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The Effect of Different Hydrodistillation Times on the Composition and Yield of Bergamot (*Citrus bergamia* Risso) Peel Essential Oil and Comparison of Cold-Pressing Method

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Abstract

Bergamot (*Citrus bergamia* Risso) is not consumed as fresh fruit as other members of *Citrus*. Bergamot essential oil has a unique aroma. Bergamot essential oil is a very valuable product used in perfumery, cosmetics, aromatherapy and food industry due to its beautiful scent and functional properties. As with other *C~~c~~itrus* species, the composition of bergamot essential oil can vary according to harvest time, climate and extraction technology.~~, and it's parameters~~. Bergamot essential oil could be obtained by cold press extraction or distillation method. Within the scope of the study, the effect of four different hydro-distillation times (5, 10, 60 and 120 minutes) on the composition of bergamot essential oil obtained from Turkish fruits was investigated. In the first 60 minutes, 96.11% of the total essential oil was obtained. Research findings have shown that the distillation time is quite effective in the composition of bergamot essential oil. Limonene, linalool and linalyl acetate, which are the main components of bergamot essential oil, varied in the range, 42.36-53.25%, 5.59-12.18% and 20.84%-26.73% respectively with respect to distillation time. In cold press method, bergamot essential oil has lower linalool/linalyl acetate ratio (0.15) compared to hydrodistillation method and a higher quality essence was obtained. ~~Fruits were cultivated in Turkey.~~

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Keywords: ~~hydrodistillation time; cold-pressing;~~ bergamot essential oil; cold-pressing; hydrodistillation time; limonene; linalool; linalyl acetate

1. INTRODUCTION

Citrus fruits rank first in the world in terms of production among fruits. They are grown commercially in more than 50 countries around the world.[1] The contribution of the *C~~c~~itrus* sector to the world economy is enormous. With the increase in health awareness among the people, the tendency to natural products increases the industrial value of these products in the world.[2] One of them is bergamot, which is mostly used in the production of essential oil.[3] In Calabria, a unique pedoclimatic region of South Italy, the world's highest quality bergamot has been produced since the middle of the 18th century.[4] Many studies were conducted on the bergamot fruits of Calabria and its applications,[5-9] often related to the essential oil composition.[10-12] A new interest is growing in Turkey for bergamot fruit.[13-14]

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Bergamot plants, despite uncertain species descriptions and phytogeographic origins, are mostly grown in the Southern Italy, Argentina and Brazil.[15] Bergamot peels are widely used in the production of essential oil as well as being used as jam in Turkey. Bergamot is mostly grown in the Mediterranean region in Turkey. It is known that about 70 tons/year of bergamot is produced in Antalya, which is located in the Mediterranean region.

Bergamot has a thick cortex that contains a large amount of aromatic oils. During *C~~c~~itrus* processing, about 25% of the total *C~~c~~itrus* weight is made of peels.[16] Bergamot essential oils are especially in demand in the perfumery and food industry. Essential oils obtained from bergamot grown in the Calabria region are known for their characteristic properties.[17] The essential oil of bergamot peel, which is defined as the "prince of *C~~c~~itrus* fruits" in Calabria, has a different composition and aroma from all other *C~~c~~itrus* peel oils.[18~~-19~~]

Terpenes, sesquiterpenes, higher alcohols, aldehydes and esters predominate in essential oils obtained from *C~~c~~itrus* fruits. The first two types of hydrocarbons make up 95% of the total essential oil, while compounds containing oxygen make up less than 5%. However, the latter are the main sources of essential oil aroma. [16~~16~~] The situation is slightly different for bergamot peel oil. Bergamot essential oil consists of about 100 compounds. The volatile fraction consists of monoterpene (43-65%) and sesquiterpene (less than 2%), hydrocarbons and oxygenated derivatives (aldehydes, ketones, alcohols, esters and oxides) together with lesser compounds. Compared to other ~~c~~*Citrus* essential oils, bergamot oil is characterized by its high oxygen fraction. The main oxygen-containing compounds are linalool (4-26%) and linalyl acetate (24-35%). The main hydrocarbon is limonene (29-46%); significant amounts of β-pinene (5–7%) and γ-terpinene (6–8%) are also present.[10,19~~6,20~~]

