



Chamomile essential oil quality after postharvest separation treatments

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Summary: Ecological conditions of the growing areas, growing practices as well as harvest and postharvest processing influence the yield and quality of chamomile. The aim of this research was to determine the influence of chamomile separation methods, as postharvest treatments, on the essential oil quality and content, with a view to improve current primary processing of this valuable medicinal plant. In order to explain the structure of laboratory data that would give deeper assessment of similarities among various samples of chamomile essential oil, PCA was employed. Tested results showed that separation of chamomile plant material, as postharvest and pre-drying treatment, had significant influence on the number of identified compounds in the chamomile essential oil. The highest content of individual essential oil compounds had chamomile flowers with short stems, especially *α*-bisabolol, chamazulene, *Z*-spiroether and *E*-*β*-farnesene. In the essential oil obtained from chamomile flowers with long stems, content of *α*-bisabolol and chamazulene were significantly lower, while *E*-*β*-farnesene and *Z*-spiroether contents were significantly higher. Furthermore, in the essential oil obtained from chamomile plant material without separation, the lowest content of *α*-bisabolol and *Z*-spiroether, and the highest content of *E*-*β*-farnesene were recorded. The correlation analysis was performed to investigate the likeness in the amounts of the active compounds of essential oil from differently processed chamomile samples. All these results indicate the importance of precise and controlled postharvest treatments, since it clearly affects the essential oil quality and content in the primary processing of this valuable medicinal plant.

Key words: essential oil, *Matricaria chamomilla*, medicinal plants, postharvest, primary processing, separation

Introduction

Chamomile (*Matricaria chamomilla* L. syn. *Chamomilla recutita* L.) herbal tea is very popular because of its sweet, grassy, lightly fruity aroma and due to its calming, carminative and spasmolytic properties (Zadeh et al., 2014). It is extensively used in the cosmetic industry: for preparation of skin creams, oils and bath additives, due

to its effectiveness in treating skin inflammation, atopic dermatitis, peristomal skin problems, etc. It can also be found in mouthwash products, toothpaste, decorative cosmetics and shampoos (Sarkic & Stappen, 2018).

On the market, chamomile is usually sold as dry flowers (*Chamomillae flos*) and essential oil (*Chamomillae aetheroleum*). Dried chamomile flowers are bought by tea companies, producers of herbal extracts and health food shops, while pharmaceutical, cosmetic and aromatherapy companies are the most common buyers of its essential oil. In spite of the global market growth, the world is facing large discrepancy between demand and supply of high-quality flowers and essential oil of chamomile (Upadhyay et al., 2016).

Despite the fact that their production area is comparably small worldwide, chamomile belongs to the group of the minor, but highly valuable crops (Karkanis et al., 2018). It is usually produced in countries with low labor costs and exported to industrialized countries (Arslan et al., 2019). Prices are largely regulated by the global supply and demand. Quality is one of the main criteria during price formation.

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Ecological conditions of the growing areas (Letchamo, 1996; Gosztola et al., 2010), as well as growing practices (Ghasemi et al., 2016; Upadhyay et al., 2016) influence the yield and quality of chamomile. However, the major factor causing differences in the quality may be the time between harvest and distillation (Bucko & Salamon, 2007).

Since mechanically gathered, fresh chamomile flowers have a very high respiration rate, the reason of which there is a need for intensive and immediate postharvest treatment, including ventilation, cooling or drying (Bottcher et al., 2001). Pre-drying separation is usually performed by a drum separator, which divides the material into two fractions that are dried separately: (1) chamomile flowers with stem length up to 30 mm, and (2) long stems with a small quantity of flowers, mixed with weed. Separate drying of first- and second-class harvest material enables better quality and higher dryer output. Pre-drying processing, primary separation and classification, can be performed by different procedures and machines (Oztekin & Martinov, 2007). Additionally, herbal material dried in a bulk immediately after the harvest, without process of separation, is regarded as a final product, as well (Szabo et al., 2010).

