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Characteristic Aroma Components of Tosa-buntan (*Citrus grandis* Osbeck forma *Tosa*) Fruit

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The volatile components of Tosa-buntan (*Citrus grandis* Osbeck forma *Tosa*) cold-pressed peel oil were studied by GC and GC-MS. The characteristic aroma compounds were investigated by GC-olfactometry in which a semi-bore capillary column, DB-Wax, was used for effective separation of the volatile components of the oil. Hydrocarbons including mono and sesquiterpenes of the oil accounted for about 98% of the volatile components. The characteristic flavor was also present in the oxygenated fraction. Flavor dilution (FD) factors of the volatile flavor components of the cold-pressed oil were determined by aroma extraction dilution analysis. The relative flavor activity derived from FD-factor and peak area percent was used in this experiment. It was suggested that compounds with higher relative flavor activity such as decanoic acid, heptyl acetate, α-bisabolol, nonanal, *cis*, *trans*-farnesol, *trans*-nerolidol and 2-dodecenal would contribute to Tosa-buntan flavor. Careful sniff testing revealed 2-dodecenal to be a characteristic or key compound of Tosa-buntan aroma. A solution of this authentic compound below 2 ppm gave a pleasant and refreshing aroma similar to Tosa-buntan flavor.

Keywords: *Citrus*, Tosa-buntan, *Citrus grandis* Osbeck forma *Tosa*, aromagram, GC-olfactometry, characteristic aroma components, 2-dodecenal

The essential oil of citrus flavor is the largest natural fragrance product. Citrus flavor has been widely used in many foods, beverages, seasonings, dressings, cosmetics and toiletry products. Flavors such as orange, lemon, grapefruit, bergamot, mandarin, lime and nerori (orange flower oil) are among the favorite essential oils not only as food additives, but also in the field of aromatherapy. Tosa-buntan, *Citrus grandis* Osbeck forma *Tosa*, belonging to the pummelo species, is the most popular among the pummelos cultivated in Japan, and produced primarily in Kochi Prefecture on Shikoku island, Japan. The total production in 1997 was about 9200 tons. Tosa-buntan has a good taste with a nice balance of sweet and sour, and a pleasant and refreshing aroma. This fruit is commonly harvested late in December and then stored for a few months in order to improve its taste and aroma. It is sold commercially at peak quality between February and April. The volatile components of the essential oil have been quantitatively determined and reviewed (Sawamura & Kuriyama, 1988; Sawamura *et al*., 1989, 1990, 1991; Sawamura, 1994). The primary components of Tosa-buntan, aside from the monoterpene hydrocarbons limonene, γ-terpinene, myrcene and α-pinene, are decanal, nootkatone, linalol and citronellal. There have been a number of papers on the volatile components in flavor research. Citrus essential oils have been very popular materials for chemical study (Shaw, 1979; Sawamura, 2000). However, the authors believe that comprehensive evaluation of the flavor should be accomplished by simultaneous chemical and sensory analyses. In our view this is one of the goals of flavor studies. A method for aroma extraction dilution analysis (AEDA) has now been developed to determine the characteristic compounds of the distinct flavor of a food (Acree, 1993). In reported research, the characteristic aroma of the Japanese citrus fruits sudachi (*C. sudachi* Hort. ex Shirai) (Padrayuttawa *et al*., 1997), yuzu (*C. junos* Sieb. ex Tanaka) (Song *et al*., 2000a) and daidai (*C. aurantium* Linn. var. *Cyathifera* Y. Tanaka) (Song *et al*., 2000b) were studied by means of AEDA. The present study aimed to perform a general analysis of the essential oil and to determine the characteristic aroma compounds of Tosa-buntan flavor.

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Materials and Methods

Materials The fruit of Tosa-buntan (*Citrus grandis* Osbeck forma *Tosa*) was provided by the Kochi Fruit Tree Experimental Station (Kochi) in December 1998 and 1999. The essential oil was prepared by hand pressing of the flavedo as previously reported (Sawamura & Kuriyama, 1988).

