https://dx.doi.org/10.21577/0103-5053.20210099

J. Braz. Chem. Soc., Vol. 32, No. 11, 2078-2085, 2021

Article

Thermal Stability and Photostability of *Satureja montana* and *Lavandula angustifolia* Essential Oils

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The influence of light and temperature stress conditions and oxygen availability on the chemical composition of *Satureja montana* and *Lavandula angustifolia* essential oils is reported. Photostability and thermal stability were evaluated by gas chromatography-mass spectrometry (GC-MS) analysis, comparing composition before and after the applied regimes. In *Satureja montana* essential oil, the amount of thymol (13.0-11.9%) and carvacrol (10.3-9.4%) decreased at elevated temperature and in the presence of air, with a simultaneous increase of *p*-cymene (24.2-26.2%) while in an inert atmosphere the composition remained the same as in fresh oil. Light caused a dehydrogenation of α-terpinene (2.1-0.9%) and γ-terpinene (5.6-4.7%) to *p*-cymene (24.2-25.9%) and decrease of *trans*-caryophyllene (5.1-4.3%). In *Lavandula angustifolia* essential oil, compounds sensitive to elevated temperature and the presence of oxygen were *cis*-ocimene (2.8-2.2%) and *trans*-ocimene (2.6-2.0%), alloocimene (3.0-2.3%), *trans*-caryophyllene (4.3-3.6%) and β-farnesene (1.7-1.2%). Irradiated samples showed a decrease in the content of *cis*-ocimene (2.8-1.9%), alloocimene (3.0-2.0%), crypton (0.6-0.1%), cuminal (0.3-0.0%), *trans*-caryophyllene (4.3-3.5%), β-farnesene (1.7-1.1%) and germacrene-D (0.5-0.1%) and an increase of *trans*-ocimene (2.6-3.5%), β-bourbonene (0.0-0.2%) and several unidentified peaks. Both oils showed an individual response to light and temperature stress. The absence of oxygen and light is the only storage regime under which the initial composition can be preserved.

Keywords: essential oil, photostability, thermal stability, *Satureja montana*, *Lavandula angustifolia*

Introduction

Natural ingredients have been used in human history from ancient times not only for feeding purpose but also because of various health benefits. Today, growing consumers awareness on green products that encompass sustainable production and safe use, changes the trends in production of nutritional, cosmetics and pharmaceutical products.[1] The industry of natural products has been growing in last decades followed by growing research interest of new bioactive substances and products from botanical origin.[2] Chemical and biological properties of extracts from medicinal and aromatic plants have been extensively studied. One of the valuable extracts that are widely used is essential oil.

Essential oils (EOs) are a complex mixture of terpenoides, phenylpropanoides and short-chain aliphatic hydrocarbon derivatives. The major chemical classes in EOs are mono- and sesquiterpenoides. It has been observed that components in EOs are susceptible to oxidation reaction, chemical transformation and polymerization which can lead to loss of quality.[3] Changes can occur during processing, transportation or storage. Light, temperature and oxygen availability are among prime factors influencing chemistry of EOs. Products of degradation can cause organoleptic alterations but they can even be harmful for use. For example, oxygenated derivatives of limonene, linalool or caryophyllene showed allergenic and skin sensitization properties.[4,5]

