Journal of Applied Research on Medicinal and Aromatic Plants 2 (2015) 182–187

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

journal homepage: www.elsevier.com/locate/jarmap

Influence of different drying methods on drying period, essential oil content and composition of *Lippia citriodora* Kunth

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ARTICLE INFO

Article history:
Received 27 November 2014
Received in revised form 22 May 2015
Accepted 1 June 2015
Available online 22 June 2015

Keywords:
Lemon verbena (*Lippia citriodora* Kunth.)
Oven drying
Vacuum drying
Freeze drying
Essential oils

ABSTRACT

The influence of four drying methods (shade drying, freeze drying, oven drying and vacuum drying at 40, 50 and 60 °C) on the essential oil content and composition of lemon verbena (*Lippia citriodora* Kunth.) was evaluated. The essential oil content and composition of dried samples were determined by hydro-distillation using a Clevenger apparatus and gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) respectively. Results showed the highest and lowest drying times belonged to shade drying (53 h) and vacuum drying at 60 °C (3 h) respectively. Glandular trichomes were vulnerable to oven whereas vacuum drying and freeze drying preserved them. The maximum essential oil content (1.0 ml/100 g D.M.) obtained from vacuum-dried (60 °C) and oven-dried (40 °C) samples. Freeze drying preserved the highest amount of oxygenated monoterpenes, especially citral (64.7%) but the highest amounts of limonene and 1,8-cineole (8.2 and 7.2% respectively) were determined in vacuum-dried (60 °C) leaves. Apart from the standard oven drying at 40 °C, vacuum drying at 60 °C can be considered as an alternative approach to dry lemon verbena in a significantly shorter drying time.

1. Introduction

The genus *Lippia* is one of 41 genera of shrubs, herbs or trees which belong to the family Verbenaceae (Combrinck et al., 2006). This genus includes approximately 200 species that they are mainly distributed throughout South and Central America, and Tropical Africa (Pascuala et al., 2001). The word *Lippia* is derived from Auguste Lippi, an Italian botanist born in Paris in 1678 (Terblanche and Kornelius, 1996). Most *Lippia* species are utilized as gastrointestinal and respiratory remedies in traditional medicine and some of them have shown antimalarial and antiviral activities (Abad et al., 1999; Pascuala et al., 2001). The leaves from *Lippia citriodora* are utilized as seasoning for food preparations and flavoring beverages (Shahhoseini et al., 2013). Essential oil is the main active substance in *L. citriodora* and its important constituents are citral, 1,8-cineole, geraniol, linalool and limonene (Terblanche and Kornelius, 1996).

Medicinal and aromatic plants can be marketed as fresh or dried products, according to their use. Fresh plants cannot be supplied in a profitable way to all world-wide locations (Ghasemi Pirbalouti et al., 2013). The main purpose of drying medicinal plants is to extend product shelf life, minimize packaging requirements and reduce shipping weights (Hamrouni Sellami et al., 2011). Drying is used to stop the growth of microorganisms and preserve the quality of agricultural products (Lin et al., 2011). Drying techniques have already been applied to reduce the moisture content of some of medicinal and aromatic plants and their effects on yield and composition of essential oil have been studied by other researchers, such as *Chamaemelum nobile* L. (Omidbaigi et al., 2004), *Matricaria recutita* L. (Azizi et al., 2009), *Satureja hortensis* L. (Sefidkon et al., 2006), *Ocimum basilicum* L. (Ebadi et al., 2013), *Laurus nobilis* L. (Hamrouni Sellami et al., 2011), *Mentha longifolia* L. (Asekun et al., 2007).

