Multi-experimental study of γ-radiation impact on oregano (*Origanum vulgare* L.)

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Summary

The influence of γ-irradiation at doses from 5 kGy to 30 kGy on oregano (*Origanum vulgare* L.) was studied by the conventional microbiological analysis tests and by the combination of GC-FID; GC-MS; GC-Olfactometry (GC-O); EPR and UV-VIS spectroscopy. Microbiological analysis proved that γ-irradiation at 7.5 kGy was sufficient to achieve the microbiological decontamination of oregano samples, persisting even after 8 months of storage. The study of γ-irradiation impact on essential oils' composition and on organoleptic quality changes, evaluated using GC-FID, GC-MS, and GC-O involving the Aroma Extract Dilution Analysis (AEDA) method revealed no changes in the chemical composition and non-considerable changes in the content of volatile oils' compounds at samples γ-irradiated up to 10 kGy. Only non-significant changes of flavours upon the radiation treatment even at dose of 30 kGy were noticed. The analysis of EPR spectra confirmed the presence of two signals in a reference sample, attributed to Mn^{2+} ions and to stable semiquinone radicals. In addition, the dose-dependent formation of radicals of different origin (mostly cellulose and carbohydrate) showing the diverse thermal stability and life-time was noticed in γ-irradiated samples. UV-VIS experiments confirmed that the antioxidant activity of oregano ethanolic extracts was only slightly affected by the absorption of γ-radiation.

Keywords

oregano; γ-irradiation; microbiological contamination; organoleptic quality; chemical composition; sensorial analysis

Herbs and spices are traditionally used in gastronomy as flavourings, but they revealed also beneficial effects on human health [1–4]. Moreover, antioxidants present in them are capable of acrylamide reduction in foods into which the spices are added [5, 6]. During their harvesting, handling, transportation and storage, they frequently undergo microbiological contamination. As follows from several older studies, commercial spices are generally contaminated with 10^5 to 10^8 microorganisms per gram [7]. To ensure the consumer safety, microbiological contamination level should not exceed an acceptable limit of 10^4 microorganisms per gram [8].

Different methods have been used to reduce the microbiological contamination in spices. Fumigation with volatile microbicide fumigants, e.g. with ethylene oxide, propylene oxide and methyl bromide, was found to be effective but cannot be used in a wider range due to toxicity and carcinogenicity of these agents [8]. Radiation disinfection by means of low doses of γ-rays, X-rays or electron beams was recognized as a means to effectively control foodborne pathogens such as *E. coli* or *Vibrio vulnificus* [9]. From all of the previously mentioned methods, γ-irradiation treatment of foods and plant products, in particular of herbs and spices, is nowadays accepted as a standard and safe sterilization technique. It lowers the risk of microbiological contaminations to minimum and prolongs the durability of products [10]. Utilisation of γ-irradiation was recently accepted also by international authorities [11]. The Directive 1999/3/EC established a Community list of foods and food ingredients that may be treated with ionizing radiation [12].

Toxicological and nutritional tests proved the safety of foods irradiated at doses below 10 kGy. This value was also accepted by Codex Alimentarius General Standard for irradiated foods as the maximum legal or allowed average absorbed dose for dried aromatic herbs, spices and vegetable seasonings sterilization, with an exception for cases when higher dose application is necessary

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to achieve a legitimate technological purpose [9, 12–15]. On the other hand, US Food and Drug Administration (FDA) set the maximum allowed dose for culinary herbs, seeds, spices, vegetable seasonings and blends of these aromatic vegetable substances to 30 kGy [16].

However, ionizing radiation treatment may alter chemical composition and flavour of spices. An unambiguous detection of irradiation and the determination of the absorbed doses, even a long time after the radiation process, is required [17–19].

Oregano (*Origanum vulgare*, L.) is one of the most frequently used spices world wide. It contains numerous different antioxidants, according to phytochemical databases, their number reaches up to 34 [20]. Carvacrol and thymol are, in dependence on oregano variety, the major ones, most significantly influencing its taste and aroma properties [21–25].

