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Research Paper / Araştırma Makalesi

Effect of Different Drying Methods and Distillation Times on Essential Oil Composition and Antioxidant Content of Rosemary

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ABSTRACT

The aim of this study is to determine the effect of different drying methods and distillation times on the antioxidant and essential oil contents of rosemary. The essential oil content of rosemary extracts was determined using hydrodistillation and its composition by GC-FID/MS. The antioxidant content of deoiled materials was quantified on the LC-MS/MS. Carnosol and carnosic acid contents (LC/MS-MS) and essential oil compositions (GC-MS) were determined after each treatment. The highest essential oil (2.34%), carnosol (0.62%), and carnosic acid (0.85%) contents of dried samples were determined at 45°C. Some variations were also observed in the essential oil composition of dried samples. These quality parameters showed significant variations over different distillation times. And, the distillation time of 120 min was determined more appropriate to obtain essential oils and antioxidant compounds at a high yield. To produce rosemary extracts with a high content of essential oils and antioxidant compounds, it is highly recommended that the fresh plants should be first dried at 45°C, and then dried samples should be processed into essential oils by hydrodistillation for 120 min. The remnants of plant materials from the production of rosemary essential oil could be used to produce carnosol and carnosic acid. Eventually, essential oils and antioxidant compounds should be extracted from rosemary in an integrated manner.

Keywords: *Rosmarinus officinalis*, Drying, Distillation time, Essential oil, Antioxidant

Farklı Kurutma Yöntemi ve Damıtma Süresinin Biberiyenin Uçucu Yağ Bileşimi ve Antioksidan İçeriği Üzerine Etkisi

ÖZ

Bu çalışmanın amacı farklı kurutma yöntemleri ve distilasyon sürelerinin biberiyenin antioksidan ve uçucu yağ içeriği üzerine etkilerini belirlemektir. Biberiye özütünün uçucu yağ içerikleri hidrodistilasyon yöntemi, uçucu yağların bileşimi de GC-FID/MS cihaz ile belirlenmiştir. Uçucu yağı alınanan örneklerin antioksidan bileşen analizleri LC-MS/MS kullanılarak tespit edilmiştir. Her uygulamadan sonra karnosol ve karnosik asit içerikleri (LC/MS-MS) ve uçucu yağ bileşen (GC-MS) analizleri gerçekleştirilmiştir. Kurutulan örneklerde en yüksek uçucu yağ (%2.34), karnosol (%0.62) ve karnosik asit (%0.85) içerikleri 45°C'de kurutlmuş örneklerde belirlenmiştir. Kurutma sıcaklığına göre örneklerin uçucu yağ bileşiminde bazı farklılıklar tespit edilmiştir. Örneklerin analizi yapılan kalite parametreleri distilasyon sürelerine göre de önemli farklılıklar göstermiştir. Distilasyon sürelerine göre en yüksek uçucu yağ oranı ve antioksidan içeriği 120 dakikalık süresinde tespit edilmiştir. Sonuç olarak biberiyeden hem uçucu yağ hem de antioksidan (karnasol ve karnosik asit) üretmek için 45°C'de kurutmanın uygun olduğu, kurutulan örneklerden uçucu

yağ elde etmek için de 120 dakikalık hidrodistilasyon uygulamasının yeterli olduğu ortaya konulmuştur. Biberiye uçucu yağı üretiminden arta kalan bitkisel materyalin daha sonra karnosol ve karnosik asit üretiminde kullanılabileceği tespit edilmiştir. Sonuçlar biberiyeden uçucu yağ ve antioksidan üretiminin entegre bir şekilde yapılabileceğini ortaya koymuştur.

Anahtar Kelimeler: *Rosmarinus officinalis*, Kurutma, Distilasyon süresi, Uçucu yağ, Antioksidan

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is a valuable essential oil and spice plant of the Lamiaceae family. It grows in mainly Mediterranean countries and its dried or fresh form is used as a spice in culinary. Rosemary is also processed for essential oil and antioxidant production [1].

