Qualitative and Quantitative Evaluation of Essential Oil of Catnip (*Nepeta cataria* L.) Under Different Drying Conditions

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Abstract

Background: Drying is the most common way to preserve quality of aromatic and medicinal plants. Chosen drying methods can affect on the essential oil content and composition of medicinal plants.

Objective: This study aimed to assess the changes in essential oil constitutes of catnip aerial part under various drying methods.

Methods: The experiment was performed as the randomized complete block design (RCBD) with 10 treatments and 3 replications. The treatments were included the freshly harvested plants, sun-drying, shade-drying, oven-drying at 35, 45, and 55 °C, microwave-drying at 100 and 200 W, and initial sun-drying followed by subsequent shade-drying and oven-drying at 45 °C.

Results: The result indicated that the various drying methods had a significant effect ($p \leq 0.01$) on the essential oil content and compositions. The highest amount of essential oil was observed in the oven-drying at 55°C. GC-MS analysis revealed the presence 9 compounds in the essential oil of catnip, which three isomers of nepetalactone with a predominance of 4a-*α*, 7-*α*, 7a-*β*-nepetalactone were major essential oil components. Although, the amount of 4a-*α*, 7-*α*, 7a-*β*-nepetalactone were reduced by oven-drying at 55 °C, but other the isomers of nepetalactone were increased. However, the total content of three isomers of nepetalactone were equal in the fresh catnip with the dried plants by the oven drier at 55°C.

Conclusion: In general, the oven-drying at 55°C is recommended as an effective method to dry the catnip.

Keywords: ***Nepeta cataria*** **L., Drying methods, Essential oil, Nepetalactone, Oven-drying**

Introduction

Post-harvest respiration and growth of microorganism are a reason for corrupting plants and change in composition active ingredients. Moisture elimination from the plant inhibits the growth of microorganisms, thus, many harmful reactions are stopped due to decreasing of available moisture. So, medicinal plants drying is an important process for those storages without loss their active ingredients [1].

Plant materials can be dried by natural or artificial methods. Natural methods are like drying in sun or shade, and artificial drying procedures are include using drying machines or new technologies such as vacuum and microwave radiation [2]. Today, the factors such as the volume of production, new technologies, and medical standards are considered for medicinal plants drying [3]. The most important factor in the drying process of the medicinal herb is drying temperature. Some advantages of increasing temperature are included decreasing drying time, partly energy saving, and more use of the dryer. However, it has been reported that high temperature can lead to some changes in the active ingredients of plants. These changes at different temperatures are dependent on plant tissue, the active ingredient kind and location storage of the active ingredient. Thus, different plants with the same active ingredient do not follow of the general rule temperature for drying [3].

There are many benefits of reducing drying time such as reduce energy consumption, increase the dryer capacity, etc. Use some new technology such as microwave is capable of reducing drying time without increasing the temperature. The microwave technique in addition to reducing drying temperature can help to increase the drying rate and improve the dried plant quality [4]. Microwave radiations spread to the plant material quickly and effectively, thus, the energy consumption is reduced. In this method, the heat produces inside the plant material and then distributed to the outside, while in the other drying methods the heat is developed from the plant material surface to the depth [5, 6]. This issue is important for drying of aromatic herbs because their active ingredient located on the surface of leaves. Therefore, the microwave is preferred for drying herbs. Hence, the essential oil dissipation is reduced due to the leaf surface contact with the low heat, enhancing the drying speed and reducing energy input [7, 8].

Some studies showed chosen drying methods for the medicinal plants can be affected on the essential oil content and components [9, 10, 11, 12]. Chan et al. (2009) expressed different drying methods reduced the leaves total phenol content and antioxidant activity in four species of the *Zingiber* genus. So, the greatest and the lowest decrement of these traits attributed to sun and microwave-drying, respectively [13]. Figiel (2009) reported that microwave-drying more than oven-drying at 70°C reduced the time drying of garlic flakes. Microwave as an agent for discoloration of plant materials has been known, but this change in the high power of microwave radiations (720 W) was lower [14].

