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Changes in the Volatile Compounds and in the Chemical and Physical Properties of Snake Fruit (*Salacca edulis* Reinw) Cv. *Pondoh* during Maturation

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During the maturation of snake fruit (*Salacca edulis* Reinw) *Pondoh*, the contents of sucrose, glucose, fructose, and volatile compounds changed drastically. The glucose, fructose, and volatile compounds contents showed their maximum levels at the end of maturation; however, the sucrose content decreased. During maturation, the flesh firmness tended to increase; however, at the end of maturation (6 months), the flesh became soft. The major volatile aroma in solvent-assisted flavor evaporation (SAFE) and solvent extracts were identified to be methyl esters of butanoic acids, 2-methylbutanoic acids, hexanoic acids, pentanoic acids, and the corresponding carboxylic acids. Furaneol (4-hydroxy-2,5-dimethyl-3(2*H*)-furanone**)** was also identified as a minor aroma constituent in the SAFE residue. The methyl esters were found to increase dramatically during stages 4−6 (5−6 months after the pollination) to exceed the amounts of carboxylic acids, whereas the acid amount increased gradually until stage 5 (5.5 months after the pollination) to reach the maximum at stage 6 (6 months after the pollination).

KEYWORDS: ***Salacca edulis;* snake fruit; *Pondoh*; volatile compounds; chemical composition; firmness; solvent-assisted flavor evaporation (SAFE)**

INTRODUCTION

Snake fruit (*Salacca edulis* Reinw) belongs to the class of *Salacca* originated from southeast Asia. The fruit is egglike in shape, and skin of the fruit is brown and looks like a snake skin; therefore, the fruit was named snake fruit. The fruit contains three pieces of seeds covered with white flesh. In Indonesia there are many snake fruit cultivars; however, most of them have an astringent taste and are not sweet. In Yogyakarta province, a new cultivar was recently discovered, Cv. *Pondoh*, which is sweet even before full maturation. At this stage, sandlike materials were observed on the flesh, and the fruit became easier to release from the bunch. The fruit is usually picked 5.5−6.0 months after pollination (between stages 5 and 6; see Materials and Methods).

Originally the *Pondoh* cultivar was grown only in Yogyakarta province; however, this cultivar has already been planted in many areas in Indonesia due to its attractive taste even at the immature stage. The production of snake fruit *Pondoh* cultivar in Yogyakarta in 1999 had increased by 100% within five years to reach 28 666 tons. Most of the fruit is freshly consumed, and some are processed into fruit juices, canned fruit, or jam. The fruit has pineapple-, pear-, and banana-like aroma. Although the fruits are very popular among Indonesians, some non-native consumers do not prefer the aroma due to its sweaty odor. The volatile constituents produced from an unknown cultivar of *S. edulis* Reinw were reported to be composed of esters, lactones, and the corresponding carboxylic acids (*1*).

This paper describes the change in the chemical and physical properties of snake fruit flesh during maturation. We also present the qualitative and quantitative change in the volatile compounds during the maturation process.

MATERIALS AND METHODS

Chemicals. All chemicals used in the experiment were analytical grade, supplied by Sigma. The authentic standards of methyl 3-methylpentanoate, methyl 3-methyl-2-pentenoate, methyl 3-hydroxy-3-methylpentanoate, and their corresponding carboxylic acids were synthesized by T. Hasegawa Co. Ltd., Japan.

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Plant Materials. Snake fruits (*Salacca edulis* Reinw) *Pondoh* were harvested in the experimental field in Pakem-Yogyakarta, Indonesia, during August 2000 at the each maturation stage, defined as follows: stage 1, 3.5 months after pollination, 31 g/fruit; stage 2, 4.0 months after pollination, 40 g/fruit; stage 3, 4.5 months after pollination, 46 g/fruit; stage 4, 5.0 months after pollination, 49 g/fruit; stage 5, 5.5 months after pollination, 54 g/fruit; stage 6, 6.0 months after pollination, 62 g/fruit. The fruits were shipped to Japan by airplane and utilized immediately after they arrived.

