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Phytochemical Studies of Citrus unshiu Peels from Fort Beaufort Farm in the Eastern Cape Province, South Africa

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Abstract

Citrus unshiu, commonly called Satsumas and locally known as Naartjies in South Africa, is a medium-sized, mostly seedless citrus fruit. Due to Satsuma's easy peeling and sweet taste, its byproducts are often discarded indiscriminately, posing serious environmental hazards. However, aromatic plants, including citrus byproducts, have become valuable raw materials due to their phytochemical content, including essential oil compounds and other secondary metabolites. Thus, the peel of three varieties of *C. unshiu*cultivated mainly for export purposes, Satsuma owari (SO), Satsuma mihowase (SM), and Satsuma okitsu (Sok), were investigated for their essential oils (EOs) and other phytochemicals, with a view to identifying their chemical constituents. Extraction of the EOs was performed by hydro-distillation using a Clevenger apparatus, and the EOs were analyzed by gas chromatography-mass spectrometry (GC-MS). The plant parts were also subjected to phytochemical screening and quantitative determinations using standard procedures.

The phytochemical analysis results showed that both the SM and Sok peels contained high amounts of flavonoids, with 18.49% and 16.07%, respectively, while SM demonstrated high levels of terpenoids and alkaloids, at 11.0% and 3.91%, respectively. All the peel samples were found to be rich in terpenoids, with limonene dominating the oil with amounts ranging from 74.0% to 79.6%, with SO proving to be the richest of all the peels' essential oils. These results demonstrate that satsuma varieties' peel waste can be used as a source of bioactive compounds for different medical and pharmaceutical applications.

Introduction

Citrus fruit, popular for its tasty juice, shelf price, flavors, aromas, and most importantly, its nutrients (Zou et al., 2016), is grown in different parts of the world, including South Africa, mainly cultivated for export purposes. South Africa is amongst the leading citrus producers and exporters in the world, with over 97000 hectares of land dedicated to the cultivation of various citrus cultivars, including oranges, soft citrus, grapefruit, lemons, and limes (Nkomfe, 2026). Substantial amounts of citrus fruits are used in the food processing industry, whilst their oils are widely used in pharmaceuticals and cosmetics for their aromas (Olmedo-Galarza et al., 2025). Satsuma mandarin (*Citrus unshiu*) peel extract has been used as a reducing and capping agent in the biogenic synthesis of silver nanoparticles in South Korea (Basavegowda & Lee, 2013). Citrus fruits are also known to be a rich source of phenolic compounds, flavonoids, vitamins, minerals, dietary fibres, essential oils, and carotenoids (Saini et al., 2022).

Citrus unshiu, commonly called Satsuma mandarin, is mainly produced in Argentina, Australia, China, Peru, Spain, South Africa, and Uruguay. It is a hybrid of mandarin, pummelo, and sweet orange (Nicolosi et al., 2000). One of the distinguishing features of the satsuma, locally known as Naartjies in South Africa, is the thin, leathery skin dotted with large, prominent oil glands that are loosely attached to the fruit, which accounts for its easy peeling compared to other citrus fruits. The most popular Satsuma variety that are exported in South Africa includes mihowase and Owari, whilst varieties such as Kuno, Miyagawa, Okitsu, Sugiyama unshiu, and Miyagawa Wase are also grown (Glenneis, 2026). Reports have

it that approximately 18% of South African 'Satsuma' mandarins produced in the Eastern and Western Cape provinces are discarded due to climatic damage on the peel, thereby making them unable to meet the strict export standards (Duvenage et al., 2004). Also, due to the rapid growth of the food processing industry, peels are discarded as waste, posing a hazardous threat to the environment.

The peel essential oil quantitative analysis sampled from China using GC/MS yielded monoterpenes with significant amounts of limonene (67.44%), β-myrcene (7.15%), 3-carene (4.4%), α-pinene (2.52%), p-cymene (2.43%), β-pinene (1.46%), sabinene (0.77%), terpinolene (0.47%) and α-thujene (0.45%). The identified oxygenated monoterpenes were found to be 6.33%, whilst the sesquiterpene amounted to be 3.43% (Lt et al., 2008). (Elmacı & Altuğ, 2005) studied the components found in Satsuma (*Citrus unshiu*), along with Bodrum (*Citrus deliciosa*) and Clementine (*Citrus reticulata*) from Turkey. The headspace extraction technique was used, followed by GC-MS analysis, to determine chemical constituents in these fruit oils. The major components detected in the essential oil of satsuma samples were limonene (80.88%), γ-terpinene (7.24%), and myrcene (3.51%), accounting for over 90% of the total oil, with the highest amount of limonene and myrcene found in Satsuma (Qiao et al., 2007) investigated the aroma components in the oil of Guoqing 1, Miyagawa Wase, and Owari growing in China, from three different Satsuma mandarin varieties. Headspace solid phase microextraction (HS-SPME) combined with GC-MS was used for this analysis. The major components in the order of abundance in all the fruits were limonene, linalool and g-terpinene, constituting more than 91% of the components of the oil. The Mihowase variety was found to have the most of these compounds, with limonene at 83.57%, followed by the Owari variety, which contained 80.99% limonene.