The *Nepeta cataria* plant (catnip or catmint) is a perennial herb belongs to *Nepeta* genus of the Lamiaceae family. *Nepeta* is a

genus composed of perennial or annual herbs, which spread in Asia, Europe and North Africa [15]. *Nepeta* genus consists about 150 species that *N. cataria* is the most well-known them [16]. This plant has carminative, tonic, diaphoretic, refrigerant, and antispasmodic properties. The nepetalactone is a major component of the catnip essential oil, which show antibacterial, fungicidal, and antiviral activities [17, 18]. Klimek and Modnick (2005) determined the *N. cataria* yellow essential oil consisted of nepetalactone (50%), nepetalic acid (33%), and neutral (14%). Ursolic acid, flavonoids, and phenolic acids were also detected in the *N. cataria* [19].

Various drying techniques widely used in the food industry for drying fruits and vegetables, but limited research evaluated effects of these methods on phytochemical traits of medicinal plant [20]. Therefore, this study has evaluated the effects of different drying methods on the qualitative and quantitative of catnip essential oil and the effective drying method was introduced eventually.

Materials and Methods

This study was conducted to assess the qualitative and quantitative changes of catnip essential oil under various drying methods in the Institute of Medicinal Plants affiliated with the Academic Center for Education Culture and Research (ACECR) in Karaj. The fresh aerial parts of catnip were harvested from the research farm in the flowering stage, accidentally. The first cutting plants were used for all treatments to avoid the impact of harvesting time on the quantity and quality of essential oil. The samples were collected on 25 July 2014 on a sunny morning (8 to 9 am). Following that, the samples were transported to the laboratory to the drying process.

The treatments were included freshly harvested plants, sun-drying, shade-drying, oven-drying at 35, 45, and 55°C, microwave-drying at 100 and 200 W, shade-drying after 3 hours of sun-drying, oven-drying at 45 °C after 3 hours of sun-drying. A sufficient amount of sample was taken for each treatments. For the sun-drying, plants were spread on a white cloth in the open area under the sun. Also for the shade-drying treatment, the catnip sample was flattened inside the room without direct sunlight penetration at 25°C. For oven-drying, the sample was spread in a thin layer on the tray. Oven temperature was regulated on operating temperatures, which were included 35, 45 and 55 °C. A microwave oven (Samsung, model MC35J8055CK) with a maximum power output of 2250 watts at 2450 MHz, which equipped with a swivel tray and a digital setting power and time was used for the drying catnip. The plant material was poured into a glass container and then placed inside the microwave cavity. For sample-drying with microwave, radiations power were regulated on 100 and 200-watt. The employed conditions of drying in each of these methods were selected after conducting trials to achieve a moisture content of ≤ 12%. The moisture content of dried samples were determined in triplicates after drying for 72 h in a lab oven at 75◦C.

Essential oils analysis

Aerial parts of *Nepeta cataria* L. were submitted to hydro-distillation with a

Clevenger-type apparatus according to the European pharmacopoeia [21]. The essential oil was extracted with 1000 ml of water for 3 hours (until no more essential oil was obtained). After cooling, the essential oil was collected by use of a syringe. Dry sodium sulfate was added to remove water from the oil, then the sample was transferred into a capped brown bottle and stored at 4 °C until analyzed by GC-MS and GC instruments. Each experiment was repeated at least three times and their mean was reported.

GC/MS analysis was carried out on an Agilent Instrument coupled with a 5973 Mass system equipped with flame ionization detector (FID) and a BPX5 capillary column (30 m×0.25 mm; 0.25 μm film thicknesses). Temperature program includes: oven temperature held for 2 minutes at 50 ºC and was enhanced to 130 ºC with 3 ºC per min rate. Then, temperature enhancement was programmed up to 270 ºC with 5 ºC per min rate and this temperature held for 3 minutes. Other operating conditions include: carrier gas was He with a flow rate of 1 ml min^{-1}; injector and detector temperatures were 280 ºC, and split ratio, 1:10. Mass spectra were taken at 70 eV. The essential oil components were identified through comparison their mass spectra and retention indices to those published in the literature and presented in the MS computer library [22, 23].

