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HORTICULTURAL STUDIES

RESEARCH PAPER

The Effect of Hydrodistillation Times and Cold Pressing on Yield and Composition of Sweet Orange (*Citrus sinensis*) Peel Essential Oil

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Abstract

Within the scope of the study, the effect of hydro-distillation times on *Citrus sinensis* (Navelina) fresh peel essential oil composition was investigated. For this purpose, five different distillation times (10, 15, 30, 60, and 120 min.) were evaluated. Research findings showed that the distillation time was not effective on the orange essential oil composition. It was determined that the most important components of *C.sinensis* peel essential oil were limonene (96.52-96.61%) and myrcene (2.03-2.06%). In addition, hydrodistillation (HD) and cold press (CP) essential oils were compared in terms of yield and some physical and chemical properties. In terms of oil yield and optical activity, the values of the oil obtained by hydrodistillation method were higher than those obtained by cold press, and the refractive index and density values were found to be lower. In terms of component ratios, it was seen that there was no significant difference between the two methods.

1. Introduction

The most important reason why the orange has a significant share in the world citrus production is that it is the most preferred citrus type, especially by the fruit juice industry. According to USDA data, a total of 49 million tons of oranges were produced in the world as of the 2020/21 season. Türkiye has some advantages in citrus cultivation in terms of geographical location and climatic conditions. Türkiye ranks 8th in world orange production with a production amount of 1.4 million tons annually. Most of orange production (85%) in Türkiye is carried out in the Mediterranean Region (Aygören, 2021).

Orange is the most widely consumed citrus fruit and accounts for 60% of the world's citrus production. During the processing of the juice, a large amount of by-products is obtained, mainly the peels, representing about 45% of the total mass. Studies have pointed out that orange peel waste may cause many environmental problems, especially water pollution (Hilali et al., 2019).

The consciousness of consumers about nutrition and health has led the food industry to develop food and food supplements enriched with bioactive compounds. Additionally, in order to protect the environment and open up a more efficient use area, the new trend consists of recycling valuable compounds found in industrial food processing wastes (Angiolillo et al., 2015).

The peels form about 25% of the citrus weight. The quality of essential oil obtained from citrus peel largely depends on many factors such as origin, soil type, climate, and citrus variety, but the way the fruit is processed also has a significant impact (Ferhat et al., 2016). Essential oils obtained from citrus peel consist of terpenes, sesquiterpenes, higher alcohols, aldehydes, and esters (Shan, 2016). As reported in the studies, the chemical composition of orange peel essential oil mainly consists of limonene (86%-97%), which is a hydrocarbon compound (Hosni et al., 2010; Gölükcü et al., 2015; Ferhat et al., 2016; Manaila et al., 2016; Sawi et al., 2019). The fact that limonene is one of the most common compounds found in the essential oils of aromatic plants can be attributed to its leading role in the biosynthesis of other monoterpenes and its protective role against herbivores. Limonene is widely used as a flavor and fragrance additive in consumer products such as perfumes and beverages (Erasto and Viljoen, 2008). In addition, limonene is an important natural compound with a wide range of uses due to its antimicrobial, antioxidant, anti-inflammatory, antinociceptive, anticancer, and insecticidal effects (Karr and Coats, 1988; Erasto and Viljoen, 2008; Vieira et al., 2018; Ngan et al., 2020; Maurya et al., 2021).

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 (Zheljazkov et al., 2013; Gölükcü et al., 2018; Durazzini et al., 2019; Anastasopoulou et al., 2020). Within the scope of the research, the effect of five different hydrodistillation periods on the yield and composition of sweet orange peel essential oil was investigated. In addition, the essential oil compositions obtained by hydrodistillation and cold press methods were compared in the study.

2. Material and Methods

2.1. Plant material

Fresh *Citrus sinensis* Navelina orange peels were used as material in the study. The samples were taken from 20-25-year-old orange trees in Aksu location of the Batı Akdeniz Agricultural Research Institute (BATEM) on December 23, 2021. After the samples were harvested, they were sent to the BATEM Medicinal Aromatic Plants Center Laboratory for analysis. First of all, the fruit weight and peel ratio of the fruits were determined. For this purpose, 18 fruits were used for each replication.

