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Effect of Gamma and e-Beam Radiation on the Essential Oils of *Thymus vulgaris thymoliferum*, *Eucalyptus radiata*, and *Lavandula angustifolia*

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The microbiological contamination of raw plant materials is common and may be adequately reduced by radiation processing. This study evaluated the effects of γ- and e-beam ionizing radiations (25 kGy) on three plants used as food or as medicinal products (*Thymus vulgaris* L., *Eucalyptus radiata* D.C., and *Lavandula angustifolia* Mill.) as well as their effects on extracted or commercial essential oils and pure standard samples. Comparison between irradiated and nonirradiated samples was performed by GC/FID and GC/MS. At the studied doses, γ and e-beam ionizing radiation did not induce any detectable qualitative or quantitative significant changes in the contents and yields of essential oils immediately after ionizing radiation of plants or commercial essential oils and standards. As the maximum dose tested (25 kGy) is a sterilizing dose (much higher than doses used for decontamination of vegetable drugs), it is likely that even decontamination with lower doses will not modify yields or composition of essential oils of these three plants.

KEYWORDS: Essential oil; γ-ionizing radiation; *e*-beam ionizing radiation; GLC, GC/MS

INTRODUCTION

One of the major problems associated with storage and export of dry samples of plants is their susceptibility to heavy microbial contamination and insect infestation, resulting in inferior quality of the product with lower market acceptability (*1*). Under the prevailing production and handling conditions, herbs and other vegetables contain a large number of microorganisms capable of causing spoilage and often human diseases (*2*−*3*). Radiation exposure offers an effective alternative means of reducing microbiological contamination and insect infestation. γ- or e-beam radiation processing is an attractive alternative method for sterilization and decontamination. Its main advantages include high penetrating power for X- and γ-rays and high intensity for electron beam, low measurable residues, small temperature rise, and fewer variables to control (dose, temperature, dryness, ...). It can be applied on the final packaged product and is applicable to heat-sensitive compounds (*4*). There are many articles concerning the effect of γ-ionizing radiations on vegetable products or on the volatile oil constituents of several spices (*5*−*9*), whereas the effects of e-beam ionizing radiation have been less studied (*10*).

Because of the increasing use of ionizing radiation for microbial decontamination, we thought it of interest to study changes in essential oils content when samples are exposed to γ- or e-beam ionizing radiation at higher doses (25 kGy) than the dose used for decontamination and to compare the influence of the different environment on the stability of the various constituents. Thus, we report here the effect of these types of ionizing radiation on aromatic plants (*Thymus vulgaris* and *Eucalyptus radiata* air-dried leaves and *Lavandula angustifolia* air-dried flowers), their essential oils, and some pure components.

The decision tree for sterilization choices for nonaqueous liquid, semisolid, or dry powder products (European Agency for the Evaluation of Medicinal Products (EMEA)) gives an order of preference to sterilization methods. Among these, thermal sterilization is referred to as the best choice, and radiation sterilization is cited right afterward and precedes all other methods: it is the recommended alternative method to thermal sterilization. Radiation sterilization is based on the exposure of the drug to ionizing radiation: γ-rays or electron beams. In solid state, the drugs are radioresistant until 25 kGy, and numerous products of degradation appear in traces (*12*). However, aqueous solutions are completely degraded after doses ranging from 0.5 to 1.5 kGy.

EXPERIMENTAL SECTION

Samples. Pure essential oil standards were obtained from Fluka (Deisenhofen, Germany): ±-linalool, (-)-α-pinene, eucalyptol, linalyl acetate, lavandulol, *p*-cymene, limonene, terpinene-4-ol, R-(-)-phellan-

