

Changes of the Volatile Profile and Artifact Formation in Daidai (*Citrus aurantium*) Cold-Pressed Peel Oil on Storage

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The chemical changes and artifact formation in daidai (*Citrus aurantium* L. var. *Cyathifera* Y. Tanaka) cold-pressed peel oil upon storage at 20, 5, and -21 °C for 3, 6, and 12 months were investigated using capillary gas chromatography (GC) and GC-MS. Major changes occurred in the oil stored at 20 and 5 °C. No changes were found at -21 °C. Monoterpene hydrocarbons decreased from 98.0 to 66.4% upon 12 months at 20 °C, while sesquiterpene hydrocarbons and alcohols increased from 0.1 to 2.4% and from 0.3 to 7.9%, respectively. Notable decreases of germacrene D, myrcene, linalyl acetate, and limonene occurred. Prominent increases of *cis*-carveol, *trans*- β -farnesene, *trans*-*p*-2,8-menthadien-1-ol, linalool, and β -caryophyllene were found. Thirty-four artifact compounds constituting 17.0% of the total volatile compounds were formed upon 12 months at 20 °C. The artifacts consisted of 13 alcohols (6.0%), five carbonyl compounds (5.3%), seven esters (4.9%), three epoxides (0.4%), four hydrocarbons (0.3%), and two unidentified. The prominent artifact compounds were (+)-carvone, *trans,trans*-farnesyl acetate, sabinene hydrate, 1-octen-3-ol, *cis,cis*-farnesyl acetate, and dihydrocarveol acetate. The results could be applied for monitoring and control of the flavor quality of daidai essential oil and related products.

KEYWORDS: Daidai; *Citrus aurantium* L. var. *Cyathifera* Y. Tanaka; cold-pressed peel oil; storage temperature; storage time; volatile compound; compositional change; artifact compound; storage stability

INTRODUCTION

Stability of the volatile chemical constituents of essential oils during handling and storage is an important consideration for their various applications, such as food processing, cosmetics, perfumery, fragrances, and aromatherapy. *Citrus* oils are complex mixtures of volatile compounds, mainly the unsaturated monoterpene and sesquiterpene compounds, which are generally labile to heat and chemical reactions. The resultant compositional changes and artifact formation are important aspects of flavor chemistry in view of the possible off-flavor development and reduced quality and economic value of the product. Moreover, the presence of artifact compounds in laboratory samples of essential oils could limit the value of the analytical information obtained. Artifacts may be formed in stored oils upon oxidation, metal catalysis, or heat or chemical interactions of the naturally present compounds. Studies on *Citrus* essential oils have mostly dealt with characterization of the components present and given less attention to compositional changes influenced by handling and storage parameters. Investigations on storage stability of special cold-pressed oils (CPOs) are,

therefore, very important. *Citrus* peel oils are much more steady than *Citrus* essence oils concerning storage stability. The better storage stability of the CPOs refers mainly to the nonvolatile residues (2–15%). These residues are coumarins, psoralens, and polymethoxylated flavones, which have antioxidative properties. These components and their quantity have to be considered by reflecting the stability of *Citrus* peel oils.

Daidai (*Citrus aurantium* L. var. *Cyathifera* Tanaka) is a variety of the sour orange (*C. aurantium* L.) produced mainly in Japan and some parts of China. The actual quantities of daidai peel oil produced in China and Japan are not clearly known. The sour orange has been used for salad dressings, culinary, seasonings, and flavoring of beverages such as tea. Reported studies on volatile components of daidai oil have indicated a large proportion of monoterpene hydrocarbons (98.0%). Limonene (*p*-mentha-1,8-diene) is the most dominant hydrocarbon, and linalyl acetate is the main oxygenated compound of the oil (1, 2). The ester in conjunction with β -elemol has been indicated as the characteristic odor compound of daidai oil (2). *Citrus* oils are generally prone to oxidation, polymerization, and structural rearrangement during handling, analysis, processing, or storage, with associated changes of the volatile profile and aroma properties (3). If a major disturbance of the natural composition of the oil occurs, such that the relative concentration of the constituents is distorted, this would affect the subtlety of

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the aroma and cause deterioration (4). Oils from different types of *Citrus* fruits exhibit some unique constituents that would influence their storage stabilities (5). Variations of sample preparation and handling methods could also affect the oil composition and influence the storage stability (6–8). The cold-pressing method of *Citrus* oil preparation avoids usage of chemical reagents, heat, or silica gel and ensures that the oil retains intact the natural antioxidants present, which would influence the storage stability of the oil (9–11). The present study investigated the compositional changes and formation of artifact compounds in daidai cold-pressed peel oil upon storage for 3, 6, and 12 months at -21 , 5 , and 20 °C. Knowledge of the chemical changes of popular *Citrus* oils as influenced by storage conditions would be important for monitoring the freshness and quality authenticity of the products.

