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Traditional Food Science: Recent Advances in Flavour Science and Sensory Evaluation

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Research Article

Quality Evaluation of Three Kinds of Hickories Based on Grey Relational Analysis and Entropy-Weight Theory

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In this paper, the nutritional ingredient, aroma component, and texture of three kinds of hickories, including American hickory, Chinese Linan hickory, and Chinese Hunan hickory, were tested by instruments. The quality of different hickory varieties was analyzed at three levels by using the grey entropy correlation analysis, namely, the single nutrient composition analysis; nutritional composition and texture analysis; nutrient composition, texture, and aroma analysis. Through the analysis of nutritional composition, American hickory gets the highest score (80.6945), followed by Linan hickory (74.9987), and Hunan hickory has the lowest score (58.5925). Through the analysis of nutrition composition and texture, Linan hickory has the highest score (80.89), American hickory is the second (71.77), and Hunan hickory is last (61.62). Through the analysis of nutrition composition, texture and aroma, Linan hickory has the highest score (75.91), followed by American hickory (74.17), and Hunan hickory has the lowest score (64.20). Finally, the comprehensive evaluation of Linan hickory quality index score is the highest. The main factors contributing to the high score of Linan hickory include superior fatty acid spectrum, aminogram and higher initial chewing hardness, moderate crispness of secondary chewing, optimal palatability, and unique aroma components ((S)-2-methyl-1-butanol, 3-methyl-2-pentene, (+/−)-2-methylbutyric acid methyl ester ethyl butyrate, ethyl 2-methylbutyrate, methyl phthalate, decene, (1S)-(−)-β-pinene). The research results provide a basis for consumers to understand the quality differences of different hickories.

1. Introduction

Hickory is a wild nut, a natural pollution-free green food, also one of the many varieties with high nutritional value of dry fruit. Hayes et al., using a series of data sets and statistical methods, studied unique fatty acids and polyphenols of walnut. The results show that walnut may be considered to be a safe potential nutrient or drug. For cardiovascular disease, age-related nervous system diseases and even cancer, people can often eat walnuts as part of a healthy diet [1]. At present, there are many kinds of hickories in the Chinese market, including hickories from America, hickories from Linan, and hickories from Hunan. All three hickories have a large market share, but people lack sufficient knowledge of their quality characteristics and differences. Lillywhite et al. investigated 1009 American consumers based on the Internet group survey, and examined the population of hickory consumers, their nut nutrition knowledge and purchasing preferences. Most of the respondents could correctly identify various nuts while they could not determine the specific nutritional characteristics [2].

Consequently, our purpose is to find a suitable method to comprehensively evaluate their quality differences according to the ingredients of different kinds of hickories. Thus, consumers can understand the differences among various hickories and purchase them in terms of their preferences.

At present, the research on the quality of hickory mainly focuses on testing nutrient ingredient of hickory by chemical methods and simple evaluation and comparison of their quality. Esteki et al. used pattern recognition to classify and identify Iranian walnuts from different geographic locations by analysis of fatty acid fingerprint based on gas chromatography [3]. Li et al. used cable-gas chromatography to

measure the total fat content and fatty acid composition of thirty-seven kinds of walnut [4]. Zhai et al. compared and analyzed the content of mineral elements and essential amino acids in *Juglans sigillata* and *J. regia* walnut kernel, examined their influence on human health, and sorted the mineral and amino acid contents [5]. Yi et al. established an infrared spectroscopy prediction model and measured and evaluated the moisture, protein, and fat content of walnut powder [6]. Prado et al. checked for the chemical composition of fatty acid, tocopherol, total oxidation stability index of phytosterol and peroxide value, analyzed composition, color and luminosity of hickory shell, tested extracts of total phenol, condensed tannins and antioxidant activity for hickory nut [7]. In addition to testing hickory kernels, some scholars have conducted research on hickory derivatives. Medina-Juarez et al. evaluated the phenolic content, total flavonoid content, concentrated tannin content, and antioxidant capacity of two varieties of hickories extract oil [8].

Physical properties of walnut kernels also have an effect on consumers' selection. Gharibzahedi et al. studied the differences in chemical, physical, and mechanical properties of three varieties Persian walnuts (Toyserkan, Chaboksar, and Karaj), which is mainly due to the individual characteristics of these varieties as well as the environment and cultivation conditions, The data obtained from these differences can be used for harvesting, transportation, sorting, sorting, and packaging [9].

Objective examination and comparison is difficult to reflect consumers' subjective feelings. There are flavor differences among different varieties of hickories. Magnuson et al. discussed the sensory differences in raw and baked eight kinds of hickory [10]. Miller and Chambers also evaluated seven black walnut varieties by sensory analysis. The trained seven members in a group developed a set of vocabulary for the black walnut and rated the sample of 22 flavor attributes [11].

Although many scholars have studied the nutrient ingredients of hickories, there is little deep and comprehensive research on the quality of hickories. The comprehensive evaluation methods, such as grey correlation degree (GCD), coefficient of variation method (CVM), analytic hierarchy process (AHP), fuzzy comprehensive evaluation (FCE), and DEA, have been applied in other fields, but there are few studies in the field of nuts or crops. For example, Veisi et al. used AHP to establish an ethics-based approach for sustainable agricultural indicator evaluation [12]. Abdollahzadeh et al. applied AHP to select management strategies of rice stem borer [13]. Yang and Mak proposed a multilayer FCE method which provides a classroom acoustic environment evaluation model to make reasonable sound processing suggestions for colleges and universities and improve the sound quality of the educational environment [14]. Chen et al. made comprehensive evaluation of environmental and economic benefits of anaerobic digestion technology for integrated food waste biogas plants based on fuzzy mathematical model [15]. Li et al. analyzed China's agricultural total factor energy efficiency based on the DEA and Malmquist indices [16]. Kao et al., based on dynamic and network DEA model, evaluated the cloud service industry. For the cloud service industry, three NDEA models were built and solved by using multiobjective programming techniques [17]. Sun combined grey relational analysis and entropy models to empirically evaluate business performance [18].

As is discussed above, scholars have conducted many research studies about nutrients, volatile substances, and physical structure on hickory. However, few were paid attention to comprehensive evaluation. Consequently, our contribution is that the quality of hickory, nutrition ingredients, texture, and aroma are evaluated comprehensively by grey correlation analysis and entropy, which will provide buying reference for consumers.

The structure of this paper is as follows: in section 2, the experimental materials and research methods are presented. In section 3, the nutritional components, texture, and aroma of three kinds of hickories were analyzed, and the important indexes affecting the quality difference of hickories are discussed. The last part draws the conclusion of this paper.

2. Materials and Methods

2.1. Sample Collection. Samples are divided into Linan hickory, Hunan hickory, and U.S. hickory, from Qingliangfeng town of Linan, Huaihua of Hunan, and the United States of America. Each species is taken by 10 kg. On this basis, according to weights of samples, they are divided into three categories—big seeds, medium seeds, and small seeds, then their quantity and proportion are calculated, respectively. Concrete layering is shown in Table 1.

In order to calculate the appearance index of hickories, a total of 259 samples of hickories were drawn. Finally, according to the smallest sample size, 45 samples were taken for each variety. As experimental data, the distribution of samples is shown in Table 2.

2.2. Sample Detection Method. The quality of hickory was studied mainly from the aspects of nutrition, aroma, and texture. The corresponding index data were obtained by instrumental analysis. The quality indicator system of hickory is composed of three parts: nutritional composition, aroma, and texture. With a total of 81 indicators, among which 21 indicators were selected from nutritional composition, 50 indicators were selected from aroma, and 10 indicators were selected from texture.

For nutrients, according to GB 5009.124-2016, GB 5009.168-2016, GB 5009.3-2016, GB 5009.6-2016, GB/T 15686-2008, etc. (China Criterion), the fatty acids of Linan hickory, U.S. hickory, and Hunan hickory were tested by Zhejiang Gongzheng Testing Center Inc. (the third party inspection institution), a total of 14 kinds of fatty acid monomer components were detected. According to the detection for free amino acids and hydrolyzed amino acid of three kinds of original seeds of Linan hickory, American hickory, and Hunan hickory, 15 amino acid monomer components were detected. Excluding some indicators with less content, 21 nutrient indicators were selected.

Table 1: Laying data of samples.

Variety		Big seeds	Medium seeds	Small seeds	Sum
Linan hickory	Quantity	316	1630	730	2676
	Proportion	11.81%	60.91%	27.28%	
Hunan hickory	Quantity	153	458	769	1380
	Proportion	11.09%	3319%	55.72%	
U.S. hickory	Quantity	147	1346	273	1766
	Proportion	8.32%	76.22%	15.46%	

Seed is classification by its weight. For Linan hickory, big seed >4.2 g, medium one (2.5, 4.2), and small one <2.5. For Hunan hickory, big one >10 g, medium one (7.14, 10), and small one <7.14. For American hickory, more than big one ≥ 6.5 g, medium one (4.3, 6.5), and small one <4.3.

Table 2: Source and distribution of samples.

Variety	Big seeds	Medium seeds	Small seeds
Linan hickory	5	28	12
U.S. hickory	4	34	7
Hunan hickory	5	15	25
Sum	14	77	44

For aroma, Linan hickory, American hickory, and Hunan hickory were fried by the same process. By SPME (solid-phase microextraction) and GC-MS (gas chromatography-mass spectrometry) analysis, 50 kinds of aromatic components were identified, including 3 kinds of aldehyde, 7 kinds of alcohol, 9 kinds of olefin, 6 kinds of ketones, 9 kinds of olefin, 6 of ester, 5 of aromatic hydrocarbon, 1 kind of steroids, 1 kind of furan, 1 kind of monoterpene, 1 kind of alkyne, and 1 kind of carboxylic acid.

For texture, five kinds of probes were selected for texture analysis, namely 1/2 shearing head, three-point bending special probe, P2E puncture probe, P100 pressure plate probe, HDPVB probe, and the simulated chewing scheme was used to test and extract data.

2.3. Research Method. This paper analyzed the grey correlation degree of hickory quality based on the entropy weighting method. We used grey correlation analysis to calculate the correlation degree of each hickory as the hickory quality score because the grey correlation analysis does not require too much sample size, nor does it require typical distribution rules. Also, the computation amount is less, whose results are consistent with qualitative analysis results. Considering that the subjective weight method will artificially affect the results of the index, we used the objective assigning method to determine the weight of each index in evaluating the quality of different hickories, that is, the entropy weight method. The calculation steps are as follows:

(1) Establish the original evaluation matrix. According to the index system (nutrient, aroma, and texture), an $m * n$ original evaluation matrix is established that m is the evaluation object and n is the evaluation index. X_{ij} represents the index value of the jth evaluation index of the ith evaluation object, and the original evaluation matrix is shown in Equation (1),

$$X = \begin{bmatrix} x_{11} & \cdots & x_{1n} \\ \vdots & x_{ij} & \vdots \\ x_{m1} & \cdots & x_{mn} \end{bmatrix}, \quad (1 \le i \le m,\ 1 \le j \le n). \tag{1}$$

(2) Set up the reference sequence R_0. Set the length of R_0 consistent with the number of columns in the evaluation matrix of $m * n$. The reference sequence R_0 is the row vector composed of the ideal optimal values of each index. Add the reference sequence to row 0 of the original evaluation matrix to form a new evaluation matrix.

$$R_0 = \{x_{01}, x_{02}, \ldots\ldots, x_{0j}\}. \tag{2}$$

In (2): x_{0j} is the optimal value of the jth column.

(3) Calculate the correlation coefficient. As a reference sequence R_0, calculate the correlation coefficient r_{ij} of each index of three kinds of hickories according to the following Equation (4):

$$r_{ij} = \frac{\min_i \min_j \left|x_{0j} - x_{ij}\right| + \mu \max_i \max_j \left|x_{0j} - x_{ij}\right|}{\left|x_{0j} - x_{ij}\right| + \mu \max_i \max_j \left|x_{0j} - x_{ij}\right|}, \tag{3}$$

where μ is the discrimination coefficient, $\mu \in [0,1]$, take 0.5.

(4) Calculate the weight. According to the theory of entropy weight method, the entropy value H_j of the jth index is calculated by Equation (4). Also, the weight of the j_{th} index, ω_j is calculated by Equation (5),

$$H_j = -k \sum_{i=1}^{m} f_{ij} \ln f_{ij}, \quad 1 \le j \le n, \tag{4}$$

$$\omega_j = \frac{1 - H_j}{n - \sum_{j=1}^{n} H_j}, \quad 1 \le j \le n, \tag{5}$$

where in Equation (4), $k = (1/\ln n)$ and $f_{ij} = x_{ij} / \sum_{i=1}^{m} x_{ij}$, when $f_{ij} = 0$ and $f_{ij} \ln f_{ij} = 0$,
where in Equation (5), $0 \le \omega \le 1$ and $\sum_{j=1}^{n} \omega_j = 1$.

(5) Calculate the comprehensive score. The score Y_i is calculated by Equation (6),

$$Y_i = \sum_{i=1}^{n} \left(r_{ij} \times \omega_j\right). \tag{6}$$

3. Results and Discussion

In the study of the hickory quality, everyone feels different, some people pay attention to nutrition; some people are attracted by the aroma; some people also pay attention to the taste. Therefore, our study judges the quality of hickories from three levels: firstly, considering nutrients and providing consumers with a reference on nutrients. Secondly, considering the nutrients and texture, namely taking into account the nutrients and stimulating the crisp chewing

perception of hickories. Finally, making a comprehensive analysis to the effects of nutrient, texture, and aroma on the quality of hickories as reference for consumers.

3.1. Consider Nutrients Only. The fatty acid and amino acid components of the nutrient components were detected, and a total of twenty-nine indicators were detected. Twenty-one kinds of indexes were selected after excluding the minimal components and other factors. According to the calculation of Equation (6), American hickory has the highest score, with the evaluation score of 80.6945, followed by Linan hickory with 74.9987, and Hunan hickory with the worst score is 58.5925 (see Table 3).

