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Original Article

Volatile aroma components and MS-based electronic nose profiles of dogfruit (*Pithecellobium jiringa*) and stink bean (*Parkia speciosa*)

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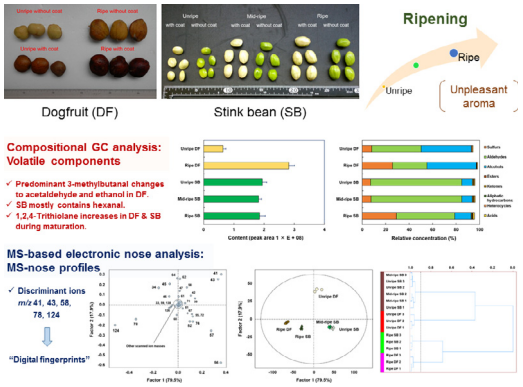
GRAPHICAL ABSTRACT



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ABSTRACT

Dogfruit (*Pithecellobium jiringa*) and stink bean (*Parkia speciosa*) are two typical smelly legumes from Southeast Asia that are widely used in the cuisines of this region. Headspace/gas chromatography/flame ionization detection analysis and mass spectrometry (MS)-based electronic nose techniques were applied to monitor ripening changes in the volatile flavor profiles of dogfruit and stink bean. Compositional analysis showed that the ripening process greatly influenced the composition and content of the volatile aroma profiles of these two smelly food materials, particularly their alcohol, aldehyde, and sulfur components. The quantity of predominant hexanal in stink bean significantly declined ($P < 0.05$) during the ripening process, whereas the major volatile components of dogfruit changed from 3-methylbutanal and methanol in the unripe state to acetaldehyde and ethanol in the ripe bean. Moreover, the amount of the typical volatile flavor compound 1,2,4-trithiolane significantly increased ($P < 0.05$) in both ripened dogfruit and stink bean from 1.70 and 0.93%, to relative amounts of 19.97 and 13.66%, respectively. MS-based nose profiling gave further detailed differentiation of the volatile profiles of dogfruit and stink bean of various ripening stages through multivariate statistical analysis, and provided discriminant ion masses, such as *m/z* 41, 43, 58, 78, and 124, as valuable "digital fingerprint" dataset that can be used for fast flavor monitoring of smelly food resources.

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Introduction

Dogfruit (*Pithecellobium jiringa*) and stink bean (*Parkia speciosa*) are popular smelly legumes from Southeast Asia that possess unpleasant aroma characteristics but that are commonly consumed in various local cooked dishes [1–3]. Dogfruit derives from the Mimosa family (Mimosaceae). It has round-flattened, horse chestnut bean shape and grow in large dark purple pods [1]. On the other hand, stink bean which belongs to pea or bean family (Fabaceae), is formed in dry, longitudinal dehiscent, straight or twisted green pods [4]. Dogfruit and stink bean are commercially available in the markets most of the year and are known under different local names across the region: dogfruit is called as jengkol, jering, krakos, yiniking, niang-yai, and ma-niang, whereas stink bean is also known as smelly bean, petai, sataw, sotor, chou-dou, and u'pang. The unfavorable aspects of these beans are their anti-nutritional components and toxicities if they are excessively consumed or improperly cooked, and in some severe cases, these undesirable properties can cause acute and chronic health effects [1,5,6]. On the other hand, the beans contain various bioactive compounds that possess potent beneficial functionalities, for example, the antifungal and antibacterial activities of dogfruit lectins and the antidiabetic and antihypertensive potentials of stink bean sterols and peptides, respectively [7–9]. In spite of the drawbacks, dogfruit and stink bean are regarded as regional delicacies, and these food resources have been used as raw materials in the production of various valuable semi-processed or processed food products, such as flours and cookies [3,10,11].

Agricultural crops, including those of legumes, are distinguishable, not only by their primary appearance or physico-chemical traits but also by other important quality attributes, such as sensory perception [12,13]. Moreover, ripening makes critical biochemical contributions to the metabolite development of volatile constituents and other nutritional components of horticultural products that might differentiate their potential food applications [14,15]. The alteration of volatile aroma components, particularly, has an important direct effect on the appeal of raw or cooked foods, as a whole or indirectly, by influencing other flavor properties and thresholds [13,15,16]. Consequently, maturity could be used as a potent indicator for the progression of volatile aroma composition and flavor characteristics in agricultural crops, which might lead to a distinction in their perceived aroma and consumer acceptance [17,18].

Numerous innovative analytical techniques have been developed to complement the use of conservative methods with common analytical instruments for evaluating food quality traits [12,19,20]. The improved analytical approaches include reliable techniques for both qualitative and quantitative measurements, and they are most often combined with robust chemometric statistical analysis to discriminate samples. Electronic nose measurement technologies, such as gas sensor arrays, fast gas chromatography (GC), and mass spectrometry (MS), have also been effectively used for distinguishing the volatile flavor profiles of various food resources and products [20–22]. The MS-based electronic nose is a non-targeted volatile-profiling technique for differentiating evaluated samples without a chromatography peak separation requirement. This profiling technique works based on the selection of ion masses needed for statistical analysis by pattern-recognition learning methods, and it can display discriminant ion masses of samples' volatile components as valuable "digital fingerprints" [19,21,23].