In another study in Uruguay, (Verzera et al., 2000) investigated satsuma mandarin oils from its peels for their chemical composition using High-Resolution Gas Chromatography coupled with Mass Spectrometry (HRGC/MS). The major components quantified were limonene (90.98%), γ-terpinene (3.34%), and myrcene (2.01%), accounting for over 95% of the total oil components. A comparison between Satsuma Owari and newly developed clones was made by (Ozkaya et al., 2018) using gas chromatography coupled with mass spectrometry and flame ionization detection (GC/MS/FID) in a quest to develop new, good-quality flavor hybrids that could be cultivated in Turkey. Owari proved to be richer in flavors compared to the new clones, with 90.01% d-limonene, 4.12% γ-terpinene, and 0.91% β-elemene, accounting for over 95% of the total constituents of the essential oil.

Additional studies conducted by various researchers investigated secondary metabolites in citrus. These include, but are not limited to, carbohydrates, alkaloids, tannins (Anagnostopoulou et al., 2006), fixed oils and lipids, reducing sugars, proteins and amino acids, terpenoids and steroids, flavonoids (Kim et al., 2011), alkaloids and terpenoids (Ghongade, 2013). The difference observed in the quality and quantities of chemical components of the same species might be associated with climatic variation, the geographical location, and fruit positional differences in a tree, as this determines the individual fruit’s exposure to light and temperature, to name a few (Verreynne et al., 2004).

It is clear that, except for South African species, researchers worldwide have conducted extensive studies on Citrus unshiu in various countries to determine its therapeutic properties. It is thus crucial to profile the citrus grown in South Africa for therapeutic uses and reduce environmental emissions from discarded citrus peel and leaves.

Experimental

Sample collection: Fruits of three Satsuma mandarin cultivars, Satsuma Owari (SO), Satsuma Mihowase (SM), and Satsuma Okitsu (Sok) were collected from Fort Beaufort farm in the Eastern Cape Province, South Africa. Sampling times at the farms ranged from 8:00 in the morning to 17:00 in the afternoon on a single day. They were placed in bags and delivered to iYunivesithi Walter Sisulu's natural products lab, where they were washed and peeled. For the extraction of fresh and air-dried plant peels, the samples were split into two halves. The peels were air-dried at room temperature for dry extraction until a constant weight was reached, with a moisture content of less than 10%. (Nyalambisa et al., 2017).

Essential oil extraction

Essential oils from fresh and dried satsuma mandarin peels were extracted by hydro-distillation using the Clevenger apparatus, according to (Miya et al., 2021). About 500 g of fresh satsuma mandarin peels were introduced into a round-bottomed flask, and distilled water was added to a level just above the citrus material. This was followed by placing the flask on the heating mantle set at 100 and was connected to a condenser (Fig. 1). At the start of boiling, the temperature was lowered to 70, and boiling was allowed to continue for 4 hrs. The EOs were stored in a refrigerator until further analysis.

Phytochemical screening of peels

Preparation of the plant material for phytochemical screening

The fruit samples were carefully peeled and then sliced into tiny pieces. The peels were ground into small pieces after being dried for two weeks at room temperature. They were measured and then placed into two separate 1-L conical flasks for fresh and dried samples, respectively.

After soaking the peels and leaf samples in flasks for 2 days, aqueous (Aq) and ethanolic (EtOH) extracts were produced. The flasks were covered to prevent evaporation, then shaken for another 48 hours. The extracts were then filtered, and their fraction was used for phytochemical screening, while the remainder was evaporated to make the crude extract.

Qualitative Phytochemical screening of Aqueous and Ethanolic extracts

The qualitative and quantitative analyses were carried out on the peels for the presence of tannins, flavonoids, steroids, terpenoids, saponins, alkaloids, and glycosides, using standard methods as described by (Miya et al., 2021).

Quantitative phytochemical screening

Saponins, tannins, flavonoids, and alkaloids were quantified using the standard methods described by (Saibu et al., 2021).