Bergamot essential oil, which has antibacterial, antiviral and antioxidant effects, is a product used in perfumery, cosmetics, aromatherapy, food and beverages due to its beautiful smell.[20~~1~~-27]

Traditional methods such as cold-press, hydrodistillation and steam distillation and advanced methods (microwave-assisted hydrodistillation, solvent free microwave extraction, single drop micro- extraction and headspace solid phase microextraction) can be used in the extraction of essential oils and volatile substances.[28,29]
~~Traditional extraction techniques are (1) soxhlet extraction, (2) maceration, and (3) hydrodistillation.~~[~~28~~] Essential oils of medicinal-aromatic plants are generally obtained by distillation. Hydrodistillation is one of the most popular and oldest ways to recover essential oil.[30,31~~28-29~~] If *Citrus* peel volatiles will be used as aroma, the cold pressing method is preferred. However, both volatile and non-volatile compounds are extracted by cold-press. Additionally, many aroma components can be thermally degraded during hydrodistillation. It is also requires large amounts of samples, long time, and high energy.[29]

In the hydrodistillation method, most of the important fragrance components of essential oils such as alcohols, aldehydes and esters are lost by heating, which affects the quality of distilled essential oils.[30,31~~16-17~~] The distillation process means that the oil is stripped of all non-volatile components, including pigments.[32~~0-31~~] In *C~~c~~itrus* fruits, essential oils of the peel are mostly obtained by cold pressing method together with hydrodistillation.

Extraction of essential oils from natural products is a competitive field of study for researchers. The selection of the extraction method depends on the properties of the components desired to be obtained.[33~~2~~] Essential oil production may differ according to factors such as distillation method and extraction device.[34-38~~3-37~~] It is stated that in the production of essential oil from some medicinal and aromatic plants, products with different compositions can be obtained depending on the distillation time.[39-45~~38-44~~]

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However, no study has been found on the effects of such an application on the composition of essential oil in *C~~c~~itrus* oils. Within the scope of the research, the effect of 4 different hydrodistillation periods on the composition and yield of bergamot peel essential oil was investigated. In addition, essential oil compositions obtained by hydrodistillation and cold press method were compared.

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2 EXPERIMENTAL

2.1 Plant material

Fresh bergamot peels were used as material in the research of the cultivar Yerli A-41 registered by Republic of Türkiye Ministry of Agriculture and Forestry Varitey Registiration and Seed Certification Center.[46~~5~~] The samples were taken from bergamot trees (30 years old, 6 decares, 120 trees) located in Batı Akdeniz Agricul~~-~~tural Research Institute *C~~c~~itrus* orchard on 23 March 2022 in Antalya/Türkiye~~urkey~~ (36º 56' 25" N; 30º 53' 10" E). Fruit genetic resouses (bergamot) are preserved ex-situ in field gene collections (BATEM). After the samples were harvested, they were brought to the Western Mediterranean Agricultural Re~~-~~search Institute (BATEM), Medicinal Aromatic Plants Center Laboratory for analysis. First of all, weight and peel ratio of the fruit were determined. For this purpose, 15 fruits were used for each replication.

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2.2 Hydrodistillation (HD) process

The essential oil yield~~amounts~~ of the samples were determined by hydrodistillation method in the Clevenger device for 2 h.[47~~4~~] For this purpose, 50 g of fresh fruit peel was crushed with 150 ml of distilled water in a blender (Waring 8011ES, USA) and then distilled in a Clevenger apparatus (Wisd WiseTherm, South Korea). Distillation times; It were set to be 0-5 minutes, 0-10 minutes, 0-60 minutes, and 0-120 minutes. The determined distillation times were kept from the beginning of boiling.

