spore suspension, the organism was grown on tryptic−soy agar at 37 °C for 10 days to get 100% sporulation. A loopful from the culture plate was suspended in sterile saline containing Tween 80 (0.01%). The cells were pelleted at 10000*g* for 10 min and resuspended in Tween 80 saline. The procedure was repeated twice. Finally, the cells were suspended in 0.85% saline and heated in a boiling water bath for 10 min to get a pure spore suspension. Spore densities of 10^3 cfu/mL were used for the test.

Preparation of Aqueous Spice Extracts. Powdered spices (10 g), including turmeric, chili, and black pepper, were homogenized with sterile saline (30 mL) using an Omnimixer. The slurry was centrifuged at 5000*g* for 15 min. The supernatant was passed through a filter paper (Whatman No. 541) before sterilization by passage through a 0.22 μm bacterial filter. The sterile aqueous extracts were used for testing the potential radioprotective effect on the bacterial cells and spores.

Testing of Radiosensitivity of Bacteria. To test the radiosensitivity, an aliquot (1 mL) of the suspension of the vegetative cells of the test organism (10^7 cfu/mL) was added to 9 mL of the aqueous extract of the spice, giving a final concentration of 10^6 cfu/mL of the test bacterium in the aqueous spice extract. The final suspension was distributed (1 mL) in microfuge tubes. The suspension of the test organism without the spice extract served as control. Similarly, an aliquot (1 mL) of the spore suspension of *B. pumilus* (10^4 cfu/mL) was added to 9 mL of the aqueous extract of the spice, giving a final concentration of 10^3 cfu/mL of the spores in the aqueous spice extract.

γ-Irradiation. γ-Irradiation was carried out in a research irradiator (Gamma Cell 220, AECL, dose rate = 21 Gy/min). The cell suspensions were irradiated at doses of 0, 0.05, 0.1,

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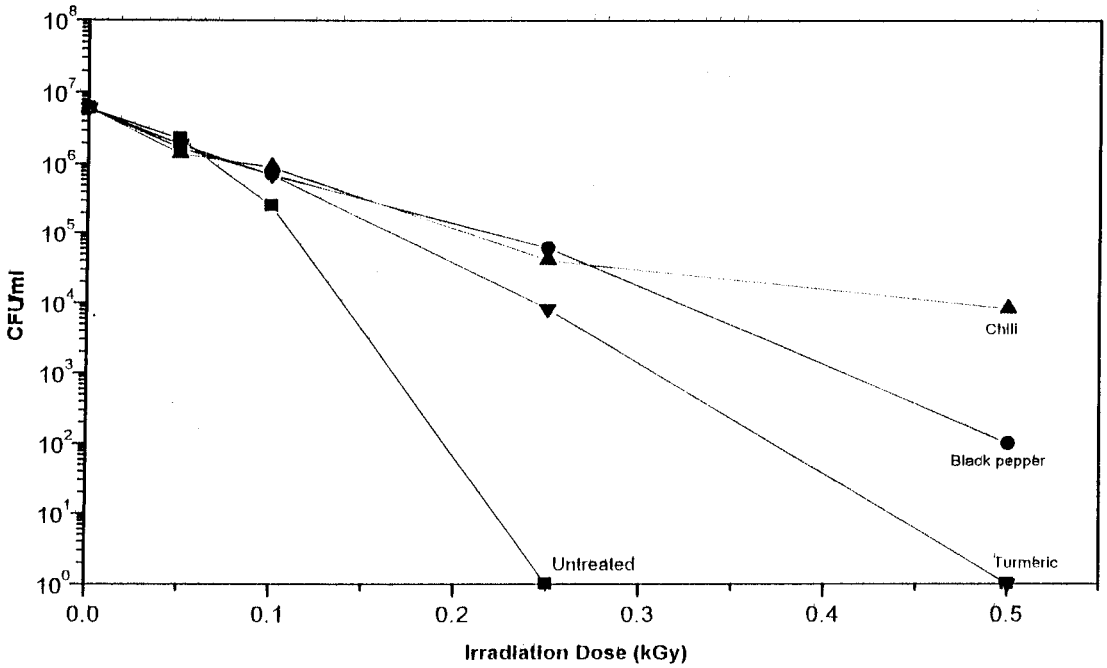

Figure 1. γ-Radiation survival curve of *B. megaterium* in 0.85% saline with and without the extract of black pepper, chili, and turmeric.

