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VARIATION OF BIOACTIVE COMPOUNDS IN ORGANIC *OCIMUM BASILICUM* L. DURING FREEZE-DRYING PROCESSING

Violeta Alexandra ION, Florin NICOLAU, Andrei PETRE, Oana-Crina BUJOR, Liliana BĂDULESCU

Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author emails: oana.bujor@qlab.usamv.ro; liliana.badulescu@qlab.usamv.ro

Abstract

Common basil (Ocimum basilicum L.), one of the most important aromatic perennial herbs due to its essential oil composition, belongs to Lamiaceae (Labiatae) family. Basil is an economically important herb and it is considered one of the finest aromatic herbs, being widely used as flavor in food industry. Basil samples were characterized in terms of chlorophyll content, total polyphenols, antioxidant activity, and volatile oil content. The fresh harvested leaves and the processed powder from leaves were hydro-distilled for 3 h in a Clevenger-type apparatus. The volatile oil was measured and collected for further GC-MS analysis. As drying technology, freeze-drying was used until the samples reached a loss of 85% from the fresh weigh, with the final dry matter content of 95.86%. Variations for the main constituents of volatile oil: 1,8-cineole, linalool, methyl chavicol, eugenol, α-bergamotene, and α-epi-cadinol were observed after processing.

Key words*: organic basil leaves, drying process, volatile oil, total phenolic content, DPPH antioxidant capacity.*

INTRODUCTION

Common basil (*Ocimum basilicum* L.), is an aromatic perennial herb, belonging to Lamiaceae (Labiatae) family. *Ocimum basilicum* L. can be marketed as fresh or dried products, according to their intended use and the supply chain. Consumer demand for organic processed products that keep more of their original fresh plant characteristics has increased in the last years. Fresh herbs (especially Lamiaceae) usually contain 75–80% water, and these water levels need to be lowered to less than 15% for preservation (Ghasemi Pirbalouti et al., 2013). Both the fresh and dried leaves are widely used to enhance the flavor of foods such as salads, pasta, tomato products, vegetables, pizza, meat, soups, marine foods, and other food products (Attokaran, 2017). *Ocimum basilicum* L. can also be used as a medicinal plant (Lee et al., 2005), along with other plants like *Arnica montana* L. (Nikolova et al., 2013) and *Matricaria chamomilla* L. (Baglou et al., 2017), or for microencapsulation in food products (Alexe et al., 2014).

Drying is by far the most widely used preservation method, as drying inhibits microbial growth, and is also the easiest way to preserve chemical composition. Different types of drying have been applied for herb processing, such as: shade-drying, sun-drying, hot air drying, freeze drying (Ghasemi Pirbalouti et al., 2013), CO_2 drying (Bušić et al., 2014), vibrofluidization (Lima-Corrêa et al., 2017) and convective-pre-drying and vacuum microwave finish-drying (Calín-Sánchez et al., 2012). The quality standard for dried products is freeze drying, which preserves the overall appearance of the original product (Telfser et al., 2019). However, the drying processes can affect the nutritional quality of the herbs, fruits and vegetables. Their phytochemical components like carotens, phenolic compounds and essential oils are of further interest due to their antioxidant and anti-inflammatory activities (Złotek et al., 2016).

The chemical composition of *Ocimum basilicum* L. consists of a wide and varying array of volatile compounds, depending on variations in chemotypes, leaf and flower colors, aroma and origin of the plants (Lal Saran et al., 2017; Vînătoru et al., 2019). Methyl eugenol, methyl chavicol, methyl cinnamate, eugenol, and linalool are generally

the main constituents of the basil essential oil Pistelli et al., 2020).

This study assessed the variation in bioactive compounds (chlorophyll content, total phenolic content, antioxidant activity and volatile oil content) using freeze-drying technology as the processing method for organic basil leaves.

