Research Article

Flavor Profile in Fresh-squeezed Juice of Four Thai Lime Cultivars: Identification of Compounds that Influence Fruit Selection by Master Chefs

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Received: 18 December 2019; Revised: 25 December 2019; Accepted: 26 December 2019; Published online: 11 February 2020

Abstract
The flavor and sensory profiles that influenced the selection of 4 commercial Thai lime cultivars (*Citrus aurantifolia* Swingle cv. 'Pan Rumpai', 'Pan Puang', and 'Pan Pijit' and *Citrus latifolia* Tanaka cv. 'Tahiti') by Thai chefs were examined. Twenty-eight volatiles (7 monoterpenes, 13 sesquiterpenes, 4 monoterpene alcohols, 1 aldehyde, 2 monoterpene aldehydes, and 1 monoterpene ester) and 9 non-volatiles (citric acid, malic acid, succinic acid, ascorbic acid, sucrose, fructose, glucose, limonin, and naringin) contributing to the flavor of Thai lime juice were identified using dynamic headspace-gas chromatography-olfactometry-mass spectrometry and high-performance liquid chromatography, respectively. An interview of master chefs and an acceptance test of culinary students revealed that Pan Puang was the most preferred lime cultivar owing to its moderate sour taste and its unique floral aroma contributed by terpinolene and linalool, along with its low content of β-myrcene, which contributes to balsamic and pungent aroma notes.

Keywords: Aroma, Taste, Volatile, Non-volatile, Flavor profile, Lime

1 Introduction

Citrus fruits are one of the most commonly consumed fruits worldwide and are used to prepare beverages and as cooking ingredients owing to their unique flavor and health benefits. Acidic citrus fruits such as lime originated in Southeast Asia in the Indo-Malayan region around 4,000 BCE before spreading to other continents

Please cite this article in press as: J. Korkitpoonpol, N. Suwannaprom, S. Keeratipibul, K. Wongravee, P. Phuwapraisirisan, L. Xiao, S. Boonbumrung, and P. Ngamchuachit, "Flavor profile in fresh-squeezed juice of four thai lime cultivars: Identification of compounds that influence fruit selection by master chefs," *Applied Science and Engineering Progress*, (2020). DOI: 10.14416/j.asep.2020.02.001

[1], [2]. In Thailand, lime juice has been used as a primary cooking ingredient for more than 400 years in Thai dishes such as Tom-yum (hot and sour soup) and Som-tum (papaya salad). Limes are categorized into three groups: Mexican lime or Key lime (*Citrus aurantifolia* Swingle), Tahiti or Persian lime (*Citrus latifolia* Tanaka), and sweet lime (*Citrus limetta*). The 'Pan' cultivar derived from the Mexican lime group is the most cultivated Thai lime, accounting for 74% of the total lime cultivation in Thailand [3]. The most frequently commercially grown cultivars in Thailand are Pan Rumpai, Pan Puang, Pan Pijit (all cultivars of *C. aurantifolia* (*Christm & Panz*.) Swingle) and Tahiti (*C. latifolia* Tanaka 'Tahiti'), with Pan Puang and Pan Rumpai being the most common commercially available cultivars. Interestingly, the Pan Pijit cultivar has not been well received by consumers despite its resistance to insects and the citrus canker disease in addition to its lower price. Pan Pijit is abundantly available in markets only during Thailand's dry season (March–April), during which the Pan Rumpai and Pan Puang cultivars are generally unavailable. Apart from the Pan cultivars, the Tahiti cultivar, which is a seedless Persian lime variety, is also widely used in the beverage industry. To date, studies on the compounds that contribute to the flavor of citrus fruits have mainly focused on commercial fruits that are common in the western cuisine such as lemon and orange. Less information is available for lime, which is a citrus fruit used primarily in Asian cuisine. Moreover, the differences in perceived flavor among these Thai lime cultivars have not been reported.