0.25, and 0.5 kGy. For *B. pumilus* spores, the doses given were 0, 0.5, 1.0, 1.5, and 2.5 kGy.

Standard Plate Count. The viable bacterial counts were determined by serially diluting the irradiated and nonirradiated suspensions of the test organism and spread plating on LB agar plates. The plates were incubated at 37 °C for 24 h before the colony-forming units were counted. For *B. pumilus* spores tryptic−soy agar plates were used.

Preparation of Plasmid DNA. Large-scale preparation of pUC18 plasmid DNA was carried out using an *E. coli* DH 5∝ clone as per the standard procedures using ethidium bromide−cesium chloride ultracentrifugation (Sambrook et al., 1989).

Testing of Radiosensitivity of DNA. *(i) Monitoring Hyperchromacity (Absorbency, A_{260}).* The spice extracts were prepared by suspending 4 g of the spice powder in 20 mL of sterile distilled water and centrifuging at 10000*g* for 10 min. The supernatant was used as the aqueous spice extract in the experiments. The plasmid pUC18 DNA (11 μg/mL) was treated with aqueous extracts of black pepper (5 μL/mL), chili (8 μL/mL), and turmeric (20 μL/mL) and irradiated in the dose range of 0−1 kGy. The DNA solution without the spice extracts served as the control. The absorbency at 260 nm (A_{260}) of the irradiated and nonirradiated (control) solutions was recorded using a UV−visible spectrophotometer.

(ii) Agarose Gel Electrophoresis. For treatment with the spice extracts 20 μL of plasmid DNA (0.5 mg/mL) was treated with 5 and 20 μL of black pepper and turmeric extract, respectively, and irradiated at doses of 1, 2, and 5 kGy. In the case of nonirradiated samples similar concentrations of the spice extracts were added and allowed to incubate for 30 min before loading on the gel.

In another experiment, because curcumin and piperine are poorly soluble in water, a saturated solution of the two compounds was prepared in Tris−EDTA buffer (pH 8.0) and centrifuged (5000*g* for 5 min). The DNA was then suspended in aliquots (1 mL) of the supernatant and exposed to a 2 kGy dose of γ-radiation. Agarose gel electrophoresis was carried out in 1% agarose gel at 4 V/cm using Tris acetate−EDTA (TAE) buffer (pH 8.0) (Sambrook et al., 1989).

RESULTS AND DISCUSSION

Figure 1 shows the survival of *B. megaterium* cells at the various doses of γ-radiation (0−0.5 kGy) without and with the aqueous extracts of black pepper, chili, and turmeric. The cells in saline without the spice extract (control) decreased with an increase in the dose of γ-radiation. Thus, at 0.25 kGy in saline without the spice extract (control) no colony forming units were detected, indicating complete elimination of microflora (Figure 1). On the contrary, in the presence of chili and black pepper extracts, only a 2 log cycle reduction in microflora was observed. In the presence of turmeric extracts, a 3 log cycle reduction in cell number was observed. At a higher dose of 0.5 kGy no colony forming units were detected in the presence of turmeric extract, indicating lack of survivors (Figure 1). However, in the presence of black pepper and chili extracts, the population was reduced only by 4 and 2 log cycles, respectively, indicating a protective effect of these spices. The D_{10} dose in saline was found to be 71 Gy (Table 1). In the presence of spice extracts the D_{10} was increased to 82, 108, and 192 Gy, respectively, with turmeric, black pepper, and chili extracts. Thus, the maximum protection was offered by chili extracts, which increased the D_{10} value to 192 Gy by >2-fold over the controls without the extract.

Table 1. D_{10} Values for *E. coli*, *B. megaterium*, and *B. pumilus* Spores in the Presence of Various Spice Extracts

	D_{10} value (Gy)		
	E. coli	*B. megaterium*	*B. pumilus* (spores)
control (without extract)	85	71	1250
black pepper	125	108	1180
chili	161	192	1730
turmeric	92	82	1314

Figure 2 shows the survival of *E. coli* cells at the various doses of γ-radiation (0−0.5 kGy) without and with the aqueous extracts of black pepper, chili, and turmeric. The cells in saline without the spice extract (control) decreased with an increase in the dose of γ-radiation. Thus, at 0.25 kGy in saline without the