Essential oil analysis: The essential oil was analyzed using a Bruker 450 GC equipped with a 300-triple quadrupole mass spectrometer and Bruker Workstation 7 software. A HP-5 MS fused silica capillary column (30m x 0.25mm, 0.25µm film thickness) was used with a 5% phenylmethylsiloxane stationary phase. The column temperature was programmed from 50°C to 310°C at 5°C/min, with a final hold at 450°C for 66.67 minutes. Helium was the carrier gas (1 mL/min), with a split ratio of 100:1. Scanning occurred over 78 minutes (35–450 amu). One microliter of diluted oil (in hexane) was injected for analysis. Identification of constituents was based on retention indices (Kovat Index) compared to literature values and authentic samples. (Adams, 1989); (Moss, 1999). Retention indices were calculated by comparing the sample to a standard series of alkanes (C8-C30) analyzed under identical conditions.

Results and Discussion

Qualitative Phytochemical screening of peels of Satsuma Mihowase, Satsuma Owari and Satsuma Okitsu

Qualitative phytochemical screening of the *peels of Citrus unshiu varieties'* solvent extracts showed the presence of tannins, saponins, flavonoids, steroids, terpenoids, alkaloids, glycosides, phenolics, and proteins in both aqueous and ethanolic extracts, except proteins, which were not detected in the ethanolic extract due to their polar nature.

Table 1 outlined the results of the qualitative phytochemical analysis of the peel extracts of Satsuma varieties. A very strong presence of flavonoids was observed in both peel samples, with higher levels in ethanolic extracts than in aqueous media. This is attributed to the fact that extraction efficiency is directly proportional to solvent polarity, with ethanol showing higher concentrations of extracted flavonoids than water, indicating the polarity of these compounds (Chaves et al., 2020). Tannins were most abundant in the satsuma mihowase variety and were observed to generally extract more ethanol owing to their polar structure characteristic of these compounds (Molnar et al., 2024).

Table 1
Phytochemical constituents of Satsuma Mihowase and Satsuma Owari

Phytochemicals	Aqueous extracts		Ethanolic extracts	
	Satsuma Mihowase peel	Satsuma owari peel	Satsuma Mihowase peel	Satsuma owari peel
Tannins	+	-	++	+
Saponins	-	+	-	-
Flavonoids	++	++	+++	+++
Steroids	-	-	-	-
Terpernoids	+	++	+++	++
Alkaloids	-	+	-	-
Glycosides	+++	+++	+	++
Phenolics	-	+	+	++
Proteins	-	-	-	-
(-) = absence; (+) = fair presence; (++) = strong presence; (+++) = very strong presence				

Plant phenolics are naturally occurring compounds synthesised by food plants and other living organisms. They are mainly characterised by their aromatic ring that contains one or more hydroxyl groups attached. They have diverse molecular structures, ranging from a simple phenol with one aromatic ring and one hydroxyl group to macromolecules such as tannins and lignins (Piironen & Salmenkallio-Marttila, 2009). Other phenol groups include quinones, xanthones, coumarins, anthraquinones, naphthoquinones, and flavonoids. They can act as polymers (lignin), aroma compounds (flavonoids and carotenoids), UV screens (flavonoids), and defense response chemicals (tannins) (Piironen & Salmenkallio-Marttila, 2009). In addition, their role in animals includes anti-aging (Kazeem et al., 2024), anti-inflammatory (Działo et al., 2016), antioxidant (Działo et al., 2016), and anti-proliferative activities (Rahman et al., 2021), which have been well documented.

Flavonoids form the largest group of phenolics with the basic C6-C3-C6 structural skeleton, consisting of two benzene rings separated by a propane unit (D'Amelia et al., 2018). These have health benefits, such as antioxidant (Kumar, 2013), anticancer (Terahara, 2015), and anti-allergic (Tapas et al., 2008) properties, amongst others.

Quantities of some phytochemicals found in the peel extracts of satsuma varieties

SO, SM, and SOk peel contained 14.3% w/w, 11.6% w/w, and 10.5% w/w saponins, respectively. It was also observed that both SO and SM were rich in saponins, suggesting that these plants are promising candidates for wound healing. Saponins are known to exhibit antimicrobial activity, helping prevent wound infections, as well as anti-inflammatory effects that regulate the healing process. Furthermore, they promote fibroblast proliferation, collagen synthesis, and angiogenesis, all of which are essential for tissue repair. The relatively higher saponin content observed in the peel extracts, particularly in SO and SOk, suggests a potentially greater wound-healing efficacy for these samples. Saponins were found to stimulate wound resurfacing with a new epithelial layer and effectively reduce inflammatory reactions during the early phase of the wound healing process (Moses et al., 2014).

