

Changes in the Volatile Composition of Yuzu (*Citrus junos* Tanaka) Cold-Pressed Oil during Storage

Simon M. Njoroge, H. Ukeda, and M. Sawamura*

Department of Bioresources Science, Faculty of Agriculture, Kochi University, B-200 Monobe, Nankoku City, Kochi 783, Japan

The changes of 34 components constituting a relative 97.6% of the total volatiles of yuzu (*Citrus junos* Sieb. ex Tanaka) cold-pressed peel oil and the possible artifacts during storage at -21 , 5 , and 20 °C were investigated after 1, 3, 6, 9, and 12 months. Major qualitative changes were found after 12 months at 20 °C. Total monoterpene hydrocarbons underwent a large decrease (from 93.3 to 37.7%), with major losses of limonene, γ -terpinene, myrcene, and α -pinene, while notable increases occurred in *p*-cymene as well as the monoterpene alcohols (from 2.1 to 13.2%). Bicyclogermacrene, the main sesquiterpene hydrocarbon of the fresh oil (1.0%), was virtually depleted. Sesquiterpene alcohols were formed as dominant artifacts (36.1%) and constituted the second most abundant volatile group of the oil after the monoterpene hydrocarbons. (–)-Spathulenol (decahydro-1,1,7-trimethyl-4-methylene-1*H*-cyclopropylazulen-7-ol) was the main artifact among the 14 identified. The alcohol constituted the second major component of the oil after limonene from the ninth month and accounted for more than 75% of the sesquiterpenic alcohols at 20 °C. The spathulenol was found for the first time to be an artifact in yuzu oil. The compound could be a useful index for monitoring the quality and freshness of yuzu flavor during storage.

Keywords: *Citrus junos* Sieb. ex Tanaka; cold-pressed oil; volatile component; storage time; storage temperature; relative composition; sesquiterpenes; artifact; spathulenol

INTRODUCTION

Essential oils of *Citrus* peel are natural flavoring materials of commercial importance. They have been used in beverages, confectionery, pharmaceuticals, cosmetics, and perfumery industries (Pisano, 1986). The quality, freshness, and uniqueness of *Citrus* oils are major considerations pertaining to their value and applications. However, being mixtures of various volatile chemical substances including unsaturated compounds, the oils are generally unstable and tend to change with time and conditions of storage (Sinclair, 1984). Most of the deterioration may occur by oxidation in air, polymerization, and rearrangement and cyclization of some labile constituents under the influences of heat, light, moisture, and catalysts (Usai et al., 1992; Gopalakrishnan, 1994; Dieckmann and Palamand, 1974). The likely losses of some components through evaporation, polymerization, or transformations into other components or artifacts would cause direct changes of the weight percentages of the affected components. Analysis of qualitative changes of the composition of an oil using relative percentage data may not easily predict the occurrence of polymerization and other losses or the compounds that might change quantitatively. Absolute quantitation would require the use of response factors and internal standards to correct for the possible variations of detector responses and the loss of components during analysis. However, qualitative information on the relative changes of the whole profile of the eluted volatiles may be obtained more easily than quantitative data and serve conveniently for quality evaluation.

Reported studies have indicated the occurrence of degradative losses of monoterpene hydrocarbons, in-

cluding limonene, γ -terpinene, α -pinene, β -pinene, sabinene, and myrcene, during storage of essential oils, while *p*-cymene and oxygenated monoterpenes generally undergo increases (Gopalakrishnan, 1994; Usai et al., 1992; Dieckmann and Palamand, 1974). Oils from different *Citrus* species may show different storage stabilities depending on the nature of the volatile components present and the method of extraction. *Citrus* oils extracted by cold-pressing contain higher amounts of coextracted nonvolatile materials than the oils isolated by solvent extraction and steam distillation methods (Shaw, 1979; Lund et al., 1981), which may influence their stability during storage.

Yuzu is the most popular among the sour *Citrus* fruits cultivated in Japan and has a unique, strong, and desirable aroma. The volatile components of yuzu oil have been investigated by many researchers (Nishimura et al., 1968; Shinoda et al., 1970; Kusunose and Sawamura, 1980; Watanabe et al., 1983; Tajima et al., 1990; Yang et al., 1992; Njoroge et al., 1994), reporting large dissimilarities in the identified constituents. Bicyclogermacrene, germacrene D, and germacrene B, first isolated in cold-pressed yuzu oil by Nishimura et al. (1969), have not been reported by many of the other workers, who have, in contrast, indicated bicyclogermacrene, *T*-cadinol, spathulenol, globulol, and eudesmol as the prominent sesquiterpenoids. The reported differences could occur from variations of sample preparation methods, storage, and analytical conditions. A recent study has confirmed bicyclogermacrene occurring as the main sesquiterpene hydrocarbon of the whole cold-pressed yuzu oil, while oxygenated sesquiterpenes were virtually absent (Njoroge et al., 1994). Although essential oils from the same botanical species may show qualitative and quantitative variations attributed to environmental conditions and plant age (Okamoto et al., 1981), the influence of storage conditions may cause profound compositional changes important to quality.

* Author to whom correspondence should be addressed (fax +81-0888-64-5200).

Yuzu flavor is known by users to undergo rapid deterioration during storage. The associated alterations of the chemical composition of the oil have not been systematically studied. The aim of this study was to investigate the relative compositional changes and the possible artifacts of yuzu cold-pressed peel oil after storage for 1, 3, 6, 9, and 12 months at -21 , 5 , and 20 °C.