In Serbia, chamomile is grown as a field crop; the annual amount exported is over 300,000 t of dried flowers (2016) with the tendency to grow. Apart from domestic varieties “Banatska” and “Tetraploidna”, there are others being grown as well exclusively for export to Germany, “Mabamille”, “Zloty Lan”, “Lutea” and “Manzana” (Acimovic et al., 2018; Acimovic et al., 2021).

The aim of this research was to determine the influence of chamomile separation methods, as postharvest treatments, on the essential oil quality and content, with a view to improve current primary processing of this valuable medicinal plant.

Material and methods

Plant material

German chamomile (*Matricaria chamomilla* L.) variety ‘Mabamille’ was cultivated at plantation field of commercial tea company, MACVAL GROUP, located in Čoka (44°56'N; 20°08'E), Serbia. Sowing was performed during September 2019, and the harvest was done during May 2020.

Postharvest pre-drying treatments

One part of the harvested material was dried immediately after the harvest, without further separation, while the other went through the process of pre-drying separation in a drum separator. Two fractions were obtained during this process: flowers with short stems (first class) and raw material with long stems with and small quantity of flowers (second class). All obtained fractions were dried in a conveyor dryer with five drying belts at 40 °C. The scheme in Figure 1 shows postharvest pre-drying treatments of chamomile.

Essential oil extraction and analysis

Dried chamomile samples were subjected to hydro-distillation using an all-glass Clevenger-type apparatus to extract essential oil according to the method outlined by the Ph. Eur. 8.0 (2013). In order to extract the essential oil, 100 g of analyzed plant material was placed in a 1000 ml conical flask with 300 ml of distilled water and connected to the Clevenger apparatus. The steam in combination with the essential oils was distilled into a graduated cylinder for 4 h and then separated from the aqueous layer. Collected oil was kept refrigerated until required for further analysis.

Gas chromatographic-mass spectrometric analysis was performed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 Network mass selective detector (MSD) (both Agilent, Santa Clara, USA), in positive ion-electron impact (EI) mode. The separation was effected using Agilent 19091S-433 HP-5MS fused silica capillary column with 30 m × 0.25 mm i.d., 0.25 µm film thickness. The GC oven temperature was programmed from 60 °C to 285 °C at a rate of 3 °C/min. Helium was used as carrier gas; inlet pressure was 20.3 kPa; linear velocity was 1 ml/min at 210 °C. Injector temperature: 250°C; injection mode: splitless. MS scan conditions: MS source temperature, 230 °C; MS Quad temperature, 150 °C; energy, 70 eV; mass scan range, 40–550 amu. The identification of components was carried out based on retention index and by comparison with reference spectra (Wiley and NIST databases).

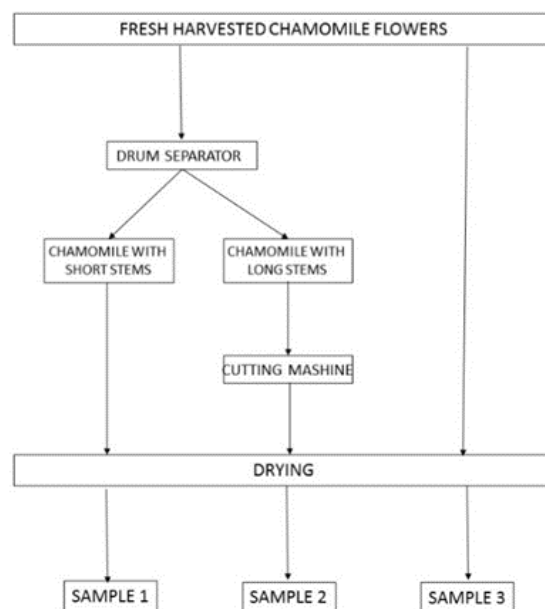


Figure 1. Scheme of the postharvest pre-drying treatments of chamomile raw material

Statistical analysis

Principal component analysis (PCA) was done for testing the effect of postharvest pre-drying treatments on chamomile essential oil chemical profile and content. By comprehending the PCA plot of the gathered samples, the perspective trend for a deeper understanding of the essential oil quality profile could be realized. The correlation analysis was performed to investigate the likeness in active compounds content of the various samples. Statistical analysis of the data was performed using the Statistica for Windows 10 software (StatSoft, Inc.). To investigate the visual similarities between different samples, the correlation analysis was performed by the R software 4.0.3 (64-bit version).