The flavonoid content of SO, SM, and SOk peel ranged from 14% w/w to 19% w/w, with the SO peel having its highest content, while SOk had elevated alkaloid quantities compared to SM. Saponin and alkaloid levels were higher in the satsuma owari variety in this study than in the other cultivars investigated. The observed differences could be due to variations in secondary biosynthesis of metabolites that normally lead to differences in citrus unshiu phytochemical profiles (Jeong et al., 2024).

Table 2
Quantity of some phytochemicals found in the peel extracts of Satsuma owari and Satsuma mihowase

Phytochemicals	**Saponins**	**Flavonoids**	**Tannins**	**Terpenoids**	**Alkaloids**
%w/w					
Citrus sample					
Satsuma Owari peel	14.30	18.49	2.76	9.10	4.82
Satsuma Mihowase peel	11.55	14.50	1.04	11.0	3.91
Satsuma Okitsu peel	12.56	16.07	1.29	7.31	3.63

Furthermore, alkaloids and tannins were found at fairly high concentrations in the SOk peel samples, with higher flavonoid levels than the mihowase variety, although still lower than those of the Satsuma owari variety. (Qaderi et al., 2023) lists altitude, rainfall, climate, and other environmental conditions as influences of plant growth, and thus, the quality of secondary metabolites synthesized by plants results in major variations in the bioactive compounds present.

Results of Essential oil analysis of the three satsuma varieties

The essential oils from satsuma varieties (SM, SO, SOk) were diluted with hexane and analyzed by GC/GC-MS. Chemical analysis results of the fresh and dried Satsuma peel essential oils and the results of fresh and dried Satsuma owari peel samples' essential oils with concentrations ≥ 0.05% are shown in Table 3 below.

The major constituents identified in satsuma mihowase peel in Table 3 were d-limonene, γ-terpinene, and β-myrcene, with amounts up to 78.7%, 9.74%, and 3.52%, respectively. Other minor compounds identified included α-pinene, linalool, and γ-terpinene. The hydrocarbon monoterpenes quantified in these peel samples dominated the peels' essential oil with up to 94%, while the oxygenated monoterpenes were present at relatively low amounts in fresh samples but increased on drying. Sesquiterpenes such as carveol, β-elemene, and geranyl acetate were observed in trace amounts. Drying slightly decreased the proportion of oxygenated monoterpenes from 3.04% to 5.28%, suggesting biochemical transformations that favor compounds with greater aroma.

Table 3
Chemical composition Satsuma mihowase peel essential oil

GSM FP	**GSM DP**	**Fragmentation ions (m/z)**			
R_T	**COMPOUND NAME**	**CAS NUMBER**	**%**	**%**	
5.281	α-Thujene	926	0.45	0.37	**136**, 93, 91, 77
5.409	α-Pinene	940	-	2.09	**136**, 93, 91, 92
6.180	Sabinene	972	0.13	-	**136**, 93, 91, 77
6.237	β-Pinene	981	1.05	1.03	**136**, 93, 91, 69
6.538	β-Myrcene	992	3.21	3.52	**136**, 93, 69, 91
6.782	α-Phellandrene	999	0.34	0.32	**136**, 93, 77, 91
7.060	α-Terpenene	1020	0.43	0.42	**136**, 121,93, 91
7.182	p-Cymene	1019	-	0.26	**136**, 119, 134, 91
7.403	D-Limonene	1030	78.67	74.00	**136**, 68, 67, 93
7.669	β-Ocimene	1044	0.16	0.35	**136**, 93, 91, 79
7.934	γ-Terpinene	1060	9.27	9.74	**136**, 93, 91, 77
8.433	Terpinolene	1086	0.72	0.94	**136**, 121,93, 91
8.640	Linalool	1100	0.76	1.36	**154**, 71, 93, 55
8.724	Nonanal	1102	-	0.14	**142**, 57, 56, 55
9.486	Z-β-Terpineol	1189	0.12	0.22	**154**, 71, 69, 121

(R_T= Eluting times in mins times; CAS Number=Literature reference number of individual compounds; RSM = Riverside Satsuma Mihowase; FP = Fresh Peels, DP = Dried Peel; Fragment ion = M^+ peak, three prominent fragment ions peaks)