MATERIALS AND METHODS

Yuzu Fruits. The fruits (*Citrus junos* Sieb. ex Tanaka) were cultivated at the Kochi Prefectural Agricultural Research Centre, Fruit Tree Experiment Station, Japan. Samples were harvested at maturity, in November 1993. The fruits were kept at 2 °C, and the peel oil was extracted within 1 week of harvest.

Extraction of Cold-Pressed Oil. The fruit mesocarp and albedo layers were peeled off and discarded. The flavedo layer with exposed oil sacs was maintained on ice and was pressed by hand to express the oil. The crude oil was collected on ice, saturated with brine, and centrifuged at $2000g$ for 15 min at 4 °C. The supernatant was decanted, dried with anhydrous sodium sulfate for 24 h at 5 °C, and filtered to obtain the cold-pressed oil for the study.

Standard Chemicals. Analytical standards were procured from commercial sources, including Fluka Fine Chemicals, Buchs, Switzerland; Wako Pure Chemical Industries, Osaka; Nacalai Tesque Inc., Kyoto; and Aldrich Chemical Co., Milwaukee, WI. Some standards were presented as gift by Ogawa Flavour Co., Tokyo.

Storage Studies. One milliliter samples of the freshly prepared oil were put in 5 mL airtight amber glass bottles in three sets, each with duplicate samples. The sample vials were closed tightly with Teflon screw caps. One set of the samples was kept at ambient temperature maintained constantly at 20 °C, another set in a refrigerator at 5 °C, and the third set in a freezer at -21 °C. The commonly used laboratory procedure to store experimental samples of essential oils involves the exclusion of air by having a small headspace in the storage vials and purging the same with inert gases, such as nitrogen or argon. However, during commercial processing, handling, storage, and usage practices, essential oils and the products containing their fragrances may encounter conditions allowing some contact with air over long periods. In this study, a limited supply of air was provided by the headspace and when aliquots were drawn for analysis. The qualitative composition of the samples was analyzed when fresh and upon storage for 1, 3, 6, 9, and 12 months at each temperature.

Gas Chromatography (GC). GC analyses were 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 wall coated with Thermo 600T, a polar liquid phase (Shimadzu). The composition of this highly polar liquid phase has not been open to users, but is known to be similar to that of PEG 20M, popularly used for separation of essential oil volatiles. 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 detector temperatures were each at 250 °C. An oil sample of $0.5\text{ }\mu\text{L}$ was injected (split ratio, 50:1). Nitrogen was the carrier gas at a flow rate of 1.0 mL/min . The flow rate of nitrogen make-up gas was 50 mL/min . Relative peak area percentages of the eluted volatile components were integrated by a Shimadzu C-R6A Chromatopack integrator (Shimadzu). The analyses were triplicated.

Gas Chromatography–Mass Spectrometry (GC–MS). GC–MS analyses were conducted using a Shimadzu GC 17A, linked with a QP 5000 MS (Shimadzu). Oil samples of $0.1\text{ }\mu\text{L}$ were injected (split ratio, 32:1). Helium was the carrier gas at a flow rate of 1.0 mL/min . The GC column and separation conditions were similar to those of the GC 14A, indicated above. Mass spectra were obtained with an ionization voltage of 70 eV . The scanning time was 1 s over a range of m/z 30–320. Qualitative analysis of the components was based on their mass spectra similarities with those of standard com-

pounds and the NIST spectral library of the GC–MS and further confirmed by comparing the experimental Kovats indices with those of standards and published data (Grant et al., 1987; Le Quere and Latrasse, 1990; Njoroge et al., 1994).

RESULTS AND DISCUSSION

The yield of cold-pressed peel oil from yuzu fresh fruit was 0.09% (w/w). The oil has recently been reported as containing 77 volatile components; in general terms, the main chemical groups were monoterpene hydrocarbons (95.6%), sesquiterpene hydrocarbons (2.1%), and monoterpene alcohols (2.2%), while aldehydes, esters, and sesquiterpene alcohols were virtually absent (Njoroge et al., 1994). In this study, the relative compositional changes of 34 components, accounting for 97.6% of the fresh oil volatiles, were analyzed upon storage periods at three temperature levels, and the artifacts formed were identified. The compounds were chosen because of their relative prominent levels in the fresh oil and their notable changes on storage. The representative capillary GC chromatograms of the oil when fresh and upon storage for 6 months at 20 °C are presented in parts A and B, respectively, of Figure 1. The injected sample volume ($0.5\text{ }\mu\text{L}$) and the split ratio (50:1) were well resolved and adequate for the analysis. Clear differences between the chromatogram of the fresh oil and that of the oil after 6 months at 20 °C are noticeable in Figure 1. Several new peaks of artifact compounds were formed at 20 °C. The peak identities and their relative area percentages, and the artifacts identified, are listed in Table 1 according to functional group classes. Quantitation of the individual components by use of internal standards and detector response factors was not made in this study. The reported data are relative to the overall aromatic profile, which may serve conveniently for quality evaluation. However, a change in one or a few components would cause a relative percentage change of the whole components of the oil, thus making it difficult to predict the chemical changes that could have occurred. Significant changes of the components were found at 20 °C with prolonged storage, and to a lesser extent at 5 °C, but were minimal at -21 °C.

