Influence of Two Sterilisation Ways, Gamma-Irradiation and Heat Treatment, on the Volatiles of Black Pepper (*Piper nigrum* L.)

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Abstract

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The objective of this study was to investigate and compare the effects of ionising irradiation with different doses of gamma-rays (5 kGy, 10 kGy, and 30 kGy) versus the effect of heat sterilisation (dry steam, 130°C, 3 min) on the microbiological quality of powdered black pepper. Subsequently, the aim was to determine the impact of these sterilisation ways on the possible changes in the chemical composition and sensory quality (flavour) of black pepper essential oils. Methods of gas chromatography (GC/FID, GC/MS) were utilised for the evaluation of the essential oils compositions. The volatile constituents of black pepper extracts were studied with regard to their particular contribution to the overall aroma by the technique of gas chromatography-olfactometry (GC/O) using the method of Aroma Extract Dilution Analysis [AEDA]. Qualitative compositions of volatile oils obtained from the control sample (0 kGy), samples irradiated at various doses, and heat treated sample were identical. The most significant changes were observed in the contents of volatile compounds after ionising radiation treatment with 30 kGy and heat treatment, respectively. These changes caused a remarkable decrease in the overall aroma of heat sterilised black pepper. Additionally, microbiological analysis showed that the heat treatment was insufficient for an effective reduction/elimination of the polluting microflora present in the analysed sample of black pepper.

Keywords: food irradiation; spices; essential oils; GC; GC/MS; sensory quality; olfactometry

Contamination of food with microorganisms (MO), particularly pathogenic non-sporeforming bacteria, is one of the most significant public health problems and an important cause of human suffering all over the world.

While the thermal pasteurisation of liquid foods is a well-established and satisfactory method of terminal decontamination/disinfection of such commodities, it has been shown to be inappropriate for solid foods and dry ingredients, or fresh foods, whose raw characteristics must be maintained to fulfil specific market requirements. Due to these reasons and keeping in mind the universality of the food spoilage problem, food irradiation has become one of the most promising programmes that attracted many countries during the movement to use "Atoms for Peace" (Boisseau 1994). However, practical limitations precluded an early industrial and commercial development and the application of these concepts. Only recently, a significant industrial utilisation of food irradiation has become widespread.

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Nowadays, food irradiation is increasingly recognised as a method for reducing post harvest food losses, ensuring hygienic quality, and facilitating wider trade in foodstuffs. Radiation pasteurisation with low doses of gamma rays, X-rays, and electrons effectively controls the foodborne pathogens. It leads to the destruction of pathogenic non-spore forming foodborne bacteria and parasitic organisms, such as trichina. As a consequence, it protects the consumers from microorganisms – related diseases such as salmonellosis, hemorrhagic diarrhoea caused by *Escherichia coli*, or gastroenteritis due to *Vibrio vulnificus* (Thayer *et al*. 1996). The application of ionising radiation in food processing is mainly based on the principle that ionising radiation causes a very effective disruption of DNA molecules in the nuclei of cells (Dhiel 1995) rendering them inactivated. Therefore, MO insect gametes and plant meristems are prevented from their reproduction, which consequently results in various preservative effects as a function of the absorbed radiation dose (Table), while chemical or other radiation-induced changes in food are minimal (Thayer 1996). An important reason for the relatively high sensitivity of DNA to the effects of ionising radiation is the fact that DNA molecules are much larger than other molecular structures inside the cell. The damage is either direct, caused by reactive oxygen-centred ($^•OH$) radicals originating from the radiolysis of water, or indirect. In the case of an indirect hit, the damage occurs to nucleic acids when the radiation ionises an adjacent molecule, which in turn reacts with the genetic material. In view of the fact that water is a major component of most foods and microbes, it is often the adjacent molecule that ends up producing a lethal product (Grecz *et al*. 1983). According to the paper published by Arena (1971), ionising radiation causes the water molecule to loose an electron producing H_2O^+. This product immediately reacts with other water molecules to produce a number of compounds, including hydrogen and hydroxyl radicals ($OH^•$), molecular hydrogen, oxygen, and hydrogen peroxide (H_2O_2). Hydroxyl radicals are very reactive and are known to interfere with the bonds between nucleic acids within a single strand, or between opposite strands. Although biological systems have a capacity to repair both single-stranded and double-stranded breaks of the DNA backbone, the damage occurring from ionising radiation is random and extensive (RazskazovskiY *et al*. 2003). Therefore, the recovery processes in bacteria after their radiation damage are unlikely to occur. The differences in sensitivity to radiation between MO are related to the differences in their chemical and physical structures, and in their ability to recover from the radiation injury. Consequently, the amount of radiation energy required to control MO in food varies, depending on the resistance of the particular species and the number of organisms present. Besides the inherent abilities of MO, several environmental factors such as the composition of the medium, moisture content, temperature during irradiation, presence or absence of oxygen, and others, significantly influence their radiation resistance, particularly in the vegetative cells. Thus, the actual dose employed is a balance between that what is needed and that what can be tolerated by the product without objectionable changes (e.g. off-flavours, texture changes, flavour alterations). According to the Codex General Standard for Irradiated Foods (CAC 2003), ionising radiation foreseen for food processing is limited to high energy photons (gamma rays of radionuclides ^{60}Co) and, to a much smaller extent ^{137}Cs, or X-rays from machine sources with energies up to 5 MeV, or accelerated electrons with energies up to 10 MeV produced by electron accelerating machines. These types of radiation are chosen because:

Table 1. Directives for dose requirements in various applications of food irradiation (Farkas 2006)

Preservative effects and types of application	Dose requirements (kGy)
Killing and sterilising insects (disinfestations of food)	0.2–0.8
Prevention of reproduction of food-borne parasites	0.1–3.0
Decrease of after-ripening and delaying senescence of some fruit and vegetables; extension of shelf-life of food by reduction of microbial populations	0.5–5.0
Elimination of viable non-sporeforming pathogenic microorganisms (other than viruses) in fresh and frozen food	1.0–7.0
Reduction or elimination of microbial of microbial population in dry food ingredients	3.0–10.0

– produce the desired food preservative effects;
– do not induce radioactivity in foods or packaging materials;
– are available in quantities and at costs that allow commercial use of the irradiation process (Farkas 2004).

The radiation treatment causes only minimal temperature rise in the product. The inner temperature of the product can rise by max. 5°C at the dose of 10 kGy, and this treatment can be applied through packaging materials including those that cannot withstand heat. This also means that the radiation treatment can be performed also after packaging, thus re-contamination or re-infestation of the product is avoided. Long-term animal feeding studies have demonstrated that radiation-pasteurised or -sterilised foods are safe and nutritious also for humans (Thayer *et al.* 1996); toxicological (WHO 1981) and nutritional tests have confirmed the safety of foods irradiated at doses below 10 kGy (Thayer 1996; Smith & Pillay 2004).

In this context, Directive 1999/3/EC of the European Parliament and of the Council of 22. 2. 1999 establishes a Community initial positive list of food and food ingredients that may be treated with ionising radiation with the maximum dose 10 kGy (EC 1999). Dried aromatic herbs, spices, and vegetable seasonings are authorised for the irradiation treatment, because they are frequently contaminated and infested with MO and their metabolites, which are harmful to public health. However, the progress in commercialisation of the food irradiation process and consumer demand for clear labelling of irradiated food highlighted the need for the testing and intensive development of the detection methods.

In the last years, the irradiation of food and agricultural products has been authorised in about 40 countries, in order to extend the shelf-life of foodstuffs and reduce food loss. It was shown that the food irradiation treatment improves the quality of the products, since it inhibits – besides pathogens – the replication of insects, parasites, bacteria, saprophytic moulds, and yeasts (Bendini *et al.* 1998).

Dried spices and herbs

Spices are frequently exposed to insects and MO during cultivation and storage, and may be potential contamination sources in foods even when added in small amounts. Commercial spices are generally contaminated with 10^5 to 10^8 MO per gram (Kiss 1982). Spices often originate in developing countries where the harvest and storage conditions are not adequately controlled with respect to food hygiene. Thus, they may be exposed to high levels of natural contamination by mesophylic, sporogenic, and asporogenic bacteria, hyphomycetes, and faecal coliforms (Bendini *et al.* 1998). Most spices are dried in the open air and can become seriously contaminated by air- and soil-borne bacteria, fungi, and insects.

MO of public health significance such as *Salmonella*, *Escherichia coli*, *Clostridium perfringens*, *Bacillus cereus*, and toxigenic moulds can also be present. Bacterial plate counts of one to 100 million per gram of spice are usual (Bendini *et al.* 1998). Good manufacturing practices during harvest and processing could improve their hygienic quality, but frequently not to an extent sufficient to obtain an acceptable microbiological purity level (WHO 1999). To ensure the consumer safety, the microbiological contamination level should not exceed an acceptable limit of 10^4 MO per gram (Farkas 1988).

