Improved Bacterial Turbidimetric Method for Detection of Irradiated Spices

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A microbiological method for the detection of irradiated spices is proposed. The method involves harvesting of residual microflora from the spice sample and allowing it to grow in a nutrient medium. After a fixed interval of incubation, turbidity developed is measured as absorbency at 600 nm (A_{600}). The differences between the A_{600} values obtained from irradiated and nonirradiated samples are large enough to allow identification of the former. The growth profiles of the harvested microflora from irradiated spices were found to be different. A dose-dependent increase in lag phase was observed with the harvested residual microflora from irradiated samples. The proposed method is much simpler and less cumbersome compared to the DEFT/APC method recommended currently for detection of irradiated spices.

Keywords: *Irradiated spices; detection methods; microbiological; turbidimetry*

INTRODUCTION

Spices are natural vegetable products that are used for flavoring, seasoning, and imparting aroma to foods. However, postharvest processing and handling of spices in the countries of their origin are often inadequate. This often leads to contamination of spices with pathogenic and spoilage microbes. When added to prepared food, outgrowth of these microbes could lead to serious quality control problems in the food industry (Farkas, 1988; Giese, 1994). Spice traders, particularly exporters, often find it difficult to meet the stringent quality standards of the importing countries. Microbial decontamination of spices with moist or dry heat is primarily not feasible because of the volatile nature of the aromatic constituents of spices. Fumigants such as ethylene oxide and methyl bromide are being phased out due to concerns related to health, worker safety, and the environment (Dickman, 1991; EPA, 1996).

Many countries have now permitted irradiation of spices for disinfestation and hygienization. An overall average dose of 10 kGy is prescribed for microbial decontamination of spices by a number of countries. However, some countries have permitted doses >10 kGy and up to 30 kGy (IAEA, 1996). Under the World Trade Organization (WTO) agreement, the member countries can trade in irradiated spices (Loaharanu, 1998).

As irradiated foods cannot be identified by sight, smell, or taste, the only way the consumer can know if the commodity has been treated by radiation is through a label on the package that clearly announces the treatment. Physical, chemical, and biological methods currently available for the identification of irradiated foods have been reviewed by a number of authors (Bogl, 1990; Delincee, 1993; Raffi and Kent, 1996; McMurray et al., 1996). Many of these methods are cumbersome and require sophisticated instrumentation. Therefore, simple and reliable detection methods could be of immense help to the regulatory agencies to check for compliance with the labeling requirements and facilitation of trade in irradiated commodities (Delincee, 1993).

Among the microbiological methods, the direct epifluorescent filter technique (DEFT) in combination with aerobic plate counts (APC) has been proposed as a screening method for the detection of irradiated spices and frozen poultry (Wirtanen et al., 1993). Besides being time-consuming, the method requires a laboratory equipped with a fluorescent microscope and a trained microbiologist for carrying out the test. A simple and relatively fast bacterial turbidimetric method for the detection of some radurized foods has been described recently (Gautam et al., 1998). In this paper, we describe modification and extension of this method for detection of irradiated spices.

MATERIALS AND METHODS

Irradiation of Spices. Whole as well as ground (powdered) spices, namely, black pepper, chili pepper, coriander, cumin, fennel, and turmeric, were procured from a local market. Each spice was distributed in 4 g quantity in biaxially oriented polypropylene (BOPP) bags and sealed. Irradiation was carried out at a dose of 10 kGy in a ^{60}Co gamma irradiator (Gamma Cell 220, AECL, 22 Gy/min). Black pepper and coriander samples were studied also at lower doses of 1, 4, and 8 kGy. The unexposed samples of the spices served as controls. The control as well as irradiated samples were stored at ambient temperature (26 ± 2 °C). In each set of experiments, samples were analyzed in triplicate.

Recovery of Residual Microflora. The test sample (4 g) was suspended in 20 mL of sterile distilled water containing Tween 80 (0.0001%) and incubated on a rotary shaker (150 rpm) for 30 min at ambient temperature. The suspension was filtered through a presterilized Whatman No. 541 paper. The filtrate was centrifuged at 5000*g* for 10 min. While the supernatant was discarded, the pellet containing surface microflora from the test samples was suspended in 20 mL of nutrient broth (Difco Laboratories, Detroit, MI). For spice powders the above method was used with a minor modification. The test sample was suspended in 50 mL of sterile distilled water containing Tween 80 (0.0001%), instead of 20 mL, and was shaken for 2 min (the higher water absorbing capacity of the spice powders compared to the whole spices required a

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Table 1. Surface Microbial Load (log cfu/g) of Nonirradiated (Control) and Irradiated (10 kGy) Whole Spice Samples[a]

	control	irradiated
black pepper	6.05 ± 0.23	nil
chili	4.36 ± 0.25	nil
coriander	3.47 ± 0.33	nil
cumin	3.04 ± 0.17	nil
fennel	2.78 ± 0.16	nil
turmeric	5.41 ± 0.04	nil

[a] Each reading represents the average of three replicates. ± values represent standard deviations.