GSM FP	GSM DP	Fragmentation ions (m/z)			
R_T	**COMPOUND NAME**	**CAS NUMBER**	**%**	**%**	
10.107	Terpinen-4-ol	1174	0.73	0.85	**154**, 71, 111, 55
10.348	α-terpineol	1189	1.43	2.50	**154**, 59, 93, 121
10.604	Decanal	1205	0.17	0.23	**142**, 57, 55, 70
10.869	Z-(+)-Carveol	1229	-	0.24	**152**, 109, 84, 83
13.657	Geranyl acetate	1385	-	0.11	**196**, 69, 68, 93
13.855	β-Elemene	1392	0.19	0.25	**204**, 93, 67, 81
16.761	g-Eudesmol	1630	-	0.09	**222**, 189, 161, 204
16.953	Neointermedeol	1641	-	0.15	**222**, 189, 161, 204
% Phytochemicals identified		**97.83**	**99.22**		
Hydrocarbon Monoterpenes		**94.43**	**93.04**		
Oxygenated monoterpenes		**3.04**	**5.28**		
Sesquiterpenes		**0.19**	**0.49**		
Other		**0.17**	**0.41**		

(R_T= Eluting times in mins times; CAS Number=Literature reference number of individual compounds; RSM = Riverside Satsuma Mihowase; FP = Fresh Peels, DP = Dried Peel; Fragment ion = M^+ peak, three prominent fragment ions peaks)

Table 4 illustrates the peel essential oil data for the owari variety. The owari variety presented a richer, stronger profile, with d-limonene dominating the oil at up to 79.6%. Similarly to the mihowase variety, γ-terpinene (8.7), myrcene (3.22), α-pinene (3.22), and linalool (1.94) were also observed in fair amounts. The dried peel sample was observed to have lower hydrocarbon levels than the fresh peel sample, while the amount of oxygenated monoterpenes increased compared to the mihowase variety. A slight increase in the number of compounds, such as perillaldehyde and farnesene, was also observed. These results reveal a more stable, richer aroma profile, suggesting improved suitability for various essential oil applications.

Table 4
Chemical composition of Satsuma owari peel essential oil

R_T	COMPOUND NAME	Kovats Index	RSO FP %	RSO DP %	Fragmentation ions (m/z)
5.282	α-thujene	926	0.40	0.42	**136**, 93, 91, 77
5.405	α-Pinene	940	2.10	1.97	**136**, 93, 91, 92
6.181	Sabinene	978	0.14	0.23	**136**, 93, 91, 77
6.232	β-Pinene	990	0.95	0.98	**136**, 93, 91, 69
6.528	Myrcene	989	3.04	3.22	**136**, 93, 91, 69
6.778	α-Phellandrene	999	0.30	0.45	**136**, 93, 91, 77
7.046	α-Terpinene	1020	0.33	0.34	**136**, 93, 121, 91
7.391	D-Limonene	1033	79.62	78.19	**134**, 93, 68, 67
7.667	β-Ocimene	1044	0.23	0.17	**136**, 93, 91, 79
7.907	γ-Terpinene	1060	8.70	7.86	**136**, 93, 91,77
8.434	Terpinolene	1086	0.64	0.63	**136**, 93, 121, 136
8.639	Linalool	1100	1.12	1.94	**154**, 71, 93, 55
10.107	Terpinen-4-ol	1174	0.54	0.58	**154**, 71, 111, 93
10.350	α-terpineol	1189	1.43	1.09	**154**, 59, 93, 121
10.604	Decanal	1205	0.20	0.29	**142**, 57, 55, 70
10.864	Z-(+)-Carveol	1229	0.22	0.14	**152**, 109, 84, 83
11.854	Perillaldehyde	1271	-	0.23	**150**, 68, 79, 67
13.855	β-Elemene	1392	0.43	0.36	**204**, 93, 67, 81
15.214	Germacrene-D	1481	0.14	-	**204**, 161, 105, 91
15.496	(E,E)-α-Farnesene	1500	0.20	0.18	**204**, 93, 69, 107
% Phytochemicals identified		**99.99**	**100**		
Hydrocarbon Monoterpenes		**93.85**	**96.75**		
Oxygenated monoterpenes		**4.94**	**2.842**		

(R_T= Eluting times in mins times; CAS Number=Literature reference number of individual compounds; RSO = Riverside Satsuma Owari, FP=Fresh Peel, DP = Dried Leaves; Fragment ion = M^+ peak, three prominent fragment ions peaks)

R_T	COMPOUND NAME	Kovats Index	RSO FP %	RSO DP %	Fragmentation ions (m/z)
Sesquiterpenes		0.76	0.17		
Other		0.44	1.26		