This study deals with the γ-radiation impact on microbiological quality of oregano. In addition, the effect of irradiation on changes in chemical composition was investigated by means of several analytical and spectroscopic techniques, e.g. by GC-FID, GC-MS, EPR and UV-VIS spectroscopy. Essential oils' composition was investigated with the accent on the determination of aroma active compounds by GC-Olfactometry (GC-O), from the possible γ-radiation induced flavour changes point of view, as well.

MATERIAL AND METHODS

Samples characterisation

Commercially available samples of oregano leaves (dry matter content, 90.1%) from Cambidi – Izmir, Turkey, were purchased from Mäspoma, Zvolen, Slovakia; packed into the 75 g polyethylene/paper bags (simulation of retail packing) and subsequently irradiated using the ^{60}Co source at average doses of 5 kGy, 7.5 kGy, 10 kGy and 30 kGy (doses rate 2 kGy.h^{-1}) according to commercial practices at Artim, Prague, Czech Republic. After the radiation treatment, all the samples were stored in closed bags in the darkness on dry place at ambient conditions.

Microbiological analysis

Elementary microbiological analysis of the reference, non-irradiated oregano sample and of samples γ-irradiated at doses mentioned above was carried out following the STN ISO 4833, STN ISO 4832 and STN ISO 7954 standards four times: immediately after the irradiation and after 4, 6 and 8 months of post-irradiation storage [26–28]. Total counts of microorganisms, presence of coliforming bacteria, yeasts and moulds was determined and expressed as colony forming units per gram (CFU.g^{-1}). Results obtained are presented in Tab. 1.

Extracts preparation

Essential oils for GC-MS, GC-FID and GC-O analysis were isolated from 10 g of dried oregano leaves by simultaneous distillation extraction using Likens-Nickerson apparatus and diethyl ether as a solvent. Two parallel isolations were performed from each sample. Extracts used in UV-VIS experiments were prepared as described previously [29].

GC-MS analysis

GC-MS analyses were performed on Hewlett-Packard HP 5971A mass-selective detector directly coupled to HP 5890II gas chromatograph (Hewlett-Packard, Waldbronn, Germany). Fused silica capillary column Ultra1 (Hewlett-Packard), 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 the gradient of 1.7 °C.min^{-1}. The ionizing voltage (*EI*) was set to 70 eV.

Tab. 1. Microbiological analysis of a reference, non-irradiated oregano sample and of samples γ-irradiated at different radiation doses.

Radiation dose [kGy]	Total count of microorganisms [CFU.g^{-1}]		Coliforming bacteria [CFU.g^{-1}]		Yeasts [CFU.g^{-1}]		Moulds [CFU.g^{-1}]	
	2 days	4 months[a]	2 days	4 months	2 days	4 months	2 days	4 months
0	1.4×10^6	1.0×10^6	1.1×10^5	6.2×10^5	1.0×10^4	<10	1.0×10^2	1.4×10^4
5	9.5×10^2	2.5×10^3	<10	<10	<10	<10	<10	3.0×10^1
7.5	<10	<10	<10	<10	<10	<10	<10	1.0×10^1
10	<10	<10	<10	<10	<10	<10	<10	<10
30	<10	<10	<10	<10	<10	<10	<10	<10

Analysis was performed immediately after the irradiation and after 4, 6 and 8 months of the post-irradiation storage. CFU – colony forming units, a - status after 4, 6 and 8 months remained unchanged.

GC-FID analysis

Hewlett-Packard HP 5890II gas chromatograph coupled with FID detector was used for the determination of volatile compounds' relative percentage composition and to assess their linear temperature programmed retention indices. The extracts were analysed on Ultra1 fused silica capillary column 50 m × 0.32 mm × 0.50 μm at the temperature programmed from 35 °C up to 250 °C with the gradient of 2 °C.min^{-1}. Linear velocity of the carrier gas hydrogen was set to 36 cm.min^{-1} (measured at a column temperature of 143 °C). Linear retention indices (*RI*) were calculated using the Van den Dool and Kratz equation [30] with C_7–C_{17} alkanes as reference standards.