Table 2. Surface Microbial Load (log cfu/g) of Nonirradiated (Control) and Irradiated (10 kGy) Spice Powders[a]

	control	irradiated
black pepper	6.69 ± 0.29	nil
chili	5.52 ± 0.06	nil
coriander	5.14 ± 0.01	nil
cumin	4.34 ± 0.11	nil
turmeric	6.09 ± 0.15	nil

[a] Each value represents the average of three replicates. ± values represent standard deviations.

larger volume for suspension). After 2 min of shaking, the suspension was filtered through a presterilized Whatman No. 541 paper. The filtrate (25 mL) was centrifuged at 5000*g* for 10 min. The supernatant was discarded, the bacterial pellet was suspended in nutrient broth medium, and the turbidimetric and SPC determinations were carried out as above. The bacterial pellet from chili pepper samples was washed once with 20 mL of sterile distilled water to remove excess coloring matter that interfered with the absorbency measurements.

Turbidimetry and Standard Plate Count (SPC). At the start of incubation a 1 mL aliquot of the medium was used for absorbency measurement at 600 nm (A_{600}) using a spectrophotometer (Chemito, Japan) and another 1 mL aliquot was employed for the determination of SPC by serial dilution method using Luria−Bertani (LB) agar plates. The plates were incubated for 48 h at ambient temperature before the colony forming units (cfu) were counted. The remaining bacterial pellet−nutrient broth suspension was allowed to incubate on a rotary shaker (150 rpm) for 10 h at 37 °C. For studying the growth profile, 1 mL aliquots of the medium were drawn for the measurement of bacterial turbidity at different time intervals.

RESULTS AND DISCUSSION

Table 1 shows the microbial load on whole spices, nonirradiated as well as irradiated (10 kGy). It was found that all irradiated spices were free from microbial contamination. However, the microbial load varied from spice to spice in the case of nonirradiated spices. The black pepper samples were found to be the most contaminated, with counts of >6 log cfu/g followed by turmeric, chili pepper, and others. Similar results were observed with spice powders (Table 2). The powdered samples of the spices were found to have a higher level of microbial contamination than the whole samples, but none of the irradiated samples, even of the powdered spices, showed the presence of viable microbes. The range of microbial contamination found in the spice samples was similar to that reported in the earlier studies from this laboratory and from elsewhere globally (Sharma et al., 1984, 1989; Munasiri et al., 1987; Farkas, 1988).

Figure 1 gives the absorbency (A_{600}) values representing the turbidity developed by the bacterial pellet recovered from nonirradiated and irradiated spices after

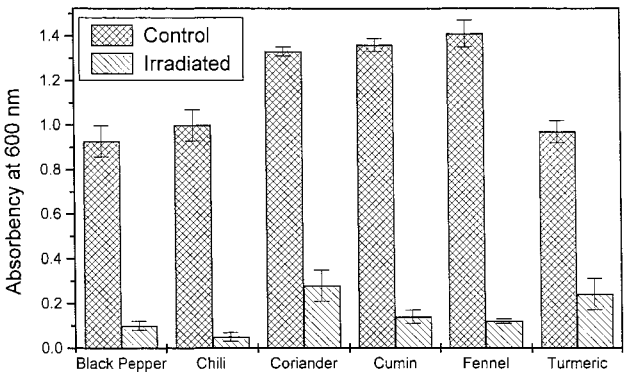

Figure 1. Absorbency (A_{600}) in nutrient broth with harvested microflora from nonirradiated (control) and irradiated (10 kGy) whole spices after 10 h of incubation on a rotary shaker (150 rpm) at 37 °C. Period of incubation for black pepper samples was 6 h. Error bars in the figure represent standard deviations.

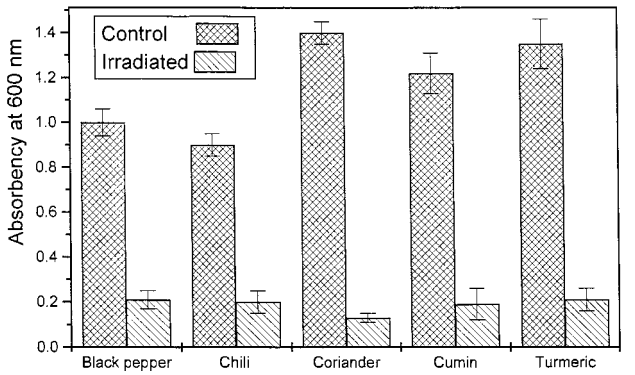

Figure 2. Absorbency (A_{600}) in nutrient broth with harvested microflora from nonirradiated (control) and irradiated (10 kGy) powdered spices after 10 h of incubation on a rotary shaker (150 rpm) at 37 °C. Error bars in the figure represent standard deviations.

it was suspended in the nutrient broth medium. The absorbency values at the beginning of incubation (0.5 h), for the irradiated as well as nonirradiated samples, were similar and ranged between 0.04 and 0.2 (data not shown). At the end of incubation (10 h), the nonirradiated samples showed severalfold higher (A_{600}) values compared to the irradiated ones. The A_{600} values in the case of control samples ranged between 0.9 and 1.4; on the other hand, the irradiated samples showed absorbency values ranging between 0.1 and 0.3.