Fractionation of Tosa-buntan peel oil The essential oil was fractionated into hydrocarbon and oxygenated fractions with a silica gel column (25 cm × 2 cm i.d.) packed with Wako gel Q-100 (Wako Pure Chemical Industries, Osaka). About 1 g of the cold-pressed oil was applied onto the column. About 700 ml of *n*-hexane was eluted to obtain hydrocarbons until no yellow spot was detected on a filter paper with bromine. Then, 700 ml of the second solvent, ethyl acetate, was used to collect oxygenated compounds. Each fraction was concentrated under reduced pressure at room temperature. The oil extracts were kept at –25°C until analyzed.

GC and GC-MS GC was a Shimadzu GC-14A equipped with a flame ionization detector, and GC-MS was a Shimadzu GC-MS QP-5000 equipped with a Shimadzu GC-17A. The analytical conditions were described earlier (Sawamura *et al*., 1999). The column was a fused silica capillary column, DB-Wax (60 m × 0.53 mm i.d., 0.25 μm in film thickness; J & W Scientific, Folsom, CA). The operating conditions were as follows: injector and detector temperatures, 250°C; column temperature, programmed from 70°C to 230°C at 2°C/min after being held at 70°C from 2

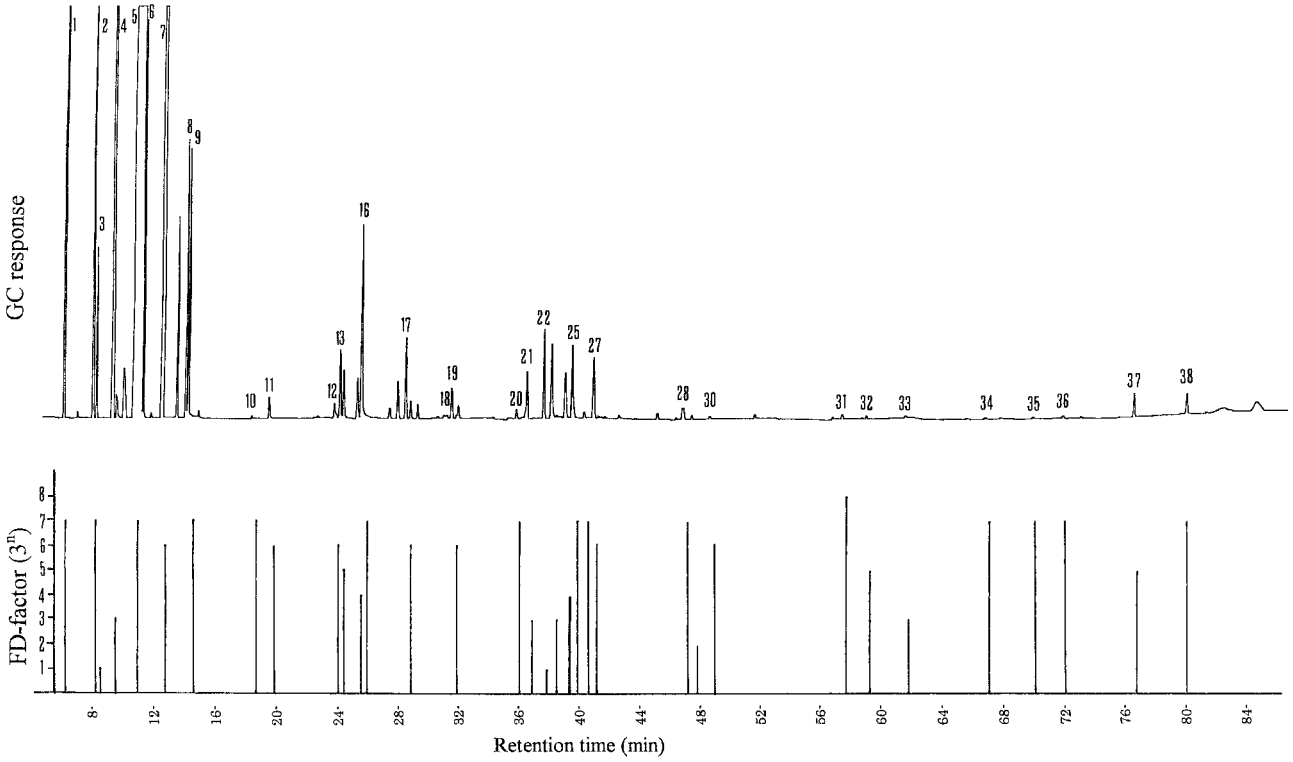

Fig. 1. Gas chromtogram (top) and aromagram (bottom) of volatile flavor components of Tosa-buntan peel oil.

min, then finally kept at 230°C for 20 min. The carrier gas (nitrogen) flow rate was 1 ml/min and the split ratio was 1:50 v/v. Identification was made using GC-MS and Kováts retention indices. The indices were estimated using two fused silica capillary columns under the same GC conditions as follows: DB-Wax (60 m × 0.22 mm i.d., 0.25 μm in film thickness; J & W Scientific, Folsom, CA) and DB-1 (30 m × 0.22 mm i.d., 0.25 μm in film thickness; J & W Scientific, Folsom, CA). The quantitative measurement was triplicated.

GC-olfactometry GC was a Shimadzu GC-8A equipped with a flame ionization detector. The column was a semi-bore capillary column, DB-Wax (60 m × 0.53 mm i.d., 0.25 μm in film thickness). The flow rate of carrier gas (nitrogen) was 5 ml/min. The oven condition and injector and detector temperatures were the same as those given above for GC. The outlet of the column was split into two ways using a GlassSeal "Y" connector (SUPELCO, Bellefonte, PA): one