Effect of Ozonation on the Essential Oil Composition of Dried Aromatic Plants

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Abstract. Ozonation as an alternative method for the disinfection of dried herb material shows promising results concerning the microbial load reduction. However, there is not enough data about the effect of the method on the essential oil quality. The aim of this study was to investigate the effect of ozone on the essential oil content and composition of dried oregano, thyme and lemon verbena. Quantitative and qualitative essential oil measurements were performed before and after ozonation. The results showed that in cases of oregano and lemon verbena, no statistically significant difference was observed either in total essential oil content or on any of their compound concentration. However ozonation may affect the concentration of some components since in the case of thyme the concentration of 8 compounds decreased.

Keywords: oregano, thyme, lemon verbena, volatile components

1 Introduction

Aromatic plants and essential oils are widely used by food, cosmetic and pharmaceutical industry due to their organoleptic characteristics and effective bioactive compounds (Arraiza Bermudez-Cañete et al., 2010). Pre- and post- harvest conditions may affect the quality of the dried aromatic plants and therefore their market value (Tanko et al., 2005). According to European Spice Association (2013), among other indicators of quality, the microbial load, the color and the composition and concentration of aromatic plants essential oil play an important role.

Ozone application seems to be the most promising technique since it leaves no residues and it is environmental friendly, in comparison to other methods developed for the microbial load reduction of aromatic plants (Brodowska et al., 2014; Torlak et al., 2013; Rice, 2002). However, ozone as a highly oxidizing agent (Greene et al., 2012) could possibly cause the deterioration of the essential oil of dried herbs when used for a long time period and/or in high concentrations. This work aims to the investigation of the effect of ozone application on the essential oil content and composition of three dried aromatic plants.

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2 Materials & Methods

2.1 Dried Plant Material

Efficient amount of dried plant material, namely oregano (*Origanum vulgare* ssp. *hirtum*), thyme (*Thymus vulgaris*) and lemon verbena (*Aloysia triphylla* syn. *Lippia citriodora*) was provided by local producers of Magnesia (Thessaly, Greece). The plant material was harvested during spring and summer of 2015, dried naturally under shade and was stored under ambient conditions until the experiments implementation.

2.2 Ozone Treatment

The ozonation device used for the dried plant material disinfection, consisted of the oxygen tank, the ozone generator and the airtight chamber. Inside the chamber a sensor was placed in order to measure the O_3 concentration. To avoid the accumulation of O_3 above the treated plant material, a fan was placed in the upper side of the chamber. Three samples of 100 g from each plant species were placed inside 1 mm mesh sieves (27 cm diameter). Sieves were placed inside the chamber and ozone was produced by providing dry oxygen to the ozone generator. The ozone concentration was adjusted to 4 ppm for a time period of 30 and 60 min. These values were set in accordance to Torlak et al. (2013), who stated that ozonation with less than 3 ppm O_3 (even for 90 min period) was ineffective for sufficient microbial reduction, whereas a higher concentration of 5 ppm O_3 for 120 min was effective but color degradation was observed.

2.3 Essential Oils Extraction

The essential oils of the above mentioned plant material, were extracted by water-steam distillation using Clevenger type apparatus. The distillation time and respectively the amount of plant material for each species are shown in Table 1. The volume of the essential oil yield was measured, and stored at 4˚C after the addition of the proper quantity of anhydrous Na_2SO_4.

Table 1. Weight of plant material samples and distillation time.

Plant Species	Weight (g)	Distillation Time (min)
Origanum vulgare ssp. *hirtum*	20	50
Thymus vulgaris	40	60
Aloysia triphylla	50	100

2.4 Gas chromatography/mass spectrometry (GC-MS) analysis

The essential oils were analyzed by GC-MS on a fused silica DB-5 column, using a Gas Chromatograph 17A Ver. 3 interfaced with a mass spectrometer Shimadzu QP-5050A supported by the GC/MS Solution Ver1.21 software, using the method described previously (Sarrou et al., 2013). The identification of the compounds was based on comparison of their retention indices (RI) relative to n-alkanes (C7-C22), with corresponding literature data and by matching their spectra with those of MS libraries.

2.5 Statistical Analysis

The data were statistically analyzed by analysis of variance (ANOVA) using Statgraphics Centurion XVI. Duncan's multiple range test was used at a significance level of 0.05.

3 Results and Discussion

Essential oil content of each plant material was measured before and after O_3 treatment. Ozonation for 30 min, was an adequate period to reduce the microbial load of oregano samples, whereas a period of 60 min was needed for lemon verbena and thyme samples. Distillation was performed only to the samples where the ozonation was effective in microbial load reduction. The essential oil content of the above mentioned aromatic plants was within range according to Goliaris et al. (2002), Kokkini (1997), Marzec et al. (2010) and Kizil et al. (2016). As shown in Table 2, the essential oil content of oregano and lemon verbena before and after treatment did not show a statistically significant difference. However the content of thyme essential oil was increased. This may be attributed to the reduction of the water content of the samples during ozonation, since the relative humidity inside the chamber was increased. This probably indicates that an amount of water was removed from the plant material to chambers atmosphere (Table 3). Accordingly, 40g of thyme plant material taken during sampling after the treatment possibly had lower water content and therefore higher content in essential oil. Consequently, ozonation does not seem to affect negatively the total essential oil yield of the treated plant material when applied under the above mentioned conditions. In order to verify this assumption, water content measurements of the samples must also be taken before and after O_3 treatment.