Similar results were obtained with spice powders (Figure 2). The absorbency values at the beginning of incubation (0.5 h), for the irradiated as well as nonirradiated samples, were similar and ranged between 0.08 and 0.4 (data not shown). However, at the end of incubation (10 h), the nonirradiated samples showed severalfold higher values compared to irradiated ones. The A_{600} values in the case of control nonirradiated samples ranged between 1 and 1.4. On the other hand, the irradiated samples showed absorbency values ranging between 0.1 and 0.2.

In nonirradiated control spices the turbidity development was a function of the microbial load, its recovery in the pellet, and growth of the harvested cells in nutrient broth during incubation. The turbidity from irradiated samples was generated by the recovery of residual viable microflora, if any, in the pellet and its growth in the nutrient medium during incubation. The exponential nature of microbial death by γ-rays, on the one hand, and the exponential nature of microbial

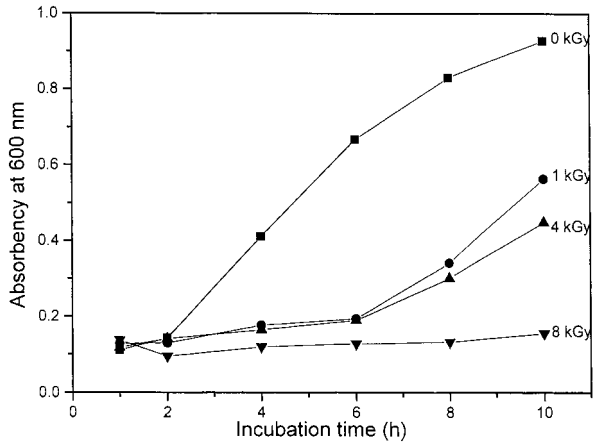

Figure 3. Growth profile (A_{600}) at different time intervals in nutrient broth containing harvested microflora from nonirradiated and irradiated (1, 4, and 8 kGy) black pepper samples. Experiments were carried out in duplicate.

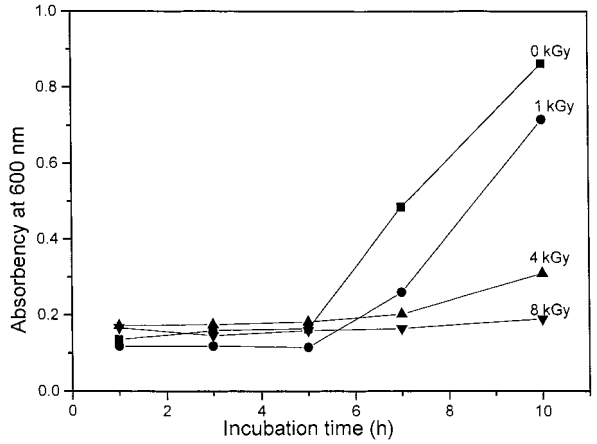

Figure 4. Growth profile (A_{600}) at different time intervals in nutrient broth containing harvested microflora from nonirradiated and irradiated (1, 4, and 8 kGy) coriander samples. Experiments were carried out in duplicate.

growth in a nutrient medium, on the other, magnify the differences in the turbidity produced by the microflora recovered from irradiated and nonirradiated samples so as to allow their clear identification.

Figures 3 and 4 show the growth kinetics of the residual microflora recovered from nonirradiated and irradiated (1, 4, and 8 kGy) whole black pepper and coriander samples, respectively. The growth was quantified by measuring the absorbency at 600 nm at different time intervals during the period of incubation. Microflora from nonirradiated samples showed the shortest lag period compared to the irradiated samples. The lag period in irradiated samples increased with the increase in the dose of γ-radiation and was longest in $\geq$8 kGy exposed samples. Microflora from nonirradiated black pepper samples showed relatively shorter lag compared to that from nonirradiated coriander. This could be attributed to the higher initial microbial load in black pepper compared to that in coriander (Tables 1 and 2).

A simple bacterial turbidimetric method has been described for foods such as mushroom, fish, chicken, and lamb (Gautam et al., 1998). However, the method could not be applied to dry commodities such as spices, in which the microbial burden largely comprised spores rather than vegetative cells of bacteria (Munasiri et al., 1987). Moreover, in the earlier method the nutrients from the food itself were sufficient for supporting microbial growth and generation of turbidity. Spices do not provide any nutrients for supporting microbial growth. On the contrary, they contain substances inhibitory to microbes. Therefore, the earlier method was modified to include harvesting of residual microflora, and its growth in a separate nutrient medium was employed for generating turbidity. The final turbidity development in the nutrient medium by the harvested microflora and the rate of its growth could provide a very good index for the detection of radiation treatment of spices. The currently recommended microbiological method (DEFT/APC) for the detection of irradiated spices (Wirtanen et al., 1993) needs sophisticated equipment and a trained analyst. The bacterial turbidimetric method proposed here is simpler and quicker and does not need special equipment or trained manpower, while providing information essentially similar to that provided by the DEFT/APC method.

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