Statistical analysis

The experiment was performed as the randomized complete block design (RCBD) with 10 treatments and 3 replications. The difference between treatments means was compared by Duncan's Multiple Range Test at 5% confidence interval. All the data were subjected to statistical analysis (one-way ANOVA) using SAS software.

Results

The results showed that the drying methods had a significant effect ($p \leq 0.01$) on the catnip essential oil content and components (Table 1). The catnip essential oil content was reduced under the sun-drying, shade-drying, microwave-drying at 100 W, and sun-drying (3 hours) followed by subsequent shade-drying compared to the fresh plant. In contrast, the amount of catnip essential oil was increased under different temperatures of oven-drying and microwave-drying at 200 W. However, the highest essential oil amount (1.6 %) was observed in oven dried sample at 55 °C and its minimum (0.02 %) was obtained in microwave dried catnip at 100 W (Table 2).

GC/MS analysis identified the 9 compounds in the essential oil, which included sabinene, *β*-pinene, *Z*-*β*-ocimene, *E*-*β*-ocimene, trans-caryophyllene, caryophyllene oxide and three isomers of iridoid nepetalactone. These compounds comprised 99.3% of the essential oil components. The nepetalactone isomers were included (4a-*α*,7-*α*,7a-*α*)-nepetalactone (6.25 – 16.83 %), (4a-*α*,7-*α*,7a-*β*)-nepetalactone (85.41 - 71.4 %) and (4a-*α*,7-*β*,7a-*α*)-nepetalactone (4.96 - 7.24%). These isomers were the major constituents of essential oil in fresh and dried catnip and the amount of (4a-*α*, 7-*α*, 7a-*β*) -nepetalactone was predominant (Table 2).

Table 1- Analysis of variance for effects of drying methods on phytochemical traits of catnip

S.O.V.	Df	Mean Square					
		Essential oil content	Sabinene	*β*-pinene	*Z*-*β*-Ocimene	*E*-*β*-Ocimene	trans-Caryophyllene
Replication	2	0.045*	0.0032**	0.0093^{ns}	0.0009*	0.042**	0.004^{ns}
Treatments	9	0.96**	0.026**	0.18**	0.011**	0.087**	0.47**
Error	18	0.008	0.00015	0.0061	0.00008	0.001	0.023

ns: non significant differences; *: significant at P<0.05; **: significant at P<0.01

Table 1- Continued

S.O.V.	Df	Mean Square					
		Caryophyllene oxide	Nepetalactone (4a-*α*,7-*α*,7a-*α*)	Nepetalactone (4a-*α*,7-*α*,7a-*β*)	Nepetalactone (4a-*α*,7-*β*,7a-*α*)	Monoterpen hydrocarbon	Oxygenated monoterpen
Replication	2	0.0004^{ns}	1.092^{ns}	0.077^{ns}	0.005^{ns}	0.081**	54.43**
Treatments	9	0.025**	33.82**	49.14**	2.21**	0.815**	3.61^{ns}
Error	18	0.0002	0.93	4.16	0.18	0.0068	6.82

ns: non significant differences; *: significant at P<0.05; **: significant at P<0.01

Table 2- Means comparison for drying methods effects on phytochemical traits of catnip

