

# Fatty Acid Composition and Applications of *Eriobotrya japonica* Seed Oil

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**Abstract:** The loquat (*Eriobotrya japonica*) is commonly found in Japan. Its fruits are consumed raw or used in processed foods, and its leaves are used as a traditional medicine and in the manufacturing of cosmetics. Additionally, its seeds have several industrial applications. Therefore, this study aimed to estimate the fatty acid composition of loquat seed oil, and to evaluate its potential application as a deodorant. Palmitic acid, linoleic acid, behenic acid, and lignoceric acid were found to be the primary fatty acids present in the seeds, among which linoleic acid was involved in the deodorization of allyl methyl sulfide. Based on these results, loquat seed oil has potential for use in deodorant production.

**Key words:** fatty acid, deodorizing activity, seed oil, *Eriobotrya japonica*

## 1 Introduction

*Eriobotrya japonica* is an evergreen fruit of the rosaceae family found in central and southern China; it was transported to Japan during the Edo period (1603-1868 CE)<sup>1)</sup>. In recent years, it has been grown in temperate limestone regions, spanning from the Boso peninsula to southern Kyushu in Japan. The plant produces white flowers with a sweet fragrant odor that bloom between late autumn and early winter. In early summer, the flowers develop into small spherical or pear-shaped fruits containing one to several large seeds. Its fruits may be consumed raw, and have also been widely used in processed foods such as jams and jellies. Dried loquat leaves are used in traditional medicine to treat coughs and indigestion<sup>1,2)</sup>. In Japan, loquat leaves have been widely used since the Edo period as a traditional medicine known as shiniseihaito, which is prescribed to patients with symptoms such as chronic rhinitis and olfactory disorders<sup>2,3)</sup>. In addition, since loquat leaves have anti-inflammatory and anti-allergenic properties, they are also used in the manufacture of cosmetics<sup>2)</sup>. Loquat seeds are further used as a traditional medicine to treat cough and indigestion<sup>2)</sup>. Amygdalin has been extensively studied as one of the primary active ingredients responsible for these pharmacological effects<sup>4)</sup>. In addition to

amygdalin, numerous studies have been conducted on other ingredients derived from loquat seeds, and their physiological effects have been elucidated<sup>5-8)</sup>. However, the amygdalin in loquat seeds may lead to hydrocyanic acid poisoning when large quantities are ingested. To date, there have been few studies on components other than amygdalin present in loquat seeds.

In recent years, we have focused on the seeds of various fruits, and have analyzed the oils extracted from the seeds of mango (*Mangifera indica*) and yamamomo (*Morella rubra*), further examining their potential application as deodorizing substances<sup>9)</sup>. In this study, we analyzed the oil contained in the seeds of "Tanaka Biwa," a readily available variety of loquat, and determined its chemical properties. Furthermore, in consideration of possible industrial uses, the deodorizing effect of the oil on substances generally classified as malodorous was investigated.

## 2 Experimental Procedure

### 2.1 Materials

*E. japonica* fruits were collected in 2016 from a local market in the Akitsu district of the Hiroshima prefecture.

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Accepted March 15, 2019 (received for review August 29, 2018)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/> <http://mc.manuscriptcentral.com/jjocs>

After removing the pulp from the fruit, the seeds were washed with water and dried in the shade. Tetrahydrofuran (THF) was distilled from sodium/benzophenone under an argon-rich atmosphere. Other solvents and reagents were used without further purification.

## 2.2 Preparation of loquat seed oil

Loquat seed powder (15.1 kg) was extracted using n-hexane (15.5 L) over 2 weeks. The sample was then filtered to separate the solid and liquid fractions. The filtrate was evaporated under a low-pressure atmosphere to obtain a solvent-free oil; 35.3 g of a light-yellow solidified oil was obtained.