Chemical composition is the main characteristics of rosemary essential oil [2]. As it is well known, the essential oil composition of MAPs (medicinal and aromatic plants) depends on physiological variations, genetic factors, environmental conditions, post-harvest processing and storage conditions [3]. Drying and distillation among post-harvest processes have generally significant effects on essential oil yield and composition of MAPs [4]. Fresh MAPs commonly contain 75-80% moisture, and if they are not processed fresh, moisture levels must be lower then 15% for protection from biochemical alteration andmicrobial spoilage of MAPs. Drying is a considerably used treatment for this purpose. On the other hand, essential oil yield and composition of any MAPs could be varied depending on drying methods and process parameters [5]. Significant variations were examined in the essential oil content and composition of rosemary according to applied drying methods [6]. The drying process has also important effects on the concentration of compounds with the antioxidant effects like rosmarinic acid, carnosol and carnosic acid in rosemary depending on the applied method parameters [7]. Commonly, low drying temperatures (30-50°C) are advised to preserve susceptible active constituents of MAPs [8]. Then, dried plant materials could be processed for essential oil or phenolic compounds. Rosemary essential oil is commonly obtained by steam or hydro-distillation methods [9]. Essential oil yield and composition of any MAPs could also be varied significantly according to distillation time [10]. Essential oil composition (active constituents' concentration) is an important factor to determine the commercial economic value for any essential oil. Furthermore, the usage area and activity performance of any essential oil could be varied in relation to active components concentration [11]. The plant residue after essential oil production is not generally evaluated effectively. It is thought that the marc of rosemary leaves, after essential oil production, can be a rich source of some antioxidants, especially water-insoluble ones. Rosemary is also used in rosmarinic acid (water soluble), carnosol and carnosic acid (water insoluble) production. These natural antioxidants contents in rosemary could be varied according to the growing region, harvesting time, plant parts [12, 13]. Moreover, essential oil distillation parameters could also influence their concentration. Together with these compounds, there has been increasing interest in natural biologically active compounds. Rosmarinic acid which is naturally occurring in several plants including rosemary, displays several biological activities together with anti-oxidant. There are many studies, including anti-oxidant properties of rosmarinic acid. However, the changes in the tricyclic carnosol and carnosic acid in rosemary due to different process parameters and the situation in the activities are not considered in detail. Additionally, there is no comprehensive study on both essential oil and diterpenoids (carnosol and carnosic acid) contents of rosemary concerning drying processes and distillation time. Hence, the objective of this paper is to assess the impact of drying temperatures and distillation time intervals on essential oil profile and carnosol and carnosic acid contents for rosemary leaves. The goal of this study is to present essential oil and antioxidants (carnosol and carnosic acid) which could be produced from the same raw material.

MATERIALS and METHODS

Materials

The rosemary (*Rosmarinus officinalis*) leaves were obtained from Tarsus-Mersin (coordinates: 36°58'05″ N; 34°48'30″ E, altitude: 254 m), located in the Mediterranean region of Turkey, in September 2017. The samples were brought to the Medicinal and Aromatic Plants Laboratory of Western Mediterranean Research Institute as soon as possible after harvesting and then they were allowed for drying processes afterward separating leaves from stems. In order to avoid raw material differences, all leaves were mixed and then randomly divided into three batches for each replication.

Methods

The drying process was carried out in the shade and tray dryer at three different temperatures (35, 45 and 55°C) using the convective hot-air oven up to the remaining moisture content of about 10%.

Essential Oil Content and Composition

The essential oil content of the samples was determined with hydrodistillation by using the Clevenger apparatus (Isotex, 98-IV-B, China). Deionized water (300 mL) was added to the Clevenger apparatus which contained a 20 g sample and extraction was carried out for 3 hours. The results were expressed as mL volatile oil content of 100 g sample (%, v/w) [14].

The essential oil composition of samples was analyzed by gas chromatography (Agilent 7890A, USA) coupled to a flame ionization detector and mass spectrometry (Agilent 5975C, USA) using capillary column (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 µm) [15]. Essential oils were diluted at a 1:100 ratio with hexane. GC-FID/MS analysis was carried out at a split mode of 50:1. Injection volume and temperature were adjusted to 1 µL and 250°C, respectively. Helium (99.9%) was the carrier gas at a constant flow rate of 0.8 mL/min. The oven temperature was programmed as follows; 60°C for 10 min, increased at 4°C min^{-1} to 220°C and held for 8 min at 220°C. MS-spectra were monitored between 35-450 amu and EI ionization mode was used at 70 eV. The relative percentage of the components was calculated from GC-FID peak areas. The components were identified by using Wiley7n, NIST05 and Adams mass spectrum libraries. Some pure standards (*α*-pinene, 1,8-cineole, borneol, etc.) were also injected at the same procedure to compare and check the results for correct identification of the compounds. Moreover, the identification of each component was evaluated both by matching their mass spectra in the libraries and retention index values were calculated against to C_8-C_{24} alkane series.