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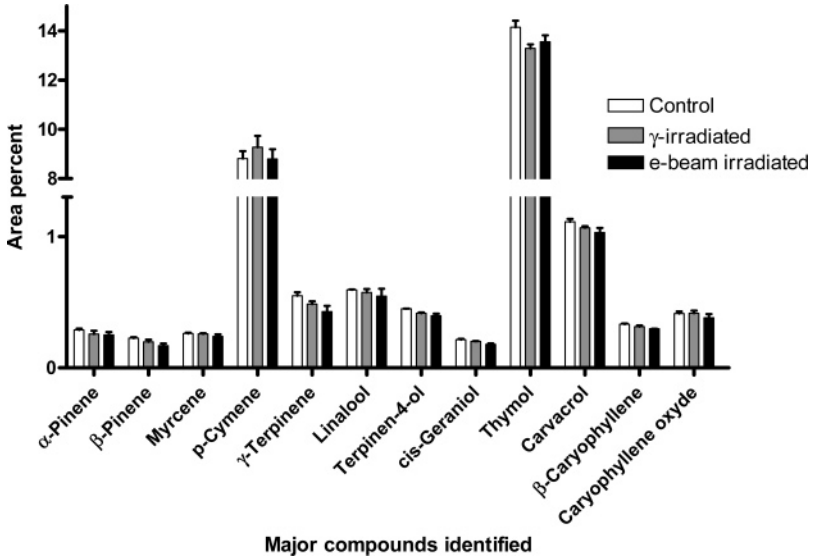

Figure 1. Composition of major constituents identified in the essential oils obtained from control, γ-irradiated and e-beam irradiated *T. vulgaris* air-dried leaves. Identification of compounds was made by both GC-FID and GC-MS; quantification of compounds was made by GC-FID. No significant differences were noticed ($P > 0.05$) between the groups before and after treatment.

Table 1. Percent Yields of Essential Oil Obtained by GC-FID from Nonirradiated and Irradiated Samples[a]

	γ-irradiated		e-beam irradiated	
plant	control	25 kGy	control	25 kGy
E. radiata	0.84 ± 0.07	0.85 ± 0.03	0.83 ± 0.03	0.84 ± 0.05
L. angustifolia	0.44 ± 0.04	0.44 ± 0.03	0.45 ± 0.02	0.44 ± 0.02
T. vulgaris	1.11 ± 0.06	1.12 ± 0.08	1.12 ± 0.06	1.12 ± 0.05

[a] No significant differences were noticed ($P > 0.05$) between the groups before and after treatment.

drene, and methyl palmitate. Commercial essential oils from *T. vulgaris thymoliferum* L., *E. radiata* L., and *L. angustifolia* P. Miller were obtained from Aromalys (Rixensart, Belgium). The dried plant samples (*T. vulgaris* L., *E. radiata* D.C., and *L. angustifolia* Mill.) came from Tilman (Baillonville, Belgium, batches no. 01L17/8345, 01L06/B307, 05K22, respectively).

Isolation Of Essential Oils From Plants. Triplicate 30 g portions of air-dried leaves (*T. vulgaris thymoliferum* and *E. radiata*) or flowers (*L. angustifolia*) from control and irradiated samples were hydrodistilled for 2 h in a Clevenger-type apparatus as described in the European Pharmacopoeia (*11*) to produce the essential oils, which were stored at +4 °C until analysis.

Ionizing Radiation Treatment. Standards and commercial essential oil samples in sealed glass vials protected from light and air-dried plants in plastic package were irradiated in independent experiments. For the air-dried plants, a third of the quantity was submitted to γ- or e-beam ionizing radiation. The remaining quantity was kept as a nonirradiated control sample. All batches were analyzed for volatile oils within 1 week of storage at 4 °C.

Different γ-rays or e-beam facilities were used for ionizing radiation. The γ-ray facility used was a panoramic ^{60}Co chamber (UCL, Louvain-La-Neuve, Belgium). The dose rate was 300 Gy h^{-1}, and to reach the exposed dose, several days are required. The e-beam facility used was a double-beam linear electron accelerator (LINAC) (Mölnlycke, Waremme, Belgium). The beam power of each electron generator was about 20 kW. The accelerated electrons were delivered in pulses of 474 and 478 Hz, respectively. The dose of 25 kGy was given in a few seconds; the dose rate of the double LINAC was in an order of magnitude of 6.3×10^4 kGy/h. An internal standard, a polymethylmethacrylate (PMMA) film, was used to control the delivered dose when irradiating the sample. Its absorbency was measured afterward. The dosimetry was determined by certified radiation centers.

