DOI: http://dx.doi.org/10.32571/ijct.1586145 E-ISSN: 2602-277X

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Effects of different rootstocks on the essential oil composition in the peel and leaf of rio red grapefruit

Musa TÜRKMEN[1*], Müge UYSAL KAMİLOĞLU[2], Durmuş Alpaslan KAYA[1], Celil TOPLU[2]

[1]*Field Crops Department, Faculty of Agriculture, Hatay Mustafa Kemal University, Antakya, Hatay 31000, Türkiye*
[2]*Horticulture Department, Faculty of Agriculture, Hatay Mustafa Kemal University, Antakya, Hatay 31000, Türkiye*

Received: 15 November, 2024; Accepted: 23 December 2024

*****Corresponding author e-mail: turkmenmusa@hotmail.com

Citation: Türkmen M.; Uysal Kamiloğlu M.; Kaya D.A.; Toplu C. *Int. J. Chem. Technol.* **2024**, 8 (2), 213-217.

ABSTRACT

The use of rootstocks in citrus production is an increasingly common in practice. The selection of the appropriate rootstock is important in many aspects such as the adaptation of the variety to environmental conditions, growth, fruit set, flowering, fruit yield and fruit quality. In the present study, essential oil yields and components determined in the peels and leaves of Rio Red grapefruit grafted onto 7 different citrus rootstocks were compared. While essential oil yields from the peels of rootstocks were identified ranged between 0.5% and 1.5%, measurements could not be made due to trace amounts in the leaves. The essential oil composition was characterized using GC-MS, with limonene being the main identified compound in all the peels with 93.39 and 95.44% in Carrizo citrange and Volkameriana, respectively. However, in the leaves of rootstocks, besides limonene, major compounds were changed. In the leaves of Brazilian sour orange, β-sinensal was detected at significantly higher ratio (33.53%), sabinene was found at high ratios in Smooth Flat Seville sour orange (27.62%).

Keywords: Rio Red Grapefruit, essential oil composition, citrus, rootstock.

1. INTRODUCTION

Citrus fruits are a plant community belonging to the Citrus genus, a member of the Aurantroideae Subfamily of the Rutaceae Family, which requires a subtropical climate and consists of varieties with high economic value such as orange, tangerine, lemon, grapefruit, bitter orange, kumquat, citron, shaddock and bergamot.[1] Global citrus fruit production areas and yields have shown continuous growth from 2017 to 2021.[2] Citrus fruit production has an important place in the world and in Turkiye. Approximately 70% of Turkiye's fresh fruit exports consist of citrus fruits. In recent years, there has been a significant increase in the exportation of citrus fruits, especially grapefruit.[3,4] Pink and red varieties of the grapefruit, one of the important citrus species, obtained as a result of natural and artificial mutations, have become an important fruit species with the increasing demand and appreciation of the consumer. 'Star Ruby' and 'Rio Red' are among the most produced colored grapefruit varieties in Turkiye.[4] Rio Red is a variety mutated from the Redblush variety and has a very attractive appearance in general. The fruit peel is thin and has many red areas. The fruit flesh is dark red.[5]

Rootstock plays an important role in citrus production for commercial marketing and industry. Optimum rootstock selection is crucial for the adaptation of the cultivar to the environmental conditions, growth, fruit setting, flowering, fruit yield, fruit quality, plant nutrient uptake, photosynthetic activity and economic life.[6,7] The citrus rootstock, which is widely used in Turkey, is a rootstock with superior qualities in terms of its general characteristics.[4] Citrus cultivation in Turkiye, Sour orange is widely used (95%) followed by Trifoliates such as Carrizo and Troyer citranges. In the Mediterranean region, which accounts for approximately 90% of total citrus production in Turkey, Sour orange is the commonly used rootstock with superior qualities in general terms. The effect of rootstocks on fruit quality has been widely discussed by many authors.[7] Because of the effects of various diseases and ecological factors, studies have been focused on a new alternative rootstock for citrus in Turkiye, as in other Mediterranean countries. In addition to the edible parts of citrus fruits, the peels of citrus fruits, which make up about 30-60% of the fruit weight[8,9] have been found to contain significant levels of essential oil, polysaccharides, sugars, and important phytochemicals with antioxidant properties beneficial for

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human health in previous studies.[8,10-17] However, there is still a need for research to economically and effectively convert these wastes into higher value products and use them in a suitable industrial field. In addition to this phenomenon, the leaves of many plants were commonly used in folk medicine, traditional medicine.[18-20] In this respect, it is important to determine and evaluate the content of the leaves as well as the peels. This study hypothesizes that the type of rootstock significantly affects the essential oil yield and composition in Rio Red grapefruit peels and leaves.