(R_T= Eluting times in mins times; CAS Number=Literature reference number of individual compounds; RSO = Riverside Satsuma Owari, FP=Fresh Peel, DP = Dried Leaves; Fragment ion = M^+ peak, three prominent fragment ions peaks)

The key constituents in the okitsu peel in Table 5, even though slightly lower compared to the other two varieties, were d-limonene, which also improved with drying. Other identified compounds quantified were γ-terpinene, β-myrcene, and linalool with up to 8.34%, 3.28 and 2.42%, respectively. The okitsu peel's essential oil presented a unique profile as it also contained aldehydes as well as citronellal and citronellol. The highest linalool content contributes to the floral, calming, sweet scent of the peel (Letizia et al., 2003) while the presence of the aldehydes presented green and fresh aroma notes. Other hydrocarbon monoterpenes quantified in these peel samples were β-ocimene, α-pinene, sabinene, and α-terpinolene. Major oxygenated monoterpenes observed were linalool, α-terpineol, perillaldehyde, and carveol. Sesquiterpenes such as α-farnesene, β-Elemene, and α-cadinol was observed in SOk peel samples.

Table 5
Chemical composition of Satsuma Okitsu peel essential oils from Riverside farm

R_T (mins)			RSOk FP	RSOk DP	Fragmentation ions (m/z)
	COMPOUND NAME	Kovats Index	%	%	
3.110	Hexanal	800	0.41	-	**100**, 56, 57, 72
5.282	a-thujene	926	0.45	0.43	**136**, 93, 91, 77
5.405	α-Pinene	940	2.00	1.90	**136**, 93, 91, 92
6.181	Sabinene	978	0.25	0.23	**136**, 93, 91, 77
6.239	β-Pinene	991	1.03	1.04	**136**, 93, 91, 69
6.538	Myrcene	981	3.28	3.12	**136**, 93, 91, 69
6.751	n-Octanal	1000	1.17	0.82	**144**, 57, 56, 55
7.046	α-Terpinene	1020	0.32	-	**136**, 93, 121, 91
7.391	Limonene	1030	76.27	77.79	**134**, 93, 68, 67
7.685	β-Ocimene	1044	0.13	0.16	**136**, 93, 91, 79
7.919	γ-Terpinene	1060	7.84	8.34	**136**, 93, 91,77
8.437	Terpinolene	1086	0.64	0.67	**136**, 93, 121, 136
8.655	Linalool	1100	2.42	2.01	**154**, 71, 93, 55
8.719	Nonanal	1102	0.32	0.27	**142**, 57, 56, 55
9.641	Citronellal	1150	0.16	0.27	**154**, 69, 95, 55
10.107	Terpinen-4-ol	1174	0.60	0.52	**154**, 71, 111, 93
10.351	α-terpineol	1189	1.29	0.86	**154**, 59, 93, 121
10.604	Decanal	1205	0.43	0.39	**142**, 57, 55, 70
10.864	Carveol	1229	0.10	0.19	**152**, 109, 84, 83
11.019	L-Citronellol	1230	-	0.17	**152**, 69, 67, 55
11.854	Perillaldehyde	1271	0.34	0.19	**150**, 68, 79, 67
13.855	β-Elemene	1504	0.37	0.36	**204**, 93, 67, 81
15.499	(E,E)-g-Farnesene	1500	0.16	0.15	**204**, 93, 79, 107

(R_T= Eluting times in mins times; CAS Number=Literature reference number of individual compounds; RSO = Riverside Satsuma Owari, FP=Fresh Peel, DP = Dried Leaves; Fragment ion = M^+ peak, three prominent fragment ions peaks)

R_T (mins)			RSOk FP	RSOk DP	Fragmentation ions (m/z)
	COMPOUND NAME	Kovats Index	%	%	
16.958	α-Cadinol	1650	-	0.11	**222**, 81, 95, 135
% Phytochemicals identified		**99.99**	**100**		
Hydrocarbon Monoterpenes		**93.85**	**96.75**		
Oxygenated monoterpenes		**4.94**	**2.842**		
Sesquiterpenes		**0.76**	**0.17**		
Other		**0.44**	**1.26**		

(R_T= Eluting times in mins times; CAS Number=Literature reference number of individual compounds; RSO = Riverside Satsuma Owari, FP=Fresh Peel, DP = Dried Leaves; Fragment ion = M^+ peak, three prominent fragment ions peaks)

Conclusions

This study demonstrated that citrus unshiu varieties, such as Owari, Mihowase, and Okitsu, were rich in monoterpene hydrocarbons, including d-Limonene and γ-terpinene. These findings are consistent with the chemical profile previously reported for citrus essential oil. The mihowase peel essential oil has the highest d-limonene level characteristic of its citrusy fresh note. The owari cultivar proved to be richer in phytochemicals than the mihowase variety, indicating higher maturity levels that lead to increased bioaccumulation, whereas the mihowase variety showed moderate amounts of these compounds. The okitsu variety's peel essential oil showed fewer components but higher amounts of linalool, implying a metabolic shift towards terpene formation. The linalool amounts formed result from the enzymatic transformation of monoterpenes such as limonene. The reduced terpenoid content in this variety suggests biochemical transformation of some of these, thereby favoring the increase in phenolic compounds such as flavonoids, which was observed to be fairly higher than that of the mihowase variety.

