Effects of Harvesting Time and Isolation Techniques on The Physicochemical Characteristics of Grapefruit (*Citrus paradisi,* Macfadyen), Bitter Orange (*Citrus aurantium,* Linnaeus) and Kumquat (*Fortunella margarita,* Swingle) Essential Oils

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Recieved: November 18, 2025
Accepted: January 7, 2026

The essential oil (EO) yield and physicochemical parameters of four grapefruit cultivars, a bitter orange, and a kumquat cultivar were evaluated in relation to harvest time and isolation technique. EO samples were obtained by hydrodistillation (HD) and cold pressing (CP). The chemical composition of the EOs was determined by gas chromatography-mass spectrometry (GC-MS/FID). EO yield across the samples ranged from 1.24% to 2.83%. The isolation method significantly influenced the relative density, optical rotation, and refractive index of the oils. The cultivar also had a statistically significant effect on refractive index and optical rotation. Oils obtained by CP had higher relative densities (0.8473-0.8524) compared to those obtained via HD (0.8385-0.8413). CP oils also exhibited higher refractive index values (1.4749-1.4765) than HD oils (1.4721-1.4733). In contrast, HD-isolated oils had higher optical rotation values (95.48-99.13°) than CP (91.55-94.50°). While some variation in EO composition was observed based on cultivar, harvesting time, and extraction method, these differences were mostly insignificant. Limonene was identified as the major component, ranging from 93.7% (Rio Red as grapefruit) to 96.0% (Nagami as kumquat). Cultivar and harvest time effects on limonene content were statistically significant, whereas the effects of the isolation method were not ($p>0.05$). β myrcene was the second main component, ranging from 1.9% to 2.1%. While harvest time and isolation method had a significant impact on its concentration, the cultivar effect was not important. The study demonstrated a small variation in the composition of these citrus essential oils. Harvest time appears to be a less critical factor for these specific properties.

Keywords: citrus essential oil, cultivar, harvest time, isolation method, limonene.

1. INTRODUCTION

World citrus production has shown significant increases in recent years. Production, which was 105,930,647 tons across 7,449,973 hectares of land in 2000, reached 169,388,810 tons across 10,554,846 hectares of land in 2023. China is the main citrus producing country (28%), followed by Brazil, Türkiye, Spain, and Egypt. Türkiye accounted for 7,877,982 tons of global production in 2023 [1]. A significant portion of citrus production consists of oranges, mandarins and lemons. Additionally, citrus such as grapefruit, sour orange and kumquat are also produced. Citrus are used both for fresh consumption and, significantly, in the food process. During the evaluation of citrus fruits in the food industry, representing approximately 50-65% of the fruit weight, waste is generated, and a significant portion of this is peel [2]. Notably, citrus waste is rich in several beneficial components, including flavonoids, carotenoids, dietary fiber, soluble sugars, and essential oils [3, 4].
Aromatic compounds known as essential oils are occurring in large quantities within oil sacs located at different depths in the flavedo part of the peel.

Citrus peel oils contain many bioactive components, the main one being limonene [5, 6], which has various functional properties [7]. Citrus oils are used in many areas such as pathogen inhibition, insect control, medicine, cosmetics and food because they are Generally Recognized as Safe (GRAS) [8, 9]. Consequently, citrus peel oils find applications in numerous sectors such as the food industry [10, 11], cosmetics, perfumery, pharmaceuticals, and the production of cleaning products [12,13,14]. A key characteristic of these oils is their high limonene content, which, in citrus essential oils from peels of orange, mandarin, bergamot, and bitter orange, can range from 36.54% to 96.10% [15]. While the primary components of essential oils largely determine their biological activities, minor components can also play a significant role through synergistic, antagonistic, or additive interactions [16,17]. Limonene (p-mentha-1,8-diene) itself is industrially important and used extensively in food, medicine, and cosmetics. Its aromatic properties and high absorption make it valuable in the pharmaceutical and cosmetic industries. Due to its aroma, limonene is also commonly used in the food industry and is GRAS-listed by the FDA [18, 19]. It is important to note that the essential oil composition of citrus peel oils can change by citrus type and cultivar, as well as the isolation method employed (cold press, hydrodistillation) [20, 21].
While most research on citrus essential oils concentrates on oranges, relatively few studies specifically examine grapefruit, bitter orange, and kumquat. In Türkiye, which possesses significant raw material potential, the production of essential oils from these and various citrus fruits holds importance for both the global and national economy. The purpose of this research was to reveal certain quality properties and volatile oil compositions of peel essential oils isolated from four grapefruit, a bitter orange and a kumquat cultivar using two different methods across four different harvest periods. Therefore, the compositional data of these oils, extracted through both cold-pressing and hydrodistillation, can offer valuable insights for researchers, producers, and the industry. Furthermore, evaluating citrus peels, which are currently considered waste, can contribute to the growth of both producers and the processing industry. It is important to note that the quality of volatile oil is affected by factors such as the citrus cultivars used, the harvest time of the material, and the isolation method employed.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