2. MATERIALS AND METHODS

2.1. Plant materials and growing conditions

The research was conducted at the Research Station of Mustafa Kemal University, Agricultural Faculty, Citrus Experimental Station, Dörtyol (36° -09' E; 36° -51' N; 9 m altitude). The trial area has sandy-loamy soil and a Mediterranean climate with hot, dry summers and mild, rainy winters. The 'Rio Red' grapefruit variety was grafted onto different citrus rootstocks 'local sour orange' (Citrus aurantium L.), 'Carrizo' and 'Troyer' citranges (Citrus sinensis Osb.×Poncirus trifoliata (L.) Raf), 'Smooth Flat Seville sour orange' (Citrus spp. hybrid of uncertain origin), 'Brazilian sour orange' (Citrus aurantiam L. var. 'Brasilian'), 'Volkameriana' (Citrus volkameriana Tan. and Pasq.) and 'Calamondin' (possibly Citrus reticulate var. austere×Fortunella hybrid, Swingle). The study was conducted on 13-year-old trees. Fruits were harvested at the end of December or at the beginning of January. Random samples of 25 fruits from each tree were collected for fruit peel essential oil analysis.

The content of essential oils in leaf samples taken from the middle of fruitless shoots on the main direction of the trees was examined. The fruits were hand-peeled and the materials (peels, leaves) were weighed fresh. The peels and leaves from each collection were processed the same day they were picked.

2.2. Isolation of the essential oil

A total of 100 g leaf and fruit peel samples were extracted by hydrodistillation with 1 L distilled water for 3 h using Neo-Clevenger apparatus. The oils were dried over anhydrous sodium sulfate and then stored in dark color (amber) glass bottles, at 4 °C ready for GC-MS analysis.[21]

2.3. GC-MS analysis of the essential oil

Analysis of the essential oil was carried out by using Thermo Scientific Focus Gas Chromatograph equipped with MS, auto sampler and TG-WAX MS-A (5% Phenyl Polysilphenylene-siloxane, 0.25 mm x 30 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250° C, MS Ionization source 319 temperature was 220° C, the injection port temperature was 220° C. The samples were injected with 250 split ratio. The injection volume was 1 µl. Oven temperature was programmed in the range of 50 ° C to 220 ° C at 3° C /min. The structure of each compound was identified by comparison with their mass spectrum (Wiley). The data were handled using Xcalibur software program.

3. RESULTS AND DISCUSSION

3.1. Essential Oil Yield

The essential oil yields from the peels of Rio Red grapefruit varieties grafted onto different rootstocks are shown in Table 1. The highest essential oil yield was found to be 1.5% in 'Smooth Flat Seville sour orange'. The essential oil yield of 'Troyer citrange' was determined to be 1.24%, followed by Volkameriana. The lowest essential oil yield were obtained from 'Carrizo citrange' (0.5%). Kademi and Garba[22] reported typical essential oil yield of 150 g citrus peels as 1.4% in their study. In addition, researchers pointed out the essential oil yield of different citrus varieties ranged from 0.2% to 1.07%. Another study showed the essential oil content in the range of 0.5–5.0% (w/v) in different citrus species.[23] Therefore, the results obtained in the present study were found to be compatible with these results.

Table 1. Essential oil yields from the peels of the grafted citrus rootstocks

Citrus Rootstocks	Carrizo citrange	Smooth Flat Seville Sour Orange	*Troyer citrange*	Sour orange (31-31)	Brazilian sour orange	Calamondin	Volkameriana
Essential oil yield %)	0.5	1.5	1.24	1.15	1.0	1.0	1.20

Essential oils obtained from the leaves of 'Rio Red' grapefruit varieties grafted onto the rootstocks were extracted with cyclohexane, but measurements could not be made due to trace amounts.