GC-O Aroma Extract Dilution Analysis (AEDA)

Aroma active compounds of oregano essential oils were determined by GC-O, involving the AEDA method. Hewlett-Packard HP 5980II gas chromatograph equipped with FID detector, Ultra1 fused silica capillary column 50 m × 0.32 mm × 0.50 μm, the column effluent splitter 1 : 1 and a sniffing port were used. Analyses were carried out in splitless mode. The temperature was programmed from 35 °C (0.5 min) up to 250 °C, with a gradient of 2 °C.min^{-1}.

Flavour dilution (*FD*) factors and odour descriptions were determined by sniffing of compounds eluting from the capillary column. For the *FD* factors explanation, refer e.g. to [31]. The extracts were diluted with diethyl ether stepwise, 1 : 10, 1 : 100, 1 : 500, 1 : 1000, 1 : 2000 and 1 : 10000, respectively. Sensory evaluations were performed by a panel of 5 trained judges.

EPR experiments

Thin-wall quartz EPR tubes (internal diameter of 3 mm, length of 150 mm, and wall thickness about 0.1 mm) were used for the measurements of solid samples, enabling the formation of cylindrical samples with identical dimensions. Oregano sample (100 mg) was placed in the tube (sample column height, (5.2 ± 0.2) cm; and then inserted into a standard TE_{102} (ER 4102 ST) rectangular cavity of an EMX X-band EPR spectrometer (Bruker, Karlsruhe, Germany). The EPR spectrum was recorded at 298 K. The temperature-dependent measurements were carried out using a Bruker temperature control unit ER 4111 VT assembled to EMX EPR spectrometer. The response and settings of EPR spectrometers were checked by means of solid 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Strong pitch standards (Bruker) daily before the experiments, as described elsewhere [29, 32–34, 36, 37].

The experimental EPR spectra processing, evaluation and simulation were carried out using WIN EPR and SimFonia programs (Bruker) as described e.g. in [29, 32–34, 36, 37].

UV-VIS experiments

Double-beam UV-VIS spectrometer Specord M40 (Carl Zeiss, Jena, Germany) with an appropriate equipment was used for the monitoring of antioxidant properties. All experiments were carried out in the same square quartz UV-VIS transparent cells (path length, 1 cm). The monitoring of oregano extract antioxidant ability was realized as described previously [29]. DPPH radical-scavenging assay, thiobarbituric acid reactive substances value (*TBARS*), ferric reducing power and total phenolic compounds content (*TPC*) of extracts were evaluated [29].

Statistical analysis

Influence of spice treatments on the relative composition of volatiles was compared using one-way Analysis of Variance (ANOVA). Holm–Sidak test was used for pair-wise comparisons (overall significance level, 0.05) if the data passed the test of normality and equal variance. Otherwise, ANOVA on ranks was calculated and Tukey test was used for pair-wise comparisons (overall significance level, 0.05).

Results of EPR experiments were statistically evaluated as described previously [29, 32–34, 36, 37]. Statistical analysis of UV-VIS results was performed using one-factor ANOVA at the significance level of 0.05 as described previously [29].

RESULTS AND DISCUSSION

Microbiological analysis

Decrease of microbiological contamination up to the acceptable level of 10^4 CFU.g^{-1} [8] or total decontamination is the primary aim of spice irradiation. Results of the microbiological analysis confirmed that total count of 1.4×10^6 CFU.g^{-1} present in the reference oregano sample decreased to less than 10 CFU.g^{-1} when exposed to radiation dose of 7.5 kGy. Total elimination of other pathogens was also achieved (Tab. 1). It was also proved that this status remained unchanged even after 8 months of post-irradiation storage.

GC analysis of oregano essential oils

GC-FID and GC-MS analysis

As follows from the GC-FID and GC-MS analysis, the steam-volatile oregano oils consist from numerous different volatile compounds,

Tab. 2. Influence of γ-irradiation on potent odorants of oregano.