Citrus fruits used in the research were harvested from the experimental area of the Aksu-Central Unit of the Batı Akdeniz Agricultural Research Institute (Antalya, Türkiye), the Ministry of Agriculture and Forestry between 2021-2023 production seasons. Mature fruits for each cultivar of grapefruit (*Citrus paradise*; Red Blush, Rio Red, Star Ruby, Marsh Seedless), bitter orange (*Citrus aurantium*) and kumquat (*Fortunella margarita*) were harvested in four different harvest periods (Table I). Approximately 15-20 fruits were collected from four sides of every tree. Fruits were taken to the laboratory on the same day.
Upon arrival at the laboratory, the initial analysis focused on fruit weight and peel ratio. For each of the three repetitions, ten fruits were individually weighed, and their peels were also weighed separately, both with a precision of 0.01 g. The reported fruit fresh weight and peel/fruit ratio represent the average values calculated from these ten fruits within each repetition.

2.2. HYDRODISTILLATION (HD) AND COLD PRESS (CP) PROCESS

The essential oil in the samples was extracted using hydrodistillation with a Clevenger-type apparatus, following the ISO 6571 standard [22]. The resulting essential oil amount is expressed as a volume percentage in relation to the fresh fruit peel weight (mL/100 g, %). For the collection of essential oil via cold pressing, the method described by Bozova et al. [23] was followed. The grated citrus peel was squeezed manually with a kitchen-type apparatus. The water-oil extract was centrifuged (15294 × g at 20°C for 20 min) to obtain cold pressed oil. Essential oil yield was determined from 100 g of fresh peel (mL/100 g, %).

Table I - Grapefruit, bitter orange and kumquat cultivars and harvest times

Species	Cultivars	1th Harvest	2nd Harvest	3th Harvest	4th Harvest
Citrus paradisi	Red Blush	01 December	20 December	10 January	30 January
	Rio Red	01 December	20 December	10 January	30 January
	Star Ruby	10 November	30 November	20 December	10 January
	Marsh Seedless	01 December	20 December	10 January	30 January
Citrus aurantium	Yerli Turunç	20 January	10 February	02 March	20 March
Fortunella margarita	Nagami	20 December	10 January	30 J January	20 February

2.3. PHYSICOCHEMICAL PROPERTIES OF THE OIL SAMPLES

Physicochemical characteristics are crucial indicators of the quality and purity of essential oils. Relative density, optical rotation, and refractive index, all of which are fundamental attribute parameters outlined in the European Pharmacopoeia. Relative density was determined according to ISO 279 [24], while refractive index was determined at 20°C by a refractometer (A. Krüss Optronic, DR6000 model) following ISO 280 [25]. The optical rotation values were specifically determined for citrus peel essential oils by a polarimeter (Optical Activity Ltd., PolAAR 31 model) according to ISO 592 [26].

2.4. ESSENTIAL OIL COMPOSITION

Gas chromatography–mass spectrometry (GC-MS) analysis was performed with an Agilent 7890A system coupled with a mass spectrometer (Agilent 5975C) and a flame ionization detector (FID), following the methodology of Özek et al. [27]. For analysis, samples were diluted as 1:50 using hexane. Capillary column (HP Innowax, 60.0 m × 0.25 mm × 0.25 µm) was used in the analysis. Helium was used as the carrier gas (0.8 mL/min). The sample was injected as 1 µL with a split ratio of 50:1, and an injector temperature was adjusted to 250°C. The column temperature was kept at 60°C for 10 minutes, increased to 250°C at a rate of 20°C/minute, and kept at 250°C for 10.5 minutes, resulting in a total analysis time of 60 minutes. The mass detector operated with a scanning range of m/z 35-500 and electron bombardment ionization at 70 eV. The essential oil components were identified by comparing their mass spectra with those in the Wiley and OIL ADAMS GC/MS Libraries. Relative Retention Indices (RRI) were calculated by C8-C40 alkane series (Sigma, USA) as standard samples. The relative percentage of the separated compounds were detected by analyzing FID chromatograms.

2.5. STATISTICAL ANALYSES

The research employed a randomized parcel trial design with three replications [28]. The analyzes were repeated twice and the obtained data were tested according to variance analysis (ANOVA) and the Duncan Test by the SAS statistical program. Data are presented as mean±standard error (SE).