3.2. Essential oil components

Totally 56 essential oil components were determined in the peels and leaves of Rio Red grapefruit varieties grafted onto 7 rootstock citrus (Table 2). It was seen that peel and leaf essential oil components differ from each other. It was observed that the components in the essential oils of peels were quite different than leaves in terms of composition and numbers.

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Table 2. Essential oil components obtained from fruit peels and leaves of the grafted citrus rootstocks

	Carrizo citrange		'Smooth Flat Seville sour orange'		Troyer citrange		Sour orange (31-31)		'Brazilian sour orange'		Calamondin		Volkameriana	
Compound Name	**P**	**L**	**P**	**L**	**P**	**L**	**P**	**L**	**P**	**L**	**P**	**L**	**P**	**L**
α-Pinene	0.27	0.21	0.38	1.15	0.35	-	0.32	-	0.34	-	0.34	0.48	0.31	0.35
Cyclohexane	-	-	0.36	0.21	-	-		-	0.10	-	-	-	-	-
β-Pinene	-	0.41	0.12	2.16	0.10	-	0.10	-	-	-	-	0.68	-	0.62
Sabinene	0.90	12.21	1.30	27.62	1.25	1.00	1.05	0.17	1.14	-	0.99	16.09	0.66	15.66
Myrcene	0.83	1.15	0.93	2.67	0.90	0.21	0.88	0.47	0.91	0.31	0.91	1.27	0.85	1.03
α-Terpinene	-	0.27	-	0.72	-	-	-	0.13		-	-	0.19	-	0.22
Limonene	93.39	15.44	94.06	20.48	94.77	2.73	94.42	6.90	94.34	9.05	94.69	48.26	95.44	5.97
β-Phellandrene	0.24	0.31	0.19	0.61	1.25	-	0.21	3.09	0.19	1.76	0.20	0.39	0.19	0.30
cis-Ocimene	-	11.48	-	0.43	-	-	-	7.09		-	-	0.25		10.0
Ocimene	-	-	-	-	-	-	-	-	0.24	1.48	-	-	0.21	-
γ-Terpinene	-	0.86	-	1.36	-	0.33	-	0.57	-	0.24	-	0.63	-	0.67
3-Carene	-	-	-	12.04	-	-	-	-	-	-	-	-	-	-
α-Ocimene	0.28	-	0.27	-	0.26	-	0.25	-	-	-	-	-	-	-
β-Ocimene	-	-	-	-	-	2.82	-	-	-	-	-	-	-	-
Terpinolene	-	0.23	-	0.31	-	-	-	0.18	-	-	-	0.17	-	0.19
cis-Sabinene hydrate	-	0.15	-	-	-	0.31	-	0.23	-	-	-	-	-	-
trans Sabinene hydrate	-	0.35	-	0.10	-	0.39	-	0.32	-	0.25	-	-	-	0.16
Octanal	0.33	-	0.18	-	0.18	-	0.25	-	0.32	-	0.17	-	0.22	-
cis-Linalool Oxide	-	-	-	-	0.10	-	-	-	0.13	-	0.12	-	0.11	-
Linalool Oxide	0.15	-	-	-	-	-	0.12	-	-	-	-	-	-	-
Citronellal	-	7.74	-	3.92	-	3.76		8.36	-	0.44		0.53	-	5.48
Decanal	0.12	0.11	0.10	0.09	-	0.28	0.11	0.15	0.11	-	-	0.22	0.10	-
Camphor	0.14	-	0.12	-	-	-	0.11	-	-	-	-	-	-	-
Linalool	0.29	6.34	0.13	7.96	0.14	13.27	0.17	15.14	0.20	1.10	0.16	4.06	0.15	11.65
1-Terpineol	-	-	-	-	-	0.30	-	0.21	-	-	-	-	-	-
trans-Caryophyllene	0.16	4.95	0.11	0.52	0.12	2.83	0.12	3.51	0.12	0.84	0.15	0.16	0.12	1.07