Therefore, the aim of this study was to determine the volatile aroma components of dogfruit and stink bean of different ripening stages and to differentiate their volatile profiles through compositional and MS-based nose datasets (Fig. 1). The volatile constituents of dogfruit and stink bean were examined by using GC with flame ionization detection (GC-FID), and the volatile characteristics were discriminated by using MS-based electronic nose and chemometric analyses. This is the first report on the volatile and MS-based nose profiles of these two smelly plant resources at different stages of maturity.

Methods

Sample preparation and standards

Fresh samples of two dogfruits (unripe and ripe) and three stink beans (unripe, mid-ripe, and ripe), which originated from the same farming source, were collected from a local market at Bandung, Indonesia, in July 2013. The plant species were authenticated by Dr. Kusumiyati (Laboratory of Horticulture, Faculty of Agriculture, Padjadjaran University), in terms of the perceived visual and physical properties of entire pods and beans. Bean type was morphologically characterized for average weight, size, and color (Table 1 and Fig. 2). The dogfruits and stink beans were peeled from their pods and shells, and the beans were cut into small pieces (about 5 mm^2) and stored at −30 °C prior to analysis. Authentic standards (carbon disulfide, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, acetaldehyde, propanal, 2-methylpropanal, butanal, 2-methylbutanal, 3-methylbutanal, pentanal, hexanal, heptanal, 2-hexenal, octanal, 2-heptenal, nonanal, 2-octenal, benzaldehyde, 2-nonenal, methanol, ethanol, 3-methylbutanol, pentanol, hexanol, octane, acetone, 2-pentanone, ethyl acetate, hexyl acetate, acetic acid, and hexanoic acid) used for the identification of volatile aroma components were purchased from Sigma–Aldrich (St Louis, MO, USA) and Tokyo Chemical Industry (Tokyo, Japan).

Volatile aroma composition analysis

The composition of the volatile aroma components of dogfruit and stink bean were examined by using an Agilent 7890A GC-FID system equipped with an Agilent G1888 headspace sampler and a fused silica capillary DB-Wax column (60 m × 0.25 mm internal dimensions, 0.25 μm film thickness, Agilent J&W, Santa Clara, CA, USA) [24]. The volatile aroma compounds were extracted from a 2 g sample, which was placed in a 20 mL headspace vial, at 80 °C for 20 min, and subsequently pressurized at 11 psi for 0.3 min into the injection port. The sample loop and transfer line were set at 170 and 210 °C, respectively. The injector and FID were both programmed at 250 °C, and the injection split ratio was 1:10. The oven was initially held for 5 min at a temperature of 40 °C, which was then raised to 200 °C at a rate of 5 °C/min and was isothermally maintained for 3 min. Helium was used as the carrier gas, and the flow rate was programmed at 23 cm/s.

The volatile compounds were identified by comparison with the linear retention indices (RIs) of a homologous series of *n*-alkanes (C5–C20) and by assessment of the MS patterns of the samples and authentic standards with MS data obtained from the National Institute of Standards and Technology (NIST) MS Library, Version 2008. For MS detection, an Agilent 5975C mass spectrometer was used with the same headspace extraction, column, and oven conditions as those described above. The electron-impact ion source and interface were both programmed at 230 °C, the electron ionization at 70 eV, and the mass acquisition range (*m*/*z*) at 29–300 amu. The relative amounts (%) of the volatile compounds were determined by measurement of the peak area response. All analyses were carried out in triplicate.

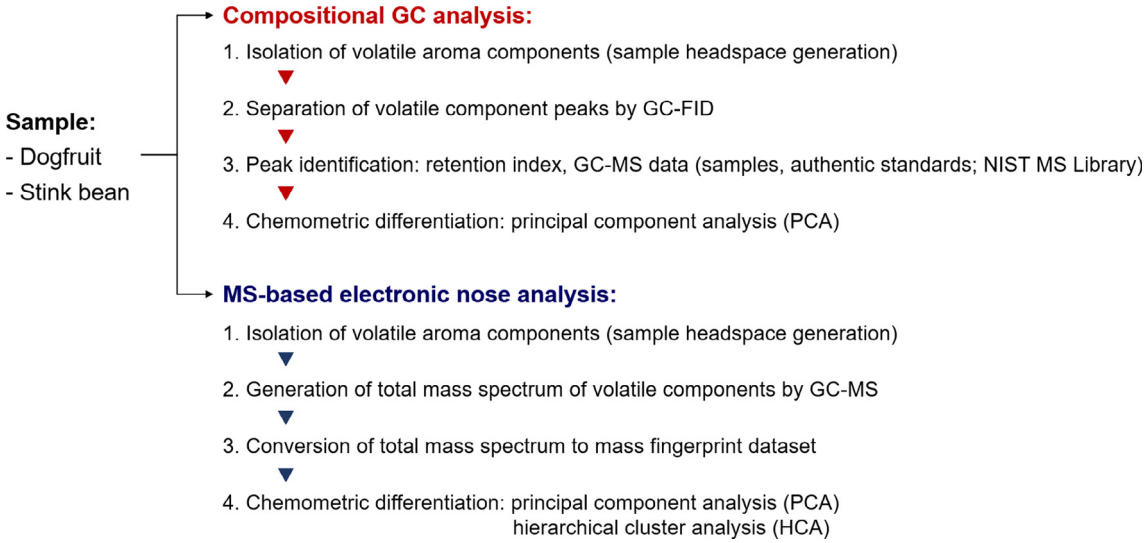

Fig. 1. Workflow of volatile aroma composition and MS-based electronic nose analyses of dogfruit and stink bean.