Because the contaminated dry plant ingredients have been causing serious troubles in the food processing industry, different methods have been used to reduce their microbiological contamination. Many commercial food processors fumigated spices with methyl bromide to eliminate insects or with ethylene oxide to eliminate bacteria and moulds. However, it has been found that both methyl bromide and ethylene oxide are extremely toxic compounds. Moreover, methyl bromide is potentially capable of depleting the atmospheric ozone layer. Ethylene oxide has been banned in Europe because of the safety and environmental concerns, and its use for the treatment of ground spice has been banned in the United States (Loaharanu 1994) .

Nowadays, the following approaches to antimicrobial treatment of spices can be taken into consideration: heat sterilisation, microwave treatment, and radiation treatment (so-called "cold sterilisation").

However, the published data significantly differ in the evaluation of particular methods used for the assessment of treated spices properties. For example, the authors Emam *et al.* (1995) have affirmed the suitability of the use of the microwave treatment for 40 s and 75 s at a frequency of

2450 MHz and a power output of 750 W for the decontamination of black pepper. The results were similar to those obtained by gamma irradiation at recommended doses (up to 10 kGy).

For all that, the authors have quoted that the microwave treatment is less effective on the volatile oil constituents, than gamma rays and that the microwave radiation increases the concentrations of major terpenes relevant to flavour with a smaller loss of aromatic compounds. On the other hand, other authors (Plessi *et al.* 2002) have recognised that one of the most common sanitising treatments is gamma rays treatment, that affects the bacterial DNA reducing its presence by 90%, although it alters the flavour remarkably. In particular, terpenes can be subjected to configuration changes in the positions of double bonds, causing a reduction or a change in the flavour of the product (Decareau 1985; Schiffmann 1986; Piggot & Othman 1993; Antonelli *et al.* 1998).

In fact, spices, herbs, and dried vegetable seasonings are currently treated with ionising radiation to eliminate microbial contamination, this, however, may alter chemical composition and subsequently the flavour of spices in the dependence on the radiation dose used. According to the authors (Wilkinson & Gould 1998), these commodities are commonly dry products and they are relatively resistant to ionising radiation; in general, they can tolerate doses up to 10 kGy, without any significant changes in flavour. Farkas (1998) has detected the threshold doses, causing organoleptic changes of black pepper, which ranged from > 9.0 kGy to 10.0 kGy.

Comparing the gamma irradiation with the heat treatment, it has been unambiguously confirmed that the treatment with ionising energy is more effective against bacteria than the thermal treatment and does not leave chemical residues in the food product (Tjaberg *et al.* 1972; Loaharanu 1994; Byun *et al.* 1996; Thayer *et al.* 1996; Olson 1998). Thus, ethylene oxide and methyl bromide treatments can be effectively replaced by food irradiation, which is less harmful to the spices than heat sterilisation, which implicates the loss of thermolabile aromatic volatiles and/or causes additional thermally induced changes (e.g. thermal decomposition or production of thermally induced radicals). The practice of food processing industry points out that the heat treatment of spices significantly reduces the content of essential oil by one third on average. Since essential oils are responsible for the organoleptic quality of spices (taste and odour), this fact is a weighty argument for the use of irradiation technology for the purposes of spices sterilisation.

Chemical composition of spices essential oils irradiated at various doses have been studied in several publications (Emam *et al.* 1995; Farag *et al.* 1996; Antonelli *et al.* 1998), but till now no work has been devoted to sensory evaluation of individual components of essential oils and their contribution to the overall aroma.

MATERIAL AND METHODS

Investigated material. A sample of dried spice marked as Vietnamese powdered black pepper 550 (ρ = 550 g/dm^3) was obtained from Mäspoma, s.r.o., Zvolen, Slovak Republic. The spice moisture content (11.87%) was determined by the STN 580110 method, article 32. Portions of 80 g each were packed into polyethylene/paper bags (simulation of retail packing).

Heat sterilisation. Heat sterilised sample of black pepper was prepared in the company Mäspoma s.r.o., Zvolen, manufacturing facility Dvory nad Žitavou. Black pepper berries were treated by dry steam at a temperature of 130°C for 3 min (technological norm of the company) whereby maximum inner temperature of berries was 98°C. Subsequently, the berries were ground. Portions of 80 g were packed into polyethylene/paper bags and stored in the darkness in a dry place under ambient conditions.

