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Study on the Use of Ionizing Radiation for the Preservation of Spices

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Abstract: *Black pepper (Piper nigrum L.) and clove (Eugenia caryophyllata) were treated with gamma radiationdoses (5.0, 10.0, 20 kGy)andstored for six months. The results showed an increase in the dry matter content of black pepper during the storage period withvalues of 91.61±2.36%, 92.59±2.39%, and 92.62±2.37 %w/w at 5.0, 10.0 and 20.0 kGy, respectively, compared with91.41±2.25%w/w for the non-irradiated sample. Also, there was an increase in the dry matter content ofcloves, withvalues of91.34±2.35%, 92.55±2.39%, and 92.87±2.37%w/wat 5.0, 10.0 and 20.0kGy, respectively, compared with a value of 91.34±2.35%w/w for the non-irradiated sample. However, a significant decrease in the total of essential oil which detected with increasing irradiation dose during the storage periods for black pepper and clovewith values of 97.42±0.22%, 98.65±0.27% and 96.88±0.23% at 5.0, 10.0 and 20.0 kGy, compared with 98.80±0.22% for the non-irradiated control. also,94.33±0.34%, 94.20±0.34 % and 94.10±0.35% at 5.0, 10.0 and 20.0 kGy, compared with 95.98±0.39% for the non-irradiated control samples,respectively .Anirradiation dose of 10.0 kGy with three months of storage led to the highest increase in the amount of total essential oils of black pepper and clovewith values 98.89±0.25% and 95.94±0.34%, respectively. A dose of 10 kGy eliminated the total bacterial count, as well as the yeast, while a dose of only 5 kGy was required to reduce the contamination. During six months of storage,the radiated spices were found to retain good microbiological quality.*

Keywords: *Black pepper,chemical composition, clove,microbial quality, Radiation,spices.*

I. Introduction

Spices are widely used to improve the taste and flavour of food, and they have a range of medicinal properties; some are known as antioxidants. Among the spices, black pepper (*Piper nigrum L.*) and clove (*Eugenia caryophyllata*) have been traditionally used for the treatment of many ailments[1]. Black pepper is acommonly used spice worldwide, especially in India and Southeast Asia. It is composed of several chemical constituents and includes some alkaloids,such as piperine and its three stereoisomers, isopiperine, chavicine and isochavicine[2][3] . Cloves are the aromatic dried flowers of a tree in the family Myrtaceae. The important chemical compounds in cloves are eugenol, tannins, flavonoids, triterpenoids and several sesquiterpenes[1]. However, spices present a potential source of microbial pollution in foodstuffs to which they are added. This primarily occurs in developing countries where harvest and storage conditions are poorly controlled with respect to food hygiene. Thus, the foodstuffs may have been exposed to a high level of contamination by mesophiles, hyphomycetes, faecal coliforms, and sporogenic and asporogenous bacteria [4] .

Irradiation is a method that uses radiant energy to forusefulpurposes, such as disinfection, shelf life improvement by the inactivation of spoilage organisms, and the improvement of the safety of spices by inactivating food-borne pathogens [5].Y-Ray irradiation is now internationally recognized as an effectivestandard and safe sterilization technique for maintaining the long-term quality of spices [6].This method decreases the risk of microbiological contamination and prolongs the shelf life of foodstuff [7]. In addition, it reduces post-harvest losses, ensures hygienic quality and facilitates food product trading[8] .The established community list provided by the Directive 1999/3/EC itemizes food ingredients and foods that can be treated by ionizing radiation and gives the maximum overall average absorbed dose as 10 kGy for dried aromatic herbs, spices and vegetable seasonings[9]. The U.S. Food and Drug Administration (FDA) limit for culinary spices, herbs, seeds, vegetable seasonings cannotsurpass 30 kGy [4][10] .No significant chemical changesfound inirradiatedblack pepper and clove with doses of 7 and 10 kGy.However, the microbiological results revealed that the aerobic platecount was reduced by 2.5 to 4.0 log cycles (mean values) with a dose of 7 kGy, while 10 kGy provided the most satisfactory hygienic results,to maintain a good market condition for at least one year[11]. Most significant changes in the chemical composition and in the microbiological quality of black pepper were observed after an ionizing radiation treatment of up to 30 kGy. The highest dose of irradiation used (30 kGy) affected many compounds by increasingthe ratio of many