Results and discussion

As already confirmed (Abbas et al. 2021), drying methods significantly influence the number and content of essential oil identified compounds. However, additional processing could add-up to the existing loss of volatile compounds, due to extensive operations with the material.

The tested results showed that separation of chamomile plant material, as postharvest and pre-drying treatment, had significant influence on the number of identified compounds in the chamomile essential oil (Table 1). Chamomile flowers with long stems had the highest number of identified essential oil compounds (57), followed by chamomile plant material without separation (53) and chamomile flowers with short stems (47).

The highest content of individual essential oil compounds had chamomile flowers with short stems

(Sample 1), especially *α*-bisabolol (42.9%), chamazulene (16.0%), *Z*-spiroether (11.8%) and *E*-*β*-farnesene (9.3%). In the essential oil obtained from chamomile flowers with long stems (Sample 2), content of *α*-Bisabolol and chamazulene were significantly lower (36.8% and 12.0%, respectively), while *E*-*β*-farnesene and *Z*-spiroether contents were significantly higher (19.1% and 12.3%). Furthermore, in the essential oil obtained from chamomile plant material without separation (Sample 3), the lowest content of *α*-Bisabolol (32.2%) and *Z*-spiroether (9.3%), and the highest content of *E*-*β*-farnesene (24.9%) were recorded. In addition, Samples 2 and 3 had similar content of chamazulene, contrary to Sample 1 which had 3-4% higher content of this compound (Table 1).

In order to explain the structure of laboratory data that would give deeper assessment of similarities among various samples of chamomile essential oil, PCA was employed, and the results are presented in the Figure 2. The first two PCs explained 100% of the total variance in the experimental data. According to results, Sample 1 was characterized by the increased values of C3, C5, C8, C30, C40, C42, C44, C45, C48, C55, C59-61, C63 and C65-C69. Sample 2 showed the augmented values of C29, C32, C51 and C53, while sample 3 was indicated by high concentrations of C1, C6, C11, C12, C15, C17, C20 and C21.

The correlation analysis was performed to investigate the likeness in the amounts of the active compounds of essential oil from differently processed chamomile samples (chamomile flowers with short and long stems and chamomile plant material without separation), and

Table 1. Chamomile essential oil quality. RI – Retention Index; Sample 1 – chamomile flowers with short stems; Sample 2 – chamomile flowers with long stems; Sample 3 – chamomile herbal material without separation; NI – Not Identified; nd – not detected. Results marked with different letters in superscript indicate statistically significant difference between mean values (p<0.05, Tukey’s HSD test).

No	Compound	RI	Sample 1	Sample 2	Sample 3
C1	Sabinene ^{MT}	970	tr	0.1±0.0 ^a	0.1±0.0 ^a
C2	<i>E</i> - <i>β</i> -Ocimene ^{MT}	1044	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
C3	<i>γ</i> -Terpinene ^{MT}	1055	0.1±0.0 ^a	tr	tr
C4	Artemisiaketone ^{OMT}	1057	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
C5	Artemisiaalcohol ^{OMT}	1080	0.1±0.0 ^a	nd	nd
C6	<i>δ</i> -Elemene ST	1335	nd	0.3±0.0 ^a	0.4±0.0 ^b
C7	NI	1367	nd	0.1±0.0 ^a	nd
C8	Decanoicacid ^O	1377	1.1±0.1 ^a	nd	nd
C9	<i>α</i> -Isocomene ST	1384	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
C10	NI	1390	nd	tr	0.1±0.0 ^a
C11	<i>E</i> -Caryophyllene ST	1417	0.2±0.0 ^a	0.4±0.0 ^b	0.6±0.1 ^c
C12	<i>β</i> -Copaene ST	1427	nd	0.2±0.0 ^a	0.2±0.0 ^a
C13	NI	1442	nd	0.1±0.0 ^a	0.1±0.0 ^a
C14	NI	1451	nd	0.1±0.0 ^a	0.1±0.0 ^a
C15	<i>E</i> - <i>β</i> -Farnesene ST	1458	9.3±0.7 ^a	19.1±1.3 ^b	24.9±1.9 ^c
C16	dehydro-Sesquicineole ^{OST}	1469	0.2±0.0 ^a	0.2±0.0 ^a	0.2±0.0 ^a
C17	Germacrene D ST	1482	2.5±0.2 ^a	3.8±0.4 ^b	5.2±0.1 ^c
C18	<i>β</i> -Selinene ST	1485	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
C19	NI	1494	0.1±0.0 ^a	0.2±0.0 ^b	0.2±0.0 ^b
C20	Bicyclgermacrene ST	1496	0.9±0.1 ^b	0.7±0.1 ^a	1.1±0.1 ^c
C21	<i>E,E</i> - <i>α</i> -Farnesene ST	1507	0.9±0.1 ^a	2.5±0.2 ^b	3.2±0.1 ^c