3. RESULTS AND DISCUSSION

The ANOVA and Duncan results for the fruit weight, peel ratio of the fruit, and peel essential oil content of the four grapefruit, a bitter orange and a kumquat cultivar were evaluated within the scope of the study given in Table II and Table III. The influence of the cultivar (C) on the fruit weight of the samples determined was significant, while the influence of harvest time (HT) and C×HT interaction was insignificant. The Marsh Seedless cultivar had the highest average fruit weight among the grapefruit cultivars examined within the scope of the research. Kumquat and bitter orange fruit weights are considerably lower than grapefruit cultivars (Table III). The average fruit weights of the citrus cultivars generally increased from the first harvest to the last harvest time except for Nagami (kumquat). The influence of the cultivar on the peel ratio was also important, while the impact of HT and C×HT was insignificant. It was observed that the peel ratios of Star Ruby were lower than other grapefruit cultivars. Bitter orange and kumquat had a higher peel ratio than grapefruit.
The variation in the essential oil yield of the samples according to the cultivar and harvest time were subjected to statistical evaluation based on the hydrodistillation method data. While the effect of the cultivar and C×HT interaction on the essential oil content of the samples was statistically important, the influence of the harvest time remained insignificant. Within the cultivars, the peel's essential oil ratios of Yerli Turunç as bitter orange was higher than grapefruit cultivars and Nagami as kumquat. Among the analyzed cultivars, the one with the lowest essential oil content was Marsh Seedless. When the effect of harvest time is examined on a cultivar basis, these differences can reach significant levels. For instance, in Star Ruby, the amount of essential oil, which was 1.80% at the beginning of the harvest, decreased by approximately 30% to 1.24% in the fourth harvest period. Fruit weights, peel quantities and essential oil content in the peel are taken into consideration, showing that it is more appropriate to harvest Mars Seedless and Red Blush in the fourth harvest period, Rio Red, Yerli Turunç and Nagami in the third harvesting period and Star Ruby in the first three harvest periods. Hydrodistillation yielded a considerably larger amount of essential oil, ranging

Table II - ANOVA results for physical properties of fruits and essential oil content

	Fruit weight		Peel ratio		Essential oil content	
	F value	p value	F value	p value	F value	p value
Cultivar (C)	149.05	0.0001	201.09	0.0001	7.44	0.0001
Harvest time (HT)	6.50	0.0022	0.83	0.4893	0.44	0.7230
C×HT	0.90	0.5748	1.36	0.2416	2.58	0.0066
Coefficient of variation	12.9351		6.7376		14.7538	

from 4.24 to 7.87 times the quantity obtained through cold pressing. Kaanin-Boudraa et al. [29] found the essential oil ratio to be 0.33% for grapefruit. Ghani et al. [30] determined the essential oil content of grapefruit to be between 0.14-0.43%, depending on harvesting time. In the research carried out by Miya et al. [31], the essential oil yields of three different cultivars were determined to be in the range of 0.21-0.34%. Farahmandfar et al. [32] determined the essential oil content for fresh bitter orange peel to be 1.0%. Sicari and Poiana [33] found the essential oil content of oval kumquat to be 0.95% using the hydrodistillation method. Lakache et al. [34] determined the essential oil ratio to be 0.30% using the hydrodistillation method for fresh oval kumquat. Our study findings differ from the literature findings, and it is thought that this is due to the difference in the materials studied.

The variance and the Duncan results for physical characteristics of grapefruit cultivars, bitter orange and kumquat based on harvesting times and extraction techniques are presented in Table IV, and Table V. Relative density, refractive index and optical rotation values of essential oils are of critical importance for industrial application, quality control, and chemotaxonomy. These physical and chemical properties provide fundamental information about an oil's purity, quality, and botanical identity. The relative density values of the citrus cultivars in relation to harvest times and isolation techniques ranged between 0.8385-0.8524. The variance analysis revealed that the isolation method was the only factor with a significant effect on the relative density of the volatile oils. Specifically, hydrodistillation yielded samples with lower relative density values compared to those obtained through the cold press method. Cold-pressed oils may contain less volatile, heavier molecules (such as waxes, coumarins, carotenoids, fatty acids) or a higher concentration of compounds that rotate light less, compared to distilled oils. This strongly indicates that the two methods yield different chemical profiles. In ISO standards, the

Table III - Duncan test results of physical properties of fruits according to cultivar and harvesting time (mean±SE)