oxygenated compounds (trans-sabinene hydrate, 3,4 dimethylstyrene, cyclohexenol, p-cy-men-8-ol, terpinen-4-ol, α-terpineal,eucarvon, piperitenon, α-terpineol,piperiton,spathulenol, and undecanone) compared with the control[12] [13] .Theresearchgoal is to study the effects of gamma rays at doses of 5, 10, and 20 kGyduring the storage periods on the chemical analysis and microbial quality of black pepper and clove.

II. Materials And Methods

2.1 Spice samples

- The flowering vine, black pepper (*Piper nigrum*), is in the family Piperaceaeand is cultivated for its fruit[12][14].
- The aromatic dried flower, clove (*Eugenia caryophyllata*), is from a tree in the family Myrtaceae[15].
- The samples were dried and used as a spice and seasoning[16][17].

The samples were purchased from a local market in Riyadh, Saudi Arabia.Then, they were washed and thoroughly dried by exposing them to cold air.The samples were subsequentlyground and placed in polyethylene bags (250 g per bag).The bags were separatedinto groups forthe irradiation process, the chemical analysis and the evaluation of the microbial quality (five replicates for each group)[6].

2.2 Irradiation process

The irradiation process was achieved using 60-cobalt gamma raysin a gamma cell-220 at King Abdul Aziz City for Science and Technology (KACST) in Riyadh. The dose rate was 14.2514 kGy/h at the time of the experiment. The samples were exposed to different gamma radiation doses of 5, 10, and 20 kGy; in addition, there was an untreated control.

2.3Chemical analysis

2.3.1 Preparing samples for chemical analysis

Samples of black pepper and clove werestored for chemical analysis under laboratory conditions (dark ,at 6 ℃, humidity of 60% for 0, 3 and 6 months).

2.3.2 Dry matter content

The dry matter content was estimated usinga 10-g sample, which was dried in a hot air oven at 130°C ± 1°C in pre-weighed dishes until reaching a constant weight. Each dish containing the dried sample was transferred to a desiccator and was cooled to room temperature. Then, the samples were weighed, and the dry matter content (percent) was calculated fromthe weight loss [18] (Association of Official Analytical Chemists1990).

2.3.3 Essential oil content

The essential oils were extracted from the treated samples of black pepper and clove, which were then injected into a gas-liquid chromatograph (GLC).

2.3.4 Extraction of essential oils

Double–distilled water was added to a flask containing the powder samples. A continuous steam distillationwas performed for 3 h. The oil and the steam distillate were isolated; the oil was dried over anhydrous sodium sulphate [19].

2.3.5GLC analysis of essential oils

Authentic essential compounds were acquired from Dragoc (Holzminden, Germany). The essential oil was analysed using a PyeUnicam gas chromatograph with dual Flame Ionization Detectors (FID) andthe chromatograph fitted with a coiled glass column (1.5 mx 4 mm) .It packed with Diatomite C100–120 mesh and coated with 10% PEGA. Theoven temperature was set to increase from 60°C to 180°C at a rate of 4°C per minute. The isothermal operation was held at 180°C for 15 minutes.The detector temperature was 220°C,and the injector temperaturewas 30°C. The gas flow rates forhydrogen, nitrogen, and air were 33,30, and 30 ml/min,respectively. The extracted essential oils were mixed with their major components and injected into the GLC to verify the resultant peaks[20]. The samples were analysed in triplicate, and the areas under the peak were calculated [21] .

2.4 Microbial content assay

According to the A.P.H.A 1985 technique, the microbial content of the spice samples was evaluated usingthe total plate count (TPC) of the microbial content of bacteria, and yeast. The estimation was performed using 10 g of each spice and with the addition of90 ml of a sterilized physiological substance (saline) to obtain a 1/10 dilution.

