Effect of Gamma-Irradiation on Microbial Decontamination and Organoleptic Quality of Black Pepper (*Piper nigrum* L.)

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Abstract: The influence of ionizing radiation treatment with different doses (5 kGy, 10 kGy and 30 kGy) of γ-rays on the microbial decontamination of powdered black pepper was investigated. It was found out that a dose of 5 kGy is sufficient to achieve a total viable count of microbial contamination. Subsequently the effect of γ-irradiation dose on the possible changes in composition of the black pepper essential oil and simultaneous potential alterations in its organoleptic quality (flavour) were studied. No significant changes in the volatile oil compounds content were observed with the radiation doses of 5 kGy and 10 kGy. Ionizing dose of 30 kGy resulted in triple increase of caryophyllene oxide concentration in compare with an untreated sample. The olfactometric analysis showed non-significant changes in flavour.

Keywords: gamma-irradiation; black pepper; volatile oil; GC; olfactometry

INTRODUCTION

Radiation pasteurization with low doses of γ-rays, X-rays and electrons effectively controls foodborne pathogens and can protect public from diseases such as salmonellosis, hemorrhagic diarrhea caused by *Escherichia coli*, and gastroenteritis from *Vibrio vulnificus* [1]. Ionizing radiation does not leave chemical residues on a product. Toxicological and nutritional tests have confirmed the safety of foods irradiated at doses below 10 kGy [2, 3]. Spices and dried herbs, used for food preservation and flavouring, are particularly exposed to biological contamination and are often potential microbial pollution sources for food they are added to. Their contamination level is generally as high as 10^5–10^8 microorganisms per gram. Black pepper (*Piper nigrum* L.) is the most widely used of all condiments, and in general, belongs to the most highly contaminated spices. Irradiation replaces the use of toxic and carcinogenic ethylene oxide and methyl bromide for elimination of microbiological contamination and is less harmful to the spice than the heat sterilization, which implicates the loss of thermolabile aromatic volatiles. Quality of black pepper depends considerably on occurrence and quantity of the alkaloid piperine and essential oil. Piperine and its derivates (nonvolatile with water steam) are responsible for pungency and hotness of black pepper, whereas the essential oil (obtained from powdered black pepper berries by water steam distillation and extraction) is responsible for characteristic flavour of the spice. Ionizing radiation is a potential method for microbial decontamination of spices, however it may alter chemical composition and flavour. Chemical composition of black pepper essential oils γ-irradiated at various doses has been studied [4], however no work was devoted to sensory evaluation of individual components and their contribution to the overall aroma. The aim of the present study was to determine γ-irradiation dose, which is required to achieve a total viable count of microorganisms in powdered black pepper and subsequently to investigate effect of recommended doses (5 kGy, 10 kGy) and exceeding dose (30 kGy) of γ-rays on the black pepper flavour, employing GC, GC/MS and aroma extract dilution analysis (AEDA) [5].

EXPERIMENTAL

Material. A sample of dried spice marked as Vietnamese powdered black pepper 550 (ϱ = 550 g/dm^3) was obtained from the supplier: Mäspoma, s. r. o.,

Zvolen, SK. The spice moisture content was 11.87%, the samples were dried at 100°C during 6 hours (by the STN 580110 standard, article 32). Portions of 80 g were stored in polyethylene and paper bags at ambient temperature on a dry place.

Irradiation. The packed samples were irradiated with 5 kGy, 10 kGy and 30 kGy at a rate of 2 kGy/h using a γ- rays Co-60 irradiator at ARTIM, s. r. o., Praha, CZ.

Microbiological analysis. Elementary microbiological investigation of spice samples untreated, heat treated and γ-irradiated at doses of 5 kGy, 10 kGy and 30 kGy was carried out under STN ISO 4833, STN ISO 4832, STN ISO 7954 immediately after irradiation and after 3 months of storage.

Extracts. Black pepper essential oils for GC/MS, GC/FID and GC-olfactometry were isolated from powdered spice untreated and treated at stated doses of γ-irradiation by simultaneous distillation extraction using Likens-Nickerson apparatus and diethyl ether as extraction solvent.

Total essential oils contents from powdered untreated, heat treated and irradiated black pepper were determined by a method European Pharmacopoeia 4 with xylene as an extraction solvent.