Aroma and taste play important roles in flavor perception and originate from complex mixtures of volatile and non-volatile compounds, respectively, in the food matrix. The unique taste of citrus stems from a balance between organic acids and sugars [4]–[6]. The perception of sweetness is caused by the presence of glucose, fructose, and sucrose, whereas that of sourness is caused by the presence of organic acids, mainly citric acid. Apart from organic acids and sugars, naringin and limonin are flavor constituents that contribute to bitterness in lime [5]–[8]. The volatile compounds in citrus mainly consist of monoterpene hydrocarbons, especially limonene, γ–terpinene, and myrcene; sesquiterpene hydrocarbons such as α-bergamotene; and oxygenated compounds, primarily aldehydes such as decanal [6], [9]–[11]. Moreover,

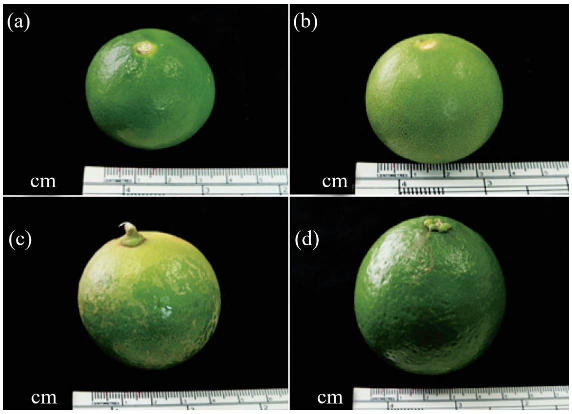

Figure 1: Lime fruits from four Thai cultivars: *Citrus aurantifolia* (Christm&Panz) Swingle 'Pan Rumpai' (a), 'Pan Puang' (b), 'Pan Pijit' (c), and *Citrus latifolia* Tanaka 'Tahiti' (d).

several other factors have been reported to affect the volatile profiles of citrus fruit including the cultivar, environmental conditions, geographical origin, season, fruit maturity, and extraction method [6], [7], [11], [12]. Although the chemical, volatile, and non-volatile profiles of citrus are well established, the correlation between the chemical profiles contributing to sensory properties and the preference of users such as chefs has remained unexplored. The objective of this study was to identify the compounds contributing to the flavor of lime juice from four Thai lime cultivars that influences selection by chefs.

2 Materials and Methods

2.1 *Plant material*

Four cultivars of lime fruits (Figure 1) were harvested in Thailand at commercial maturity from two commercial orchards in Phetchaburi province (for Pan Rumpai, Pan Puang, and Pan Pijit) and Nakhon Nayok province (for Tahiti). The ages of commercial maturity were 5 and 4 months after flowering for the Pan and Tahiti cultivars, respectively.

The harvesting areas were selected based on the area of the most commercial production for each lime cultivar. The lime fruits were harvested in September (2017), which is in a rainy season of Thailand and is the optimal harvesting period. Generalized monsoon index (GMI) is the index to determine the severity levels of agricultural drought. In September (2017), Phetchaburi province and Nakohn Nayok province has $GMI_{percentile}$ of 82 and 61, respectively, which indicated high humidity.

All lime fruits were transported to a pilot plant at Chulalongkorn University on the same day. The fruits from each cultivar were visually sorted to discard damaged and defective fruits and then washed with clean water.

The lime fruits from each cultivar were used for juice analyses including physicochemical properties evaluation, volatile and non-volatile compounds profiling, and sensory analyses (a descriptive analysis and a chefs' preference test). In each analysis of each lime cultivar, three replicates comprising ten fruits per replicate were analyzed, except for the chefs' preference test, in which 150 lime fruits were used. The lime fruits were stored at 6°C and used for analyses within five days.

2.2 *Chemicals*

For the identification of volatile compounds, a standard compound (2-methyl-3-heptanone) and n-alkanes standard solution (C_8–C_{20}) were purchased from Sigma-Aldrich (St. Louis, MO, USA). For the identification of non-volatile compounds, high-performance liquid chromatography (HPLC)-grade limonin, naringin, L-(−)-malic acid, and D-(−)-fructose were purchased from Sigma-Aldrich. D-(+)-glucose and sucrose were purchased from Supelco Analytical (Bellefonte, PA, USA). Citric acid, L-(−)-ascorbic acid, and succinic acid were purchased from Fisher Scientific (Pittsburgh, PA, USA). HPLC-grade acetonitrile was purchased from RCL Labscan (Bangkok, Thailand). Sodium hydroxide was purchased from Merck & Co., Inc. (Kenilworth, NJ, USA) and 98% sulfuric acid was purchased from QReC chemical (New Zealand). All standard aroma compounds for descriptive analysis references are commercial flavors obtained from Firmenich (Thailand) Ltd. (Bangkok, Thailand).