Different drying methods	Essential oil content	Sabinene (MH)	*β*-Pinene (MH)	*Z*-*β*-Ocimene (MH)	*E*-*β*-Ocimene (MH)	trans-Caryophyllene (SH)	Caryophyllene oxide (SO)	N1 (MO)	N2 (MO)	N3 (MO)
Freshly harvested catnip	0.4^{c}	0.12^{e}	0.41^{d}	0.08^{c}	0.26^{d}	0.89^{e}	0.29^{b}	6.25^{d}	85.41^{a}	4.96^{d}
The sun-drying	0.06^{d}	0.15^{d}	0.58^{c}	0.128^{b}	0.36^{bc}	2.01^{a}	0.34^{a}	16.32^{ab}	71.4^{d}	6.87^{ab}
Shade-drying	0.06^{d}	0.21^{c}	0.84^{a}	0.201^{a}	0.63^{a}	1.99^{a}	0.32^{a}	15.54^{ab}	73.19^{bcd}	5.25^{d}
Oven-drying at 35 ° C	1^{b}	0.17^{d}	0.57^{c}	0.121^{b}	0.4^{b}	1.58^{b}	0.12^{f}	16.70^{ab}	71.85^{cd}	7.24^{a}
Oven-drying at 45 ° C	1^{b}	0.17^{d}	0.66^{c}	0.095^{c}	0.32^{c}	1.7^{b}	0.25^{c}	12.68^{c}	75.53^{bc}	7.12^{a}
Oven-drying at 55 ° C	1.6^{a}	0.24^{b}	0.84^{a}	0.061^{d}	0.15^{e}	1.19^{d}	0.1^{f}	16.83^{a}	72.73^{bcd}	7.1^{a}
Microwave at 200 W	1^{b}	0.27^{a}	0.81^{ab}	0.121^{b}	0.39^{b}	1.61^{b}	0.15^{e}	16.01^{ab}	73.05^{bcd}	6.2^{bc}
Microwave at 100 W	0.02^{d}	0^{f}	0.18^{e}	0^{e}	0.07^{f}	0.9^{e}	0.16^{e}	16.74^{a}	73.99^{bcd}	6.8^{ab}
Shade-drying after sun-drying	0.07^{d}	0^{f}	0.18^{e}	0^{e}	0.07^{f}	1.27^{cd}	0.33^{a}	14.88^{b}	76.37^{b}	5.68^{cd}
Oven at 55 ° C after sun-drying	1^{b}	0.2^{c}	0.68^{bc}	0.079^{c}	0.25^{d}	1.47^{bc}	0.22^{d}	11.64^{c}	75.89^{b}	7.18^{a}

Means with the same letters in each column indicate no significant difference between treatments at the 5% level of probability. N1: Nepetalactone (4a-*α*,7-*α*,7a-*α*), N2: Nepetalactone (4a-*α*,7-*α*,7a-*β*), N3: Nepetalactone (4a-*α*,7-*β*,7a-*α*), MH: Monoterpene hydrocarbon, MO: Oxygenated monoterpene: SH: Sesquiterpene hydrocarbon, SO: Oxygenated sesquiterpene

All drying methods were enhanced the 4a-*α*,7-*α*,7a-*α*-nepetalactone content. Thus, its minimum and maximum amount were observed in the sample of fresh catnip (6.25 %) and oven-drying at 55 °C (16.83 %), respectively. Excluding shade-drying and oven-drying at 45 °C after sun-drying, 4a-*α*, 7-*α*,7a-*α*-nepetalactone amount had no significant differences among other drying methods. The content of 4a-*α*, 7-*α*,7a-*β*-nepetalactone fell under different drying methods. The highest content of 4a-*α*, 7-*α*,7a-*β*-nepetalactone was obtained in the fresh catnip (85.41 %), while its lowest acquired in the catnip dried under the sunshine (71.4%). Also, the content of 4a-*α*, 7-*β*, 7a-*α*-nepetalactone was increased in all drying methods. The greatest and lowest content of 4a-*α*,7-*β*,7a-*α*-nepetalactone were respectively observed in the oven-dried samples at 45 °C after sun-drying (7.18 %) and fresh catnip (4.96 %). However, this compound content had no a significant difference in fresh catnip with dried catnip in the shade (5.25 %), and shade-drying after sun-drying (5.68 %) (Table 2).