Relative Changes of the Monoterpene Hydrocarbons. The relative percentage of the monoterpene hydrocarbons decreased to 37.7% on storage for 12 months at 20 °C. Each of the individual components, except *p*-cymene, showed a decreasing trend. The losses at -21 and 5 °C were far less than at 20 °C, and the group decreased to 88.3 and 92.0% , respectively. Of the observable relative losses of the individual monoterpene hydrocarbons after 12 months at 20 °C, those of limonene (from 78.1 to 31.5%), γ -terpinene (from 9.9 to 1.5%), myrcene (from 2.1 to 0.6%), terpinolene (from 0.4 to 0.1%), α -terpinene (from 0.2% to a trace), α -pinene (from 1.8 to 0.7%), β -pinene (from 0.7 to 0.3%), and sabinene (from 0.2 to 0.1%) were prominent. On the other hand, *p*-cymene increased steadily and constituted the second most abundant compound in the group (5.2%) after limonene (51.9%), from the ninth month. At 5 °C, the *p*-cymene increased to twice its original level. However, the relative changes of the peak areas may not fully reflect the actual quantitative changes of the individual components.

The observed changes of monoterpene hydrocarbons in the yuzu oil were in accordance with reports on storage of other oils, such as that of hop (Tressl et al., 1978), Thompson navel orange (Usai et al., 1992), and cardamom and clove bud (Gopalakrishnan, 1994). The

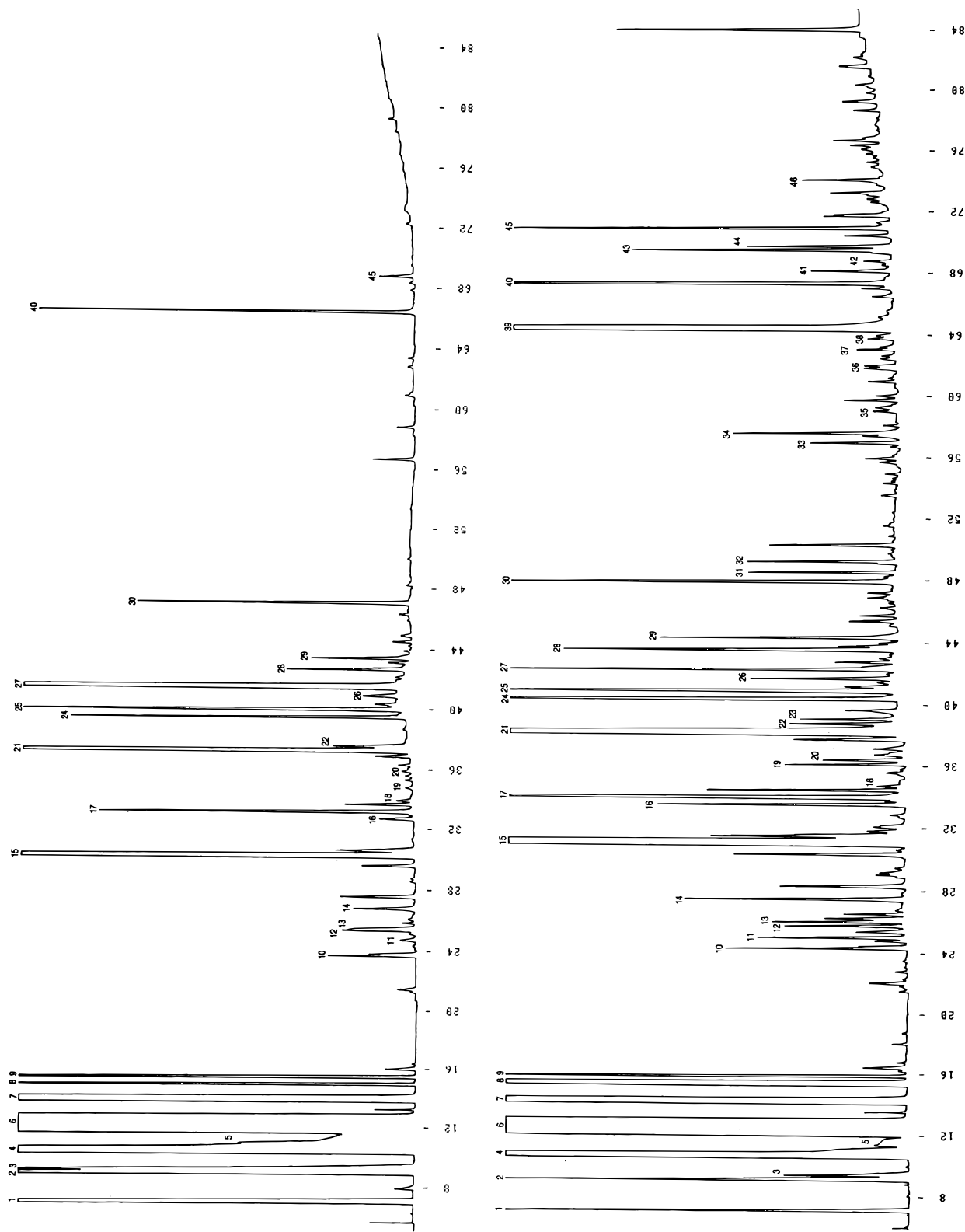


Figure 1. Capillary gas chromatograms of freshly prepared yuzu cold-pressed peel oil (A, top) and of yuzu cold-pressed peel oil stored for 6 months at 20 °C (B, bottom). Peak numbers correspond to those in Table 1.