Gas chromatography/Mass spectrometry (GC/MS). GC/MS analyses were performed on Hewlett-Packard HP 5971A mass-selective detector directly coupled to HP 5890II gas chromatograph. Fused silica capillary column Ultra 1 (HP), 50 m × 0.20 mm × 0.33 μm was employed with helium as a carrier gas. The samples were injected by the split technique at 250°C. The column temperature was programmed from 35°C to 250°C with gradient 1.7°C/min. The ionizing voltage (EI) was 70 eV.

Gas chromatography (GC). Hewlett-Packard HP 5890II gas chromatograph with FID was used for determination of relative percentage composition of volatile compounds and of their linear temperature programmed retention indices. The samples of extracts were analyzed on Ultra 1 (HP), fused silica capillary column 50 m × 0.32 mm × 0.50 μm, at the temperature programmed from 35°C up to 250°C with gradient of 2°C/min. Linear velocity of the carrier gas hydrogen was 36 cm/min (measured at column temperature 143°C). The linear retention indices (RIp) were calculated after Van den Dool and Kratz [6] equation. *n*-Alkanes C_6–C_{18} were used as the reference standards.

Gas chromatography/olfactometry – aroma extract dilution analysis (AEDA). For the AEDA [5] Hewlett-Packard HP5980II gas chromatograph equipped with FID, Ultra1 (HP), the fused silica capillary column 50 m × 0.32 mm × 0.50 μm and the column effluent splitter 1:1 and sniffing port were used. The temperature was programmed from 35°C up to 250°C with a gradient of 2°C/min. The flavour dilution (FD) factors [5] and odour descriptions were determined by sniffing of compounds eluting from the capillary column. The extracts for AEDA were diluted with diethyl ether stepwise 1:10, 1:100, 1:200. Sensory evaluations were performed by a panel of 3 trained judges.

RESULTS AND DISCUSSION

Decrease of microbial contamination level up to the total elimination of present microorganisms (MO) is the primary aim of the spice irradiation treatment. The microbiological results confirmed

Table 1. Microbiological analysis of untreated, heat treated and irradiated black pepper immediately after irradiation and after 3 months of storage

Irrad. dose (kGy) / Storage	Total MO (KTJ/g)		Coliforms (KTJ/g)		Yeasts (KTJ/g)		Moulds (KTJ/g)	
	without	3 months	without	3 months	without	3 months	without	3 months
0	1.0×10^6	2.5×10^6	1.0×10^1	2	< 10	< 10	1.0×10^1	< 10
5	< 1	< 1	< 1	< 1	< 10	< 10	< 10	< 10
10	< 1	< 1	< 1	< 1	< 10	< 10	< 10	< 10
30	< 1	< 1	< 1	< 1	< 10	< 10	< 10	< 10
Heat treated*	1.6×10^5	1.6×10^6	< 1	< 1	< 10	< 10	< 10	< 10

*heat sterilization of black pepper berries by dry steam at 130°C and subsequent mulling
KTJ/g = count of colonies per gram

that the total count of MO 10^6 colonies in the untreated sample of black pepper decreased using 5 kGy irradiation dose to less than 1 KTJ/g (Table 1). It was found out by the heat treatment of black pepper berries with dry steam at 130°C and subsequent mulling, the total MO count was lower by one order as the control sample. In the control sample number of MO has increased 2.5-times during 3 months storage. The status in γ-irradiated samples was unchanged within 3 months storage. In the heat treated sample during 3 months the count of MO multiplied by one order. The extracts from untreated and γ-irradiated samples of powdered black pepper were pale yellowish liquids with characteristic, terpenic, powerful odour black pepper note. The steam-volatile black pepper oil consisted primarily of monoterpene and sesquiterpene hydrocarbons, and oxygenated compounds. The GC/MS and GC/FID analyses of volatile constituents of spice extracts revealed more than sixty compounds from which we were able to identify more than fifty ones using the mass spectra and the published retention indices. α- and β-pinene, sabinene, δ-3-carene, limonene were the important components of the monoterpene fraction. β-Caryophyllene is the major sesquiterpene and it was the main compound of volatile black pepper oil according to the quantity. Qualitative composition of volatile oils obtained from control samples and from irradiated samples of powdered black pepper at various doses was identical. No significant changes were observed in the volatile oil compound content, expressed as a relative percentage, at radiation doses 5 kGy and 10 kGy (toxicologically and nutritionally confirmed as safe maximal dose). The most important change was possible to observe at ionizing of 30 kGy