Carnosol and Carnosic Acid Contents

Before the extraction, dried materials were ground by a grinder (Retsch GM200, Germany) at 10000 rpm for 20 s. Thereafter, the sample was extracted by ethyl alcohol in an ultrasonic water bath (Bandelin Sonorex, Germany) for 15 min. Then, centrifuged by ultracentrifuge (Sigma, 2-16KL,) at 5000 rpm at 4°C for 5 min. Finally, the supernatant was filtered from a 0.45 µm membrane filter and transferred to a liquid chromatography (Agilent, 1290 Infinity, USA)-mass spectrometer (Agilent 6430 Triple Quad, USA) (LC-MS/MS) for analysis [16].

Antioxidant compounds were quantified according to the method by Fischer *et al*. [17] by using the Zorbax RRHD Eclipse Plus C18 column (3 µm, 100 × 2.1 mm) on the LC-MS/MS (Agilent. First of all, MS optimization parameters of carnosol and carnosic acid were determined (Table 1). Then, calibration solutions for each component (1, 2, 5, 10 and 20 ppm) were prepared and the calibration curve was drawn using these determined parameters.

Table 1. MS optimization parameters for the antioxidant compounds.

Parameter	Compound	
	Carnosol	Carnosic acid
Polarity	+	-
Precursor ion	330.8	330.9
Fragmentor voltage	110	90
Product ions	266.8, 284.9	286.9, 243.9
Collision energy	18, 10	18, 20

LC-MS/MS Analysis Parameters

The HPLC elution was carried out at 35 °C with an gradient flow of mobil phases (Table 2) at a flow rate of 0.3mL $minute^{-1}$ and injection volume of 3 µL. Mobil phase: A; methanol:water (5:95(v/v), 0.01% formic acid, 5 µM ammonium formate), B; methanol (0.01% formic acid, 5 µM ammonium formate). Total analysis duration was 15 minute and, ionization was done by ESI source at 70 eV.

Table 2. Gradient elution program used in chromatographic analyses

Time (minute)	A (%)	B (%)
0.00	95	5
3.00	95	5
8.00	20	30
12.00	10	95
15.00	5	5

Statistical Analyses

The experiment was conducted in a randomized design with three replications and the results of the analyses were reported as mean ± standard error (SE). Significant differences were calculated by analysis of variance using SAS software (SAS Institute Inc., Cary, NC). Furthermore, Duncan's multiple range test was performed to determine the significance of differences between variances ($P<0.05$).

RESULTS and DISCUSSION

Rosemary leaves are dried conventionally in shade at atmospheric conditions. Otherwise, they are dried by different drying methods especially air-circulated oven dryers at different temperatures concerning raw material status. Figure 1 shows the variation of essential oil and antioxidant component contents of rosemary leaves depending on the drying processes.

The essential oil yields of the samples showed statistically significant differences ($P<0.05$) compared to the drying methods. The essential oil content of the samples dried in the shade had the lowest value at 1.84%. This process duration was one week to obtain about 10% moisture level. The second lowest value was determined as 2.03% at 55°C. The former has a long drying time and this may lead to essential oil loss. The latter has a high drying temperature compared to other applications. Thus, this may lead to similar results because of evaporation. Previous studies stated that drying at high temperatures or long time may cause a decrease in essential oil content [18, 19]. In order to avoid this result, drying is generally carried out at 30-50°C and as much as possible a short time for MAPs. Our findings showed that the highest essential oil content was determined at 35°C and 45°C. MAP's drying is very important in terms of preserving the quality and aromatic properties. As the drying temperature is increased, the amount of essential oil decreases [20]. Piga *et al*. [21] examined the effect of different drying temperatures (30°C, 38°C and 45°C) and air flow rates (300, 1250 m^3 h^{-1}) on the amount of essential oil for rosemary grown in Italy. Essential oil yields were determined as 4.05%, 3.69% and 2.98% for each temperature, respectively. Thus, the best drying temperature for the rosemary plant was mentioned as