The required dilution was prepared, and the following AJAR culture medium was used:

-Bacto plate count agar 15g
-Trypone 5g
-Bactodextrose(Glucose) 1g
-Bacto yeast extract 2.5g
-pH 7±0.2

After sterilization, the AJAR medium was placed in petri dishes thatwere prepared in advance; then, sterilized medium was placed in the dishes, and they were incubated at 35°C for 48 h. Five replicates wereprepared for each test analysis, and the total count was calculated for every 1 g of the non-radiated and radiated spice samples.

2.5 Statistical analysis

The data were subjected to analysis of variance (ANOVA) for the completely randomized block design that was used. Averages and least significant differences were calculated by the SAS system version 9.1.3 (Cary, NC).The results were expressed as the mean ± standard deviation.A value of $P < 0.05$ was considered significant [22]

III. Results And Discussion

Table 1 shows the effects of irradiation and storage on the changes in dry matter content during the storage of irradiated black pepper and clove. The dry matter content increased by approximately 4% w/w during the storage of these spices. The black pepper values were91.61±2.36%, 92.59±2.39%, and 92.62±2.37% w/w at 5.0, 10.0 and 20.0 kGy, respectively,compared with 91.41±2.25%w/w of the non-irradiated control. An increase in the clove dry matter content was also observed with values of 91.34±2.35%, 92.55±2.39%, and 92.87±2.37% w/w at 5.0, 10.0 and 20.0 kGy, respectively, compared with 91.34±2.35%w/w of the non-irradiated control.These findings are in agreement with those described bySuhaj et al., 2006, who noticed an increase in dry matter content during the storage of irradiated spice under laboratory conditions[6].Studies illustrated that radiation exposure at different doses affect the final dry matter content of this spice[23][24].The effects of gamma irradiation on the essential oils in the spice samples during thestorage period are shown in Tables 2 and 3.Table2 shows a decrease in the amount of total essential oils content inblack pepper after six months of storagewith values of 97.42±0.22%, 98.65±0.27% and 96.88±0.23% at 5.0, 10.0 and 20.0 kGy, respectively, compared with 98.80±0.22% for the non-irradiated control. The total concentrations of the identified compounds decreased gradually with increasing irradiation dose during the storage periods; a dose of 10.0 kGy and three months of storage for the samples led to the highest increase in the total essential oils of black pepper(98.66±0.25%).

InTable2, the primary compounds innone irradiated black pepper sampleswhich identified as essential oilswere Limonene (22.80±0.12%), Sabinene(22.30±0.23%) and D-carene(15.90±0.13%).Our data agree with Suhaj et al. (2006)[6]and Franco et al.(2004)[25].Limonene appears to account for the highest proportion of essential oils in the tested black pepper at different irradiation doses during the storage periods, as was observed from the results by Emam et al. (1995)[26].As shown inTable3, a reduction in the amount of total essential oils contentin clove was shown after six months of storage, with values of 94.33±0.34%, 94.20±0.34 % and 94.10±0.35% at 5.0, 10.0 and 20.0 kGy, respectively, compared with 95.98±0.39% for the non-irradiated control samples .The total concentrations of the identified compounds decreased gradually with increasing irradiation dose during the storage periods; a dose of 10.0 kGy and three months of storage led to the highestamount of total essential oils of clove(95.94±0.34). Eugenol (73.50±1.34%) and Eugenol Acetate(10.81±0.89%) were the major essential oil components in clove in none irradiation samples, and the identified compounds in clove in this study agree withthe results Abozid et al. (2013)[15]andAlma et al., (2007)[27].Eugenol appears to account for the highest proportion of the tested clove essential oils of different irradiation doses during the storage periods,as was observed from the results ofGuan et al.(2007)[28] and Chaieb et al.(2007)[29].The results demonstrated that the essential oils in spices are radiosensitive, especially at high doses. Previous studies showed that the essential oils in spices are heat sensitive,especially at temperatures above 90°C [30] .The irradiation process at low doses is used asa cold, physical treatment for food because no significant heating occursafter treating the samples. Therefore, irradiation has no direct effect on the flavour of compounds; however, it can indirectly affect the flavour by oxidation or hydroxylation of the terpene aromatic ring due tothe production of free radicals in the food[31].These radicals can react with terpenes to produce terpene alcohols, as indicated in the study on the gamma irradiation of spices[10].Terpenes, which are incorporated in most of the essential oils, have the same skeletal structure but have different functional groups, such as-CHO, -OH, or -COOH. Therefore, configurational changes can occurfollowing high dose irradiation, including changes in the locus of the double bond and the functional group,performing in different