No.	Odorants	*RI*[a]	*FD* factor					Odour quality	Identification[b]
			0 kGy	5 kGy	7.5 kGy	10 kGy	30 kGy		
1	*cis*-3-hexenal	768.8	1 000	1 000	1 000	1 000	1 000	leaf-like, green, grassy	RI, ST, A
2	1-octene-3-ol	963.2	10 000	10 000	10 000	10 000	10 000	mushroom-like, earthy	MS, RI, ST,A
3	myrcene	981.9	100	100	100	100	100	hop oil-like, slight bitter	MS, RI, ST,A
4	α-phellandrene	993.2	100	100	100	100	100	herbaceous, dill-like	MS, RI, ST,A
5	*p*-cymene	1 008.9	1 000	1 000	1 000	100	100	exotic flowery, citrus-like	MS, RI, ST,A
6	eucalyptol	1 016.0	1 000	1 000	1 000	1 000	1 000	peppermint, cool, fresh	MS, RI, ST,A
7	(Z)-β-ocimene	1 026.8	1 000	1 000	1 000	1 000	1 000	fresh, bitter	MS, RI, ST,A
8	(E)-β-ocimene	1 037.5	1 000	1 000	1 000	1 000	1 000	bitter, grapefruit, earthy	MS, RI, ST,A
9	γ-terpinene	1 046.7	100	100	100	100	100	herbaceous, metallic, gas	MS, RI, ST,A
10	guaiacol[t]	1 065.7	2 000	2 000	2 000	2 000	2 000	bitter, phenolic, smoke	RI, A
11	linalooloxide (unkn.)	1 071.3	100	100	100	100	100	plastic, woody	MS, A
12	nonanal	1 081.4	500	500	500	500	500	earthy, cucumber, waxy	RI, ST, A
13	linalool	1 083.0	10 000	10 000	10 000	10 000	10 000	flowery, citrus-like	MS, RI, ST,A
14	2,6-nonadienal (unkn.)	1 120.8	500	500	500	500	500	waxy, varnish	RI, ST, A
15	(E)-2-nonenal	1 133.0	1 000	1 000	1 000	1 000	1 000	plastic, cucumber, waxy	MS, RI, ST,A
16	(Z)-2-nonenal	1 137.9	100	100	100	100	10	flowery, iris-like	MS, RI, ST,A
17	borneol	1 144.9	1 000	1 000	1 000	1 000	1 000	dandelion, earthy-pepper	MS, RI, ST,A
18	1-terpinen-4-ol	1 158.2	100	100	100	100	10	musty, mouldy	MS, RI, ST,A
19	unknown	1 167.7	500	500	500	500	500	smoke, phenolic, ink-like	–
20	α-terpineol (unkn.)	1 169.6	500	500	500	500	500	flowery, lilac	MS, RI, ST,A
21	2,4-dimethylanisole[t]	1 214.2	10 000	10 000	10 000	10 000	10 000	balsamic, anise-like	MS, A
22	unknown	1 244.5	1 000	1 000	1 000	1 000	1 000	balsamic, phenolic	–
23	(E,Z)-2,4-decadienal[t]	1 257.4	100	100	100	100	100	B-vitamin, meaty broth	ST, A
24	carvacrol	1 275.7	10 000	10 000	10 000	10 000	10 000	herbaceous, oregano-like	MS, RI, ST,A
25	unknown	1 301.3	500	500	500	500	500	meaty broth, bitter, fish	–
26	eugenol	1 325.4	1 000	1 000	1 000	1 000	1 000	clove-like, spicy	MS, RI, ST,A
27	β-damascenone	1 369.5	10 000	10 000	10 000	10 000	10 000	fruity, dried plum	RI, ST, A
28	methyleugenol[t]	1 403.6	100	100	100	100	100	fresh, herbaceous	MS, A
29	β-caryophyllene	1 410.9	500	500	500	500	500	spicy, terpeny, woody	MS, RI, ST,A
30	unknown	1 421.1	500	500	500	500	500	sweet, fresh, herbal, dill	–
31	aromadendrene	1 431.0	100	100	100	100	100	pleasant note, citrus-like	MS, RI, ST,A
32	α-humulene	1 443.8	100	100	100	100	100	terpeny, varnish, woody	MS, RI, ST,A
33	bicyclogermacrene[t]	1 474.8	100	100	100	100	10	terpeny, carrot, parsnip	MS
34	β-bisabolene	1 498.0	100	100	100	100	100	fresh, herbal, toothpaste	MS, A
35	δ-cadinene	1 510.1	500	500	500	500	500	balsamic, spicy, thyme	MS, A
36	spathulenol[t]	1 557.1	10	10	10	10	10	pleasant, aromatic,woody	MS
37	isoaromadendrene epoxide[t]	1 647.8	100	100	100	100	100	spicy, balsamic, herbal	MS, A