Therefore, determining a suitable drying method to achieve higher active substances in medicinal plants is very important. A literature search was undertaken on the effects of different drying methods on essential oil content and composition of *Lippia* genus. Calvo-Irabien et al. (2009) compared shade drying, sun drying and oven drying at 20 and 40 °C and showed that drying treatments had no effect on qualitative or quantitative characteristics of the essential oil. Agah and Najafian (2012) reported that shade drying is suitable for preservation of limonene (7.0%), neral (24.1%) and geranial (31.9%) in *L. citriodora* essential oil. Yousif et al. (2000) reported in *L. berlandieri* that the level of thymol in vacuum-microwave-dried plants was 1.3 times the hot air-dried and they had better color. Shahhoseini et al. (2013) investigated the effect of drying air

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http://dx.doi.org/10.1016/j.jarmap.2015.06.001
2214-7861/

temperature (30, 40, and 50 °C) and air flow rate (0.5, 1, and 1.5 m/s) on the quantity and quality of the essential oil of *L. citriodora*. In that research, the maximum essential oil content and the majority of the compounds were obtained at 50 °C and a 0.5 m/s air flow.

Trichomes are described as unicellular or multicellular appendages, which originate from epidermal cells only and develop outwards on the surface of different plant organs (Werker, 2000). Plant species that have glandular trichomes usually produce comparatively large amounts of bioactive secondary metabolites. The secondary metabolites of several biochemical pathways can be found in glandular trichomes of higher plants, although terpenoids seem to predominate and are of primary interest to the pharmaceutical industry (Duke et al., 2000). For the Verbenaceae family several reports of trichomes exist (Casadoro and Rascio, 1982; Cantino, 1990; Yashodhara et al., 2001; Combrinck et al., 2006). Specifically for L. *citriodora*, Argyropoulou et al. (2010) reported the presence of five types of glandular and one type of non-glandular trichome. Essential oil may be lost due to volatilization and mechanical damage to glands during drying. Glandular trichomes of *Lippia* species are vulnerable to oil loss (Combrinck et al., 2006). According to important role of glandular trichomes in the maintenance of essential oil in lemon verbena, investigation of the effects of post-harvest processes such as drying on them is necessary.

The aim of this study was to investigate the effect of drying methods (shade drying, freeze drying, oven drying at 40, 50 and 60 °C and vacuum drying at 40, 50 and 60 °C) on the structure of glandular trichomes, essential oil content and composition of lemon verbena.

2. Materials and methods

2.1. Plant materials

Fresh leaves of lemon verbena (*Lippia citriodora* Kunth.) which used in this research were harvested from the greenhouse of Department of Horticulture, Tarbiat Modares University, Iran. The harvested leaves were randomly divided into eight batches containing three sets of 100 g each. Then they were stored in a refrigerator at a temperature of 4 ± 0.5 °C before the drying experiments. To measure the initial moisture content, the leaves were dried using an oven (105 ± 2 °C for 7 h) until there was no change in weight between the two consecutive measurements. This process was repeated five times. The initial moisture content of the lemon verbena was 62.08% on a wet basis.

2.2. Drying methods

The drying was carried out until reaching moisture content of about 9% on the wet basis in all drying treatments, the weight loss was measured using an analytical balance with 0.0001 readability (Sartorius, TE214S, Germany). All treatments were replicated 3 times.

2.2.1. Shade drying (SD)

This method was performed in a dark and dry room with appropriate ventilation. The temperature of the room was 25 ± 2 °C while the relative air humidity varied within the range of 22–27%.

2.2.2. Freeze drying (FD)

The samples were frozen by direct immersion in liquid N_2 and then were lyophilized in a freeze-drier (Zirbus, Vaco 5, Germany) for a period of 7 h at −52 °C.

2.2.3. Vacuum drying (VD)

The drying process was conducted in a vacuum oven (Memmert, GMBH D-91126, Germany). A fixed vacuum level (250 kPa) and three temperature levels of 40, 50 and 60 °C were used to dry the samples.

2.2.4. Oven drying (OD)

A convection oven (Binder, 7200 Tuttlingen, Germany). Size: 100 × 120 × 60 cm (Width × Height × Depth), capacity: 45 kg, relative humidity: 38 ± 4%. Three temperature 40, 50 and 60 °C were used to dry samples.