components [32].The results of the microbiological aspects of this study are presented in Table 4. The difference between irradiated samples and non-irradiated (control) samples indicated that most microbial counts were high for the control samples and low for the irradiated ones. Thus, the use of irradiation treatment might affect the microbial counts. It was noticed that gamma irradiation initiated a substantial reduction in all of the tested microorganisms, and this reduction was proportional to the irradiation dose. The lowest irradiation dose of 5 kGy decreased the total aerobic bacterial counts of black pepper and clove to 3.1×10^3 and 2.5×10^2, respectively, whereas the total aerobic bacterial counts decreased to < 10 with the doses of 10 and 20 kGy during the storage periods. In addition, black pepper and clove irradiated with various doses of γ-irradiation showed a considerable reduction in the total yeast and mould count,reaching< 10.Sharma et al. (1984)[33] revealed that the bacterial counts of commercially obtainable spices rangedfrom 10^2 to 10^7/g, whereas the fungal counts varied from 10^2 to 10^3/g.These findings are consistent with those stated by Farkas (1988)[32], who noticed that spices might not be suitable for the growth or long-term survival of the bacterial counts.The higher reduction in total aerobic bacterial counts in the spice samples might be due to the direct effect of radiation and the indirect effect of radiolysis, which is greater in control samples than in irradiated samples. Irradiation is used to improve the microbiological safety of foods. The irradiation showed a feasible process because the doses necessary to ensure good microbiological quality did not change the overall quality of the Spice[11]. There was an increase in the shelf life of the irradiated when compared to the non-irradiated samples [34][35][36].

IV. Conclusion

Radiation treatment increased the total of dry matter content during the storage for black pepper and clove. But it decreased the total amount of essential oils in the treated samples and In addition, Gamma irradiation caused a great reduction in all tested microorganisms, and this decrease was proportional to irradiation dose.The results of this study show a convenient method of irradiation that is used for food preservation, especially the spices.

V. Recommendation

Since no specific dose was found to be advantageous for the parameters tested, the present study recommends utilizing γ-radiation for black pepper ,clove and other spices. Further,there is a need to evaluate the in vivo assays after feeding the experimental animals the irradiated spices.

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Tables

Table 1. Dry matter contents of black pepper and clove irradiated with various doses of γ-radiation.

Dry matter (% w/w) of spices		0.0 kGy			5.0 kGy			10.0 kGy			20.0 kGy	
Storage period in months	0	3	6	0	3	6	0	3	6	0	3	6
Spices												
Black pepper	87.60 ±2.25	90.27±2.33	91.41±2.25	87.37±2.25	90.40 ±2.33	91.61± 2.36	88.40±2.28	91.41±2.35	92.59 ±2.39	87.46±2.60	90.23± 2.33	92.62± 2.37
Clove	88.40 ±2.29	91.07±2.32	91.34±2.35	88.10±2.28	91.13 ±2.35	91.34± 2.35	88.30±2.30	91.33±3.33	92.55 ±2.39	88.21±2.40	90.98± 2.35	92.87± 2.37

* All determinations are the mean of 5 samples; the results are expressed as the mean ± SD (standard deviation), ($P < 0.05$)

Table 2. Total essential oils contents of black pepper irradiated with various doses of γ-radiation