2.3 *Sample preparation*

For each cultivar, fresh lime juice used in each replicate for the analyses of the physicochemical properties, volatile compounds, non-volatile compounds, and sensory descriptive parameters was individually prepared from 10 lime fruits (approximately 100 mL). For the chefs' preference test, fresh lime juice was prepared from 150 lime fruits. The peel was removed using a ceramic knife to avoid contamination of the juice with peel components. The lime fruits for each replicate of each analysis were hand squeezed using a ceramic squeezer and then mixed together well.

Different amounts of fresh-squeezed lime juice were used in each replicate for the analyses of physicochemical properties (5 mL), volatile compounds (3 mL), non-volatile compounds (25 mL), and sensory descriptive parameters (100 mL). For the analysis of non-volatile compounds, fresh-squeezed lime juice from each replicate was centrifuged at 7000 × g for 10 min and then filtered through Whatman® no.1 filter paper. The supernatants were kept at −20°C until they were used for the UHPLC analyses. Prior to the UHPLC analyses of bitter compounds, sugars, and organic acids, the lime juice samples were diluted with the dilution factors of 1, 2, and 10, respectively, and were filtered through a 0.22-µm nylon filter.

2.4 *Physicochemical properties*

The physicochemical properties [pH, titratable acidity (%), and total soluble solids content (TSS, °Brix)] of lime juice were investigated. The pH was measured with a pH meter (CyberScan pH 1000 meter, Eutech Instruments, Singapore). The titratable acidity measurements were performed by titrating 5 g of lime juice with 0.1 M NaOH, and the results were expressed as citric acid equivalents (AOAC, 1999). A few drops of juice were collected for TSS measurements using a refractometer (Atago, Tokyo, Japan).

2.5 *Analysis of volatile compounds*

For each replicate, lime juice was freshly prepared and was immediately used for the analysis of volatile compounds. Lime juice (3 mL) spiked with 2-methyl-3-heptanone (20 µL of 6.53 mM stock solution in ethanol) as an internal standard was added into a 10 mL glass vial, which was sealed with magnetic universal screw caps and then subjected to a headspace extraction. The analyses of the volatile compounds were performed using GC-MS (7890A and 5975 inert mass single quadrupole detector; Agilent Technologies, Santa Clara, CA, USA) equipped with a thermal desorption unit (TDU) and a dynamic headspace station (Gerstel, Mülheim an der Ruhr, Germany). The lime juice sample was incubated at 40°C for 15 min and then the headspace volatile compounds were purged with nitrogen gas, with the flow rate of 20 mL/min, at 30°C for 10 min. The volatile compounds were adsorbed onto Tenax® TA sorbent coated with 2,6-diphenylphenylene oxide (Gerstel, Mülheim an der Ruhr, Germany). The trap was dried under nitrogen gas with the flow rate of 50 mL/min for 9 min to remove moisture. The trapped volatile compounds were then desorbed inside the TDU in splitless mode. The temperature of the TDU was initially 40°C. It was then heated to 300°C at a rate of 200°C/min and held for 5 min with a constant transfer line temperature of 300°C, and the samples were automatically injected into the GC-MS system. The injector was operated in split mode (5:1). Separations were performed on a DB5-MS column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). The oven was programmed to run at 50°C for 2 min, after which it was ramped up at 5°C/min to 105°C for 2 min and then to 185°C at 2°C/min, then the temperature was increased to 260°C at 25°C/min and held there for 5 min. The effluent was transferred to a 5975 inert mass selective detector (Agilent Technologies, Santa Clara, CA, USA) that was set to scan in the *m/z* value range of 15–400. The ionization in MS was performed in electron impact mode with the ionization energy of 70 eV.