C22	δ-Cadinene ST	1523	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
C23	NI	1533	nd	0.1±0.0 ^a	nd
C24	NI	1552	tr	0.1±0.0 ^a	nd
C25	NI	1555	nd	0.1±0.0 ^a	nd
C26	NI	1557	0.1±0.0 ^a	nd	0.1±0.0 ^a
C27	NI	1561	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
C28	NI	1562	tr	0.1±0.0 ^a	tr
C29	Spathuleno ^{OST}	1576	0.6±0.1 ^{ab}	0.7±0.1 ^b	0.5±0.0 ^a
C30	Caryophyllene oxide ^{OST}	1581	0.2±0.0 ^b	0.1±0.0 ^a	0.1±0.0 ^a
C31	NI	1584	nd	0.1±0.0 ^a	nd
C32	Salvial-4(14)-en-1-one ^{OST}	1589	nd	0.1±0.0 ^a	tr
C33	NI	1597	nd	0.1±0.0 ^a	nd
C34	NI	1595	nd	nd	0.1±0.0 ^a
C35	NI	1610	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
C36	NI	1614	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
C37	NI	1628	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
C38	NI	1633	nd	0.1±0.0 ^a	nd
C39	NI	1637	0.1±0.0 ^a	nd	0.1±0.0 ^a
C40	α-Bisabolol oxide B ^{OST}	1655	3.6±0.3 ^c	2.7±0.1 ^b	1.8±0.0 ^a
C41	NI	1660	0.1±0.0 ^a	0.1±0.0 ^a	nd
C42	α-Bisabolol ^{OST}	1693	42.9±2.8 ^b	36.8±2.8 ^{ab}	32.2±2.4 ^a
C43	NI	1710	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
C44	Chamazulene ST	1736	16±2.1 ^a	12.0±0.9 ^a	13.7±0.9 ^a
C45	α-Bisabolol oxide A ^{OST}	1749	4.4±0.3 ^b	2.7±0.2 ^a	2.2±0.2 ^a
C46	NI	1755	0.2±0.0 ^b	0.1±0.0 ^a	0.2±0.0 ^b
C47	NI	1764	nd	0.1±0.0 ^a	tr
C48	Benzylbenzoate ^O	1766	0.1±0.0 ^a	nd	nd
C49	NI	1788	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
C50	NI	1842	tr	0.1±0.0 ^a	tr
C51	Z-Spiroether ^D	1889	11.8±1.1 ^b	12.3±0.6 ^b	9.3±0.4 ^a
C52	NI	1890	nd	0.1±0.0 ^a	nd
C53	E-Spiroether ^D	1896	0.3±0.0 ^a	0.4±0.0 ^b	0.3±0.0 ^a
C54	NI	1920	0.1±0.0 ^a	0.1±0.0 ^a	0.1±0.0 ^a
C55	Methyl hexadecanoate ^O	1923	0.1±0.0 ^a	nd	tr
C56	NI	1944	0.7±0.0 ^b	nd	0.5±0.0 ^a
C57	NI	1949	nd	0.7±0.1 ^a	nd
C58	NI	2033	nd	tr	0.1±0.0 ^a
C59	9,12-Octadecadienoic acid (Z,Z)-, methyl ester ^O	2093	0.1±0.0 ^a	nd	tr
C60	9,12,15-Octadecatrienoic acid, methyl ester ^O	2099	0.1±0.0 ^a	nd	tr
C61	Phytol ^{OD}	2120	0.1±0.0 ^a	nd	nd
C62	NI	2141	nd	0.1±0.0 ^a	nd
C63	Z,Z-9,12-Octadecadienoic acid ^O	2147	0.2±0.0 ^b	nd	0.1±0.0 ^a
C64	NI	2152	nd	0.3±0.0 ^a	nd
C65	Tricosane ^A	2298	0.2±0.0 ^b	0.1±0.0 ^a	0.1±0.0 ^a
C66	Tetracosane ^A	2394	0.1±0.0 ^a	tr	tr
C67	Pentacosane ^A	2497	0.8±0.1 ^c	0.6±0.0 ^b	0.4±0.0 ^a
C68	Heptacosane ^A	2694	0.2±0.0 ^b	0.1±0.0 ^a	0.1±0.0 ^a
C69	Nonacosane ^A	2895	0.1±0.0 ^a	tr	tr
Monoterpene hydrocarbons (MT)			0.2	0.2	0.2
Oxygenated monoterpenes (OMT)			0.2	0.1	0.1
Sesquiterpene hydrocarbons (ST)			30.1	39.3	49.6
Oxygenated sesquiterpenes (OST)			51.9	43.3	37.0
Oxygenated diterpenes (OD)			0.1	-	-
Others (O)			1.7	-	0.1
Diacetylenes (D)			12.1	12.7	9.6
Alkanes (A)			-	0.8	0.6
NI			2.0	3.5	2.3
Total identified				99.9	99.5
Number of compounds			47	57	53