Table 1. Relative Compositional Changes in Yuzu (*C. junos* Tanaka) Cold-Pressed Oil during Storage

no. ^a	compound ^b	relative concentration ^c (%)															
		fresh oil	−21 °C					5 °C					20 °C				
			1	3	6	9	12	1	3	6	9	12	1	3	6	9	12
monoterpene hydrocarbons																	
1	α-pinene	1.84	1.91	1.94	1.91	1.70	1.52	1.81	1.78	1.69	1.57	1.46	1.95	1.94	1.61	1.45	0.66
2	β-pinene	0.69	0.74	0.73	0.77	0.64	0.61	0.72	0.67	0.68	0.66	0.62	0.74	0.74	0.75	0.59	0.30
3	sabinene	0.24	0.24	0.23	0.34	0.37	0.34	0.22	0.17	0.25	0.21	0.17	0.24	0.25	0.19	0.17	0.08
4	myrcene	2.14	2.06	1.99	2.17	2.02	1.99	2.02	1.92	1.90	1.89	1.98	2.02	1.84	1.36	1.05	0.56
5	α-terpinene	0.15	0.15	0.14	0.23	0.26	0.26	0.13	0.17	0.20	0.27	0.23	0.12	0.12	0.04	0.02	tr
6	limonene	78.13	77.68	77.01	77.03	77.11	76.70	78.02	76.14	75.87	74.54	72.00	77.06	76.54	66.70	51.87	31.49
7	γ-terpinene	9.32	10.24	10.19	10.15	10.02	9.60	10.28	10.04	10.26	10.30	10.37	10.07	9.62	8.01	2.23	1.47
8	p-cymene	0.40	0.37	0.38	0.41	0.48	0.48	0.40	0.50	0.67	0.73	0.89	0.61	0.68	4.65	5.19	3.05
9	terpinolene	0.39	0.42	0.41	0.45	0.47	0.47	0.43	0.42	0.48	0.49	0.50	0.41	0.40	0.50	0.09	0.05
10	α-p-dimethyl styrene + p-mentha-1,4,8-triene	0.04	0.04	0.04	0.04	0.04	0.03	0.04	0.04	0.04	0.05	0.06	0.04	0.04	0.08	0.16	0.06
	total	93.34	93.85	93.06	93.50	93.11	92.00	94.07	91.85	92.04	90.71	88.28	93.26	92.17	83.89	62.82	37.72
monoterpene alcohols																	
15	linalool	1.79	1.95	2.00	2.00	2.08	2.09	1.98	2.05	2.14	2.18	2.25	1.95	1.97	5.43	7.08	3.67
24	α-terpineol	0.10	0.13	0.12	0.14	0.15	0.17	0.13	0.14	0.16	0.16	0.15	0.13	0.13	0.49	1.24	1.36
30	(Z)-carveol	0.07	0.07	0.06	0.13	0.13	0.11	0.06	0.06	0.12	0.13	0.12	0.06	0.06	0.24	0.36	0.63
31	nerol														0.07	0.33	0.59
32	geraniol														0.07	0.32	0.45
34	perillyl alcohol														0.05	0.71	1.22
40	thymol	0.12	0.11	0.11	0.14	0.15	0.16	0.13	0.12	0.16	0.17	0.18	0.12	0.11	0.56	2.13	4.20
45	p-mentha-1,8-dien-10-ol	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.01	0.05	0.97	1.04
	total	2.08	2.26	2.29	2.41	2.51	2.53	2.30	2.37	2.58	2.64	2.70	2.26	2.27	6.72	10.81	9.86
oxides and epoxides																	
11	(Z)-limonene oxide	tr	tr	tr	tr	0.01	0.02	tr	tr	tr	tr	tr	tr	0.01	0.07	0.15	0.07
12	(E)-limonene oxide	0.01	0.03	0.03	0.03	0.03	0.04	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.09	0.06
33	(Z)-caryophyllene epoxide		0.01	0.01	0.02	0.02	tr	0.02	0.01	0.02	0.02	tr	0.01	0.02	0.09	0.24	0.53
35	(E)-caryophyllene epoxide		0.01	0.01	0.01	0.01	tr	0.01	0.01	0.01	0.01	tr	0.01	0.01	0.03	0.16	0.48
	total	0.01	0.05	0.05	0.06	0.07	0.06	0.06	0.04	0.06	0.06	0.03	0.05	0.07	0.22	0.64	1.14
sesquiterpene hydrocarbons																	
13	δ-elemene	0.09	0.09	0.08	0.03	0.03	0.02	0.09	0.09	0.09	0.09	0.06	0.08	0.06	0.09	0.16	0.23
14	α-copane	0.01	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.04	0.04	0.03	0.03	0.03	0.03	0.15	0.15
16	β-elemene	0.04	0.04	0.04	0.02	0.02	0.04	0.04	0.04	0.05	0.05	0.05	0.04	0.04	0.13	0.18	0.16
17	caryophyllene	0.17	0.17	0.15	0.15	0.16	0.14	0.16	0.16	0.17	0.17	0.17	0.16	0.17	0.17	0.20	0.26
18	aroma	0.01	0.01	tr	tr	tr	0.03	0.01	0.01	0.01	0.01	0.04	0.01	0.01	0.01	0.01	0.02
19	dendrene																
19	γ-elemene	0.02	0.01	0.01	tr	tr	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.05	0.05	0.30
20	allo-aroma dendrene	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.05	0.10
21	(E)-β-farnesene	0.45	0.63	0.58	0.64	0.68	0.62	0.63	0.65	0.72	0.71	0.72	0.60	0.63	2.13	2.78	2.51
22	α-humulene	0.04	0.03	0.03	0.04	0.04	tr	0.04	0.04	0.05	0.05	tr	0.03	0.04	0.06	0.19	0.20
23	δ-muurolene												tr	tr	tr	0.05	0.06
25	germacrene D	0.20	0.21	0.19	0.20	0.20	0.12	0.20	0.19	0.21	0.20	0.20	0.19	0.17	0.19	0.16	0.16
26	α-muurolene	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.05	0.04	0.15	0.04	0.03	0.04	0.22	0.13
27	bicyclogermacrene	0.99	0.94	0.84	1.02	1.07	0.94	0.92	0.87	1.03	1.02	0.97	0.82	0.52	0.31	0.14	0.03
28	δ + γ-cadinene	0.04	0.04	0.04	0.06	0.06	0.04	0.04	0.04	0.07	0.08	0.07	0.04	0.04	0.07	0.15	0.32
29	sesquiphellandrene	0.03	0.04	0.03	0.04	0.05	0.03	0.04	0.03	0.05	0.05	0.04	0.03	0.04	0.18	0.21	0.63
	total	2.14	2.29	2.07	1.98	2.40	2.07	2.28	2.27	2.57	2.53	2.52	2.10	1.80	3.51	4.70	5.26
sesquiterpene alcohols																	
36	globulol	0.01	0.01	0.01	tr	tr	tr	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.10	0.35
37	elemol													tr	0.03	0.07	0.16
38	viridiflorol														tr	0.02	0.35
39	spathulenol		tr	tr	tr	0.01	0.01	tr	0.02	0.04	0.05	0.07	0.03	0.26	2.64	14.05	28.25
41	α-cadinol		0.01	0.01	0.02	0.02	0.10	0.09	0.11	0.11	0.12	0.16	tr	0.02	0.13	0.24	1.88
42	T-cadinol									0.01	0.01	0.09			tr	0.21	0.52
43	β-eudesmol		tr	tr	tr	tr	tr	tr	tr	0.01	0.01	0.01	tr	0.02	0.18	0.47	1.91
44	α-eudesmol															tr	0.11
46	(Z,E)-farnesol						tr					0.01			tr	tr	2.55
	total	0.01	0.02	0.02	0.02	0.03	0.11	0.10	0.14	0.18	0.20	0.35	0.04	0.31	3.00	15.16	36.08
	total natural volatiles (%)	97.58	98.44	97.46	97.92	98.06	96.66	98.69	96.52	97.23	95.92	93.54	97.66	96.29	94.24	78.62	53.26
	total artifact volatiles (%)		0.03	0.03	0.05	0.06	0.11	0.12	0.15	0.30	0.22	0.34	0.05	0.34	6.32	17.79	40.04