MATERIALS AND METHODS

Sample Preparation. The daidai fruit at maturity in December was obtained from the Kochi Prefectural Agricultural Research Center, Fruit Tree Experiment Station, Japan. The fruit was in one batch and harvested at the same time; thus, standard deviations were not used. The fruit was kept at 5 °C prior to isolation of the oil, which was done within a week of harvest while the fruit was still fresh. The peel oil was isolated by the cold-pressing method as described previously (9). The flavedo was prepared by peeling off the mesocarp and albedo layers to expose the oil glands. It was then pressed by hand to express the oil that was collected on ice. The crude oil was saturated with brine and centrifuged at -4 °C at $2000g$ for 15 min. It was decanted, dried with anhydrous sodium sulfate for 24 h at 5 °C, and filtered to obtain the CPO for the study.

Storage Conditions. Samples of 1 mL of the freshly prepared CPO were put in 5 mL amber glass vials in three sets of duplicate samples. The sample vials were closed tightly with Teflon screw caps and wrapped with aluminum foil to avoid light. The first set was kept at a constant 20 °C, the second at 5 °C, and the third at -21 °C. In each sample vial, a limited supply of air was provided by the headspace. The storage conditions took into consideration the manner in which *Citrus* essential oils or products made with them were handled during commercial practice and home usage in order to establish the degree to which temperature and time could be detrimental to the flavor. Usually, essential oils are stored refrigerated (0 – 5 °C) and often (but not always) under an inert gas atmosphere. Industrially processed CPO was not used in this work. It has to be kept in mind that the storage conditions investigated with *Citrus* peel oil itself will give different results as compared to blending *Citrus* peel oil into beverages or other food. The oil samples were analyzed when fresh and upon storage for 3, 6, and 12 months at each temperature.

Gas Chromatography (GC) and GC-MS Analyses. GC analysis was performed using a Shimadzu 14A gas chromatograph (Shimadzu Corp., Kyoto, Japan) fitted with a $50\text{ m} \times 0.25\text{ mm}$ i.d., fused silica capillary column (Thermo 600T, a polar liquid phase similar to PEG 20M; Shimadzu, Kyoto) as described previously (12). The oven temperature was programmed from 70 °C (isothermal for 2 min) at an increasing rate of 2 °C/min to 230 °C (isothermal for 20 min). The injector and the flame ionization detector were each at 250 °C. Samples of $0.5\ \mu\text{L}$ were injected. The split ratio was 50:1. Nitrogen was the carrier gas at a flow rate of 1.0 mL/min . A Shimadzu C-R6A Chromatopack integrator was used for resolution of the separated components. Retention indices (RI) of the components and standard compounds were determined relative to a homologous series of *n*-alkanes (C_7 – C_{27}) under the same GC analytical conditions. The concentration of each compound was expressed as a relative % to the concentration of the entire volatiles. GC-MS was conducted using a Shimadzu GC17A-QP 5000MS. The column and temperature conditions were similar to those indicated above. Oil samples of $0.2\ \mu\text{L}$ were injected. The split ratio was 32:1. Helium was the carrier gas at a flow rate of 1.0 mL/min . The MS ionization voltage was 70 eV , and the ion source temperature was 250 °C. The scanning time was 1 s over a range of 30 – 320 m/z .

Identification of Compounds. The individual components were identified by comparisons of their RI to those of standard compounds and the mass spectra similarities to those of compounds registered in the NIST 62 and 107 spectral libraries of the GC-MS. Analytical reference standards were purchased from various manufacturers, including Wako Pure Chemical Industries, Osaka, Japan; Aldrich Chemical Co., U.S.A.; Nacalai Tesque, Kyoto, Japan; and Fluka Fine Chemicals, Switzerland. Tentative identification was based on GC-MS databases.

RESULTS AND DISCUSSION

Volatile Profile of Fresh Daidai Cold-Pressed Peel Oil.