The identification of volatile compounds was performed by matching mass spectra with those obtained from the NIST MS 14.0 library (National Institute of Standards and Technology, Gaithersburg, MD, USA) and confirmed by comparing their linear retention indices (calculated from their retention time relative to that of adjacent n-alkanes) with the literature values. Compound identification was further confirmed by the odor characteristics obtained from GC-MS equipped with an olfactory detection port (ODP3; Gerstel, Mülheim an der Ruhr, Germany) under the same experimental conditions as used for the GC-MS analyses. The peak area of the internal standard (2-methyl-3-heptanone) was used to obtain semi quantitative data of the identified compounds. The identified aroma compounds in this study were the compounds that have i) more than 80% MS score ii) ± 15 difference between the calculated retention index and those from the NIST library database within iii) odor description based on GC-O results and aroma database (Pherobase, the Good Scents Company, and Flavornet). The dose-over-threshold (DoT) or odor activity values (OAV) were calculated from the relative concentrations of the volatile compounds divided by their odor threshold in water.

2.6 *Analysis of non-volatile compounds*

A 1290 Infinity II UHPLC system (Agilent Technologies, Santa Clara, CA, USA) was used for the analyses of organic acids (citric, malic, ascorbic, and succinic acids) and bitter compounds (limonin and naringin). An Alliance 2690 system equipped with a 410 differential refractometer detector (Waters Corporation, Milford, MA, USA) was used for the analysis of sugars (glucose, fructose, and sucrose). The DoT for taste was calculated from the relative concentrations of the non-volatile compounds divided by their taste threshold in water. For the analysis of bitter compounds, a ZORBAX® Eclipse Plus C18 Rapid Resolution HD 2.1 mm × 50 mm, 1.8 μm chromatography column (Agilent Technologies, Santa Clara, CA, USA) was used. A binary gradient elution system composed of deionized water (A) and acetonitrile (B) was applied as follows: 0–3 min, 15–50% B; 3–6 min, 50–100% B; 6–9 min, 100% B. The injection volume was 5 μL per sample, the flow rate was 0.25 mL/min, and the column temperature was set at 25°C. The diode array detector was set at 220 nm for the acquisition of chromatograms.

For the analysis of organic acids, a Rezex ROA organic acid column (7.8 mm × 300 mm; Phenomenex, Torrance, CA, USA) was used. The mobile phase was 0.005 N H2SO4 with an isocratic flow rate set at 0.50 mL/min. The injection volume was 10 μL per sample, and the column temperature was set at 40 °C. The diode array detector was set at 210 nm for the acquisition of chromatograms. For the analysis of sugars, a 410 differential refractometer (Waters Corporation) was used as a detector.

2.7 *Descriptive analysis*

The sensory descriptive analysis of fresh-squeezed Thai lime juice from four cultivars was performed by eight panelists from Betagro Science Center, Bangkok, Thailand (two males and six females, ages 30–55). All subjects gave their informed consent for inclusion before they participated in the study. The study protocol was approved by the Ethics Committee for research involving human research participants, health sciences group, Chulalongkorn University (Project identification code 064/2019). The panelists were trained through six training sessions (2 h per session per day). In the first training session, the panelists were allowed to evaluate lime juice from different cultivars to become familiar with all lime cultivars used in this study and to develop essential knowledge about sensory aroma and taste attributes and their definitions. Refinement of the attributes and standards was discussed in two subsequent training sessions. In the following sessions, the panelists practiced evaluating the sensory attributes using a 15-cm unstructured line scale on a score sheet to achieve a consensus regarding how to define and rate the attributes. During the last two training sessions, the panelists practiced evaluating the lime juice samples from four cultivars. For the actual descriptive analysis sessions, each panelist evaluated three replicates from each lime cultivar. As reference standards for the taste attributes (Supplementary Table S1), 10 mL of each standard was added to a 59 mL polystyrene plastic cup with a lid, the same as for the lime juice samples. As reference standards for the aroma attributes, 45 µL of the standards were diluted in 300 mL of water, and 500 µL of each diluted standard was added to a 1 mL glass vial with a lid. A randomized block design was used to assign sample presentation sequences. The panelists were served a set of three-digit coded tasting cups covered with lids at 25°C with a coffee stirrer spoon for one taste serving, a cup of water, and a few pieces of unsalted crackers as a palate cleanser between samples. The panelists were asked to clean their palate until no taste remaining in the palate and take at least 10-min break between samples. The final attributes consisted of eleven aroma notes (oxidized, citrus, lemon, green, fruity, peely, floral, pickled lime-like, solvent-like, waxy, and ripe) and three taste attributes (sourness, sweetness, and bitterness).

