Analysis of Aroma Volatile Profile of Philippine Durian Pulp (*Durio zibethinus* Rumph. ex Murray) using Headspace Solid Phase Microextraction (HS-SPME) coupled with GC-MS

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Abstract Durian (*Durio zibethinus* Rumph. ex Murray) is an exotic tropical fruit known for its excellent flavor and strong distinctive aroma. Volatiles of durian pulp from different cultivars in Thailand, Malaysia and Indonesia were already reported. However, no study of volatiles from Philippine 'Puyat' durian is available. 'Puyat' is the most preferred by consumers in the Philippines due to its firm flesh with sweet and buttery taste. Aroma volatile compounds were analyzed by headspace solid phase microextraction (HS-SPME) method and gas chromatography – mass spectrometry (GC-MS). Volatiles were extracted for 1 hour using 65 µm PDM/DVB SPME fiber and separated by DB-WAX capillary column (30 m × 0.25 mm id × 0.25 µm film thickness). Durian pulp produced 22 volatiles composed of 15 esters, 6 sulfurs, and 1 thioacetal. Ethyl propanoate, ethyl octanoate, propyl propanoate, ethyl 2-methyl butanoate and diethyl disulfide were the major compounds identified. These compounds were also reported to be the most abundant in durian cultivars of other countries. Results of this study can then be used for future development of postharvest technologies to control the production of volatiles from Philippine durian pulp.

Keywords Durian, SPME, GC-MS, volatile compounds

1. Introduction

Durian (*Durio zibethinus* Rumph. ex Murray), known as the 'King of Tropical Fruits', is a highly prized fruit in Southeast Asian countries. Its production and export is dominated by Thailand, followed by Malaysia and Indonesia [1]. It is also one of the ten major fruit crops in the Philippines wherein it is primarily produced for domestic consumption and only a small fraction is utilized for exports [2].

Durian fruit has an excellent, unique flavor but with a strong and very distinctive aroma which makes it difficult to transport. It is prohibited in air-conditioned rooms and public transports. Its aroma can be categorized as fruity and onion-like, which are respectively linked to the fruit's ester and sulfury volatile composition [3].

Different studies have already been done to identify and analyze the volatile compounds present in durian. In 1972, Baldry *et al.* reported 26 compounds which comprise the volatile flavoring constituents of durians from Singapore and Kuala Lumpur [3]. Most of these were esters and thioesters (Singapore), and thiols (Kuala Lumpur). Then in 1980, Moser *et al.* also reported that eight sulfur compounds were identified in Thai durian, from which diethyl trisulfide and diethyl disulfide were found in high quantities [4]. From the studies of Baldry *et al.* (1972), Moser *et al.* (1980), Wong and Tie (1995); Weenen *et al.* (1996), Jaswir *et al.* (2005), and, Naf and Velluz (1996) [5], there are 137 durian volatile constituents already reported. It was also found out that the volatile

compounds present and their corresponding concentrations in durian vary among regions and cultivars [3, 6].

HS-SPME is a sampling technique that has been used to idenitfy and quantify volatiles from various fruits. Volatile profiles of some durian cultivars were analyzed using this method, especially those from Malaysia and Thailand [5, 6]. In the Philippines, HS-SPME had also been applied to 'Atabrine' durian cultivar by Neti *et al.* [7]. However, no study has yet been reported on 'Puyat' cultivar which is more preferred by Filipino consumers due to its big cylindrical fruit, firm flesh, and sweet and buttery taste [8].

We hereby report the aroma volatile compounds in the Philippine 'Puyat' durian cultivar. The resulting volatile profile was then compared with the cultivars already reported in other studies.

2. Materials and Methods

2.1. Materials

Fresh table-ripe durian fruits (*Durio zibethinus* Rumph. ex Murray) cultivar 'Puyat' were purchased from a local market in Davao City. Pulps were removed from the husk, transferred into 1000 mL polypropylene storage containers, placed in a polystyrene box and stored in commercial freezer for 24 hours prior to transport.

Frozen durian fruits were transported from Davao City, Philippines to Tsukuba City, Japan through air transport. They were then stored at -80°C in the Pomology Laboratory, University of Tsukuba until they were used for experiments.

Volatile aroma compounds used as GC standards were diethyl disulfide, propyl propanoate, ethyl propanoate, ethyl 2-methylbutanoate and ethyl octanoate which were all purchased from Sigma-Aldrich Japan K.K., Tokyo, Japan. These GC standards were reported in previous studies to be the major volatile compounds present in Malaysian and Thai durian varieties. Meanwhile, thiophene (Sigma-Aldrich, Japan) served as the internal standard (IS). All standards were prepared in 1ppm solution by diluting in methanol.