Irradiation. The packed samples of the powdered spice were irradiated using the gamma-rays ^{60}Co source at average doses of 5 kGy, 10 kGy, and 30 kGy (dose rate 2 kGy/h) according to the commercial practices at ARTIM, s.r.o., Praha, Czech Republic. After the radiation treatment, all the samples were stored in the darkness in a dry place under ambient conditions.

Microbiological analysis. Elementary microbiological investigation of the spice samples untreated (control), heat treated, and gamma-irradiated at doses of 5 kGy, 10 kGy, and 30 kGy was carried out according to ISO norms STN ISO 4832:1997 (Microbiology – General guidance for the enumeration of coliforms – Colony count technique), STN ISO 4833: 1997 (Microbiology – General guidance for the enumeration of micro-organisms – Colony count technique at 30°C) and STN ISO 7954:1997 (Microbiology – General guidance for enumeration of yeasts and moulds – Colony count technique at 25°C). All samples were investigated 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 10 g of powdered spice untreated, heat treated, and treated at appointed doses of gamma-irradiation by simultaneous distillation/extraction using Likens-Nickerson apparatus and diethyl ether as the extraction solvent. Two parallel isolations were made from each sample.

The determination of the essential oil content was executed by the method of European Pharmacopoea 4 from powdered black pepper (10 g) using xylene as the 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 using the split technique at 250°C. The column temperature was programmed from 35°C to 250°C with a gradient of 1.7°C/minutes. The ionising voltage (EI) was set to 70 eV.

GC-TOF MS and GC × GC × TOF MS. The time-of-flight mass spectrometer PEGASUS III, LECO Corp. – equipped with columns Equity 1, 60 m × 0.25 mm × 0.25 μm and Supelcowax 2.5 m × 0.1 mm × 0.1 μm – was used for the identification of unknown compounds and differentiation between the untreated and gamma-irradiated samples. Helium was employed as carrier gas. The samples were injected using the split technique at 250°C. The temperature program was kept from 40°C, 1 min, to 240°C, 3 min, with a gradient of 4°C/minutes.

Gas chromatography (GC). Hewlett-Packard HP 5890II gas chromatograph with flame-ionising detector (FID) was used for the determination of the relative percentage composition of volatile compounds as well as their linear temperature programmed retention indices. The samples were analysed 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 a gradient of 2°C/minute. The linear velocity of hydrogen as the carrier gas was 36 cm/min (measured at column temperature 143°C). The linear retention indices (RI) were calculated in accordance with Van den Dool and Kratz equation (Van den Dool & Kratz 1963). As the reference standards, *n*-alkanes C_8–C_{18} were used.

Statistical analysis. The effects of the spice treatments on the relative percentage composition of volatiles were compared using Analysis of variance (one-way, repeated measurements). When the data complied with the test of normality and equal variance, the Holm-Sidak test was used for pairwise comparisons (overall significance level of 1%). In the case that any of these assumptions failed, ANOVA on ranks (repeated measurements) was calculated and Tukey test was used for pairwise comparisons (overall significance level of 1%).

Gas chromatography/olfactometry (GC/O) – aroma extract dilution analysis (AEDA). GC/O is the technique that connects the instrumental method (gas chromatography) with sensory analysis (olfaction). One of the GC/O methods is the so-called AEDA (Aroma Extract Dilution Analysis). It is a quantitative gas chromatography – olfactometry procedure for determining the potency of odorants in food extracts. In this method, the effluent from GC column is divided by a splitter to FID (flame-ionising detector) and to the sniffing port, where the aroma character and relative aroma potency of individual volatile constituents of the food extract are determined by human nose. The results of this analysis are the so-called flavour dilution (FD) factors of aroma – active compounds. The FD factor of a compound is the highest ratio of dilution of the initial volatiles extract, at which its odour is detected by human nose. Therefore, the higher FD factor of the compound means its higher contribution to the overall aroma.

The AEDA Hewlett-Packard HP 5980II gas chromatograph equipped with FID, Ultra 1(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/minute. The flavour dilution (FD) factors (Grosch 1993) and odour descriptions were determined by sniffing the 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 5 trained judges.

RESULTS AND DISCUSSION

Total elimination of the MO present is the primary aim of the spice treatment using the heat sterilisation or irradiation. The microbiological results confirmed that the total count of 10^6 colonies MO in the untreated (control) sample of black pepper was reduced to less than 1 CFU/g using 5 kGy irradiation dose (Table 2); this irradiation level completely sterilised the tested spice.