The volatile components of the fresh daidai peel oil are presented in **Table 1**. Twenty-eight components, accounting for 99.8% of the total volatile profile, were identified and monitored during storage, by virtue of their relative prominence. The six monoterpene hydrocarbons in the oil accounted for almost the totality of the volatiles (98.0%), as reported in a previous study where the GC chromatograms regarding daidai oil with clear identification of the components had been illustrated (1). Limonene was largely the most prominent (94.7%), followed by myrcene (1.6%), β -pinene (0.7%), α -pinene (0.5%), γ -terpinene (0.3%), and sabinene (0.2%). The fresh oil was notably devoid of α -terpinene, a compound occurring in many *Citrus* oils (5). The oil was quite deficient in sesquiterpene hydrocarbons (0.1%) unlike other reported Japanese *Citrus* peel oils (1, 2, 5, 9, 13, 14). Among the five components present, germacrene D accounted for the entire percentage. β -Caryophyllene, *trans*- β -farnesene, α -humulene, and bicyclogermacrene occurred in trace quantities. The total oxygenated compounds accounted for 1.7% of the total volatiles. Among them, esters were the most prominent (1.2%) and linalyl acetate (1.1%) was the major constituent. The other esters present were octyl acetate, *p*-mentha-1,8-dien-10-yl acetate, bornyl acetate, decyl acetate, and nonyl acetate in minor quantities. The alcohol group (0.3%) was mainly comprised of linalool and *cis*-nerolidol. The other alcohols present were α -terpineol, thujol, *trans*-*p*-menthadien-1-ol, and *cis*-carveol at trace quantities. The aldehydes (0.2%) were comprised of octanal, nonal, and decanal.

Alterations of the Monoterpene Compounds Upon Storage. The alterations of the monoterpene constituents of the oil upon storage for 3, 6, and 12 months at -21 , 5 , and 20 °C are shown in **Table 1**. The constitution of individual chemical components and functional group classes relative to the whole aromatic profile without absolute changes provides reliable and convenient qualitative information (12). As shown in **Table 1**, the monoterpene hydrocarbons decreased progressively during storage at 20 °C, to as low as 66.4% upon 12 months. The observed decreases at 5 °C were much less over the same period (to 87.6%). Virtually no alterations were found at -21 °C. The prominently decreased compounds at 20 °C were β -myrcene, γ -terpinene, and limonene. In contrast, slight increases of *p*-cymene and terpinolene occurred under the same conditions. The decreases of myrcene and γ -terpinene were observed only at 20 and 5 °C in 12 months. The increase of the total oxygenated compounds was observable only at 5 and 20 °C with prolonged storage. The most notable increases were found in the ester and alcohol groups. The carbonyl compounds remained relatively unchanged. The compounds that showed sharp increases were *cis*-carveol, *p*-mentha-1,8-dien-10-yl acetate, *trans*-*p*-menthadien-1-ol, nonyl acetate, linalool, and decyl acetate. The increases of nonyl acetate and decyl acetate were observed only at 20 °C. Slight increases of α -terpineol, octyl acetate, and thujol occurred at 20 °C. Conversely, a sharp decrease of linalyl acetate was found. Only the particularly

Table 1. Compositional Changes and Artifact Volatile Compounds of Daidai (*C. aurantium* Y. Tanaka) Cold-Pressed Peel Oil When Fresh and Upon Storage for 3, 6, and 12 Months at -21 , 5, and 20 °C