The sorting results in Table 3 are mainly due to the following reasons:

According to the detection for fatty acid ingredient of three raw seeds—Linan hickory, American hickory, and Hunan hickory—there are 14 kinds of fatty acid monomer components. The main ingredients include α-linolenic acid, linoleic acid, oleic acid, palmitic acid, and stearic acid. Also, the remaining components are minor components. The functional oil components detected are α-linolenic acid, linoleic acid, and oleic acid. Among the three kinds of hickories, the content of linoleic acid and oleic acid in American hickory is the highest, 14.10% and 38.20%, respectively. Its content of α-linolenic acid (0.98%) is slightly lower than that of Linan hickory and significantly higher than that of Hunan hickories. In addition, American hickory has the highest content of hickory palmitic acid (3.88%) and stearic acid content (1.44%). Above indicators reflect the good nutrient quality and health effects of U.S. hickory.

The free amino acid with the tannins and other taste substances together form the taste index of the hickories glycine, alanine, serine, and aspartic acid have obvious sweet taste, and the aspartic acid has a certain umami taste. Lysine, arginine, histidine, phenylalanine, tyrosine, leucine, isoleucine, and methionine have a certain bitter taste. Also, proline mixes a certain sweet and bitter tastes. The content of sweet amino acid in three kinds of hickories is 0.0503 mg/100 g (Linan hickory), 0.0376 mg/100 g (Hunan hickory), and 0.0375 mg/100 g (American hickory), respectively. The content of bitter amino acid is 0.0574 mg/100 g (Linan hickory), 0.0611 mg/100 g (Hunan hickory), 0.0465 mg/100 g (American hickory), respectively. Therefore, the sweet and bitter amino of American hickory are both the lowest.

The pericarp of the nut has a strong astringent taste, which comes from the tannin. Compared with Linan and Hunan hickories, American hickory has the least amount of tannin. After the same processing technology, it has better quality in taste than Linan and Hunan hickories. Among the 21 nutritional indicators, the highest proportion of weight was fat content, accounting for 0.063, followed by linoleic acid content, accounting for 0.061. The content of these two indicators of American hickory is far greater than that of Linan and Hunan hickories. The rich unsaturated fatty acids in nuts protect the cardiovascular system and help the body slow down aging. Figure 1 shows the visualized weights of 21 indicators and their descending order.

Table 3: Scores only considering nutrients.

Variety	Evaluation score
Hunan hickory	58.59247
Linan hickory	74.99874
U.S. hickory	80.6945

3.2. Consider Nutrient Composition and Texture. Considering nutrients and textures, there are thirty-one indicators. Compared with the analysis of only a single nutrient, ten texture indicators were added. The highest core was Linan hickory, with an evaluation score of 80.89, followed by American hickory with 71.77, and the last for Hunan hickory with 61.62, which indicates that Linan hickory has a crisper taste compared with American and Hunan hickory.

The sorting results in Table 4 are mainly due to the following reasons:

The weights of the three kinds of hickories considering nutrients and texture are shown in Figure 2. The higher weights are three-probe HDPVB hardness, three-probe P2 crispness, and fat. The special probe for three-point bending tests the brittleness of the samples by a three-point bending fracture. One indicator obtained by the three-point bending probe is hardness (brittleness). The hardness sorting of the test data of the three kinds of samples is as follows: Linan (20.52 N)> Hunan (19.12 N)> U.S. (16.04 N). It can be observed from the above data that the shearing stress to break Linan hickory is the highest, that of Hunan is middle and that of U.S. is the smallest, which reflects the highest hardness of Linan hickories, followed by Hunan and American hickories. The texture of American hickory is the softest. The test data of the probe indicates that Linan hickory has a large initial chewing hardness, and it is weaker than Hunan and American hickory in the brittleness of the first chewing.

3.3. Consider Nutrients, Texture, and Aroma Comprehensively. A total of eighty-one indicators were considered, including nutrition, texture, and aroma. Linan hickory scored 75.91, slightly higher than American hickory score of 74.17 and Hunan hickory score of 64.20. After adding aroma index, American hickory narrowed the gap with score of Linan hickory, which shows that American hickory has a unique aroma (see Table 5).

The sorting results in Table 5 are mainly as follows:

It is high for entropy weights of aroma component in hickories, and the total proportion accounts for 0.752397, which has an important influence on the evaluation of hickory quality. American hickory differs from Linan hickory and Hunan hickory in that it has a unique aroma different from others. However, Linan hickory and Hunan hickory also have their own unique aroma (see Figure 3).

According to the characteristic aroma that its value (aroma component content/threshold value) is greater than 1, it is determined that the unique characteristic aroma components for the American hickory are naphthalene, 4-methyl-3-pentenoic acid, tridecane, furfural, 2,6,6-

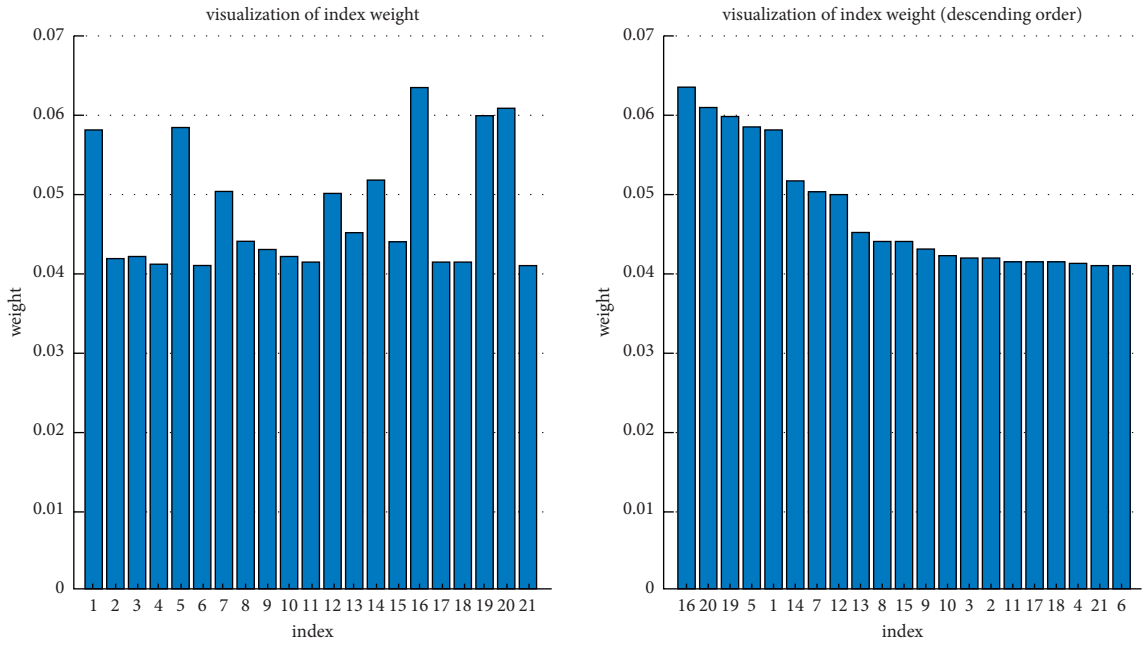

Figure 1: The weights of 21 indexes of three kinds of hickories with single nutrient component.

Table 4: Scores considering nutrient and texture.

Variety	Evaluation score
Hunan hickory	61.62219
Linan hickory	80.89055
U.S. hickory	71.77297

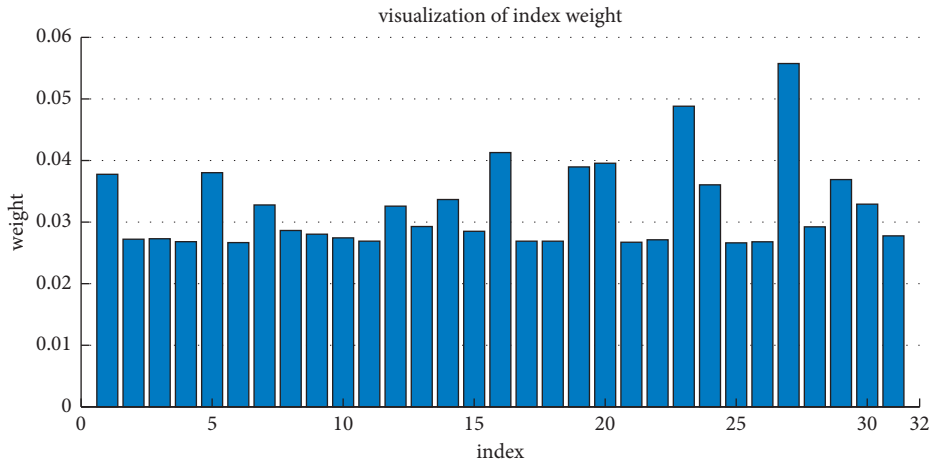

Figure 2: The weights of 31 indexes of three kinds of hickories with nutrient and texture.

Table 5: Scores of three kinds of hickories considering nutrition, texture, and aroma.

Variety	Evaluation score
Hunan hickory	64.20345
Linan hickory	75.90734
U.S. hickory	74.17492

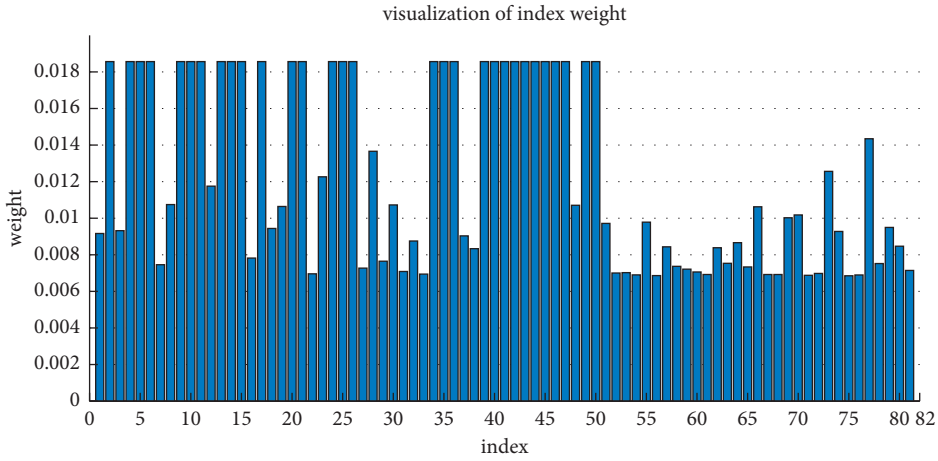

Figure 3: The weights of 81 indexes of three kinds of hickories with nutrition, texture, and aroma.

trimethyl-2-cyclohexene-1,4-dione, 2-propyl-1-heptanol, butyl butyrate, 4-methyldodecane, 2-methyl-1, 1′-biphenyl, tetradecane, 2,6-di-tert-butylphenylhydrazine, and diphenylmethane.

The unique aroma components of Linan hickory are (S)-2-methyl-1-butanol, 3-methyl-2-pentene, (+/−)-2-methyl-butyric acid methyl ester ethyl butyrate, ethyl 2-methyl-butyrate, methyl phthalate, decene, and (1S)-(−)-β-pinene.

The unique aroma components of Hunan hickory are 2-methyl-2-heptanol, 1,1′-((1-methylethylene)diethylene(oxygen))dibutane.

4. Conclusions

Our research helps consumers understand the quality of the hickories. The research report of quality is published in the media institutions by government annually. Consumers can make decisions of purchasing according to the quality scores. In this paper, we conducted a study from three aspects, and the main conclusions of the study are as follows,

(1) Nutrient: compared with Linan hickories and Hunan hickories, the content of functional oils in American hickories is higher, and the fatty acid spectrum is superior. At the same time, the content of tannins with astringency is the lowest, and amino acid contents is also better.

(2) Texture: evaluating through five kinds of texture probes from different angles, it is concluded that, compared with Hunan hickory and American hickory, Linan hickory has higher initial chewing hardness, moderate crispness of secondary chewing, and optimal palatable chewing. In terms of texture taste, Linan hickory is superior to American hickory and Hunan hickory.

(3) Aroma: relative to the Linan hickory and Hunan hickory, unique aroma composition of American hickory is naphthalene, 4-methyl-3-pentene acid, tridecane, decanal, 2,2,6-trimethyl-2-cyclohexene-1,4-dione, 2-propyl-1-heptanol, n-butyl butyrate, 4-methyldodecane, 2-methyl-1,1′-biphenyl, tetradecyl, 2,6-di-tert-butyl-p-benzoquinone, and diphenyl methane. Because of unique aroma, American hickory is different from Linan hickory and Hunan hickory. However, it also provides a reference for consumers to choose their preferred taste.

(4) The quality index system of hickory is composed of three parts, namely nutrition, texture, and aroma. The weight of the aroma is 0.752397, relatively large, and that of the nutrition is 0.165035, and that of the texture is 0.082568. The weight of the aroma is more than 75%, which determines the special quality of a kind of hickory to some extent. American hickory has outstanding aroma and superior nutrition. Linan hickory has a crisp taste and is also loved by consumers.

The experimental analysis in this paper is based on an objective evaluation method. It does not consider the subjective feelings from the perspective of consumers. On the other hand, only the grey correlation analysis method is applied. There are no other methods to do more comparisons with it. Also, it still needs studying whether other methods can be used to achieve the same conclusion or not. In the future, we will continue to improve our methods to solve more and more problems.

Data Availability

The data can be downloaded in https://figshare.com, DOI: 10.6084/m9.figshare.13084997.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Fruit Volatile Fingerprints Characterized among Four Commercial Cultivars of Thai Durian (*Durio zibethinus*)

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Ripe durian fruits produce unique volatiles of pungent odor comprising esters, alcohols, ketones, and sulfur-containing compounds. Recently, "Chanthaburi 1" hybrid bred from 2 famous commercial cultivars of "Chanee" and "Monthong" claimed to be less fragrant during ripening, but there was no report. The present study compared the volatile profiles from 3 Thai commercial cultivars of "Kanyao," "Chanee," and "Monthong" compared to "Chanthaburi 1", and the relationships of the cultivars were organized using the volatile fingerprints. Out of 41 volatile compounds detected by SPME/GC-MS in ripe durian flesh, 33 compounds were esters, but only 14 esters were found in "Chanthaburi 1." Ripe flesh of most durian cultivars contains ethyl-2-methyl butanoate and ethyl hexanoate as the active volatiles. "Chanthaburi 1" contained fewer components with low odor activity value (OAV) of the volatiles. "Chanee" ripe flesh exhibited the strongest durian smell among the four varieties, whereas "Monthong" exhibited a strong apple-like fruity odor and "Kanyao" was more green fruity. Diethyl disulfide and 3, 5 dimethyl-1, 2, 4-trithiolane contributing pungent smells of garlic or onion were found only in "Chanthaburi 1" and "Monthong." In terms of detected volatiles, "Kanyao" and "Chanee" were highly close when "Monthong" was apart. PCA analysis revealed that "Chanthaburi 1" contained ester compounds ancestrally related to the parents, "Chanee" in the component I and "Monthong" in the component II. These data could be beneficial for managing the status of Thai durians in global markets.