^a Peak numbers refer to Figure 1. ^b Components with prominent changes during storage. Z = *cis*; E = *trans*. ^c Average of three determinations. tr, trace (<0.01%). 1, 3, 6, 9, and 12 refer to months of storage.

considerable decrease of the relative percentage of limonene at 20 °C could be attributed to polymerization, evaporation, some oxidation, and possible rearrangement. Some loss of limonene occurred by oxidation, evidenced by the formation of small amounts of *cis*- and *trans*-limonene oxides as artifacts in the oil. Although limonene would also be expected to undergo losses by cyclizing to camphene, α -pinene, and β -pinene as previously noted (Dieckmann and Palamand, 1974), these transformations were not observable from the relative percentage data. Camphene was completely absent from the oil, and both α - and β -pinenes decreased at 20 °C. In general, there was not much evidence of rearrangement of the monoterpene hydrocarbons. The losses of α -pinene and β -pinene could be attributed to polymerization and possible evaporation. Although the two pinenes would likely undergo heat-catalyzed isomerization, the former isomer leading to α -terpinene and the later to myrcene (Pelter, 1969), these changes were not observed, and the possible isomerization products showed decreasing percentages. The possibility of myrcene cyclizing to α -terpinene, γ -terpinene, limonene, and terpinolene, as previously indicated (Dieckmann and Palamand, 1974), was not observed in this work, where myrcene constituted a small percentage in the fresh oil and the *p*-menthadienes decreased during storage. The observed relative decrease of sabinene was not well understood but could have possibly occurred by evaporation or polymerization. The increase of *p*-cymene could occur by dehydrogenation and rearrangement of α - and γ -terpinene, leading to the triple endocyclic double bonds of the *p*-cymene structure. *p*-Cymene could also be formed by hydrogenation and double-bond rearrangement of limonene. In general, the losses of the monoterpene hydrocarbons could be attributed to oxidation, polymerization, and evaporation. There was a noticeable slight decrease of the volume and an increase of viscosity of the oil after 12 months at 20 °C.

Relative Changes of the Monoterpene Alcohols.

The monoterpene alcohol group underwent a large relative increase and was sensitive at each storage temperature. The constitution of the group increased from 2.1, to 2.5% at -21 °C, to 2.7% at 5 °C, and to 13.2% at 20 °C after 12 months storage. At each temperature, the alcohols initially present in the oil, including linalool, α -terpineol, *cis*-carveol, and thymol, underwent some relative increase. Nerol, geraniol, perillyl alcohol, and *p*-mentha-1,8-dien-10-ol, amounting to a relative 3.3% after 12 months, were found as artifacts. Linalool, the main oxygenated constituent of the fresh oil (1.8%), increased to 2.1% at -21 °C, to 2.3% at 5 °C, and to 3.7% at 20 °C after 12 months, attaining a peak (7.1%) after 9 months, and decreased to 3.7% after 12 months. Thymol accounted for the main monoterpene alcohol of the oil upon 12 months of storage (4.2%). α -Terpineol and *cis*-carveol also increased prominently under similar conditions.