2.3 Cold press process

Obtaining essential oil by cold pressing was done according to Kırbaşlar et al.[48~~6~~] For this purpose, first of all, the albedo parts of the fruit peels containing essential oil were grated with a hand-held grater. The resulting graters were subjected to manual pressing. The water:oil emulsion obtained as a result of pressing was centrifuged at 12000 rpm for 20 minutes in an ultracentrifuge at 20 °C. The water residue in the essential oils collected in the upper phase was removed with anhydrous sodium acetate and analyzed.

2.4 GC/MS-FID analysis

Component analysis of bergamot peel essential oils obtained as a result of different distillation times was determined using GC/MS-FID (Gas chromatography (Agilent 7890A) mass spectrometry (Agilent 5975C)) and flame ionisation dedector device. For this purpose, a capillary column (HP Innowax; 60.0 m x 0.25 mm x 0.25 µm) was used. First of all, essential oil samples were diluted with n-hexane at a ratio of 1:50 to be analyzed. In the chromatographic analysis, helium gas was used as the mobile phase at a flow rate of 0.8 mL/min. Essential oils diluted with hexane were given to the device as 1 µL with a split ratio of 1:40. The device injection block temperature was set to 250 °C. In order to real-ize the essential oil component separation, the column temperature program was adjusted as 60 °C (10 minutes), from 60 °C to 220 °C at 4 °C/minute and 220 °C (10 minutes). The scanning range (m/z) of 35-450 amu was used for the mass detector and 70 eV for ioniza-tion. The data of Wiley7n, Oil Adams and Nist05 libraries were used as a basis for the identification of essential oil components of the samples. The percentages of the obtained components were made using the FID detector, and the identification was made using the MS detector.[49~~6~~] The identification accuracy of the components was also calculated using the alkane series, and the rention indices values were compared with the literature values.

2.5 Statistical analyses

The research was carried out in a randomized plot design with three replications. Essential oil component analyzes were performed as two injections per repetition. The data obtained as a result of the study were subjected to the Duncan Multiple Comparison test using the SAS package program. Obtained statistical data are presented as mean±standard error.

3 RESULTS

Yields and chemical composition of the essential oil

In bergamot with an average weight of 219.55±48.36 g (mean±standard deviation), the peel ratio was determined as 26.64%. The essential oil content~~amount~~ and essential oil~~ yield~~ ratios obtained as a result of four different distillation periods applied within the scope of the study are given in Table 1. The research findings showed that there were significant differences in the essential oil ratios obtained depending on the distillation time. In the first 60 minutes 96.11%~~82.88%~~ of the total essential oil ~~in the first 10 minutes of distillation and 96.11% in the first 60 minutes ~~were obtained.

TABLE 1 Essential oil content with respect to distillation time (mean±standard error).

Distillation time (minute)	Essential oil content (%)	Ratio in total~~Essential oil yield~~ (%)
0-5	0.95±0.03[c]	36.96
0-10	2.13±0.22[b]	82.88
0-60	2.47±0.07[ab]	96.11
0-120	2.57±0.09[a]	100.00

[1] Different letters in the same column indicate a difference of *p*<0.05 between the means.

Within the scope of the research, component analyzes of essential oils obtained at different times were also performed (Table 2). In total, 18 ~~types of essential oil~~ components have been identified. The data obtained reveal that there were significant differences between the essential oil component concentrations of the samples depending on the time. In all of the samples, limonene constitutes the most proportionally important part of the bergamot essential oil. Limonene had a tendency to increase in the first 10 minutes and then decrease. In our study, linalool tended to increase and linalyl acetate to decrease with increasing distillation time (*p*<0.05). β-Pinene ranged from 4.09% to 4.46%, and γ-terpinene was between 5.96-6.62%. Predominantly, hydrocabons monoterpenes, as well as aliphatic ester (hexyl acetate), monoterpene esters (linalyl acetate, neryl acetate, geranyl acetate), monoterpene alcohols (linalool, α-terpineol, nerol, geraniol) and hydrocarbon sesquiterpene (β-bisabolene) have been detected.