Table 1
Morphological traits of dogfruit and stink bean of different ripening stages.

Traits	Dogfruit		Stink bean		
	Unripe	Ripe	Unripe	Mid-ripe	Ripe
Bean number per pod	1–2	1–2	8–9	12–13	14–15
Coat thickness (mm)	0.45 ± 0.07	0.45 ± 0.07	0.27 ± 0.06	0.29 ± 0.03	0.40 ± 0.07
Bean weight (g)	5.04 ± 0.89	12.45 ± 1.61	1.14 ± 0.09	1.16 ± 0.10	2.81 ± 0.22
Bean length (mm)	25.33 ± 2.28	34.32 ± 2.30	17.44 ± 1.54	18.28 ± 0.24	23.80 ± 0.25
Bean width (mm)	26.86 ± 1.79	33.80 ± 2.28	15.35 ± 0.89	15.29 ± 0.52	20.35 ± 1.00
Bean height (mm)	14.01 ± 1.10	19.89 ± 1.98	7.47 ± 0.33	7.59 ± 0.25	10.74 ± 0.35
Bean color	Light yellowish cream	Deep greenish brown	Light whitish green	Light green	Deep green

Each value is expressed as the mean ± standard deviation (n = 5). Colors were determined by visual observation.

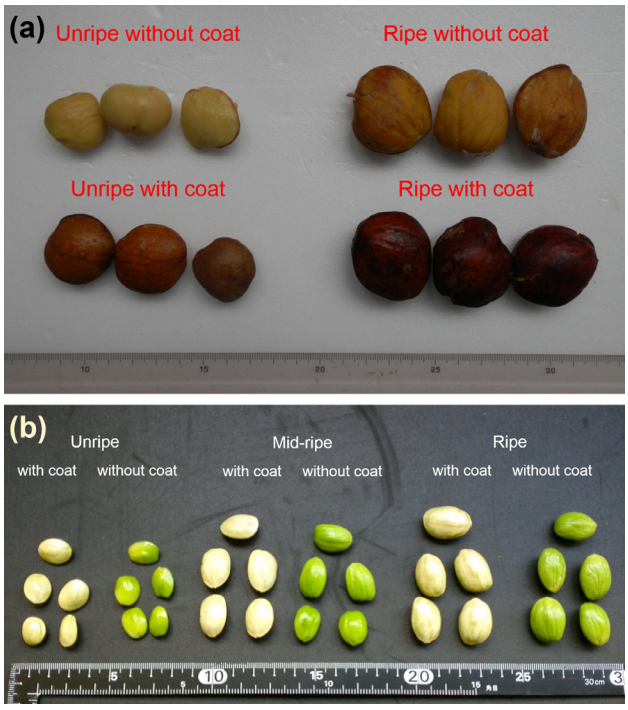

Fig. 2. (a) Dogfruit and (b) stink bean with and without bean coats of different ripening stages.

MS-based electronic nose analysis

The MS-nose profiles of dogfruit and stink bean were acquired by using a GERSTEL Chemsensor (GERSTEL, Mülheim, Germany) in an Agilent G1888 HSS-7890A GC-5975C MS system (Agilent J&W) [19]. The headspace extraction and MS conditions were set as described above, except for the ion source and interface temperatures, which were both maintained at 250 °C. Volatile compounds from the samples were passed through an HP-5MS fused silica capillary column (30 m × 0.25 mm internal dimensions, 0.25 μm film thickness, Agilent J&W). The oven was initially held for 1 min at a temperature of 40 °C, which was then raised to 250 °C at a rate of 20 °C/min and was isothermally maintained for 3 min. The total mass spectrum intensities of detected ion masses (m/z 29–300) of volatile components were converted to a mass fingerprint dataset. All analyses were carried out in triplicate.

Statistical analysis

The relative concentrations of the volatile aroma components of dogfruit and stink bean were statistically compared by using Microsoft Office Excel 2007 (Microsoft Corp., Redmond, WA, USA) by analysis of variance, followed by Fisher's least significant difference post hoc test at $P < 0.05$. The chemometric differentiation of volatile compounds in dogfruit and stink bean and a correlation of their ion masses were evaluated by mean-centered principal component analysis (PCA) by using Pirouette 4.5 software (Infometrix, Bothell, WA, USA). The connection between dogfruit and stink bean was also statistically determined through a hierarchical

cluster analysis (HCA) plot by using Pirouette 4.5 software. The MS data were preprocessed in a mean-centering structure, and the HCA plot was taken at Euclidean distance and incremental linking.

Results and discussion

Volatile aroma components of dogfruit and stink bean of different ripening stages

Dogfruit and stink bean possessed distinct volatile aroma components that accounted for 94.36–98.24% of identified compounds at different maturation stages (Table 2). The peak area relative content of these volatiles was 0.64 and 2.82 E+08 in unripe and ripe dogfruits, respectively. They ranged from 1.85 to 1.94 E+08 in stink bean during ripening. There were 24 volatile components in both unripe and ripe dogfruit, whereas stink bean had more complex profiles with 42, 41, and 32 compounds in unripe, mid-ripe, and ripe beans, respectively. The major volatile component groups of unripe dogfruit were 42.74% alcohols (4 compounds) and 42.15% aldehyde compounds (12), followed by 8.05% sulfur compounds (5). The composition due to the alcohols and sulfurs altered to 41.90 and 25.90%, respectively, during ripening, whereas the

Table 2
Relative concentrations (%) of volatile aroma compounds of dogfruit and stink bean.