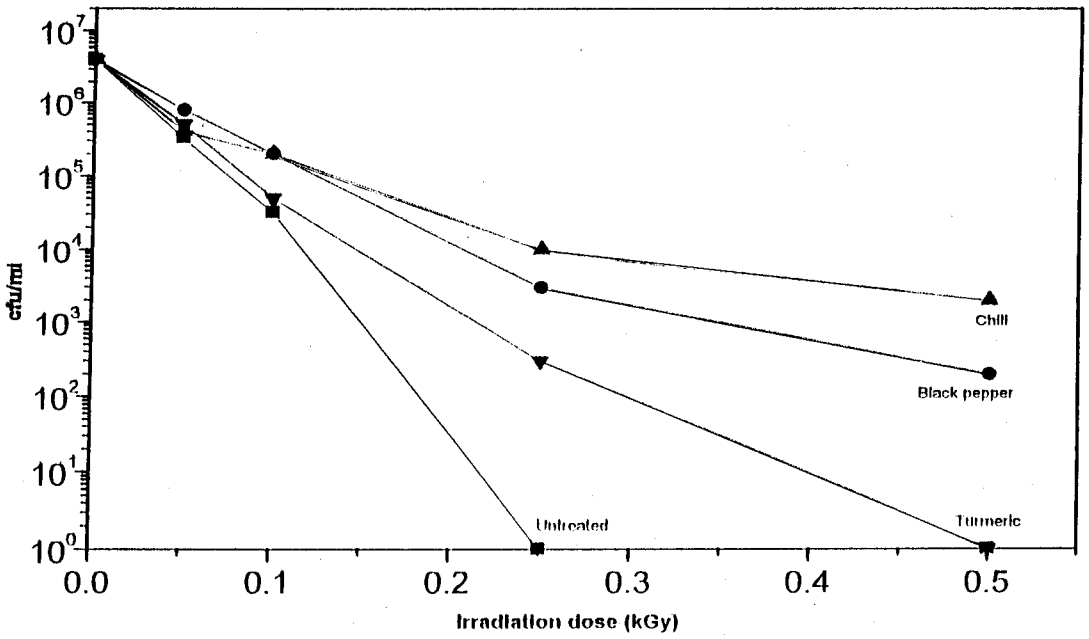

Figure 2. γ-Radiation survival curve of *E. coli* in 0.85% saline with and without the extract of black pepper, chili, and turmeric.

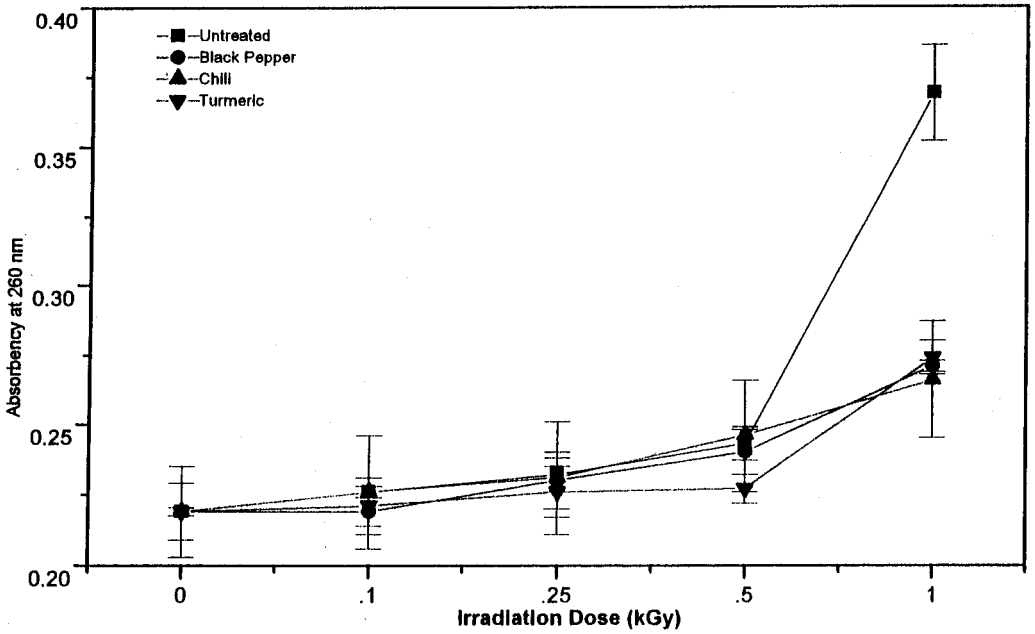

Figure 3. Absorbency at 260 nm of nonirradiated and irradiated (0.1−1 kGy) pUC18 plasmid DNA (11 μg/mL) with and without spice extracts, black pepper (5 μL/mL), chili (8 μL/mL), and turmeric (20 μL/mL).