TABLE 2 Essential oil composition of the volatile oil with respect to hydrodistillation time (%, mean±standard error)~~.~~

Compounds	RI~~[z]~~[*]	RI[y]	5 min	10 min	60 min	120 min
α-Pinene	1030	1025	0,68±0.02[a]	0.70±0.00[a]	0.73±0.02[a]	0.70±0.01[a]
β-Pinene	1122	1110	4.46±0.11[a]	4.09±0.03[b]	4.06±0.10[b]	4.44±0.06[a]

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Sabinene	1132	1122	0.80±0.02^{a}	0.71±0.01^{bc}	0.66±0.02^{c}	0.73±0.02^{b}
β-Myrcene	1170	1160	1.61±0.03^{b}	1.77±0.03^{a}	1.84±0.02^{a}	1.85±0.06^{a}
Limonene	1214	1198	**45.12±1.02^{c}**	**53.25±0.62^{a}**	**50.19±0.57^{b}**	**42.36±0.96^{d}**
cis- β-Ocimene	1242	1234	0.18±0.01^{bc}	0.15±0.00^{c}	0.23±0.01^{b}	0.32±0.03^{a}
γ-Terpinene	1260	1245	5.96±0.13^{c}	6.31±0.02^{b}	6.37±0.11^{ab}	6.62±0.07^{a}
Hexyl acetate	1279	1273	0.21±0.02^{b}	0.27±0.00^{a}	0.12±0.01^{c}	0.06±0.01^{d}
p-Cymene	1285	1270	0.80+0.15^{a}	0.32+0.00^{b}	0.24+0.03^{c}	0.24+0.01^{c}
α-Terpinolene	1298	1282	0.31±0.02^{c}	0.35±0.01^{bc}	0.37±0.01^{b}	0.44±0.00^{a}
Linalool	1549	1543	**8.53±0.17^{b}**	**5.59±0.09^{c}**	**7.73±0.34^{b}**	**12.18±0.48^{a}**
Linalyl acetate	1565	1554	**26.73±0.87^{a}**	**21.48±0.57^{b}**	**20.84±0.29^{b}**	**21.43±0.62^{b}**
α-Terpineol	1709	1694	1.46±0.08^{c}	1.48±0.02^{c}	2.07±0.09^{b}	2.90±0.20^{a}
Neryl acetate	1734	1718	0.99±0.05^{c}	1.19±0.03^{b}	1.23±0.06^{b}	1.44±0.04^{a}
β-Bisabolene	1746	1727	0.13±0.01^{b}	0.19±0.01^{b}	0.35±0.01^{a}	0.31±0.04^{a}
Geranyl acetate	1764	1751	0.97±0.06^{c}	1.08±0.00^{c}	1.35±0.06^{b}	1.70±0.09^{a}
Nerol	1804	1794	0.29±0.05^{c}	0.30±0.00^{c}	0.44±0.02^{b}	0.61±0.03^{a}
Geraniol	1848	1839	0.77±0.06^{c}	0.77±0.01^{c}	1.18±0.05^{b}	1.67±0.10^{a}

*~~z~~Different letters at the same line shows significant difference at *p*~~P~~<0.05
RI^{z}: calculated, RI^{y}: Babushok et al. [50~~47~~]

3.1 Comparasion of two extraction methods

In addition, the compositions of essential oils obtained by hydrodistillation (CLV) and cold pressing (CP) were evaluated within the scope of the research (Table 3). While the average amount of distilled oil obtained by hydrodistillation was 2.57%, cold-pressed oil ratio was found to be 0.5%. The GC/MS-FID data obtained showed that there were significant changes in bergamot peel volatile fraction~~essential oil~~ according to the extraction method. The main components of the peel volatile fraction~~essential oil~~ obtained by both methods were determined as limonene, linalool and linalyl acetate. However, there were significant changes in the ratios of linalool and linalyl acetae compared to the extraction method. While the linalool ratio in the bergamot peel extract~~ssential oil~~ obtained by cold pressing was at the level of 5.37%, the ratio of this component obtained by hydrodistillation was approximately 3 times higher than that value. On the other hand, linalyl acetate was the opposite. However, the difference here was proportionally lower than that found for linalool. The difference detected in limonene, which is also the main component of *C*~~c~~*itrus* peel oils, was lower than these two components.