The changes of the monoterpene alcohols could be related to degradations of some monoterpene hydrocarbons. The increase of linalool, and the formation of geraniol and nerol, could be attributed to autooxidation of myrcene. The three alcohols have previously been shown to occur directly by autooxidation of myrcene (Dieckmann and Palamand, 1974). Linalool could be oxidized to linalool oxide, but this change was not detected in the oil. The observed increase of thymol could be attributed to dehydrogenation and hydration of α - and γ -terpinene, in view of their closely related structures. These monoterpene hydrocarbons under-

went prominent decreases in the same oil. α -Terpineol would likely be formed by oxidation of limonene, as previously observed (Dieckmann and Palamand, 1974). This alcohol is a well-known hydration product of limonene under acidic conditions and has been suggested as a useful compound for reflection on extraction methods and storage history of *Citrus* oils (Wilson and Shaw, 1980).

Relative Changes of the Sesquiterpene Hydrocarbons. A total of 14 sesquiterpene hydrocarbons were identified in the fresh oil. Among the components, bicyclogermacrene constituted the highest relative percentage (1.0%). The others relatively notable were *trans*- β -farnesene (0.5%), germacrene D (0.2%), and caryophyllene (0.2%). The constitutions of the functional group, and of the individual components, were not much changed after 12 months at -21 °C. However, at 5 and 20 °C, the group increased to 2.5 and 5.2%, respectively. δ -Muuroleone was formed as an artifact at 20 °C. Of the individual components, bicyclogermacrene decreased by the highest proportion and was virtually absent from the oil at 20 °C after 12 months. Similarly, germacrene D decreased by an extent of 20%. On the other hand, δ -, β -, and γ -elemene, α -copaene, aromadendrene, *allo*-aromadendrene, α - and δ -muuroleone, *trans*- β -farnesene, δ - and γ -cadinene, α -humulene, and sesquiphellandrene underwent relative increases on prolonged storage. *trans*- β -Farnesene showed the largest increase (from 0.5% to 2.5%).

The observed changes in the constitution of the sesquiterpene hydrocarbons at 20 °C could be explained to some extent by the possible reactions of bicyclogermacrene and germacrene D. The two compounds constituted 55% of the sesquiterpenes in the fresh oil. Germacrene-type hydrocarbons are known to be particularly labile, undergoing oxidization, isomerization, and rearrangement under suitable conditions of air, heat, acid, light, or other agents (Nishimura et al., 1969; Yoshihara et al., 1969; Sorm, 1971). Bicyclogermacrene is thought to be the possible precursor of tricyclic sesquiterpenes (Tressl et al., 1983). Isomerization of the bicyclic sesquiterpene would likely lead to aromadendrene, *allo*-aromadendrene, and δ -cadinene, which were found to increase in the oil at 20 °C. Bicyclogermacrene has been indicated to be capable of biogenetic-type transformations leading to aromadendrene, α -gurjunene, and viridiflorene (Tressl et al., 1983). Bicyclogermacrene, previously reported as a major component of yuzu oil (Yang et al., 1992), and a possible product of Cope rearrangement of bicyclogermacrene, was not found under the conditions of the present study. Germacrene D may transform into cadinenes, amorphanes, and muurolenes (Sorm, 1971). The possible isomerization products of germacrene D under heat and silica gel chromatography, such as δ - and γ -cadinenes and α - and γ -muurolenes, and those from photoisomerization, including α - and β -bourbonenes and β -copaene, have been previously demonstrated (Yoshihara et al., 1969). The increases of δ - and γ -cadinenes and α - and γ -muurolenes at 20 °C corresponded with the decreases of germacrene D and could be attributed to its possible thermal isomerization. The increase of γ -elemene at 20 °C could occur by isomerization of germacrene B, as has previously been pointed out (Herout, 1971). Germacrene B has been reported at a trace quantity in yuzu cold-pressed oil (Nishimura et al., 1969; Njoroge et al., 1994). This compound has been demonstrated to transform to γ -elemene and selina-3,7-diene during isolation by preparative GC (Tressl et al., 1983). These possible derivatives of germacrene B were observed to increase

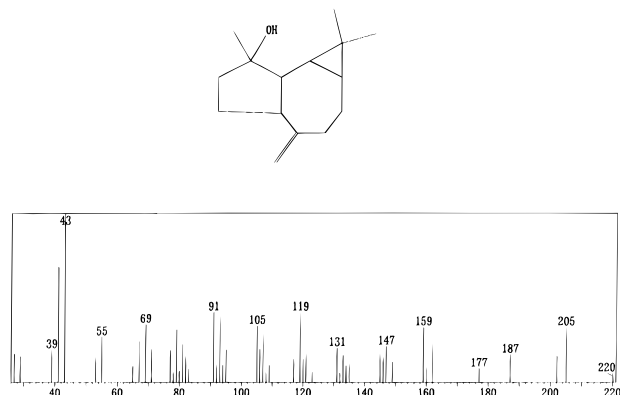


Figure 2. Mass spectrum fragmentation pattern and structure of (–)-spathulenol (decahydro-1,1,7-trimethyl-4-methylene-1*H*-cyclopropylazulen-7-ol).

by small percentages. β -Elemene has been suggested to be formed by thermal isomerization of germacrene C (Sorm, 1971), but this precursor was absent from the yuzu oil. It could be possible that some sesquiterpenes were formed by decomposition of other compounds during the analysis. The large relative increase of *trans*- β -farnesene could not be explained from changes of the other sesquiterpenes present in the oil. This compound has been reported to undergo large increases in lemon oil during fractionation with silica gel column chromatography (Chamblee et al., 1991). The occurrence of *cis*- and *trans*-caryophyllene epoxides (1.0%) could be attributed to epoxidation of caryophyllene. However, this chemical change was not reflected in the relative percentages data among the individual components.