1. Introduction

In Thailand, durian plant collection was firstly reported for 227 varieties. However, there currently are several cultivars, including "Chanee," "Kanyao," and "Monthong," in the business both in domestic and export markets [1]. "Chanee" comprises a moderate fruit size of 2.5–3 kg. The fruit shape shows swelling in the middle and is blunt at the blossom end with a big and short peduncle. When ripening, fruit is easily peeled, and the yellow flesh is a very soft fine texture, but with the thin flesh and an ample seed, it is famous for domestic markets. "Kanyao" bears a moderate fruit size of 3 kg, showing a round fruit shape and a big and long peduncle. The ripe flesh has smoothly fine texture and is yellow and sweet. "Monthong," the most famous variety, exhibits big fruit of 3–4 kg. Fruit is long, having shoulders at the stem end and protruding at the blossom end. The ripe flesh is dry and thick with a lean seed [2–4]. Ripe fruits of most typical durian varieties release a pungent solid smell, resulting in trouble for foreigners and under public assemblage. Recently, "Chanthaburi 1" (ICN × *M* 5-1-1), bred from "Chanee" and "Monthong," was officially approved and

registered as a new variety by the Department of Agriculture, Thailand, on 9 October 2006. The fruit is an early-season production with a harvesting time of 99–105 days after pollination. The average fruit weight is 2.5–3 kg, comprising bright yellow flesh and a sweet, delicate texture. The ripe fruit of "Chanthaburi 1" is claimed to have an extra-low smell [5]. Nevertheless, there is no analytical report yet for the volatile characterization of the fruit.

Aroma is a unique character of ripe durian fruit preferred by some but annoying for many people. Furthermore, it is seriously prohibited to take durian fruit/eat during public transportation or in assembly places such as hotels or convention halls. This matter would be a significant obstacle for the marketing of durians. Ethyl esters (fruity esters and general fruit) are the prominent esters in ripe "Monthong" flesh [6, 7]. Nevertheless, this sweet smell is interrupted by sulfurous smells of sulfur-containing compounds. Ethanethiol, diethyltrisulfide, diethyldisulfide, dimethyl sulfide, 2,3-butanedithiol, ethyl 1-methylethyl disulfide, 3-methyl-thiozolidine, methyl ethyl disulfide, and 1-propanethiol are such sulfur-containing compounds found in ripe durian flesh [6–8]. There is no report of the relationship of durian cultivars by the aroma volatile so far. There have been many reports of volatile components of ripe durians in "Monthong" [6–9], few in "Chanee" [6], but there is no report in "Kanyao" and "Chanthaburi 1." Furthermore, from the fruit's visual appearance, "Chanthaburi 1" fruit shape is very similar to the shape of "Kanyao," leading to confusion by visual appearance. Thus, fruit volatile profiles between the cultivars compared as volatile fingerprints were brought in the interest. Here, the present study was to identify odor characteristics of 4 commercial varieties. Volatiles of "Kanyao" and "Chanthaburi 1" were firstly reported, and the volatile relationship of these four varieties was then investigated.

2. Materials and Methods

2.1. Plant Materials and Sample Preparation. Mature durian fruits at 90% maturation from 4 cultivars, "Chanee" (1.9–2.2 kg) at 15 weeks after anthesis (WAA), "Kanyao" at 18 WAA (1.7–2.0 kg), "Monthong" at 19 WAA (2.2–2.8 kg), and 'Chanthaburi 1 at 14 WAA (1.4–1.8 kg), were harvested from commercial orchards in Chanthaburi Province, eastern Thailand, between April and June 2018. Fruits were incubated at room temperature (25°C, 70–75% RH) for natural ripening. Fruit showing initial dehiscence at the blossom end (Supplementary Figure 1), referred to as full ripening, was peeled, and the ripe flesh was used for volatile analysis. The visual appearance of the whole fruit and half-dehusked of ripe fruits of the four cultivars is shown in Figure 1.

2.2. Chemicals. The internal standard of volatile analysis was thiophene (≥99% purity) (Sigma Chemical Co., USA).

2.3. Volatile Trapping. The ripe aril of each cultivar was finely blended by using a high-speed homogenizer for 2 min. Homogenate at 5 g was put into a 20 mL glass vial sealed with a screw cap having a silicone laminated with polytetrafluoroethylene septum. The volatiles in the sample's headspace were trapped by SPME and analyzed by GC-MS modified from [10]. The volatiles in the headspace of the sample in a vial were trapped by solid-phase microextraction (SPME) coated with 65 μm of Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) (1 cm length), while heated at 50°C for 30 min.

2.4. Analysis of Volatiles in Ripe Durian Flesh. The SPME was injected into a gas chromatogram (GC 6850 series, Agilent Technologies, USA), equipped with an HP-5MS column (5% phenyl-methylsiloane capillary column, 30m × 0.248 mm I.D. with 0.25 μm thickness) and an Agilent 5913 mass selective detector with the following condition: 200°C of the injection port (splitless mode), 50°C of the column oven for 1 min and increased at a rate of 5°C·min^{-1} to 120°C and then to 250°C at a 10°C·min^{-1} rate, and 250°C of the detector. Helium was the carrier gas set to 2 mL·min^{-1} at 15.9 psi.

Thiophene at 10 μL·L^{-1} was used as the internal standard. The spectra of the volatile profile were analyzed in the electron impact (EI) mode with an electron energy of 70 eV; a mass range of m/z 45–450; a scan rate of 0.25 s/scan; and an electron multiplier (EM) voltage of 3000 V. Spectra of the volatile profile were compared to a mass spectral database from the NIST V.14 Llibrary values (Palisade Corp., Newfield, NY, USA). There were 3 replications for each analysis.

2.5. Calculation of Volatile Compounds. Each volatile compound of the clear peak from the GC-MS chromatogram was analyzed for the content compared to thiophene as the internal standard. Volatile content in ng thiophene per g fresh weight was estimated by the peak area of volatiles divided by the peak area of internal standard (thiophene) and 10 μL internal standard solution (0.5 g·L^{-1} thiophene) to 5 g durian homogenate prior to taking SPME [11].

$$\text{Volatile content}\left(\text{ng thiophene g}^{-1}\text{FW}\right) = \frac{\text{peak area of volatile/peak area of internal standard}}{\text{g durian aril homogenate}}. \quad (1)$$

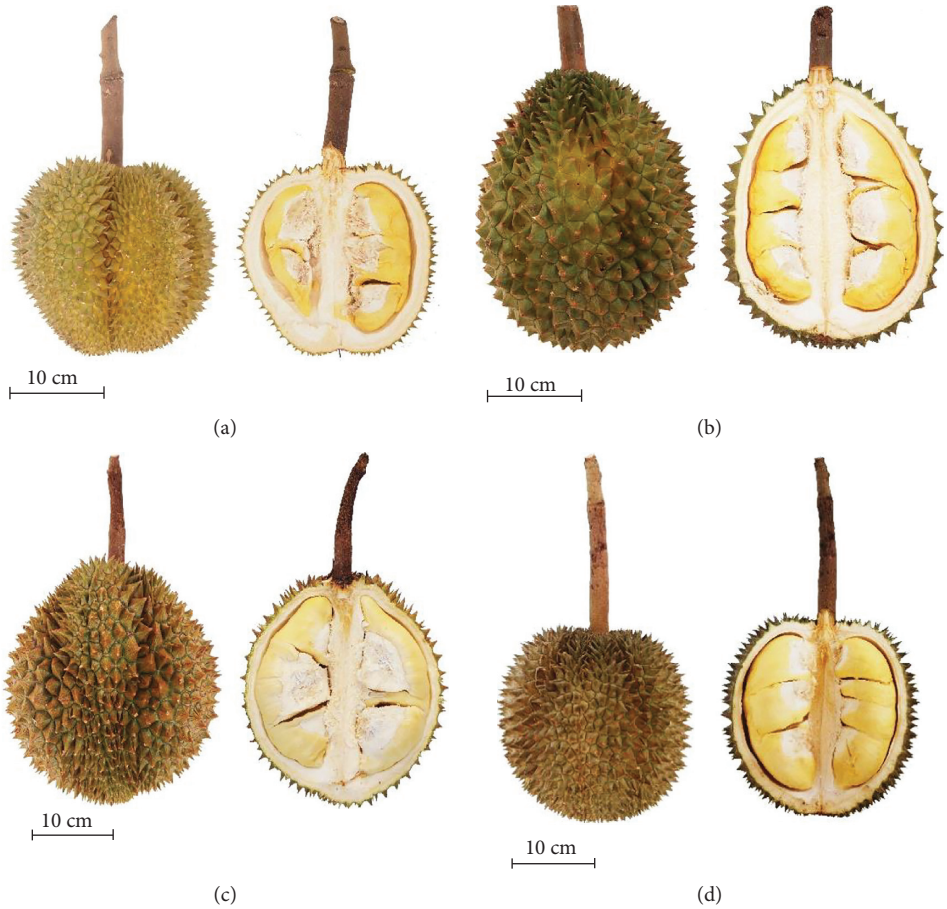


Figure 1: Appearances of the whole fruit (left) and flesh (right) of (a) "Chanthaburi 1," (b) "Chanee," (c) "Monthong," and (d) "Kanyao."

Odor activity value (OAV) was obtained by dividing the concentration of the compound in a matrix by its odor threshold in that matrix. Thus, it is generally assumed that the odorants with higher OAVs contribute more strongly to the overall aroma. OAV of each volatile compound was calculated using the following formula [12]:

$$\text{OAV} = \frac{\text{concentration of the volatile content}}{\text{odor theshold value}}. \quad (2)$$

2.6. Statistical Analysis. The volatile relationship of durian cultivars was analyzed using principal component analysis (PCA) by Minitab® program ver.17 (Minitab Ltd., UK). The contents and types of ester volatiles between cultivars were analyzed using multivariations of principal components by Minitab®.

3. Results and Discussion

3.1. Volatile Profiles in Ripe Durians. From our experience, here was the first report of volatiles contributed in ripe flesh of "Kanyao," and a new hybrid, "Chanthaburi 1" bred from "Chanee" as the female gamete and "Monthong" as the male gamete. There were 41 major volatile compounds detected in 4 cultivars, comprising 33 esters, 2 sulfur-containing compounds, 3 organic acids, 2 phenolics, and 1 aldehyde (Table 1). "Chanthaburi 1" contained major 16 volatiles of 14 esters and 2 sulfur compounds. "Chanee" contained mainly 21 compounds of 17 esters, 3 organic acids, and 1 aldehyde. "Monthong" comprised 23 compounds of 19 esters and 2 sulfur compounds and 2 organic acids. "Kanyao" found 29 volatiles, including 23 esters, 1 sulfur compound, 1 phenolic acid, 3 organic acids, and 1 aldehyde.

Fruit odor is a mixture of many volatile substances, but the main volatile component is the criteria used to determine the odor matter. Nowadays, the odor threshold value of that substance is academically used and can be described, whereas OAV is calculated from the detected substance. The OAV value greater than 1 is the more important [13]. From OAV, ethyl-2-methylbutanoate (277.3), ethyl nonanoate (225.7), ethyl octanoate (204.9), and ethyl hexanoate (115.0) were the active volatiles of "Chanthaburi 1" ripe flesh, whereas diethyl disulfide was only 4.0 (Table 2). In "Chanee" flesh, 5 ethyl esters, ethyl octanoate (3613.6), ethyl dodecanoate (1126.2), ethyl-2-methylbutanoate (923.2), ethyl hexanoate (318.5), and ethyl propanoate (117.0), were among the major active volatiles (Table 2). In "Monthong" ripe flesh, ethyl octanoate (4173.7), ethyl hexanoate (1808.8), methyl octanoate (843.3), and ethyl-2-methylbutanoate (278.3) were high in the OAV (Table 2), while ethyl octanoate (4241.9), ethyl dodecanoate

Table 1: Volatile compounds released from ripe flesh of 4 Thai durian fruits corresponded to the GC-MS chromatogram profiles.