Sugar Content. A 5-g sample was homogenized in 20 mL of aqueous ethanol (80%) and 5 mL of diethyl ether. The aqueous fraction was heated at 80 °C for 30 min and centrifuged (5000*g*, 20 min) to give supernatant, which was added with lead acetate (2 mL of 10%) and again centrifuged (5000*g*, 20 min). The latter supernatant was then evaporated in vacuo, and the residue was neutralized with aqueous oxalic acid (10%). The solution was diluted with aqueous ethanol (80%) and filtered through Sep-Pak C-18 (Waters) prior to HPLC analyses. The HPLC conditions were as follows: column, Unisil Q NH_2, 5 μm, 250 × 4.6 mm i.d. (GL Science); solvent, CH_3CN/H_2O = 65:35 at a flow rate of 1.0 mL/min, at 40 °C, equipped with RI detector (model RID-6A, Shimadzu, Japan).

Acid Content. Twenty grams of snake fruit was extracted with 20 mL of water and filtered. The filtrate was titrated with 0.1 M NaOH standard solution, with phenolphthalein as indicator. The acidity was calculated as the content of malic acid.

Texture Assessment. The firmness of the snake fruit flesh was measured using a Universal Testing Machine (Lloyd-1000, UK). A piece of flesh 1 × 1 cm^2 in size was placed onto the platform, and the top was lowered to contact with the sample. The compression speed used for the experiment was 60 mm/min. The maximal force needed to break the flesh was monitored to quantify the instrumental firmness of the flesh.

Identification of Volatile Compounds Extracted by the Solvent-Assisted Flavor Evaporation (SAFE) Method. The flesh (430 g) of snake fruit was homogenized for 1 min in an aqueous solution composed of saturated $CaCl_2$ solution (430 g) and distilled water (430 g). The homogenate was filtered through filter cloth to give 1030 mL of bright-yellow solution. A portion (300 mL) of the filtrate was subjected to distillation using the SAFE apparatus (*2*) under high vacuum of 10^{-3} Pa. The extraction was conducted three times. The volatile compounds were extracted by 125 mL of diethyl ether from an aqueous solution in the SAFE apparatus to give an ether extract. The ether fraction was concentrated with a Vigruex column after drying over anhydrous Na_2SO_4 to give extracts, later designated as the SAFE extract (1 mL). The un-extracted materials, which remained in the evaporation vessel containing compounds with higher boiling points, were extracted with 280 mL of diethyl ether. The ether fraction was concentrated as mentioned above to give a sample, the SAFE residue (1 mL). Both the SAFE extract and the SAFE residue were analyzed by GC and GC−MS as shown below.

GC Conditions for the Analyses of SAFE Extract and SAFE Residue. A Hewlett-Packard GC-6890 gas chromatograph, equipped with a split−splitless injector and a flame ionization detector (FID), was used.Both the injector and detector temperatures were 250 °C. A TC-WAX column (0.25 mm i.d. × 60 m, 0.25 μm film thickness) was used under the following temperature program: 10 min isothermal at 40 °C, then raised to 230 °C at a rate of 3 °C/min, and finally held for 10 min. The flow rate of the carrier gas (He) was 1.1 mL/min. A volume of 1 μL of each sample was injected with split ratio of 1:50.

GC−MS Conditions for the Analyses of SAFE Extract and SAFE Residue. A Hewlett-Packard 5973 GC−MS mass-selective detector (*m*/*z* 30−300, 70 eV) was used. The column type, injector, column program temperatures, and split ratio were the same as those mentioned for GC analysis. The flow rate of the carrier gas (He) was 1 mL/min. Compounds were identified using mass spectra and Kovats retention indices (*3*). Calculation of Kovats retention indices (*K* index) for individual peaks was done by using retention time data from a series of alkane standards. For identification of the dominant methyl esters of carboxylic acids and the corresponding acids, the GC−MS data were directly compared with those of authentic compounds.

Change in the Contents of Volatile Compounds during Maturation. To extract even compounds with low volatilities, the microwave extraction method (*4*, *5*) was used. To 5 g of ground sample was added 50 mL of pentane in an Erlenmeyer flask covered with a glass watch disk, and the mixture was heated in a microwave oven (Hitachi MRO-1500, 2450 MHz, 950 W) for 1 min. The extract was then filtered through anhydrous Na_2SO_4. Ethyl decanoate (10 μg/mL) was added as an internal standard before the extraction. The extraction was conducted two times. The combined filtrates were then concentrated to l mL with a Vigruex column and finally flushed with nitrogen to 20 μL before being subjected to GC and GC−MS.