2.8 *In-depth interview and chefs' preference test*

Master chefs from a Michelin-starred Thai restaurant participated in the in-depth interview study. They were asked about their overall opinions and the ideal characteristics of lime juice for use in general Thai cooking. For the chefs' preference test, a hedonic liking test involving the four lime juice samples was performed involving 117 culinary students (39 males and 78 females, ages 18–22) recruited from the Suan Dusit School of Culinary Arts, which is the oldest and the most famous training school for Thai cooking with an 80-year history. All subjects gave their informed consent for inclusion before they participated in the study. The study protocol was approved by the Ethics Committee for research involving human research participants, health sciences group, Chulalongkorn University (Project identification code 064/2019). The chefs were interviewed about the characteristics of lime juice that they would preferably select for Thai cooking and were asked to indicate their preferences of the juice samples from the four lime cultivars on a 9-point scale (1 = "extremely dislike", 5 = "neither like nor dislike", and 9 = "extremely like"). Each sample (10 mL) was added into a 59 mL polystyrene plastic cup with a lid. A randomized block design was used to generate the sample presentation sequences, and the samples were labeled with random three-digit numbers. Chefs were given a set of four lime juice samples, which were served at 25°C with a cup of water and unsalted crackers as a palate cleanser between samples. The chefs were asked to take a 10-min break between samples.

2.9 *Statistical analysis*

To assess the differences in physicochemical properties, volatile compounds, non-volatile compounds, sensory attributes, and chefs' preferences among the lime juice samples, a one-way analysis of variance and multiple comparisons of the mean values at the 95% confidence level by Fisher's least significant difference test (XLSTAT software version 2017, Addinsoft, Paris, France) were performed. Moreover, a multivariate analysis of the volatile and non-volatile compounds was performed via a heatmap and a hierarchical cluster analysis based on similarity with Ward's method using MetaboAnalyst 4.0 software (http://www.metaboanalyst.ca) [13].

3 Results and Discussion

3.1 *Flavor attributes of fresh-squeezed lime juice influencing master chefs' selection*

In the in-depth interview study, master chefs from

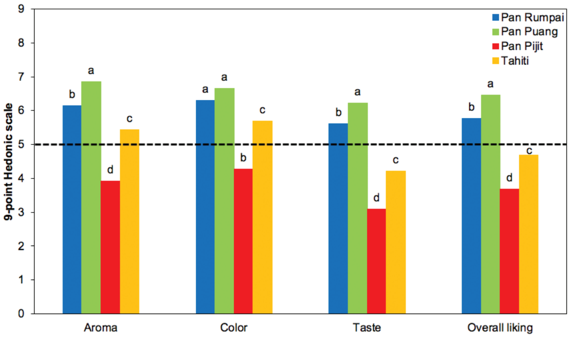

Figure 2: Mean liking score for color, taste, aroma, and overall liking. Values with the same letter were not significantly different within each attribute ($p < 0.05$).

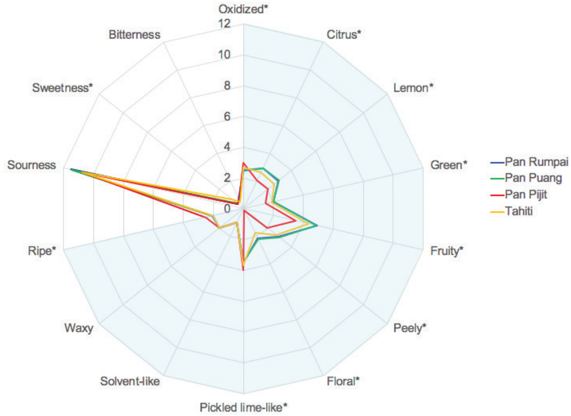

Figure 3: Flavor profiles of fresh-squeezed juice from four lime cultivars. Panelists rated the intensity of each attribute on a scale from 0 to 15 with 0 = not detectable and 15 = very strong.