Table 2. Retention Time (R_T) and Percent Content of Standards Obtained by GC-FID after γ- and e-Beam Ionizing Radiation (25 kGy)[a,b]

		area percent[a]		
standards	retention time (R_T)	control	γ-irradiated	e-beam irradiated
α-pinene	4.02	95.0 ± 2.1	95.7 ± 2.3	96.3 ± 1.3
phellandrene	5.11	98.5 ± 1.3	99.2 ± 1.6	99.8 ± 1.1
p-cymene	5.43	96.5 ± 1.5	97.6 ± 2.3	93.9 ± 2.5
eucalyptol	5.45	95.7 ± 2.1	96.2 ± 2.4	96.3 ± 1.3
limonene	5.48	98.3 ± 4.3	101.5 ± 5.1	99.6 ± 3.3
linalool	6.47	97.8 ± 2.7	102.8 ± 4.1	99.8 ± 3.5
lavandulol	7.37	99.3 ± 3.8	99.8 ± 2.8	99.0 ± 1.4
terpin-4-ol	7.81	97.9 ± 3.9	100.1 ± 2.6	102.6 ± 2.2
linalyl acetate	8.52	98.2 ± 2.7	101.0 ± 3.4	98.3 ± 2.3

[a] Data are the mean of three replicates ± standard deviation. [b] No significant differences were noticed ($P > 0.05$) between standards before and after treatments.

GC-FID Analysis. The analyses were performed on a Focus GC (ThemoFinnigan, Italy) equipped with a FID detector. Chromatographic separations were performed on a capillary column (DB-XLB; column length 15 m × 0.25 mm with a 25 μm film thickness) from J&W Scientific (Agilent Technologies, U.S.A.). Helium was used as a carrier gas at a flow rate of 1 mL/min. Samples were injected in the split mode (split ratio 1/69). The injector temperature was set at 250 °C, and the column oven programmed from 50 to 250 °C (6 °C/min) and held at final temperature for 5 min. The detector temperature was set at 250 °C.

The relative proportions of essential oil constituents were obtained by electronic integration of the FID peak area without the use of response factor correction (normalization method). For pure compounds, calibration curves were realized by injecting in the same conditions samples containing 20, 40, 60, 80, 100, and 120 μg/mL of each standard separately and 50 μg/mL methyl palmitate as the internal standard. The solvent was *tert*-butyl methyl ether.

Standards and essential oil samples were diluted in *tert*-butyl methyl ether (1% v/v) before the chromatographic injection.

GC-MS Analysis. The system used was a Trace GC 2000 series (ThermoQuest, Italy) equipped with split−splitless injector and AS 2000 autosampler (ThermoQuest) and coupled to a Trace MS mass spectrometer (ThermoQuest) operating in the electron-impact mode (70 eV). Chromatographic separations were performed as described above (GC-FID analysis).

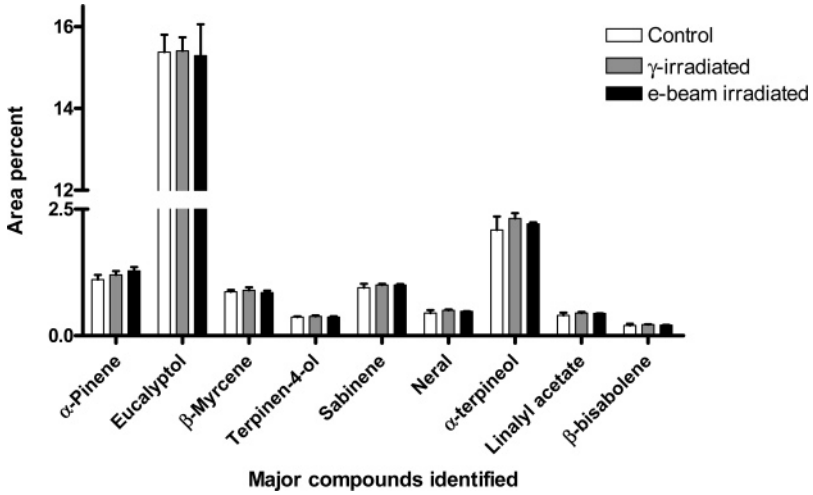

Figure 2. Composition of major constituents identified in the essential oils obtained from control, γ-irradiated, and e-beam irradiated *E. radiata* air-dried leaves. Identification of compounds was made by both GC-FID and GC-MS; quantification of compounds was made by GC-FID. No significant differences were noticed ($P > 0.05$) between the groups before and after treatment.