α-Terpineol	0.29	0.62	0.14	0.38	0.13	1.47	0.21	1.32	0.21	0.47	0.14	0.31	0.16	0.86
Terpinen-4-ol	0.10	2.52	-	1.90	-	5.07	-	3.23	-	-	-	1.33	-	2.57
α-Humulene	-	0.99	-	0.17	-	1.30	-	-	-	-	-	0.30	-	0.59
Citronellyl acetate	-	0.36	-	0.79	-	0.58	-	0.26	-	-	-	0.13	-	1.00
β-Farnesene	-	0.14	-	0.10	-	0.43	-	0.18	-	-	-	0.27	-	0.21
Z-Citral	0.13	4.64	-	1.34	-	4.50	-	7.67	-	-	-	0.30	-	4.52
Farnesol	-		-	0.18	-	0.35	-	1.32	-	0.63	-	-	-	0.49
Elixene	-	0.56	-	-	-	1.01	-	0.53	-	-	-	0.20	-	-
Neryl acetate	-	1.36	-	1.14	-	1.26	-	0.99	-	-	-	-	-	3.25
E-Citral	0.14	7.76	-	2.27	-	8.43	-	13.02	-	-	-	-	-	-
Germacrene A	-	5.25	-	-	-	8.24	-	-	-	0.89	-	1.87	-	3.28
Geranyl acetate	-	0.73	-	1.05	-	0.87	-	0.66	-	-	-	-	-	1.38
Citronellol	-	1.20	-	0.63	-	1.37	-	2.15	-	-	-	0.53	-	1.85
Nerol	-	0.67	-	0.24	-	0.67	-	1.43	-	-	-	-	-	1.04
Geraniol	-	0.50	-	0.24	-	0.68	-	1.05	-	-	-	0.11	-	0.72
Humuladienone	-	0.18	-	-	-	0.26	-	0.17	-	0.50	-	-	-	0.19
cis-Caryophyllene	-	-	-	-	-	0.35	-	0.15	-	0.43	-	0.10	-	-
Nerolidol	-	0.19	-	0.13	-	0.60	-	0.28	-	1.26	-	0.20	-	0.31
Spathulenol	-	0.61	-	0.32	-	1.54	-	0.58	-	-	-	0.64	-	0.69
Isospathulenol	-	-	-	-	-		-	-	-	2.85	-	-	-	-
β-Elemene	-	0.25	-	0.11	-	0.28	-	5.26	-	3.5	-	1.23	-	0.98
Viridiflorene	0.19	0.19	-	0.12	0.11	0.55	-	-	-	0.32	-	0.16	-	0.25
4-Vinylguaiacol	-	-	-	0.11	-		-	-	-	-	-	-		7.12
β-Sinensal	-	4.20	-	3.13	-	18.74	-	7.43	-	33.53	0.14	4.80	-	1.33
Caryophyllene oxide	-	0.29	-	0.47	-	2.72	-	1.31	-	4.23	-	0.42	-	0.47
Aromadendrenepoxide	-	0.34	-	0.26	-	0.57	-	-	-	1.97	-	0.13	-	-
Elemol	-	-	-		-	0.31	-	0.13	-	0.54	-	0.24	-	0.33
Junipene	-	0.24	0.10	0.09	-	1.22	-	0.49	-	1.12	-	-	-	-
γ-Costol	-	-	-		-	-	-	-	-	1.12	-	1.99	-	2.80
Phytol	-	1.90	-	0.58	-	3.58	-	2.62	-	10.73	-	-	-	2.80

*P: Peel, L: Leaf, ‘-‘: not detected

When the compounds obtained from the peels were examined, limonene was detected as a major component. The highest ratio (95.44%) of limonene was found at Volkameriana, the lowest ratio (93.39%) was determined for Carrizo citrange. Similar to the present study, limonene was found also main component in the previous studies in which essential oils of different citrus peels were examined.[24-27] Vieira et al.[28] reported that limonene is used in food, cosmetic and medicinal industries. Table 2 showed that the ratio of most essential oil components determined in the peels remained below 1% except limonene.