45°C for fast hot-air flow and at 38°C for low-speed hot-air flow. Jalal *et al*. [22] examined the effect of different drying practices on the essential oil content of rosemary. The highest rate of essential oil was determined as 0.9% in shade dried sample. In another study the essential oil ratios were 1.00%, 0.14% and 0.12% for mint (*Mentha piperita* L.), and 2.13% 1.62% and 1.09% for rosemary at 40, 60 and 80 °C, respectively [6]. Our results are in line with the literature concerning temperature effects. While Piga *et al*.'s [20] findings are higher than our results, Jalal *et al.* [22] report's value is lower than the present study. These differences could result from raw material (genetic factors, harvesting time, etc.), applied temperature, instrument properties (air flow, temperature increment etc.) differences as mentioned by Hernandez *et al.* [2].

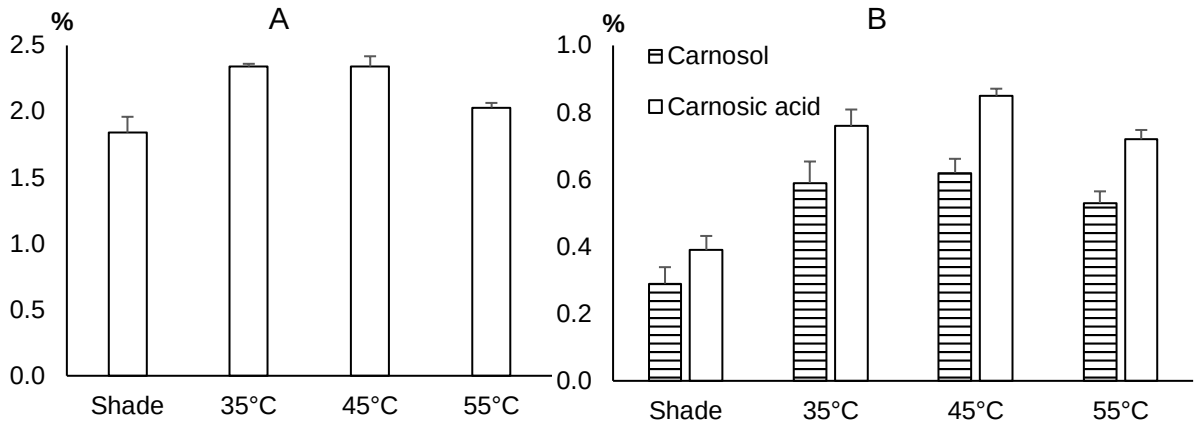

Figure 1. Essential oil (A), carnosol and carnosic acid (B) contents of the samples dried with different drying methods (means±standard deviation)

The drying process may also have an effect on some other phytochemicals like antioxidants. The main antioxidant compounds are carnosol and carnosic acid for rosemary [23]. It was observed that there were statistically significant differences in the amounts of carnosol and carnosic acid in the samples depending on the drying conditions. The lowest carnosol and carnosic acid were determined at 55°C followed by room temperature dried (in shade) sample. And, the highest values for these components were determined at 45°C. Similar results were observed in essential oil yield as aforementioned. Research showed that drying conditions may affect the chemical composition of MAPs. It has been reported that the antioxidant properties of MAPs and their extracts could be changed concerningdrying methods. Hence, it is important to choose appropriate drying methods for obtaining targeted phytochemicals with high yields [24]. Such as Orphanides *et al.* [25] found that there was a decrease in phenolic amounts when high temperatures were applied to mentha. However, the drying effects on the antioxidant compounds of rosemary are very limited. Hussain *et al*. [26] determined the total phenolic matter, rosmarinic acid content and antioxidant activity (ORAC) of six Lamiaceae herbs, including rosemary, dried by air, freeze and vacuum oven. Air-dried one had the highest values for these parameters. Mulinacci *et al*. [7] compared the antioxidant compounds content of the freeze-dried and air-dried (shade) rosemary. Freeze-dried samples had higher carnosol, carnosic acid and rosmarinic acid than shade-dried ones. Present findings showed significant differences from the literature because of process parameters and raw material differences. It is concluded that the most suitable drying process among the applied processes is 45°C oven drying in terms of carnosol, carnosic acid and essential oil yields. Within the scope of the research, the effects of drying on the essential oil composition were also examined. The essential oil composition changes of the samples are depicted in Table 3.

