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Effect of gamma irradiation on micro-organisms, essential oil content and volatile oil component of spice.

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ABSTRACT

Gamma irradiation at 5 kGy and 7.5 kGy reduced the bacterial and fungal contamination of cardamom, nutmeg and pepper to commercially acceptable levels. *Bacillius* sp, *Aspergillus* sp and *Penicillium* sp were the predominant microbial contaminants in Sri Lankan spices. Re-contamination of micro-organisms was not observed during storage for 3 months at 28 ^{0} C and at 75% RH. Gamma irradiation at 5 kGy and 7 kGy did not reveal any significant variation on the volatile oil components and essential oil contents when compared with the unirradiated cardamom , nutmeg and pepper.

Key words: Gamma irradiation, microbial contamination, volatile oil components.

INTRODUCTION

Sri Lankan spices are important export commodities. Introducing gamma irradiation technology to Sri Lanka will be beneficial to the spice industry as it will improve the quality of spices. This technology will facilitate loss reduction while extending storage life *via* the control of insect infestation and a reduction in the load of microbial contaminants (Farkas 1983).

Present microbial contamination observed in export quality pepper lies between 10^8 - 10^9 organisms g^{-1}. Indonesia, Malaysia and India are the main competitors for pepper and cardamom. The competition mainly arises on account of yield, quality and price. While yield and chemical quality vary according to cultivar and growing region, the hygienic quality of spices depends on post harvest handling and storage practices adopted by farmers and traders. Often Sri Lankan commodities command low prices and export consignments face rejection in international markets due to poor quality. Culinary practices followed abroad are different from those followed in Sri Lanka and stringent quality standards need to be maintained when spices are added as seasoning towards the end of the cooking process. The presence of extraneous matter, insect infestation and discoloration due to mould growth are not permitted. All spices should conform to international standards of microbiological quality, i.e. standards set by the International Commission for Microbiological Specifications for Foods, which permit upto 10^4 organisms g^{-1} (ICMSF 1974).

Traditional methods such as sun drying and curing treatments used in Sri Lanka for microbial disinfestation of spices are known to result in loss of volatile compounds and deterioration in colour of the product. Fumigants such as methyl bromide are being phased out of use for disinfestation. They are not effective against all microbial pathogens, and can also affect organolyptic quality and leave toxic residues in the product (Vajdi *et al.* 1973).

The present study confirmed that irradiation technology has a number of advantages over other disinfestation methods. The energy and penetration power of gamma rays and accelerated electron beams may be used effectively to kill pathogenic micro organisms at low dosages which will not affect the quality of the spices. Such treatments do not leave toxic residues and can be conducted at ambient temperature so as to prevent loss of volatile compounds. Decontamination of spice commodities adopting gamma irradiation technology, has been carried out in India (Padwal Desai *et al.* 1987; Munasiri *et al.* 1987 and Sharma *et al.* 1989) and in Korea (Cho *et al*. 1990).

MATERIALS AND METHODS

Sun - dried spice samples, packed in polyetheylene bags weighing 100g each were obtained from the Sri Lanka Standards Institute. Thirty replicates of spice commodities each weighing 10g, packed in polythylene bags (0.17 μm gauge) were used for this study. The samples were irradiated at room temperature in the 2000 curies Co^{60} radiation chamber available at the Horticultural Research and Development Institute, Gannoruwa, Peradeniya, Sri

Lanka at 3 different doses of 5 kGy, 7.5 kGy and 10 kGy respectively. Fricke dosimetry was used to measure the absorbed dose and dose calibration.

Un-irradiated (10g) and irradiated spice samples (10g) taken 24 hours after irradiation were each mixed with 99ml phosphate buffer solution. A serial dilution range was prepared by using phosphate buffer after allowing 5 min. for sedimentation.

Spread plate method was adopted to obtain total microbial counts. Potato dextrose agar medium was used for fungal cultures. Plate count agar medium was used to obtain aerobic mesophilic bacterial count, while violet red bile lactose agar was used for detection and enumeration of coliforms or enterobacteriaceae. Bacterial cultures were incubated at 37°C for 24 - 48 h, while fungal cultures were incubated at 30°C for 5 days. Identification of fungi and bacteria were carried out by studying their vegetative and asexual structures and *via* biochemical tests respectively.