## 2.3 Analysis of volatile organic compounds in the headspace of loquat seeds

For analysis, ground loquat seeds (0.5 g) were placed in a tube (10 mL), sealed using a septum-type cap, and kept in a heat block at 40°C. After allowing equilibrium to be reached over 5 min, solid-phase microextraction (SPME) fibers (Carboxen/PDMS Stable Flex-fibers, 85 µm, Sigma Aldrich, St. Louis, MO, USA) were placed in the tube for sampling. Volatile compounds were extracted by exposure to the headspace of the sample at 40°C for 30 min. The volatile compounds in the headspace gas were determined by gas chromatography (GC) using an Agilent Technology 7890A equipped with an Agilent Technology 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and a DB-WAX capillary column (60 m × 0.25 mm × 0.25 µm; Agilent Technologies). After sample adsorption, the SPME fiber was inserted into the injector operated in splitless mode at 250°C and desorbed for 10 min. The column temperature was held at 40°C for 5 min after injection, increased by 5°C min<sup>-1</sup> to 220°C, held for 10 min. Helium was used as the carrier gas, at a flow rate of 2.0 mL min<sup>-1</sup>. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV. The ion source temperature was 200°C, and the gas chromatography/mass spectroscopy (GC/MS) interface temperature was 200°C. The analysis was performed in full scan mode (mass range: 50–300 *m/z*) with a scan time of 3 scans s<sup>-1</sup>. Components were identified by matching their retention times and GC/MS data with data available in the NIST library.

## 2.4 Physicochemical characteristics

Determination of the acid value, saponification value, iodine value, hydroxyl value, and ester value of the extracted oil was carried out using standard methods for the analysis of fats, oils, and related materials established by the Japan Oil Chemists' Society<sup>10)</sup>.

## 2.5 Fatty acid composition

For the analysis of glycerides, loquat seed oil was esterified using the sodium methoxide method to increase the

concentration of volatile methyl esters. The fatty acid methyl ester composition was then determined by GC with a Shimadzu GC-17A (Kyoto, Japan) equipped with a DB-1 capillary column (30 m × 0.32 mm × 0.25 µm; Agilent Technologies) and a flame ionization detector (FID). The column temperature was held at 60°C for 3 min after injection, increased by 10°C min<sup>-1</sup> to 300°C, held for 13 min. The temperatures of the column injector and detector were 250°C and 300°C, respectively. Helium was used as the carrier gas at a flow rate of 3.3 mL min<sup>-1</sup>. Fatty acids methyl esters were identified by comparing the retention time of the samples and appropriate fatty acids methyl esters standards.

## 2.6 Gel permeation chromatography (GPC) analysis

Samples were dissolved in THF and analyzed on a GPC system using THF as the mobile phase. A Shimadzu isocratic high-performance liquid chromatography (HPLC) system equipped with a Shodex RI-201 refractive index (RI) detector was used. Three columns were used, namely a Shodex GPC KF-802 (8.0 mm I.D. × 30 mm), a Shodex GPC KF-802.5 (8.0 mm I.D. × 300 mm), and a TSKgel G1000HXL (7.8 mm I.D. × 300 mm). The flow rate was 0.7 mL min<sup>-1</sup> and the sample injection volume was 20 µL.

## 2.7 Evaluation of deodorizing activity

To evaluate the deodorizing activity of loquat seed oil, 0.05 g portions of the oil were placed in stoppered 300 mL Erlenmeyer flasks, to which the different odorous substances were added individually. Then, each tightly sealed flask was incubated for 60 min at room temperature. The odorous substances tested were ammonia (150 ppm), trimethylamine (20 ppm), acetic acid (50 ppm), isovaleric acid (50 ppm), acetaldehyde (100 ppm), hydrogen sulfide (20 ppm), and methyl mercaptan (5 ppm). After 60 min, the residual odorous compounds in the headspace were detected using a Kitagawa AP-20 gas detector (Komyo Rikagaku Kogyo, Kanagawa, Japan) equipped with a Kitagawa gas detector tube.