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Properties of EOs, such as scent and biological activity, are governed not only by the main components but also by synergism between the components. The same is worth for EOs stability because changes that occur to one substance can affect the behavior of others present inside. Consequently, alteration characteristics will be different for every individual botanical species of oil as is shown by previous research.[6-17] Turek and Stintzing[6] studied impact of different storage temperatures, light and oxygen availability on the quality of EO from *Lavandula angustifolia*, *Pinus sylvestris*, *Rosmarinus officinalis* and *Thymus vugaris*. Each oil showed characteristic changes upon same applied external conditions with *Rosmarinus officinalis* oil being most sensitive to degradation. Significant decrease of α-terpinene during storage was found only under the light but did not alter in the dark. Misharina *et al*.[7] also found individual response to compositional change for 14 studied EOs during the storage for a year in darkness and in light. It was found that light accelerates the change, weather the oil was more or less stable. Light caused changes in stored *Pimpinella anisum* EO where Miething *et al*.[8] identified photoanethole as a result of photocycloaddition between anethole and anisaldehyde followed by elimination step. In *Foeniculum vulgare* EO *trans*-anethole isomerized to toxic *cis*-anethole or oxidized to anisaldehyde under the influence of light.[9] Rowshan *et al*.[10] investigated the stability of *Thymus daenensis* Celak EO under different storage temperatures during three months (room temperature, refrigerator and freezer). Changes occurred at each temperature regime with the least alterations when oil was stored at low temperatures. Najafian[11,12] found the same for *Melissa officinalis* and for *Lavandula officinalis* EOs. Stability of *Helichrysum italicum* subsp. *italicum* and *Juniperus communis* EO was studied by us exposing the oils to different storage conditions (light, temperature and oxygen availability) during one year.[13] While *Juniperus communis* EO was found to be quite stabile, *Helichrysum italicum* subsp. *italicum* EO was more sensitive to aging, with the changes being more pronounced if it was kept in the light. Influence of accelerated light stress conditions on EOs stability were also studied by us.[14] *Helichrysum italicum* subsp. *italicum*, *Abies alba* and *Juniperus oxycedrus* EOs were exposed to UV-A irradiation in the presence of atmospheric oxygen as well as in the presence of inert gas. While *Abies alba* and *Juniperus oxycedrus* EOs were found to be photostable, composition of *Helichrysum italicum* subsp. *italicum* EO changed due to photochemical transformation of γ-curcumene. Test in the photoreactor confirmed photochemical transformations that occurred during realistic storage conditions.

In this paper, degradation characteristics of the two EOs from Bosnia and Herzegovina (BH) were assessed under the conditions that cause accelerated changes. Triplicate samples of *Satureja montana* L. (Lamiaceae) and *Lavandula angustifolia* Mill. (Lamiaceae) EOs were exposed to light and temperature stress under oxygen and nitrogen atmosphere. After exposure to different conditions, samples were analyzed by gas chromatography-mass spectrometry (GC-MS) and compared to the fresh ones in order to evaluate potential changes in chemical composition.

Experimental

Plant material and chemicals

Fresh sample of *Satureja montana* essential oil was obtained from distillery plant Halilović d.o.o., while *Lavandula angustifolia* essential oil was obtained from a plantation located near Mostar. *Satureja montana* was harvested in Herzegovina region in 2019 (Mostar, 43°14'42"N, 17°86'73"E) and subjected to steam distillation for 3 h. *Lavandula angustifolia* was harvested in Herzegovina region (Gornaci, Mostar, 43°25'48.4"N, 17°42'43.2"E) in 2020 and the oil was extracted by steam distillation for 2 h. Each oil was dried over anhydrous sodium sulfate (Sigma-Aldrich, St. Louis, MO, USA). Pentane was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Thermostability testing

Two sets of EO samples were prepared: aliquots of 1 mL were filled in 2 mL amber glass vials and closed with sealing plugs with the air left in the headspace (conditions A) while in the second set samples vials were filled up to the neck and capped (conditions B). Samples were prepared in triplicates and stored at 50 ºC in an incubator oven for 72 h and analyzed using GC-MS.[6,18,19]

Photostability testing

Essential oil samples were prepared as follows: aliquots of 1 mL were filled in 2 mL clear glass vials and closed with sealing plugs with the air left in the headspace (conditions C). The second set of samples was prepared in the same way but flushed with pure nitrogen (conditions D). Samples were prepared in triplicates. The sealed vials were irradiated for 72 h at 25 °C in a Luzchem (Luzchem Research Inc., Ontario, Canada) CCP-ICH2 photoreactor equipped with 16 lamps of the 366 nm wavelength. After

irradiation, oil samples were analyzed using GC-MS system.[6,18,19]