All samples meant for storage studies were held at ambient temperature in the laboratory for 3 months and 6 months respectively. At a fixed time interval, designated samples were analyzed for total viable bacterial and fungal counts using spread plate method.

Essential oil content was obtained from finely milled irradiated and unirradiated spice samples weighing 50g, by steam distillation in a Deryng's apparatus. Gas chromatography (carbo wax 20m) was carried out using a Shimadzu GCL - 8 A FIDC chromatograph, and using a carbo wax 20 m column to study the composition of the volatile components of the essential oils of the irradiated and unirradiated spice samples. For gas chromatography the initial temperature was 70°C and the final temperature was 230°C for pepper and nutmeg. In the case of nutmeg the initial temperature was 100°C and the final temperature was 230°C. The program rate was 5°C/min. The temperature of the detector and the injector was 250°C.

A CRD design with 10 replicates was adopted in this study. The means were analyzed using Duncan Multiple Range Test at 5% level of significance.

RESULTS AND DISCUSSION

The effect gamma irradiation on bacterial and fungal populations in pepper, cardamom and nutmeg are given in Table : 1.

A progressive increase in the dose showed a concomitant decrease in the total viable fungal and bacterial counts in all the spices. The results obtained indicate that 5 kGy gamma irradiation dose was sufficient to reduce the total viable fungal count of pepper from 4.52×10^3 cfu/g to 2.9×10^2 cfu/g. The total viable bacterial count of pepper was reduced from 3.21×10^6 cfu/g to 2.00×10^4 cfu/g. The 7.5 kGy gamma irradiation dose reduced the total viable bacterial count of pepper 3.21×10^6 cfu/g to 1.00 x

Table 1. Effect of gamma irradiation on total viable bacterial count and total viable fungal counts of pepper, cardamom and nutmeg after 24 h, 3 months and 6 months storage

Spice Commodity	Irradiation treatment kGy	24 h. Storage		3 months storage		6 months storage	
		TVBC[†] cfu/g[°]	TVFC[‡] Cfu/g	TVBC cfu/g	TVFC cfu/g	TVBC cfu/g	TVFC cfu/g
Pepper	0	3.21×10^6	4.52×10^3	4.02×10^6	1.00×10^3	4.25×10^7	1.20×10^7
	5	2.20×10^4	2.90×10^2	2.91×10^2	0	2.89×10^2	0
	7.5	1.0×10^2	0	0	0	0	0
	10	0	0	0	0	0	0
Cardamom	0	1.20×10^3	2.00×10^2	1.10×10^3	1.90×10^2	1.00×10^3	1.70×10^2
	5	30	0	28	0	26	0
	7.5	0	0	0	0	0	0
	10	0	0	0	0	0	0
Nutmeg	0	1.02×10^4	4.30×10^2	1.00×10^4	4.10×10^2	9.00×10^3	3.90×10^2
	5	3.00×10^2	0	2.9×10^2	0	2.7×10^2	0
	7.5	0	0	0	0	0	0
	10 kGy	0	0	0	0	0	0

[†]TVBC - Total Viable Bacterial Count
[°]Cfu/g - colony forming unit per gram
[‡]TVFC - Total Viable Fungal Count

Table 2. Effect of Gamma Irradiation on major essential oil v/w content of pepper, Cardamom and Nutmeg ml/100 g.

Spice Commodity	Irradiation dose kGy	Essential oil content mg 100g^{-1}
Paper	0 (control)	3.1$_a$
	5	3.1$_a$
	7.5	3.2^a
Cardamom	0 (control)	6.3$_b$
	5	6.2$_b$
	7.5	6.4$_b$
	10	6.3^b
Nutmeg	0 (control)	4.9$_c$
	5	4.9$_c$
	7.5	5.0$_c$
	10	5.5^c

Values within the column followed by the same letters are not significant according to Duncan 's Multiple Range Test at 5% probability.

Table 3. Effect of gamma irradiation on major volatile oil components or pepper, after 24h. Respective volatile oil components expressed as a percentage of the total essential oil content recorded on GLC.