	Compound	RT	Relative content (ng thiophene/g FW)			
			"Chanthaburi 1"	"Chanee"	"Monthong"	"Kanyao"
			Ester			
1	Methyl-2-methylbutanoate	0.4766	nd	nd	0.71	nd
2	Ethyl acetate	2.1226	8.81	nd	nd	nd
3	Ethyl propanoate	2.9628	nd	3.39	nd	5.70
4	Ethyl-2-methylpropanoate	3.6143	1.85	5.32	2.85	9.71
5	Ethyl butanoate	4.3402	nd	1.08	2.07	2.69
6	Propyl propanoate	4.5230	nd	nd	nd	2.38
7	Ethyl-2-methylbutanoate	5.3346	83.20	276.97	83.49	198.86
8	Methyl-2-methyl-2-butenoate	5.7576	4.67	1.11	1.08	1.07
9	Methyl hexanoate	5.7747	nd	nd	nd	7.33
10	Ethyl-3-methyl-2-butenoate	6.5005	4.74	4.98	1.51	nd
11	Propyl-2-methylbutanoate	6.5348	nd	45.52	nd	nd
12	Ethyl-2-methyl-2-butenoate	7.5865	50.87	8.30	nd	6.61
13	Pentyl-2-methylbutanoate	7.7465	26.58	nd	nd	63.31
14	Methyl-2-hexenoate	8.3524	nd	nd	0.59	nd
15	Ethyl hexanoate	9.2496	10.35	28.67	162.79	65.76
16	Methyl heptanoate	9.9754	nd	1.93	nd	nd
17	Ethyl-2-methylpentanoate	10.227	nd	5.82	nd	nd
18	Propyl-2-methyl-(E)-2-butenoate	10.296	16.93	nd	nd	3.17
19	Ethyl-2-hexenoate	10.547	1.05	nd	7.62	1.32
20	Propyl hexanoate	11.999	nd	nd	18.92	10.29
21	Ethyl heptanoate	12.096	nd	15.50	8.20	8.93
22	Methyl octanoate	12.850	nd	nd	21.08	26.94
23	Ethyl-4-octenoate	14.548	nd	nd	3.23	nd
24	Ethyl octanoate	14.931	8.20	144.54	166.95	169.68
25	Ethyl-2-methyl octanoate	15.754	nd	3.16	nd	0.80
26	Ethyl-(E)-2-octenoate	16.302	0.85	nd	11.35	3.10
27	2-Methylbutyl hexanoate	16.463	nd	nd	1.16	nd
28	Propyl octanoate	17.543	nd	5.81	10.61	16.20
29	Methyl decanoate	18.400	nd	nd	3.08	5.99
30	Ethyl decanoate	20.252	2.93	4.37	24.15	27.25
31	Methyl dodecanoate	23.424	nd	nd	nd	1.78
32	Ethyl dodecanoate	25.064	nd	2.25	nd	8.28
33	Ethyl nonanoate	25.076	2.26	nd	nd	nd
	Total		223.29	558.74	531.44	647.13
			Sulfur compound			
1	Diethyl disulfide	7.0606	4.69	nd	2.48	nd
2	3,5-Dimethyl-1,2,4-trithiolane	13.074	5.10	nd	1.14	1.66
	Total		9.79	0	3.61	1.66
			Acid			
1	Propanoic acid	2.7227	nd	1.02	nd	2.91
2	Hexanoic acid	8.8038	nd	0.72	4.45	9.92
3	Octanoic acid	14.302	nd	3.47	2.86	12.57
	Total		0	5.21	7.31	25.40
			Phenolic			
1	2,4-Di-tert-butylphenol	23.138	nd	nd	1.72	nd
	2,5-bis (1,1-Dimethylethyl phenol)	23.144	nd	nd	nd	1.80
	Total		0	0	1.72	1.80
			Aldehyde			
1	trans-2-Methyl-2-butenal	3.4086	nd	4.36	nd	9.61
	Total	3.4086	0	4.36	0	9.61

(4138.9), methyl octanoate (1077.4), ethyl hexanoate (730.6), ethyl-2-methylbutanoate (662.9), and ethyl propanoate (196.4) were the active volatiles in "Kanyao" (Table 2). There were some volatile compounds detected only in each cultivar. Ethyl acetate (10.0) and ethyl nonanoate (225.7) were only in "Chanthaburi 1," propyl-2-methylbutanoate (n/a), methyl heptanoate (6.7), and ethyl-2-methyl pentanoate (n/a) were only in "Chanee," methyl-2-methylbutanoate (n/a), methyl-2-hexenoate (n/a), ethyl-4-octenoate (n/a), and 2-methylbutyl hexanoate (n/a) were only in "Monthong," and propyl propanoate (8.5), methyl hexanoate (<1), and methyl dodecanoate (683.3) were only in "Kanyao."

Table 2: Odor characteristics of ester, sulfur, acid, phenolic, and aldehyde containing compounds from ripe flesh of 4 Thai durian fruits.

	Compound	Odor description	Aroma threshold values (ppb)	Odor activity values (OAV)				References*
				"Chanthaburi 1"	"Chanee"	"Monthong"	"Kanyao"	
			Ester					
1	Methyl-2-methylbutanoate	Sweet, fruity, apple-like odor	n/a	—	—	n/a	—	—
2	Ethyl acetate	Fruity, sweet, grape- and rum-like odor	0.88	10.014	—	—	—	D
3	Ethyl propanoate	Green, fruity apple-like odor	0.029	—	117.048	—	196.40	M
4	Ethyl-2-methylpropanoate	Fruity	0.1	18.50	53.163	28.47	97.05	O
5	Ethyl butanoate	Fruity, pineapple	0.2	—	5.38	10.35	13.44	H
6	Propyl propanoate	Sharp, chemical, pungent, sweet, fruity	0.28	—	—	—	8.48	M
7	Ethyl-2-methylbutanoate	Fruity	0.3	277.329	276.97	278.31	662.88	N
8	Methyl-2-methyl-2-butenoate	Caramel note, ethereal rum	35 (in water)	<1	<1	<1	<1	R
9	Methyl hexanoate	Fruity, pineapple, ethereal	70	—	—	—	<1	O
10	Ethyl-3-methyl-2-butenoate	n/a	n/a	n/a	n/a	n/a	—	—
11	Propyl-2-methylbutanoate	Winey	n/a	—	n/a	—	—	—
12	Ethyl-2-methyl-2-butenoate	Sweet fruity, green notes	n/a	n/a	n/a	—	n/a	—
13	Pentyl-2-methylbutanoate	n/a	12	2.22	—	—	5.28	A
14	Methyl-2-hexenoate	Fruity green banana honey	n/a	—	—	n/a	—	—
15	Ethyl hexanoate	Apple-like, fruity, aniseed-like, sweet	0.09	115.032	318.54	1,808.81	730.63	H
16	Methyl heptanoate	Sweet, fruity and green, with a waxy apple-like note	0.29	—	6.67	—	—	B
17	Ethyl-2-methyl pentanoate	Fruity, green, melon and waxy with a fatty nuance	n/a	—	n/a	—	—	J
18	Propyl-2-methyl-(E)-2-butenoate	n/a	n/a	n/a	—	—	n/a	—
19	Ethyl-2-hexenoate	Fruity, green, pulpy pineapple and apple	0.14	7.462	—	54.41	9.46	C
20	Propyl hexanoate	Sweet, fruity, juicy, pineapple, green and tropical	70	—	—	<1	<1	E
21	Ethyl heptanoate	Fruity pineapple cognac rum wine	0.24	—	64.60	34.16	37.22	D
22	Methyl octanoate	Waxy, green, sweet, orange, aldehydic, vegetable, herbal	0.025	—	—	843.29	26.94	I
23	Ethyl-4-octenoate	n/a	n/a	—	—	n/a	—	—
24	Ethyl octanoate	Pleasant, fruity, floral odor, wine apricot note	0.04	204.91	3,613.61	4,173.69	1,077.42	K
25	Ethyl-2-methyl octanoate	n/a	n/a	—	n/a	—	n/a	—
26	Ethyl-(E)-2-octenoate	Fruity, green with a fatty waxy note	n/a	n/a	—	n/a	n/a	—
27	2-Methylbutyl hexanoate	Ethereal	n/a	—	—	n/a	—	—
28	Propyl octanoate	n/a	n/a	—	n/a	n/a	n/a	—
29	Methyl decanoate	Oily, winey, fruity, floral	n/a	—	—	n/a	n/a	—
30	Ethyl decanoate	Fruity, grape-, cognac-, and brandy-like odor	0.53	5.53	8.25	24.15	51.42	F

Table 2: Continued.

	Compound	Odor description	Aroma threshold values (ppb)	Odor activity values (OAV)				References*
				"Chanthaburi 1"	"Chanee"	"Monthong"	"Kanyao"	
31	Methyl dodecanoate	Waxy, soapy nutty, coconut, mushroom	0.0026	—	—	—	683.31	B
32	Ethyl dodecanoate	Waxy, soapy, rummy, nutty, floral	0.002	—	1,126.15	—	4,138.90	B
33	Ethyl nonanoate	Slightly fatty, oily, fruity, nutty, reminiscent of cognac with a rosy fruity note	0.01	225.68	—	—	—	L
			Sulphur compound					
1	Diethyl disulfide	Onion, garlic	2	3.97	—	1.25	—	M
2	3,5-Dimethyl-1,2,4-trithiolane	Sulphury, onion, meaty	n/a	n/a	—	n/a	n/a	G
			Acid					
1	Propanoic acid	Pungent, acidic, dairy	1	—	1.02	—	2.94	P
2	Hexanoic acid	Sour, fatty, sweaty, cheesy	0.0047	—	154.23	956.57	2,132.40	Q
3	Octanoic acid	Fatty, waxy, rancid oily, vegetable cheesy	0.011	—	318.60	262.70	1,154.29	Q
			Phenolic					
1	2,4-Di-tert-butylphenol	n/a	n/a	—	—	n/a	—	—
2	2,5-bis (1,1-Dimethylethyl phenol)			—	—	—	n/a	—
			Aldehyde					
1	trans-2-Methyl-2-butenal	Strong green fruit	n/a	—	n/a	—	n/a	G

*The capital letters represented the references of odor threshold value as follows: [A][14] Allison and Katz (1919), [B][15] Backman (1917), [C][16] Berger (1985), [D][17] Cometto-Muñiz, et. al. (2005), [E][18] Fan and Xu (2011), [F][19] Ferreira et. al. (1998), [G][20] Gemert (2011), [H][21] Guth (1997), [I][22] Karl et. al. (1994), [J][23] Komthong (2006), [K][24] Rychlik (1998), [L][25] Schwarz (1995), [M][26] Nagata (2003), [N][27] Takeoka et al. (1989), [O][28] Takeoka et al. (1990), [P][29] van Thriel et al. (2006), [Q][30] Wise et al. (2007), and [R][31] Yair (2012).

Ripe flesh of most durian cultivars contains ethyl-2-methylbutanoate (fruity note) and ethyl hexanoate (fruity, apple, green, and tropical fruit odor) as the active volatiles showing high OAV. Both found in all four cultivars and most commercial durians were blended with some high-OAV compounds to characterize the flavor of each durian variety. In general, ripe durian flesh exhibits the fruity sweet fragrance of both compounds. "Chanthaburi 1" contained fewer components of volatiles as well as low OAV of the volatiles. This indicates that the flesh of "Chanthaburi 1" conducted very low intensity of odors during ripening. Ethyl heptanoate (fruity, pineapple, banana-like note) was found in every cultivar except "Chanthaburi 1." Ripe flesh of "Chanee" exhibited the strongest durian aroma among 4 varieties: "Chanee" exhibited aroma of ethyl octanoate (fruity, floral odor, wine apricot note), ethyl dodecanoate (waxy, soapy, nutty, rummy), and ethyl propanoate (green fruity, apple-like) characterized as nutty, rummy, and green apple-like, "Monthong" exhibited strong apple-like fruity, aldehydic, waxy fragrance of ethyl octanoate (fruity, floral odor, wine apricot note), ethyl hexanoate (apple-like, fruity), and methyl octanoate (waxy, green, sweet, orange, aldehydic vegetable), and "Kanyao" exhibited more complex waxy, nutty, green apple-like fruity aroma of ethyl octanoate, ethyl dodecanoate (waxy, soapy, nutty, rummy note), methyl octanoate (waxy, green, sweet orange), and ethyl propanoate (green fruity, apple-like).

Diethyl disulfide and 3,5-dimethyl-1,2,4-trithiolane found in low levels in ripe durian pulp are the key compounds in durians. Although sulfur-containing compounds exhibited low OAV, compared to the esters, they exhibit an annoying pungent smell. "Chanthaburi 1" as well as "Monthong" contained sulfur-containing compounds of diethyl disulfide and 3,5-dimethyl-1,2,4-trithiolane, which exhibit a garlic-like, onion-like, pungent smell [32]. In particular, diethyl disulfide in ripe "Monthong" showing an OAV of 1.25 would release the pungent smell of "Monthong" durian as reported by Laohakunjit et al. [8] and Niponsak et al. [9]. Previous studies in Malaysia and Indonesia found that the indigenous varieties exhibited a prominent smell of sulfur-containing compounds when fully ripe showing an unpleasant odor overall [32, 33].

In 4 cultivars of Thai durian fruit, ripe aril sharply produced a series of ethyl esters derived from ethyl alcohol and acyls CoA of straight carbons ranging from C_4–C_{10} (Table 1). Ethanol in the aril could be generated from anaerobic respiration under a partial hypoxic condition in aril tissue. Due to very high respiration of durian fruit during ripening, fruit husk behaving like a gas barrier makes low gas permeability to the aril. Under partial hypoxia, anaerobic respiration was induced in the aril, resulting in increased

ethanol [34–37]. Aliphatic and aromatic alcohols are typically found in Malaysian durians, whereas thiols are produced in Thai durians and alcohols are not typically produced in Indonesian and Filipino durians [37]. On the other hand, with a series of straight acyl CoA reacted with the ethanol, it is supposed that β-oxidation of fatty acids would be involved in the process of ripe fruits [38] as durian pulps have high contents of fatty acids such as methyl stearate (35.93%), methyl palmitate (32.91%), methyl palmitoleate (9.50%), methyl octadecenoate (4.86%), methyl oleate (4.68%), methyl myristate (2.52%), and methyl linoleate (2.20%) [39]. Furthermore, amino acid metabolism plays a crucial role in ester production in durians. For instance, ethyl-2-methylbutanoate, a primary volatile compound, is derived from 2-methylbutanoyl-CoA through isoleucine metabolism [40]. The origination of acyls CoA in the ester production could be separated into two sources from the results. When the acyl CoA of C_4 could be derived from amino acids, acyl CoA above C_6 could be from lipid oxidations. Furthermore, alcohol acyltransferase (AAT), which modifies alcohols and acyl CoA to esters, could be essentially involved in the production of esters in most durians. Although ATT has not yet been reported in durian, it was reported to be essential for ester production during ripening in many fruits [41–43]. However, as a result of fewer esters in "Chanthaburi 1," the production of esters is apparently disturbed in the fruit probably by mutant functioning of the AAT or the substrate-enzyme incompatibility.

3.2. The Relationship of Durian Cultivar Relied on Aroma Volatiles. All 4 varieties showed that ethyl esters were the major components in the ripe flesh. Ethyl acetate and ethyl nonanoate were found in "Chanthaburi 1" but not in the parent, "Chanee" and "Monthong," whereas, on the other hand, ethyl butanoate and ethyl heptanoate found in the parent were not found in "Chanthaburi 1". Methyl-2-methylbutanoate was detected only in ripe "Monthong" flesh.