The deodorizing activity of (*E*)-2-nonenal (30 ppm) was analyzed under the following conditions. The concentration of residual (*E*)-2-nonenal in the headspace gas (2 mL) was determined by GC on a Shimadzu GC-2014AF equipped with a Unisole F-200 30/60 column (2.1 m × 3.2 mm) and a FID. The column temperature was maintained at 120°C, and the injector and detector were maintained at 200°C. The carrier gas used was N<sub>2</sub>, at a flow rate of 50 mL min<sup>-1</sup>.

The deodorizing activities of allyl mercaptan (0.12 ppm), allyl methyl sulfide (29 ppm), dimethyl sulfide (4 ppm), and dimethyl trisulfide (1 ppm) were evaluated under the following analytical conditions. The concentrations of remaining sulfur-containing compounds in the headspace gas (2 mL) were determined by GC on a Shimadzu GC-2014AF equipped with a 1,2,3-tris(2-cyanoethoxy)propane (TCEP)

column (2.1 m × 3.2 mm) and a flame photometric detector (FPD). The column temperature was maintained at 50°C, and the injector and detector were maintained at 200°C. The carrier gas used was N<sub>2</sub>, at a flow rate of 40 mL min<sup>-1</sup>.

The deodorizing activity (%) was calculated according to the following equation:

$$\text{Deodorization activity (\%)} = (B - A) / B (\%)$$

where A is the detected concentration or peak, and B is the detected concentration or peak in a blank test.

### 3 Results and Discussion

#### 3.1 Chemical composition of volatiles in headspace

Dried loquat seeds were crushed and their headspace was analyzed for volatiles via SPME and GC/MS. Benzaldehyde was the most abundant volatile compound (95.78%), followed by benzyl alcohol (0.44%), hexyl acetate (0.31%), and (3Z)-3-hexen-1-yl acetate (0.21%) (Fig. 1 and Table 1). Lee *et al.*<sup>11)</sup> previously showed that apricot (*Prunus armeniaca*) seed oil contained benzaldehyde as the major volatile component (90.6%). Similar results identifying benzaldehyde as the major volatile compound have been reported for plum seed and apricot seed essential oils<sup>12, 13)</sup>. The high benzaldehyde content in the headspace of loquat seeds was determined to be due to hydrolysis caused by interactions between amygdalin and emulsin resulting from the destruction of the seed cell wall after grinding of the samples<sup>14)</sup>. The study by Lee *et al.*<sup>11)</sup> also identified benzoic acid and mandelonitrile as minor components; however, in our analysis, mandelonitrile was not found. The difference was thought to be due to differences in the methods used to collect aroma components.

#### 3.2 Characteristics of loquat seed oil

To determine the characteristics of loquat seed oil, its

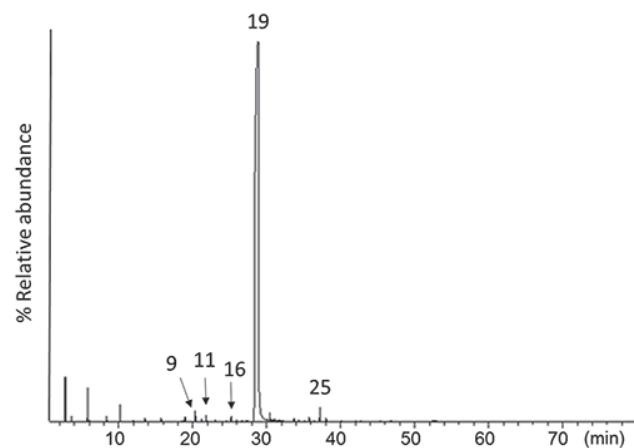


Fig. 1 Gas chromatography mass spectroscopy chromatogram of loquat seed powder.

acid value, saponification value, iodine value, hydroxyl value, and ester value were measured according to standard methods for the analysis of fats, oils, and related materials established by the Japan Oil Chemists' Society<sup>10)</sup>. The values obtained are given in Table 2. The characteristics of loquat seed oil resembled those of cacao butter and

Table 1 Chemical constituents of the headspace derived from loquat seeds.