no	RI	constituent	concentration (relative %) ^a									
			fresh ^b	-21 °C			5 °C			20 °C		
				3 ^c	6 ^c	12 ^c	3 ^c	6 ^c	12 ^c	3 ^c	6 ^c	12 ^c
Monoterpene Hydrocarbons												
1	1036	α -pinene	0.5	0.5	0.6	0.5	0.6	0.5	0.5	0.6	0.5	0.5
2	1125	β -pinene	0.7	0.7	0.7	0.6	1.0	0.8	0.6	0.7	0.7	0.5
3	1135	sabinene	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.1
4	1169	β -myrcene	1.6	1.8	1.7	1.5	1.6	1.7	0.5	0.8	0.4	0.1
5	1228	limonene	94.7	94.1	93.8	94.9	92.1	89.0	85.8	88.5	86.1	65.1
6	1257	γ -terpinene	0.3	0.3	0.3	0.3	0.2	0.2	*	*	*	*
7	1284	<i>p</i> -cymene	tr	tr	tr	tr	*	*	*	*	*	*
8	1290	terpinolene	tr	tr	tr	tr	*	tr	*	tr	tr	0.1
		total	98.0	97.7	97.4	98.1	95.8	92.5	87.6	90.9	87.9	66.4
Sesquiterpene Hydrocarbons												
9	1605	β -caryophyllene	*	*	0.1	*	0.1	0.1	0.5	0.4	0.7	1.1
10	1664	<i>trans</i> - β -farnesene	*	*	*	*	*	*	*	0.4	0.6	1.2
11	1673	α -humulene	tr	*	*	*	*	*	*	tr	0.1	0.1
12	1713	germacrene D ^g	0.1	0.1	0.1	0.1	*	0.1	0.1	*	tr	<i>d</i>
13	1742	bicyclogermacrene ^g	*	*	*	*	*	*	*	*	*	*
		total	0.1	0.1	0.2	0.1	0.1	0.2	0.6	0.8	1.4	2.4
		total hydrocarbons ^e	98.1	97.8	97.6	98.2	95.9	92.7	88.2	91.7	89.3	68.8
Carbonyl Compounds												
14	1299	octanal	0.1	0.1	0.1	0.1	0.1	0.1	*	0.1	0.1	0.1
15	1405	nonanal	*	*	*	*	*	*	*	*	*	*
16	1503	decanal	0.1	0.1	0.1	0.1	tr	0.1	0.1	0.1	0.1	0.1
		total	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.2
Alcohols												
17	1545	linalool	0.2	0.2	0.2	0.1	0.2	0.1	0.4	1.3	1.1	1.0
18	1647	thujol	tr	tr	tr	tr	*	*	*	tr	tr	0.1
19	1664	<i>trans</i> - <i>p</i> -menthadien-1-ol ^g	*	*	*	*	0.1	*	0.5	tr	0.6	1.2
20	1695	α -terpineol	*	*	*	*	*	0.1	0.1	0.1	0.2	0.1
21	1834	<i>cis</i> -carveol	*	*	0.1	*	0.1	0.5	0.6	0.8	1.4	5.2
22	1998	<i>cis</i> -nerolidol ^g	0.1	tr	0.1	*	*	0.1	*	0.1	0.1	0.3
		total	0.3	0.2	0.4	0.1	0.4	0.8	1.6	2.3	3.4	7.9
Esters												
23	1477	octyl acetate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2
24	1561	linalyl acetate	1.1	1.1	1.2	1.0	1.6	0.8	0.7	0.9	1.0	0.5
25	1575	nonyl acetate	*	*	*	*	*	*	*	0.2	0.3	0.9
26	1585	bornyl acetate	tr	tr	tr	tr	*	*	*	*	tr	*
27	1678	decyl acetate	*	*	*	*	*	*	*	*	0.1	0.4
28	1858	<i>p</i> -mentha-1,8-dien-10-yl acetate ^g	*	*	*	tr	0.1	0.5	0.6	0.7	0.8	1.3
		total	1.2	1.2	1.3	1.1	1.8	1.4	1.4	1.9	2.4	3.3
		total oxygenated volatile compounds ^f	1.7	1.6	1.9	1.4	2.3	2.4	3.1	4.4	6.0	11.4
		total natural volatile compounds ^(e+f)	99.8	99.4	99.5	99.6	98.2	95.1	91.3	96.1	95.3	80.2
Artifact Compounds Hydrocarbons												
29	1249	<i>allo</i> -ocimene ^g			tr	tr			*	*	*	*
30	1629	aromadendrene ^g							*	*	*	0.1
31	1638	β -elemene						tr	*	*	*	0.1
32	1753	δ -cadinene ^g			tr	tr	*	*	0.1	0.1	0.1	0.1
		total			tr	tr	*	*	0.1	0.1	0.1	0.3
Alcohols												
33	1459	hexan-1-ol							*	*	*	*
34	1462	1-octen-3-ol				0.1	0.1		1.7	1.0	1.8	1.1
35	1466	sabinene hydrate			tr	0.1	tr	0.2	1.0	0.6	1.1	2.4
36	1676	<i>cis</i> -piperitol			0.1	0.1	0.1	0.1	0.2	0.4	0.3	0.5
37	1776	decanol			tr	0.1	tr	tr	*	*	0.1	0.2
38	2036	<i>trans</i> -nerolidol ^g		0.1				0.1	*	*	*	0.1
39	2075	globulol ^g							0.2	*	0.1	0.7
40	2098	viridiflorol ^g				tr			0.1	tr	*	0.1
41	2119	spathulenol ^g							0.1	tr	0.1	0.1
42	2122	cedrenol									*	*
43	2127	cedrol							0.2	*	tr	0.1
44	2233	β -eudesmol ^g							0.1	tr	*	0.1
45	2327	(<i>Z,E</i>)-farnesol							*	*	*	0.6
		total		0.1	0.1	0.4	0.2	0.3	3.6	2.0	3.5	6.0
Carbonyl Compounds												
46	1434	α -thujone					*	*	*	*	*	*
47	1437	β -thujone		*	tr		*	*	0.1		0.1	0.2
48	1749	(+)-carvone			0.1	*	*	0.1	1.1	0.5	1.3	4.8
49	1784	cumin aldehyde					tr	tr	0.1		0.1	0.3
50	2045	cinnamic aldehyde							*	tr	0.1	*
		total		*	0.1	*	0.1	0.1	1.3	0.5	1.6	5.3