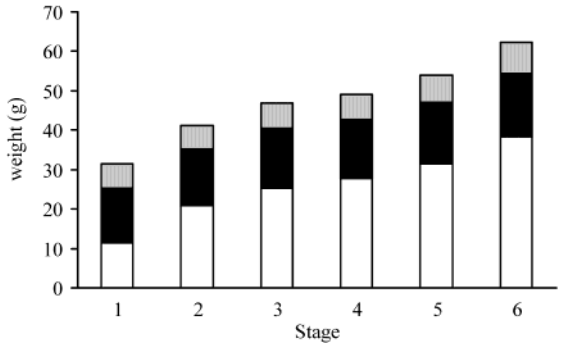

Figure 1. Change in the weight of flesh, seeds, and skin of snake fruit during development (white, flesh; black, seeds; and gray, skin). The stage definitions are described in the Plant Materials section of the Materials and Methods.

GC and GC−MS Conditions for the Quantification of the Volatile Compounds in the Flesh at Each Maturation Stage. A Hitachi G-3000 equipped with a TC-Wax column (0.25 mm i.d., 30 m length, and 0.25 μm film thickness) was used for GC analyses. GC conditions: temperature, 60 °C, elevated to 230 °C (3 °C/min), and then held for 10 min; injector temperature. 250 °C. GC−MS conditions: column, Supelco-Wax-10 (30 m length, 0.25 mm i.d., and 0.25 μm film thickness); oven temperature, from 40 to 60 °C (35 °C/0.5 min), and then elevated to 230 °C (3 °C/min), and 45 °C/min to 270 °C. Helium at a flow rate of 1 mL/min was used as carrier gas, the ionizing voltage was 70 eV, and the mass range was *m*/*z* 30−300. Quantification of the volatile compounds was done by comparing the peak areas of the compounds with that of the internal standard.

Fatty Acids Determination. Flesh of snake fruit (250 mg) at each maturation stage was placed in a glass tube with a Teflon cap in the presence of heptadecanoic acid, used as an internal standard. Lipid extraction and methylation were done according to the method of Garces and Mancha (*6*). Fatty acid compositions were determined on the basis of GC analyses, using an instrument equipped with a TC-Wax capillary column (0.25 mm i.d., 30 m length, and 0.25 μm film thickness) and an FID detector. The oven program used was 50 °C (2 min), ramped by 4 °C to 220 °C (15 min). The injector and detector temperatures were both 250 °C.

RESULTS AND DISCUSSION

As shown in **Figure 1**, the weight of the flesh increased during maturation, while the ratio of seeds and skin apparently decreased remarkably. The fruit had white flesh at stages 1 and 2, which then changed to yellowish white after stage 5. The flesh ratio sharply increased at stage 2, and gradually increased during the maturation period to reach its maximum level at more than 60% of the total weight at stage 6. The increasing weight during maturation is a common phenomenon with fruit. For peach fruit, the fresh weight sharply increased about 11-fold during periods 2−3 of the final stage of development (*7*). The seeds grew earlier than any other parts of the fruit. The seed sizes were almost unchanged during the development process, indicating that the seed likely grew sharply until stage 1.

As shown in **Figure 2**, the contents of the sucrose, glucose, and fructose were changed remarkably. The sucrose content

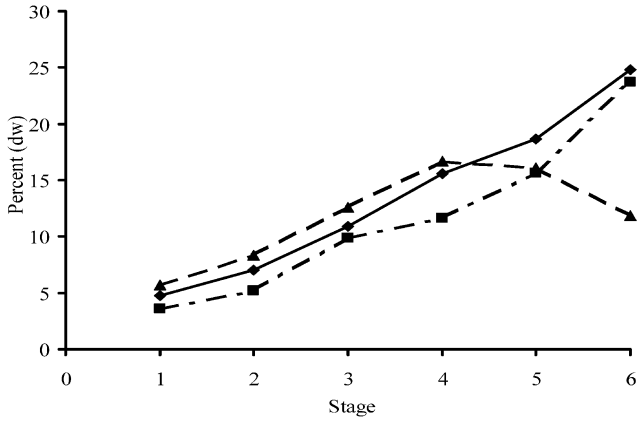

Figure 2. Changes in the contents of glucose, fructose, and sucrose of snake fruit during development (◆, fructose; ■, glucose; and ▲, sucrose). The stage definitions are described in the Plant Materials section of the Materials and Methods.