spice extract (control) no colony forming units were detected, indicating complete elimination of the microflora (Figure 2). On the contrary, in the presence of chili and black pepper extracts ~2 log cycle reductions in microflora were observed. In the presence of turmeric extracts a 3 log cycle reduction in cell number was observed. At a higher dose of 0.5 kGy no colony forming units were observed in the presence of turmeric extract, indicating lack of survivors (Figure 2). However, in the presence of black pepper and chili extracts the population was reduced by ~3 log cycles, indicating a protective effect of these spices. The D_{10} value of *E. coli* in saline was found to be 85 Gy. In the presence of spice extracts it was found to be 92, 125, and 161 for turmeric, black pepper, and chili, respectively. Once again the data showed that the greatest protection to the organism was offered by the chili extracts (Table 1).

Manytimes foods may be contaminated with the spores of bacteria. In fact, spices and dry seasonings may add to the spore load of the food to which they are added (Munasiri et al., 1987). The effect of these spice extracts on the radiosensitivity of spores of *B. pumilus* by γ-radiation was also tested (Table 1). The D_{10} value of *B. pumilus* spores was found to be 1250, 1180, 1310, and 1730 Gy in saline and extracts of black pepper, turmeric, and chili, respectively. This indicated that black pepper had no effect and turmeric only a slight effect on the radiosensitivity of the spores of *B. pumilus*. However, chili extracts showed considerable protection (Table 1).

It is clear that the presence of spice extracts markedly reduced the sensitivity of the organism to γ-radiation. Maximum protection was found to be offered by chili followed by black pepper and turmeric.