TABLE 3 Essential oil composition of bergamot essential oil by extraction methods~~.~~

Compounds	**CLV***	**CP***
α-Pinene	0.80±0.02^{a}	0.70±0.01^{b}
β-Pinene	4.56±0.04^{a}	4.38±0.02^{b}
Sabinene	0.60±0.02^{a}	0.62±0.02^{a}
β-Myrcene	1.78±0.04^{a}	1.03±0.02^{b}
Limonene	**45.90±0.85^{a}**	**42.27±0.96^{b}**

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cis- β-Ocimene	0.17±0.01[a]	0.16±0.01[a]
γ-Terpinene	5.65±0.05[a]	5.37±0.03[b]
Hexyl acetate	0.13±0.03[a]	0.10±0.01[a]
p-Cymene	0.21±0.03[a]	0.15±0.02[b]
α-Terpinolene	0.41±0.04[a]	0.25±0.02[b]
Linalool	**15.39±0.06[a]**	**5.70±0.02[b]**
Linalyl acetate	**16.43±0.05[b]**	**37.85±0.06[a]**
α-Terpineol	2.68±0.02	udl
Neryl acetate	1.21±0.02[a]	0.61±0.01[b]
β-Bisabolene	0.37±0.03[b]	0.55±0.03[a]
Geranyl acetate	1.55±0.02[a]	0.26±0.02[b]
Nerol	0.62±0.02	udl**~~*~~
Geraniol	1.54±0.01	udl

*~~-~~Different letters at the same column shows significant difference at p<0.05
**CLV: Hydrodistillation, CP: Cold pressing
***udl: under detection level

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The limonene ratio of bergamot peel essential oil obtained by hydrodistillation was higher than that obtained by cold press. It has been determined that there are some differences in the components that are proportionally lower in bergamot peel essential oil according to the extraction method. In this sense, α-terpineol, nerol and geraniol components, which are in the structure of monoterpene alcohols, could be detected in essential oils obtained by hydrodistillation, but remained below the detection limit in sample obtained by cold pressing. As a matter of fact, linalool content, which is in the structure of monoterpene alcohol and is important in bergamot peel essential oil, was similarly lower in the sample obtained by cold pressing. This difference in composition shows that oil with different properties can be produced depending on the extraction method to be applied for bergamot peel oil.

4 DISCUSSION

In our study, it was determined that there was only a 3.89% increase in the yield~~amount~~ of essential oil at the end of 120 minutes compared to 60 minutes. According to the hydrodistillation time, it is seen that 82.88% of the essential oil can be obtained in the first 10 minutes from the beginning of the boiling point.~~it is seen that a 10-minute period was sufficient to obtain 82.88% yield to obtain bergamot essential oil in terms of yield.~~

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Anastasopoulou et al.[44]~~[32]~~ hydrodistilled *Pimpinella anisum* seed essential oil with a modified clevenger type apparatus for 1 h, 2 h and 3 h. The highest percentage of essential oil yield (80 ml/kg) was obtained after 1 hour of distillation and gradually the yield decreased to 60 ml/kg after 2 hours and to 25 ml/kg after 3 hours.
Semerdjieva et al.[51]~~[49]~~ determined the essential oil yield of *Ruta graveolens* fruit, *Hyssopus officinalis* subsp with respect to distillation time. A significant amount of the aristatus essential oil content yield (0.44%) was separated during the first 0-5 min distillation and the lowest yield (0.05%) during the 40-60 min distillation. Therefore, it has been shown that the distillation of *H. officinalis* can significantly reduce the 3-hour hydrodistillation reported in the literature.
In the study conducted by Jurevičiūtė et al.,[43]~~[31]~~ it was determined that as the hydrodistillation time increased in lemon thyme, the percentages of monoterpene decreased, while the percentages of

sesquiterpene in the essential oil increased. The results showed that hydrodistillation of essential oil from lemon thyme for more than 60 minutes was useless.