C	HT	Fruit weight (g/fruit)	Peel ratio (%)	Essential oil yield (CP, %)	Essential oil yield (HD, %)	Essential oil (HD, mL/whole fruit)
Star Ruby	1	262.35±26.59	24.07±1.415	0.26±0.120	1.80±0.084	1.14
	2	268.95±27.98	22.65±0.120	0.23±0.090	1.81±0.155	1.10
	3	307.15±19.10	22.76±1.135	0.24±0.130	1.62±0.027	1.13
	4	334.09±9.94	24.22±0.160	0.26±0.130	1.24±0.038	1.00
	mean	**293.13^{b}±14.38**	**23.43^{d}±0.440**	**0.25±0.045**	**1.62^{bc}±0.080**	**1.09**
Marsh Seedless	1	355.89±52.12	27.16±1.215	0.23±0.070	1.45±0.127	1.40
	2	341.87±20.94	28.15±0.960	0.28±0.105	1.48±0.113	1.42
	3	331.57±35.18	29.62±1.815	0.22±0.065	1.45±0.079	1.42
	4	368.13±21.01	28.29±0.335	0.24±0.020	1.60±0.188	1.67
	mean	**349.37^{a}±40.00**	**28.30^{c}±0.563**	**0.24±0.028**	**1.50^{c}±0.060**	**1.48**
Red Blush	1	283.34±25.31	28.78±2.495	0.29±0.130	1.65±0.023	1.35
	2	268.38±14.22	25.39±0.445	0.28±0.155	1.32±0.193	0.90
	3	345.41±5.55	26.71±0.870	0.28±0.125	1.50±0.046	1.38
	4	370.90±14.48	27.19±0.225	0.29±0.100	1.84±0.058	1.86
	mean	**317.01^{b}±18.59**	**27.02^{c}±0.685**	**0.29±0.049**	**1.58^{bc}±0.073**	**1.37**
Rio Red	1	293.98±41.87	28.74±0.875	0.34±0.135	1.70±0.177	1.44
	2	280.22±18.55	29.36±1.465	0.37±0.145	1.86±0.074	1.53
	3	345.15±3.65	27.01±3.350	0.37±0.125	1.92±0.043	1.79
	4	342.34±4.49	26.39±2.095	0.38±0.155	1.61±0.063	1.45
	mean	**315.42^{b}±36.89**	**27.87^{c}±0.934**	**0.36±0.053**	**1.77^{b}±0.058**	**1.55**
Yerli Turunç	1	132.16±3.28	36.32±0.050	0.49±0.060	2.22±0.110	1.07
	2	124.40±10.93	42.89±0.275	0.46±0.040	1.98±0.238	1.06
	3	149.81±19.43	40.66±1.005	0.48±0.020	2.83±0.260	1.72
	4	164.05±7.33	40.28±2.010	0.47±0.000	2.07±0.211	1.37
	mean	**142.61^{c}±20.79**	**40.04^{b}±0.992**	**0.49±0.015**	**2.04^{a}±0.098**	**1.30**
Nagami	1	10.21±0.15	49.67±2.875	0.26±0.150	1.33±0.140	0.07
	2	12.09±0.18	53.83±1.215	0.25±0.145	1.32±0.193	0.09
	3	11.53±1.14	55.45±3.024	0.29±0.140	1.89±0.227	0.12
	4	9.84±1.24	53.89±0.435	0.26±0.090	1.88±0.125	0.10
	mean	**10.92^{d}±1.34**	**53.21^{a}±1.178**	**0.26±0.051**	**1.61^{bc}±0.114**	**0.09**

A **significant difference** ($p<0.05$) between cultivar means is denoted by different letters in the same column.

relative density value for grapefruit peel essential oil extracted by CP was stated as 0.852-0.860 [35], for bitter orange as 0.840-0.860 [36], and for kumquat as 0.842 [37]. The relative density value for grapefruit essential oil isolated by CP was determined as 0.85 by Viudo-Martos et al. [38]. In parallel, density value of the peel oil of Citrus paradise isolated using the cold press technique was 0.8573 g/mL [39]. Similarly, relative density values of grapefruit peel oil isolated by hydrodistillation and microwave extraction were 0.856, 0.862, respectively [40]. While the relative density values of samples extracted by CP aligned with limit values, those derived from hydrodistillation were generally lower than literature values. This difference is thought to stem from various factors including cultivar, harvest time, climate, and cultural process differences, in addition to the oils' chemical composition.

The refractive index for the samples varied from 1.4721 to 1.4766. For HD-obtained oils, the refractive index values across cultivars are very narrow (1.4723 - 1.4731), suggesting that these properties are relatively similar among the cultivars. The values across the four harvest times are generally very close to each other, often within the average error range. This indicates that harvest time (maturation periods) alone does not have a large and consistent effect on the physical properties examined. The isolation method significantly affected the refractive index value of the volatile oil. The refractive index value of CP oil was higher than that obtained by HD. This is likely to be on account of different non-volatile chemical components such as waxes [41], carotenoids [42], and coumrains [43] in the sample instead of EO composition. Indeed, the oils produced by these two methods exhibit observable sensory differences, such as color. According to ISO standards, cold-pressed grapefruit peel oil has a refractive index limit value of 1.474-1.479 [35]. Vasek et al. [39] determined the refractive index value of grapefruit peel essential oil to be 1.4723. Conversely, Kamal et al. [44] determined the refractive index of grapefruit peel essential oil obtained by hydrodistillation as 1.4627-1.4635, depending on drying methods. In another study, the refractive index values of red grapefruit and oval kumquat peel essential oils obtained by hydrodistillation were determined to be 1.463 and 1.475, respectively [37]. The research findings revealed that, in this regard, the analyzed cultivars were generally consistent with standard data but showed partial differences from literature values. This may be due to differences in the characteristics of the cultivar studied in the literature and the region where it is grown.

Each pure essential oil has a characteristic range of optical rotation. This quality could be used as a parameter in the authentication of essential oils. The effect of cultivar (C), isolation method (IM) and C×IM interaction on optical rotation values of these citrus volatile oils was found to be statistically significant. Yerli Turunç (bitter orange) had the highest optical rotation value with respect to the cultivar. On the other hand, like other physical parameters, difference in optical rotation according to isolation method was more obvious than other factors. HD essential oils consistently show significantly higher optical rotation values than CP oils for all cultivars and harvest times. The reference range specified in the ISO standards for grapefruit peel essential oil varies between +91° and +96° values [38], and for bitter orange peel essential oil, it varies between +88° and +95° values [35]. Vasek et al. [39] determined the optical rotation value for grapefruit peel essential oil isolated by the cold press method as +90.25°. While all the essential oil obtained from grapefruit and bitter orange cultivars by CP was compatible with these limit values, the data determined for HD essential oils obtained by HD were higher than these limit values. It is thought that this difference may be due to chemical composition (carotenoids, coumarins, waxes etc.), except for the essential oil component. In fact, literature values have also shown that there may be differences in this sense.