It was noticed that the heat treatment of black pepper berries had reduced total count of MO by one order in comparison with the control sample. The number of MO increased 2.5-times in the control sample during 3 months storage. During the same time period, the count of MO was multiplied by one order in the heat treated sample. The status in gamma-irradiated samples at all particular doses remained unchanged during 3 months storage, hence the ionising radiation was shown to be a powerful sterilising tool for the spice.

The extracts from the untreated and gamma-irradiated samples of powdered black pepper were pale yellowish liquids with a characteristic, terpenic, powerful odour of black pepper trait. The extract of the heat treated sample spice was darker – coloured with less strong overall odour. The qualitative compositions of volatile oils obtained from the control, heat treated sample, and from irradiated samples of spice at various doses were identical. These results were also confirmed by GC-TOF MS analysis. The steam-volatile black pepper oil consisted primarily of monoterpene and sesquiterpene hydrocarbons, and oxygenated compounds (Figure 1). The GC/FID and GC/MS analyses revealed more than sixty compounds from which more than fifty ones could be identified by the mass spectra and linear retention indices published. α- and β-pinene, sabinene, car-3-ene, limonene are the important components of the monoterpene fraction. β-Caryophyllene is the major sesquiterpene and it is the main compound of volatile black pepper oil according to the quantity (36% rel.). Most of the compounds were affected by the heat treatment only. The heat treatment caused a significant increase (at 1% significance level) of some monoterpenes: α- and β-pinene, camphene, sabinene, myrcene, α-phellandrene, 3-carene, α-terpinene, *p*-cymene, 1,8-cineole, limonene, γ-terpinene proportions compared to the control. These changes might be due to the thermal isomerisation products of some terpenes as shown by Richard *et al.* (1971) and by Farag *et al.* (1996).

The heat treatment as well as excess irradiation dose of 30 kGy induced a significant decrease of some volatiles in comparison with the control. The control sample had significantly higher levels of β-elemene, α-guaiene, α-humulene, and β-farnesene. No exceptional changes were observed in the volatile oil compounds contents at radiation doses of 5 kGy and 10 kGy (toxicologically and nutritionally confirmed as safe maximal dose). These findings are in agreement with the results of the authors Wilkinson and Gould (1998). These have quoted that the changes in odour and flavour may occur above 15 kGy, depending upon a number of factors, including the individual product, age of seasoning, storage temperature, humidity, and packaging. The authors subsequently presented that chemical constituents of herbs and spices remain largely unchanged, and that an increase in the extractability of the irradiated products may take place which may result in an apparent increase in the volatile oil yield, lipid content, and hot water solubles in some spices.

According to our findings, many compounds were affected by the highest dose of irradiation used (30 kGy), which increased the proportion of some oxygenated compounds: trans-sabinene hydrate, 3,4-dimethylstyrene, cyclohexenol, *p*-cy-

Table 2. Microbiological analysis of untreated, heat treated, and irradiated black pepper immediately after irradiation and after 3 months of storage

Radiation dose (kGy)	Total MO(CFU/g)		Coliforms (CFU/g)		Yeasts (CFU/g)		Moulds (CFU/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