Although, sabinene compound was not detected in the essential oil of microwave samples dried at 100 W, and shade-dring after sun-drying. But other drying methods were increased the sabinene content compared to the fresh catnip. The highest sabinene amount was observed in the microwave-drying at 200 W (0.27 %). The *β*-pinene amount was enhanced in all drying methods excepted for the microwave-drying at 100 W, and shade-drying after sun-drying. The maximum content of *β*-pinene was acquired in oven-drying at 55 °C and shade-drying (0.84 %) while its minimum was observed in microwave-drying at 100 W, and shade-drying after sun-drying (0.18 %) (Table 2).

The *Z*-*β*-ocimene was no detectable in the catnip essential oil dried in the microwave-drying at 100 W, and shade-drying after sun-drying. Shade-drying had the most effect on the increment of *Z*-*β*-ocimene content (0.2 %). Compared to the fresh catnip, the *Z*-*β*-ocimene amount was increased up to 50 percentage in the oven-drying at 35 °C, but its content was reduced by temperature increasing. *E*-*β*-ocimene content was respectively increased up to 37 % and 1.7 in the samples dried under sun and shade methods compared to the fresh catnip. Also, the *E*-*β*-ocimene amount was enhanced up to 54 and 23 percent when catnip dried in the oven at 35 and 45 °C, respectively. However, increasing oven temperature to the 55 °C reduced the *E*-*β*-ocimene content to 42 % compared to fresh catnip. The highest *E*-*β*-ocimene content was obtained in the shade dried sample (63 %) while its minimum was observed in the microwave dried catnip at the 100 W, and shade-drying after sun-drying (0.07 %) (Table 2).

In comparison to fresh catnip, the caryophyllene amount was increased by all drying methods exception of microwave-drying at 100 W. The greatest content of caryophyllene was acquired in dried catnip by the sun and shade (2 and 1.99 %, respectively). However, its lowest was obtained in the fresh catnip and microwave-drying at 100 W (0.89 and 0.9 %, respectively). The content of caryophyllene oxide was reduced by either microwave powers, various temperatures of oven, and oven-drying at 45 °C after sun-drying. The highest content of caryophyllene

oxide in essential oil of catnip was observed in the sun-drying and shade-drying as well as combination of these methods (0.34, 0.32 and 0.33 %, respectively). The least content of caryophyllene oxide was obtained in the oven-drying at 34 and 55 °C (0.12 and 0.1 %, respectively) (Table 2).

Different drying methods had a significant effect ($p \leq 0.01$) on the content of monoterpene hydrocarbons while they hadn't a significant effect on the content of oxygenated monoterpene. The greatest content of monoterpene hydrocarbons was obtained in the shade dried samples (1.88 %). But on the contrary, their minimum was obtained in the microwave dried catnip at 100 W, and shade-drying after sun-drying (0.25 %). Oven-drying at various temperatures was increased the hydrocarbon monoterpenes amount compared with the control, but there was no a significant difference among various temperatures (Figure 1). Compared to freshly harvested plant, the content of monoterpene hydrocarbons were increased at 200 W of microwave-drying while, their content was reduced in microwave-drying at 100 W. Oxygenated sesquiterpene was decreased in both microwave powers. Sesquiterpene hydrocarbon was increased in microwave-drying at 200 W and unchanged in microwave-drying at 100 W (Table 2).