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Research write-up and methodological evaluations, V.G.M.;Supervisors, A.O.O, G.M.M., and A.O.O. All authors have reviewed and agreed to the published version of the manuscript.

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References

1. Adams R. p. (1989). Identification of Essential Oils by Ion trap Mass Spectroscopy.
2. Anagnostopoulou MA, Kefalas P, Papageorgiou VP, Assimopoulou AN, Boskou D. Radical scavenging activity of various extracts and fractions of sweet orange peel (Citrus sinensis). Food Chem. 2006;94(1):19–25. https://doi.org/https://doi.org/10.1016/j.foodchem.2004.09.047.
3. Basavegowda N, Lee Y. Synthesis of silver nanoparticles using Satsuma mandarin (Citrus unshiu) peel extract: A novel approach towards waste utilization. Mater Lett. 2013;109:31–3. https://doi.org/10.1016/j.matlet.2013.07.039.
4. Chaves JO, de Souza MC, da Silva LC, Lachos-Perez D, Torres-Mayanga PC, Machado APdF, Forster-Carneiro T, Vázquez-Espinosa M, González-de-Peredo AV, Barbero GF, Rostagno MA. (2020). Extraction of Flavonoids From Natural Sources Using Modern Techniques [Review]. *Frontiers in Chemistry*, *Volume 8–2020*. https://doi.org/10.3389/fchem.2020.507887

5. D'Amelia V, Aversano R, Chiaiese P, Carputo D. (2018). The antioxidant properties of plant flavonoids: their exploitation by molecular plant breeding. *Phytochemistry Reviews*, *17*. https://doi.org/10.1007/s11101-018-9568-y
6. Duvenage S, Van Lill D, Walsh C. Optimising the extraction of natural flavouring from 'Satsum' mandarin (Citrus unshiu Marc). South Afr J Plant Soil. 2004;21:182–7. https://doi.org/10.1080/02571862.2004.10635045.
7. Elmacı Y, Altuğ T. Flavor characterization of three mandarin cultivars (Satsuma, Bodrum, Clemantine) by using GC/MS and flavor profile analysis techniques. J Food Qual. 2005;28:163–70. https://doi.org/10.1111/j.1745-4557.2005.00009.x.
8. Ghongade R. (2013). PHYTOCHEMICAL ANALYSIS OF CITRUS KARNA FRUIT. *International Journal of Pharma and Bio Sciences*.
9. Glenneis K. (2026). Soft citrus production: Fruit farming in South Africa [Article]. Retrieved 3 June 2026, from https://southafrica.co.za/soft-citrus-varieties-south-africa.html
10. Jeong S-M, Kim D-S, Kim SS, Kim H-J. Effects of the Cultivation Methods on the Sensory Quality and Phytochemical Profiles of Satsuma Mandarin (Citrus unshiu). Horticulturae. 2024;10(1):54.
11. Letizia CS, Cocchiara J, Lalko J, Api AM. Fragrance material review on linalool. Food Chem Toxicol. 2003;41(7):943–64. https://doi.org/https://doi.org/10.1016/S0278-6915(03)00015-2.
12. Lt s, lt, Liu NT, Tang Y, Zhang Y, Zhang J, M., Zeng H. (2008). Chemical composition of essential oil from the peel of Satsuma mandarin. *African Journal of Biotechnology*, *7*, 1261–1264.
13. Miya G, Nyalambisa M, Oyedeji O, Gondwe M, Oyedeji A. Chemical Profiling, Toxicity and Anti-Inflammatory Activities of Essential Oils from Three Grapefruit Cultivars from KwaZulu-Natal in South Africa. Molecules. 2021;26(11):3387.
14. Molnar M, Jakovljević Kovač M, Pavić V. A Comprehensive Analysis of Diversity, Structure, Biosynthesis and Extraction of Biologically Active Tannins from Various Plant-Based Materials Using Deep Eutectic Solvents. Molecules. 2024;29(11):2615.
15. Moses T, Papadopoulou KK, Osbourn A. Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. Crit Rev Biochem Mol Biol. 2014;49(6):439–62. https://doi.org/10.3109/10409238.2014.953628.
16. Moss GP, Wilfried A, König. E.B.-Verlag, Hamburg, 1998, ISBN 3-930826-48-8, $700. *Phytochemistry*, *52*, 1385–1386.
17. Nicolosi E, Deng Z, Gentile A, La Malfa S, Continella G, Tribulato E, Nicolosi E, Deng ZN, Gentile A, Malfa Sl, Continella G, Tribulato E. Citrus phylogeny and genetic origin of important species as investigated by molecular markers. Theor Appl Genet 100: 1155–1166. *Theoretical and Applied Genetics*, *100*, 1155–1166. https://doi.org/10.1007/s001220051419
18. Nkomfe L. (2026). South Africa was world's largest citrus exporter in 2025 – CGA.
19. Nyalambisa M, Oyemitan IA, Matewu R, Oyedeji OO, Oluwafemi OS, Songca SP, Nkeh-Chungag BN, Oyedeji AO. Volatile constituents and biological activities of the leaf and root of Echinacea species from South Africa. Saudi Pharm J. 2017;25(3):381–6. https://doi.org/10.1016/j.jsps.2016.09.010.