Relative Changes of the Sesquiterpene Alcohols.

Sesquiterpene alcohols were virtually absent from the fresh oil, for which only globulol at a low percentage (trace) was detected. However, the relative constitution of the group increased on storage, and eight artifacts were identified. The group increased to 0.1% at -21°C , to 0.4% at 5°C , and to 36.1% at 20°C after 12 months. Of the artifacts, (–)-spathulenol (decahydro-1,1,7-trimethyl-4-methylene-1*H*-cyclopropylazulen-7-ol) was the main constituent (28.3%). The others were *cis,trans*-farnesol (2.6%), β -eudesmol (1.9%), α -cadinol (1.9%), *T*-cadinol (0.5%), and viridiflorol (0.5%).

The mass spectrum and structure of (–)-spathulenol are shown in Figure 2. The MS fragmentation pattern of the sesquiterpene alcohol was well matched with that of spathulenol A reported by Le Quere and Latrassé (1986). Spathulenol has previously been reported as a component of yuzu oils extracted by solvents and fractionated with silica gel column chromatography (Shinoda et al., 1970; Watanabe et al., 1983). The compound was completely absent from the fresh cold-pressed oil used in this study. (–)-Spathulenol was found for the first time to be an artifact in yuzu oil. Its geometric isomer, (+)-spathulenol, has been reported as a major constituent of blackcurrant (*Ribes nigrum* L.) bud oil isolated by preparative GC (Le Quere and Latrassé, 1986). The alcohol has also been reported to occur as a major component of hyssop (*Hyssopus officinalis* L.) oils isolated by Soxhlet extraction and supercritical carbon dioxide, the two oils also containing germacrene D, bicyclogermacrene, hedycaryol, ledol, and *T*-cadinol at abundant levels (Kerrolla et al., 1994).

The increase of the relative percentages of the sesquiterpene alcohols at 20°C could, to some extent, be attributed to the possible reactions of the germacrene hydrocarbons. Spathulenol could be formed directly

from bicyclogermacrene on air oxidation. The possible isomerization of bicyclogermacrene to aromadendrene and *allo*-aromadendrene, and the subsequent oxidation of these isomeric tricyclic sesquiterpenes, could also possibly lead to spathulenol, as has been previously demonstrated (Nishimura et al., 1969). Some studies on synthesis and reduction of sesquiterpene epoxides under stringent reaction conditions, including LiAlH_4 as reducing catalyst, have demonstrated the transformations of aromadendrene into globulol, *allo*-aromadendrene into viridiflorol, δ -cadinene into torreyol (δ -cadinol) and *T*-muurolol, and γ -cadinene into α -cadinol and *T*-cadinol (Tressl et al., 1983). In this storage study, some of these possible transformation products were found as artifacts in the yuzu oil. The possibility of thermal isomerization and pyrolyses of polymerized compounds at the injector (250°C), column (230°C), and detector (250°C), if operative, would likely cause the degradation of the components, leading to artifact formation, as has previously been noted (Horning et al., 1963). Elemol could be a possible artifact from the Cope rearrangement of hedycaryol during GC analysis (Schulz and Stahl-Biskup, 1991). However, this reaction was unlikely to occur in the yuzu oil, which did not contain the germacrenol.

Published information about qualitative or quantitative chemical changes of sesquiterpenic compounds in *Citrus* oils during storage has been scarce. The observed large increase of sesquiterpene alcohols in the stored yuzu oil was unprecedented in *Citrus* oil studies. The formation of spathulenol to the high extent at 20°C could not be explained by the relatively small amount of sesquiterpene hydrocarbons in the fresh oil. Moreover, it would also be unlikely for the cold-pressed oil to contain enzymes to convert monoterpenes to sesquiterpenes. It would be likely that the monoterpene hydrocarbons polymerized to high-boiling nonvolatile compounds not eluting from the column in large amount, thus causing large differences between the integrated relative percentage data of the eluted volatiles and the actual composition of the oil. These results indicate that the qualitative composition of yuzu oil is quite unstable on storage at 20°C from the third month. The oil could, however, retain its original quality for 6 months at 5°C and was virtually unchanged at -21°C at up to 12 months. The large and consistent increase of spathulenol at room temperature could be a suitable index for monitoring the storage quality and freshness of yuzu oil and its products.

LITERATURE CITED

- Chamblee, T. S.; Clarke, Jr., B. C.; Brewster, G. B.; Radford, T.; Iacobucci, G. A. Quantitative analysis of the volatile constituents of lemon peel oil. Effects of silica gel chromatography on the composition of its hydrocarbon and oxygenated fractions. *J. Agric. Food Chem.* **1991**, *39*, 162–169.
- Dieckmann, R. H.; Palamand, S. R. Autoxidation of some constituents of hops. The monoterpene hydrocarbon, myrcene. *J. Agric. Food Chem.* **1974**, *22*, 498–503.
- Gopalakrishnan, N. Studies on the storage quality of CO_2 -extracted cardamom and clove bud oils. *J. Agric. Food Chem.* **1994**, *42*, 796–798.
- Herout, V. Biochemistry of sesquiterpenoids. In *Aspects of Terpenoid Chemistry and Biochemistry*; Goodwin, T. W., Ed.; Academic Press: London, 1971; pp 53–90.
- Horning, E. C.; Maddock, K. C.; Anthony, K. V.; Vandenheuvell, W. J. A. Quantitative aspects of gas chromatographic separations in biological studies. *Anal. Chem.* **1963**, *35*, 526–532.
- Kerrolla, K.; Galambosi, B.; Kallio, H. Volatile components of four phenotypes of hyssop (*Hyssopus officinalis* L.). *J. Agric. Food Chem.* **1994**, *42*, 776–781.