The essential oil composition variation of Star Ruby, Marsh Seedless, Red Blush, Rio Red (grapefruit), Yerli

Table IV - ANOVA results for relative density, refractive index and optical rotaion values

	Relative density		Refractive index		Optical rotation	
	F value	p value	F value	p value	F value	p value
Cultivar (C)	1.51	0.2044	6.41	0.0001	2.42	0.0490
Harvest time (HT)	0.92	0.4405	1.35	0.2702	0.06	0.9825
Isolation method (IM)	1109.08	0.0001	2139.44	0.0001	211.02	0.0001
C×HT	0.50	0.9312	0.68	0.7876	0.86	0.6111
C×IM	1.93	0.1065	19.18	0.0001	1.02	0.4148
HT×IM	1.46	0.2365	1.24	0.3055	0.48	0.7008
C×HT×IM	1.35	0.2119	0.55	0.8993	0.49	0.9361
Coefficient of variation	0.1623		0.0235		1.5186	

Table V - Mean values for relative density, refractive index and optical rotation values of peel volatile oils by cultivar, harvesting time and isolation method (±standard error)

C	HT	Relative density		Refractive index		Optical rotation (°)	
		HD	CP	HD	CP	HD	CP
Star Ruby	1	0.8408±0.0005	0.8487±0.0012	1.4723±0.0001	1.4757±0.0001	96.45±0.250	93.68±0.275
	2	0.8410±0.0005	0.8488±0.0013	1.4721±0.0003	1.4758±0.0001	97.88±0.075	92.60±0.300
	3	0.8404±0.0022	0.8504±0.0005	1.4724±0.0002	1.4762±0.0001	99.13±0.325	93.38±0.275
	4	0.8403±0.0008	0.8502±0.0010	1.4724±0.0001	1.4760±0.0000	96.28±0.225	92.30±0.100
	mean	**0.8406±0.0005**	**0.8495±0.0005**	**1.4723±0.0001**	**1.4759±0.0001**	**97.43±0.447**	**92.99±0.231**
Marsh Seedless	1	0.8397±0.0004	0.8524±0.0004	1.4725±0.0002	1.4760±0.0004	97.28±0.175	91.58±0.480
	2	0.8398±0.0015	0.8500±0.0008	1.4723±0.0001	1.4762±0.0000	96.38±1.075	92.33±1.375
	3	0.8390±0.0005	0.8499±0.0004	1.4728±0.0005	1.4759±0.0004	95.88±1.875	92.73±0.980
	4	0.8398±0.0005	0.8507±0.0003	1.4721±0.0002	1.4761±0.0002	96.88±1.475	93.43±0.125
	mean	**0.8396±0.0003**	**0.8507±0.0004**	**1.4724±0.0001**	**1.4760±0.0001**	**96.60±0.534**	**92.52±0.418**
Red Blush	1	0.8395±0.0005	0.8507±0.0007	1.4722±0.0001	1.4759±0.0003	97.95±0.550	91.88±0.525
	2	0.8413±0.0005	0.8487±0.0007	1.4724±0.0002	1.4760±0.0002	97.53±0.625	91.58±1.425
	3	0.8399±0.0014	0.8482±0.0021	1.4724±0.0002	1.4756±0.0003	96.38±1.775	91.73±0.475
	4	0.8404±0.0008	0.8473±0.0008	1.4723±0.0000	1.4764±0.0002	96.75±1.150	92.26±0.460
	mean	**0.8403±0.0004**	**0.8487±0.000**	**1.4723±0.0001**	**1.4759±0.0001**	**97.15±0.489**	**91.86±0.327**
Rio Red	1	0.8399±0.0002	0.8484±0.0010	1.4723±0.0002	1.4762±0.0003	96.83±1.025	92.03±0.880
	2	0.8407±0.0007	0.8488±0.0015	1.4724±0.0001	1.4765±0.0003	97.05±1.050	91.85±0.250
	3	0.8398±0.0015	0.8487±0.0024	1.4725±0.0004	1.4765±0.0003	96.73±1.325	91.55±0.050
	4	0.8385±0.0007	0.8508±0.0005	1.4724±0.0001	1.4766±0.0002	96.55±0.550	92.33±0.075
	mean	**0.8397±0.0004**	**0.8491±0.0007**	**1.4724±0.0001**	**1.4764±0.0001**	**96.79±0.394**	**91.94±0.204**
Yerli Turunç	1	0.8396±0.0001	0.8493±0.0013	1.4723±0.0001	1.4751±0.0000	98.13±1.425	93.98±2.075
	2	0.8385±0.0002	0.8495±0.0013	1.4722±0.0003	1.4750±0.0003	98.05±0.250	94.18±1.775
	3	0.8407±0.0003	0.8476±0.0005	1.4723±0.0002	1.4749±0.0004	98.20±0.800	94.35±1.750
	4	0.8394±0.0001	0.8475±0.0013	1.4727±0.0003	1.4755±0.0007	95.83±0.225	93.83±1.325
	mean	**0.8397±0.0002**	**0.8485±0.0006**	**1.4724±0.0001**	**1.4751±0.0001**	**97.55±0.491**	**94.08±0.666**
Nagami	1	0.8399±0.0014	0.8507±0.0005	1.4727±0.0005	1.4753±0.0002	96.83±0.575	92.48±0.975
	2	0.8409±0.0002	0.8485±0.0010	1.4733±0.0003	1.4751±0.0002	95.48±1.875	93.45±0.850
	3	0.8401±0.0007	0.8482±0.0004	1.4733±0.0002	1.4750±0.0002	96.98±0.375	93.33±1.125
	4	0.8390±0.0012	0.8488±0.0003	1.4731±0.0001	1.4751±0.0004	98.53±0.275	94.50±2.100
	mean	**0.8400±0.0004**	**0.8490±0.0004**	**1.4731±0.0001**	**1.4751±0.0001**	**96.95±0.559**	**93.44±0.580**