Results showed that the essential oil composition of rosemary was significantly affected ($P<0.05$) by the drying applications. The main component of rosemary essential oil is 1,8-cineole. There are two types of rosemary in the European pharmacopoeia, and their chemical composition differed from each other with respect to the ratio of main components. The main components of rosemary essential oil were 1,8-cineole and camphor. There are two types of rosemary essential oil. One of them called Spanish and the other one is Moroccan and Tunisian in European Pharmacopoeia. There are major differences in essential oil composition and also some other characteristics of them [28]. Napoli *et al.* [29] classified rosemary in three chemotypes as cineoliferum (1,8-cineole rich), camphoriferum (camphor > 20%), verbenoniferum (verbenone > 15%), considering essential oil composition. The rosemary used in the present study is close to Moroccan and Tunisian types with respect to the European Pharmacopoeia and cineoliferum according to essential oil composition. The highest 1,8-cineole content was found in shade-dried sample as 58.91% followed by 55°C. The sample dried at 45°C had the lowest 1,8-cineole content among the oven-drying applications. Additionally, the highest *α*-terpineol and *δ*–terpineol contents were found in shade-dried samples, and the lowest value was obtained at 45°C. On the other hand, *α*-pinene, camphene and borneol were found as the highest at 45°C, and the lowest values were found in shade-dried samples. The data obtained showed that there were partial changes in the other essential oil

components depending on the drying process. The change in the ratio of the essential oil component during drying depends on the type of compounds, the drying time and temperature [19]. Szumny *et al*. [30] reported drying methods have significant effects on the essential oil composition of rosemary. Convective and vacuum-microwave drying combination was found more appropriate because of the short time process to decrease the adverse impact of the drying on the product. Verma and Chauhan [31] compared shade and oven drying (50°C) effects on rosemary essential oil composition. 1,8-cineole concentration was found to be 31.8% in shade drying and 32.9% in oven drying. Other major constituents, camphor and *α*-pinene yields were found to be 26.6%, 13.4% in shade drying and 31.7% and 9.2% in oven drying, respectively. The difference between our research findings and literature values is thought to be due to the difference in herbal material. However, as stated by Verma and Chauhan [31], the similarity between 50°C applications in the shade and in the oven was also seen in our findings.

Table 3. Essential oil composition of the samples dried with different drying methods (%, mean±SE)

Compounds	RI†	RI‡	Drying Conditions			
			Shade	35°C	45°C	55°C
α-pinene	1024	1025	8.20^{b}±0.268	8.67^{ab}±0.778	9.53^{a}±0.297	9.48^{ab}±0.255
Camphene	1068	1069	2.49^{b}±0.085	2.88^{a}±0.099	3.15^{a}±0.057	3.02^{a}±0.156
β-pinene	1111	1110	6.39^{b}±0.085	7.42^{b}±0.057	6.17^{b}±0.099	5.80^{c}±0.099
β-myrcene	1165	1161	1.80^{a}±0.085	1.74^{a}±0.071	1.76^{a}±0.042	1.51^{b}±0.028
α-terpinene	1182	1178	0.74^{a}±0.014	0.76^{a}±0.042	0.73^{a}±0.014	0.71^{a}±0.007
Limonene	1202	1198	1.99^{ab}±0.057	1.94^{b}±0.014	2.08^{a}±0.042	1.74^{c}±0.042
1,8-cineole	1214	1211	58.91^{a}±0.750	54.20^{b}±1.287	52.63^{b}±0.962	58.23^{a}±1.153
γ-terpinene	1248	1245	1.26^{c}±0.085	1.72^{a}±0.099	1.55^{ab}±0.085	1.41^{bc}±0.071
p-cymene	1274	1270	1.03^{a}±0.014	0.87^{b}±0.071	0.98^{ab}±0.042	0.96^{ab}±0.057
Camphor	1533	1515	2.03^{b}±0.113	2.36^{a}±0.042	1.95^{b}±0.085	1.07^{c}±0.057
Bornyl acetate	1591	1579	2.20^{c}±0.085	3.03^{a}±0.085	2.66^{b}±0.127	2.16^{c}±0.156
Terpinen-4-ol	1613	1601	3.15^{b}±0.141	4.10^{a}±0.113	3.81^{a}±0.226	2.64^{c}±0.170
δ-terpineol	1679	1679	0.91^{a}±0.042	0.81^{ab}±0.057	0.71^{b}±0.028	0.75^{b}±0.014
α-terpineol	1704	1694	4.71^{a}±0.113	4.20^{b}±0.127	3.94^{b}±0.255	4.09^{b}±0.085
Borneol	1709	1700	4.21^{d}±0.127	5.30^{c}±0.057	8.34^{a}±0.099	6.41^{b}±0.339