way leading to the detector and the other to the sniffing port. The split ratio of the detection to the sniffing was 1 to 4. The sniffing port was always heated with a flexible heater at 250°C to avoid condensation of the volatile materials in the glass tube. The carrier gas stream for sniffers was moistened at the outlet for sniffing to prevent the nose from drying. The panel was composed of three previously trained individuals. The cold-pressed Tosa-buntan oil stepwise 3-fold diluted with acetone until the sniffers could not detect any significant odor in a run (Acree, 1993).

Results and Discussion

Volatile components of Tosa-buntan peel oil A chromatogram and aromagram of Tosa-buntan peel oil are shown in Fig. 1. Thirty-eight peaks of the essential oil detected were identified by GC-MS, retention indices on a polar and an apolar column and co-injection with authentic compounds. The relative peak area percents of those compounds are given in Table 1. The monoterpene hydrocarbons such as limonene, ocimene, myrcene, α-pinene, β-pinene, sabinene, γ-terpinene and terpinolene accounted for 97.18% on the basis of relative peak area percent, higher than the weight percent reported previously (Sawamura *et al.*, 1989, 1991). The percent of sesquiterpenes β-elemene, α-humullene and germacrene D was 0.14%.

Among oxygenated compounds the aliphatic aldehydes octanal, nonanal, decanal, dodecanal, 2-dodecenal and hexadecanal were detected, and they were relatively rich in the peel oil, accounting for 0.65%. Alcohols such as linalol, α-terpineol, citronellol, perillyl alcohol, *trans*-nerolidol, elemol, α-bisabolol and *cis*, *trans*-farnesol accounted for 0.52%. Octyl acetate was a major component of esters. Nootkatone is an important indicator for identifying the species of *C. grandis* and *C. paradisi* (Sawamura *et al.*, 1991, 1994b), as well as one of the character-impact compounds of grapefruit (MacLeod & Buigues, 1964; Nursten, 1979; Demole & Enggist, 1983). Thirteen additional compounds identified in the CPO analysis of the oxygenated fraction were: hexyl acetate, allyl caproate, isopulegol, camphene hydrate, menthol, isooctanol, citronellyl acetate, dodecanal, geranyl propionate, *cis*-caryophyllene oxide, α-eudesmol, isopinocampherol and β-ionol.