Ester compounds as the major volatiles were taken to calculate the relationship between cultivars. The differences in essential substances between durian species may be due to genetics and the environment. Genetic factors influence the formation of precursors, enzymes, and odor generation [44]. The durian of "Chanthaburi 1," a hybrid variety, has overall odor characteristics related to the parent variety, "Chanee," and the father species is "Monthong." Nevertheless, by considering the odor, "Chanthaburi 1" has a mild odor while still unripe, similar to the odor of "Kanyao." Although identifying the essential substances in "Chanthaburi 1" durian exhibited a more minor odor type than the strong aroma varieties, the essential substances (OAV) in the "Chanthaburi 1" exhibited characteristics related to both "Chanee" and "Kanyao." The relative content of the ester was obtained according to the dendrogram (Figure 2) of each essential substance. The volatile contents in "Chanthaburi 1" were related to "Monthong" when considering the ester composition. The ester compounds in "Chanthaburi 1" were correlated well with the "Monthong" variety, consistent with the species characteristics that 'Monthong' was the father. However, the relationship of ester compounds in "Chanee" was close to that in "Kanyao."

Principal component analysis (PCA) using the ester compounds from Table 1 was operated to correlate and classify the essential components of the four durian varieties. Ester compounds were classified in the same component, with an eigenvalue greater than 1, and the component was equal to 2 (data not shown) with Minitab© 17 displayed in the score plot and biplot (Figure 3). The main component and the secondary components were associated with the ester compounds of the four durian varieties. When looking at the main components, "Chanthaburi 1" durian was related to "Chanee," and from the secondary, "Chanthaburi 1" was, on the other hand, related to the "Monthong" variety which corresponds to the ester characteristics of the parents. But, "Kanyao" has characteristics that are clearly different from those of "Chanthaburi 1" by both components. In addition, the ester characteristics of "Chanthaburi 1" as shown in Figure 3(c) were ethyl acetate, ethyl nonanoate, and methyl-2-methyl-2-butenoate, which exhibit a rum-like, grape, and cognac, as well as caramel note. For "Chanee," it can be seen from Figure 3(c) that the distinctive esters were propyl-2-methylbutanoate, ethyl-2-methyl pentanoate, and methyl heptanoate showing winey, apple, pineapple, green, melon and waxy flavors, cognac rum wine, intensely fruity, and orris-like. In "Monthong," the ester characteristics were methyl-2-methylbutanoate, ethyl-2-hexenoate, and methyl-2-hexenoate. The scent characteristics are sweet fruity, apple-like odor, green, pineapple, apple, green, banana, honey. On the other side, "Kanyao" exhibited a distinctive scent of methyl hexanoate, propyl propanoate, and methyl dodecanoate, showing fruity, pineapple, complex fruity odor, apple and banana, waxy soapy, nutty, and coconut mushroom. When considering the OAV value of each durian species, if the OAV is greater than 1, it can be expected to exhibit a unique aroma. The OAV value of "Chanthaburi 1" was clearly similar to that of "Chanee," the mother variety, and close to that of "Kanyao" (Table 2 and Figure 4). The OAV values showed that "Chanthaburi 1" had the dominant esters, ethyl acetate (10.0) and ethyl nonanoate (225.7), which exhibited fruity, sweet, grape and rum-like, slightly fatty, oily, fruity, scent characteristics of nutty, reminiscent of cognac with a rosy fruity note. Nevertheless, "Kanyao" has outstanding OAV values of ethyl octanoate (4,241.9) and ethyl dodecanoate (4,138.9) at high, which is likely to be another distinctive scent, characterized by long stems showing fruity, fatty, floral odor (wine apricot note), waxy, sweet, musty, pineapple, dairy, sweet, waxy soapy rummy, and nutty floral. The distinctive OAV value is of methyl heptanoate (6.7) because it is found only in "Chanee," showing sweet, fruity, and green, with a waxy apple-like note. The higher levels of OAV were found in "Monthong" and "Kanyao" durians, but less common in "Chanthaburi 1" was ethyl octanoate (204.9), which showed a pleasantly fruity, floral odor (wine apricot note). The OAV values were different from the ester relative content, which was the relative content of the volatile compounds present in each durian species, indicating that "Chanthaburi 1" was

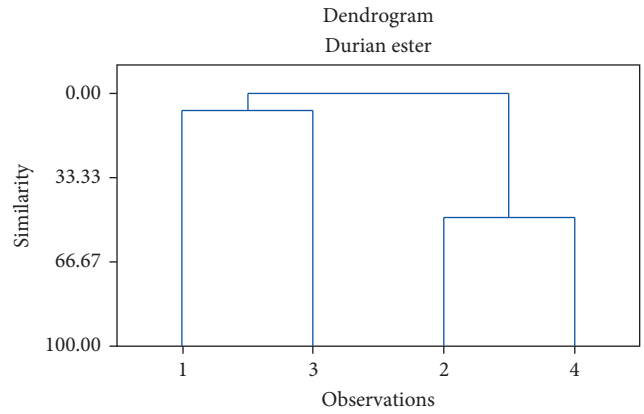
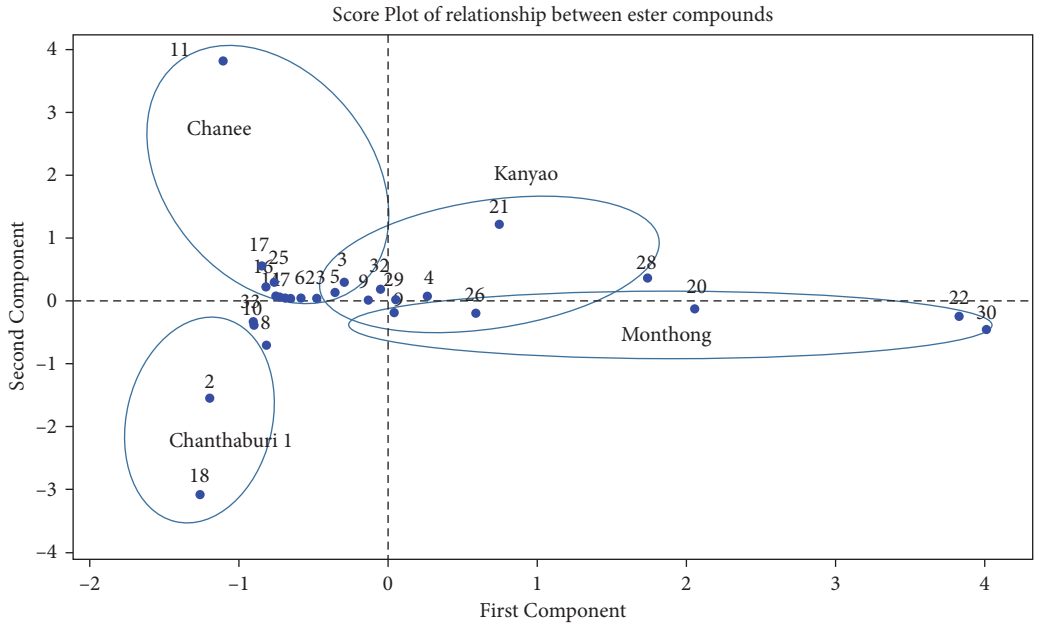
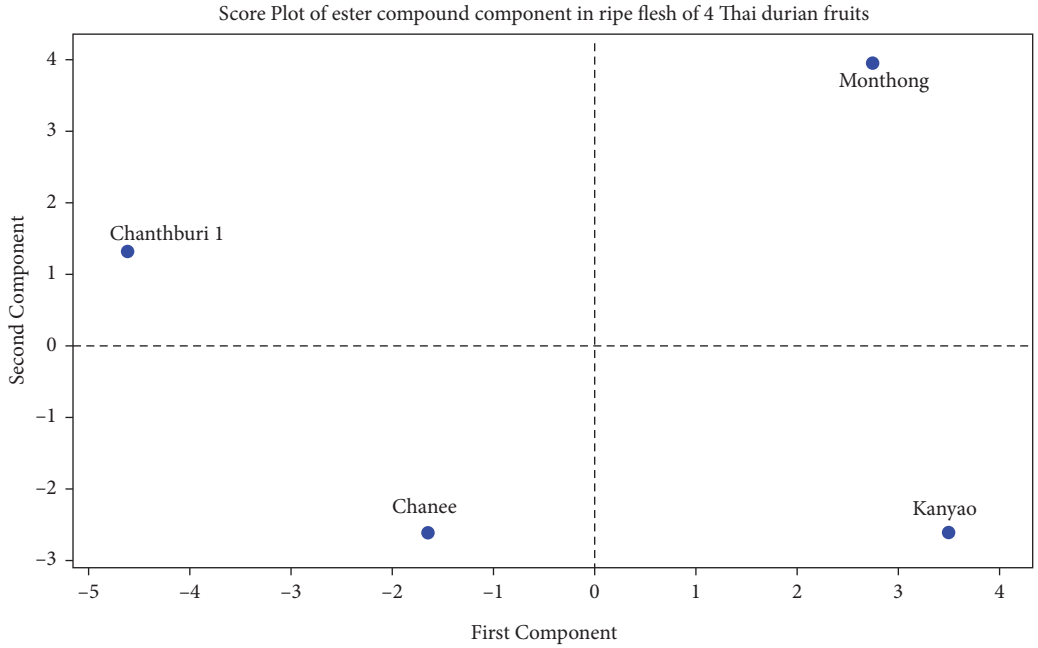


Figure 2: Dendrogram of the relationship in the ester compound produced in 4 Thai durian cultivars (1 = "Chanthaburi 1," 2 = "Chanee," 3 = "Monthong," and 4 = "Kanyao").

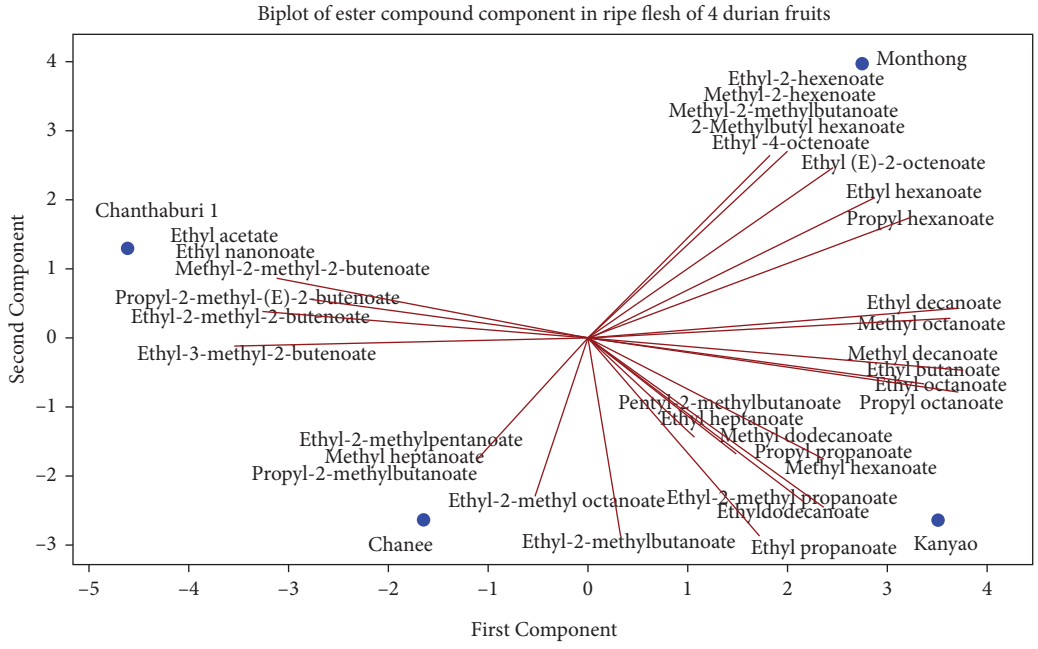


(a)

Figure 3: Continued.



(b)



(c)

Figure 3: Principal component analysis (PCA) relationship between 4 Thai durian cultivars. (a) Score plot of the relationship between 4 Thai durian cultivars using the ester compounds. (b) Score plot of the ester compound component in ripe flesh of 4 Thai durian cultivars. (c) Biplot of the ester compound component relationship with 4 Thai durian cultivars.

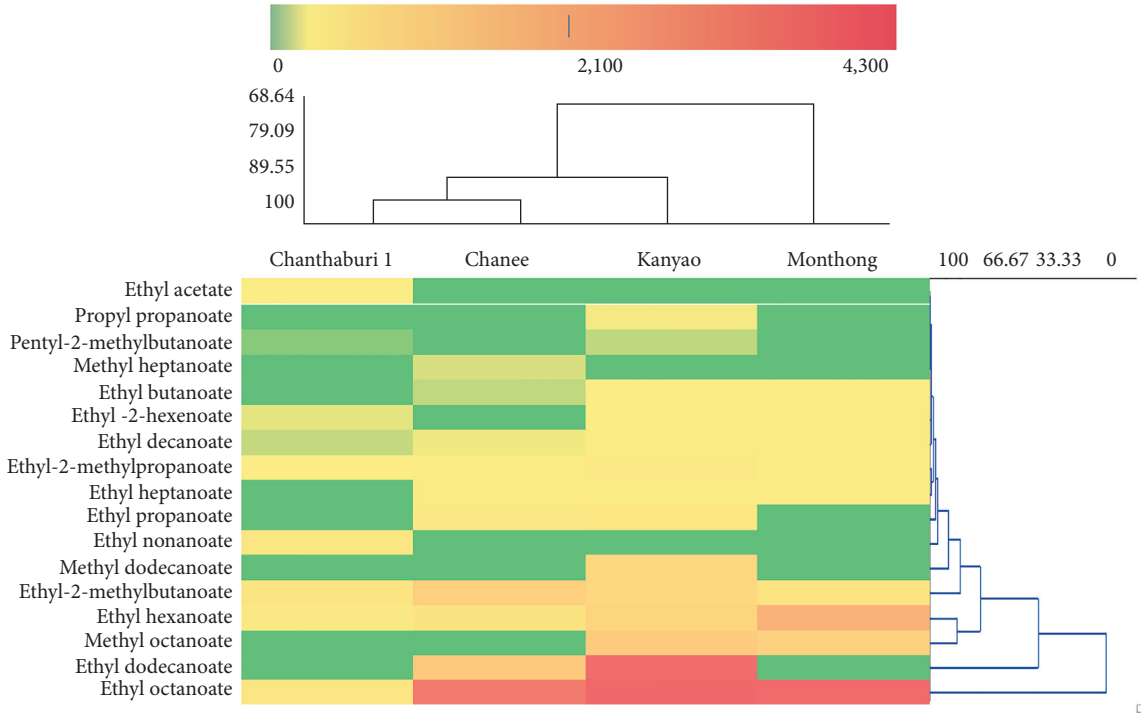


Figure 4: Heat map of odor activity value (OAV), which is greater than 1 of each durian species.

consistent with "Monthong" and "Chanee," the father and mother, respectively. Nevertheless, if the OAV value was considered, "Chanthaburi 1" was close to "Kanyao" more than "Monthong" (Figure 4). According to the observation from the odor characteristics, the odor of "Chanthaburi 1" is mild, similar to that of "Kanyao," which the OAV value can explain to some extent. Based on information on the composition of these essential substances, it could greatly benefit the status of Thai durians in terms of the choice of eating fresh fruit and the choices to use ripe durian pulp as an ingredient of food or dessert which requires the durian odor. The study could increase the opportunities of Thai durian transport channels to the world.