Table 2. Essential oil content before and after ozonation.

Plant Species	Essential oil content (% dry weight)		
	0 min	30 min	60 min
Origanum vulgare ssp. *hirtum*	3.30±0.13	3.30±0.18	-
Thymus vulgaris	0.92±0.04[a]	-	1.35±0.02[a]
Aloysia triphylla	0.61±0.03	-	0.66±0.02

The results obtained were expressed as Mean ± SD, n = 3.
Mean values followed by the same letters at the same row denote statistically significant difference at a probability of $P < 0.05$.

Table 3. Temperature (T, ˚C) and relative humidity (RH, %) in the chamber during ozonation.

Plant Species	Ozonation Conditions					
	0 min		30 min		60 min	
	T	RH	T	RH	T	RH
Origanum vulgare ssp. *hirtum*	33.0	36.0	37.2	27.0	-	-
Thymus vulgaris	35.1	28.6	36.5	32.6	37.0	29.8
Aloysia triphylla	31.2	36.3	34.7	32.5	35.4	30.7

The results concerning essential oil composition revealed that ozonation of dried lemon verbena material did not affect its essential oil chemical composition (Table 4). The major constituents were β-citral ranged from 25.91 to 26.73%, α-citral ranged from 18.55 to 19.22% , limonene ranged from 12.29 to 13.31% and 1,8 cineol ranged from 8.32 to 8.07%, before and after the application respectively. However, no statistically significant differences were observed between the above mentioned values before and after treatment. Citral a and citral b contributed more than 40% of the total essential oil content which is in agreement with Vogel et al. (1999) results. As stated by Kizil et al. (2016), different essential oil composition, is probably due to the different geographical and ecological factors effect. Growth stage and cultivation methods may also affect the essential oil composition (Argyropoulou et al., 2007). Moreover, the statistical analysis of the concentration of the total identified compounds (12) of *Aloysia triphylla* essential oil, did not indicate any significant difference.

Table 4. Essential oil composition of *Aloysia triphylla* before and after ozone treatment

No	Compounds	Concentration (%)	
		0 min	60 min
1	sabinene	1.41±0.21	1.36±0.10
2	6-methyl-5-hepten-2-one	1.54±0.32	1.02±0.08
3	limonene	12.29±0.64	13.31±0.70
4	1,8-cineol	8.32±0.78	8.07±0.69
5	linalool	0.67±0.05	0.61±0.04
6	α-terpineol	1.40±0.06	1.29±0.05
7	α-citral	18.55±0.42	19.22±0.74
8	β-citral	25.91±0.57	26.73±0.80
9	β-caryophyllene	1.55±0.29	1.68±0.14
10	ar-curcumene	5.08±0.77	5.24±0.90
11	spathulenol	3.43±0.48	3.73±0.55
12	caryophyllene oxide	4.89±0.61	5.06±0.79

The results obtained were expressed as Mean ± SD, n = 3.

As shown on Table 5, the main components of thyme essential oil where p-cymene ranged from 31.97 to 32.01%, thymol ranged from 30.65 to 31.5%, carvacrol ranged from 13.11 to 14.5% and γ-terpinene 6.05%. These components contributed more than 80% of the total essential oil content. However, the concentration of none of these components showed significant difference after the ozonation. The composition of thyme's essential oil presented in this work was in accordance to that referred by Raal et al. (2005). Consequently, this essential oil could be classified as thymol chemotype and specifically to the subgroup p-cymene> thymol> γ-terpinene (Marzec et al. 2010). Nevertheless, statistical analysis of the rest of the components concentration showed that 8 compounds out of 23 in total, decreased significantly. This decrease can be attributed to the high ozone oxidation efficiency. As stated by Brodowska et al. (2015) high ozone doses among with long treatment time, resulted in 50% reduction of α-pinene compared to control samples of berries. Same as α-pinene, all of the compounds of thyme that where reduced (1,8-cineol, cis-sabnene hydrate, linalool, borneol, thymol methyl ether, carvacrol methyl ether, β-caryophyllene and γ-cadinene) contain 3 or more -CH_3 groups that could be alkylated after long contact with ozone. Also, linalool, β-caryophyllene and γ-cadinene contain 2 or more double bonds, which can be broken. In addition borneol, which is secondary alcohol, can be easily oxidated to the ketone camphor. Despite that these 8 components were identified at low concentration, there is not scientific indication that this can affect the quality of the essential oil.