MATERIALS AND METHODS

Chemicals

Folin - Ciocalteau reagent (2 N), DPPH (1,1-diphenyl-2- picrylhydrazyl), and anhydrous sodium carbonate were purchased from Sigma-Aldrich Company. Trolox ((±)-6-hydroxy-2,5,7,8 - tetramethyl chromane-2-carboxylic acid) was purchased from Acros Organics, Fisher Scientific (Geel, Belgium). Methanol and hexane were purchased from Honeywell (Riedel-de Haën, Seelze, Germany). Gallic acid was purchased from Carl Roth, and acetone was purchased from Chemical Company.

Organic basil materials

Organic basil leaves (*O. basilicum* var. *crispum*) were purchased from Vegetables Research and Development Station of Buzau at commercial maturity, in September 2019. Two types of leaf samples were used for analysis: a) fresh leaves and b) freeze-dried leaves.

For the freeze-drying of the samples, the batches of basil leaves (approximately 20 g each) were frozen in an ultra-low temperature freezer MDF-594-PE from Panasonic Corporation (Osaka, Japan) at −80°C for 24 h. After freezing, the samples were freeze-dried in an Alpha 2-4 LSCplus (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) freeze drier at a pressure of 0.5 mPa and with the shelf temperature of −55°C.

Moisture determination

Moisture content was determined when the sample was received and after drying. An amount of 0.5 g of fresh sample was dried until constant mass in a Sartorius thermobalance at 105°C.

Determination of chlorophylls a and b, and of total carotenoids content

Extraction of chlorophyll content was made based on Lichtenthaler & Wellburn (1983) using acetone 80% as solvent and expressed as µg/mL of extract:

$$C_a = 12.21A_{663} - 2.8A_{646} \quad (1)$$

$$C_b = 20.13A_{646} - 5.03A_{663} \quad (2)$$

$$C_{x+c} = \frac{1000\ A_{470} - 3.27C_a - 104C_b}{229} \quad (3)$$

The results were further calculated for mass and final extraction volume and the final results expressed as mg per g of dry matter (mg/g DM).

Essential oil extraction and GC-MS analysis

The fresh harvested leaves (300 g) and freeze-dried powders (60 g) were hydro-distilled for 3 h in a Clevenger-type apparatus. The obtained oil samples were diluted with hexane and analyzed by Gas Chromatography coupled with Mass Spectrometry (GC-MS). Analysis of the essential oils was performed on an Agilent 6890 GC coupled with a 5973 Network single quadruple mass spectrophotometer detector in Electron Ionization (EI) mode and 7673 injector on a HP-5MS capillary column (30 m × 0.25 mm id, 0.25 µm film thicknesses). The following operating initial conditions were employed: 50°C for 8 min, then a 4°C/min ramp to 280°C. Helium was used as carrier gas with a constant flow of 1.0 mL/min, injection volume 3 µL with a split ratio 50: 1. The temperatures for inlet, MS transfer line and ion source was 250°C, 250°C and 230°C, respectively. The GC column was coupled directly to the spectrometer in EI mode at 70 eV with the mass range of 50-550 amu at 2 scan/s.

Extraction of polyphenols

Extraction of polyphenols from basil samples was based on the method described by Stan et al. (2017). To 1 g of dried sample or 0.2 g of fresh sample, 10 mL of 70% aqueous methanol were added and the samples were incubated in the dark overnight at 4°C. After that, the extracts were shaken at 500 rpm for 1 h and then centrifuged at 5000 rpm, 4°C, for 10 min. The supernatant was recovered in a 50 mL centrifuge tube and the residue was re-extracted two more times with 10 ml of 70% aqueous methanol. All three supernatants were combined and then the volume of each extract

was adjusted to 30 mL with the extraction solvent.

Total phenolic content (TPC)

The total phenolic content of the extract solutions was determined by the Folin - Ciocalteu spectrophotometric method described by George et al. (2005). A 2.5 mL of water-diluted Folin - Ciocalteu reagent (1/10) was added to 0.5 mL methanolic extract. The mixture was incubated for 2 min at room temperature, and after incubation, 2 mL of sodium carbonate (7.5%) was added. The mixture was heated for 15 min at 50°C and finally cooled in a water-ice bath. A mixture of solvent and reagents was used as a blank.