Table 1. (Continued)

no	RI	constituent	fresh ^b	concentration (relative %) ^a								
				-21 °C			5 °C			20 °C		
				3 ^c	6 ^c	12 ^c	3 ^c	6 ^c	12 ^c	3 ^c	6 ^c	12 ^c
Esters												
51	1374	heptyl acetate	tr	tr		*	*	*	*	*	*	0.1
52	1661	citronellyl acetate			tr	*	*	*	*	*	*	*
53	1994	dihydrocarvyl acetate ^g			*		0.2	0.2	0.1	0.1	0.1	0.8
54	2151	cedryl acetate						*	tr	*	*	0.1
55	2263	<i>cis,cis</i> -farnesyl acetate						*	tr	*	*	0.9
56	2265	<i>cis,trans</i> -farnesyl acetate					*		tr	*	*	tr
57	2269	<i>trans,trans</i> -farnesyl acetate	tr	tr		*	0.1	0.1	*	0.2	0.2	3.0
		total	tr	tr		*	0.3	0.3	0.1	0.3	0.3	4.9
Oxides and Epoxides												
58	1385	<i>trans</i> -2,3-epoxy-butane ^g				tr	tr	*	*	0.2	0.1	
59	1957	<i>cis</i> -caryophyllene epoxide ^g				*	*	0.1	0.1	*	0.1	
60	1968	<i>trans</i> -caryophyllene epoxide ^g				*	0.1	0.1	*	*	0.3	
		total		tr		*	0.1	0.2	0.1	0.2	0.5	
Unidentified Compounds												
61	1327	unidentified I						*	*	*	*	
62	1339	unidentified II						*	*	*	*	
		total % artifacts	0.1	0.1	0.4	0.4	0.8	5.7	3.0	6.0	17.0	
		total number of artifacts	4	10	12	14	17	32	30	32	34	

^a Mean of triplicate determinations. ^b Freshly prepared CPO. ^c Months of storage. ^d Not detected. ^e Sum of monoterpene and sesquiterpene hydrocarbons. ^f Sum of alcohols, esters, and carbonyl compounds. tr, detected but not quantitated; *, not less than 0.005% and less than 0.05%. ^g Tentatively identified.

evident changes were considered in this work, based on relative percentage changes of the components. The chemical configurations of some of the prominently altered monoterpene compounds are illustrated in **Figure 1**. Monoterpene compounds readily undergo structural modifications and chemical reactions at elevated temperatures during storage (12). The observed decreases of limonene (5), sabinene (3), α -pinene (1), myrcene (4), linalyl acetate (24), and γ -terpinene (6) and the corresponding increases of *p*-cymene (7), *p*-mentha-1,8-dien-10-yl acetate, *trans-p*-menthadien-1-ol (19), linalool (17), terpinolene (8), α -terpineol (20), and *cis*-carveol (21) were in accordance to previous reports on *Citrus* oils (12, 15). Structural rearrangements of limonene, sabinene, γ -terpinene, and α - and β -pinene (2) influenced by heat and oxidation would lead to *p*-cymene and related compounds of the *p*-menthadiene skeleton, such as terpinolene and α - and β -phellandrene. The phellandrenes were absent from the daidai oil. The increases of linalool could be attributed to autoxidation of myrcene (16). α -Terpineol is readily formed upon oxidation of limonene under acidic conditions (12, 16). The observed sharp increase of linalool in the early months followed by a decline was in accordance with a previous paper (12). The decreases among the major monoterpene compounds could be a result of structural rearrangement, cyclization, isomerization, decomposition, artifact formation, or direct loss on prolonged storage. Limonene, being particularly susceptible to oxidation, could lead to *cis*-carveol, as illustrated previously (17). *cis*-Carveol could also be formed by hydration of the related carvones present in the oil. Thujol (18) could be formed by structural rearrangement and hydration of the closely related sabinene (17). The sharp increase of *p*-mentha-1,8-dien-10-yl acetate could be attributed to oxidation of the structurally related *p*-mentha-1,8-diene (limonene). Similarly, the notable increase of *trans-p*-menthadien-1-ol could occur through oxidation and hydration of related *p*-menthadiene compounds such as α - and γ -terpinene, limonene, *p*-cymene, and terpinolene. The loss of linalyl acetate by possible degradative reactions leading to compounds such as linalool could negatively impact the perceived aroma of the oil. The observed large alteration of limonene (from 94.7 to 65.1%) as compared to oxygenated