Flavor dilution analysis of cold-pressed oil The cold-pressed oil was directly provided to the sniff testing in AEDA. The flavor dilution factor (FD-factor) was expressed by powers of three. An aromagram together with a gas chromatogram is shown in Fig. 1 (below). It will be seen that there is a great difference between the responses of GC and sensory evaluation. A

very small peak like heptyl acetate on the gas chromatogram, for instance, was detected as a predominant peak on the aromagram. As shown in Table 1, the range of the factors of each peak was between 1 and 8. *trans*-Nerolidol was the highest FD-factor as 8, while sabinene and α-terpineol were the lowest. Components which had an FD-factor of 7 were as follows: four terpene hydrocarbons, α-pinene, β-pinene, limonene and α-humullene; four aldehydes, octanal, decanal, geranial and 2-dodecenal; two alcohols, α-bisabolol and *cis*, *trans*-farnesol; two esters, heptyl acetate and geranyl acetate; and one ketone, nootkatone. Most components showed higher factors than 4; these higher FD-factors are often related to the top note of the aroma. However, the FD-factor also depends on the concentration. A case often occurs where the higher the content of a compound is, the higher the FD-factor is. Therefore, the FD-factor does not always represent a significant contribution to aroma. We proposed the following equation: relative flavor activity=log $3^n/S^{0.5}$, where 3^n is FD-factor and *S* is peak area percent (Song *et al*., 2000a).

The FD-factor is considered the value in the oil sample, and appears to be equivalent to the odor activity or odor unit of an authentic compound in a standard solution (Blank & Grosch, 1991). The theoretical odor unit (Guadagni *et al*., 1963; Nursten, 1979) represented by the quotient of threshold value and concentration is applicable only in cases where all the compositions in a given sample are preliminarily identified. The threshold value may, however, be impossible to determine unless the authentic compound is available. In contrast to the principle of the odor unit, the FD-factor can be defined whether the compound is known or not, or available or not, if the peak is detected by GC and separated properly on the chromatogram. Therefore, the concept of relative flavor activity can be applied to a wide range of flavor investigation. The peak area percents in Table 1 are rounded to two decimal places. However, when the relative flavor activity of each component was figured out, the peak area percents were taken to three places.

The overvaluation resulting from concentration may be reduced by this calculation. Limonene is, for instance, the most predominant component and its FD-factor is as high as 7. However, the low relative flavor activity (0.4) supports the notion of its low importance in Tosa-buntan flavor. The compounds decanoic acid, heptyl acetate, α-bisabolol, nonanal, *cis*, *trans*-farnesol, *trans*-nerolidol and 2-dodecenal, with higher relative flavor activity, would contribute to the Tosa-buntan flavor when a fragrance of Tosa-buntan is artificially created.

Sniff testing of Tosa-buntan oil Sniff testing is used not only for AEDA, but also for expressing the aroma character of each component. Organoleptic response to a compound is, in general, dependent upon sample concentration. A whole compo-

Table 1. Volatile flavor components and flavor dilution analysis of Tosa-buntan peel oil.