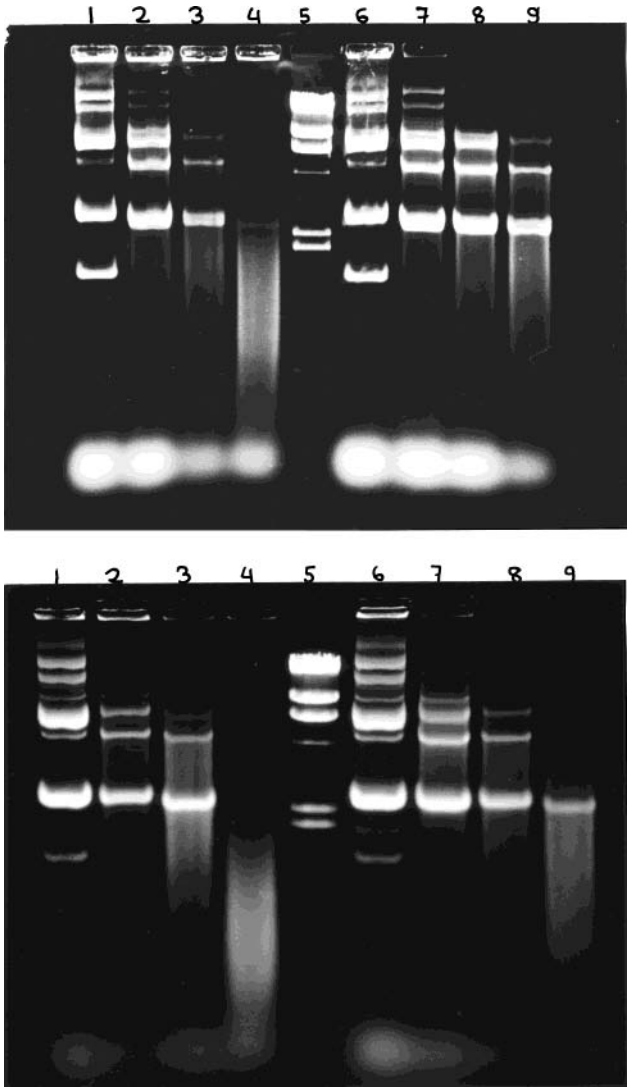

Figure 4. Agarose gel electrophoresis of pUC18 plasmid DNA. (a, top) Effect of aqueous extracts of black pepper on the susceptibility of pUC18 plasmid DNA to γ-irradiation: (lanes 1−4) plasmid DNA without the extract of black pepper at 0, 1, 2, and 5 kGy, respectively; (lane 5) λ *Hin*dIII digest marker; (lanes 6−9) plasmid DNA with the extract of black pepper at 0, 1, 2, and 5 kGy, respectively. (b, bottom) Effect of aqueous extracts of turmeric on the susceptibility of pUC18 plasmid DNA to γ-irradiation: (lanes 1−4) plasmid DNA without the extract of turmeric at 0, 1, 2, and 5 kGy, respectively; (lane 5) λ *Hin*dIII digest marker; (lanes 6−9) plasmid DNA with the extract of turmeric at 0, 1, 2, and 5 kGy, respectively.

Figure 3 shows the effect of spice extracts on the susceptibility of pUC18 DNA to γ-radiation. Increase in absorbency at 260 nm (A_{260}), indicating hyperchromacity, a measure of DNA degradation, was monitored in the presence and absence of spice extracts. It was found that the increase in absorbency (A_{260}) was less in the DNA treated with the spice extracts. The effect was more prominently seen at higher doses. The percent change in the control untreated samples was 69%, almost 3-fold compared to the treated samples at 1 kGy. It was found to be 22, 24, and 25%, respectively, with chili, black pepper, and turmeric. These results also showed that chili offered more protection to DNA than the other spices.

Figure 4a shows the gel profile of plasmid pUC18 DNA irradiated with and without the extracts of black pepper at 1, 2, and 5 kGy doses of γ-radiation. The

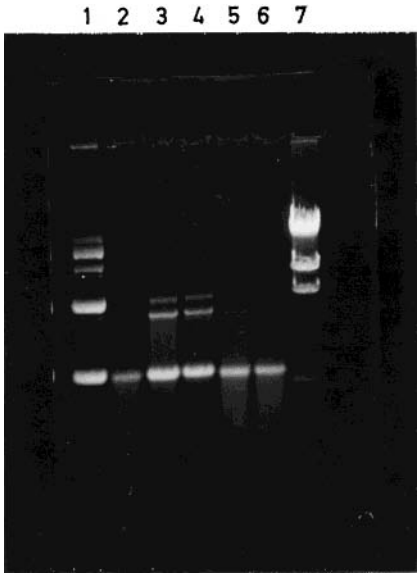

Figure 5. Effect of curcumin and piperine on the susceptibility of pUC18 plasmid DNA to γ-irradiation: plasmid DNA without treatment (lane 1), exposed to 2 kGy dose of γ-radiation (lane 2), before irradiation treated with fully saturated solution of curcumin in TE (lane 3) and with the same diluted again 1:1 in TE (lane 4); treated with fully saturated solution of piperine in TE (lane 5) and with the same diluted again 1:1 in TE (lane 6); λ *Hin*dIII digest marker (lane 7).

pUC18 is a 2.69 kb plasmid with an ampicillin resistance marker on it. Higher doses were selected for this experiment to magnify the monitorable degradative effect on the plasmid. It is clear from the figure that the plasmid DNA was protected during irradiation in the presence of the spice extracts, as indicated by its lesser degradation (lanes 6−9) compared to that in lanes 1−4, which was without the spice extracts. Similar results were obtained with the extracts of turmeric (Figure 4b). As the chili extracts interfered with the ethidium bromide staining of DNA during gel electrophoresis, the data could not be included.

The appearance of more than one band of plasmid in gel electrophoresis (Figure 4) is due to the presence of concatamers or multimeric forms of the plasmid. A single band of linear plasmid is obtained after digestion of the multimeric plasmid with a restriction endonuclease having a single site on the plasmid (Old and Primrose, 1989).