2.3. Scanning electron microscopy (SEM)

The samples were mounted on double-sided carbon tape on stubs, plasma-coated with 10 μm gold (KYKY-SBC12, Beijing, China) and viewed with a scanning electron microscope (KYKY-EM3200, Beijing, China). Resolution 6.0 nm, magnification 15*x*–250,000*x*, accelerating voltage: 0–30 kV, three electron magnetic lens system.

2.4. Essential oil isolation

The essential oils (EO) from lemon verbena leaves were extracted by hydro-distillation using Clevenger type apparatus. Approximately 50 g of sample was placed in a round-bottomed flask containing 500 ml of distilled water. Distillation was continued for approximately 3 h and the collected EO content was determined on the basis of dry matter (ml/100 D.M.). The EOs were dried over anhydrous sodium sulfate to eliminate traces of moisture and stored in dark glass bottles at 4 °C until analysis.

2.5. Gas chromatography (GC)

EOs were analyzed by GC, using a Thermo-UFM ultra-fast gas chromatograph equipped with a Ph-5 fused silica column (10 m × 0.1 mm i.d., film thickness 0.4 μm). Oven temperature was held at 60 °C for 5 min and then programmed to 285 °C at a rate of 80 °C/min. Detector (FID) temperature was 280 °C and injector temperature was 280 °C; helium was used as carrier gas with a linear velocity of 0.5 ml/min. The percentages of compounds were calculated by the area normalization method, without considering response factors.

2.6. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were carried out on a Varian 3400 GC–MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm); oven temperature was 50–240 °C at a rate of 4 °C/min, transfer line temperature 260 °C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1:60, ionization energy 70 eV, scan time 1 s, mass range 40–300 a.m.u. The EO components were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature (Adams, 2007; Davies, 1990). Mass spectra from the literature were also compared (Adams, 2007; Stenhagen et al., 1974). The retention indices were calculated for all volatile constituents, using a homologous series of *n*-alkanes.

2.7. Data analysis

The research was conducted using completely randomized block design with three replicates. Differences of means were tested by using a Duncan's Multiple Range Test (SAS) at 5% level of significance.

3. Results and discussion

3.1. Effect of drying methods on drying period

Drying method had significant effect on drying time (Fig. 1). Shade drying showed the longest drying time (53 h) while the shortest (3 h) belonged to vacuum drying at 60 °C. As expected, with an increase of temperature in vacuum and oven drying, drying time reduced significantly. In the oven drying, drying times of the verbena leaves were 25, 14 and 6 h at the temperature levels of 40, 50 and 60 °C, respectively. In other words, drying times of oven drying at 40 and 50 °C were about 4.2 and 2.3 times longer than oven drying at 60 °C. In the vacuum drying, drying times of samples were 13, 8 and 3 h at the temperature levels of 40, 50 and 60 °C, respectively, and drying time at 60 °C was shortened by a factor of 4.3 and 2.6 compared with the drying processes conducted at 40 °C and 50 °C, respectively. Vacuum drying approximately was two times faster than oven drying irrespective of drying temperature. Although the drying time for the freeze drying was shorter than for the vacuum drying at 50 °C (7 vs 8 h), the difference was not statistically significant. Drying time depends on amount of plant material, its moisture content, temperature and humidity (Sefidkon et al., 2006). Long drying period causes degradation of plant materials and also increases energy consumption and drying costs (Sagar and Suresh Kumar, 2010). The present results based on reducing the drying time by increasing the temperature are in agreement with previous works (Ghasemi Pirbalouti et al., 2013; Azizi et al., 2009; Ebadi et al., 2013; Figiel et al., 2010; Shahhoseini et al., 2013).