Peak no.	Compound	RI[a]		Peak area %[b]	FD-factor (3^n)	Relative flavor activity[c]
		DB-Wax	DB-1			
1	α-pinene	1027	925	1.13	7	3.1
2	β-pinene	1117	978	0.50	7	4.7
3	sabinene	1126		0.14	1	1.3
4	myrcene	1161	986	1.81	3	1.1
5	limonene	1219	1030	87.07	7	0.4
6	*cis*-β-ocimene	1222		0.22		
7	γ-terpinene	1253	1023	6.04	6	1.2
8	terpinolene	1285	1069	0.26	7	4.7
9	octanal	1289	1041	0.26		
10	heptyl acetate	1373	1116	tr[d]	7	59.0
11	nonanal	1393	1107	0.02	6	50.6
12	*trans*-limonene oxide	1469	1143	0.03	6	18.5
13	octyl acetate	1475	1146	0.08	5	4.97
14	citronellal	1479	1154	0.06		
15	copaene	1493	1375	0.05	4	5.0
16	decanal	1497	1210	0.26	7	6.5
17	linalol	1549	1096	0.26	6	5.6
18	β-elemene	1592	1385	0.01		
19	caryophyllene	1597	1415	0.04	6	13.2
20	α-humullene	1671	1446	0.01	7	28.3
21	neral	1682	1237	0.07	3	5.4
22	α-terpineol	1700	1184	0.12	1	1.4
23	germacrene D	1709		0.12	3	4.2
24	dodecanal	1725	1409	0.07	4	7.0
25	geranial	1734	1264	0.11	7	9.9
26	geranyl acetate	1747	1382	0.01	7	11.1
27	citronellol	1758	1225	0.09	6	15.9
28	2-dodecenal	1859	1452	0.02	7	23.6
29	perillyl alcohol	1869	1290	0.01	2	13.9
30	perillyl acetate	1889		0.01	6	34.5
31	*trans*-nerolidol	2053	1533	0.01	8	46.0
32	elemol	2082	1549	0.01	5	29.8
33	hexadecanal	2129		0.01	3	19.5
34	α-bisabolol	2240	1685	tr	7	55.7
35	decanoic acid	2304	1376	tr	7	71.2
36	*cis, trans*-farnesol	2347	1744	0.01	7	49.2
37	dodecanoic acid	2449		0.03	5	13.6
38	nootkatone	2527	1799	0.12	7	9.6

[a]RI: Retention indices, [b]Mean of triplicate, [c]Relative flavor activity = log 3^n/(peak area %)$^{0.5}$, [d]Trace (relative peak area percent <0.005%).

sition or some of the components will lead to development of characteristic aroma research on an aroma material like citrus essential oil. It has been reported that the characteristic flavor of grapefruit is nootkatone and its derivatives (Demole & Enggist, 1983) and 1-*p*-menthen-8-thiol (Demole *et al*., 1982). Citral is known to be the typical aroma of lemon (Nursten, 1979). The authors reported that eighteen and six compounds of the essential oils of yuzu (*C. junos* Sieb. ex Tanaka) (Song *et al*., 2000a) and daidai (*C. aurantium* Linn. var. *Cyathifera* Y. Tanaka) (Song *et al*., 2000b) contributed significantly to their characteristic aromas, respectively. FD-factor or relative flavor activity may be useful criteria for reconstruction of the original aroma. However, it has no relation to the aroma character of each compound. Thus, the sniff testing of the original essential oil by on-line GC is an effective means of determining the characteristic or key compounds of an aroma. We performed the sniff testing of Tosa-buntan cold-pressed oil and successfully identified one component, which smells impressively like Tosa-buntan aroma: 2-dodecenal of peak no. 28, as seen in Table 1 and Fig. 1. This result was confirmed in analysis of an oxygenated fraction prepared from the cold-pressed oil. This peak fractionated with GC was also identified as 2-dodecenal by GC-MS. Finally, a chemical reagent of 2-dodecenal was examined organoleptically. A concentrated solution of the compound gives us an unpleasant odor associated with a stinking noxious weed, *Houttuymia cordata*. However, a solution of 2-dodecenal diluted below 2 ppm has a pleasant and refreshing aroma with a typical Tosa-buntan flavor.