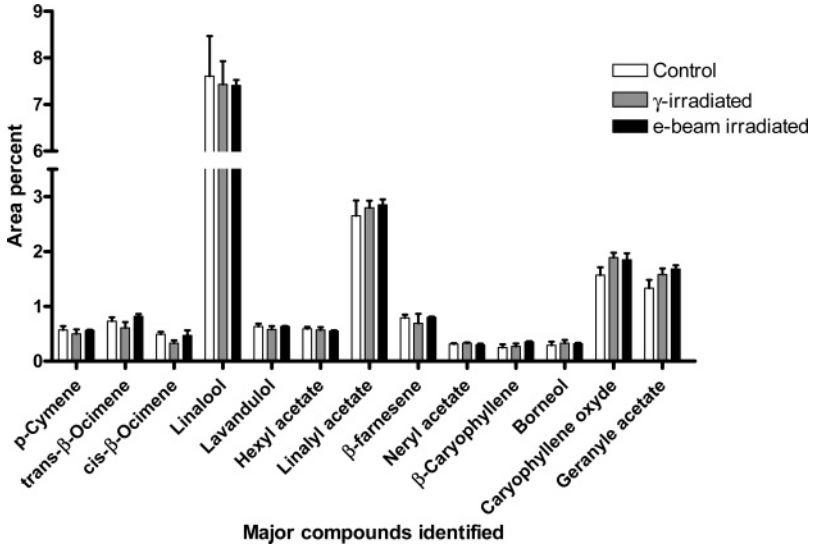

Figure 3. Composition of major constituents identified in the essential oils obtained from control, γ-irradiated, and e-beam irradiated *L. angustifolia* air-dried flowers. Identification of compounds was made by both GC-FID and GC-MS; quantification of compounds was made by GC-FID. No significant differences were noticed ($P > 0.05$) between the groups before and after treatment.

Qualitative GC-MS analyses were made by identifying the individual constituents by comparison of their retention times and/or mass spectra with analytical standards and by computer searching, matching mass spectral data with those held in the NIST (National Institute of Standards and Technology, 1998, v 1.6) library. The data were submitted to statistical analysis (ANOVA) using the *GraphPad Prism* (v 4.00, 2003, GraphPad Prism Software incorporated, web: http.//www.graphpad-.com) program on a personal computer to determine significant sources of variation. Differences between means were analyzed using the Student's t-test and considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

We decided to study plants and plant essential oils with the new analytical sensitivity of hyphenated chromatography using a large dose (the dose for sterility) in order to be able to detect some chemical changes. Usual doses used for different applications are well-known; 25 kGy is the reference dose for drug sterilization (*13*).

The hydrodistillation of the aerial parts of *L. angustifolia* and *E. globulus* gave pale yellow oils, whereas *T. vulgaris* gave orange oil. The percent yields of volatile oil obtained from nonirradiated, γ-irradiated, and e-beam irradiated samples are given in **Table 1**. As shown in this table, no differences were observed for the 3 plants. **Table 2** gives the percent content of irradiated and nonirradiated pure standards, whereas **Figures 1**, **2**, and **3** show the percentages of identified compounds in the essential oils of nonirradiated and irradiated samples of *T. vulgaris*, *E. globulus*, and *L. angustifolia*.