No	RI	Compound	Dogfruit		Stink bean			Identification[#]
			Unripe	Ripe	Unripe	Mid-ripe	Ripe	
1	525	Hydrogen sulfide	0.16 ± 0.04^{d}	0.19 ± 0.03^{d}	2.32 ± 0.02^{b}	1.92 ± 0.13^{c}	5.59 ± 0.39^{a}	RI, MS
2	670	Methanethiol	0.24 ± 0.08^{c}	0.21 ± 0.01^{c}	3.25 ± 0.46^{b}	4.00 ± 0.39^{b}	9.63 ± 0.93^{a}	RI, MS
3	724	Carbon disulfide	nd.	0.05 ± 0.01^{a}	tr.	tr.	tr.	RI, MS, Std
4	739	Dimethyl sulfide	5.84 ± 0.21^{a}	1.12 ± 0.04^{b}	0.20 ± 0.01^{c}	0.11 ± 0.01^{c}	tr.	RI, MS, Std
5	1023	Thiophene	tr.	tr.	0.05 ± 0.00^{a}	0.07 ± 0.01^{a}	0.05 ± 0.00^{a}	RI, MS
6	1071	Dimethyl disulfide	nd.	nd.	0.15 ± 0.00^{b}	0.20 ± 0.02^{a}	0.11 ± 0.01^{c}	RI, MS, Std
7	1112	1-(Methylthio)pentane	tr.	tr.	0.05 ± 0.03^{a}	tr.	tr.	RI, MS
8	1391	Dimethyl trisulfide	tr.	nd.	0.02 ± 0.01^{a}	0.03 ± 0.00^{a}	tr.	RI, MS, Std
9	1406	*S*-Ethyl hexanethioate	nd.	0.03 ± 0.00^{a}	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	tr.	RI, MS
10	1560	2-Pentylthiophene	tr.	tr.	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	tr.	RI, MS
11	1675	2,3,5-Trithiahexane	nd.	tr.	0.04 ± 0.01^{a}	0.02 ± 0.00^{a}	tr.	RI, MS
12	1716	1-Methyl-3-(methylthio)benzene	0.11 ± 0.02^{b}	4.34 ± 0.25^{a}	0.02 ± 0.00^{b}	tr.	0.09 ± 0.00^{b}	RI, MS
13	1785	1,2,4-Trithiolane	1.70 ± 0.52^{c}	19.97 ± 0.40^{a}	0.93 ± 0.20^{c}	1.12 ± 0.23^{c}	13.66 ± 1.08^{b}	RI, MS
		Total sulfurs	8.05	25.90	7.10	7.52	29.13	
14	698	Acetaldehyde	7.36 ± 1.22^{d}	29.02 ± 0.24^{a}	15.01 ± 1.08^{c}	20.72 ± 1.12^{b}	6.96 ± 0.23^{d}	RI, MS, Std
15	782	Propanal	0.18 ± 0.01^{c}	0.06 ± 0.00^{e}	0.25 ± 0.01^{a}	0.21 ± 0.00^{b}	0.12 ± 0.01^{d}	RI, MS, Std
16	807	2-Methylpropanal	5.53 ± 0.44^{a}	tr.	0.09 ± 0.00^{b}	0.07 ± 0.01^{b}	tr.	RI, MS, Std
17	867	Butanal	tr.	tr.	0.19 ± 0.01^{b}	0.20 ± 0.01^{a}	0.14 ± 0.01^{c}	RI, MS, Std
18	908	2-Methylbutanal	4.07 ± 0.30^{a}	tr.	0.04 ± 0.00^{b}	0.04 ± 0.00^{b}	tr.	RI, MS, Std
19	912	3-Methylbutanal	22.13 ± 2.44^{a}	tr.	0.05 ± 0.00^{b}	0.04 ± 0.00^{b}	tr.	RI, MS, Std
20	974	Pentanal	0.31 ± 0.01^{c}	0.07 ± 0.00^{d}	3.70 ± 0.18^{a}	3.58 ± 0.12^{a}	3.00 ± 0.15^{b}	RI, MS, Std
21	1078	Hexanal	1.39 ± 0.17^{d}	0.12 ± 0.00^{d}	56.03 ± 1.52^{a}	50.28 ± 1.08^{b}	38.79 ± 2.41^{c}	RI, MS, Std
22	1151	2-Methylhexanal	0.12 ± 0.02^{b}	0.04 ± 0.02^{b}	1.52 ± 0.14^{a}	1.40 ± 0.05^{a}	0.14 ± 0.02^{b}	RI, MS
23	1179	Heptanal	0.10 ± 0.02^{c}	tr.	0.20 ± 0.01^{a}	0.17 ± 0.00^{a}	0.14 ± 0.01^{b}	RI, MS, Std
24	1216	2-Hexenal	0.50 ± 0.13^{a}	nd.	0.05 ± 0.00^{b}	0.04 ± 0.00^{b}	0.03 ± 0.00^{b}	RI, MS, Std