4. Conclusions

Thirty-three esters and three sulfur-containing compounds were the main volatiles found and affected the flavor character of the ripe pulp of four varieties of Thai durians, "Chanee," "Monthong," "Kanyao," and "Chanthaburi 1." Ethyl esters were the major esters as ethyl-2-methylbutanoate and ethyl hexanoate were the crucial essential substances found in all four varieties. The overall aroma character of the durian was a mixture combined of fruity-like apple/pineapple with rum, butter, oily, and waxy odors. Although ripe durians produced few sulfur-containing volatiles, the compounds exhibit a sulfurous pungent smell. Using the volatile ester profiles, "Chanthaburi 1" correlated with "Chanee," the mother breed, and "Monthong," the father breed. "Kanyao" was different from "Chanthaburi 1." However, with high OAV values concerned, "Chanthaburi 1" was obviously associated with "Chanee," but the odor character was more similar to "Kanyao" than "Monthong."

Data Availability

The data used to support the findings of this study are included within the supplementary information file.

Conflicts of Interest

There are no conflicts of interest in this study.

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Supplementary Materials

Supplementary Figure 1: naturally ripe fruit at the initial dehiscence (red circle) at the blossom end. (*Supplementary Materials*)

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Research Article

Characterization of the Key Aroma Compounds in the Fruit of *Litsea pungens* Hemsl. (LPH) by GC-MS/O, OAV, and Sensory Techniques

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The key aroma compounds in the fruit of *Litsea pungens* Hemsl. (LPH) were concentrated through solvent-assisted flavor evaporation (SAFE) and characterized by gas chromatography-mass spectrometry-olfactometry (GC-MS/O), quantitative descriptive analysis (QDA), odor activity values (OAVs), and addition test. The results showed that LPH contained 31 aroma-active compounds (flavor dilution, FD = 9). Among them, 30 odorants were quantified by the standard curve method. The OAV analysis results showed that 25 odorants had OAVs ≥ 1, which could be considered as the potent odorants. D-Limonene and 3,7-dimethyl-2,6-octadienal had the highest OAVs (OAV = 9803 and 8399), followed by (*Z*)-3,7-dimethylocta-2,6-dienal (OAV = 1893), *β*-myrcene (OAV = 1798), (*E*)-3-phenyl-2-propenoic acid ethyl (OAV = 1603), and *β*-caryophyllene (OAV = 1129). Addition experiments further confirmed that 3,7-dimethyl-2,6-octadienal, (*Z*)-3,7-dimethylocta-2,6-dienal, and D-limonene contributed to lemon attribute, *β*-myrcene contributed to green attribute, citronellal contributed to mint and fresh note, and eucalyptol contributed to eucalyptus-like note were the key odorants.

1. Introduction

Litsea pungens, as a genus belongs to Lauraceae's family, is an evergreen or deciduous tree or shrub with about 200 species distributed worldwide (mainly distributed in tropical and subtropical regions of Asia and America) [1]. In China, there are 72 species of *Litsea pungens* Hemsl. (LPH) distributed in 20 provinces (Figure 1). Among them, Yunnan Province has the most species (37), followed by Guangdong (24), Sichuan (18), Guizhou (15), and Hunan Province (12). The fruit, root, branch, and leaves of LPH have a wide range of applications in traditional Chinese medicine, fragrance industry [2, 3], cosmetics industry, and food industry due to the functional compounds existing inside, such as the aromatic compounds (essential oil), flavonoids, terpenoids, butanolides, and butenolactones steroids, lignans, amides, and alkaloids. The LPH, rich in citral, is an important raw material for ionone and damascene [4, 5]. Due to the large and broad market demand, several decades ago, LPH species had been industrially cultivated, especially in Yunnan, Hubei, Hunan, Sichuan, Chongqing, and Guizhou provinces of China.

The therapeutic effects of LPH include removing dampness, regulating spleen deficiency, helping digestion, dysmenorrhea, expelling cold, and analgesia. All these benefits have been widely recorded in ancient Chinese medicine books such as "Guizhou folk medicine," "Chongqing Herbs," and "Hunan yaowuzhi." Modern molecular biology technologies have also elucidated that the functional compounds in the fruits, roots, branches, and leaves of LPH have anti-inflammatory activity, antimicrobial activity, hepatoprotection, antidiabetic, antiasthma activity, anticholelithiasis activity, immunomodulation, and miscellaneous bioactivities [3]. This information confirmed the

Figure 1: The area distribution of *Litsea pungens* Hemsl. [3–19]. Numbers presented in each province in the map of China represent the *Litsea pungens* Hemsl. species.

abundance of pharmacological properties of LPH. According to the Chinese natural spices classification standard (GB/T 21725-2017), LPH with a strong aroma is characterized as one of the 20 pungent type spices [20, 21]. Based on its unique characteristics of citrus, sweet lemon, camphor wood, and green aroma, LPH is also a vital fragrance applied to many southwestern Chinese dishes, such as cooking beef or lamb, chili sauce, and pickled vegetables, especially the traditional Zhijiang Blood Duck (Huaihua, Hunan province) [6–8]. The LPH is an important pharmaceutical/food resource; therefore, elucidating the flavor chemistry of LPH is meaningful to the application of standardized LPH.

Up to now, five extraction methods have been applied to extract the aroma compounds in LPH's fruits, and more than 277 aroma compounds in LPH's fruit have been reported [4–19]. Most of them were terpenoids, which were the biomarkers of fruit freshness and ripeness [9]. However, only a small part (1~3%) was the key odorants perceived in human olfaction [22, 23]. Steam distillation (SD) was the most popular extraction method [11], followed by solid-phase microextraction (SPME) [17], supercritical CO_2 fluid extraction (SFE) [12], molecular distillation extraction (MDE) [18], and simultaneous distillation extraction (SDE) [10]. By applying SD coupled with gas chromatography-mass spectrometry (GC-MS), (*Z*)-3,7-dimethyl-2,6-octadienal (α-citral), (*E*)-3,7-dimethyl-2,6-octadienal (β-citral), D-limonene, terpinen-4-ol, (3Z)-3,7-dimethyl-2,6-octadiene-1-ol (nerol), 1,8-cineole, linalool, caryophyllene oxide, and 6-methyl-5-hepten-2-one were detected as the dominant aroma compounds in LPH's fruit [11–16]. Qiao et al. elucidated that α-citral, β-citral, nerol, D-limonene, and 6-methyl-5-hepten-2-one accounted for 90% of essential oil (extracted by SFE) of LPH's fruit, and citral was most prevalent (60%). However, in fresh LPH's fruit [17], α-citral and β-citral were most prevalent (60.72%), followed by D-limonene (24.17%), α-pinene (3.15%), β-myrcene (2.92%), and β-pinene (2.11%). Additionally, Qiao et al. [17, 18] reported that D-limonene and citronellal accounted for 25.46% and 19.41% in LPH essential oils, respectively, by SPME-GC-MS analysis. Wang et al. confirmed that MDE has the higher extraction efficiency for fruit essential oils than other extraction methods suitable for industrial production [19]. Although the aroma compounds in LPH were qualitatively analyzed by mass spectrometry and quantified by area normalization method, the gas chromatography-olfactometry (GC-O) analysis and quantification by constructing the standard curves were not applied to screen the aroma-active compounds. Therefore, more efforts should be strengthened to characterize the key aroma compounds in LPH's fruit. Solvent-assisted flavor evaporation (SAFE) is the most suitable and useful extraction method for LPH's aroma analysis due to its high lipid content [24–26].

The objectives of this work are to identify the key aroma compounds in LPH's fruit by (1) isolating the volatile compounds from LPH's fruit by SAFE; (2) characterizing the aroma-active compounds by gas chromatography-mass spectrometry-olfactometry (GC-MS/O); and (3) quantifying the aroma-active compounds and calculating their OAVs. (4) Confirming the key odorants by addition experiment.

2. Materials and Methods

2.1. Sample Preparation. The dried LPH's fruit (August 2019) was purchased from Guiyang Qingshanbulao *Litsea pungens* Hemsl. Oil Factory (Guiyang, Guizhou, China). The moisture of dried LPH's fruit (7.08%) was detected by a high-speed moisture analyzer (IR35M-000230 V1, Denver Instrument GmbH, Göttingen, Germany) reference to our previous work [27, 28]. Before determination, the LPH's fruit was powdered by grinder (Bangjie Machinery Equipment Factory, Wuxi, China).

2.2. Chemicals. 1,2-Dichlorobenzene and dichloromethane with GC grade were purchased from Thermo Fisher (Beijing, China). 3-Methylbutyric acid, 4-methylacetophenone, D-limonene, 2-octanol, decanoic acid, caryophyllene oxide, β-caryophyllene, β-myrcene, 6-methyl-5-hepten-2-one, phenylacetic acid, and methional with purity over 99% were purchased from J&K (Beijing, China). Ethyl p-methoxycinnamate, (*E*)-3-phenyl-2-propenoic acid ethyl, and safrole were bought from Beijing Zhongke Quality Inspection Biotechnology Co., Ltd. (Beijing, China). 3,7-Dimethyl-2,6-octadienal, fenchone, (3*Z*)-3,7-dimethyl-2,6-octadien-1-ol, citronellal, eucalyptol, linalool, (E)-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, 4-(1-methylethyl)-benzaldehyde, 2-(3-methyl-2-butenyl)-3-methylfuran, methyleugenol, D-carvone, carveol, (*R*)-2-methyl-5-((*S*)-6-methylhept-5-en-2-yl)-cyclohexa-1,3-diene, estragole, (+)-2-bornanone, anethole, and (*Z*)-3,7-dimethylocta-2,6-dienal were purchased from Toronto Research Chemicals (Beijing, China).

Eugenol and *p*-cresol were bought from Sigma-Aldrich (Beijing, China).

2.3. Sensory Evaluation. Quantitative descriptive analysis (QDA) was used to evaluate the aroma profiles of LPH's fruit. Twelve panelists with no rhinitis and no smoking (6 females and 6 males, age of 22–30) were recruited from our laboratory. The sensory evaluation room temperature was 23~25°C, the humidity was 50~55%, and filament lamp (36 W) was used. All panelists were informed of the aim, detailed experimental steps, and requirements of sensory evaluation before participating in this experiment. They were trained for 3 weeks before the QDA analysis: (1) all panelists were requested to sniff and describe the aroma characteristics of 54-aroma kit (Le Nez du Vin®, France) with 3 times a week (each training lasted for 30 min); (2) then, panelists were requested to analyze the aroma profiles of LPH's fruit descriptively. The final 6 aroma attributes (lemon, floral and sweet, mint and fresh, green, eucalyptus-like, and sour) were determined according to the frequency of descriptors, and their corresponding referenced standards were D-limonene, nerol, menthol, 1-hexanol, 1,8-cineole, and propionic acid, respectively; (3) finally, 12 panelists were qualified to score the intensity of 6 aroma attributes on a scale from 1 to 9 (1–3, weak; 4–6, medium; 7–9, strong). The LPH's fruit sample (4.00 g) loaded in 200 mL transparent glasses was presented to the panelist.

2.4. Isolation of the Volatile Compounds by SAFE. Dried LPH's fruit (20.00 ± 0.20 g) and dichloromethane solvent (80 mL) were loaded in a conical flask (250 mL) and extracted for 15 min by ultrasonication (KH-500 DE, Jiangsu, China) in 500 W at 10°C. Then, the organic phase was collected after filtration. After 3 extractions, the collected solvents were combined and submitted to the SAFE apparatus for volatile isolation. Isolation of the volatile compounds from the solvents was reference from our previous work with some modifications [25, 26]. The recycled water in SAFE apparatus was (40 ± 1)°C; the distillation flask was bath at (40 ± 1)°C; the collection flask was immersed in liquid nitrogen; the extraction system was operated under vacuum (10^{-5}~10^{-6} Pa) via molecular turbine pump (Edwards, England), and the filtrate was added dropwise to the distillation flask. Then, the extract was concentrated to 1~2 mL with rotary evaporation instrument (EYELA N-1100, Tokyo Physical and Chemical Equipment Co., Ltd, Japan) after drying with anhydrous sodium sulfate. Finally, the concentrate was reduced to 1.00 mL by nitrogen (99.99%) before GC-MS and GC-MS/O analysis. All analyses were repeated in triplicate.

2.5. GC-MS and GC-MS/O Analysis. The identification and quantification of the aroma compounds were conducted by a single quadrupole gas chromatograph-mass spectrometer (GC-MS) (Thermo Fisher Trace 1310, Thermo Fisher Technology Co., Ltd, USA) in a split ratio of 50 : 1 (optimized in our lab). The aroma-active compounds were screened by GC-MS equipped with a sniffing port (ODP3, Gerstel, Germany) (GC-MS/O). The temperature of sniffing port was 220°C, and the humidifier with flow rate of 10 mL/min (nitrogen, 99.999%) was used to humidify the air at sniffing port. The GC effluent was split at a ratio of 1 : 1 between the MS and sniffing port for the GC-MS/O's special structure in splitless injection. Separation of the aroma compounds in LPH's fruit extract was achieved on TG-5MS and TG-WAX columns (both $30\,\text{m} \times 0.25\,\text{mm}$ i.d. $\times 0.25\,\mu\text{m}$, Thermo Fisher). Helium (99.999%) was the carrier gas, and the carrier gas flow rate was constant at 1.200 mL/min and 2.000 mL/min in GC-MS and GC-MS/O, respectively.