A 65 µm polydimethylsiloxane-divinylbenzene (PDMS/DVB) (Supelco Co., Bellefonte, PA, USA) SPME fiber was used for manual extraction. This fiber was considered to be the most appropriate to use for durian [9, 10]. Standard calibration curve for peak area to concentration of the internal standard (ISTD) thiophene was constructed.

2.2. Isolation of volatile compounds

HS-SPME was used to extract volatile compounds from durian pulp. Conditioning of the fiber was done at 250°C for 30 min per manufacturer recommendation.

Durian pulp sample was placed in a 1.6 L glass desiccator and thiophene (1ppm), as ISTD, was added into the sample for every set-up. The set-up was equilibrated for 30 min at 20°C. Afterwards,

headspace sampling was performed by inserting the SPME device into the desiccator for 1 hr at the same temperature. Equilibration and sampling times chosen were based on the results of Zhang and Li [9]. Measurements were done in triplicate. Equilibration and sampling times were kept constant.

2.3. Gas Chromatography – Mass Spectrometry conditions

The fiber was then injected into the gas chromatography – mass spectrophotometer (Thermo Fisher Scientific, Austin, TX, USA) at 240°C for 5 min in a splitless mode to desorb the analytes from the fiber coating. Separation of analytes was performed using DB-WAX capillary column (30 m × 0.25 mm id × 0.25 µm film thickness). Oven temperature program was initially set at 40°C for 10 min, increased to 240°C at 5°C/min and held at this temperature for 5 min.

Volatile compounds were identified using the National Institute of Standards and Technology (NIST) library and quantified by peak area comparison of analytes with thiophene as the IS.

3. Results and Discussion

A total of 22 volatile compounds were identified from the headspace of table-ripe Philippine ‘Puyat’ durian pulp using HS-SPME and GC-MS methods. These were composed of 15 esters, 6 sulfurs, and 1 thioacetal. These volatiles have been reported in earlier reports using other durian cultivars [3,4], [11,12,13,14], [5], [15], [6], [10], [7]. Some volatiles such as alcohols, aldehydes and ketones were not found in this study but were previously reported in other durian cultivars.

Figure 1 shows a typical chromatogram where the data (such as retention time, RT, and average area, AA) of durian volatile compounds were obtained. This represents the volatile profile of Philippine ‘Puyat durian. Meanwhile, Table 1 lists the volatiles present in Philippine ‘Puyat’ durian, with their retention times obtained from the experiments and corresponding odor descriptions.

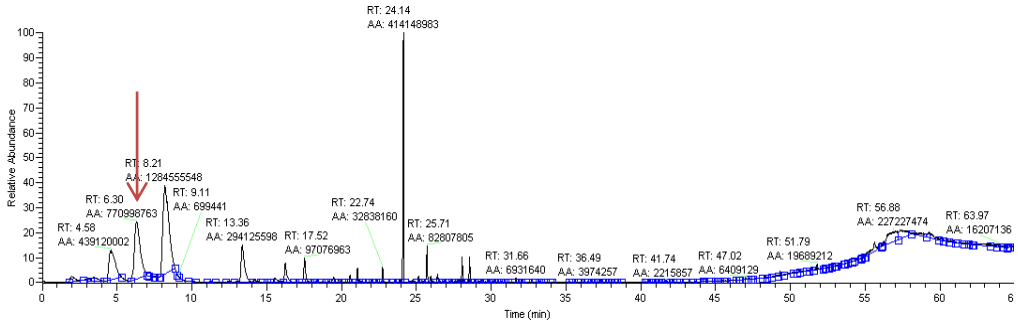

Figure 1. ‘Puyat’ durian representative chromatogram obtained by headspace SPME GC-MS Analysis. Arrow points to the internal standard.

Table 1. Volatile compounds identified from Philippine ‘Puyat’ durian pulp.