25	1319	Octanal	tr.	tr.	0.05 ± 0.00^{a}	0.04 ± 0.01^{b}	0.03 ± 0.01^{b}	RI, MS, Std
26	1326	2-Heptenal	nd.	nd.	0.21 ± 0.01^{a}	0.18 ± 0.00^{b}	0.09 ± 0.00^{c}	RI, MS, Std
27	1395	Nonanal	0.23 ± 0.04^{a}	0.03 ± 0.00^{d}	0.15 ± 0.01^{b}	0.11 ± 0.01^{c}	0.13 ± 0.01^{bc}	RI, MS, Std
28	1449	2-Octenal	tr.	tr.	0.16 ± 0.01^{a}	0.14 ± 0.00^{b}	0.11 ± 0.01^{c}	RI, MS, Std
29	1637	Benzaldehyde	tr.	tr.	tr.	0.04 ± 0.00^{a}	tr.	RI, MS, Std
30	1655	2-Nonenal	tr.	tr.	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	tr.	RI, MS, Std
31	1683	2,4-Nonadienal	0.23 ± 0.01^{a}	tr.	tr.	tr.	tr.	RI, MS
		Total aldehydes	42.15	29.33	77.72	77.31	49.67	
32	895	Methanol	34.16 ± 0.93^{a}	13.89 ± 0.33^{b}	6.23 ± 0.30^{d}	6.21 ± 0.18^{d}	10.93 ± ± 0.71^{c}	RI, MS, Std
33	933	Ethanol	7.26 ± 0.18^{b}	27.78 ± 0.64^{a}	0.85 ± 0.05^{c}	0.81 ± 0.01^{c}	0.73 ± 0.26^{c}	RI, MS, Std
34	1207	3-Methylbutanol	0.94 ± 0.06^{a}	0.08 ± 0.01^{b}	tr.	tr.	nd.	RI, MS, Std
35	1250	Pentanol	0.38 ± 0.01^{c}	0.15 ± 0.00^{d}	1.45 ± 0.03^{a}	1.32 ± 0.03^{b}	1.32 ± 0.08^{b}	RI, MS, Std
36	1353	Hexanol	tr.	tr.	0.38 ± 0.10^{b}	0.43 ± 0.12^{b}	0.99 ± 0.06^{a}	RI, MS, Std
		Total alcohols	42.74	41.90	8.90	8.77	13.97	
37	792	Octane	tr.	0.03 ± 0.00^{b}	0.04 ± 0.00^{b}	0.03 ± 0.00^{b}	0.16 ± 0.02^{a}	RI, MS, Std
38	1436	(*Z*)-3-Ethyl-2-methyl-1,3-hexadiene	nd.	nd.	0.17 ± 0.01^{a}	0.13 ± 0.01^{b}	0.10 ± 0.01^{c}	RI, MS
		Total aliphatic hydrocarbons	–	0.03	0.21	0.17	0.25	
39	810	Acetone	nd.	0.12 ± 0.00^{c}	0.13 ± 0.01^{bc}	0.15 ± 0.00^{b}	0.38 ± 0.01^{a}	RI, MS, Std
40	971	2-Pentanone	0.08 ± 0.00^{d}	0.03 ± 0.00^{d}	0.89 ± 0.11^{b}	1.03 ± 0.07^{a}	0.37 ± 0.02^{c}	RI, MS, Std
		Total ketones	0.08	0.15	1.03	1.18	0.75	
41	880	Ethyl acetate	tr.	0.04 ± 0.00^{b}	0.04 ± 0.01^{bc}	0.03 ± 0.00^{c}	0.08 ± 0.00^{a}	RI, MS, Std
42	1291	Hexyl acetate	tr.	0.09 ± 0.00^{a}	0.07 ± 0.00^{b}	0.09 ± 0.02^{a}	0.07 ± 0.00^{b}	RI, MS, Std
		Total esters	–	0.13	0.11	0.12	0.14	
43	947	2-Ethylfuran	tr.	tr.	0.06 ± 0.00^{a}	0.05 ± 0.01^{b}	0.03 ± 0.00^{c}	RI, MS
44	1239	2-Pentylfuran	0.82 ± 0.05^{a}	0.32 ± 0.10^{bc}	0.25 ± 0.03^{c}	0.31 ± 0.04^{c}	0.43 ± 0.05^{b}	RI, MS
		Total heterocycles	0.82	0.32	0.32	0.36	0.47	
45	1456	Acetic acid	0.53 ± 0.14^{b}	0.47 ± 0.03^{b}	0.53 ± 0.11^{b}	0.63 ± 0.11^{b}	0.93 ± 0.12^{a}	RI, MS, Std
46	1858	Hexanoic acid	tr.	tr.	0.16 ± 0.05^{b}	0.21 ± 0.02^{b}	0.31 ± 0.06^{a}	RI, MS, Std
		Total acids	0.53	0.47	0.70	0.84	1.24	
		Total identified	94.36	98.24	96.08	96.26	95.62	
		Total content (peak area 1 × E + 08)	0.64	2.82	1.94	1.82	1.85	