2-Dodecenal and nootkatone The authors have focused on the level of nootkatone from the aspect of a characteristic compound of the pummelo and its relatives such as Tosa-buntan, Suisho-buntan, natsudaidai and grapefruit (Sawamura *et al*., 1991). Moreover, the statistical analysis based on the essential oil components and isozyme pattern of peroxidases and esterases was performed to identify the pummelo species and its relatives from the aspect of *Citrus* taxonomy (Zheng *et al*., 1993, 1994, 1996). In the present study, 2-dodecenal has been demonstrated

Table 2. Samples of the *Citrus* Genus[a].

No.	Species	Scientific name	Common name	Relative peak area percent[c]	
				2-Dodecenal	Nootkatone
1	*C. grandis*	*C. grandis* Osbeck forma *Banhakuyu*	Banhakuyu	0.01	tr
2	*C. grandis*	*C. grandis* Osbeck forma *Egami*	Egami-buntan	0.04	0.06
3	*C. grandis*	*C. grandis* Osbeck forma *Hirado*	Hirado-buntan	0.01	0.03
4	*C. grandis*	*C. grandis* Osbeck forma *Honda*	Honda-buntan	0.03	0.10
5	*C. grandis*	*C. grandis* Osbeck forma *Mato*	Mato-buntan	0.01	0.36
6	*C. grandis*	*C. grandis* Osbeck forma *Suisyo*	Suisho-buntan	0.03	0.06
7	*C. grandis*	*C. grandis* Osbeck forma *Tosa*	Tosa-buntan	0.02	0.12
8	*C. grandis*	*C. grandis* Osbeck forma *Benikawa*	Uchimurasaki	0.03	0.09
9	*C. grandis*	*C. grandis* Osbeck forma *Uwa-pummelo*	Uwa-pummelo	0.02	0.03
10	*C. paradisi*	*C. hassaku* Hort. ex Tanaka	Hassaku	0.01	0.03
11	*C. paradisi*	*C. natsudaidai* Hayata	Natsudaidai	0.01	0.01
12	*C. paradisi*	*C. sulcata* Hort. ex Tanaka	Sanbokan	0.02	0.01
13	*C. paradisi*	*C. paradisi* Macfadyen	Grape fruit (Marsh)	0.01	0.02
14	*C. paradisi*	*C. paradisi* Macfadyen forma *Redblush*	Grape fruit (Redblush)	0.01	0.05
15	*C. ichangensis*	*C. Wilsonii* Tanaka	Ichang lemon	tr[d]	0.03
16	*C. ichangensis*	*C. sphaerocarpa* Hort. ex Tanaka	Kabosu	tr	—
17	*C. ichangensis*	*C. inflata* Hort. ex Tanaka	Mochiyuzu	tr	—
18	*C. ichangensis*	*C. taguma-sudachi* Hort. ex Y. Tanaka	Naoshichi	0.01	—
19	*C. ichangensis*	*C. sudachi* Hort. ex Shirai	Sudachi	—	—
20	*C. ichangensis*	C. *yuko* Hort. ex Tanaka	Yuko	—	—
21	*C. ichangensis*	*C. junos* Sieb. ex Tanaka	Yuzu (Japanese)	—	—
22	*C. ichangensis*	*C. junos* Sieb. ex Tanaka	Yuzu (Korean)	—	—
23	*C. ichangensis*	*C. junos* Sieb. ex Tanaka	Seedless yuzu	—	—
24	*C. aurantifolia*	*C. aurantifolia* Swingle	Mexican lime	tr	0.01
25	*C. aurantifolia*	*C. latifolia* Tanaka	Tahiti lime	0.01	0.04
26	*C. aurantifolia*	*C. bergamia* Risso et Poit forma *Balotin*	Bergamot (Balotin)	0.02	0.04
27	*C. aurantifolia*	*C. bergamia* Risso et Poit forma *Fantastico*	Bergamot (Fantastico)	tr	0.04
28	*C. limon*	*C. limon* Burmann forma *Eureka*	Eureka lemon	0.01	—
29	*C. limon*	*C. limon* Burmann forma *Lisbon*	Lisbon lemon	0.01	—
30	*C. aurantium*	*C. aurantium* Linn. var. Cyathifera Y. Tanaka	Daidai	0.04	—
31	*C. aurantium*	*C. aurantium* Linn. forma *Kabusu*	Kabusu	0.04	0.01
32	C. *aurantium*	*C. sp.*	Kiyookadaidai	—	—
33	C. *aurantium*	*C. neo-aurantium* Tanaka	Konejime	0.02	—
34	C. *aurantium*	*C. aurantium* var. *figaradia* Hooker	Sour orange	0.03	0.01
35	C. *sinensis*	*C. sinensis* Osbeck var. *sanguinea* Tanaka forma *Tarocco*	Tarocco blood orange	—	—
36	*C. sinensis*	*C. sinensis* Osbeck forma *Valencia*	Valencia orange	—	—
37	*C. sinensis*	*C. tamurana* Hort. ex Tanaka	Hyuganatsu	0.01	—
38	*C. sinensis*	*C. iyo* Hort. ex Tanaka	Iyokan	0.02	—
39	*C. sinensis*	*C. ujukitsu* Hort. ex Shirai	Ujukitsu	0.01	—
40	*C. reticulata*	*C. unshiu* Marcov. forma *Imamura*	Imamura unshu	0.01	—
41	*C. reticulata*	*C. unshiu* Marcov. forma *Miyagawa-wase*	Miyagawa-wase unshu	0.01	—
42	*C. reticulata*	*C. reticulata* Blanco	Ponkan	—	—
43	*C. tachibana*	*C. tachibana* Tanaka	Tachibana	0.01	—
44	Unidentified	Unidentified	Ozu	—	—
45	*Fortunella japonica*	*Fortunella japonica* Swingle[b]	Kinkan or Kumquat	0.06	—