Dose response of irradiation of essential oils		0.0 kGy			5.0 kGy			10.0 kGy			20.0 kGy	
Storage period in months	0	3	6	0	3	6	0	3	6	0	3	6
CompoundsIn Black Pepper, %												
∝-Tujene	1.69 ±0.13	1.09± 0.13	1.0±0.10	0.64± 0.09	0.62± 0.10	0.6±0.10	1.53±0.15	1.54± 0.14	1.0±0.13	1.29± 0.14	1.89± 0.14	1.75±0.12

∝-Pinene	8.2±0.60	8.22±0.61	8.11±0.69	8.0±0.40	8.01±0.43	7.95±0.41	6.01±0.91	6.1±0.89	6.01±0.92	6.8±0.97	6.95±0.80	6.0±0.79
Sabinene	22.3±0.23	22.29±0.17	22.31±0.21	22.05±0.14	22.0±0.12	21.9±0.12	22.46±0.25	22.49±0.23	22.5±0.29	21.9±0.23	21.0±0.22	22.0±0.22
ββ-Pinene	8.23±0.23	8.25±0.22	8.24±0.22	8.22±0.20	8.19±0.23	8.0±0.16	8.07±0.21	8.3±0.21	8.29±0.23	7.83±0.21	7.63±0.20	7.43±0.20
D-carene	15.9±0.13	15.21±0.11	15.15±0.12	15.85±0.14	15.85±0.14	15.8±0.10	15.1±0.14	15.5±0.16	15.25±0.16	15.5±0.14	15.9±0.15	15.75±0.14
Limonene	22.8±0.12	22.75±0.22	22.81±0.21	23.8±0.16	23.75±0.20	23.8±0.20	23.6±0.20	23.62±0.23	23.6±0.26	23.4±0.20	23.81±0.22	23.72±0.22
Linalool	1.3±0.07	1.45±0.08	1.52±0.11	1.15±0.08	1.13±0.07	1.12±0.07	1.46±0.08	1.64±0.11	1.51±0.12	1.81±0.09	1.13±0.10	1.0±0.10
∝-Copaene	6.85±0.22	6.86±0.22	6.8±0.24	6.85±0.20	6.82±0.20	6.8±0.19	6.71±0.21	6.75±0.21	6.95±0.23	6.35±0.20	6.41±0.19	6.32±0.19
BCaryophyllene	12.14±0.12	12.34±0.13	12.35±0.13	12.0±0.10	12±0.11	11±0.11	12.24±0.12	12.24±0.13	12.19±0.15	12.03±0.12	12.18±0.12	12.0±0.13
Terpinen-4o1	0.5±0.22	0.55±0.21	0.51±0.19	0.5±0.22	0.5±0.20	0.45±0.20	1.34±0.23	1.36±0.23	1.35±0.24	1.07±0.20	1.0±0.22	0.91±0.22
Total	99.91±0.02	99.01±0.21	98.8±0.22	99.06±0.17	98.87±0.18	97.42±0.22	98.52±0.25	98.89±0.25	98.65±0.27	97.98±0.25	97.9±0.23	96.88±0.23

All determinations are the mean of 5 samples; the results are expressed as the mean ± SD (standard deviation), ($P < 0.05$

Table 3. Total essential oils contents ofclove irradiated with various doses of γ-radiation