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, unkn. - unknown isomer.

primarily of monoterpene and sesquiterpene hydrocarbons, and of oxygenated compounds. Compounds α-thujene, camphene, α-pinene, sabinene, 3-carene, *p*-cymene, limonene, myrcene, α-phellandrene, *cis*-, *trans*-β-ocimene and γ-terpinene [21] were identified as dominant components of the monoterpene fraction. Besides them, oxygenated monoterpenoids, mainly linalool and hotrienol, but also eucalyptol, carvone, camphore, borneol, eugenol or *cis*-β-terpineole were detected [21]. Linalool and hotrienol are the major oxygenated monoterpenoids being also the main compounds of oregano essential oil, representing approx. 50% of the whole essential oils composition.

The substantial part of essential oils is represented by phenolic compounds thymol and carvacrol [4, 21], reaching up to approx. 30% of the whole volatile fraction.

These analyses revealed that majority of the identified oregano volatile constituents were only slightly or negligibly affected by the radiation treatments, as the qualitative compositions of volatile oils present in reference oregano sample and in samples γ-irradiated at doses from 5 up to 30 kGy remained unchanged. Only in an oregano sample γ-irradiated at a dose of 10 kGy, significantly increased contents of linalool, hotrienol and sabinenhydrate were noticed in comparison to the reference. In addition, γ-irradiation at higher doses (10 kGy and 30 kGy) caused a conspicuous increase of *p*-methoxypyridine, α-terpinolene and two linalooloxides contents. On the other hand, decreased bicyclogermacrene contents in all the γ-irradiated samples was detected. In accordance with previously published data [35], the observed changes probably come from the γ-radiation induced oxidation or hydroxylation of terpenes' aromatic rings. As a result of radiation treatment of foods with low moisture contents (up to 10%), the increased production of alcohols was detected by URBAIN [35]. Besides that, the production of free radicals, multiplying the primary effect of irradiation on terpenes, was observed.

Sensorial analysis

GC-O analysis

Gas chromatography-olfactometry analysis revealed the presence of 37 key odorants with *FD* factors ranging from 10 up to 10000. As the most potent odorants, 1-octene-3-ol (2), linalool (13), 2,4-dimethylanisole (21), carvacrol (24) and β-damascenone (27), each of them with *FD* = 10000, were identified (Tab. 2). For the majority of aroma active compounds, no effect of irradiation on *FD* factors was proven. Non-significant differences in *FD* factors were observed only for compounds No. 5, 16, 18 and 33, just in one dilution step (Tab. 2). However, these differences did not influence the overall aroma profile of oregano volatile oils in any way.

EPR experiments

Fig. 1 shows the EPR spectrum of the reference oregano sample and of a sample irradiated at a dose of 30 kGy, measured one week after the radiation process. The analysis of EPR spectra of the reference sample confirmed the natural presence of paramagnetic Mn^{2+} ions and of stable semiquinone radical structures produced by the oxidation of polyphenolic compounds present in plants, in accordance with previously published data [25, 29, 32–37]. These radicals are practically not affected either by thermal treatment of sample at temperatures from 293 K to 353 K or by the storage [29, 32–34].