2.2. Hydrodistillation (HD) process

The essential oil amounts of the samples were determined by the hydrodistillation method in the clevenger device (TSE, 2011). 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 (Isotex, Türkiye). Determined distillation times were given considering the boiling point. The oil yield obtained in 120 minutes was accepted as 100% and the oil yield was calculated based on 100 g of fruit peel.

2.3. Cold press process

The essential oil was obtained by cold pressing according to Kırbaşlar et al. (2001). For this purpose, first of all, the flavedo 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 essential oils collected in the upper phase were taken into eppendorf tubes and analyzed.

2.4. GC/MS-FID analysis

Component analysis of orange peel essential oils obtained as a result of different distillation times for hydrodistillation and cold press was determined using a gas chromatography (Agilent 7890A) mass detector (Agilent 5975C) device (GC/MS-FID). Analyzes were carried out with reference to the method used by Özek et al. (2010). First of all, orange peel essential oil samples were diluted with hexane at a ratio of 1:50. In the chromatographic analysis, a capillary column (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 μm) was used as the column, and helium gas at a flow rate of 0.8 mL min^{-1} was used as the carrier gas. The injection block temperature was set to 250°C. 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 data of Wiley7n, Oil Adams, and Nist05 libraries were used to identify the essential oil components of the samples. The percentage of each essential oil compoenents was determined by using flame ionization dedector values.

2.5. Physical analysis

Relative density (TS, 2012a), refractive index (TS, 2009), and optical activity (TS, 2012b) analyzes were made according to the Turkish Standard.

2.6. Statistical analysis

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 and Discussion

The essential oil ratios obtained as a result of five different distillation times (10, 15, 30, 60, and 120 minutes) applied within the scope of the study and the ratios of these data to the total amount of essential oil (yield) are given in Table 1. Research findings show that 80.33% of the total essential oil is obtained in the first 15 minutes of distillation and 100% in 60 minutes. In the 60-120 minute interval,

Table 1. Essential oil content and yield at different distillation times (mean±standard error).

Distillation time (minute)	Essential oil content (%)	Essential oil yield (%)
0-10	1.05±0.00 c	34.43
0-15	2.45±0.00 b	80.33
0-30	2.65±0.00 ab	86.89
0-60	3.05±0.00 a	100.00
0-120	3.05±0.00 a	100.00

*Different letters in the same column indicate a difference of P<0.05 between the means.

Table 2. Essential oil composition at different distillation times (%, mean±standard error).

Essential oil composition	RI^z	RI^y	10 min	15 min	30 min	60 min	120 min
α-pinene	1030	1025	0.52±0.01 b	0.53±0.01 ab	0.55±0.02 ab	0.57±0.01 a	0.55±0.00 ab
Sabinene	1122	1110	0.19±0.01	0.19±0.01	0.17±0.03	0.18±0.00	0.18±0.02
δ-3-carene	1132	1122	0.28±0.03	0.23±0.01	0.30±0.03	0.26±0.02	0.28±0.02
Myrcene	1170	1160	2.03±0.02	2.04±0.02	2.05±0.01	2.06±0.00	2.05±0.01
Limonene	1214	1198	96.57±0.05	96.61±0.01	96.52±0.02	96.56±0.02	96.56±0.01
β-phellandrene	1242	1234	0.29±0.00	0.29±0.01	0.29±0.01	0.28±0.01	0.29±0.00
Linalool	1260	1245	0.14±0.02	0.10±0.01	0.12±0.02	0.10±0.01	0.10±0.00

*Different letters at the same line show significant difference at P<0.05.
RI^z: Calculated from alkane series retention times, RI^y: Babushok et al. (2011).

Table 3. Essential oil composition and some quality parameters of Navelina peel oil obtained by different extraction methods.