Compounds	Retention	Odor description

	time (min)	
Esters		
Methyl propanoate	3.57	Fruity odor reminiscent of rum [a]
Ethyl propanoate	4.59	Odor reminiscent of rum and pineapple [a]
Ethyl butanoate	7.31	Fruity odor with pineapple undertone [a]
Methyl 2-methyl butanoate	6.21	Sweet fruity, apple-like odor [a]
Propyl propanoate	7.74	Complex fruity odor reminiscent of apple banana [a]
Ethyl 2-methyl butanoate	8.15	Powerful green, fruity apple-like odor [a]
Propyl butanoate	12.45	Pineapple and apricot-like odor [a]
Propyl 2-methyl butanoate	13.39	Sweet, fruity [e]
Ethyl hexanoate	17.54	Powerful fruity odor with pineapple-banana note [a]
Propyl hexanoate	20.58	Ether-like odor with pineapple, blackberry undertone [a]
Ethyl heptanoate	21.07	Fruity odor reminiscent of cognac; winey-brandy odor [a]
Methyl octanoate	22.74	Powerful winey, fruity, orange-like odor [a]
Ethyl octanoate	24.30	Pleasant fruity floral odor with wine and apricot note [a]
Propyl octanoate	26.40	Winey, oily-fruity, brandy-like [e]
Ethyl decanoate	29.38	
Sulfur-containing compounds		
Ethanethiol	1.94	Onion, rubber odor [d]
1-Propanethiol	2.61	Cabbage, sweet onion-like odor [d]
Diethyl disulfide	16.38	Sulfury, roasty, cabbage-like odor [c]
Diethyl trisulfide	25.71	Sweet and alliaceous odor [b]
3,5-dimethyl 1,2,4-trithiolane	28.05	Sulfury, heavy cocoa odor [c]
Methyl ethyl disulfide	13.04	
Thioacetal		
1,1-bis (ethylthio)-ethane	31.64	Burnt, rubbery, alliaceous odor [a]

[a]Burdock (2002) [5]
[b]Naf ang Velluz (1996) [5]
[c]Weenan et al. (1996) [5]
[d]Goniak and Noble (1987) [5]
[e]El-Sayed AM (2012) [17]

Ethyl propanoate, ethyl octanoate, propyl propanoate, ethyl 2-methyl butanoate and diethyl disulfide were the major compounds identified from 'Puyat' durian pulp, considering the sampling procedure and extraction conditions used in this study. Table 2 presents these compounds with their retention time, peak area, similarity factor and concentration obtained from experiments. All five compounds were also common in most durian cultivars, [5], [15], [10]. Chawengkijwanich et al. [6] and Chin et al. [16] had the same results except for ethyl octanoate. Neti et al. [7] also found the similar compounds in Philippine 'Atabrine' cultivar except for propyl propanoate.

Ethyl propanoate and ethyl 2-methyl butanoate were also among the most abundant volatiles in 'Mon Thong' [6, 10] and Malaysian durian cultivars [3, 15, 5]. In other reported studies, 1-propanethiol was one of the major volatile compounds identified from durian [5]. However, in this cultivar, it was detected at relatively low concentrations. The same has been reported by Voon et al. [15] in Malaysian durian D2 which contained significantly lower concentration ($p<0.01$) of 1-propanethiol than other cultivars.

Durian fruits contain mostly ester or sulfur compounds [15], but sulfurs are the compounds generally responsible for the onion-like odor which is usually perceived as offensive. Table 2 shows that diethyl disulfide is the only sulfur compound and the rest were esters. Despite the fact that most durian cultivars seem to produce strong onion-like odor, some sulfur compounds were not usually detected or were present in only small relative concentrations, as in the case of 1-propanethiol, diethyl disulfide and 3,5-dimethyl 1,2,4-trithiolane in this study. In fact, 3,5-dimethyl 1,2,4-trithiolane is an example of thiols regarded as the most odorous among all the aroma compounds despite its very low concentration15]. This can be explained by the characteristic low odor threshold of sulfur-containing volatiles which causes their offensive smell even at very low amounts [10].

Table 2. Major volatile compounds found in Philippine 'Puyat' durian.

	Ethyl propanoate	Propyl propanoate	Ethyl 2-methyl butanoate	Ethyl octanoate	Diethyl disulfide
Retention time (min)	4.59	7.74	8.15	24.3	16.38
Peak area [f]	4.70E+08	1.81E+09	3.08E+09	1.94E+10	4.32E+09
Similarity factor (%) [g]	96	94	90	92	97
Concentration (µg/g) [h]	5.27 ± 1.70	0.10 ± 0.05	0.78 ± 0.20	0.08 ± 0.04	0.24 ± 0.17

[f] Peak area measured by GC-MS when spiked with standards
[g] Similarity factor according to NIST Library
[h] Concentration (mean ± S.E) obtained from the durian pulp when spiked with thiophene

4. Conclusion

The volatile profile of the 'Puyat' durian cultivar of the Philippines was mostly composed of esters and sulfurs. The compounds identified from Philippine 'Puyat' durian were comparable to other cultivars in Malaysia and Thailand. Observations and findings of this study will serve as basis for future endeavor in postharvest technologies and management for Philippine durian cultivars.

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