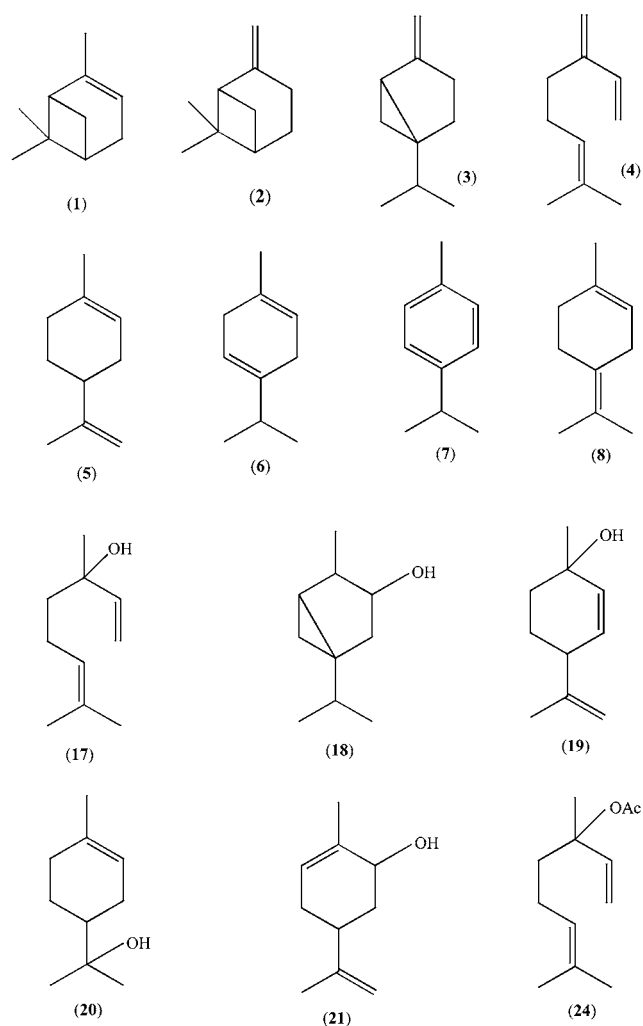


Figure 1. Structural illustrations of some monoterpene volatile compounds of daidai (*Citrus aurantium* L. var. *Cyathifera* Y. Tanaka) cold-pressed peel oil. The number against each structure refers to the identity of the compound as indicated by the same number in **Table 1**.

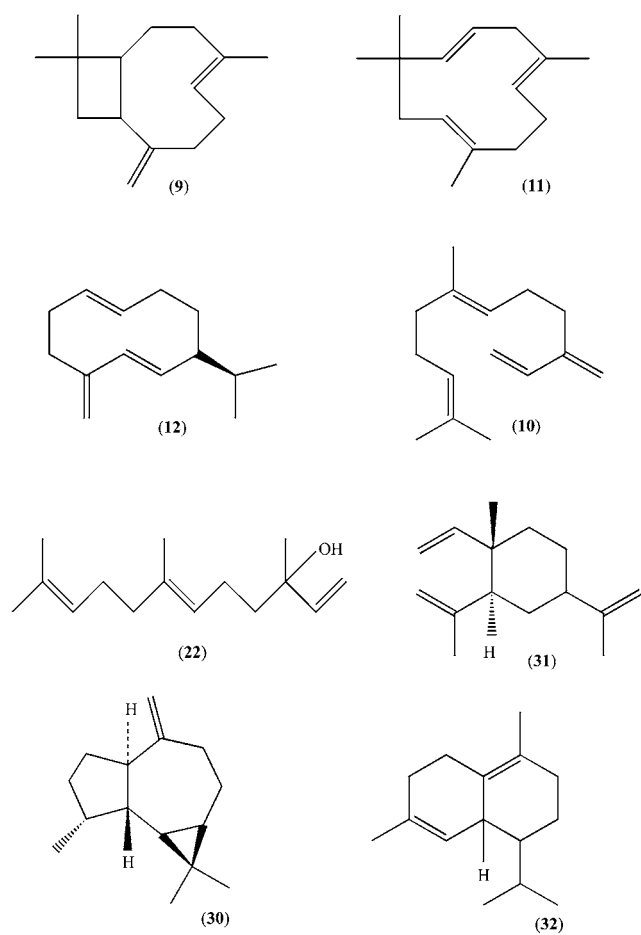


Figure 2. Structural illustrations of some sesquiterpene volatile compounds of daidai (*C. aurantium* L. var. *Cyathifera* Y. Tanaka) cold-pressed peel oil. The number against each structure refers to the identity of the compound as indicated by the same number in Table 1.

compounds could not be explained, and it could be possible that transformations into artifacts or nonvolatile matter occurred.