Compounds	HD	CP
α-pinene	0.55±0.01	0.52±0.01
Sabinene	0.16±0.01	0.16±0.01
δ-3-carene	0.32±0.01	0.29±0.02
Myrcene	2.04±0.02	2.04±0.01
Limonene	96.61±0.05	96.53±0.07
β-phellandrene	0.29±0.01	0.29±0.00
Linalool	0.11±0.01	0.09±0.01
Oil content (%)	3.05±0.05 a	0.93±0.02 b
Relative density (20°C)	0.8315±0.00 b	0.8422±0.00 a
Refractive index (20°C)	1.4725±0.00 b	1.4739±0.00 a
Optical activity (20°C)	98.10±0.20 a	96.45±0.25 b

*Different letters at the same line show significant difference at P<0.05.
HD: Hydrodistillation. CP: Cold pressing

which is the last stage of distillation, there was no increase in the total amount of essential oil. The results showed that 60 minutes of Navelina orange essential oil is sufficient for hydrodistillation.

Within the scope of the research, component analysis of essential oils obtained at different distillation times were also performed (Table 2). α-pinene, sabinene, δ-3-carene, myrcene, limonene, and β-phellandrene from monoterpene hydrocarbons and linalool from monoterpene alcohols were detected in orange peel essential oil. Limonene constituted the most important part of the essential oil composition with 96% in all of the samples. The second highest component is myrcene. According to the data obtained, the amount of limonene and myrcene did not change during the times. Monoterpenes (C_{10}) in the orange essential oils have boiling points in the range of 154 to 176°C. It may be due to the fact that the components in the essential oil composition are monoterpene and their chemical structure and properties are close to each other.

In addition, the compositions of essential oils obtained by hydrodistillation (HD) and cold pressing (CP) were evaluated within the scope of the research (Table 3). The data obtained showed that there were significant changes in Navelina orange peel essential oil according to the extraction method. The main components of the peel essential oil obtained by both methods were determined as limonene and myrcene. It was determined that the essential oil component ratios in the Navelina peel essential oil obtained by cold pressing showed similarities with the component ratios of the samples obtained by hydrodistillation, and there were no statistically significant differences between the applications (P>0.05). It was determined that there were significant differences in the amount of oil obtained, density, refractive index, and optical activity values between the essential oil obtained by hydrodistillation and the obtained by cold press (P<0.05). In our study, it was determined that there was no increase in the amount of essential oil at the end of 120 minutes compared to 60 minutes. In this study, in terms of yield according to the hydrodistillation times, it is seen that a 60-minutes period is sufficient to obtain 100% yield to obtain Navelina orange peel essential oil.

In a study conducted on lemon thyme (*Thymus × citriodorus*) by Jurevičiūtė et al. (2019), it was shown that hydrodistillation time longer than 60 minutes is useless. In the study by Zhelzajkov et al. (2013), 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. Semerdjieva et al. (2019), in their study to determine the essential oil yield of *Hyssopus officinalis* subsp aristatus, obtained a significant amount of essential oil (0.44%) during the first 0-5 minutes of distillation.

In distillation time studies conducted by Jurevičiūtė et al. (2019), Semerdjieva et al. (2019), and Zhelzajkov et al. (2013), it was determined that distillation times could be shorter in terms of yield, similar to our study. It has been observed that hydrodistillation studies, which require a longer time compared to other traditional methods, can be performed in shorter periods. In this way, determining the optimum distillation time specific to the product will also provide an advantage in terms of energy efficiency.

In the study conducted by Ferhat et al. (2016) with HD in 3 varieties of *Citrus sinensis* (Tarocco, Valencia Late, Washington Navel), it was determined that the chemical composition of the essential oil consists mainly of hydrocarbon compounds limonene and β-myrcene. In the essential oil obtained in 180 min HD of three samples, limonene ranged from 92.49% to 95.48%, and β-Myrcene ranged from 1.65% to 1.87%.

In a 3-hour hydrodistillation study with sweet orange (*Citrus sinensis* Osbeck) in a clevenger type device made by Hosni et al. (2010), limonene, β-pinene, and linalool were detected between 96.0-97.3%, 1.45-1.82%, and 0.22-0.04% respectively. It has been reported that the yield and composition differences between sweet orange essential oils can vary depending on genetics, even though they are harvested and processed under the same conditions.

Sawi et al. (2019), in a 4-hour HD study, pointed out that in sweet orange *C. sinensis* (Egypt) dry peel oil, the monoterpene hydrocarbon group, with limonene (86.02%) and myrcene (4.42%) being the main components, is the dominant group in the composition of the essential oil.