Each value is expressed as the mean ± standard deviation (n = 3), obtained by GC-FID analysis; nd.: not detected; tr.: trace amount (<0.01%); values in the same row followed by the same letter are not significantly different (P < 0.05).

[#] RI: identification based on retention index; MS: identification based on the NIST MS library; Std: identification based on pure standards analyzed by mass spectrometry.

aldehyde content declined from 42.15 to 29.33%, with only half the number of identified compounds remaining. On the other hand, the stink beans predominately contained aldehydes, although the proportion declined from 77.72 to 49.67% during ripening; this was accompanied by elevations in the alcohol and sulfur components from 8.90 to 13.97% and 7.10 to 29.13%, respectively. These results indicated that various biochemical reactions, including lipid and carbohydrate degradations, as well as amino acid and phenylpropanoid metabolic changes, occur to a large extent during final ripening of these beans and can alter their volatile flavor profiles [13,14]. Conversely, maturation development from the early to intermediate ripening stage has less impact on the overall volatile flavor profile of stink bean, which indicates that slower volatile component generation occur while the plant is using more nutrients for enlarging its size and weight [13,15].

In detail, the predominant volatile components of unripe dogfruit were methanol and 3-methylbutanal (34.16 and 22.13%, respectively), the amounts of which were significantly higher ($P < 0.05$) than those in ripe dogfruit and other smelly beans (Table 2). The composition also comprised intermediate amounts of acetaldehyde, ethanol, dimethyl sulfide, and 2-methylpropanal ranging from 5.53 to 7.36%. These volatile components may provide green, malty, pungent, and sulfurous smells to unripe dogfruit [25,26]. Moreover, unripe dogfruit contained significantly higher minor amounts of 3-methylbutanol, 2-pentylfuran, 2-hexenal, and nonanal than other materials and was the only sample containing 2,4-nonadienal. Conversely, ripe dogfruit had significantly higher acetaldehyde, ethanol, and 1,2,4-trithiolane levels, at 29.02, 27.78, and 19.97%, respectively. These predominant volatiles contribute pungent and ether odors to the characteristic ripening of this food material [26]. Sulfuric 1,2,4-trithiolane, in particular, is known to be one of the key aroma components in shiitake mushrooms that provide the woody and fresh shiitake-mushroom perceptions [27]. However, both unripe and ripe dogfruits lacked dimethyl disulfide, 2-heptenal, and (*Z*)-3-ethyl-2-methyl-1,3-hexadiene which might exclude sour-putrid cabbage, soap-fat, and nutty characteristics from their volatile flavor profiles, respectively [25,26,28].

Stink bean had a remarkably higher amount of hexanal, which may specify green and grassy aroma traits [29], than that in dogfruit. In spite of that, the amount of this volatile aldehyde significantly and gradually declined during ripening, from 56.03 to 50.28% in unripe and mid-ripe beans, respectively, and it then reached 38.79% in the ripe stage. Moreover, stink bean had about 15.01% acetaldehyde in the unripe stage, which significantly increased to a level of 20.72% in the mid-ripe period but then dropped to 6.96% during the final ripening process. On the other hand, unripe and mid-ripe stink beans comprised steady intermediate amounts of methanethiol (3.25–4.00%) and methanol (6.21–6.23%), which were then significantly enhanced to 9.63 and 10.93%, respectively. The ripening process also remarkably improved concentrations of hydrogen sulfide and 1,2,4-trithiolane from 1.92 and 0.93% to 5.59 and 13.66%, respectively. The large portion of sulfuric compounds in the compositional result of the present study is in agreement with the previously reported volatile profile of Malaysian stink beans [2]; these compounds are also important constituents in other strong-aroma plant materials and products, including leeks, onions, and dried mushrooms [27,30]. Taken together, the sulfurous, putrid, cheesy, woody, and shiitake odor characteristics from sulfuric volatile components are enhanced in stink bean during ripening and may impact on the sensory flavor perception when it is consumed or used as a food ingredient [26,27,29].

The distinctiveness of the volatile flavor profiles of dogfruit and stink bean of different ripening stages was also shown from the useful arrangement for the first two principal component (PC) factors in PCA plots that were derived from the relative concentrations of the volatile aroma components (Fig. 3). The factor loadings plotted several distinct volatile components for the first two PC factors that might explain the volatile composition variations of dogfruit and stink bean (Fig. 3a). They were methanol, 3-methylbutanal, 2-methylbutanal, 2-methylpropanal, and dimethyl sulfide, which were plotted in the positive quadrant of both factors, whereas ethanol, acetaldehyde, 1,2,4-trithiolane, and 1-methyl-3-(methylthio)benzene were only positively related to factor 1. On the other hand, hexanal was clearly separated in the outlying negative quadrant of factor 1, along with methanethiol, hydrogen sulfide, and pentanal, but the latter compounds were close to the plot center where other volatile compounds were loaded. These center-loaded plots indicated compositional likeness of the volatiles in dogfruit and stink bean and, thus, suggest common base aroma formations to the two bean materials regardless of the maturity stage. Moreover, the score plots showed opposite separation of the materials to the first PC factor (69.4%), in which dogfruit was recorded in the positive quadrant and stink bean in the negative (Fig. 3b). Therefore, the second PC factor (25.5%) could separate unripe and ripe dogfruit but failed to distinguish the volatile-profile variations in stink bean during ripening. This PCA outcome thus clearly