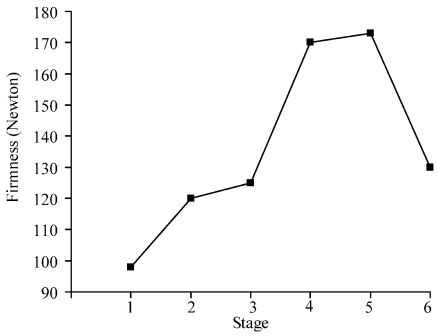

Figure 3. Change in the firmness of snake fruit during development. The stage definitions are described in the Plant Materials section of the Materials and Methods.

increased during maturation, from stages 1 to 4, and then decreased, whereas the glucose and fructose contents increased throughout the maturation. The decrease in the content of sucrose during stages 5 and 6 was in accordance with the increasing of glucose and fructose contents. It must be ascribed to the hydrolysis of sucrose by sucrase to yield glucose and fructose after stage 4. The increment of reducing sugar made the texture of the flesh sandy, because sandlike material was observed on the flesh. The flesh became sweet after stage 3, when the ratio of sugar to acid was about 4.6.

Fruit firmness is a very important factor to evaluate the physical properties of the tissue, which directly affect the texture. A change in the firmness may be caused by modification of the chemical structure of the cell wall. The massive breakdown of the cell wall of avocado must be ascribed to hydrolysis of polysaccharides by the action of hydrolytic enzymes (*8*). As shown in **Figure 3**, the energy needed to break the snake fruit flesh varied from 98 to 175 N during maturation. At stage 1, the energy was less than 100 N, indicating that the flesh was soft. During the development, the flesh firmness increased until stage 5, to reach the maximum level (170 and 172 N) at stages 4 and 5, respectively, and then the flesh became soft (130 N) again at stage 6. As the fruit was soft enough at stage 6 to be damaged during handling and transportation from the field to the market, it was suggested that the snake fruits be picked during stages 4 and 5, when the contents of sugars and the firmness showed the highest values.

As shown in **Table 1**, methyl esters of C4, C5, C6, C7, and C8 carboxylic acids were dominant (68.5%) in the SAFE extract

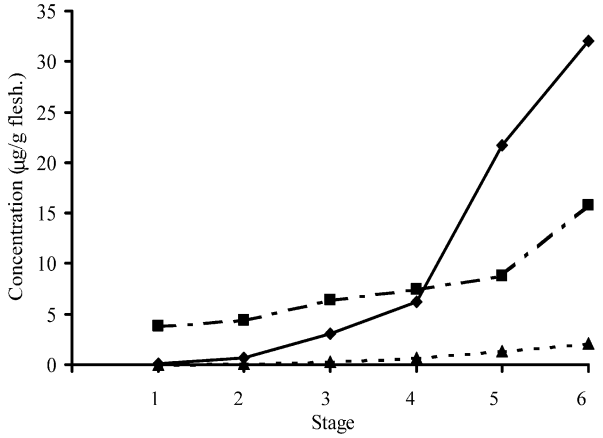

Figure 4. Change in the concentrations of esters, alcohols, and acids in the snake fruit during maturation (◆, esters; ■, acids; and ▲, alcohols). The stage definitions are described in the Plant Materials section of the Materials and Methods.