Chromatographic analysis of the different essential oils from whole plants irradiated at 25 kGy indicated that there were no substantial changes in the constituents after ionizing radiation, and the same results were obtained for irradiated commercial standards and essential oils (data not shown). These results are not in good agreement with the literature data which indicate that linalool showed a great sensitivity to γ-radiation (*7*), whereas in our case, no significant qualitative or quantitative differences could be observed in the GLC profile of linalool. Previous investigations have suggested that treatment of spices with γ-radiation doses higher than 6 kGy produces a dose-

dependent reduction effect on essential oil contents, particularly in the cases of black pepper (*14*) and red chili pepper (*15*). Nevertheless, some authors have demonstrated that, at a dose of 10 kGy, dry ginger was found to be sterile without affecting its flavor quality (*16*). These results show that specific effects could be observed for different essential oil extracts and that the same compound present in essential oils from different plants may behave differently even if submitted to the same treatment (*17*).

There is relatively little information available comparing γ-rays and e-beam in the radiolysis of plants. The main difference is the dose rate: the same ionizing radiation dose needs several hours for γ-radiation and only a few seconds for e-beam radiation. Therefore, the number of simultaneous tracks of ionizing particles will be different, and overlapping of spurs (center of reactive species) is possible (*18*). In the liquid state, the number of the reactive species diffusing from the tracks may decrease with the dose rate (*19*).

Our results and the radiation resistance of essentials oils could be explained by the presence of antioxidant compounds in essential oils or plants studied. This may be important, since ionizing radiation generates radical cations which react with molecules, producing free radicals. These radicals, in liquid state, can induce chemical changes. Essential oils extracted from *Lavandula angustifolia* and several *Thymus* species showed appreciable antioxidant activity (*20*). Furthermore, it has been shown that antiradical activity in some investigated plant extracts was also correlated with their content of phenolic compounds. For example, flavonoid aglycones were shown to be responsible for most of free radical scavenging activity of *Lavandula* extract, whereas different *Thymus* extracts were also very effective *in vitro* (*20*−*22*).

Considering the chemical potency, we did not observe any significant change in the irradiated pure standards as compared to nonirradiated ones. However, the study of trace impurities could show the appearance of radiolytic products, depending on the ionizing radiation mode (γ-rays or e-beams), and indicating the complexity of secondary mechanisms. Nevertheless, it has been shown that the quantity of radiolytic products produced during ionizing radiation may be less than 1% (*12*).

Our results demonstrate that γ- and e-beam exposures at 25 kGy, a dose higher than the dose used for decontamination of vegetable drugs, had no significant effect on either the qualitative or quantitative composition of the extracted and commercial essential oils of *T. vulgaris*, *E. globulus*, and *L. angustifolia* or on their yields as compared to the equivalent nonirradiated ones. So, it is likely that decontamination with lower doses will not modify essential oil yields or composition for these three plants. Nevertheless, it would be interesting to study the effect of such high ionizing doses on cell structures, since studies have shown that, in some cases, plants underwent rapid spoilage after ionizing radiation, probably due to textural damage (*23*). In addition, it seems to be interesting to study whether a modification of the structure of some pure compounds (even in trace) could lead to formation of toxic, long-lived radicals.

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LITERATURE CITED

(1) Govindarajan, V. S. Ginger—chemistry, technology and quality evaluation: Parts 1 and 2. CRC. *Crit. Rev. Food Sci. Nutr.* **1982**, *17*, 1−96 and 189−258.

(2) Uzuegbu, J. O.; Emifoniye, A. T. Postharvest fungal spoilage of some Nigerian fruits and vegetables. *Nigeria Food J.* **1984**, *2*, 153−155.

(3) Obuekwe, C. O.; Ogbimi, A. O. Prevalence of *Bacillus cereus* and some other gram-positive bacteria in Nigerian dried food condiments. *Nigeria Food J.* **1989**, *7*, 11−19.

(4) Reid, B. D. Gamma processing technology an alternative technology for terminal sterilization of parenterals. *PDA J. Pharm. Sci. Technol.* **1995**, *49*, 83−89.

(5) Chatterjee, S.; Variyar, P. S.; Gholap, A. S.; Padwal-Desai, S. R.; Bongirwar, D. R. Effect of gamma-irradiation on the volatile oil constituents of tumeric (*Curcuma longa*). *Food Res. Int.* **2000**, *33*, 103−106.