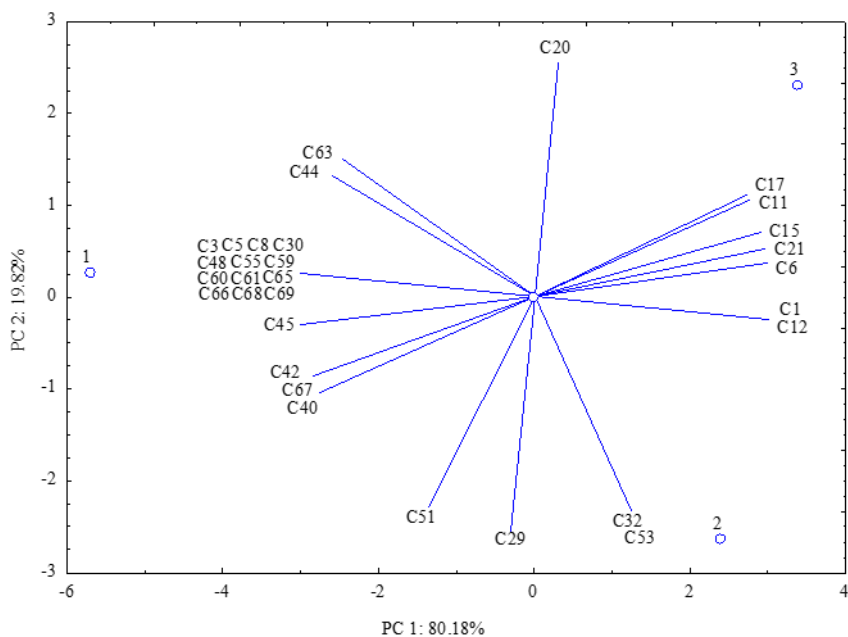


Figure 2. The PCA biplot diagram, showing the relationships among volatile compounds of different chamomile samples. The shown compound titles (C1-C69) were explained in Table 1.

the results were shown in the Figure 3. The darker the color of the squares, the stronger is the correlation between these compounds, i.e. likeness in the amounts of active compounds. At the same time, lighter color suggests a specific dissimilarity in active compounds content.