Gas chromatography-mass spectrometry analysis

The analysis of the oils was carried out using Shimadzu (Japan) GC/MS QP2010 system equipped with an AOC-20i autosampler, using fused silica capillary column Inert Cap (5% diphenyl-95% dimethylpolysiloxane, 30 m × 0.25 mm internal diameter, film thickness 0.25 μm). 1.0 μL of solution diluted 1:500 v/v in pentane was injected in splitless mode with helium as carrier gas. For *Satureja montana* EO, the operating conditions were as follows: injection temperature 260 °C; helium flow rate, 1.14 mL min^{-1}; oven temperature program: 45 °C (4 min), 45-70 °C (1 °C min^{-1}), 70-140 °C (2 °C min^{-1}), 140-250 °C (6 °C min^{-1}). MS electron impact (EI) conditions: ion source temperature: 250 °C, interface temperature: 250 °C, ionization voltage: 70 eV, mass range: *m/z* 40-400 u, scan time: 0.5 s. The operating conditions for *Lavandula angustifolia* EO were as follows: injection temperature 260 °C; helium flow rate, 1.11 °C min^{-1}; oven temperature program: 50 °C (2 min), 50-120 °C (2 °C min^{-1}), 120-220 °C (5 °C min^{-1}). MS (EI) conditions: ion source temperature: 200 °C, interface temperature: 280 °C, ionization voltage: 70 eV, mass range: *m/z* 40-400 u; scan time: 0.5 s. GCMS solution 2.5 (Shimadzu) was used to handle data. GC-MS analyses were performed in triplicate and the results are represented as mean values ± standard deviation.

Identification of oil components was based on (*i*) retention indices on a non-polar column relative to a homologous series of *n*-alkanes (C8-C40), (*ii*) on the comparison of their mass spectra and retention indices with the Wiley 7 and NIST spectra libraries and with those reported in the literature.[20,21]

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) test and Tukey's post-test to explore significance of differences ($\alpha = 0.05$) in relative amount of compound after exposure to different conditions. In addition to the changes that are statistically significant, the results also show changes related to new peaks in the chromatograms. New peaks are found in very small quantities or in traces, which is why they do not represent a statistical difference.

Results and Discussion

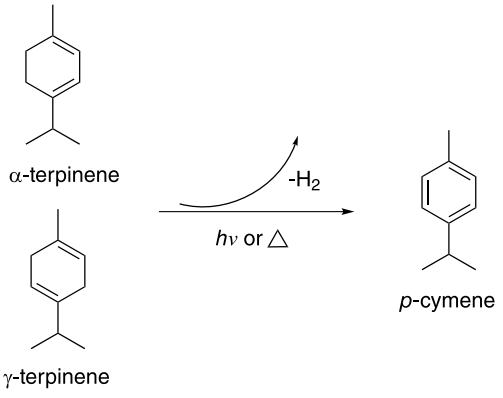
Effect of increased temperature was studied by storing oil samples in an incubator oven at 50 °C under the air atmosphere (sample A) and without air (sample B) for 72 h. Samples of fresh oils were irradiated in a photoreactor at a wavelength of 366 nm in order to imitate the UV-A fraction of sunlight. One set of samples was irradiated in an air atmosphere (sample C) and the other set was exposed to light under nitrogen (sample D) for 72 h. Tables 1 and 2 show compounds from EOs whose share has changed during storage.

Stability of *Satureja montana* essential oil

The main compounds found in *Satureja montana* EO were *p*-cymene (24.2%), thymol (13.0%), carvacrol (10.3%), carvacrol-methyl-ether (6.4%), γ-terpinenene (5.6%) and *trans*-caryophyllene (5.1%).

The influence of elevated temperature on compositional changes was greater in half-filled bottles containing air, showing a decrease of thymol and carvacrol and an increase of *p*-cymene (Table 1). The reduction of thymol and carvacrol under the applied conditions may be associated with their antioxidant effect since they possess a phenolic ring.[22,23]

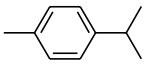
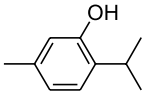
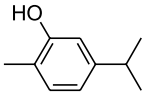
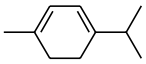
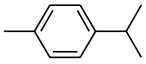
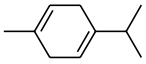
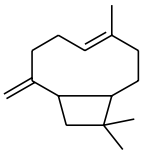
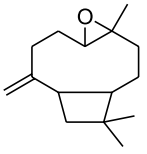
Upon light exposure, the share of *p*-cymene increased, followed by a simultaneous decrease in γ-terpinene and α-terpinene (Table 1). Observed changes are found to be more pronounced in the oxygen atmosphere (sample C) than with the inert gas (sample D). *p*-Cymene is a typical product of aged essential oils.[24-27] The reaction that could lead to the formation of *p*-cymene is the oxidative dehydrogenation of α- and γ-terpinene. These unsaturated monoterpenes possess allylic hydrogen atoms that can be readily abstracted leading to resonance stabilized radicals.[19,28] Another dehydration step leads to aromatic *p*-cymene (Scheme 1).