Alterations of Sesquiterpene Compounds Upon Storage.

The total sesquiterpene hydrocarbons increased to 0.6% and 2.4% upon storage for 12 months at 5 °C and 20 °C, respectively. No changes were found at -21 °C. The compounds whose percentage levels increased prominently were *trans*- β -farnesene, β -caryophyllene, and α -humulene. Conversely, sharp decreases of germacrene D and bicyclogermacrene occurred, and the two compounds were depleted from the oil upon 12 months at 20 °C. The chemical configurations of some of the prominently altered sesquiterpene compounds are illustrated in Figure 2. β -Caryophyllene (9), a bicyclic sesquiterpene hydrocarbon, usually co-occurs with the monocyclic sesquiterpene, α -humulene (11) (also called α -caryophyllene), in many essential oils (18). The two compounds increased prominently in the daidai oil upon storage, as reported for other oils (12, 17). The increases of these compounds were not explainable from the observed relative changes of other components present. The polyunsaturated acyclic sesquiterpene hydrocarbon, *trans*- β -farnesene (3,7,11-trimethyl-1,3,6,10-dodecatriene) (10), exists in the α - and β -forms and four geometric isomers: *cis,cis*; *cis,trans*; *trans,cis*; and *trans,trans* (19). Ring opening of germacrene D (12) could form the farnesene structure (19) and lead to increases of *trans*- β -farnesene. The losses of germacrene D and bicyclogermacrene at the higher temperatures and prolonged storage could be attributed to isomerization, ring opening, and oxidation. Germacrene D readily converts to

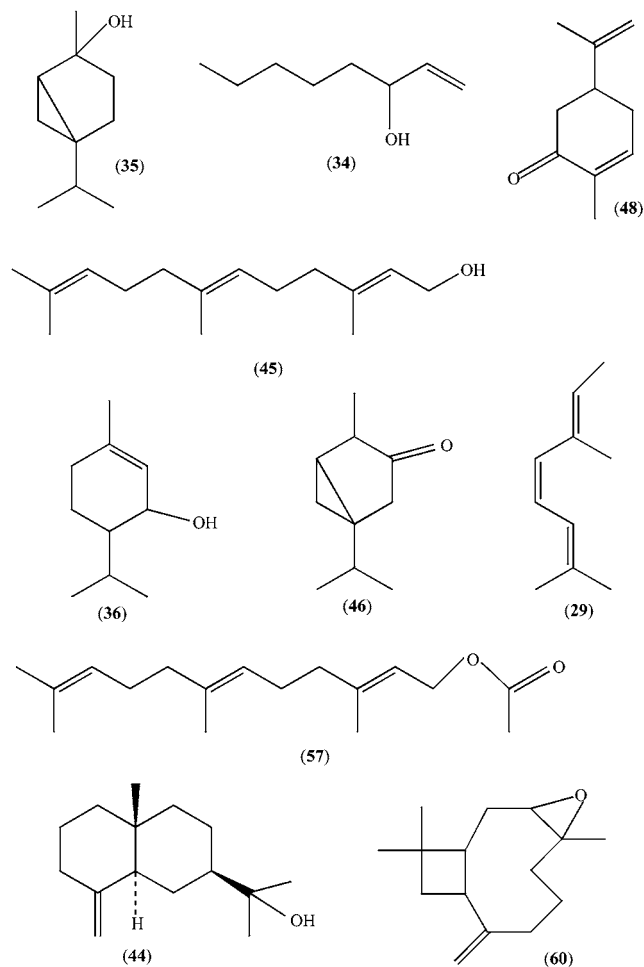


Figure 3. Structural illustrations of some artifact volatile components of daidai (*C. aurantium* L. var. *Cyathifera* Y. Tanaka) cold-pressed peel oil upon storage. The number against each structure refers to the identity of the compound as indicated by the same number in Table 1.

cadinenes, muurolenes, and elemene-type sesquiterpene hydrocarbons (20). Bicyclogermacrene rearranges to the tricyclic aromadendrene, *allo*-aromadendrene, and viridiflorene (12, 20). These alterations were evident in the stored daidai oil. *cis*-Nerolidol (22), an acyclic sesquiterpene alcohol and the only oxygenated sesquiterpene compound found in the fresh daidai oil, underwent a moderate increase upon prolonged storage at 20 °C. Its related hydrocarbon, bisabolene (20), was however not detected in the oil. The observed increase of *cis*-nerolidol could be attributed to structural rearrangement and hydration of the closely related β -farnesene.