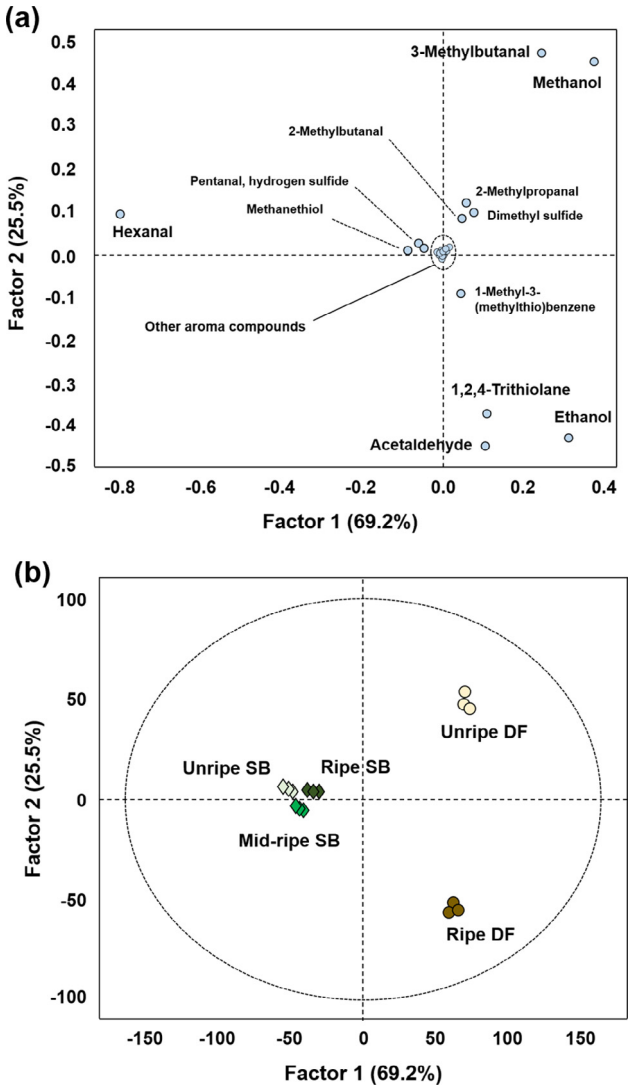

Fig. 3. (a) Factor loadings and (b) principal component score plots of the relative concentrations of the volatile aroma compounds of dogfruit (DF) and stink bean (SB), obtained by GC-FID analysis.

showed separation of the two beans according to their volatile aroma components as discriminatory loading factors. However, stink beans at different maturity stages might be recorded as a single material when stink bean and dogfruit are evaluated together. On the other hand, the maturity stage allowed differentiation of the volatile profile of dogfruit, as indicated by significantly higher amounts of 3-methylbutanal and methanol in the unripe material, whereas the prominent volatiles were acetaldehyde, ethanol, and 1,2,4-trithiolane in fully ripened beans.

MS-based electronic nose profiles of dogfruit and stink bean of different ripening stages

The volatile aroma profiles of dogfruit and stink bean were also differentiated through a PCA plot from MS-nose analysis that accounted for 97.4% in the first two PC factors (Fig. 4). The score plot outlined a separation of the unripe or mid-ripe dogfruit and stink bean from their fully ripened beans that was clearly bordered by the zero line of PC factor 1 (Fig. 4b). Moreover, unripe dogfruit was solely positively associated with both factors and was clearly separated from ripe dogfruit and any stink bean. However, unlike the result in the volatile compositional PCA plot, ripened stink bean was distinctly plotted from unripe and mid-ripe beans (Fig. 3b versus Fig. 4b). This improved volatile-profile separation was due to the MS intensities of influential discriminatory ions that were captured from scanned ion masses with a much larger number of loaded variables than that of the volatile compositional method (272 ions [recorded from *m*/*z* 29–300] versus 46 identified compounds) (Fig. 3a versus Fig. 4a) [19,21]. The potential association had been found between discriminant ion masses with MS fragmentation. These may derive from the samples' volatiles through comparison of each discriminant ion with the MS fragmentation patterns (target and qualifier ions) of the identified volatiles listed in Table 2 and corresponding authentic standards, analyzed by compositional GC method.

In detail, the corresponding loading plot showed important scattered ions, such as *m*/*z* 39, 41, 42, 43, 58, 62, and 71, that positively associated with both PC factors, whereas *m*/*z* 60, 78, 124, and 126 were oppositely positioned (Fig. 4a). These discriminatory ion masses revealed key qualifier ions for associated aroma compounds and might be suitable for distinguishing dogfruit and stink bean during ripening. For instance, *m*/*z* 41, 43, and 58 which may derive from predominant 3-methylbutanal might contribute to the separation of unripe dogfruit from other beans (Fig. 4b and Table 2). Conversely, *m*/*z* 78 and 124, which are qualifier ions for 1,2,4-trithiolane, clearly indicate the ripened beans, and the significantly greater relative concentration of this sulfuric compound in ripe dogfruit located it at a more distant negative plot within PC factor 1. In addition, other recorded ion masses were only positively associated with factor 1, including *m*/*z* 55, 56, 57, 67, 72, 76, 81, and 82. These prominent scattered ions might further indicate the influence of hexanal as the predominant compound in unripe and mid-ripe beans, as the qualifier ions *m*/*z* 55, 56, 57, 67, and 82 are linked to this green-grassy aroma emitting aldehyde.