[a]Classified by Swingle, [b]Another genus in the Rutaceae family comprising the *Citrus* genus, [c]Column was fused silica capillary (60 m × 0.25 μm i.d., 0.25 μm in film thickness) coated with DB-Wax, [d]Trace (relative peak area percent < 0.005%).

to be a primary characteristic compound of Tosa-buntan aroma. Forty-four kinds of citrus fruits consisting of nine species and a kind of kumquat based on Swingle's taxonomy were examined for levels of both 2-dodecenal and nootkatone. The results are shown in Table 2. The pummelo species from no. 1 to no. 9, and the grapefruit species of no. 10 to no. 14 contained both compounds simultaneously in various proportions. The pummelo,generally has a flavor very similar to that of Tosa-buntan. It is suggested that 2-dodecenal is essential to the formation of the characteristic flavor of the pummelo. On the other hand, one of the characteristic flavors of a certain species of grapefruit is nootkatone. The flavor is something between pummelo and grapefruit.

Some other species also contained both or either compound of 2-dodecenal and nootkatone. The content of sour citrus fruits such as daidai, konejime, kabusu and sour orange accounted for 0.02 to 0.04% at higher levels of 2-dodecenal with no or only a trace amount of nootkatone. It seems that species other than pummelo do not give a Tosa-buntan like flavor even if the level of 2-dodecenal is nearly ten times that of pummelo, probably due to their volatile composition backgrounds being different from those of the pummelo groups. We suggested that the increase of Tosa-buntan flavor during storage of the fruits would be due to an increase of the nootkatone level (Sawamura *et al*., 1989). We detected then only a trace amount of 2-dodecenal during storage. Wilson and Shaw (1980) also did not detect 2-dodecenal in cold-pressed grapefruit oil. It is certain that recent improvement of analytical conditions such as apparatus and column would have yielded more accurate and reliable results.

In conclusion, a key compound of Tosa-buntan aroma is 2-dodecenal. When we reconstruct the Tosa-buntan flavor, 2-dodecenal is an essential component. It is also suggested that the overall flavor of Tosa-buntan is a result of its unique essential peel oil composition, which includes 2-dodecenal, nootkatone and several compounds with high relative flavor activity.

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