As in peels, limonene was also found as one of the main component in the leaves of ‘Rio Red’ grapefruit varieties grafted onto the rootstocks. The ratios of limonene determined in the leaves varied; the highest ratio (48.26%) was determined in Calamondin and the lowest (2.73%) in Troyer citrange (Table 2). In the leaves of Brazilian sour orange, β-sinensal was detected at significantly higher ratio (33.53%). β-Sinensal was also determined in the leaves of Troyer citrange at a high ratio (18.74%). Sharon-Asa et al.[29] was also found β-sinensal as a major compound in their studies about citrus flavor. As presented in Table 2, sabinene was found at high ratios in Smooth Flat Seville sour orange (27.62%), Calamondin (16.09%), Volkameriana (15.66) and

DOI: http://dx.doi.org/10.32571/ijct.1586145 E-ISSN: 2602-277X

Carrizo citrange (12.21%), respectively. In agreement with the present study, Matuka et al.[30] found sabinene as the main compound in the leaf of *Citrus sinensis* L. It was stated that sabinene a bicyclic unsaturated monoterpene is used in cosmetic, flavor and medicinal industries because of pleasant odor and antimicrobial and antiflammatory effects.[31] cis-Ocimene was found at highest ratio (11.48%) in Carrizo citrange followed by Volkameriana (10.00%) and Sour orange (7.09%). According to Table 2, 3-carene was detected only in the essential oil of Smooth Flat Seville sour orange leaves at a ratio of 12.04%. While citronellal was detected at high ratios in Sour orange (8.36%), Carrizo citrange (7.74%), Volkameriana (5.48%), Smooth Flat Seville sour orange (3.92%) and Troyer citrange (3.76%), lower in Calamondin (0.53%) and Brazilian sour orange (0.44%). Unlike the peels, linalool was found to be clearly higher in the leaves. It was detected in Sour orange, Troyer citrange, Volkameriana, Smooth Flat Seville sour orange, Carrizo citrange, Calamondin and Brazilian sour orange at a ratio of 15.14%, 13.27%, 11.65%, 7.96%, 6.34%, 4.06% and 1.10%, respectively (Table 2). Trans-Caryophyllene was identified at highest ratio (4.95%) in Carrizo citrange followed by Sour orange (3.51%), Troyer citrange (2.83%) and Volkameriana (1.07%). It was below 1% in the leaves of other varieties. Citral known as main component of citrus fruit's oil was detected in the present study cis (Z-citral) and trans (E-citral) forms. Z-Citral and E-citral both was identified at highest ratios in Sour orange (7.67 and 13.02%, respectively). As shown in Table 2, they were not detected in Brazilian sour orange. Citral was detected at higher ratios in leaves than peels. Bhuiyan et al.[32] examined the essential oil of *Citrus Medica* L. peel and leaf was reported that citral composition is higher in the peel than leaf and it was determined as one of the major components in the peel. While germacrene A was detected highest (8.24%) in the leaves of Troyer citrange, β-elemene was in Sour orange (5.26%). Phytol was determined significantly higher in the leaves of Brazilian sour orange at a ratio of 10.73% than other varieties. It was reported that phytol was the major compound in *Citrus unshiu* and it was used as anticarcinogenic and antiproliferative agent.[33]

4. CONCLUSION

In this study, essential oil yields and components identified in the peels and leaves of Rio Red grapefruit grafted onto 7 different citrus rootstocks were compared. The results showed that the compounds detected in the leaves were higher in number and diversity than in the peels. While limonene was determined as a main component in all the peels, the main components identified in the leaves varied. In the peels, the ratio of most essential oil components remained below 1% except limonene. On the other hand, various components were detected at high ratios in the leaves. These components, individually or in combination, could potentially enable...Rio Red grapefruit grafted onto citrus rootstocks peels and leaves to be used in medicinal treatments as well as food and cosmetic industries. In addition, the present study provides the opportunity to make production in accordance with the purpose by guiding Rio red grapefruit producers on the basis of variety.

Conflict of Interest

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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