Peak No. <sup>a)</sup>	R.T. <sup>b)</sup>	Compounds	% Area <sup>c)</sup>
1	13.56	Hexanal	0.12
2	13.86	Undecane	0.01
3	17.32	Dodecane	0.03
4	18.44	Butyl butyrate	0.03
5	18.62	(3E)-3-Hexenal	0.04
6	18.81	2-Pentylfuran	0.02
7	18.99	Ethyl caproate	0.15
8	19.88	Styrene	0.02
9	20.34	Hexyl acetate	0.31
10	20.93	Tridecane	0.02
11	21.52	(3Z)-3-Hexen-1-yl acetate	0.21
12	23.04	1-Hexanol	0.06
13	24.06	(3Z)-3-Hexen-1-ol	0.01
14	24.56	Tetradecane	0.05
15	24.69	(2E,4E)-2,4-Hexadienal	0.02
16	25.22	Hexyl butyrate	0.20
17	25.85	Ethyl caprylate	0.02
18	26.69	(3E)-3-Hexen-1-yl butyrate	0.08
19	28.80	Benzaldehyde	95.78
20	31.86	Acetophenone	0.04
21	32.22	Ethyl benzoate	0.03
22	34.33	Naphthalene	0.04
23	35.09	Methyl salicylate	0.02
24	35.75	1-Phenyl-1,2-propanedione	0.13
25	37.18	Benzyl alcohol	0.44
26	38.05	2-Phenylethanol	0.11
27	40.05	Biphenyl	0.02
28	41.07	Cinnamaldehyde	0.01
29	42.72	Mandelic acid	0.05
30	52.35	Benzoic acid	0.01
		Others	1.92
Total			100.00

a) The peak number correspond to numbers in Fig. 1.

b) Retention time (min)

c) Peak area percentage of total ion chromatogram.

**Table 2** Chemical characteristics of loquat seed oil and other oils.

Chemical properties	Loquat seed oil	Cacao butter	Beef tallow
Acid value (KOH mg/g)	50.6	—	—
Saponification value	277.2	~199–202	~190–196
Iodine value (I <sub>2</sub> g/100 g)	42.0	~29–38	~45–53
Hydroxyl value (KOH mg/g)	16.5	—	—
Ester value (KOH mg/g)	226.6	—	—

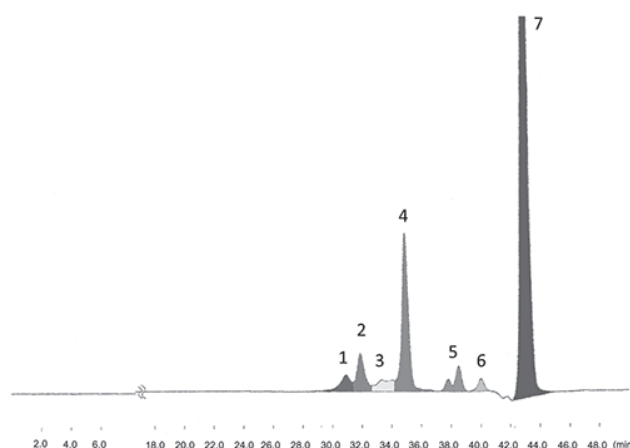
beef tallow. In addition, because the acid value of the oil was 50.6 KOH mg/g, the oil was determined to be unsuitable as food<sup>15)</sup>. In addition, loquat seed contains amygdalin<sup>4)</sup>, and is also known to be unsuitable as food for this reason. Therefore, hexane was used to extract the loquat seed oil. Since amygdalin is a glycoside, it is generally extracted with a polar organic solvent such as methanol or a mixture of water and methanol. On the other hand, it is not extracted with non-polar solvents such as the hexane used in this study. For this reason, it is considered that the possibility of hydrocyanic acid production from the extracted oil is low. However, there is a possibility that mandelonitrile, which is a decomposition product of amygdalin, will be present in loquat seed oil and further decompose to generate hydrocyanic acid. For this reason, we attempted to detect hydrogen cyanide gas present in the headspace of loquat seed oil using a gas detection tube, and it was found to be below the detection limit (data not shown). Based on this result, it was considered that hydrocyanic acid was not present in the extracted oil obtained in this study.