According to the results of a study by Manaila et al. (2016), which aimed to compare the properties of essential oil obtained from the fresh peels of five citrus plants by hydrodistillation method, limonene was found in orange (*C. sinensis*) at a rate of 97% and yield of 1.08%. In our study, the main component of Navelina orange peel essential oil was 96% limonene in all of the samples. Similar results have been reported in other studies on orange (Hosni et al., 2010; Dugo and Bonaccorsi, 2014; Ferhat et al., 2016; Gölükcü et al., 2018).

In the 3-hour hydrodistillation study of Hosni et al. (2010), the subspecies of *C. sinensis* Osbeck are Meski (2.31%), Valencia Late (1.89%), and Thomson Navel (1.49%), and Maltaise Blanc (2%) stated that the difference between the yields of dry peel oils of sweet oranges is due to genotype.

Although Ferhat et al. (2016) reported in that the temperature applied in the hydrodistillation method and the long extraction time may change the oil composition, no significant change was detected between the hydrodistillation and cold pressed essential oil compositions in our study. Significant differences were determined in terms of yield in the study carried out by hydrodistillation and cold pressing method on fresh fruit peels *of Citrus sinensis* (L.) Osbeck's Washington Navel, Valencia Late, and Tarocco. It was determined that the yield obtained by hydrodistillation method was higher than that obtained by cold press. In our study, the amount of oil obtained by hydrodistillation method was determined as 3.05% in the fresh peel at 120 minutes, while this rate remained at 0.93% in the cold press.

A measure of essential oil quality is properties such as refractive index, density, and optical activity. Besides the essential oil composition, these values may differ according to the non-volatile components in the chemical composition of the oil. The refractive index, optical activity, and density values of lemon peel oil obtained by hydrodistillation and cold pressing were determined by Ferhat et al. (2007). The results of the study showed that the refractive index, optical activity and density values of the sample obtained by cold pressing were higher than those obtained by hydrodistillation ($P<0.05$). Our findings differed from the literature in terms of optical activity value.

In our study, it was determined that the refractive index and density of the essential oil obtained by hydrodistillation were significantly lower than that obtained by cold pressing ($P<0.05$). The waxes and pigments contained in the orange peel are transferred to the oil during cold pressing. In the peel, which consists of a tissue rich in pigments (chlorophyll and carotenoids), sebaceous glands containing essential oil are unevenly distributed (Giacomo and Raymo, 2014). Ferreira et al. (2020) also reported that oils obtained by cold pressing contain components such as non-volatile carotenoids, and flavonoids. As a matter of fact, while the oil obtained by hydrodistillation was colorless, it had a yellow-orange color obtained by cold pressing. It is thought that these differences may be due to chemical composition differences. As a matter of fact, it was determined by Li et al. (2021) that non-volatile component groups such as coumarin and furanocoumarin were considerably higher in oils obtained by cold pressing compared to those obtained by hydrodistillation. In addition, these values could be changed by any adulteration. Dilution of the most valuable citrus oils with inexpensive sweet orange terpenes results in increased optical rotation (Bonaccorsi et al., 2014).

It was determined that there were significant differences in the amount of oil obtained, density, refractive index, and optical activity values between the essential oil obtained by hydrodistillation and the obtained by cold press ($P<0.05$). This difference shows that depending on the extraction method to be applied for sweet orange peel oil with different properties can be produced.

5. Conclusion

Essential oil compositions in raw plant materials can be significantly affected by factors such as the age of the plant, the used part, the harvest time, the type of harvest, and the drying method, especially the species and variety. Standardized limonene is an important issue in the essential oil industry. Limonene can be obtained from the peels of sweet oranges, which contain as high as 96% limonene. The standardized product can be used in many areas.

Within the scope of the research, the effect of distillation time on orange essential oil composition was investigated. Findings revealed that when distillation time is taken into account, a product with a different composition in terms of limonene was not obtained. It has been determined that there are significant differences in parameters such as density, refractive indices, optical activity, and color of the oils obtained by hydrodistillation and cold pressing, and it has been determined that different extraction methods can be selected according to the purpose of use.

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