3.2. Effect of drying methods on glandular trichomes

Plants have both glandular and non-glandular and glandular trichomes that play different roles. Glandular trichomes are one of the most common secretory structures that produce and store essential oils in plants (Biswas et al., 2009). Leaves of L. *citriodora* have one type of non-glandular and at least five types of glandular trichomes (Argyropoulou et al., 2010). The results of SEM (Fig. 2) showed that the glandular trichomes were vulnerable to drying condition. Increasing of temperature in oven drying and prolongation of the vacuum drying damaged the glandular trichomes and the lowest amount of damage was observed in the shade and freeze drying methods. Deformation and cracking of glandular trichomes were quite obvious in oven and vacuum drying conditions. The current findings about the negative impact of increasing temperature on trichomes are in agreement with previous reports (Combrinck et al., 2006; Antal et al., 2014).

3.3. Effect of drying methods on the EO content

The results of EO content analysis in different drying methods are presented in Fig. 3. The results showed that the drying method had a significant effect on EO content ($p < 0.05$). The highest EO contents (1.0 ml/100 g D.M.) occurred with vacuum drying at 60 °C and oven drying at 40 °C whereas shade-dried and oven-dried samples at 50 °C were characterized by appreciable EO contents (0.9 ml/100 g D.M.) and no significant differences were found between them. The lowest EO contents (0.7 ml/100 g D.M.) were obtained by vacuum drying at 40 °C and oven drying at 60 °C. EO contents obtained from vacuum drying compared to those after oven drying showed that despite the use of the same temperatures in both methods, oven drying resulted in higher EO contents.

The results showed that increasing drying temperature in vacuum drying (from 40 to 60 °C) resulted in a significant increase in the EO content. This could be attributed to the vacuum condition which increased evaporation of EO. When drying temperature in vacuum drying decreased (for example: 40 °C), time of presence of plant material in vacuum condition increased (chapter 3.1.). Increasing the presence of plant material in vacuum condition will cause the increasing the possibility of damage to the trichomes. An operating pressure below atmospheric pressure can cause EO loss due to volatility, polarity and chemical structure of each constituent (Argyropoulos and Müller, 2014). *Lippia* species bear EO in glandular trichomes which are thought to be more vulnerable than subcutaneous glands and which may be damaged by vacuums, causing the release of volatile compounds (Combrinck et al., 2006). Results of this investigation showed that freeze drying could not preserve EO content in the leaves of the lemon verbena. Previous studies have mentioned that low pressure in freeze dryer chamber strongly affects the glandular trichomes and results in significant loss of EO from the trichomes (Antal et al., 2014). Based on our results, with increase of drying temperatures in oven drying, the EO content decreased significantly and these results are in agreement with Shahhoseini et al., 2013; who reported that when drying temperature increased (30–50 °C), EO content of *L. citriodora* were gradually decreased. Similar results were reported by other researchers in medicinal plants such as *Mentha piperita* L. (Blanco et al., 2002), *Artemisia dracunculus* L. (Arabhosseini et al., 2006) and *Salvia officinalis* L. (Hamrouni Sellami et al., 2013). The reason for this phenomenon could be evaporation of EO as a result of increase the temperature (Ghasemi Pirbalouti et al., 2013; Argyropoulos and Müller, 2014; Hamrouni Sellami et al., 2011). Several researchers reported that increasing of drying temperature can damage to glandular trichomes in medicinal plants (Díaz-Maroto et al., 2003 Yousif et al., 2000). In contrast, some studies reported that increasing drying temperature could induce an increase in the EO content of medicinal plants such as *S. hortensis* L. (Sefidkon et al., 2006) and *Lippia alba* (Mill.) (Castro et al., 2000). These opposite results may be due to differences in plant species, secretory structures and chemical composition of EO (Hamrouni Sellami et al., 2012).

3.4. Effect of drying methods on EO composition

In the current experiment, 24 compounds were identified in the EOs of *L. citriodora* affected by different drying methods which represented 97.4–98.7% of the EOs present. The chemical compounds of the EOs can be seen in Table 1. The main components of the EO in all drying methods were geranial (32.0–38.5%), neral (32.0–38.5%), limonene (5.0–8.2%), 1,8-cineole (4.5–7.3%), γ-elemene (4.6–6.5%) and spathulenol (3.9–5.5%).