Artifact Formation. Artifact compounds of various chemical groups were formed in the oil upon prolonged storage at the higher temperatures, as shown in Table 1. Although many of these compounds could be natural constituents of some other essential oils, they were completely absent from the fresh daidai oil. The numbers and relative amounts of the artifacts formed in the oil were influenced by temperature and time, as shown in Table 1. A total of 34 artifact compounds constituting a relative 17.0% of the total volatiles were formed upon 12 months at 20 °C. Similarly, 32 artifact compounds amounting to 5.7% were found at 5 °C and 11 constituting 0.4% were formed at -21 °C. The chemical configurations of some of the prominent artifacts are illustrated in Figure 3 and some in Figure 2. Artifacts of the alcohol group were the most prominent, accounting for 0.4, 3.6, and 6.0% upon 12 months at -21, 5, and 20 °C, respectively. The main components were sabinene

hydrate (35), 1-octen-3-ol (34), globulol, *cis,trans*-farnesol (45), and *cis*-piperitol (36). The other notable alcohol artifacts were spathulenol, viridiflorol, *trans*-nerolidol, and β -eudesmol (44). The five constituents of the carbonyl group artifacts accounted for 5.3% of the total volatiles upon 12 months at 20 °C. Among them, (+)-carvone (48) was the most prominent. The others were cumin aldehyde, β -thujone, α -thujone (46), and cinnamic aldehyde. The prominent ones among the seven ester group artifacts were *trans,trans*-farnesyl acetate (57), *cis,cis*-farnesyl acetate, and dihydrocarvyl acetate. The others were heptyl acetate, cedryl acetate, *cis,trans*-farnesyl acetate, and citronellyl acetate at minor quantities. The four artifacts of the hydrocarbon group were aromadendrene (30), β -elemene (31), δ -cadinene (32), and *allo*-ocimene (29). Thermal decomposition of α -pinene and linalyl acetate would lead to ocimene, which readily isomerizes to *allo*-ocimene (21). The epoxide artifacts were *trans*-2,3-epoxybutane, *cis*-caryophyllene epoxide, and *trans*-caryophyllene epoxide (60). Two artifact compounds remained unidentified. Formation of the artifacts of various chemical categories in the stored daidai oil could occur due to many factors, including chemical reactions and structural modifications influenced by time and temperature. (+)-Carvone is a rare component of *Citrus* oils (1, 2, 5, 6, 9, 13–15). Formation of this compound as an artifact in daidai oil could be attributed to oxidation of limonene and dehydrogenation of α -terpineol, by virtue of their closely related structures (17). The observed formation of dihydrocarvyl acetate could be attributed to reactions of the corresponding carveol and carvone present in the oil. Sabinene hydrate could be formed by reduction and hydration of sabinene. Structural rearrangement of sabinene would form α -phellandrene, which on oxidization and hydration would lead to piperitone and piperitol, respectively (17). *trans*-Nerolidol could be formed by rearrangement of *cis*-nerolidol. The formation of 1-octen-3-ol, an unusual component of *Citrus* oils, could be attributed to reactions of the related octanal and octyl acetate present in the oil. The oxygenated artifacts of the farnesene group, *cis,trans*-farnesol, *cis,cis*-farnesyl acetate, *cis,trans*-farnesyl acetate, and *trans,trans*-farnesyl acetate could be formed from the related hydrocarbon, *trans*- β -farnesene by oxidative transformations and isomerization (19). β -Eudesmol is a bicyclic sesquiterpene alcohol of the eudesmane group that includes eudalene, β -selinene, and nootkatene (18). β -Elemene was formed in the oil as an artifact. Its cyclization and structural rearrangement could lead to β -selinene (18), which upon oxidation could form β -eudesmol. Formation of the tricyclic sesquiterpene alcohols, globulol, viridiflorol, and spathulenol, as artifacts in the oil could be attributed to oxidation of the germacrene type hydrocarbons, aromadendrene, *allo*-aromadendrene, and bicyclogermacrene, respectively (12, 15, 18–20). The caryophyllene epoxide artifacts could be formed by epoxidation of β -caryophyllene (19). This study showed that storage of daidai oil at –21 °C was efficient in maintaining the compositional stability for up to 12 months while higher temperatures could cause major alterations within 3 months. The compositional alterations and formation of several artifacts in daidai oil stored at 5 and 20 °C could lead to notable deterioration of the sensory quality of the oil. As shown in this work, low temperature storage could protect the oil from deterioration. The results could be applied for monitoring the quality of daidai flavor and related products during storage.

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