According to the analyzed results, the correlation among active C40-C69 compounds concentration found

in Samples 1, 2 and 3 was mostly positive, while the correlation among C1-C32 compounds concentration was mainly negative. However, the correlation among tested samples for each individual compound was positive (Figure 3), which indicates that except the variation in the amounts of the major compounds, these compounds were still presented in similar ratios when different processing methods were applied. Yet,

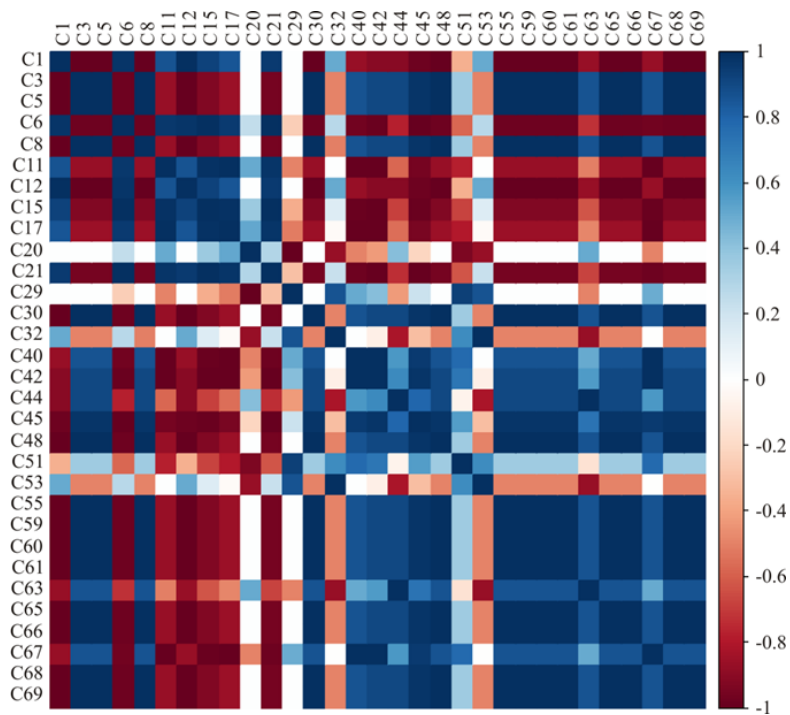


Figure 3. Correlation between volatile compounds of different *Matricaria chamomilla* samples. Colors blue and red indicate positive and negative correlation, respectively. A hue indicates the correlation among concentrations (positive or negative).

according to statistical analysis (Table 1), that variation in the amounts of the individual constituents is statistically significant and points to a higher quality of the essential oil from the Sample 1, obtained from the first-class chamomile (flowers with short stems) material. Sample 1 essential oil contains the highest contents of α -bisabolol, α -bisabolol oxides A and B, and chamazulene, which indicates the highest pharmacological value.

Essential oil yield and composition in chamomile are affected by genetic, agronomical and environmental factors (Kumar et al., 2020). In general, decreased duration of drying time leads to desired essential oil content and composition (Mahmoudi et al., 2020).

α -Bisabolol is a naturally occurring oxygenated sesquiterpene alcohol, the most valuable compound from chamomile essential oil. However, the highest content of α -Bisabolol is noted in ligulate flowers (Pekic et al. 1999). Oxygenated sesquiterpenes, such as α -Bisabolol have higher boiling points compared to hydrocarbon sesquiterpenes, so this compound does not evaporate easily during manipulation of plant material process (Hazrati et al. 2021). This could explain the highest content of this compound in Sample 1 which mostly consists of chamomile flowers. α -Bisabolol is used in a wide range of cosmetic formulations, as a skin conditioning agent, because of its anti-inflammatory and anti-allergic properties (Madhavan, 1999). However, it is also appreciated agents from naturally occurring dietary phytochemicals with gastro protective effects, antitumor activity and many others pharmacological properties (de Siqueira et al., 2014; Javed et al., 2020).