In each row different letters mean significant differences between applications (P<0.05).
† Calculated from retention times of each compound compared by alkane series' retention time.
‡ Retention indices mentioned in Babushok *et al*. [27].

Distillation time is another factor that has an effect on essential oil, carnosol and carnosic acid amounts. For this purpose, samples were taken and analyzed at 10, 20, 30, 60, 120 and 180 min intervals from the beginning of boiling in the hydrodistillation process to compare the findings. Carnosol and carnosic acid were determined in the plant marc (waste for essential oil process) during this process. Figure 2 shows the essential oil, carnosol and carnosic acid contents of each sample with respect to distillation time.

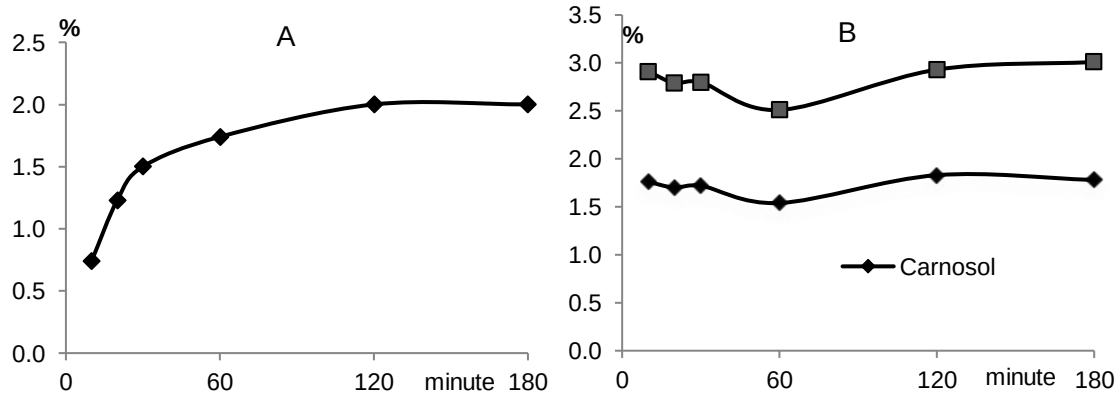

Figure 2. Essential oil (A), carnosol and carnosic acid contents (B) of the samples with respect to distillation time

Some differences were observed in each antioxidant component depending on the distillation times. While these differences were statistically insignificant for carnosol (P > 0.05), 60 min of distillation for carnosic acid differed statistically from other applications. The highest carnosol content was determined in 120 min of distillation, followed by 180 min. Along with this, the highest carnosic acid was found in 180 and 120 min applications. On the other hand, statistically significant differences were found in essential oil content according to the distillation times. When the essential oil amounts are compared, 37% of the total essential oil is in the first 10 min, 61.5% in 20 min, 75% in 30 min, 87% in 60 min, and the whole in 120 min. As a result, it was determined that the 120 min distillation time is sufficient for the production of essential oil from rosemary during this