Absorbance was measured in a Specord 210 Plus UV-Vis spectrophotometer (Analytik Jena, Jena, Germany) at 760 nm. The amount of total phenolic content was expressed as mg Gallic acid equivalents per g dry matter (mg GAE/g DM). Triplicates of independent extract solutions were analyzed.

DPPH radical scavenging activity

The DPPH test was adapted from a method described by Bujor et al. (2016) with some modifications. Briefly, 0.1 mL of the sample extract was added to 2 mL of 0.2 mM solution of DPPH in methanol (prepared daily, protected from the light and kept in ice). The solutions mixture was put under dark and shaking at 500 rpm (IKA KS 260 homogenizer) for 30 minutes. Then the absorbance was measured at 515 nm. Methanol was used as a blank reference. The results were expressed as micromoles of Trolox equivalents per gram of dry matter (μM TE/g DM).

RESULTS AND DISCUSSIONS

Fresh samples showed a moisture content loss of 86.8% after freeze-drying. The remaining powder (13.2% of the fresh sample), showed a residual moisture of 4.14%. The same range of basil leaves moisture contents were obtained by Ghasemi Pirbalouti et al. (2013) (80.72% for purple and 83.97% for green basil) and Bušić et al. (2014) (89.72% and 90.45% for fresh leaves, and 6.05% for freeze-dried leaves). As mentioned by Bušić et al. (2014), the European Spice Association (2018) recommends that the maximum moisture content of dried basil to be up to 12%. So in the case of the present study, more freeze-drying time was required in order to ensure a good quality to the product.

Content of leaves' pigments

The analysis of photosynthetic pigments (total chlorophyll, total carotenoids) showed a variation between dried and fresh leaves (Table 1). The chlorophyll a content decreased with 16.5% and chlorophyll b with 2.7% after drying, resulting in an approximately total decrease of 20% of total chlorophyll content in freeze-dried leaves. The carotenoids content decreased 43% in freeze-dried leaves compared to fresh leaves.

Table 1. Determination of foliar pigments (chlorophyll a, chlorophyll b, carotenoids)

	Fresh leaves	Freeze-dried leaves
Chlorophyll a (mg/g DM)	6.31 ± 0.63	5.27 ± 0.39
Chlorophyll b (mg/g DM)	2.12 ± 0.11	2.06 ± 0.20
Total chlorophyll (mg/g DM)	8.43 ± 0.74	7.33 ± 0.59
Total carotenoids (mg/g DM)	1.53 ± 0.15	1.07 ± 0.08

Variations in the essential oil composition

The identification of the individual compounds in leaves of organic basil for fresh and freeze-dried material was carried out using mass spectra and their identities were confirmed by comparing their mass spectra with NIST Mass Spectral Library and literature as showed in Table 2.

The essential oil (EO) extraction yield of the basil samples was 0.067% w/w for fresh leaves and 0.158% w/w for powdered leaves. EO composition is reported in Table 2, with 61 compounds identified in both fresh and freeze-dried leaves. More than 50% of the total chemical composition of fresh leaves essential oil (Figure 1A) was composed of linalool 27.59%, methyl chavicol 11.43%, α-epi-cadinol 10.52% and eugenol 7.30%. For freeze dried leaves the concentration (Figure 1B) of the main constituents varied compared to fresh leaves: linalool 18.14%, α-epi-cadinol 14.30%, eugenol 9.11%, γ-cadinene 5.28%, and methyl chavicol, 4.93%.

This chemical composition reveals that essential oil of *O. basilicum* L. processed by freeze-drying shows a decrease in monoterpenes hydrocarbons (1.11%) and oxygenated monoterpenes (20.42%). The

compound with higher molecular mass and boiling point maintained its concentration, whereas sesquiterpene hydrocarbons decreased with 14.43% and oxygenated sesquiterpenes with 5.85%. Other chemical compounds maintained similar concentrations for fresh leaves (9.61%) and freeze dried leaves (10.86%), as showed in Table 2.