20. Olmedo-Galarza V, Pinto-Mosquera N, Pineda-Flores H, Manosalvas-Quiroz L. Citrus Waste Valorization: Unconventional Pathways for Sustainable Biomaterials and Bioactive Products. Sustainability. 2025;17(24):10887.
21. Ozkaya O, Yabaci S, Incesu M, Yesiloglu T. The general and volatile properties and the quality of two newly selected Satsuma clones (11/1 İzmir and 30/ İzmir) grown under Mediterranean ecological conditions. Food Sci Technol. 2018;39. https://doi.org/10.1590/fst.33017.
22. Piironen V, Salmenkallio-Marttila M. (2009). CHAPTER 7: Micronutrients and Phytochemicals in Wheat Grain. In (pp. 179–222). https://doi.org/10.1094/9781891127557.007
23. Qaderi MM, Martel AB, Strugnell CA. Environmental Factors Regulate Plant Secondary Metabolites. Plants (Basel). 2023;12(3). https://doi.org/10.3390/plants12030447.
24. Qiao Y, Xie B-j, Zhang Y, Zhou H-y, Pan S-y. Study on Aroma Components in Fruit From Three Different Satsuma Mandarin Varieties. Agricultural Sci China. 2007;6(12):1487–93. https://doi.org/https://doi.org/10.1016/S1671-2927(08)60012-7.
25. Saibu G, Adeyemo G, Adu O, Oguntibeju O, Oyedeji O, Oyedeji AO, Nakin V. Proximate and Phytochemical analysis of Aqueous Leaf Extract of Solanum nigrum from Lagos, South-West Nigeria. Afr J Biomedical Res. 2021;24(3):397–402.
26. Saini RK, Ranjit A, Sharma K, Prasad P, Shang X, Gowda KGM, Keum YS. Bioactive Compounds of Citrus Fruits: A Review of Composition and Health Benefits of Carotenoids, Flavonoids, Limonoids, and Terpenes. Antioxid (Basel). 2022;11(2). https://doi.org/10.3390/antiox11020239.
27. Tapas DA, Sakarkar DM, Kakde R. Flavonoids as Nutraceuticals: A Review. Trop J Pharm Res. 2008;7. https://doi.org/10.4314/tjpr.v7i3.14693.
28. Terahara N. Flavonoids in Foods: A Review. Nat Prod Commun. 2015;10:521–8. https://doi.org/10.1177/1934578X1501000334.
29. Verreynne JS, Rabe E, Theron KI. Effect of bearing position on fruit quality of mandarin types. South Afr J Plant Soil. 2004;21(1):1–7. https://doi.org/10.1080/02571862.2004.10635014.
30. Verzera A, Trozzi A, Cotroneo A, Lorenzo D, Dellacassa E. Uruguayan essential oil. 12. Composition of Nova and Satsuma mandarin oils. J Agric Food Chem. 2000;48(7):2903–9. https://doi.org/10.1021/jf990734z.
31. Zou Z, Xi W, Hu Y, Nie C, Zhou Z. Antioxidant activity of Citrus fruits. Food Chem. 2016;196:885–96. https://doi.org/https://doi.org/10.1016/j.foodchem.2015.09.072.

Figures



Figure 1

Schematic representation of the method followed for EO extraction and analysis.