Another multivariate statistical analysis also confirmed volatile aroma profile differentiation of dogfruit and stink bean from their recorded ion masses in an HCA dendrogram clustering tree (Fig. 5). Out of the five beans of different ripening stages, four volatile groups were formed at a component similarity of 0.900, wherein stink beans at the unripe and mid-ripe stages comprised a mixed cluster. Moreover, ripe dogfruit and stink bean were presented as closest group to one another and were split from their immature forms, indicating the comparable progression of their volatile component profiles during the bean maturation process. This clustering outline clearly provides a better general view of the volatile aroma component discrimination in plant resources during ripening of different origins, including dogfruit and stink bean [21,22]. These

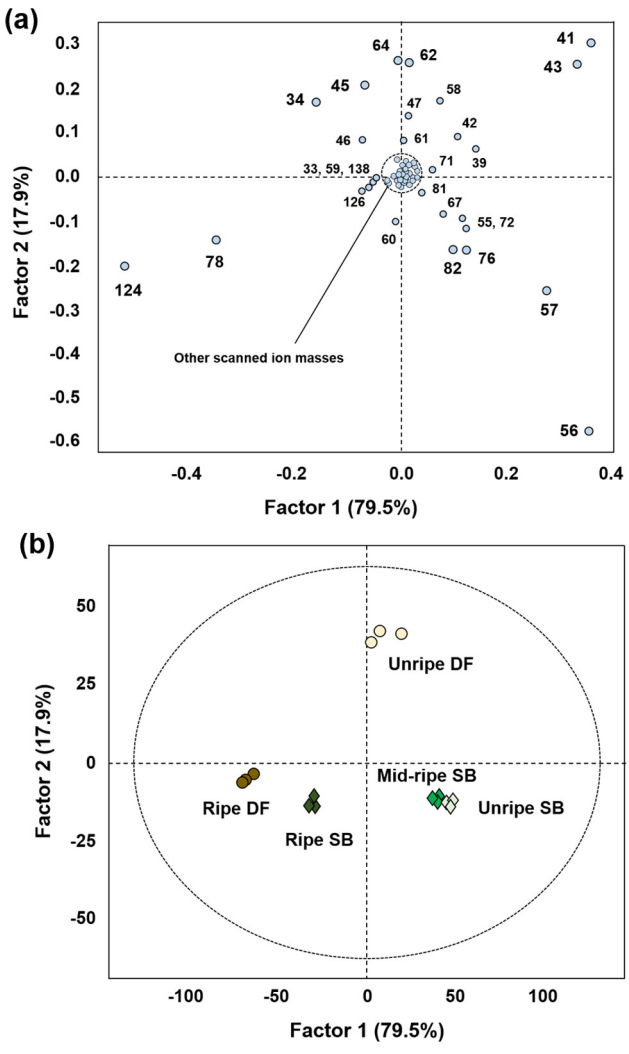

Fig. 4. (a) Factor loadings and (b) principal component score plots of the volatile profiles of dogfruit (DF) and stink bean (SB), obtained by MS-nose analysis.

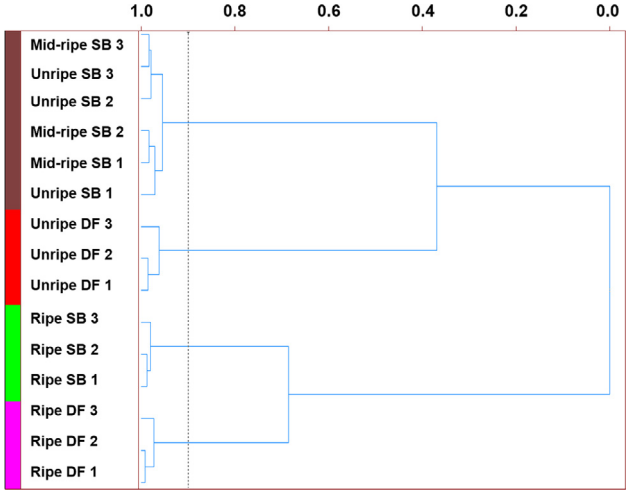

Fig. 5. HCA dendrogram of the volatile profiles of dogfruit (DF) and stink bean (SB), obtained by MS-nose analysis. The volatile component similarity was obtained as 0.900.

MS-based nose results detailed the volatile-profile differentiations and provided an important chemical markers in a form of discriminative MS dataset as "digital fingerprints" for dogfruit and stink bean during maturity for further development of rapid measurement technology on volatile alterations evaluation of these legumes or their derivative products [21]. The MS-based electronic nose method and chemometric data analysis might thus be applied for monitoring the flavor quality of smelly plant materials in a faster and thorough manner than compositional GC measurement, which confirms the advantageous use of MS-based e-nose profiling technique on differentiation of food flavor [19–21,31,32].

Conclusions

Dogfruit and stink bean had distinctive compositions and contents of volatile aroma components that varied greatly in the alcohol, aldehyde, and sulfur compounds, but stink bean comprised a greater number of volatiles than that of dogfruit. Stink bean mostly contained hexanal at all maturity stages, whereas unripe dogfruit was primarily predominated by 3-methylbutanal and methanol, which then altered to acetaldehyde and ethanol in ripe dogfruit. There were significant changes in the amount of 1,2,4-trithiolane in both dogfruit and stink bean during maturation. The compositional dataset constructed a multivariate PCA plot that displays separation only for dogfruit during ripening. The non-targeted MS-based electronic nose and chemometric analyses further distinguished the volatile profiles of dogfruit and stink bean on an ion-mass basis, and detailed the differentiation of these smelly materials through PCA and HCA arrangements. The MS-based nose technique also provided a valuable recorded MS dataset and discriminative ion masses which may be derived from samples' volatile components, such as m/z 41, 43, 58, 78, and 124, that could be used as "digital fingerprints" for monitoring volatile flavor changes in dogfruit and stink bean during ripening.

Conflicts of interest

Authors have declared no conflicts of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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