Verbena also contained at some percentages 1,8-cineol, linalool and β-caryophyllene while oregano contained cis-sabinene hydrate, linalool, carvacrol methyl ether and β-caryophyllene. In contrast to thyme samples, no reduction was observed for these compounds. This might be attributed to the low ozonation time of oregano samples and to the differences of the plant material (leaf surface and size) among verbena and thyme samples that were treated for the same time.

As a consequence, more studies need to be conducted in order to identify the appropriate O_3 concentration and ozonation period so that the high quality of the essential oil can be maintained.

Table 5. Composition of essential oil of *Thymus vulgaris* before and after ozone treatment

No	Compounds	Concentration (%)	
		0 min	**60 min**
1	α-pinene	0.46±0.27	0.85±0.00
2	camphene	0.40±0.20	0.68±0.00
3	β-pinene	0.44±0.04	0.28±0.12
4	β-myrcene	0.63±0.26	0.81±0.01
5	α-terpinene	1.02±0.29	1.20±0.01
6	p-cymene	31.97±0.90	32.01±0.37
7	β-phellandrene	0.52±0.12	0.60±0.01
8	1,8-cineol	0.85±0.04 [a]	0.76±0.02 [a]
9	γ-terpinene	6.05±0.67	6.05±0.16
10	cis-sabinene hydrate	1.19±0.07 [b]	0.92±0.48 [b]
11	linalool	2.24±0.01 [c]	1.92±0.08 [c]
12	camphor	0.39±0.02	0.34±0.01
13	borneol	1.31±0.03 [d]	1.18±0.05 [d]
14	terpinen-4-ol	0.56±0.00	0.55±0.03
15	thymol methyl ether	0.31±0.02 [e]	0.24±0.00 [e]
16	carvacrol methyl ether	0.83±0.04 [f]	0.65±0.02 [f]
17	thymol	30.65±1.32	31.50±0.60
18	carvacrol	13.11±0.90	14.15±0.24
19	β-caryophyllene	3.39±0.38 [g]	2.44±0.09 [g]
20	β-bisabolene	0.36±0.08	0.25±0.00
21	δ-cadinene	0.39±0.14	0.24±0.02
22	γ-cadinene	0.25±0.10 [h]	0.00 [h]
23	caryophyllene oxide	0.53±0.21	0.38±0.01

The results obtained were expressed as Mean ± SD, n = 3.
Mean values followed by the same letters at the same row denote statistically significant difference at a probability of $P < 0.05$.

Essential oil analysis of oregano (Table 6), showed a typical composition of carvacrol chemotype oregano, as referred by Vokou et al. (1993). The main constituents before and after the ozone treatment were two isomeric phenols namely carvacrol ranged from 63.26 to 64.55% and thymol ranged from 5.06 to 4.58%, and their precursors namely p-cymene ranged from 13.11 to 11.9% and γ-terpinene ranged from 8.15 to 8.37%. These components contributed more than 80% of the total essential oil content. The statistical analysis did not point out any difference among the constituents before and after treatment. These results show that probably ozone treatment did not affect the chemical composition of oregano's essential oil.

Table 6. Composition of essential oil of *Origanum vulgare* ssp. *hirtum* before and after ozone treatment

No	Compounds	Concentration (%)	
		0 min	30 min
1	α-thujene	0.40±0.19	0.37±0.26
2	α-pinene	0.61±0.30	0.53±0.37
3	camphene	0.14±0.10	0.14±0.10
4	β-pinene	0.10±0.07	0.09±0.07
5	1-octen-3-ol	0.33±0.05	0.33±0.03
6	3-octanone	0.13±0.01	0.13±0.01
7	β-myrcene	1.45±0.53	1.30±0.68
8	α-phellandrene	0.19±0.06	0.15±0.10
9	α-terpinene	1.49±0.42	1.34±0.56
10	p-cymene	13.11±0.13	11.90±0.38
11	β-phellandrene	0.48±0.13	0.42±0.16
12	γ-terpinene	8.15±0.08	8.37±0.09
13	cis-sabinene hydrate	0.32±0.04	0.34±0.01
14	borneol	0.29±0.05	0.26±0.02
15	terpinen-4-ol	0.34±0.03	0.32±0.03
16	carvacrol methyl ether	0.29±0.01	0.29±0.02
17	thymol	5.06±0.48	4.58±0.40
18	carvacrol	63.26±0.03	64.55±1.04
19	β-caryophyllene	1.73±0.26	1.75±0.22
20	α-humulene	0.32±0.25	0.17±0.03
21	β-bisabolene	0.62±0.05	0.69±0.13

The results obtained were expressed as Mean ± SD, n = 3.

4 Conclusions

The results of the present study revealed that O_3 application for the disinfection of oregano and lemon verbena dried plant material did not reduce the essential oil amount and the concentration of the main constituents. In contrast, ozonation may affect the amount of some components, since in the case of thyme the concentration of 8 compounds was reduced. However, there is not enough scientific evidence that this reduction can affect the essential oil quality. More studies need to be conducted in order to identify the appropriate O_3 concentration and ozonation period in order to maintain the high quality of the essential oil.

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