The results showed that drying methods caused some variation in main components of EO and they changed the chemical profiles of lemon verbena EO. Freeze drying preserved the most amount of citral (geranial + neral) (64.7%) but the lowest amount of this compound (55.4%) was observed in oven dried at 40 °C sample. With the increase of the temperature from 40 to 60 °C in oven drying, the amount of citral was increased. However, this was reversed with the vacuum-drying technique and increasing temperature had negative effect on amount of citral. The changes in the concentrations of the volatile compounds during drying depend on several factors, such as the drying method, the type of plant (Asekun et al., 2007), volatility and chemical structure of the constituent (Venskutonis, 1997). These variations occur from losses or increase in oil constituents due to the formation of new compounds by oxidation, glycoside hydrolysis, esterification, and/or other processes (Ghasemi Pirbalouti et al., 2013). Moreover, Gershenzon et al. (2000) suggested that there may be membranes selectively more permeable to certain volatiles or separate compartments for the synthesis of emitted volatiles and stored substances.

Amounts of limonene and 1,8-cineole were reduced with the increase of the temperature from 40 to 60 °C in oven drying, but reverse results were obtained in vacuum drying. Lower contents of γ-elemene and spathulenol were observed for the freeze dried

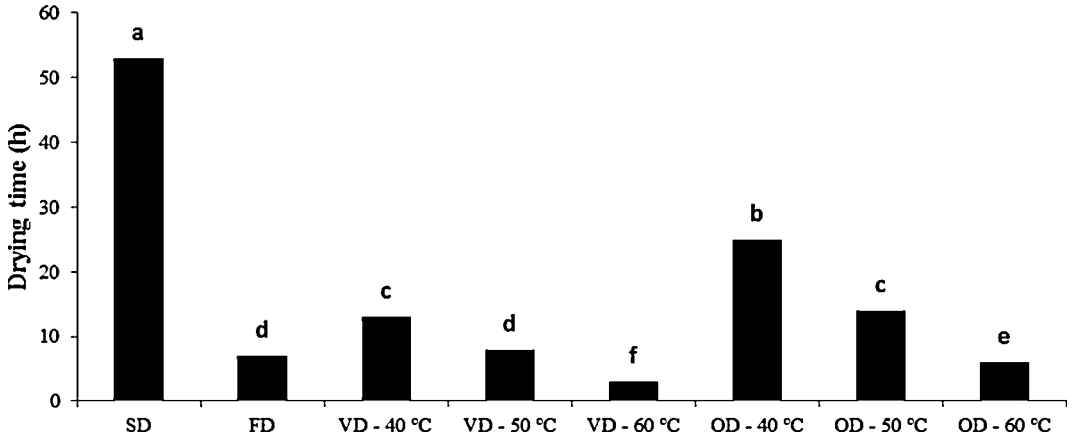

Fig. 1. Effect of different drying methods on drying time of lemon verbena leaves (SD: shade drying, FD: freeze drying, VD: vacuum drying, OD: oven drying).