### 3.3 Fatty acid composition of loquat seed oil

Loquat seed oil was methyl esterified using a solution of boron trifluoride and methanol, and was subsequently analyzed by GC (Table 3). Methyl esters of palmitic acid (C16:0), linoleic acid (C18:2), behenic acid (C22:0), and lignoceric acid (C24:0) were detected. Next, the oil was decomposed with methanol using a solution of 28% sodium methoxide in methanol and analyzed by GC. Using this method, methyl esters of stearic acid (C18:0) were identified in addition to the fatty acid methyl esters confirmed by the GC/MS analysis described above. Methyl esters of fatty acids with twenty or more carbon atoms, such as arachidic acid (C20:0), erucic acid (C22:1), and tricosanoic acid (C23:0) were also identified. These results suggest that loquat seed oil contained large concentrations of benzaldehyde, behenic acid, and lignoceric acid. Behenic acid is known to be a component of the oil contained in seeds of *Moringa oleifera* Lam, and a minor component of rapeseed and peanut oils<sup>16–18)</sup>. Although it has lower bioavailability than oleic acid, behenic acid can increase blood cholesterol levels. Therefore, it is considered necessary to pay attention to possible overdose<sup>19)</sup>. Lignoceric acid is also

**Table 3** Fatty acid composition of loquat seed oil based on GC/MS analysis.

Peak No.	Compounds	Peak area (%)
1	Benzaldehyde	70.7
2	Methyl benzoate	1.5
3	Benzoic acid	0.4
4	Methyl palmitate	1.8
5	Stearic acid	0.4
6	Methyl linoleate	4.0
7	Methyl stearate	0.2
8	Methyl arachidate	0.2
9	Methyl erucate	0.5
10	Methyl behenate	2.4
11	Methyl lignocerate	1.9
	Others	16.0
Total		100.0

**Fig. 2** GPC chromatogram of loquat seed oil.

contained as a minor component in peanut oil<sup>16)</sup>, but its physiological effects have not been clarified.

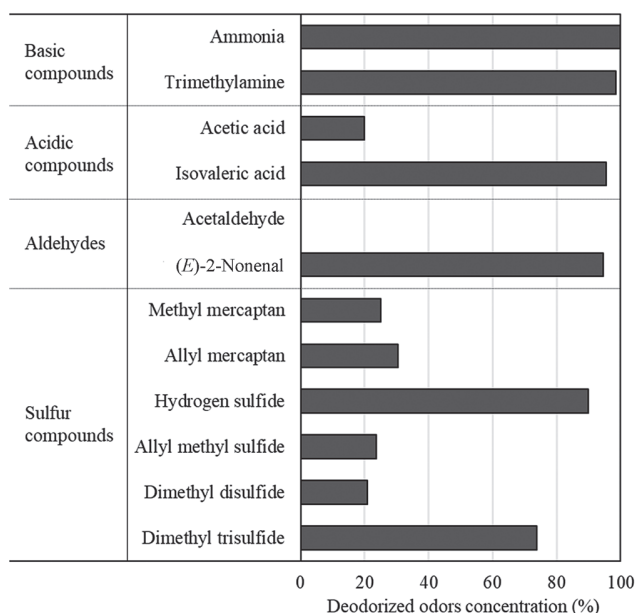
### 3.4 GPC analysis

As shown in Fig. 2, it was found that approximately 70% of the components of loquat seed oil were low-molecular weight compounds. Triglycerides, diglycerides, and fatty

**Table 4** Composition of loquat seed oil based on GPC analysis.

Peak No. <sup>a)</sup>	Composition	Peak area (%)
1	Triglycerides	2.90
2	Diglycerides	4.63
3	Monoglycerides or high-molecular weight fatty acids	2.84
4	C <sub>16</sub> ~C <sub>18</sub> Fatty acids	15.81
5	Low-molecular weight compounds	3.42
6	Benzoic acid	1.16
7	Benzaldehyde and volatile compounds	69.17
	Others	0.07
Total		100.00

a) The peak number correspond to numbers in Fig. 2.