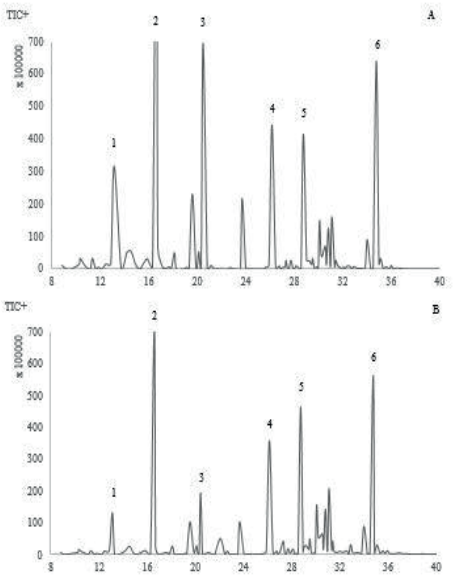

Figure 1. Essential oil chromatographic profile of fresh basil leaves (A) and freeze-dried basil leaves (B): 1) 1,8 Cineole; 2) Linalool; 3) Methyl chavicol; 4) Eugenol; 5) α-Bergamotene; 6) α-epi-Cadinol

Variation of total phenolic content and antioxidant activity

The influence of freeze-drying process on total phenolic content and antioxidant activity of basil leaves are presented in Figure 2. In this study, the highest TPC was found for freeze-dried leaves (4.76 mg GAE/g DM) compared to only 0.32 mg GAE/g DM for fresh leaves (Figure 2A).

In the case of the antioxidant activity (Figure 2B), the trends are similar to the one observed for the total phenolic content. Freeze dried leaves remains the samples which display higher antioxidant activity (295.00 µM TE/g DM) compared to fresh leaves (42.76 µM TE/g DM). These results are in agreement with those of Bušić et al. (2014) who determined that both TPC and antioxidant activity of fresh samples were lower than freeze dried basil samples.

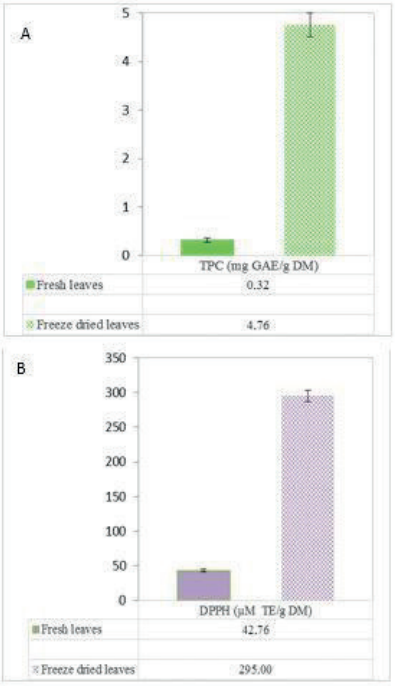

Figure 2. Total phenolic content (A) and antioxidant activity (B) of common basil leaves

Moreover, these findings are not surprising, since freeze-drying is known as the best solution to preserve foods' quality (Raponi et al., 2017). Recent work of Pistelli et al. (2020) also reported results of TPC in accordance with the results of our study (3.75 and 4.25 mg GAE/g DM for natural dried leaves of basil). Zlotek et al. (2016) founded slightly higher TPC and DPPH results compared to present study, but this could be attributed to the different type of solvents, method of extraction and sample origin.

CONCLUSIONS

The changes in volatile oil constituents during freeze-drying vary due to the different boiling points of the compounds. Although freeze drying is one of the most recommended techniques for herbs drying, significant changes can occur in the chemical composition of the essential oil of *Ocimum basilicum* L.

Table 2. Variation in chemical composition of *Ocimum basilicum* L. leaves after processing