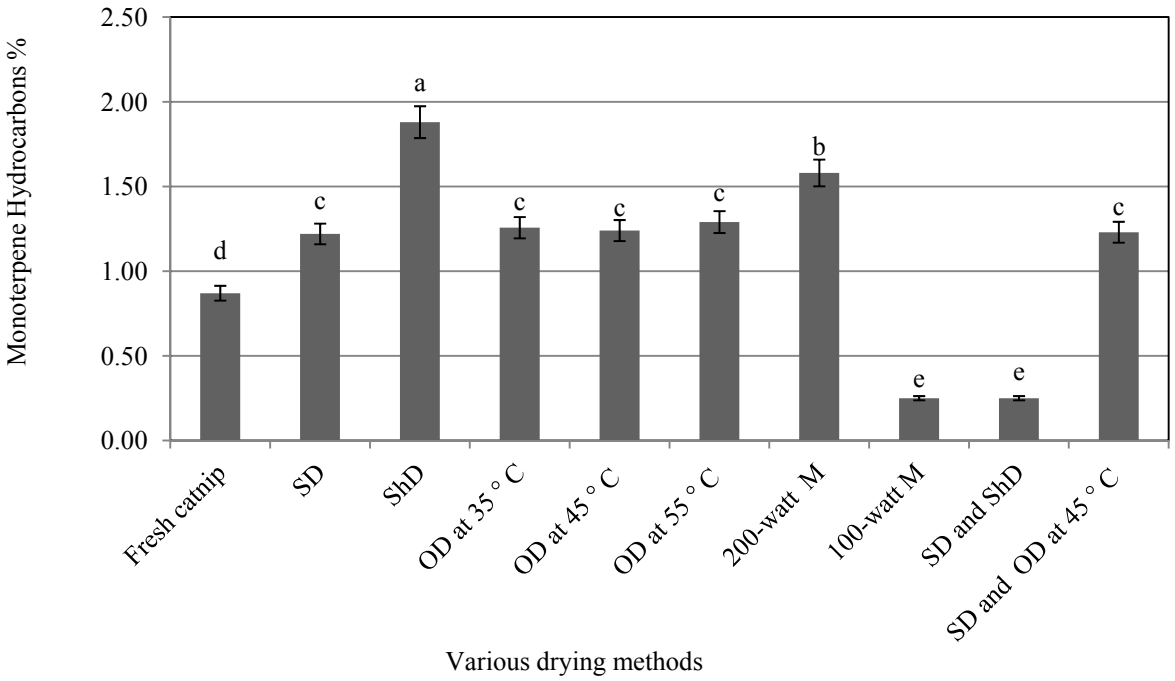

Figure 1- Influence of different drying methods on monoterpene hydrocarbon content. (SD: The sun-drying, ShD: shade-drying, OD: Oven-drying, M: Microwave)

Discussion

The results showed that the natural drying methods as sun and shade-drying led to a reduction in the content of catnip essential oil compared to freshly harvested catnip. However, the content of catnip essential oil was increased by artificial drying methods as variant oven temperature and microwave at the 200 W. Moreover, the highest content of catnip essential oil was obtained in the oven-drying at 55 °C. This result is compliance with another study [24, 25]. However, this finding is contrary to the results of some studies [26 - 30]. The temperature and duration of drying process are effective factors on physical and phytochemical features in the dried herb [31]. Besides, an increasing the temperature reduces the drying time [32]. Therefore, fast drying at high temperatures due to reducing energy input, wrinkles and appearance of the hardening phenomenon on the plant tissue surface, can help reduce evaporation of active and aromatic ingredients [27]. It seems, increasing the content of catnip essential oil in artificial drying methods be due to reducing time drying. Hence, a small amount of essential oil is likely evaporated.

Three isomers of nepetalactone were detected in the catnip essential oil which 4a-*α*, 7-*α*,7a-*β*-nepetalactone was major isomer. The sum amount of three nepetalactone isomers were no a considerable variation among different drying methods (94 - 97.5 %), but the conversion of the isomers to each other is varied. The 4a-*α*, 7-*α*, 7a-*β*-nepetalactone content reduced under drying operation. However, 4a-*α*, 7-*α*, 7a-*α*-nepetalactone and 4a-*α*, 7-*β*, 7a-*α*- nepetalactone amount increased by drying process and their maximum content were observed in the oven-drying at 55 °C. Therefore, the 4a-*α*,7-*α*,7a-*β*-nepetalactone in catnip essential oil is sensitive to drying and converted to other isomers.