from the flesh at stage 5. The major components were methyl 3-methyl-2-pentenoate (35.2%), methyl 3-methylpentanoate (13.3%), methyl 3-hydroxy-3-methylpentanoate (7.5%), methyl 3-methyl-2-butenoate (1.9%), methyl hexanoate (4.7%), methyl 4-hydroxy-3-methyl-2-pentenoate (2.6%), methyl 3-hydroxy-2-methylbutaboate (0.4%), and other methyl esters. Carboxylic acids with C2, C4, C5, C6, C7, C8, and C12 were also dominant (27.3%), of which 2-methylbutanoic acid (16.6%) and 3-methylpentanoic acid (3.8%) were the major components. No others hydroxyl derivatives of carboxylic acids were found in the SAFE extract. Besides those compounds, lactones (2.3%), alcohols (1.1%), and other minor components were detected. In the SAFE residue, the ratio of carboxylic acids (41.4%) was comparable to the level of the methyl esters (47.6%). Among the methyl esters, the amount of the hydroxy derivatives was remarkable. Interestingly, furan and pyran derivatives, such as tetrahydro-4-methyl-2*H*-pyran-2-one (0.47%), tetrahydro-6-methyl-2*H*-pyrane-2-one (0.15%), 3,4-dihydro-2*H*-pyran-2-carbaldehyde (0.55%), 4-methyl-2(5*H*)-furanone (0.02%), and 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (Furaneol, 0.02%), were detected. Among these compounds, Furaneol is well documented as having a sweet, caramel-like flavor (*25*, *26*). We have to note that the concentration of the total volatile compounds in the SAFE extract was almost 2 times that for the SAFE residue (**Table 1**). The total number of volatile compounds in those extracts was almost double that previously reported (*1*). Since the snake fruit used in the previous report was not described in detail, i.e., variety and stage of the fruit, the differences in the extraction method and the environment of the fruit growth are the most plausible reasons for the differences in the volatile compounds compositions. Application of SAFE method to a model solution of selected aroma compounds resulted in higher yields compared to high-vacuum distillation (*2*). As shown in **Table 1**, by SAFE method we could observe the presence of a higher number of volatile compounds: 42 methyl esters were detected. Among these, 24 compounds marked with a plus in the table were not reported previously (*1*). Higher numbers of carboxylic acids (18 compounds marked with a plus) were newly detected. As already suggested, esters are commonly occurring and important aroma components of many fruits, especially tropical fruits (*28*). We may be able to suggest that these methyl esters and lactones are strong contributors to the characteristic aroma of the snake fruit, because those compounds have been

Table 1. Concentration, Odor Character, and Odor Threshold of Volatile Compounds on SAFE Extract and SAFE Residue from Snake Fruit at Stage 5 (% Area)