Irradiation Dose (kGy)	Pinene L (%)	Pinene B (%)	Sabinene (%)	Limonene (%)	Caryophyllene (%)
0 (control)	15.82$_a$	23.04$_b$	13.12$_c$	18.73$_d$	18.16$_c$
5	16.0$_a$	21.09$_b$	12.85$_c$	18.76$_d$	17.96$_c$
7.5	15.91$_a$	20.90$_b$	12.89$_c$	18.75$_d$	17.81
10	16.03	21.88	13.10	19.10	17.81

Values within the column followed by the same letters are not significant according to Duncan 's Multiple Range Test at 5% probability.

Table 4. Effect of gamma irradiation on volatile oil components of cardamom.

Does	Pinene (%)	Sabinene (%)	Limonene (%)	Terpineol (%)	Linalyl Acetate(%)	Terpenyl Acetate (%)
0 kGy (control)	2.29^a	1.08^b	46.54^c	2.10^d	3.00^e	32.90^f
5 kGy	2.60^a	1.18^b	48.95^c	2.00^d	3.16^e	30.98^f
7.5 kGy	2.62^a	1.166^b	48.82^c	2.03^d	3.00^e	30.71^f
10 kGy	2.56^a	1.168^b	50.05^c	2.00^d	3.01^e	30.02^f

Values within the column followed by the same letters are not significant according to Duncan 's Multiple Range Test at 5% probability.

Table 5. Effect of gamma irradiation on volatile oil components of nutmeg.

Doses	Sabinene (%)	Phellandrene (%)	Cineole (%)	Terpineol (%)	Elemicene (%)
5 kGy	49.20^a	5.47^b	6.50^c	5.91^d	2.72^e
5 kGy	43.41^a	5.12^b	6.40^c	7.00^c	3.20^c
7.5 kGy	47.95^a	4.91^b	5.50^c	6.18^d	3.30^c
10 kGy	50.06^a	4.41^b	5.91^c	6.02^d	2.92^c

Values within the column followed by the same letters are not significant according to Duncan 's Multiple Range Test at 5% probability.

10^2 cfu/g, and it completely eliminated the fungi from the samples. A dose of 10 kGy values within the column followed by the same letters are not significant according to Duncan's Multiple Range Test at 5% Probability.

Effectively eliminated all bacetria and fungi from pepper. These observations are in agreement with the observation of Munasiri *et al*(1987).

The 5 kGy gamma irradiation reduced the total viable bacterial count of cardamom from 1.20×10^3 cfu/g to 30 cfu/g. It was evident from this study that gamma irradiation was effective in the elimination of fungi from these samples.

The results obtained in this study indicate that the total viable bacterial count of nutmeg was reduced from 1.02×10^4 cfu/g to 3.00×10^2 cfu/g at 5 kGy gamma irradiation. This dose completely eliminated the fungi from nutmeg samples. A dose of 7.5 kGy was effective in eliminating all bacteria and fungi from cardamom and nutmeg resulting in zero counts(Table 1).

It is evident from Table 1 that there was only little variation in total viable bacterial counts and fungal counts of irradiated spice samples following 3 months and 6 months storage. However in unirradiated samples fungal and bacterial counts were significantly lower after 3 months and 6 months storage. It is possible that the anaerobic environment created by CO_2 build up within the packs due to respiratory activity of the surviving micro organisms was responsible for the slight decline in total viable microbial counts in these instances. Observation thus indicate that gamma irradiation reduced the total viable microbial load of the spice samples considered in this study, and enabled quality maintenance of spices, during storage periods extending to 6 months with no microbial decontamination.

It was observed that in unirradiated pepper samples, the total viable bacterial and fungal count increased with storage time. The total viable bacterial and fungal counts were high in unirradiated pepper due to the high relative humidity and temperature (28°C - 30°C) in the ambient storage environment.

The fungi associated with pepper, cardamom and nutmeg were identified as *Aspergillus niger, Aspergillus flavus, Pencillium ittalicum*, and *Rhizopus* sp., while bacterial contamination was attributed to *Bacillus* sp, *Pseudomonas* sp, *E. coli* and *Streptococcus sp.*

Results indicate that there was no significant difference between the essential oil content and volatile oil components of irradiated and unirradiated pepper, cardamom and nutmeg samples as presented in Table: 2, 3, 4 and 5. This study indicates that gamma irradiation technology could be beneficial to the spice industry in reducing microbial contamination and in storage quality maintenance.

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