- Kusunose, H.; Sawamura, M. Aroma constituents of some sour citrus oils. *Nippon Shokuhin Kogyo Gakkaishi* **1980**, *27*, 517–521.
- Le Quere, J. L.; Latrasse, A. Identification of (+)-spathulenol in the essential oil of blackcurrant buds (*Ribes nigrum* L.). *Sci. Aliments* **1986**, *6*, 47–59.
- Le Quere, J. L.; Latrasse, A. Composition of the essential oils of blackcurrant buds (*Ribes nigrum* L.). *J. Agric. Food Chem.* **1990**, *38*, 3–10.
- Lund, E. D.; Shaw, P. E.; Kirkland, C. L. Composition of rough lemon leaf oil. *J. Agric. Food Chem.* **1981**, *29*, 490–494.
- Nishimura, K.; Shinoda, N.; Hirose, Y. A new sesquiterpene, bicyclogermacrene. *Tetrahedron Lett.* **1969**, 3097–3100.
- Njoroge, S. M.; Ukeda, H.; Kusunose, H.; Sawamura, M. Volatile components of Japanese yuzu and lemon oils. *Flavour Fragrance J.* **1994**, *9*, 159–166.
- Njoroge, S. M.; Ukeda, H.; Kusunose, H.; Sawamura, M. Japanese sour *Citrus* fruits. Part III. Volatile constituents of sudachi and mochiyuzu oils. *Flavour Fragrance J.* **1995**, *10*, 341–347.
- Okamoto, R. A.; Ellison, B. O.; Kepner, R. E. Volatile terpenes in *Sequoia sempervirens* foliage. Changes in composition during maturation. *J. Agric. Food Chem.* **1981**, *29*, 324–326.
- Pelter, A.; (in part) Harper, S. H. Bicyclic monoterpenoids and related compounds. In *Rodd's Chemistry of Carbon Compounds*, 2nd ed., Part C; Coffey, S., Ed.; Elsevier: Amsterdam, 1969; pp 136–249.
- Pisano, R. C. The future of natural essential oils in the flavor and fragrance industry. *Perfum. Flavor.* **1986**, *11*, 35–41.
- Schulz, G.; Stahl-Biskup, E. Essential oils and glycosidic bound volatiles from leaves, stems, flowers and roots of *Hyssopus officinalis* L. (Lamiaceae). *Flavour Fragrance J.* **1991**, *6*, 69–73.
- Shaw, P. E. Review of quantitative analyses of citrus essential oils. *J. Agric. Food Chem.* **1979**, *27*, 246–257.
- Shinoda, N.; Shiga, M.; Nishimura, K. Constituents of yuzu (*Citrus junos*) oil. *Agric. Biol. Chem.* **1970**, *34*, 234–242.
- Sinclair, B. W. Chemical composition of essential oils. In *The Biochemistry and Physiology of the Lemon and Other Citrus Fruits*; Sinclair, B. W., Ed.; Division of Agriculture and Natural Resources; The Regents of the University of California: Los Angeles, 1984; pp 249–287.
- Sorm, F. Sesquiterpenes with ten-membered carbon rings—a review. *J. Agric. Food Chem.* **1971**, *19*, 1081–1087.
- Tajima, K.; Tanaka, S.; Yamaguchi, T.; Fujita, M. Analysis of green and yellow yuzu peel oils (*Citrus junos* Tanaka). Novel aldehyde components with remarkably low odor thresholds. *J. Agric. Food Chem.* **1990**, *38*, 1544–1548.
- Tressl, R.; Friesel, L.; Fendessack, F.; Köppler, H. Studies on the volatile composition of hops during storage. *J. Agric. Food Chem.* **1978**, *26*, 1426–1431.
- Tressl, R.; Engel, K. H.; Kossa, M.; Köppler, H. Characterization of tricyclic sesquiterpenes in hop (*Humulus lupulus*, var *Hersbrucker Spät*). *J. Agric. Food Chem.* **1983**, *31*, 892–897.
- Usai, M.; Arras, G.; Fronteddu, F. Effects of cold storage on essential oils of peel of Thompson navel oranges. *J. Agric. Food Chem.* **1992**, *40*, 271–275.
- Watanabe, I.; Yanai, T.; Furuhashi, A.; Awano, K.; Kogami, K.; Hayashi, K. Volatile components of yuzu (*Citrus junos*). In *Proceedings of the 9th International Congress of Essential Oils*, Singapore, 1983; pp 78–82.
- Wilson, C. W.; Shaw, P. E. Glass capillary gas chromatography for quantitative determination of volatile constituents in cold-pressed grapefruit oil. *J. Agric. Food Chem.* **1980**, *28*, 919–922.
- Wyllie, S. G.; Cook, D.; Brophy, J. J.; Richter, K. M. Volatile flavor components of *Annona atemoya* (custard apple). *J. Agric. Food Chem.* **1987**, *35*, 768–770.
- Yang, R.; Sugisawa, H.; Nakatani, H.; Tamura, H.; Takagi, N. Comparison of odor quality in peel oils of acid citrus. *Nippon Shokuhin Kogyo Gakkaishi* **1992**, *39*, 16–24.
- Yoshihara, K.; Sakai, T.; Hirose, Y. Germacrene D, a key intermediate of cadinene group compounds and bornenenes. *Tetrahedron Lett.* **1969**, 2263–2264.

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