For each factor, different letters in the same column indicate a significant difference between the mean values (p<0.05).

C: Cultivar, HT: Harvest Time, HD: Hydrodistillation, CP: Cold-pressed

Turunç (bitter orange) and Nagami (kumquat) cultivars for three harvest times and two isolation methods are shown in Table VI.

Partial differences were examined in the essential oil composition of the Star Ruby, Marsh Seedless, Red Blush and Rio Red cultivars based on harvesting time and isolation technique. The amount of limonene varied between 93.7% and 95.1% in these grapefruit cultivars. These data indicate that these cultivars are a valuable source of limonene. β-myrcene ranked second proportionally (1.9-2.0%), followed by sabinene (0.3-0.9%) and α-pinene (0.5-0.6%) for these grapefruit cultivars (Table VI). Sabinene is a significant monoterpene hydrocarbon that is especially present in grapefruit cultivars. Among the samples evaluated in the scope of the research, Rio Red peel oil had the highest number of identified components, with 19 components. Citronellal, (E)-α-farnesene, and β-sinensal were detected only in Rio Red among the grapefruit cultivars.

Table VI - Essential oil composition (%) of the citrus cultivars.

Component	RRI	SR	MS	RB	RR	YT	N
α-pinene	1030	0.5-0.6	0.5-0.6	0.5-0.6	0.5-0.6	0.5-0.6	0.4-0.5
β-pinene	1122	-	-	-	-	0.1-0.3	-
Sabinene	1132	0.5-0.8	0.4-0.7	0.4-0.6	0.3-0.9	0.1-0.2	0.1-0.1
β-myrcene	1170	1.9-2.0	2.0-2.0	1.9-2.0	1.9-2.0	1.9-2.0	1.8-2.0
Limonene	1214	94.4-95.0	94.1-94.8	94.3-95.0	93.7-95.1	95.4-95.9	95.4-96.0
β-phellandrene	1223	0.3-0.3	0.3-0.3	0.3-0.3	0.3-0.3	0.3-0.3	0.2-0.3
γ-terpinene	1260	0.2-0.3	0.2-0.3	0.1-0.3	0.1-0.3	0.1-0.2	-
Octanal	1304	0.3-0.4	0.3-0.4	0.3-0.4	0.3-0.4	0.1-0.1	-
Citronellal	1481	-	-	-	0.1-0.1	-	0.1-0.1
α-copaene	1491	udl-0.1	0.1-0.2	0.1-0.2	0.1-0.1	-	-
Decanal	1501	0.1-0.3	0.3-0.3	0.1-0.2	0.3-0.3	udl-0.1	-
Linalool	1549	0.1-0.2	0.1-0.1	0.1-0.1	0.1-0.1	0.1-0.4	-
1-Octanol	1557	0.1-0.1	udl-0.1	udl-0.1	udl-0.2	-	-
Linalyl acetate	1561	-	0.1-0.3	udl-0.1	-	udl-0.5	-
β- caryophyllene	1596	0.3-0.4	0.1-0.3	0.2-0.4	0.1-0.2	-	-
(*E*)-α-farnesene	1663	-	-	-	0.1-0.2	-	-
α-terpineol	1709	udl-0.1	0.1-0.3	udl-0.2	udl-0.1	udl-0.1	-
β-bisabolene	1746	udl-0.1	0.1-0.1	0.1-0.1	0.1-0.1	-	0.9-1.5
Geranial	1748	udl-0.1	udl-0.1	-	0.1-0.1	-	-
δ-cadinene	1751	0.1-0.1	0.1-0.2	0.1-0.2	0.1-0.1	-	-
Geranyl acetate	1767	-	-	-	-	0.1-0.1	0.2-0.3
Citronellol	1776	-	-	-	-	-	0.1-0.2
Nerolidol	2047	-	-	-	-	udl-0.1	-
β-sinensal	2221	-	-	-	udl-0.2	-	-
Unidentified		0.1-0.4	0.1-0.4	0.1-0.6	0.0-0.5	0.2-0.5	0.0