The oven temperature of TG-WAX column analyzer was initially held at 40°C for 2 min, increased to 100°C (temperature rise rate, 4°C/min) and held for 1 min, and then increased to 175°C (temperature rise rate, 2°C/min) and held for 1 min, finally increased to 230°C (temperature rise rate, 5°C/min). The oven temperature of TG-5MS column analysis was initially held at 40°C, then increased to 100°C (temperature rise rate, 3°C/min), increased to 170°C (temperature rise rate, 1°C/min) and held for 1 min, and finally increased to 230°C (temperature rise rate, 5°C/min). The temperature of the sniffing port was kept at 230°C. The injector temperature was 250°C, and the ion source temperature was 280°C. The electronic-impact mass spectra ionization mode with ionization energy of 70 eV was used. The full scan mode (m/z range from 40 to 350 amu) was used.

2.6. Gas Chromatography-Olfactometric (GC-O) Analysis. The aroma frequency, combined with the aroma dilution method, was used in the GC-O study. Firstly, the concentrated organic extract was diluted to 1 : 9 with dichloromethane solvent. The diluted sample was then submitted to the GC-MS/O with the TG-WAX column to screen the aroma-active compounds with flavor dilution (FD) factor over 9. The diluted sample was repeated 3 times by 3 trained panelists. Only the aroma compounds detected over 5 of 9 were recorded. Panelists underwent GC-MS/O training by sniffing 31 standards aroma compounds in dichloromethane solvent (1,000 μg/L) three times before this experiment.

2.7. Identification and Quantification. The identification of the aroma compounds was based on comparing the mass spectra (MS) database NIST 2020, with retention indexes (RIs, on nonpolar and polar GC columns), pure standards (S), and the odor characteristics (O). All quantifications of key odorants were performed by constructing standard curves. The abscissa was referred to the ratio of the peak area of each compound to the three internal standards (1,2-dichlorobenzene, 2,500 μg/mL; 2-octanol, 2,900 μg/mL; 3-methylacetophenone, 3,000 μg/mL) that are obtained by GC-MS and the ordinate was the concentration ratio of aroma compounds to the three internal standards [29]. Each quantified aroma compound referenced from the specifically internal standard was labeled in Table 1. Each of the internal standards (100 μL) was added when LPH's fruit was extracted by dichloromethane solvent.

Table 1: Odor activity values (OAVs) of aroma-active compounds (FD = 9) in *Litsea pungens* Hemsl. fruit.

No.	Compounds	CAS	Concentration (μg/kg)	Threshold (μg/kg)	OAV	Standard curves	R^2
5	D-Limonene[c]	5989-27-5	333,321.19 ± 1,339.09	34 [30]	9803	$y = 0.0539x - 0.0014$	0.9932
20	3,7-Dimethyl-2,6-octadienal[a]	5392-40-5	251,978.13 ± 683.24	30 [31]	8399	$y = 0.0851x + 0.0288$	0.9936
17	(*Z*)-3,7-Dimethylocta-2,6-dienal[a]	106-26-3	189,366.87 ± 7,528.88	100 [31]	1893	$y = 0.074x + 0.0085$	0.9958
3	β-Myrcene[a]	123-35-3	179,800.17 ± 4,978.31	100 [32]	1798	$y = 0.0896x - 0.0085$	0.9975
28	(*E*)-3-Phenyl-2-propenoic acid ethyl[b]	4192-77-2	6,913.31 ± 913.99	4 [31]	1603	$y = 0.1055x + 0.0146$	0.9975
26	β-Caryophyllene[b]	87-44-5	76,771.87 ± 1,296.43	64 [30]	1129	$y = 0.068x + 0.0212$	0.9837
12	Citronellal[a]	106-23-0	25,397.45 ± 1,483.47	31 [30]	819	$y = 0.0487x + 0.008$	0.9972
7	Linalool[a]	78-70-6	360,841.19 ± 7,836.63	500 [32]	722	$y = 0.073x + 0.0067$	0.9944
4	6-Methyl-5-hepten-2-one[a]	110-93-0	29,118.53 ± 9,49.40	50 [32]	582	$y = 0.094x - 0.0003$	0.9999
2	Methional[a]	3268-49-3	890.48 ± 177.02	1.8 [33]	494	$y = 0.4585x + 0.004$	0.995
13	Estragole[b]	140-67-0	13,376.54 ± 3,255.45	35 [31]	382	$y = 0.0875x - 0.004$	0.9964
6	Eucalyptol[b]	470-82-6	81,581.26 ± 1,136.85	230 [31]	355	$y = 0.0613x + 0.0025$	0.9987
11	2-(3-Methyl-2-butenyl)-3-methylfuran[c]	15186-51-3	23,794.84 ± 4,610.74	100 [31]	238	$y = 0.0384x + 0.0058$	0.9979
21	Anethole[b]	104-46-1	7,133.33 ± 551.68	50 [31]	143	$y = 0.062x + 0.0085$	0.9993
23	Eugenol[b]	97-53-0	10,249.40 ± 824.78	90 [31]	114	$y = 0.0988x - 0.0039$	0.9882
18	D-Carvone[c]	2244-16-8	6,998.14 ± 682.58	160 [31]	44	$y = 0.1175x + 0.0262$	0.9936
25	Methyleugenol[b]	93-15-2	2,529.33 ± 467.25	68 [31]	37	$y = 0.0696x + 0.011$	0.9977
9	*p*-Cresol[b]	106-44-5	3,229.82 ± 41.03	100 [32]	32	$y = 0.0429x + 0.0248$	0.9928
22	Safrole[b]	94-59-7	2,328.99 ± 390.14	160 [31]	15	$y = 0.0797x + 0.0141$	0.9978
16	4-(1-methylethyl)-benzaldehyde[b]	122-03-2	2,500.23 ± 157.00	177 [31]	14	$y = 0.0612x + 0.0183$	0.9961
15	(3*Z*)-3,7-Dimethyl-2,6-octadien-1-ol[a]	106-25-2	3,747.99 ± 643.73	300 [30]	12	$y = 0.1214x - 0.006$	0.9983
31	Ethyl *p*-methoxycinnamate[b]	24393-56-4	5,422.92 ± 244.75	500 [34]	11	$y = 0.1576x + 0.0407$	0.9956
30	Caryophyllene oxide[b]	1139-30-6	3,829.05 ± 630.81	400 [31]	10	$y = 0.0412x + 0.0156$	0.9837
27	(+)-2-Bornanone[d]	464-49-3	3,802.95 ± 770.73	1360 [31]	3	$y = 0.0482x - 0.007$	0.9962
1	3-Methyl-butryicacid[a]	503-74-2	156.40 ± 3.15	100 [31]	1.5	$y = 0.1128x - 0.0033$	0.9982
10	Fenchone[c]	1195-79-5	97.44 ± 11.68	440 [31]	<1	$y = 0.0482x - 0.007$	0.9962
14	Carveol[c]	99-48-9	665.79 ± 637.26	4000 [35]	<1	$y = 0.4792x - 3E{-}19$	0.9999
24	Decanoic acid[a]	334-48-5	2,043.84 ± 670.91	2200 [32]	<1	$y = 0.084x + 0.0068$	0.9943
29	(*R*)-2-Methyl-5-((*S*)-6-methylhept-5-en-2-yl)-cyclohexa-1,3-diene[c]	495-60-3	9,925.30 ± 535.49	—	—	$y = 0.0875x - 0.004$	0.9964
19	Phenylacetic acid[c]	103-82-2	—	—	—	—	—
8	(*E*)-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol[c]	7212-40-0	10,040.07 ± 468.85	—	—	$y = 0.0765x - 1E - 04$	0.9991

The alphabet a, b, c, and d represent compounds calibration by internal standard compounds of 2-octanol, 1,2-dichlorobenzene, and 4′-methylacetophenone, respectively; "—" represents the aroma compounds were lower than the quantitative limitation.

2.8. Calculation of the Odor Activity Value (OAV). The OAVs of aroma-active compounds were measured by dividing their concentration detected in the LPH's fruit sample by their odor threshold detected in water. Each threshold value was referenced from the corresponding literature studies and book, which labeled in Table 1 [30–33, 36, 37]. The aroma-active compounds with OAV ≥ 1 are considered to be the potent key odorants of LPH fruit.

2.9. Addition Experiment. The addition tests were conducted to validate the potent odorants and elucidate their specifically contributions with high OAV to LPH's fruit sample by adding the aroma compounds to the LPH's fruit sample (5.00 g) based on the detected concentration. Two original LPH's fruit samples (5.00 g) and one aroma added sample were subjected to the panelists. Panelists were requested to evaluate the difference by triangle tests and quantitative descriptive analysis (Section 2.3) [38] as shown in Table 2.

3. Results and Discussion

3.1. Sensory Evaluation. The QDA result data of LPH's fruit were plotted on a spider diagram shown in Figure 2, suggesting that lemon note was the strongest, followed by floral and sweet, mint and fresh, eucalyptus-like, and green characteristics. The sour note of LPH's fruit had the lowest intensity. This result also elucidated the LPH's fruit had a potent flavor enhancer or improving ability.

3.2. Aroma Compounds in LPH's Fruit. In this work, a total of 159 volatile compounds including 55 olefins, 34 alcohols, 10 aldehydes, 8 acids, 18 ketones, 14 esters, 7 phenolics, 4 furans, 3 alkanes, 3 ethers, and 3 sulfur compounds were detected by SAFE-GC-MS analysis (Table S1). Among the 55 olefins, terpenoids were the most prevalent substances, including the monoterpenes (C10), sesquiterpenes (C15), and diterpenes (C20). These plant-derived compounds were biosynthesized from the active isoprene C5 units

Table 2: Results of the addition tests.

No.	Omitted compounds	Correct numbers	Significance[a]	Contribution aroma
1	D-Limonene	9/12	*	Lemon
2	3,7-Dimethyl-2,6-octadienal	12/12	**	Lemon
3	(*Z*)-3,7-Dimethylocta-2,6-dienal	12/12	**	Lemon
4	*β*-Myrcene	10/12	**	Green
5	(*E*)-3-Phenyl-2-propenoic acid ethyl	5/12		
6	*β*-Caryophyllene	5/12		
7	Citronellal	12/12	**	Mint and fresh
8	Linalool	11/12	*	Floral and sweet
9	6-Methyl-5-hepten-2-one	8/12		
10	Methional	7/12		
11	Estragole	5/12		
12	Eucalyptol	11/12	**	Eucalyptus-like
13	2-(3-Methyl-2-butenyl)-3-methylfuran	8/12		
14	Anethole	8/12		
15	Eugenol	6/12		

[a] "**" and "*" represent the significance at $P < 0.001$ and $P < 0.05$, respectively.

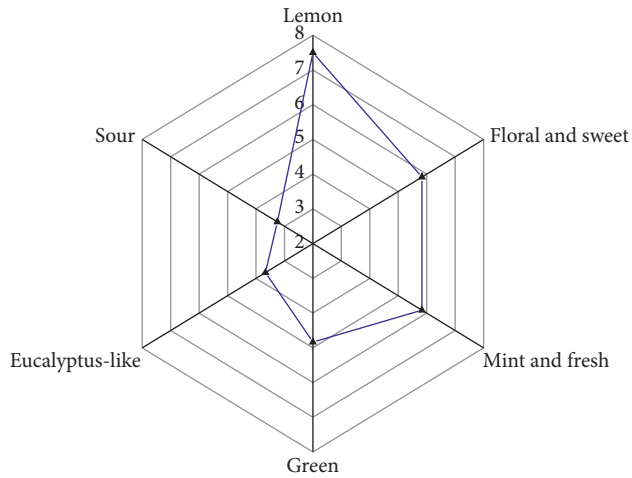


Figure 2: Aroma profiles of *Litsea pungens* Hemsl. fruit by quantitative descriptive analysis.

dimethylallyl diphosphate and isopentenyl diphosphate, which belong to the most diverse family of natural products [39, 40]. As the results showed, *α*-pinene, camphene, *β*-myrcene, *α*-phellandrene, 3-carene, D-limonene, (*Z*)-3,7-dimethyl-1,3,6-octatriene, *γ*-terpinene, *p*-mentha-1,4(8)-diene, *β*-ocimene, and *o*-cymene were monoterpenes (C10); (*Z*)-*α*-bergamotene, humulene, copaene, caryophyllene, guaia-1(10),11-diene, (*E*,*E*)-1-methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene, (*E*)-*β*-famesene, *α*-cubebene, *α*-guaiene, and *β*-bisabolene were sesquiterpenes (C15). On the contrary, only one diterpene (2,6,11,15-tetramethyl-hexadeca-2,6,8,10,14-pentaene) was detected in LPH's fruit. These results elucidated that the most of the volatile terpenoids were mono and sesquiterpenes with only a few diterpenes [40, 41]. Applying isotope-labeled pathway-specific precursors and green fluorescent protein-labeled terpene synthases have shown that monoterpenes' biosynthesis was located in plastids and fueled by the methylerythritol phosphate pathway. In contrast, the biosynthesis of sesquiterpenes was located in the cytosol and fueled by the cytosolic/peroxisomal mevalonic acid pathway and, under certain conditions, the methylerythritol phosphate pathway via the abovementioned metabolic crosstalk [42]. Acyclic (geraniol, linalool, and myrcene), monocyclic (D-limonene and *α*-terpineol), and bicyclic (car-3-ene and *α*-pinene) monoterpenes were biosynthesis from geranyl diphosphate by terpene synthases that shared a coupled isomerization-cyclization reaction sequence [42]. These compounds were usually found in plant-derived monoterpenes. Carvone could be generated by a series of enzyme-catalyzed synthesis, including geranyl diphosphate synthase, (−)-limonene synthase, (−)-limonene 6-hydroxylase, and (−)-*trans*-carveol dehydrogenase [43]. *β*-Caryophyllene was derived from farnesyl diphosphate by sesquiterpene cyclization reactions leading to volatile sesquiterpene hydrocarbons [44].