The content of monoterpene hydrocarbons in the catnip essential oil included ocimene isomers, *β*-pinene, and sabinene were increased under various drying methods excepted to the microwave at 100 W and combined drying methods. The highest content of ocimene isomers and *β*-pinene were acquired in the shade-drying. However, the highest content of sabinene was observed in microwave-drying at 200 W. Several studies have shown that different drying methods can be effective on the essential oil components [9 - 11]. Sefidkon et al. (2006) reported the highest amount of essential oil and carvacrol were observed in the oven-drying at 45 °C while the maximum content of *γ*-terpinene was obtained in the sun-drying sample. Ocimene, *β*-pinene, and sabinene derived of geranyl diphosphate by specific enzymes include *β*-ocimene synthase, *β*-pinene synthase, and sabinene-hydrate synthase, respectively. Hence, it seems some drying method may be increased the enzymes activity of monoterpene hydrocarbons biosynthesis during drying.

Caryophyllene content as sesquiterpene hydrocarbon was increased through the various drying process. But amount of caryophyllene oxide as oxygenated sesquiterpene was increased only via the sun and the shade-drying and it reduced under other drying methods. Changes of sesquiterpene amount during drying process do not determine so that, identical methods

have been a contradiction effects on sesquiterpene content in different herbs. Thus, the results acquired in this study were similar to some studies [33] and are contrary to numerous scientists findings [34, 35].

High quality in dried aromatic and medicinal plant is obtained to the heat between 30 to 60°C [36]. The findings revealed the essential oil content of *β*-pinene, sabinene, and 4a-*α*, 7-*α*, 7a-*α*- nepetalactone were increased via increment the oven temperature from 35°C to 55°C. But the ocimene isomers and caryophyllene content were reduced. Asekun et al. (2007) expressed the menthone and 1, 8-cineol amount in essential oil of *Mentha longifolia* was reduced by increasing the oven temperature [30].

Enhancing microwave radiation power from 100 to 200 W had no a significant effect on nepetalactone isomers (Oxygenated monoterpenes) and caryophyllene oxide (Oxygenated sesquiterpene) content while the essential oil content and other its components (Monoterpene and sesquiterpene hydrocarbons) were boosted. However, microwave-drying had no a significant effect on the total content of oxygenated monoterpenes, but the nepetalactone isomers conversion to each other was changed. Sesquiterpenes and monoterpene hydrocarbons alterations depended on microwave power. Both microwave powers decreased content of caryophyllene oxide compared to freshly harvested plant whereas content of caryophyllene was increased at 200 W microwave. But its content at 100 W microwave was unchanged. Monoterpene hydrocarbons were decreased in low microwave power (at 100 W) but their content was increased in high microwave power (at 200 W). The results were an agreement to other studies [3, 37]. Microwave radiation is an electromagnetic radiation class with long waves. When the waves pass through the food, the polar molecules such as water and salts will start to vibrate thus, this vibration converts the microwave radiation energy to heat [38, 39, 40]. Time drying in the microwave at 100 W was longer than the microwave at 200 W. Hence, it is possible that increasing the terpenoids degradation or vaporization at 100 W of microwave were due to long contact of catnip with electromagnetic radiation or heat.

Essential oil of Lamiaceae plants is stored in glandular trichome on the leaves surface [41]. Some medicinal plants that the active ingredient located on the leaves surface are sensitive to high temperatures. So, the microwave-drying is recommended for the type of medicinal plants drying [27]. Because microwaves radiation without damage to the external surface of plant tissues, can remove the moisture from them [42]. The microwave radiation due to the rapid diffusion of a product has been introduced to reduce the moisture content of agricultural products [43].

Conclusion

The drying methods of *Nepeta cataria* L. shoots had a significant effect on essential oil quantity and quality. The highest essential oil amount was observed in the oven dried sample at 55°C. Although the amount of 4a-*α*,7-*α*,7a-*β*-nepetalactone as the predominant catnip

essential oil component reduced in the oven-drying at 55 ° C, the other isomers of nepetalactone were increased. Also, the total nepetalactone isomers amount in the fresh catnip were equal with the oven dried plant at 55 °C. Thus, oven-drying at 55 °C was the effective method for the drying catnip.

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