Zhelzajkov et al.[40~~38~~] in the study in which the yield and composition of lavender essential oil were examined according to the distillation time, the maximum oil yield was reached at 60 minutes. It was determined that linalool acetate was at its highest rate at 30 minutes. It was showed that the essential oil yield did not increase after the 60th minute.

To evaluate the effect of hydrodistillation time on essential oil content from fresh leaves of *E. pyriformis* by Durazzini et al.,[42~~0~~] four extractions were performed at four different times. The obtained results revealed that the relative ratio of chemical compounds, as well as the essential oil yield, vary significantly depending on the species, extraction time and method. An extraction time of 120 minutes has been shown to be ideal for obtaining the highest oil yield.

Ibrahim et al.,[45~~3~~] different distillation times (0-240 min) were used to generate fractions of *Myristica fragrans* essential oil with different chemical compositions and bioactivity. The highest oil yield among the fractions was obtained in the distillation period of 10-30 minutes. In general, it was determined that *M. fragrans* seed oil yields increased with increasing distillation time.

In the studies conducted by Jurevičiūtė et al.,[43~~1~~] Semerdjieva et al.[51~~48~~] and Zhelzajkov et al.[40~~38~~] with distillation, it was determined that distillation times could be shorter in terms of yield, although the material studied was different, similar to our study.

In our study, it was determined that limonene, linalool and linalyl acetate, which are the main components of bergamot essential oil, ranged between 42.36-53.25%, 5.59-12.18% and 20.84-26.73% respectively. In all of the samples, limonene, linalyl acetate and linalool constitute the most proportionally important part of the bergamot essential oil composition. Similar results have been reported in studies on bergamot.[3,10,32,48~~0,46~~] Beta pinene ranged from 4.09-7.50%, and gamma-terpinene was between 5.96-10.11%. Predominantly hydrocabons monoterpene, as well as aliphatic ester (hexyl acetate), monoterpene esters (linalyl acetate, neryl acetate, geranyl acetate), monoterpene alcohol (linalool, alpha-terpineol, nerol, geraniol) and hydrocarbon sesquiterpene (beta-bisabolene) have been detected.

D-limonene is a flavor and fragrance additive especially used in perfumes, food and beverages. It also has antioxidant, antidiabetic, anticancer, anti-inflammatory, cardioprotective, gastroprotective, hepatoprotective, immune modulatory, anti-fibrotic, anti-genotoxic properties[52]. Limonene is a valuable ingredient used as an antibacterial agent in the food industry.[36,53~~4,50~~] Limonene, γ-terpinene and linalyl acetate have the larvicidal toxicity. The cold pressed essential oil of bergamot showed better larvicidal toxicity than the distilled one due to the high linalyl acetate ratio.[54]

Similar to the study conducted by Jurevičiūtė et al.[43~~1~~] in lemon thyme, in this study, it was found that the essential oils obtained at all hydrodistillation times had determined high monoterpene (57.70-67.65%) and less sesquiterpene hydrocarbons (0.13-0.35%).

Geraniol, nerol, geranyl acetate, neryl acetate, α~~alpha-~~terpineol constituted 1.67%, 0.61%, 1.70%, 1.44%, and 2.90% of bergamot essential oil, respectively (Table 2). The results showed that distillation time can affect the percentage of geraniol and nerol, similar to the work done by Jurevičiūtė et al.[43~~1~~] on lemon thyme. These components reached their highest values at 120 min ($p<0.05$). Limonene and *p*-cymene were found to decrease with increasing distillation time, similar to the study of Ibrahim et al.[45~~3~~]