CFU/g – colony forming unit per gram

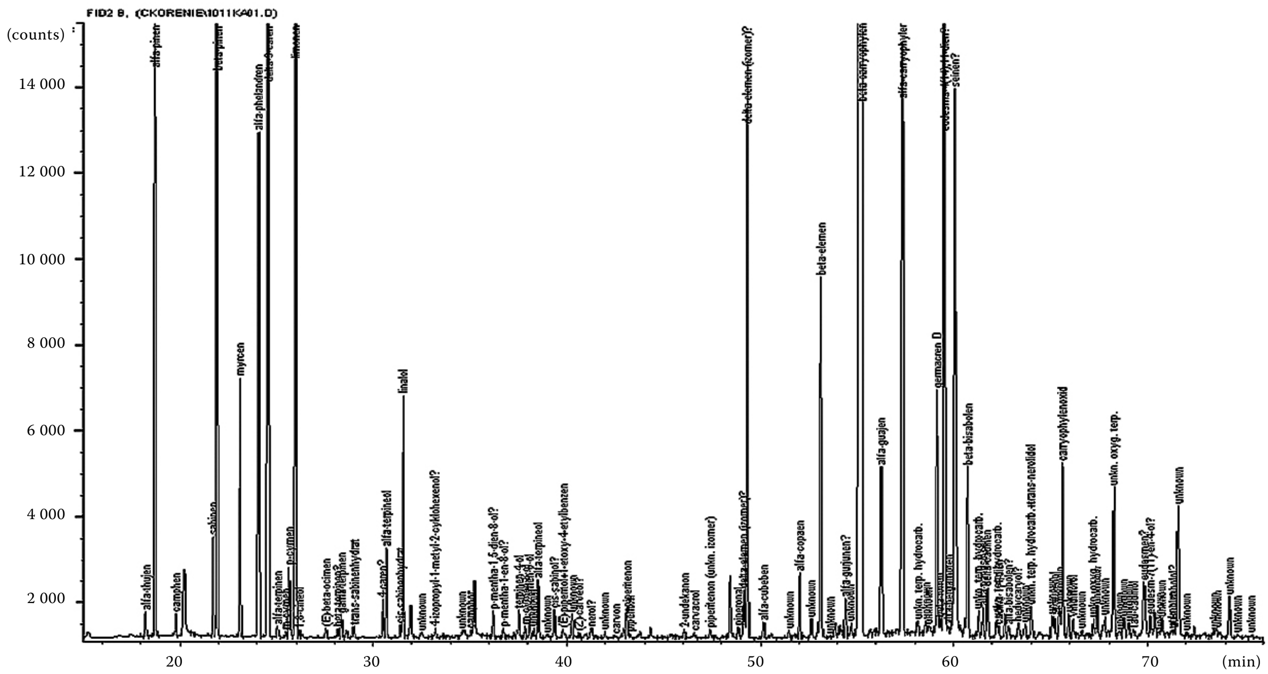

Figure 1. Chromatogram of the black pepper essential oil volatile components – untreated sample (control)

men-8-ol, terpinen-4-ol, α-terpineal, α-terpineol, eucarvon, piperitenon, piperiton, undecanone, and spathulenol as compared with the control. The most important change could be observed at an ionising dose of 30 kGy (3-times excess of the authorised dose) resulting in triple increase of caryophyllene oxide in comparison with the control. On the contrary, the equipollent loss of β-caryophyllene was registered.

The effect mentioned was observed in the spice irradiated by 30 kGy as well as in essential oil spread on neutral carrier (Na_2SO_4) irradiated by 30 kGy. These changes can be explained through the irradiation effects on terpenes. The irradiation process at low doses is considered to be a cold physical treatment for food, because a significant heating of product is omitted (the temperature of the product may rise by max. 5°C at the dose of 10 kGy). Therefore, irradiation does not show any thermal effect on the flavour compounds but via one of the following reaction pathways, i.e. via oxidation or hydroxylation of the aromatic ring of terpene, or via an indirect effect, which can generate free radicals from the water contained in spices (approx. 10%) (Urbain 1986). These radicals can react with terpenes to produce terpene oxides and terpene alcohols. On the other hand, terpenes, which are incorporated in most of the essential oils, have the same skeleton structure but differ in their functional groups, such as OH, CHO, or COOH. Therefore, configurational changes can occur by high doses, including changes in the positions of the double bonds and the functional groups to produce different compounds.

Gas chromatography-olfactometry analyses (AEDA) of volatile extracts revealed 22 potent odorants with FD factors in the range from 10 to 200. It was found that 3-methylthiopropanal, oct-1-en-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 further eight unknown compounds are responsible for the characteristic flavour of black pepper essential oils untreated, heat treated, and irradiated at the appointed doses (Table 3). The identification of these compounds was performed on the basis of EI mass spectra, linear retention indices, and aroma character, as compared to those of reference compounds or published data. The effect of irradiation on the FD factors was not proved in a majority of the individual compounds. The most potent odorants are linalool (9) with a flowery aroma character and an unknown compound (22) with spicy, typical black pepper-like aroma, both of them with FD = 200. The influence of irradiation on components 2, 7, 15, 16 (marked as unknowns) is not significant (Table 3). The differences between the FD factors obtained are only in one dilution step, however, no

Table 3. Influence of heat treatment and radiation treatment on potent odorants of black pepper

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

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

important changes were perceived in the overall aroma of the appointed black pepper volatile oils. In connection with the decrease of some thermolabile volatiles caused by the spice heat treatment, their FD factors were changed in comparison with both the control and appointed radiation doses. The greatest alterations occurred in context with linalool (FD = 10), piperitone (FD = 10), β-damascenone (FD = 10), and an unknown compound (22) with FD = 10 (Table 3). The noticeable decrease of FD factors of the aroma-active compounds studied produced a significant effect on the overall aroma of heat treated black pepper.

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