	compound name[a]	SAFE[b]	residue[b]	KI index	odor quality (ref)	threshold[c] (ref)
			Methyl Esters			
C-4	methyl butanoate+	t		983	pungent (*12*)	5 (*10*)
	methyl 3-methylthiopropanoate	0.04	0.01	1516		
	dimethyl succinate	0.07	0.16	1568		
C-5	methyl 3-methyl-2-butenoate+	1.92	0.11	1148		
	methyl 2-methylbutanoate	0.03		1007	fruity (*10*)	
	methyl 3-methylbutanoate	0.21	t	1016	fruity (*10*)	
	methyl pentanoate+	0.01		1048		
	methyl 2-methyl-2-butenoate	0.82	0.03	1184		
	methyl 3-hydroxy-3-methylbutanoate	0.38	0.61	1358		
	methyl 3-hydroxy-2-methylbutanoate|1+	0.42	1.38	1509	woody (*11*)	
	methyl 3-hydroxy-2-methylbutanoate|2+	0.06	0.22	1523		
	methyl 2-hydroxy-3-methylbutanoate+		0.08	1396		
	methyl 3-methylpentanoate	13.33	0.37	1115	snake fruit character[d]	
C-6	methyl 4-methylpentanoate+	0.12		1136		
	methyl 3-methyl-4-pentenoate+	t		1140		
	methyl hexanoate	4.73	0.13	1174	pear, ester (*9*,*12*)	
	methyl 3-methylenepentanoate+	0.06	t	1200		
	methyl 3-methyl-3-pentenoate #1	0.07	t	1211		
	methyl 4-methyl-2-pentenoate+	t		1229		
	methyl 3-methyl-3-pentenoate #2	0.19	0.01	1246		
	methyl 3-methyl-2-pentenoate	35.22	1.16	1252	snake fruit character[d]	
	methyl 4-methyl-3-pentenoate+	t		1270		
	methyl 2,4-hexadienoate+	0.02		1319		
	methyl 3-hydroxy-3-methylpentanoate	7.53	11.52	1448		
	methyl 2-hydroxy-4-methylpentanoate+	0.14	0.07	1470		
	methyl 2-hydroxy-3-methylpentanoate″ 1	0.02	0.08	1484		
	methyl 2-hydroxy-3-methylpentanoate″ 2	0.02	0.01	1489		
	methyl 3-hydroxy-2-methylpentanoate	t				
C-7	methyl 3-oxohexanoate+	t		1561		
	methyl 3-hydroxyhexanoate	0.25	0.74	1626		
	methyl 4-hydroxy3-methylpentanoate+		1.25	1760		
	methyl 3-methyl-4-oxopentanoate+	0.05	0.08	1532		
	methyl 4-hydroxy-3-methyl-2-pentenoate+	2.61	29.29	1984		
	methyl 5-hydroxyhexanoate+	0.03	0.29	1827	woody (*11*)	
	methyl heptanoate	0.02		1273		
	methyl benzoate+	0.03		1613		
	methyl 4-methylenehexanoate+	t		1345		
C-8	methyl octanoate+	0.02		1377	roasted coconut (*9*)	
	methyl (*Z*)-5-octenoate+	0.02		1395		
	methyl phenylacetate	0.07	0.01	1760	honey-like (*13*)	
	methyl 3-hydroxyoctanoate+		0.03	1850		
C-other	methyl dodecanoate+		0.08	1791		
			Other Esters			
	ethyl formate+	t	0.01	821		
	ethyl acetate+	0.03	0.06	884	fruity (*20*)	
	ethyl 3-methylpentanoate+	0.01		1168		
			Acids			
C-2	acetic acid+	0.01	0.07	1424	sour (*14*, *15*)	32 300 (*17*)
C-4	butanoic acid+	t	0.04	1621	sweaty (*14*, *16*)	240 (*16*)
C-5	2,2-dimethylpropanoic acid+	0.07	0.04	1568		
	2-methylbutanoic acid	16.61	24.99	1644	sweaty (*14*, *16*)	540 (*10*)
	3-methylbutanoic acid	3.7		1659	sweaty (*15*)	560 (*17*)
	pentanoic acid+	0.02	0.06	1738	sweaty (*14*)	
	3-methyl-2-butenoic acid+	0.04	0.29	1793		
	2-methyl-(*E*)-2-butenoic acid	0.8	2.37	1838		
C-6	2-methylpentanoic acid+	t		1769		
	3-methylpentanoic acid+	3.82	5.95	1783		
	4-methylpentanoic acid+	0.07	0.24	1803		
	3-methyl-3-pentenoic acid+	0.6		1982		
	2-methyl-2-pentenoic acid	0.25	1.15	1909		
	(*E*)-3-methyl-2-pentenoic acid+		1.32	1892		
	2-methyl-3-pentenoic acid+	0.03		1924		
	3-hydroxy-3-methylpentanoic acid+	t	0.33	2288		
	4-hydroxy-3-methylpentanoic acid+		0.66	2642		
	hexanoic acid	1.21	1.96	1835	sweaty (*10*, *14*)	3000 (*19*)
	cis-3-hexenoic acid+		0.15	1922		
C-others	2-ethylhexanoic acid+	0.09	0.07	1937		
	heptanoic acid	0.03		1941	rancid (*22*)	
	octanoic acid+	0.04	1.27	2048	sweaty (*18*)	
	decanoic acid+		0.5	2256	waxy (*35*)	
	hexadecanoic acid+	t		2894		

Table 1 (Continued)

compound name[a]	SAFE[b]	residue[b]	KI index	odor quality (ref)	threshold[c] (ref)
		Lactones			
3-methyl-4-pentanolid+ 1	1.44	4.09	1590		
3-methyl-4-pentanolide+ 2	0.36	1.42	1691		
4-hexanolide	0.27	1.04	1710		
2-methyl-5-pentanolide	0.02		1728		
5-hexanolide	0.18	0.01	1789		
3,4-dimethyl-2-butenolide+		0.04	1844		
4-heptanolide+		0.01	1811		
4-octanolide+	t		1968		
		Furans and pyrans			
tetrahydro-6-methyl-2H-pyran-2-one		0.15	1787		
tetrahydro-4-methyl-2H-pyran-2-one		0.47	1803		
4-methyl-2(5H)-furanone		0.02	1909		
3,4-dihydro-2H-pyran-2-carbaldehyde		0.55	1992		
furaneol		0.02	2012		
		Alcohols and Others			
methanol+	t		898		
2-propanol+	0.02		925		
2-butanol+	0.31	0.01	1022		
propanol+	t		1038		
3-methyl-2-butanol	0.04	0.05	1083		
2-pentanol+	0.03		1094		
butanol+	t		1129		
2-methylbutanol+	0.57		1188		
3-methyl-2-butenol+	t	t	1282		
2-butanone	t		901		
2-pentanone	t		975		
3-methyl-2-pentanone		t	1014		
2-methylbutanal		t	913		
methyl amyl ketone+		t	1183		
limonene+		t	1192	citrus-like (*15*)	
2-methyl-2-butenol+	0.04	t	1309		
3-methylpentanol+	0.11	0.01	1314		
1-(2-methoxy-1-methylethoxy)-2-propanol+		0.01	1478		
1-(2-methoxypropyl)-2-propanol+		0.01	1509		
(*Z*)-3-hexenol+	t		1371	pungent (*23*)	
benzyl alcohol+	0.01	0.01	1875		
2-phenylethyl alcohol	t	0.02	1909		
dodecanol+		0.01	1953		
ethylene malericanhydrate+		0.03	1600		
heptadecane+		0.33	1700		
8-heptadecene+		0.02	1716		
1-heptadecene+		0.01	1746		
2-undecanone+		0.02	1594		
diethyleneglycol monomethyl ether+		0.03	1620		
		Unidentified Peaks			
	0.47	2.24			