E- β -farnesene is a common volatile component of chamomile and many higher plants (Satyal et al., 2015). Its biosynthesis originates from farnesyl diphosphate (FPP) via terpene synthases, and different levels of expression of terpene synthases influence accumulation (Block et al., 2017). Bearing in mind that highest content of E- β -farnesene was in the sample dried in a bulk, immediately after harvest, without further cleaning, the accumulation of this compound could be a consequence of high activity of terpene synthases during fermentation, due to high respiration rate (Tippmann et al., 2016). This compound is a pheromone in several insect species could have importance in ecological pest control.

Spiroethers from chamomile are important due to their unique biological activity profiles. In this study, the highest content of Z-spiroether is noted in sample of chamomile flowers with long stems and leaves. It is known that the leaves have the highest concentration of spiroether (Ma et al. 2007), as well as that spiroether content increases during drying process (Abbas et al., 2021). Of the two spiroether isomers, the *cis* form exhibits more potent activity (Ma et al., 2007), such as high antimicrobial activity (Kazemi, 2015), and also completely inhibits the production aflatoxin G1 (AFG1) by *Aspergillus parasiticus* and 3-acetyldeoxynivalenol (3-ADON) by *Fusarium graminearum* (Yoshinari et al., 2008).

Conclusions

Considering that essential oil obtained from the first-class chamomile (flowers with short stems) contain the α -Bisabolol as the main compound, its pharmacological value is the highest in comparison to other two samples. Essential oil from chamomile sample with long stems has high content of Z-spiroether, while essential oil obtained from chamomile plant material without separation has high content E- β -farnesene. All these results indicate the importance of precise and controlled postharvest treatments, since it clearly affects the essential oil quality and content in the primary processing of this valuable medicinal plant.

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Uticaj tretmana posležetvene separacije na kvalitet etarskog ulja kamilice

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Sažetak: Ekološki uslovi proizvodnog područja, tehnologija gajenja kao i žetva, ali i posležetveni tretmani značajno utiču na prinos i kvalitet kamilice. Cilj ovog istraživanja je bio da se odredi uticaj metoda separacije, kao posležetvenog tretmana, na kvalitet etarskog ulja, sa ciljem da se unapredi process primarne prerade ove lekovite biljke. Da bi se objasnila struktura laboratorijskih podataka, procenile i sagledale sličnosti i razlike između uzoraka etarskih ulja kamilice dobijenih primenom različitih metoda separacije, korišćena je PCA metoda. Dobijeni rezultati pokazuju da separacija sirovine kamilice kao posležetveni tretman koji prethodi sušenju, ima značajnog uticaja na broj identifikovanih komponenti u etarskom ulju, ali i njihov udeo. Najmanji broj pojedinačnih komponenti u etarskom ulju je imao uzorak cvetnih glavica sa kratkom drškom, pri čemu je zabeležen najveći sadržaj *a*-bisabolol, hamazulen, *Z*-spiroeter i *E*- β -farnesen. U etarskom ulju dobijenom od biljne sirovine sa većim udelom stabla kamilice, sadržaj *a*-bisabolola i hamazulena je bio značajno niži, dok je sadržaj *E*- β -farnesena i *Z*-spiroetera bio značajno viši. Dalje, u etarskom ulju dobijenom od bioljnog materijala bez separacije, zabeležen je najniži sadržaj *a*-bisabolola i *Z*-spiroetera, i najveći sadržaj *E*- β -farnesena. Analiza korelacije je izvedena da bi se ispitala sličnost u sadržaju aktivnih komponenti u etarskom ulju dobijenom primenom različitih procesa separacije kamilice. Svi rezultati ukazuju na značaj primene posležetvenih tretmana, jer se jasno vidi njihov uticaj na kvalitet etarskog ulja ove veoma važne lekovite biljke.

Ključne reči: etarsko ulje, lekovito bilje, *Matricaria chamomilla*, posležetvena prerada, primarna prerada, separacija

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