**Fig. 3** Deodorizing effect of loquat seed oil.

acids were also detected. Based on these results, it is possible that the acid value shown in **Table 4** is high due to the high content of fatty acids and benzoic acids. Likewise, the high hydroxyl value could be attributed to the high triglyceride and diglyceride content. Palm oil and rice oil are known to contain large amounts of free fatty acids, which was determined to be due to lipase activity in the fruits and seeds of the respective plants<sup>19)</sup>. Lipase activity in loquat seeds has not been clarified, but it is possible that fatty acid contents may be increased during processes such as seed crushing and extraction.

### 3.5 Deodorizing effect of loquat seed oil

Previous studies have reported that fruit seed oil often has strong deodorizing effects<sup>9, 20)</sup>. In this study, we measured the deodorizing effect of loquat seed oil on twelve odorous compounds (**Fig. 3**). The oil showed a strong de-

odorizing ability, with a deodorizing effect of 90% or greater against ammonia, trimethylamine, isovaleric acid, (*E*)-2-nonenal and hydrogen sulfide. The deodorizing action against ammonia and trimethylamine was thought to be due to neutralization reactions caused by the high fatty acid and benzoic acid contents. Wu *et al.*<sup>9)</sup> showed that mango seed oil exhibits a deodorizing effect against (*E*)-2-nonenal, a compound which causes age-related changes in body odor<sup>21)</sup>. In the present study, it became clear that loquat seed oil was more effective than mango oil in deodorizing (*E*)-2-nonenal. However, the deodorizing effects of the oil against acetic acid and the sulfur-containing compounds methyl mercaptan, allyl mercaptan, allyl methyl sulfide, and dimethyl disulfide, were approximately 20–30%.

### 3.6 Deodorizing effect of model fatty acid composition

To confirm which components contributed to the deodorizing effect, the typical fatty acids contained in loquat seed oil were added at different concentrations to a model fatty acid solution (**Table 5**). As shown in **Table 6**, Samples (6)–(8), which did not contain palmitic acid or linoleic acid, exhibited relatively lower deodorization rates against ammonia. Furthermore, no deodorizing effects against trimethylamine, hydrogen sulfide, dimethyl disulfide, or dimethyl trisulfide were observed in these samples. Based on these results, it was suggested that palmitic acid and linoleic acid were effective deodorants against nitrogen-containing compounds (ammonia, trimethylamine), hydrogen sulfide, dimethyl disulfide, and dimethyl trisulfide. In addition, the presence of palmitic acid and linoleic acid markedly increased the deodorization rate against (*E*)-2-nonenal. However, when the proportions of palmitic acid and linoleic acid were increased, no improvements in deodorization rate were observed.

In this study, we found that the fatty acids contained in loquat seeds may not exert strong deodorizing effects against sulfur-based compounds (hydrogen sulfide, methyl



**Table 5** Fatty acid concentrations in model samples.

Sample No.	Fatty acid (mg)			
	Palmitic acid	Linoleic acid	Behenic acid	Lignoceric acid
(1)	30.0 <sup>a)</sup>	30.0	150.0	30.0
(2)	30.0	30.0	30.0	150.0
(3)	20.0	100.0	20.0	100.0
(4)	20.0	100.0	100.0	20.0
(5)	20.0	20.0	100.0	100.0
(6)	–	–	120.0	120.0
(7)	–	–	200.0	40.0
(8)	–	–	40.0	200.0