[a] A plus after the name indicates that the compound was not detected in the previous report. [b] t, trace. [c] Threshold in micrograms per liter of water. [d] Preliminary sniffing.

reported to show a fruity note (*10*). Carboxylic acids such as 2-methylbutanoic acid, 3-methylbutanoic acid, and hexanoic acid cause an unpleasant sweaty odor. Those three carboxylic acids have odor thresholds of 540, 560, and 3000 μg/kg, respectively (**Table 1**). The presence of 2-methylbutanoic acid in snake fruit, for example, was about 30 times higher than its odor threshold, although those values are not reported for methyl esters. Further research results will be communicated elsewhere. It is worth noting that the variation of the carboxylic acid part of the methyl esters was different from that of the carboxylic acids, indicating the substrate specificity of the enzymes being involved in the methyl ester formation.

A change in the volatile compounds content during maturation of the snake fruit was investigated. Pentane extracts issued from microwave extraction were quantitatively and qualitatively analyzed by GC−MS. The compositions of volatile compounds were similar to those in the SAFE extracts and the residue. As shown in **Figure 4**, the dominant volatile compounds were carboxylic acids of 3.86−6.37 μg/g flesh until stage 3. The level increased gradually until stage 5 to reach 8.84 μg/g flesh, and then remarkably increased to the maximum level (15.8 μg/g flesh) at stage 6. For the esters, the levels were much lower (0.09−3.07 μg/g flesh) than those of carboxylic acid until stage 3, and then they rapidly increased after stage 4 to exceed the level of carboxylic acids. The ratios of esters/carboxylic acids were 0.02−0.83 until stage 4, and 2.45 and 2.03 at stages 5 and 6, respectively. Thus, the esters, important contributors to snake fruit aroma, were mainly biosynthesized between stages 4 and 6 to reach the high level (21.7 and 32.0 μg/g flesh at stages 5 and 6, respectively). A similar phenomenon were observed in those levels in guava (*28*), peaches (*7, 29*), strawberries (*30*), and grapes (*31*).

Methyl ester formation on fruits has been reported in strawberries (*32*), bananas (*33*), and melons (*34*). There, acyl-CoAs were involved in this process. Research on ester formation in snake fruit is an important subject, and the results will be

reported elsewhere. The acid moieties of the esters present in snake fruit may be originated from degradation products of fatty acids. During maturation, the total fatty acids present in the flesh showed a maximum level at stage 3 (0.55%) and then decreased to 0.29 at stage 6 (data not shown). Unsaturated fatty acids observed in snake fruit were $C_{18(1)}$, $C_{18(2)}$, and $C_{18(3)}$. The average contributions among the total fatty acids were about 43.9%, 16.4%, and 7.17%, respectively. The unsaturated fatty acids would be oxidized to yield short-chain carboxylic acids. These carboxylic acids are well known as contributors to sweaty, sour, and/or body odors, which prevent consumers outside of Indonesia from tasting this fruit. The sniffing analysis should be done to ascertain those malodor contributors and to discover any other possibility to modify the odor.

In conclusion, we have analyzed the physical and chemical changes of snake fruit during the maturation process. On the basis of the sugar contents and firmness as well as the aroma composition, it is suggested that the fruits be consumed at stage 5 (fruit picked up 5.5 months after the pollination), when the flesh showed high firmness and accumulated a high amount of sugars and aroma compounds. Sensory evaluation tests on the fruit at each stage are required.

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