SR: Star Ruby, MS: Marsh Seedless, RB: Red Blush, RR: Rio Red, YT: Yerli Turunç, N: Nagami

RRI: Relative Retention Index, udl: under detection limit

Fifteen compounds were identified in Yerli Turunç (*Citrus aurantium*) essential oil from the peel, also known as bitter orange. The limonene content, which is the main component in bitter orange peel essential oils, varied between 95.4% and 95.9% based on the harvest time and isolation method. As can be understood from the findings, no significant variation occurred in this regard. The β-myrcene content showed a variation between 1.9% and 2.0%. Among the samples evaluated in the scope of the research, Nagami (kumquat) peel essential oil had the lowest number of identified components. In total, nine different components were identified. This indicates that kumquat peel oil has a simpler chemical profile compared to the other samples. In these samples, limonene was again detected as the main component, with its concentration ranging from 95.1% to 96.0%. As in many other samples analyzed, β-myrcene proportionally followed limonene in these samples. The third-ranked component, unlike in many other species and cultivars, was β-bisabolene, and its amount varied between 0.9% and 1.5%, depending on the harvest time and extraction method. Nagami also contains components like citronellol, which are absent in the others. Partial differences were also observed in the percentages of other identified components depending on the harvest time and essential oil extraction method.

The ANOVA and Duncan Test results for the ratios of β-myrcene and limonene, which are found at higher levels than other components in the peel volatile oil composition of the samples, are presented in Table VII and Table VIII. β-myrcene content is significantly influenced by both the harvest time and isolation method, and, crucially, the effect of harvest time varies depending on the specific cultivar (Table VII). The lowest β-myrcene content was detected in the Yerli Turunç (bitter orange) peel oil during the first harvest period, whereas, conversely, it was found in the Nagami (kumquat) during the fourth harvest period. Among the grapefruit cultivars, the lowest β-myrcene content was found in the fourth harvest period for all cultivars except for Marsh Seedless. When evaluating the β-myrcene content based on isolation methods, it will be observed that essential oils obtained via HD (hydrodistillation) are higher than those obtained via CP (cold pressing). While the cultivar and harvesting time had a significant effect on the content of the main component, limonene, the influence of the isolation method and all interactions remained insignificant. The cultivars with the lowest limonene content were Rio Red (94.41%) and Marsh Seedless (94.42%). The average limonene content was determined to be 94.73% for Star Ruby and 94.76% for Red Blush. The limonene content of Yerli Turunç (bitter orange) and Nagami (kumquat) peel oils was higher compared to grapefruits. While not as strong as the cultivar effect, it implies that harvest time can influence limonene levels to some extent. The highest limonene content, based on average data, was observed in the second harvest period sample.

Miya et al. [31] studied the composition of three grapefruit peel volatile oils extracted by hydrodistillation. Their research findings revealed that the essential oil composition varied among the different cultivars. In all three cultivars, limonene was identified as the main component, distributed within a range of 86.70-89.90% in the samples. It was also found that myrcene, which was present in higher proportions compared to other components, varied between 2.43-3.57%. Kırbaşlar et al. [45] performed essential oil component analyses of fully maturated grapefruit (unknown cultivar, harvested in November) peel oils obtained by cold press. In the study, it was determined that the ratios of the main components limonene, myrcene and β-pinene were 92.5%, 2.6% and 0.6%, respectively. Ghani et al. [30] investigated the effect of harvesting time on the peel essential oil composition of grapefruit (Red Blush), obtained by hydrodistillation. Within the scope of the research, fruits were harvested at four different periods: September 26 (green stage), October 28, December 5, and February 3 (pinkish yellow stage). The study findings showed that the contents of limonene and myrcene varied between 90.92-93.82% and 1.22-1.88%, respectively, depending on the harvest time. Ou et al. [46] studied the effect of the isolation method on grapefruit peel volatile oil. Their research findings demonstrated that the isolation method significantly impacted the essential oil's composition. The essential oil obtained via hydrodistillation had limonene and myrcene contents of 96.06% and 2.06%, respectively. In contrast, the sample obtained by cold press contained 91.83% limonene and 3.06% myrcene, in the same order. Sutour et al. [47] studied on the composition of volatile oils obtained from five kumquat species by hydrodistillation methods. Limonene was detected as the main constituent in oval kumquat as 93.1%, followed by germacrene D (2.4%) and (1.4%) myrcene. Sicari and Poiana [33] obtained oval kumquat peel oil

Table VII - ANOVA results for β-myrcene and limonene

	β-myrcene		Limonene	
	F value	p value	F value	p value
Cultivar (C)	1.54	0.1490	25.93	0.0001
Harvest time (HT)	4.93	0.0046	2.89	0.0448
Isolation method (IM)	4.60	0.0371	0.66	0.4199
C×HT	4.01	0.0001	1.25	0.2731
C×IM	0.14	0.9833	0.85	0.5194
HT×IM	0.03	0.9927	0.36	0.7793
C×HT×IM	0.34	0.9875	0.28	0.9953
Coefficient of variation	1.9438		0.4599	