Dose response of irradiation of		0.0 kGy			5.0 kGy			10.0 kGy			20.0 kGy	
Storage period in months	0	3	6	0	3	6	0	3	6	0	3	6
Compounds in Clove %												
Eugenol	73.5±1.34	73.5±1.34	73.29±1.89	72.95±1.59	72.94±1.58	72.9±1.53	73.66±1.60	73.65±1.60	71.96±1.61	73.6±1.49	73.2±1.5	73.05±1.
Eugenol acetate	10.81±0.89	10.80±0.89	10.78±0.88	10.68±0.82	10.67±0.82	10.6±0.86	10.92±0.88	10.9±0.88	10.90±0.89	10.71±0.86	10.69±0.	10.5±0.8
β-Caryophllene	4.56±0.36	4.41±0.36	4.39±0.39	4.35±0.37	4.3±0.39	4.22±0.43	4.40±0.33	4.41±0.33	4.40±0.35	4.26±0.36	4.33±0.3	4.30±0.3
Caryophyllene oxide	2.57±0.20	2.5±0.19	2.33±0.17	2.25±0.20	2.2±0.18	2.2±0.18	2.41±0.20	2.43±0.20	2.41±0.19	2.37±0.18	2.67±0.2	2.50±0.2
∝ - Humulene	1.56±0.12	1.5±0.11	1.79±0.11	1.5±0.12	1.5±0.10	1.59±0.13	1.34±0.13	1.35±0.12	1.35±0.11	1.34±0.13	1.54±0.1	1.42±0.1
∝- Pinene	0.91±0.09	0.85±0.08	0.81±0.06	0.66±0.09	0.65±0.07	0.62±0.05	0.85±0.06	0.82±0.05	0.81±0.06	0.61±0.09	0.80±0.0	0.81±0.0
Ledol	1.49±0.11	1.53±0.10	1.53±0.10	1.45±0.12	1.44±0.12	1.41±0.11	1.33±0.10	1.33±0.10	1.33±0.12	1.31±0.17	1.39±0.1	1.37±0.1
Linalool	0.89±0.07	0.89±0.07	0.88±0.08	0.75±0.03	0.71±0.04	0.65±0.05	0.86±0.03	0.85±0.03	0.86±0.02	0.60±0.03	0.67±0.0	0.61±0.0
Methyl salicylate	0.14±0.11	0.16±0.11	0.15±0.12	0.12±0.10	0.12±0.09	0.11±0.09	0.13±0.11	0.15±0.11	0.15±0.13	0.11±0.11	0.12±0.1	0.13±0.1
2-Nonanone	0.02±0.001	0.04±0.002	0.03±0.001	0.05±0.002	0.05±0.001	0.03±0.002	0.04±0.003	0.05±0.006	0.03±0.006	0.05±0.003	0.04±0.0	0.04±0.0
TOTAL	96.45±0.33	96.18±0.32	95.98±0.39	94.76±0.34	94.58±0.33	94.33±0.34	95.94±0.34	95.94±0.34	94.20±0.34	94.96±0.34	95.45±0.	94.10±0.

All determinations are the mean of 5 samples; the results are expressed as the mean ± SD (standard deviation), ($P < 0.05$

Table 4. Microbiological quality of spices irradiated with various doses of γ-radiation

Spices	Dose (kGy)	*Escherichia coli ATCC35218* (log cfu/g)			*Staphylococcus aureus ATCC25923* (log cfu/g)			*Klebsiella pneumoniaeATCC* (log cfu/g)			*Yeast Candida albicans* (log cfu/g)		
		Periods of storage in month											
		0	3	6	0	3	6	0	3	6	0	3	6
Black Pepper	0	3.5×10^5	2.5×10^5	2.5×10^5	2.6×10^5	2.5×10^5	2.6×10^5	3.1×10^5	3.5×10^5	3.0×10^5	6.4×10^5	6.4×10^5	6.2×10^5
	5	1.3×10^3	1.5×10^2	1.2×10^2	2.0×10^3	1.5×10^2	0.9×10^2	1.0×10^3	1.5×10^2	1.3×10^2	3.0×10^1	<10	<10
	10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	20	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Clove	0	1.5×10^4	1.5×10^4	1.0×10^4	2.5×10^4	2.5×10^4	1.5×10^4	2.0×10^4	1.5×10^4	1.5×10^4	3.9×10^4	3.5×10^3	3.5×10^3
	5	0.5×10^2	0.5×10^1	0.5×10^1	1.5×10^2	1.5×10^1	0.5×10^1	1.2×10^1	0.9×10^1	0.5×10^1	1.0×10^1	<10	<10
	10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	20	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10