Table 2. Influence of γ-irradiation on potent odorants of black pepper

No.	Compound	RIp[a] Ultra1	FD factor				Aroma character	Identification[b]
			0 kGy	5 kGy	10 kGy	30 kGy		
1	3-methylthiopropanal	861.3	100	100	100	100	cooked potato-like	RI,ST,A
2	unknown	900.7	100	100	10	10	musty, burnt, mousy	–
3	1-octen-3-one	954.0	100	100	100	100	mushroom-like	RI,ST,A
4	myrcene	981.9	10	10	10	10	hop oil-like, herbaceous	MS,RI,ST,A
5 + 6	1,8-cineole + unknown	1016.0	10	10	10	10	peppermint, cool, fresh	MS,RI,ST,A
7	unknown	1031.2	10	10	10	10	herbaceous, earthy, bitter	–
8	unknown	1054.7	100	100	100	10	smoke, terpeny	–
9	α-terpinolene[t]	1076.0	10	10	10	10	vegetable, bitter, green	MS
10	linalool	1083.1	200	200	200	200	flowery	MS,RI,ST,A
11	unknown	1142.0	100	100	100	100	thiamin, meaty consommé	–
12	*p*-cymen-8-ol	1156.2	10	10	10	10	phenolic, bitter, fuel-like	MS,RI,A
13	*cis*-sabinol[t]	1177.1	10	10	10	10	earthy, muddy, musty	MS
14	unknown	1191.4	10	10	10	10	rancid fat-like	–
15	piperitone[t]	1220.8	100	100	100	100	balsamic, sweet, anise	MS
16	unknown	1288.6	10	10	100	100	terpeny, almond	–
17	unknown	1292.4	10	10	100	100	rancid fat-like	–
18 + 19	unk. + β-damascenone	1375.5	100	100	100	100	fruity, prune-like	RI,A
20	β-farnesene	1446.0	10	10	10	10	terpeny, spicy	MS,RI,ST,A
21	germacrene D[t]	1469.6	10	10	10	10	flowery	MS
22	β-bisabolene[t]	1497.8	100	100	100	100	terpeny, earthy, celery	MS
23	δ-cadinene	1509.9	100	100	100	100	thyme, sweet, terpeny	MS,A
24	unknown	1716.9	200	200	200	200	spicy, black pepper-like	—

[a]linear retention index; [b]means of the identification: MS-EI – mass spectrum, RI – retention index, ST – sniffing of standard compound, A – known character; [t]tentative identification

(3-times exceeded authorized dose) resulting in triple increase of caryophyllene oxide concentration in compare with the untreated sample. This effect was observed in 30 kGy irradiated spice and also in 30 kGy irradiated essential oil on neutral carrier (Na_2SO_4). Gas chromatography-olfactometry analyses (AEDA) of volatile extracts revealed 24 potent odorants with FD factors in the range from 10 to 200. It was found out that 3-methythiopropanal, 1-octen-3-one, myrcene, 1,8-cineole, linalool, β-damascenone, β-farnesene, δ-cadinene, α-terpinolene, *p*-cymen-8-ol, piperitone, germacrene D, β-bisabolene and eight unknown compounds are responsible for characteristic flavour of black pepper essential oils untreated and irradiated at the stated doses (Table 2). Identification of these compounds was performed on the basis of EI mass spectra, retention indices and aroma character, compared to that of reference compounds or published data. The most potent odorants are linalool (10) with flowery aroma character and an unknown compound (24) with spicy, typical black pepper-like aroma, either of them with FD = 200. 3-Methylthiopropanal, 1-octen-3-one, tentatively identified piperitone and β-bisabolene, β-damascenone and δ-cadinene had FD = 100. In the majority of individual compounds effect of irradiation on the FD factors was not proven. The influence of γ-irradiation on components (2, 8, 16, 17) marked as unknowns, is not very significant. Differencies of obtained FD factors are in one dilution step only. Aroma characters of these constituents are described as burnt, musty, mousy, smoke, rancid fat, terpeny, almond. The identification of the unknown odorants is in progress.

CONCLUSIONS

γ-Irradiation at recommended doses produced non-significant effect on the steam-distilled oil content of black pepper, its volatile compounds composition and simultaneously slight potential alterations in aroma-activity of some essential oils components, although the overall organoleptic quality of stated volatile oils were not considerably changed. It is possible to suppose via influence of irradiation dose higher like triple of authorized value (30 kGy) that ionizing radiation generates radical reactions in oxidation of β-caryophyllene under formation of caryophyllene oxide. However, no important changes in overall aroma of black pepper were perceived.

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