Curcumin, piperine, and capsanthin in turmeric, black pepper, and chili, respectively, have been shown to possess strong antioxidant activities (Srinivas et al., 1992; Noguchi et al., 1994; Tipsrisukond, 1998). It was therefore interesting to ascertain if these antioxidants were involved in the protection of DNA. As these compounds have poor solubility in water, saturated solutions of these antioxidants in Tris−EDTA buffer were used in these studies. Figure 5 shows the effect of curcumin and piperine on the sensitivity of plasmid DNA to γ-irradiation. Lane 1 contains the nonirradiated pUC18 plasmid DNA, and lane 2 contains the DNA exposed to a 2 kGy dose of γ-irradiation. Most of the plasmid DNA is seen to be degraded here. Lanes 3 and 4 are curcumin-treated, whereas lanes 4 and 5 are piperine-treated, pUC18 DNA exposed to 2 kGy of γ-radiation. In both the curcumin- and piperine-treated samples, the destruction of DNA was visibly less than in the control. However, the curcumin showed better protection compared to piperine. Thus, it was clear that

the two known antioxidants offered protection to DNA against DNA damage by γ-irradiation. However, these compounds may not be the sole protecting agents present in spices.

These results show that the spices added to a food can significantly alter the susceptibility of its microflora to γ-radiation. Interestingly, and contrary to expectations, all of the major spices tested including chili, turmeric, and black pepper were found to offer protection to the two bacteria tested. However, maximum protection was found to be offered by the extracts of chili. These results also show that the observed protection of microbes may essentially be due to the protection of their DNA by the constituents of spices. Many of the spices have been reported to have antibacterial and antifungal principles (Sharma et al., 1984; Shelef, 1983). Therefore, it could probably be assumed that in food some spices can act synergistically with γ-radiation in the destruction of microbes. However, this may not be the case with the major spices tested in this study. These spices have been found to show no significant antibacterial activity (Shelef, 1983; Sharma et al., 1984). Moreover, in the presence of antibacterial activity, one would see a synergistic effect, hence, more kill. However, we have observed the opposite effect.

These studies show the importance of spices in food as the dose-modifying factors during radiation processing. From another angle it may be interesting to further explore the radioprotective effects of chili. Radioprotective agents are of interest for their potential use in preventing injury not only during exposure to radiation but also during radiation therapy for protecting normal tissue.

LITERATURE CITED

Farkas, J. *Irradiation of Dry Food Ingredients*; CRC Press: Boca Raton, FL, 1988.

Giese, J. Spices and seasoning blends: A taste for all seasons. *Food Technol*. **1994**, *48*, 87−98.

Josephson, E. S., Peterson, M. S., Eds. *Preservation of Food by Ionising Radiations*; CRC Press: Boca Raton, FL, 1983.

Loaharanu, P. Trends in global acceptance and trade in irradiated food. In *Workshop on Harmonisation of Procedures and Regulations on Food Irradiation for Asia and the Pacific*, Seoul, Republic of Korea, April 27−29, 1998; ICGFI Document 26; Vienna, Austria, 1998.

Munasiri, M. A.; Parte, M. N.; Ghanekar, A. S.; Sharma, A.; Padwal-Desai, S. R.; Nadkarni, G. B. Sterilization of ground pre-packed indian spices by gamma irradiation. *J. Food Sci.* **1987**, *52*, 523−525.

Nair, P. M.; Sharma, A. Food Irradiation. *Encycl. Agric. Sci.* **1994**, *2*, 293−303.

Noguchi, N.; Komuro, E.; Etsuo, N.; Willson, R. L. Action of curcumin as an antioxidant against lipid peroxidation. *J. Jpn. Oil. Chem. Soc.* **1994**, *43*, 1045−1051.

Old, R. W.; Primrose, S. B. *Principles of Gene Manipulation*; Blackwell Scientific Publications: London, U.K., 1989.

Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1989.

Sharma, A.; Ghanekar, A. S.; Padwal-Desai, S. R.; Nadkarni, G. B. Microbial status and antifungal properties of irradiated spices. *J. Agric. Food Chem*. **1984**, *32*, 1061−1064.

Sharma, A.; Padwal-Desai, S. R.; Nair, P. M. Assessment of microbiological quality of some gamma irradiated spices. *J. Food Sci*. **1989**, *54*, 589−590.

Shelef, L. A. Antimicrobial effects of spices. *J. Food Saf*. **1983**, *6*, 29−44.

Srinivas, L.; Shalini, V. K.; Shylaja, M. Turmerin: A water soluble antioxidant peptide from turmeric (*Curcuma longa*). *Arch. Biochem. Biophys*. **1992**, *292*, 617−623.

Tipsrisukond, N.; Fernando, L. N.; Clark, A. D. Antioxidant effects of essential oil and oleoresin of black pepper from supercritical carbon dioxide extractions in ground pork. *J. Agric. Food Chem*. **1998**, *46*, 4329−4333.

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