The application of γ-radiation on oregano samples led to the dose-dependent formation of additional EPR signals, attributed to 'carbohydrate'and 'cellulosic' radical species [25, 29]. These radicals originate mostly from cleavage processes of cellulose matter and/or of other polysaccharides forming the skeleton of plant structures and their cells. Although the 'cellulosic' EPR signal was declared as a marker of γ-irradiation of natural cellu-

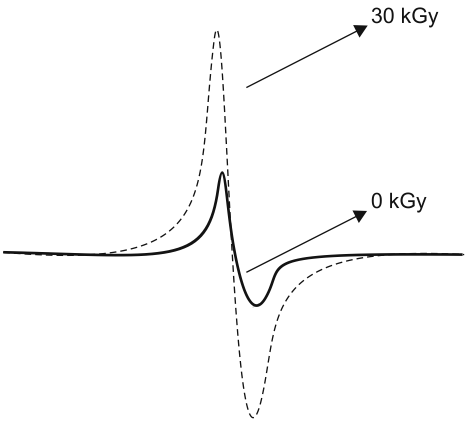

Fig. 1. Experimental EPR spectra of a reference, non-irradiated sample and oregano sample irradiated at a dose of 30 kGy.
EPR spectra were recorded one week after γ-radiation treatment using the magnetic field width, SW = 8 mT at 298 K.

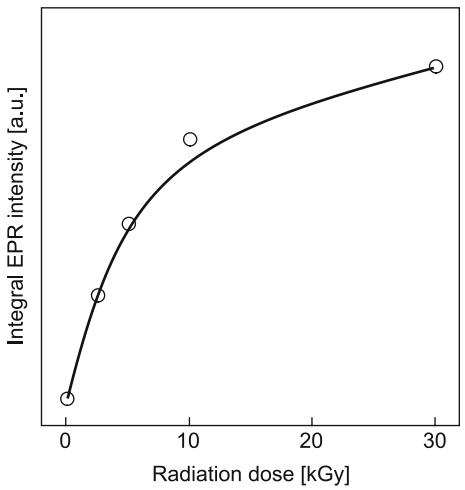

Fig. 2. The dependence of integral EPR intensity of oregano samples on γ-radiation dose evaluated for EPR spectra measured at 298 K using 0.633 mW microwave power one week after the irradiation.

lose-containing materials, its intensity in oregano samples is minimal [29, 38]. The dependence of integral EPR intensity of oregano samples on the absorbed dose of γ-radiation, evaluated from experimental EPR spectra measured one week after the radiation process, is depicted on Fig. 2.

As was previously published by HORVÁTHOVÁ et al. [29], the radiation-induced radicals detected in oregano samples reveal only a limited lifetime and their stability is also strongly temperature-dependent. The lowest lifetime and thermal stability was estimated for 'cellulosic' radical structures, followed by 'carbohydrates' [29, 32].

UV-VIS experiments

Results of UV-VIS experiments proved that γ-irradiation only in a negligible way influences both the ability of oregano extract to terminate DPPH free radicals and to reduce ferric ions to ferrous ones. Slightly increased *TBARS* values and *TPC* were noticed in extracts prepared from irradiated samples immediately after the radiation process. In addition, during 5 months of the post-irradiation storage, the differences in *TBARS* values still remained, while the *TPC* content differences gradually disappeared [29].

CONCLUSION

The study of γ-irradiation impact on oregano is a complex problem, as it consists of numerous well-balanced chemical constituents. It was proven that γ-irradiation even at a dose of 7.5 kGy is an effective microbiological decontamination tool. The effects of decontamination still persist after 8 months of post-irradiation storage. GC-FID, GC-MS and GC-O techniques confirmed the presence of numerous different compounds in oregano essential oils, some of them being active as potential odorants, but no significant changes were detected neither in their quantitative composition nor in their odour quality, upon the radiation treatment at various doses up to 30 kGy. As a result of γ-irradiation, the dose-dependent formation of different radical structures of a limited lifetime and thermal stability was detected. All oregano extracts revealed significant ability to terminate DPPH free radical. Similarly like *TPC* contents and *TBARS* values, it was only slightly influenced by the absorption of γ-radiation.

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