process. Essential oil content could be decreased with increasing distillation times because of the essential oil volatile characteristics. According to the encountered literature, there are no available records on together with antioxdant eficiency and essential oil contents of rosemary leaves with respect to distillation times. However, detailed research has been carried out on the essential oil and antioxidant content of rosemary individually. Zhelijazkov *et al*. [10] studied the effect of steam distillation time on essential oil yield and composition of rosemary. They reported that there were no increments in essential oil yield after 40 min distillation. Essential oil yield was found as 0.51% at this time and then started to decrease to 0.38% at 160 min. Jalal *et al.* [22] determined the essential oil yield of rosemary with respect to distillation time. They found the total essential oil yield as 1.8% at 3 hours hydro-distillation. And, 75% of the oil was obtained in 20 minutes. The essential oil content was somewhat higher than the reported by Zhelijazkov *et al.* [10] and Jalal *et al*. [22] This could be sourced from rosemary genetic differences and also distillation procedure differences (boiling rate, size reduction, plant/water ratio etc). Hence, important differences were seen in essential oil composition for each material and also in distillation methods. According to Wollinger *et al*. [32] distillation time also affects antioxidant compounds concentration in both water and leaves residue. They found that the highest rosmarinic acid in the water residue for 150 minutes of distillation due to its water solubility. On the other hand, carnosic acid content of the water residue was low because of the low solubility of this compound in water. These findings show that it would be appropriate to produce essential oils and antioxidants from rosemary in an integrated manner.

Essential oil compositions were determined to ascertain the effect of distillation times on the quality of the oil. Duncan's results of essential oil components are given in Table 4.

Table 4. Essential oil composition of the samples with respect to distillation time (%, mean±SE)

Compounds	Distillation time (min)					
	10	20	30	60	120	180
α-pinene	12.70^{a}±1.259	10.44^{bc}±1.032	9.21^{c}±0.339	10.41^{bc}±0.608	12.13^{ab}±0.354	13.33^{a}±0.099
Camphene	4.55^{a}±0.453	3.35^{b}±0.325	3.21^{b}±0.311	3.31^{b}±0.368	3.69^{ab}±0.368	3.87^{ab}±0.339
β-pinene	4.21^{a}±0.410	3.25^{b}±0.325	3.15^{b}±0.311	3.44^{ab}±0.339	3.91^{ab}±0.382	3.97^{ab}±0.269
β-myrcene	1.26^{b}±0.127	1.26^{b}±0.127	1.27^{b}±0.127	1.35^{b}±0.127	1.57^{ab}±0.156	1.67^{a}±0.057
α-terpinene	0.54^{ab}±0.057	0.50^{b}±0.049	0.51^{b}±0.057	0.53^{ab}±0.057	0.66^{ab}±0.071	0.67^{a}±0.071
Limonene	1.84^{bc}±0.184	1.75^{c}±0.170	1.92^{bc}±0.184	1.94^{bc}±0.198	2.31^{ab}±0.226	2.47^{a}±0.184
1,8-cineole	57.79^{abc}±1.344	60.96^{a}±1.018	57.62^{abc}±1.895	60.05^{ab}±1.711	56.39^{bc}±1.442	54.72^{c}±1.739
γ-terpinene	1.04^{a}±0.141	0.86^{a}±0.127	0.90^{a}±0.099	0.83^{a}±0.113	1.06^{a}±0.156	1.03^{a}±0.141
p-cymene	1.81^{a}±0.170	1.80^{a}±0.255	1.95^{a}±0.276	1.94^{a}±0.269	2.11^{a}±0.184	2.26^{a}±0.325
Camphor	1.73^{a}±0.240	1.66^{a}±0.240	1.92^{a}±0.269	1.53^{a}±0.212	1.52^{a}±0.212	1.36^{a}±0.198
Bornyl acetate	2.15^{a}±0.141	1.92^{a}±0.269	2.26^{a}±0.325	1.75^{a}±0.247	1.95^{a}±0.276	1.65^{a}±0.141
Terpinen-4-ol	3.95^{a}±0.141	3.12^{ab}±0.438	4.13^{a}±0.580	2.55^{b}±0.354	3.31^{ab}±0.283	3.74^{a}±0.523
δ-terpineol	0.44^{b}±0.057	0.67^{ab}±0.099	0.83^{a}±0.113	0.72^{a}±0.099	0.65^{ab}±0.092	0.63^{ab}±0.085
α-terpineol	1.97^{b}±0.283	3.57^{a}±0.509	4.61^{a}±0.651	4.04^{a}±0.566	3.62^{a}±0.481	3.60^{a}±0.297
Borneol	4.02^{b}±0.566	4.88^{ab}±0.905	6.49^{a}±0.919	5.60^{ab}±0.792	5.13^{ab}±0.240	5.03^{ab}±0.849

In each row different letters mean significant differences between applications (P<0.05).