Scheme 1. Aromatization of α-terpinene and γ-terpinene.

Light and oxygen caused a decrease of *trans*-caryophyllene with a simultaneous increase of caryophyllene oxide, a well-known secondary oxidation product formed by epoxidation of the caryophyllene double bond

Table 1. Chemical composition and GC-MS relative peak area of characteristic compounds during exposure of *Satureja montana* essential oils to the elevated temperature and to the light

Structure	Compound	RI[a]	RI[b]	Fresh[c] / %	A[d] / %	B[d] / %
	*p*-cymene	1020	1020	24.2 ± 0.3[a]	26.2 ± 0.3[b]	24.5 ± 0.2[a]
	thymol	1293	1289	13.0 ± 0.2[a]	11.9 ± 0.2[b]	13.2 ± 0.1[a]
	carvacrol	1299	1307	10.3 ± 0.2[a]	9.4 ± 0.1[b]	10.4 ± 0.3[a]
					C[d] / %	D[d] / %
	α-terpinene	1010	1014	2.1 ± 0.2[a]	0.9 ± 0.2[b]	1.3 ± 0.2[b]
	*p*-cymene	1020	1020	24.2 ± 0.3[a]	25.9 ± 0.2[b]	24.9 ± 0.1[c]
	γ-terpinene	1051	1054	5.6 ± 0.2[a]	4.7 ± 0.1[b]	5.0 ± 0.1[b]
	*trans*-caryophyllene	1407	1419	5.1 ± 0.1[a]	4.3 ± 0.1[b]	5.2 ± 0.1[a]
	caryophyllene oxide	1572	1573	0.4 ± 0.0[a]	0.9 ± 0.1[b]	0.8 ± 0.1[b]

[a]RI: retention indices on apolar column; [b]RI: retention indices on apolar column reported in the literature; [c]values (mean ± standard deviation) in the same row with different letters indicate statistical differences ($p < 0.05$) in relative amount of compound after exposure to different conditions; [d]storage condition A: 50 °C, 72 h, air; B: 50 °C, 72 h, nitrogen; C: light, 72 h, air; D: light, 72 h, nitrogen.

Table 2. Chemical composition and GC-MS relative peak area of characteristic compounds during exposure of *Lavandula angustifolia* essential oils to the elevated temperature and to the light

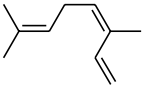
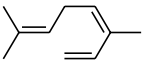
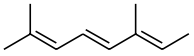
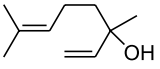
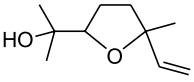
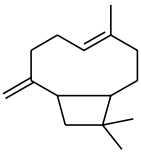
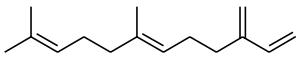
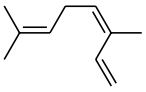
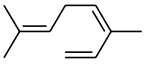
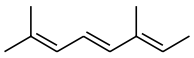
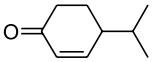
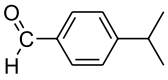
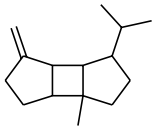
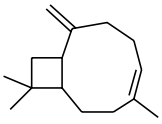
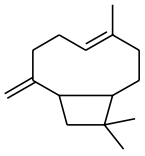
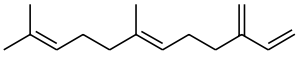
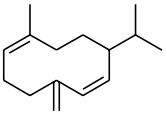
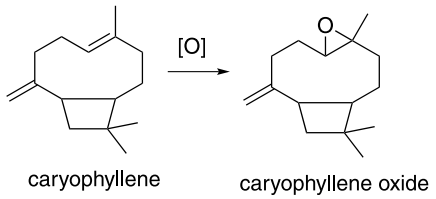
Structure	Compound	RI[a]	RI[b]	Fresh[c] / %	A[d] / %	B[d] / %
	*cis*-ocimene	1033	1032	2.8 ± 0.1[a]	2.2 ± 0.1[b]	2.9 ± 0.1[a]
	*trans*-ocimene	1043	1044	2.6 ± 0.1[a]	2.0 ± 0.1[b]	2.6 ± 0.1[a]
	alloocimene	1126	1131	3.0 ± 0.1[a]	2.3 ± 0.1[b]	3.1 ± 0.1[a]
	linalool	1103	1095	24.1 ± 0.3[a]	25.9 ± 0.2[b]	24.3 ± 0.2[a]
	linalool oxide	1182	1171	–	tr	–

Table 2. Chemical composition and GC-MS relative peak area of characteristic compounds during exposure of *Lavandula angustifolia* essential oils to the elevated temperature and to the light (cont.)