Table VIII - Mean percentages of β-myrcene and limonene contents (±SE)

	β-myrcene	Limonene
Star Ruby	1.95^{ab}±0.009	94.73^{b}±0.114
Marsh Seedless	1.97^{a}±0.005	94.42^{c}±0.104
Red Blush	1.96^{ab}±0.006	94.76^{b}±0.104
Rio Red	1.97^{a}±0.009	94.41^{c}±0.156
Yerli Turunç	1.94^{b}±0.007	95.62^{a}±0.0063
Nagami	1.96^{ab}±0.023	95.60^{a}±0.072
1^{th} HT	1.97^{a}±0.008	94.84^{b}±0.150
2^{nd} HT	1.96^{a}±0.006	95.11^{a}±0.095
3^{th} HT	1.97^{a}±0.009	94.97^{ab}±0.102
4^{th} HT	1.93^{b}±0.012	94.77^{b}±0.171
HD	1.97^{a}±0.006	94.96±0.105
CP	1.95^{b}±0.007	94.89±0.085

Different letters within the same column (for each factor) indicate a significant difference between means ($p<0.05$).

by hydrodistillation, solvent and supercritical carbon dioxide extraction methods. Limonene and β-myrcene were detected as the main components in the samples and it was determined that these components were distributed in the ranges of 96.08-96.91% and 1.63-1.77%, respectively. Another study by Peng et al. [48] studied the effect of distillation and cold press methods on the essential oil composition of kumquat peel. The study found that volatile oil isolated by steam distillation had limonene and myrcene content of 94.71% and 1.88%, respectively. In contrast, the sample obtained by cold press contained 95.06% limonene and 2.02% myrcene. The findings showed that these extraction methods showed similarities with regards to volatile oil composition. Farahmandfar et al. [32] also investigated the impact of drying treatments applied to the peel on the composition of bitter orange peel essential oil. Limonene was detected as the highest constituent in all citrus samples, followed by myrcene. The proportions of these components ranged from 81.19-88.7% and 3.07-3.81%, respectively. Bourgou et al. [49] also determined that there could be significant differences in bitter orange peel essential oil composition based on harvest time. In their study, limonene, the main component of the bitter orange peel volatile oil extracted by hydrodistillation, was determined to have a wide distribution, ranging from 67.90% to 90.95%. For the study, fruits were collected during three periods: pre-ripening (green), semi-ripe (yellow), and ripe (orange). The origin, climate, and extraction method are all key factors that can cause substantial variations in the compositions of volatile oils. This variability is also observed in oils extracted from variable cultivars of citrus fruits [50]. The limit values of limonene and β-myrcene in ISO standards were stated as 92-96% and 1.5-2.5%, respectively for CP grapefruit essential oil [35], and 93-95% and 1,5-3%, respectively for Mediterranean type CP bitter orange essential oil [36]. Evaluation of the limit data showed that the study's cultivars were incompatible with the established limit values.

4. CONCLUSIONS

As a result of the research, when the analysis findings obtained were evaluated, it was determined that the citrus peel essential oil ratio showed significant differences depending on the cultivar and C×HT interaction. The highest essential oil yield was found as 2.83% for bitter orange at the third harvest time. The cultivar contributes to variations in refractive index and optical rotation. The isolation method (HD vs. CP) has a consistent and significant impact on all three physicochemical properties. While CP oils have higher relative density and refractive index, HD oils have higher optical rotation. This is a crucial finding, indicating that the chosen isolation technique profoundly affects the physical characteristics of the volatile oil. Conversely, the cultivar, isolation method, and harvest time do not appear to significantly influence the relative density, refractive index, or optical rotation values. While the volatile oil composition showed slight variations based on cultivar, harvest time, and extraction method, these variations were generally statistically insignificant. For all samples analysed within the scope of the research, limonene was the dominant component, followed by ⍰-myrcene at an average proportion of 2%. The research findings indicated that essential oils with high limonene content (93.7-96.0%) could be produced from these citrus cultivars across four different harvest periods using two different isolation methods. Cold press application is a widely preferred method in citrus peel essential oil on an industrial scale. Furthermore, evaluating the two isolation methods, particularly concerning limonene, a key functional component of citrus oils, revealed that producing these essential oils via hydrodistillation did not negatively affect their limonene content. On the other hand, the existing standards for citrus peel oils were established for essential oils obtained by the cold-press method. However, in non-integrated facilities, essential oil production is also carried out from citrus peels using the hydrodistillation method. Consequently, the study findings have revealed the necessity of creating an international standard for citrus peel oils obtained through hydrodistillation, especially considering their physical properties.

Acknowledgments
This project is financially supported by the General Directorate of Agricultural Research and Policies [TAGEM], Republic of Türkiye, Ministry of Agriculture and Forestry. In this study, data from project number TAGEM/TBAD/B/21/A7/P6/2370 were used.

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