The essential oil compositions are significantly affected by the distillation times. 1,8-Cineole, having a monoterpene ether structure and also named eucalyptol, is the main component of rosemary essential oil. The biological activities of the essential oil are attributed to mainly monoterpenes such as 1,8-cineole, borneol, pinene [33]. In particular, 1,8-cineole is widely used for cough treatment, muscular pain, neurosis, rheumatism, asthma, and urinary stones and also in the cosmetic industry [34]. It ranged from 54.72% (180 min) to 60.96% (20 min) depending on distillation time. There is a decrease in the amount of this main component after 20 min of distillation time. The second highest component was found as *α*-pinene for this oil. Contrary to 1,8-cineole, the highest value for *α*-pinene was determined at 180 minutes' distillation time. The average concentration of *α*-pinene decreased to a minimum degree of 9.21% at the 30th min and then started to increase, reaching 13.33% at the 180th min. The other major components were camphene, *β*-pinene, terpinen-4-ol, *α*-terpineol and borneol. There was a change in camphene and *β*-pinene similar to the *α*-pinene concentration with regard to distillation time. On the other hand, terpinen-4-ol, *α*-terpineol and borneol amounts varied irregularly considering distillation times. The concentrations of *β*-myrcene, *α*-terpinene, limonene, *p*-cymene components in the essential oil varied between 1.26-1.67%, 0.50-0.67%, 1.75-2.47%, 1.80-2.26%, respectively. These component concentrations increased by 33%, 24% 34%, and 25% from the beginning to the end of the distillation period, in the same order. This is related to the boiling point and solubility degree in water of each component of essential oil [35]. Sadeh et al. [36] reported that essential oil components were separated with respect to water solubility degree than boiling points.

Zheljazkov *et al.* [10] applied eight distillation times from 1.25 min to 160 min with a steam distillation method to compare the yield and composition of rosemary essential oil. Major components were determined as *α*-pinene, 1,8-cineole and camphor. They reported that, while *α*-pinene, 1,8-cineole yields decreased with increasing in distillation time, camphor increased during distillation. Likewise, 1,8-cineole content ranged between 18.9-23.3%, and the highest ratio was determined at 2.5 min distillation time. Jalal *et al.* [22] reported that 75% of the total essential oil was obtained

at 20 minutes' distillation time and highest 1,8-cineole with 60,13% at this time for rosemary. There is no more available record about essential oil composition and antioxidant content for rosemary applied at different distillation time according to encountered literature. However, some research has been conducted on this topic for different MAPs. One of them is on bay laurel and its main component is 1,8-cineole. For this purpose, the effect of five different hydro-distillation times (10-120 min) on the essential oil composition of bay laurel was investigated. It was found that the distillation time was quite effective in the laurel essential oil composition. It was determined that 1,8-cineole, which is one of the important components of laurel essential oil, varies between 57.7-79.4% depending on the application time, the highest 1,8-cineole ratio was determined with 79.4% in 10 min of distillation time application [37]. Toker *et al*. [38] determined differences in essential oil composition for oregano as to distillation times. The main component is carvacrol for this plant, ranging between 62.92-84.35%, and the highest value was determined for 30 minutes' distillation. Some changes were also observed in studies on the effect of distillation time on plants such as fennel [39]. There are some differences between the present study and the literature. This could be the result of raw materials, processing techniques and process parameters. To compare the yield of antioxidant components, there are no available results on these compounds regarding distillation times.

CONCLUSION

Drying is the most common and fundamental method for post-harvest preservation and processing of any MAP's. It was determined that the most suitable drying temperature for rosemary was 45°C in an air circulation oven of essential oil and antioxidant content. Rosemary can be evaluated in different ways following the drying process, and one of the products that can be produced in this sense is essential oil. Within the scope of the study, depending on the duration of the distillation process used in the production of essential oil, the essential oil yield and composition, as well as carnosol and carnosic acid contents, were analyzed and the optimum distillation time was tried to be revealed. In this sense, it was observed that a two-hour hydrodistillation application under laboratory conditions gave the most successful results in terms of essential oil. Besides, the biomass residue after hydro-distillation (essential oil production) could be evaluated to obtain antioxidant substances called carnosol and carnosic acid. Moreover, results revealed that compositional differences for the essential oil could be obtained by accounting for distillation process times. As a conclusion, essential oil and antioxidant production from rosemary should be done in an integrated manner.

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