Structure	Compound	RI[a]	RI[b]	Fresh[c] / %	A[d] / %	B[d] / %
	*trans*-caryophyllene	1411	1419	4.3 ± 0.2[a]	3.6 ± 0.1[b]	3.6 ± 0.1[b]
	β-farnesene	1456	1446	1.7 ± 0.1[a]	1.2 ± 0.0[b]	1.3 ± 0.1[c]
					C[d] / %	D[d] / %
	*cis*-ocimene	1033	1038	2.8 ± 0.1[a]	1.9 ± 0.1[b]	2.2 ± 0.1[b]
	*trans*-ocimene	1043	1049	2.6 ± 0.1[a]	3.5 ± 0.1[b]	3.5 ± 0.1[b]
	alloocimene	1126	1130	3.0 ± 0.1[a]	2.0 ± 0.0[b]	2.2 ± 0.1[c]
	crypton	1180	1184	0.6 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.1[b]
	cuminal	1235	1239	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	β-bourbonene	1374	1384	not detected	0.2 ± 0.0[b]	0.2 ± 0.0[b]
	*cis*-caryophyllene	1393	1408	not detected	0.1 ± 0.0[b]	0.1 ± 0.0[b]
	*trans*-caryophyllene	1411	1419	4.3 ± 0.2[a]	3.5 ± 0.1[b]	3.5 ± 0.1[b]
	not identified	1429	–	not detected	tr	tr
	β-farnesene	1456	1446	1.7 ± 0.1[a]	1.1 ± 0.0[b]	1.1 ± 0.1[b]
	not identified	1463	–	not detected	tr	tr
	germacrene-D	1475	1480	0.5 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.1[b]

[a]RI: retention indices on apolar column; [b]RI: retention indices on apolar column reported in the literature; [c]values (mean ± standard deviation) in the same row with different letters indicate statistical differences ($p < 0.05$) in relative amount of compound after exposure to different conditions; [d]storage condition A: 50 °C, 72 h, air; B: 50 °C, 72 h, nitrogen; C: light, 72 h, air; D: light, 72 h, nitrogen. tr = traces < 0.1%.

(Scheme 2).[29] The amount of caryophyllene remained the same when oxygen was absent.



Scheme 2. Oxidation of *trans*-caryophyllene.

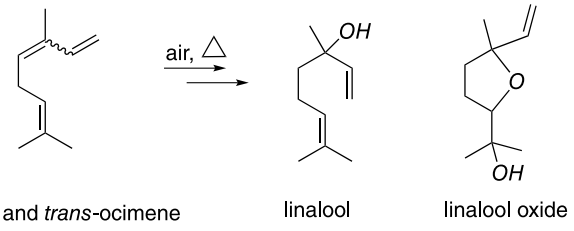
Upon irradiation a shift in color occurred for *Satureja montana* EO from colorless to pale yellow (sample C) and to yellow-orange (sample D). Color change can be attributed to polymerization reactions. Light stimulates polymerizations, especially when the sample is flushed with nitrogen since the absence of oxygen allows polymerization reactions to take place to a greater extent.[30,31]

Changes in the chemical composition of *Satureja montana* essential oil caused by light are more pronounced than changes caused by elevated temperature. The inert nitrogen atmosphere reduces changes caused by light and inhibits changes under elevated temperature.

Stability of *Lavandula angustifolia* essential oil

Fresh *Lavandula angustifolia* EO contained linalyl acetate (26.1%) and linalool (24.1%) as the main compounds, followed by lavandulol acetate (7.1%), 4-terpineol (5.4%) and *trans*-caryophyllene (4.3%).