	Compound	Chemical class	*RT	Fresh leaves (%)	Freeze-dried leaves (%)	**Ref
1	Camphene	Monoterpenes hydrocarbons	8.86	0.19	0.13	Pistelli et al., 2020; Tshilanda et al., 2016; Ghasemi Pirbalouti et al., 2013; Amaral-Baroli et al., 2016
2	Sabinene	Monoterpenes hydrocarbons	10.27	0.26	0.16	Amaral-Baroli et al., 2016; Sonmezdag et al., 2018
3	β-Pinene	Monoterpenes hydrocarbons	10.36	0.55	0.37	Koroch et al., 2017; Sonmezdag et al., 2018
4	1-Octen-3-ol	Alkenyl alcohol	10.78	0.16	0.12	Tshilanda et al., 2016; Ghasemi Pirbalouti et al., 2013
5	2,3-Dehydro-1,8-cineole	Pyrans	11.18	0.02	0.03	
6	β-Myrcene	Monoterpenes hydrocarbons	11.38	0.54	0.31	Tavallali et al., 2020
7	3-Octanol	Aliphatic alcohol	11.64	0.03	0.02	Amaral-Baroli et al., 2016
8	α-Phellandrene	Monoterpenes hydrocarbons	11.94	0.10	0.09	Tavallali et al., 2020
9	α-Terpinene	Monoterpenes hydrocarbons	12.52	0.27	0.29	Sonmezdag et al., 2018; Tavallali et al., 2020
10	p-Cymene	Alkylbenzene	12.88	0.25	0.26	Tavallali et al., 2020
11	1,8 Cineole	Oxygenated monoterpenes	13.16	5.22	3.34	Koroch et al., 2017
12	trans-β-Ocimene	Monoterpenes hydrocarbons	13.75	0.04	0.03	Sonmezdag et al., 2018; Tshilanda et al., 2016; Tavallali et al., 2020
13	β-Ocimene	Monoterpenes hydrocarbons	14.21	0.84	0.28	Tavallali et al., 2020
14	γ-Terpinene	Monoterpenes hydrocarbons	14.57	0.89	0.66	Sonmezdag et al., 2018; Tshilanda et al., 2016
15	cis-Sabinene hydrate	Oxygenated monoterpenes	14.91	0.26	0.11	Pistelli et al., 2020; Tshilanda et al., 2016
16	1-Octanol	Primary alcohol	15.35	0.04	0.04	Al-Maskri et al., 2011
17	Terpinolene	Monoterpenes hydrocarbons	16.00	0.05	0.32	Tavallali et al., 2020
18	cis-β-Terpineol	Oxygenated monoterpenes	16.30	0.12	0.05	Anand et al., 2019
19	Linalool	Oxygenated monoterpenes	16.61	27.59	18.14	Sonmezdag et al., 2018; Tavallali et al., 2020
20	Nonanal	Aldehyde	16.77	1.06	0.58	Jiang et al., 2016
21	1-Octen-3-ol acetate	Ester	17.20	0.06	0.04	Tavallali et al., 2020
22	(E)-p-2-Menthen-1-ol	Oxygenated monoterpenes	17.33	0.07	0.07	
23	4-Acetyl-1-methylcyclohexene	Oxygenated monoterpenes	17.72	0.12	0.16	
24	Camphor	Oxygenated monoterpenes	18.14	0.84	0.67	Sonmezdag et al., 2018; Tavallali et al., 2020
25	trans-2-Nonen-1-al	Primary alcohol	19.00	0.05	0.03	
26	Isoborneol	Oxygenated monoterpenes	19.10	0.07	0.05	Amaral-Baroli et al., 2016;
27	α-Terpineol	Oxygenated monoterpenes	19.20	0.09	0.08	Calín-Sánchez et al., 2012
28	Terpinen-4-ol	Oxygenated monoterpenes	19.62	3.80	2.60	Tshilanda et al., 2016
29	α-Terpinol	Oxygenated monoterpenes	20.16	0.88	0.69	Koroch et al., 2017
30	Methyl chavicol	Oxygenated monoterpenes	20.50	11.43	4.93	Koroch et al., 2017; Tshilanda et al., 2016
31	n-Octyl acetate	Ester	21.17	0.19	0.16	Pistelli et al., 2020;
32	Linalyl acetate	Oxygenated monoterpenes	22.76	0.10	0.28	Tavallali et al., 2020
33	L-α-Bornyl acetate	Oxygenated monoterpenes	23.73	3.58	2.61	Tavallali et al., 2020
34	exo-2-Hydroxycineole acetate	Oxygenated monoterpenes	25.68	0.10	0.08	Özcan et al., 2002
35	δ-Elemene	Sesquiterpene hydrocarbons	25.94	0.19	0.23	Tavallali et al., 2020
36	Eugenol	Allylbenzene	26.19	7.30	9.11	Koroch et al., 2017; Tavallali et al., 2020
37	α-Copaene	Sesquiterpene hydrocarbons	26.80	0.17	0.27	Koroch et al., 2017; Tavallali et al., 2020
38	β-Cubebene	Sesquiterpene hydrocarbons	27.28	0.14	0.24	Koroch et al., 2017; Tavallali et al., 2020