The chain acids, such as acetic acid, 3-methyl-butyric acid, and decanoic acid, were derived from the degradation of fatty acids. Phenylacetic acid was generated from L-phenylalanine via the shikimic acid pathway under the action of phenylalanine ammonia-lyase. Esters in the plants mainly generated from two ways: alcohol acyltransferase and carboxylesterase, belonging to the biosynthesis of amino acid-derived odor compounds [45]. Three kinds of ketone, including sesquiterpenes ketones, furan ketones, and chain ketones, were detected in the LPH's fruit. Sesquiterpenes ketones were mainly derived from the biosynthesis of the terpenoid pathway; furan ketones were carbohydrate-derived or carotenoid-derived volatile compounds, and chain ketones generated from lipids degradation [46]. The phenolic compounds were mainly derived from L-phenylalanine. Cinnamic acid was generated under the action of a phenylalanine ammonia-lyase, and then cinnamic aldehyde produced though reduction reaction [47]. Aldehydes with fresh green characteristics were mainly derived from the fatty acids (linolenic acid) by the lipoxygenase pathway [45]. Generally, the fatty acids were stored in plants as triacylglycerides and were liberated by lipases before they are acted as direct precursors for various volatiles. The sulfur compounds were derived from amino acid degradation [36]. Most of the alcoholics (monoterpenes), such as (3*Z*)-3,7-

Table 3: Qualitative analysis results of aroma-active compounds (FD = 9) in *Litsea pungens* Hemsl. fruit.

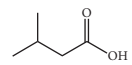
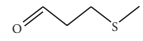
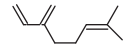
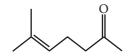
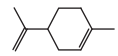
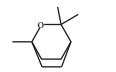
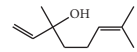
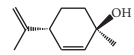
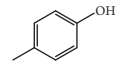
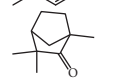
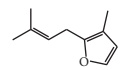
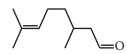
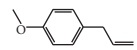
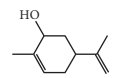
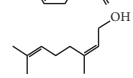
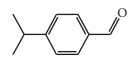
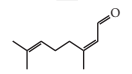
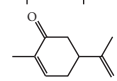
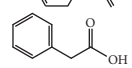
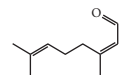
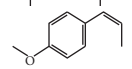
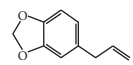
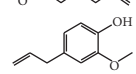
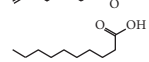
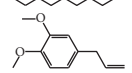
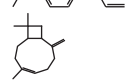
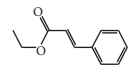
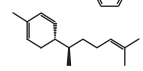
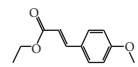
No.	Compounds	CAS	Structure	RI (TG-5MS/ TG-WAX)	Aroma descriptions	Identification
1	3-Methyl-butryicacid	503-74-2		863/859	Sour, sweaty, cheese	MS/RI/S/O
2	Methional	3268-49-3		903/906	Cooked potato	MS/RI/S/O
3	β-Myrcene	123-35-3		989/990	Spicy, peppery, green	MS/RI/S/O
4	6-Methyl-5-hepten-2-one	110-93-0		991/986	Citrus, green, musty	MS/RI/S/O
5	D-Limonene	5989-27-5		1027/1039	Citrus, orange, fresh, sweet	MS/RI/S/O
6	Eucalyptol	470-82-6		1032/1029	Eucalyptus, camphor, medicine	MS/RI/S/O
7	Linalool	78-70-6		1104/1116	Floral, sweet, woody, green	MS/RI/S/O
8	(*E*)-1-Methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol	7212-40-0		1105/1127	Fresh, minty, woody	MS/RI/S/O
9	*p*-Cresol	106-44-5		1085/1076	Phenolic, leather, animal	MS/RI/S/O
10	Fenchone	1195-79-5		1090/1083	Fresh, woody	MS/RI/S/O
11	2-(3-Methyl-2-butenyl)-3-methylfuran	15186-51-3		1093/1100	Caramel, sweet, floral	MS/RI/S/O
12	Citronellal	106-23-0		1151/1156	Sweet, floral, citrus	MS/RI/S/O
13	Estragole	140-67-0		1196/1204	Spicy, green, herbal, fennel	MS/RI/S/O
14	Carveol	99-48-9		1225/1210	Spearmint, caraway	MS/RI/S/O
15	(3*Z*)-3,7-Dimethyl-2,6-octadien-1-ol	106-25-2		1236/1225	Sweet, floral, citrus, magnolia	MS/RI/S/O
16	4-(1-Methylethyl)-benzaldehyde	122-03-2		1239/1233	Spicy, cumin, green, herbal	MS/RI/S/O
17	(*Z*)-3,7-Dimethylocta-2,6-dienal	106-26-3		1242/1254	Floral, sweet, citral, lemon	MS/RI/S/O
18	D-Carvone	2244-16-8		1246/1255	Spicy, mint, green	MS/RI/S/O
19	Phenylacetic acid	103-82-2		1262/1274	Sweet, honey, floral	MS/RI/S/O
20	3,7-Dimethyl-2,6-Octadienal	5392-40-5		1276/1290	Lemon, sweet, citura	MS/RI/S/O
21	Anethole	104-46-1		1279/1298	Anise, licorice, medicinal	MS/RI/S/O
22	Safrole	94-59-7		1287/1264	Spicy, woody, floral, anise	MS/RI/S/O
23	Eugenol	97-53-0		1348/1361	Clove, sweet, spicy, woody	MS/RI/S/O
24	Decanoic acid	334-48-5		1373/1423	Rancid sour, fatty, waxy	MS/RI/S/O
25	Methyleugenol	93-15-2		1404/1411	Sweet, fresh, spicy, clove, cinnamon	MS/RI/S/O
26	β-Caryophyllene	87-44-5		1420/1424	Clove, sweet, woody, spicy	MS/RI/S/O

Table 3: Continued.

No.	Compounds	CAS	Structure	RI (TG-5MS/ TG-WAX)	Aroma descriptions	Identification
27	(+)-2-Bornanone	464-49-3		1443/1143	Camphor, minty, herbal, woody	MS/RI/S/O
28	(*E*)-3-Phenyl-2-propenoic acid ethyl	4192-77-2		1474/1463	Floral, honey, balsamic, wine	MS/RI/S/O
29	(*R*)-2-Methyl-5-((*S*)-6-methylhept-5-en-2-yl)-cyclohexa-1,3iene	495-60-3		1500/1494	Spicy, fresh, sharp woody	MS/RI/S/O
30	Caryophyllene oxide	1139-30-6		1561/1543	Sweet, fresh, woody, spicy	MS/RI/S/O
31	Ethyl p-methoxycinnamate	24393-56-4		1773/1748	Cinnamon, sweet, wine	MS/RI/S/O

"MS," compounds were identified by mass spectra (NIST 14); "RI," compounds were identified on TG-WAX and TG-5MS columns; "S," compounds were identified by standards; "O," compounds were identified by GC-O.

dimethyl-2,6-octadiene-1-ol, (*E*)-linalool oxide, (*E*)-4-thujanol, and (*E*)-p-mentha-2,8-dien-1-ol were derived from the precursor of granyl diphosphate, whereas the sesquiterpenes alcoholics including nerolidol and decahydro-1,4-dimethyl-7-(1-methylvinyl)azulen-4-ol were derived from the precursor of (*E*,*E*)-farnesyl diphosphate [47].

These volatile compounds with the properties of sweet, floral, citrus, green, herb, fresh, eucalyptus, phenolic, spicy, and sour characteristics also had an antimicrobial activity or other biological functional activities. They were detected in LPH's fruits and existed in the roots, stems, and leaves, which played an important role in plant-animal interactions, including the attraction of pollinators and seed disseminators, protecting the plants from pathogen attack, and repellence of herbivores [36].

3.3. Quantitation and Calculation of the Odor Active Values (OAVs). A total of 31 aroma-active compounds (FD = 9) were detected by gas chromatography-olfactory. Among them, terpenoids (16) were the most prevalent. *β*-Myrcene, linalool, and citronellal were acyclic monoterpenes. D-limonene, carveol, eucalyptol, and D-carvone were monocyclic monoterpenes; (+)-2-bornanone and fenchone were bicyclic monoterpenoids; (3*Z*)-3,7-dimethyl-2,6-octadien-1-ol (nerolidol) was acyclic sesquiterpenes; *β*-caryophyllene, caryophyllene, and (*R*)-2-methyl-5-((*S*)-6-methylhept-5-en-2-yl)-cyclohexa-1,3-diene oxide were bicyclic sesquiterpenes. These compounds with spicy, floral, green, fresh, woody, citrus, and herb spearmint characteristics consist of the main aroma profiles of LPH's fruits. The other compounds, such as acids, phenolics, and esters, also played an important role in the overall aroma profiles.

To confirm the potent aroma compounds in the overall aroma profiles of LPH's fruit, the aroma-active compounds were quantified, and their odor activity values (OAVs, the ratio of concentration to odor threshold) were also calculated. The standard curves of each aroma-active compound are shown in Table 1. All the calibration curves obtained had good linearity ($R^2 > 0.99$). Quantification results showed (Table 3) that linalool had the highest concentration (360,841.19 *μ*g/kg), followed by D-limonene (333,321.19 *μ*g/kg), 3,7-dimethyl-2,6-octadienal (251,978.13 *μ*g/kg), (*Z*)-3,7-dimethylocta-2,6-dienal (189,366.87 *μ*g/kg), *β*-myrcene (179,800.17 *μ*g/kg), eucalyptol (81,581.26 *μ*g/kg), and *β*-caryophyllene (76,771.87 *μ*g/kg). Fenchone (97.44 *μ*g/kg) and 3-methyl-butyric acid (156.40 *μ*g/kg) had the lowest concentrations.

As the OAV analysis results shown above, there were 25 odorants with OAVs ≥ 1 in LPH's fruit, but 3 compounds (fenchone, carveol, and decanoic acid) had OAVs < 1, indicating that they were not the potent odorants in the overall aroma of LPH's fruit. The odor threshold value of (*E*)-1-methyl-4-(1-methylvinyl) cyclohex-2-en-1-ol and (*R*)-2-methyl-5-((*S*)-6-methylhept-5-en-2-yl)-cyclohexa-1,3-diene was not found; therefore, their OAVs were not calculated. Moreover, the concentration of phenylacetic acid was lower than the limit of quantitation. The remained 25 odorants with OAVs ranged from 1.5 to 9803 were the potent odorants for LPH's fruit aroma. Among them, D-limonene had the highest OAV (OAV = 9803) for its significant high concentration and low threshold (34 *μ*g/kg), followed by 3,7-dimethyl-2,6-octadienal (OAV = 8399), (*Z*)-3,7-dimethylocta-2,6-dienal (OAV = 1893), *β*-myrcene (OAV = 1798), (*E*)-3-phenyl-2-propenoic acid ethyl (OAV = 1603), *β*-caryophyllene (OAV = 1129), citronellal (OAV = 819), linalool (OAV = 722), 6-methyl-5-hepten-2-one (OAV = 582), methional (OAV = 494), estragole (OAV = 382), eucalyptol (OAV = 355), 2-(3-methyl-2-butenyl)-3-methylfuran (OAV = 238), anethole (OAV = 143), and eugenol (OAV = 114). Interestingly, most of the terpenoids exhibited higher OAVs, which are similar to other species, for example, *Toona sinensis* (A. Juss.) and pepper (*Zanthoxylum bungeanum*) [30, 37]. The results of aroma characteristics quality, OAV analysis, and the sensory evaluation elucidated that D-limonene, citronellal, 3,7-dimethyl-2,6-octadienal, (*Z*)-3,7-dimethylocta-2,6-dienal, (*E*)-3-phenyl-2-propenoic acid ethyl, *β*-myrcene, linalool, methional, 6-methyl-5-

hepten-2-one, 2-(3-methyl-2-butenyl)-3-methylfuran, β-caryophyllene, estragole, anethole, eucalyptol, and eugenol might be the key odorants of LPH's fruit.

3.4. Addition Experiment. Addition tests results elucidated that 3,7-dimethyl-2,6-octadienal and (*Z*)-3,7-dimethylocta-2,6-dienal had high significant ($P < 0.001$) contribution to the lemon attribute of LPH's fruit. Besides, D-limonene also had contribution to lemon attribute ($P < 0.05$). β-Myrcene had high significant ($P < 0.001$) contribution to the green attribute of LPH's fruit. Citronellal had high significant ($P < 0.001$) contribution to the mint and fresh attribute. Eucalyptol had high significant ($P < 0.001$) contribution to the eucalyptus-like note of LPH's fruit. In summary, 3,7-dimethyl-2,6-octadienal and (*Z*)-3,7-dimethylocta-2,6-dienal, D-limonene, β-myrcene, citronellal, and eucalyptol were confirmed as the key odorants of LPH's fruit. As the significant difference results elucidated, 3,7-dimethyl-2,6-octadienal and (*Z*)-3,7-dimethylocta-2,6-dienal were greater than that of D-limonene.

4. Conclusions

The aroma compounds in LPH's fruit were isolated by SAFE. By application of frequency combined with aroma dilution analysis, 31 aroma-active compounds were detected, and 30 of them were further quantified by the external standard method. OAVs ≥ 1 were obtained for 25 odorants among which D-limonene (OAV = 9803) and 3,7-dimethyl-2,6-octadienal (OAV = 8399) had the highest OAV value. (*Z*)-3,7-dimethylocta-2,6-dienal (OAV = 1893), β-myrcene (OAV = 1798), (*E*)-3-phenyl-2-propenoic acid ethyl (OAV = 1603), and β-caryophyllene (OAV = 1129) had OAV over 1000. These results elucidated that they played an important role to the overall aroma profiles if LPH's fruit. Based on the addition tests, 3,7-dimethyl-2,6-octadienal and (*Z*)-3,7-dimethylocta-2,6-dienal and D-limonene contributing to lemon attribute, β-myrcene contributing to green attribute, citronellal contributing to mint and fresh note, and eucalyptol contributing to eucalyptus-like note were confirmed as the key odorants in LPH's fruit.

Data Availability

All the date used in this work could be found in the manuscript and the supplemental materials.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Supplementary Materials

Table S1: the aroma compounds isolated by solvent-assistant evaporation extraction in *Litsea pungens* Hemsl. fruit. (*Supplementary Materials*)

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