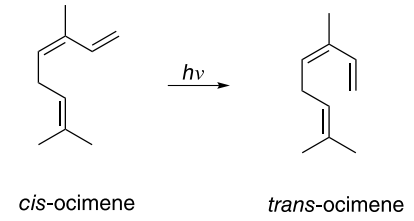
When the elevated temperature was applied in the presence of air (conditions A), content of *cis*-, *trans*-ocimene and alloocimene decreased (Table 2) while their amount remained the same in the absence of oxygen. The double bonds and allylic positions present in the *cis*- and *trans*-ocimene could be oxidized.[32] Possible oxidation products could be linalool whose share increased and linalool oxide which was found in traces (Scheme 3).



Scheme 3. Oxidation of ocimene.

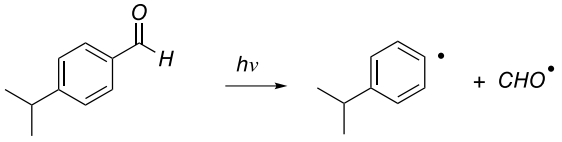
The reactions responsible for alloocimene decline could be dimerization, polymerization and oxidation.[33] Among sesquiterpenes, the amount of *trans*-caryophyllene and β-farnesene decreased in the presence and absence of oxygen, but degradation products have not been identified. For *trans*-caryophyllene it can be assumed that its strained four-membered ring break at elevated temperature or even in the applied GC-MS conditions. The pale yellow color of fresh lavender oil remained unchanged after temperature stress.

When *Lavandula angustifolia* oil was exposed to light, both with and without air, *cis*- and *trans*-ocimene underwent different changes then under the influence of elevated temperature. It was observed that the amount of *cis*-ocimene decreased with a simultaneous increase in *trans*-ocimene which can be attributed to photoisomerization (Scheme 4).[30,31]



Scheme 4. Photoisomerization of *cis*-ocimene.

The reduction in conjugated unsaturated monoterpene alloocimene can be attributed to polymerization reactions. Crypton and cuminal disappeared completely under the influence of light. Cuminal, an aromatic aldehyde is a photoreactive compound which can act as a photoinitiator when irradiated. Dissociation pathways depend on the reaction conditions, but the most likely reaction could be α-cleavage (Scheme 5).[34,35]



Scheme 5. α-Cleveage of cuminal.

Crypton is an α,β-unsaturated ketone, so the main disappearance route could be [2 + 2]-photocycloaddition reaction with double bonds present inside.[30,31]

Among sesquiterpenes, the amount of *trans*-caryophyllene, germacrene-D and β-farnesene decreased in both applied conditions and in the equal extent (Table 2). A small amount of *cis*-caryophyllene was detected in irradiated samples, but no other products that could be related to *trans*-caryophyllene transformation were identified. Bourbonene is also identified as a new peak in the chromatogram. It can be associated with the reduction of germacrene-D since this sesquiterpene is known to convert to a number of products under the influence of light, including bourbonene.[36] β-Farnesene is a polyunsaturated

sesquiterpene prone to cyclization and oxidation reactions. Numerous farnesene epoxy derivatives can be formed in the presence of oxygen. Without the presence of oxygen, cyclization can take place in a greater extent leading to bicyclic structures and polymerization products.[37] Two new peaks on the chromatogram with retention indices 1429 and 1463 can be associated with the transformation of β-farnesene, according to signals in mass spectra, but the products remained unidentified.

The color of *Lavandula angustifolia* oil changed from light yellow to yellow only by lightening under nitrogen. This can be attributed to polymerization which can occur in a greater proportion when competing oxidation reactions are absent. The cuminal present in this oil can act as a photoinitiator for polymerizations.

Conclusions

Photostability and thermal stability investigation on two selected EOs demonstrated the individual character of these oils in response to irradiation and elevated temperature. *Satureja montana* and *Lavandula angustifolia* showed interesting chemical changes of terpenes under the applied conditions. *Satureja montana* is more sensitive to light than to elevated temperature, especially in an oxygen atmosphere. *Lavandula angustifolia* showed various chemical changes induced by light while elevated temperature caused changes only when oxygen is available. Both oils showed the greatest stability when light and oxygen were absent.

Acknowledgments

This work was supported by Federal Ministry of Education and Science, Bosnia and Herzegovina (grant No. 05-39-2629-1/19).

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Submitted: March 5, 2021
Published online: July 8, 2021