The content of limonene in bergamot oil is low compared to other *C~~e~~itrus* oils, while linalyl acetate and linalool are relatively high. The linalool/linalyl acetate ratio is considered as an important quality criterion for bergamot peel oils. It is reported that this value is 0.3 in the samples of Reggio Calabria region of Italy, which is the production center of bergamot and its products.[55~~0~~] It is known that the linalool/linalyl acetate ratio affects the aroma of bergamot essential oil. In a good essence, this ratio is low~~er than 0.3~~.[20] In our study, the ratio of linalool/linalyl acetate varied as 0.26 at the 10th minute, 0.37 at the 60th minute and 0.57 at the 120th minute. According to this study, 10 minutes from the beginning of the boiling is considered adequate~~sufficient~~ to obtain a quality bergamot essential oil in

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terms of a yield of 82.88% and a linalool/linalyl acetate ratio of 0.26. It was determined that the essential oil quality decreased with increasing distillation time.

In our study, the ratio of limonene in the composition of the essential oil obtained in the first 10-minute period of distillation was at the highest level with 53.25%. However, at the end of the distillation period of 120 minutes, 20.45% loss occurred in the ratio~~amount~~ of limonene. It is stated that significant losses may occur when distillation is applied for an excessively long time.[30,31~~20~~] Linalyl acetate reached its highest value with 26.73% in the first 5 minutes, and linalool with 12.18% at the end of 120 minutes. In our study, linalool tended to increase and linalyl acetate to decrease with increasing distillation time ($p<0.05$).

Eleni et. al.[54] determined 30 components in cold pressed and distilled essential oil of the bergamot rind from Greece. The main component for cold press oil was linalyl acetate while for distilled oil the main compound was limonene. Other important constituents were linalool, limonene and γ-terpinene. Comparison between the bergamot peel sample treated by cold press or hydrodistillation revealed that the percentage of linalyl acetate falls from 40.5% to only 10.7% in the distilled oil. The thermal treatment for the distillation procedure leads to decomposition of linalyl acetate. Similarly, in our study, ratio of linalyl acetate decreased from 26.73% to 21.43% in the distilled oil.

In this study, it was determined that the relative percentages of some components such as γ-terpinene, linalool, and α-terpineol increased significantly with increasing distillation time ($p<0,05$). ~~In this study, it was determined that the of γ-terpinene with a boiling point of 183 °C and compounds with a boiling point above 183 °C (linalool BP: 198 °C, α-terpineol BP: 219 °C, neryl acetate BP: 238 °C, geranyl acetate BP: 245 °C, nerol BP: 224 °C, geraniol BP: 230 °C, linalyl acetate BP: 220 °C) percentage compositions were higher at 120 minutes. However, it is understood that the boiling point cannot be very decisive in the essential oil composition, since the situation of other components with lower boiling points cannot be generalized.~~ Similar to our study, in the study conducted by Ibrahim et al.[45~~3~~] in *M. fragrans* essential oil, it was determined that the concentration of compounds with higher boiling points was higher in the later fractions, while the concentration of compounds with low boiling points was generally higher in the initial fractions.

Parameters such as boiling point and polarity of each component can be effective on these changes.[45,56~~4,52~~] It has been reported from studies that the composition of bergamot peel essential oil depends on many factors such as geographical cultivation area, production year, variety, age of the plant, agronomic techniques (fertilization, irrigation), harvest date of the fruits, storage conditions and period.[3,10~~20~~] ~~The data obtained show that the distillation time for each component may have a different level and direction effect.~~The data obtained shows that distillation time affects the ratios of the components.

Kırbaşlar et al.[48~~6~~] evaluated the essential oil compositions of the fruits obtained at three different harvest times by cold pressing. It has been determined that there are some differences in the composition of the samples according to the harvest time. The percentage composition of limonene and linalyl acetate of the sample harvested at the yellow stage was higher than that of green-yellow, fully green peel color. Linalool content was lowest at full maturity stage. The samples analyzed within the scope of our study were also analyzed at full maturity stage, and the component ratios are similar to our study. Researchers also reported that the essential oil compositions of the samples obtained by cold press and steam distillation showed differences. While the linalool/linalyl acetate ratio was 0.15 in our cold press study, it was found to be 0.94 in our hydrodistillation study, which was well above the limit value of 0.30 (Table 3). It shows why vacuum distillation is preferred because long-term heat application deteriorates the essence quality. Similar to the study of Kırbaşlar et al.,[48~~6~~] linalyl acetate was found to be higher (more than 2 times higher) and linalool (up to 1/3) lower than distillation in the cold press method.