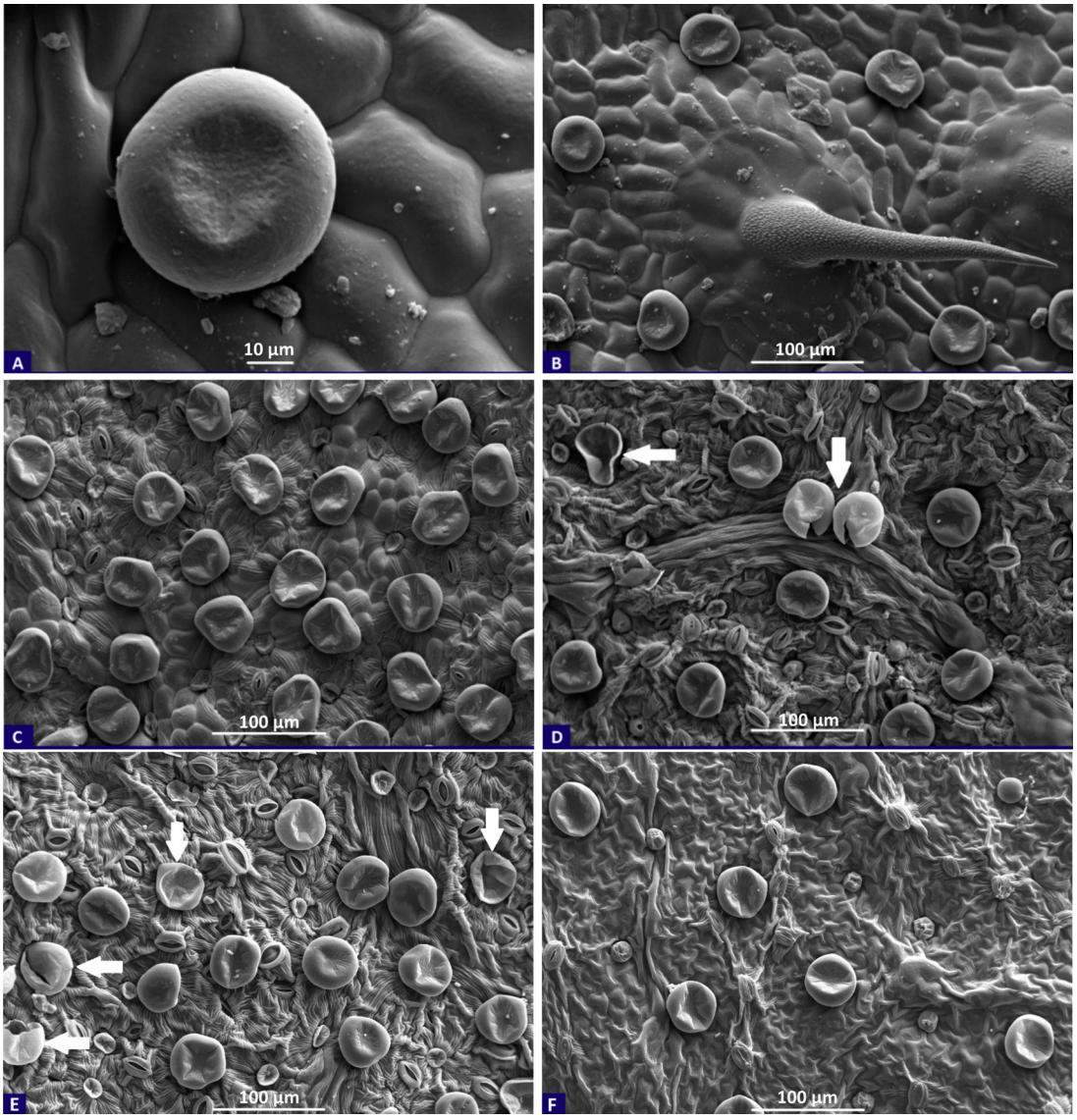

Fig. 2. (A) Glandular trichome on leaf of *Lippia citriodora* Kunth. (B) Cystolithic trichome. (C) Distribution of glandular trichomes on leaf lower surface (abaxial) in freeze dried leaves. (D) Damaged glandular trichomes by oven drying at 60 °C. (E) Damaged glandular trichomes by vacuum drying at 40 °C. (F) Glandular trichomes in shade dried leaf. Scale bars: A = 10 μm, B–F = 100 μm.