a) Unit: mg

**Table 6** Deodorizing effect (%) of the primary fatty acids in loquat seed oil.

Sample No.	Nitrogen-containing compounds		Short-chain fatty acids		Aldehydes	
	Ammonia	Trimethyl amine	Acetic acid	Isovaleric acid	Acetaldehyde	(E)-2- Nonenal
(1)	<b>98.7</b>	<b>98.5</b>	0.0	85.0	0.0	64.9
(2)	<b>99.0</b>	<b>98.5</b>	0.0	84.0	0.0	66.3
(3)	<b>99.3</b>	<b>98.5</b>	4.0	88.0	0.0	49.3
(4)	<b>98.7</b>	<b>98.5</b>	2.0	84.0	0.0	40.3
(5)	<b>98.7</b>	<b>98.5</b>	0.0	76.0	0.0	46.8
(6)	<b>80.0</b>	0.0	0.0	44.0	0.0	11.0
(7)	<b>88.0</b>	0.0	40.0	0.0	0.0	18.1
(8)	<b>70.0</b>	0.0	<b>90.0</b>	0.0	0.0	8.8

Sample No.	Sulfur-containing compounds					
	Hydrogen sulfide	Methyl mercaptan	Allyl mercaptan	Allyl methyl sulfide	Dimethyl disulfide	Dimethyl trisulfide
(1)	25.0	0.0	0.0	30.4	8.2	14.9
(2)	0.0	0.0	0.3	36.7	6.1	9.8
(3)	0.0	14.0	0.0	<b>80.2</b>	11.5	34.5
(4)	20.0	10.0	0.0	69.2	12.3	29.5
(5)	0.0	10.0	7.1	36.1	1.3	3.6
(6)	0.0	10.0	9.0	9.0	0.0	0.0
(7)	0.0	0.0	10.3	14.6	0.0	0.0
(8)	0.0	0.0	7.1	19.0	0.0	0.0

mercaptan, allyl mercaptan, allyl methyl sulfide, dimethyl disulfide, and dimethyl trisulfide). However, we observed an overall improvement in the deodorization rate against allyl methyl sulfide in Samples (3) and (4), which had higher concentrations of linoleic acid. In particular, Sample (3), which contained a high concentration of lignoceric acid, showed the highest deodorization rate against allyl methyl sulfide (80.2%). Based on these results, we determined that linoleic acid was involved in the deodorization of allyl methyl sulfide. In addition, these results suggest that an

increase in the concentration of lignoceric acid contributed to an improvement in the deodorization rate.

#### 4 Conclusions

The chemical composition of loquat seed oil was found to be similar to that of cacao butter and beef tallow. In addition, because the acid value of the oil was 50.6 KOH mg/g, the oil was considered unsuitable as food. Through GC

analysis, it was found that loquat seed oil had properties similar to those of peanut oil. In addition, a direct analysis of the seed oil revealed that benzaldehyde accounted for approximately 70% of the oil (by peak area percentage), with fatty acids comprising a small proportion. The dominant fatty acids in the oil were palmitic acid, linoleic acid, behenic acid, and lignoceric acid.

In our previous studies, we found that some seed oils exhibit deodorizing effects; therefore, we investigated the deodorizing effect of loquat seed oil against substances considered to be the causes of unpleasant odors in daily life. The oil showed strong deodorization effects (greater than 90%) against ammonia, trimethylamine, hydrogen sulfide, isovaleric acid, and (*E*)-2-nonenal. Furthermore, to identify which fatty acids were responsible for the deodorization effects, samples containing varying concentrations of palmitic acid, linoleic acid, behenic acid, and lignoceric acid were prepared. It was found that palmitic acid and linoleic acid were the most important fatty acids for the deodorization of ammonia, trimethylamine, hydrogen sulfide, dimethyl disulfide, and dimethyl trisulfide. In addition, it is concluded that careful attention should be paid to cyanide compounds when using loquat seeds. Overall, loquat seed oil may have useful applications in deodorants.

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