In our study, the main components of the bergamot peel essential oil obtained by both methods (CLV and CP) were determined as limonene, linalool and linalyl acetate. There were significant changes in the ratios of linalool and linalyl acetate compared to the extraction method. Similary Mohammadhosseini et al.[28] revealed that there were significant differences for the major constituent

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components common in all profiles of *Salvia limbata*, among the different extraction methods. Nekoei and Mohammadhosseini[29] determined the main volatile components of fruit peel of *C. sinensis* which isolated by solid phase micro-extraction, single drop micro- extraction and cold-press were the same but in different ratios.

Baser et al.[57~~3~~], determined the chemical composition of bergamot peel essential oil obtained by cold pressing. In the study, the main components of bergamot peel essential oil were determined as limonene (32.28%), linalyl acetate (37.39%) and linalool (16.27%). While the linalyl acetate ratio was similar to the value determined in our study, it was observed that the limonene ratio was lower than our findings, and the linalool ratio was higher. This difference is thought to be related to the fruit harvest time. As a matter of fact, the study conducted by Kırbaşlar et al.,[48~~6~~] showed that the harvest time is effective in this sense. In the study conducted by Sawamura et al.,[55~~1~~] the ratios of limonene, linalyl acetate and linalool, which are the main components, were reported as 37.24%, 30.06% and 8.82%, respectively, in the samples of the Calabria Region, which is the origin of bergamot production. Our study findings partially show similarities with these data. In the study conducted by Costa et al.,[32~~0~~] the ratio of limonene, linalool and linalyl acetate obtained by cold pressing were 38.89%, 6.55% and 37.00% respectively. These data showed significant similarities with our study. Two different application results showed that there could be significant differences in bergamot peel essential oil according to the extraction method.

5 CONCLUSIONS

The increasing awareness of consumers about the relationship between nutrition and health has led the food, pharmaceutical and cosmetic industry to develop products enriched with bioactive compounds. In this context, it has been observed that the extraction method and~~,~~ hydrodistillation time may be important in terms of yield and obtaining different composition ratios.~~ in obtaining a product with a composition suitable for the purpose.~~

Hydrodistillation method, which is one of the oldest distillation methods, can be completed in a shorter time considering the time and energy constraints.~~ in today's conditions in obtaining the essential oil.~~ It has been observed that essential oil with a linalool/linalyl acetate ratio of 0.26 can be obtained in 10 minutes of hydrodistillation from the beginning of the boiling point.~~It can be said that 10 minutes hydrodistillation time is the most suitable time in terms of 82.88% yield and 0.26 lialool/linalyl ratio content.~~ It is seen that a higher quality essence can be obtained with the ratio of 0.15 linalool/linalyl acetate in cold pressing method compared to hydrodistillation method. In the future studies, distillation methods applied under vacuum at low temperatures may also provide the appropriate linalool/linalyl acetate ratio. It is needed to separate oil granules from *Citrus* peel before cold-press in terms of volatiles yield.

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Formattato: Rientro: Sinistro: 0 cm, Sporgente 0,75 cm

Formattato: Tipo di carattere: Non Corsivo

Formattato: Tipo di carattere: Corsivo

Formattato: Tipo di carattere: Non Corsivo

Formattato: Tipo di carattere: Non Corsivo

Formattato: Rientro: Sinistro: 0 cm, Sporgente 0,63 cm

Formattato: Tipo di carattere: Corsivo

Formattato: Tipo di carattere: Corsivo

Formattato: Tipo di carattere: Non Corsivo

Formattato: Tipo di carattere: Non Corsivo

Formattato: Sottolineato