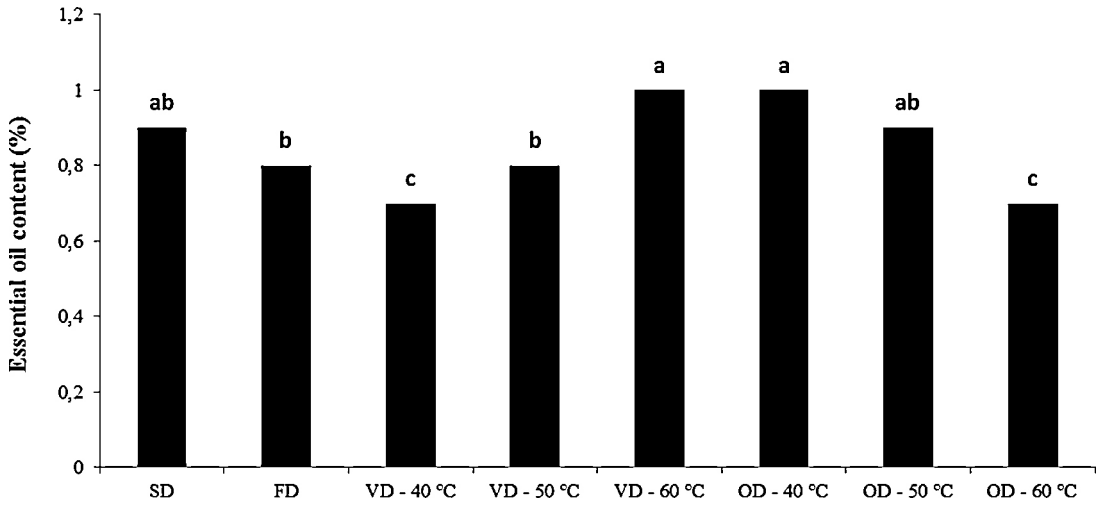

Fig. 3. Essential oil content of lemon verbena (V/W%) in different drying methods (SD: shade drying, FD: freeze drying, VD: vacuum drying, OD: oven drying).

Table 1
Chemical compositions of lemon verbena essential oils after treatment by different drying methods.

No.	Compound	RI*	Essential oil constituents (%)							
			Shade dried	Freeze dried	Oven dried			Vacuum dried		
					40°C	50°C	60°C	40°C	50°C	60°C
1	α-Pinene	938	–	–	–	–	–	–	–	0.2
2	Camphene	953	0.5	–	0.4	0.5	0.5	0.5	0.4	0.3
3	Sabinene	981	2.4	1.8	2.3	1.5	1.7	2.1	2.1	2.4
4	Limonene	1028	5.8	5.0	6.8	6.5	6.4	5.8	6.5	8.2
5	1,8-Cineole	1031	6.2	4.5	7.3	6.4	6.1	6.0	6.2	7.2
6	γ-Terpinene	1062	0.4	0.3	0.4	0.5	0.5	0.5	0.4	0.4
7	Terpinolene	1090	0.6	0.6	0.8	0.6	0.6	0.9	0.6	0.3
8	Transpinocarveol	1140	0.3	0.4	0.6	0.7	0.6	0.3	0.4	0.5
9	*cis*-Sabinol	1143	0.7	0.8	1.4	1.7	1.7	0.7	1.1	1.0
10	Citronellal	1152	0.7	1.7	1.8	2.0	1.9	1.0	1.8	1.4
11	α-Terpineol	1190	0.7	0.7	0.9	1.0	1.1	0.9	1.0	1.0
12	Nerol	1228	0.2	–	0.1	–	–	0.1	–	–
13	Neral	1238	23.3	26.2	22.2	24.0	23.9	23.3	24.8	23.5
14	Geranial	1267	35.9	38.5	32.3	32.2	32.8	34.6	33.4	32.0
15	Neryl acetate	1360	1.4	1.3	1.3	1.2	1.3	1.4	1.4	1.3
16	α-Copaene	1379	0.5	0.4	0.6	0.5	0.6	0.6	0.5	0.6
17	α-Gurjunene	1410	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
18	E-caryophyllene	1421	1.0	1.1	1.3	1.3	1.4	1.2	1.2	1.6
19	γ-Elemene	1439	5.0	4.6	5.8	6.2	6.5	5.7	5.8	6.3
20	α-Humulene	1456	0.6	0.6	0.5	0.5	0.5	0.6	0.5	0.6
21	Cubenol	1514	1.6	1.4	1.0	1.2	1.2	1.5	1.4	1.2
22	Spathulenol	1580	5.2	3.9	5.1	5.0	5.1	5.5	5.1	5.0
23	Globulol	1587	3.7	3.2	302	2.9	3.0	3.9	3.1	2.8
24	Epi-α-cadinol	1642	0.5	0.4	0.6	0.6	0.6	0.6	0.6	0.7
Monoterpene hydrocarbons			9.7	7.7	9.7	9.6	10.7	9.8	10	11.8
Oxygenated monoterpenes			69.4	74.1	69.4	70.2	68.8	68.3	70.1	67.9
Sesquiterpene hydrocarbons			7.3	6.9	9.2	8.7	8.4	8.3	8.2	9.3
Oxygenated sesquiterpenes			11	8.9	9.9	9.7	9.9	11.5	10.2	9.7
Total			97.4	97.6	98.2	98.2	97.8	97.9	98.5	98.7