(6) Onyenekwe, P. C.; Ogbadu, G. H.; Hashimoto, S. The effect of gamma radiation on the microflora and essential oil of Ashanti pepper (*Piper guineense*) berries. *Postharvest Biol. Technol.* **1997**, *10*, 161−167.

(7) Antonelli, A.; Fabbri, C.; Boselli, E. Modification of dried basil (*Ocimum basilicum*) leaf oil by gamma and microwave irradiation. *Food Chem.* **1998**, *63*, 485−489.

(8) Koseki, P. M.; Villavicencio, A. L. C. H.; Brito, M. S.; Nahme, L. C.; Sebastião, K. I.; Rela, P. R.; Almeida-Muradian, L. B.; Mancini-Filho, J.; Freitas, P. C. D. Effects of irradiation in medicinal and eatable herbs. *Radiat. Phys. Chem.* **2002**, *63*, 681−684.

(9) Variyar, P. S.; Gholap, A. S.; Thomas, P. Effect of gamma-irradiation on the volatile oil constituents of fresh ginger (*Zingiber officinalis*) rhizome. *Food Res. Int.* **1997**, *30*, 41−43.

(10) Owczarczyk, H. B.; Migdal, W.; Kedzia, B. The pharmacological effect of medical herbs after microbiological decontamination by irradiation. *Radiat. Phys. Chem.* **2000**, *57*, 331−335.

(11) *Pharmacopée Européenne*, 5th ed.; Conseil de l'Europe: Strasbourg, 2005.

(12) Barbarin, N.; Tilquin, B.; De Hoffmann, E. Radiation sterilization of cefotaxime: investigation of potential degradation compounds by liquid chromatography-electrospray chromatography. *J. Chromatogr.* **2001**, *924*, 51−61.

(13) Raffi, J. Comment les instances internationales de santé ont-elles reconnu l'innocuité des aliments ionisés? In *Introduction à l'ionisation des aliments*; Tilquin, B., Ed.; Nauwelaerts: Frison Roche, Belgium, 2003; pp 26−34.

(14) Uchman, W.; Fiszer, W.; Mroz, I.; Pawlik, A. The influence of radapertization upon sensory properties of black pepper. *Nahrung* **1983**, *27*, 461−468.

(15) Onyenekwe, P. C.; Ogbadu, G. H. Radiation sterilization of red chili pepper (*Capsicum frutescens*). *J. Food Biochem.* **1995**, *19*, 121−137.

(16) Andrews, L. S.; Cadwallader, K. R.; Grodner, R. M.; Chung, H. Y. Chemical and microbial quality of irradiated ground ginger. *J. Food Sci.* **1995**, *60* (4), 829−832.

(17) Farag, S. A.; Aziz, N. H.; Attia, A. Effect of irradiation on the microbiological status an flavouring materials of selected spices. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 283−298.

(18) Slegers, C.; Tilquin, B. Theoretical approach to the destruction or sterilization of drugs in aqueous solution. *Radiat. Phys. Chem.* **2004**, *72*, 363−365.

(19) Crucq, A.-S.; Slegers, C.; Deridder, V.; Tilquin, B. Radiosensitivity study of cefazolin sodium. *Talanta* **2000**, *52*, 873−877.

(20) Zygadlo, J. A.; Lamarque, A. L.; Maestri, D. M.; Grosso, N. R. Use of essential oils as natural antioxidants. *Grasas Aceites.* **1995**, *46*, 285−288.

(21) Ferreira, A.; Proençaa, C.; Serralheiroa, M. L. M.; Araújo, J. The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. *J. Ethnopharmacol.* **2006**, *108*, 31−37.

(22) Dapkevicius, A.; Venskutonis, R.; Van Beek, T. A.; Linssen, P. H. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *J. Sci. Food Agric.* **1998**, *77*, 140−146.

(23) Nayak, C. A.; Suguna, K.; Narasimhamurthy, K.; Rastogi, N. K. Effect of gamma irradiation on histological and textural properties of carrot, potato and beetroot. *J. Food Eng.* **2007**, *79*, 765−770.

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