39	β-Elemene	Sesquiterpene hydrocarbons	27.35	0.46	0.81	Koroch et al., 2017; Tavallali et al., 2020
40	α-Cubebene	Sesquiterpene hydrocarbons	27.46	0.08	0.14	Calín-Sánchez et al., 2012
41	Methyleugenol	Phenylpropene	27.76	0.45	0.47	Pistelli et al., 2020; Tavallali et al., 2020
42	Caryophyllene	Sesquiterpene hydrocarbons	28.18	0.17	0.27	Ahmed et al., 2019;
43	α-Bergamotene	Sesquiterpene hydrocarbons	28.79	6.84	11.81	Pistelli et al., 2020;
44	cis-β-Farnesene	Sesquiterpene hydrocarbons	28.98	0.14	0.21	Amaral-Baroli et al., 2016
45	Epi Bicyclosesquiphellandrene	Sesquiterpene hydrocarbons	29.05	0.29	0.55	Ahmed et al., 2019; Al-Maskri et al., 2011
46	a-Humulene	Sesquiterpene hydrocarbons	29.26	0.45	0.71	Ghasemi Pirbalouti et al., 2013
47	γ-Muurolene	Sesquiterpene hydrocarbons	29.56	0.55	1.25	Amaral-Baroli et al., 2016
48	Germacrene D	Sesquiterpene hydrocarbons	30.12	2.47	3.97	Sonmezdag et al., 2018
49	(E)-β-Famesene	Sesquiterpene hydrocarbons	30.27	0.66	1.27	Pistelli et al., 2020;
50	Bicyclogermacrene	Sesquiterpene hydrocarbons	30.60	1.17	1.65	Pistelli et al., 2020; Tavallali et al., 2020
51	α-Selinene	Sesquiterpene hydrocarbons	30.85	2.08	3.62	Amaral-Baroli et al., 2016
52	γ-Cadinene	Sesquiterpene hydrocarbons	31.14	2.65	5.28	Tavallali et al, 2020
53	δ-Cadinene	Sesquiterpene hydrocarbons	31.45	0.46	1.09	Tavallali et al., 2020
54	Cubedol	Oxygenated sesquiterpenes	32.10	0.11	0.24	Maurya et al., 2019
55	4-epi-Cubedol	Oxygenated sesquiterpenes	32.36	0.15	0.17	Tshilanda et al., 2016
56	Nerolidol	Oxygenated sesquiterpenes	32.64	0.15	0.30	Tavallali et al., 2020
57	Spathulenol	Oxygenated sesquiterpenes	32.96	0.14	0.78	Tavallali et al., 2020
58	1,10-di-epi-Cubenol	Oxygenated sesquiterpenes	34.06	1.50	2.27	Milenković et al., 2019
59	α-epi-Cadinol	Oxygenated sesquiterpenes	34.82	10.52	14.30	Koroch et al., 2017; Tavallali et al., 2020
60	β-Eudesmol	Oxygenated sesquiterpenes	35.02	0.27	0.42	Koroch et al., 2017; Tshilanda et al., 2016
61	α-Cadinol	Oxygenated sesquiterpenes	35.15	0.55	0.77	Koroch et al., 2017

*RT-retention time; **references where similar compounds were found.

No significant changes in chlorophyll content was observed. Given the high content of phenolic compounds and antioxidant activity of dried leaves, the freeze-drying is a sustainable processing technique for preservation of phenolic compounds and antioxidant activity. Further studies and trials are required in order to optimize the freezing temperature for a better understanding of the freeze-drying temperature effect on the quality of organic *Ocimum basilicum* L.

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