leaves as compared to the oven-dried and vacuum-dried samples. With the increase of the temperature from 40 to 60 °C in oven and vacuum drying, amount of γ-elemene was increased but the relative amount of spathulenol was not influenced. Khangholil and Rezaeinodehi (2008) reported that when temperature of drying increased, the monoterpenes (such as limonene and 1,8-cineole in this experiment) content were gradually decreased, but sesquiterpenes (such as γ-elemene) content were increased. They stated that monoterpenes released more rapidly because of their lower molecular weight compared to sesquitepenes and this might account for the loss of volatile compounds in leaves of lemon verbena when oven dried. Also in many medicinal plants, the extensive decrease of EO compounds dried at higher temperatures were reported: *L. nobilis* L. (Hamrouni Sellami et al., 2011), *Thymus vulgaris* L. and *S. officinalis* L. (Venskutonis, 1997), *O. basilicum* L. (Ghasemi Pirbalouti et al., 2013), *M. longifolia* L. (Asekun et al., 2007), *S. officinalis* L. (Hamrouni Sellami et al., 2012) and in *C. nobile* L. (Omidbaigi et al., 2004). Also, It seems that increasing drying temperature in the vacuum condition reduced drying time and exposure to vacuum conditions and it was the reason for the preservation of limonene and 1,8-cineole.

The compounds of EOs in all drying methods could be classed in the following main chemical groups (Table 1): monoterpene hydrocarbons (representing 7.7–11.8% of total concentration of volatiles), oxygenated monoterpenes (67.9–74.1%), sesquiterpene hydrocarbons (6.9–9.3%) and oxygenated sesquiterpenes (8.9–11.5%).

Oxygenated monoterpenes were the most important group. The maximum amount of oxygenated monoterpenes was obtained by freeze drying (74.1%) whereas leaves which were vacuum-dried and oven-dried at 50 °C were characterized by appreciable oxygenated monoterpenes (70.1% and 70.2%, respectively). On the other hand, our results showed that the concentration of the chemical groups of EO varied with the method of drying and these results are in accordance with results of studies on other medicinal and aromatic plants (Hamrouni Sellami et al., 2011; Ghasemi Pirbalouti et al., 2013; Venskutonis, 1997).

4. Conclusion

The results confirmed that the drying method significantly affected the drying time of *L. citriodora* Kunth. and a twofold reduction of drying time was determined in vacuum compared with oven drying irrespective of drying temperature. SEM evaluation of abaxile and adaxile leaf surface of L. *citriodora* showed that drying method affect glandular trichome integrity as in oven drying and vacuum damage this secretary structure of leaf. Although oven drying at 40 °C is often used for drying of medicinal plants in practice, vacuum drying at 60 °C seems to be an effective approach for the conservation of total essential oil content as well as of specific oil components in a significantly shorter drying time.

Acknowledgments

The authors would like to thank Mrs. Pema Sanders for her helpful recommendations on manuscript. This research was supported by a grant from Tarbiat Modares University, Tehran, Iran.

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