a Michelin-starred Thai restaurant revealed the characteristics of the limes and lime juice that they would preferably select for use in Thai cooking. They preferred fruits that have a broadly rounded shape (40–45 mm diameter) with a flat base, a green paper-thin peel, and juicy flesh. The fresh-squeezed juice should have a unique lime and floral aroma with moderate sourness. A light hand-squeezing method of juicing is used to minimize bitterness. Moreover, the preferences of 117 culinary students from the Suan Dusit culinary school were determined for lime juice samples from the four cultivars. As shown in Figure 2, the results indicated that they preferred Pan Rumpai and Pan Puang lime juice based on aroma, appearance, taste, and overall liking. Pan Pijit and Tahiti were not accepted by the culinary students (overall liking score < 5.0), even though the aroma and color of Tahiti were moderately preferred. Therefore, these findings implied that taste and aroma are the most important quality parameters that culinary students used to select limes for Thai cooking

3.2 *Sensory characteristics of fresh-squeezed juice of four Thai lime cultivars*

To characterize the flavor attributes of juice from the four lime cultivars, a quantitative descriptive analysis was utilized with eight trained sensory panelists. The sensory profiles of the fresh-squeezed lime juice samples are shown in Figure 3. Pan Rumpai and Pan Puang shared broadly similar flavor profiles and were characterized as having citrus, lemon, green, fruity, and floral notes. Pan Pijit showed strongly oxidized, pickled lime-like, and ripe aroma notes but had the lowest intensity of citrus, lemon, green, fruity, and peely aroma notes and lacked a discernable floral note. Tahiti had a moderate intensity for all aroma notes and showed the highest sweetness among the four lime cultivars. From the results of the quantitative descriptive analysis, we could better clarify the flavor attributes that were well received by the master chefs and the culinary students. A high intensity of desirable aroma notes (citrus, lemon, green, fruity, peely, and floral), a low sweetness, and a high sourness were the preferred characters of lime juice. The Pan Pijit lime juice sample, which was the least preferred among the four samples, might have been considered less desirable owing to having the highest intensity of undesirable aroma notes (i.e., oxidized, pickled lime-like, and ripe aroma notes). Moreover, it not only had a low intensity of desirable aroma notes but also lacked a discernable floral aroma, which was described by the master chefs as a characteristic of a desirable lime juice for use in Thai cooking.

3.3 *Non-volatile and volatile compounds and their contributions to the flavor of lime juice*

Organic acids and sugars are primary components of lime juice and play key roles in its taste. The relative ratio of acid to sugar was low in orange (10:90) and grapefruit (20:80) but higher in acid citrus fruits like

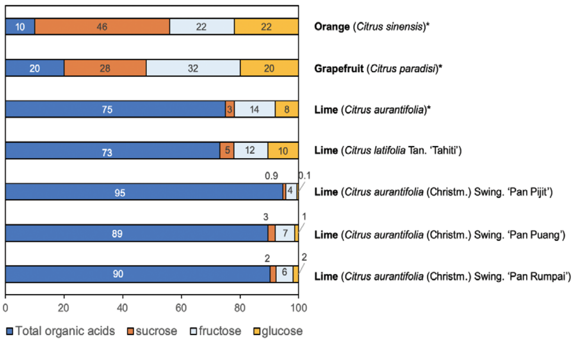

Figure 4: Relative composition of organic acids and sugars of citrus juice samples *[14].

lime (75:25) [14]. The proportion of organic acids commonly shows a negative correlation with the concentration of sugars. For *C. aurantifolia* lime juice (Figure 4), the Thai limes (Pan Rumpai, Pan Puang, and Pan Pijit) showed high relative acid to sugar ratios up to 95:5.

Organic acids are non-volatile components that contribute to sourness in citrus juice [15]. In this study, citric acid was the most predominant organic acid, followed by malic acid, and trace amounts of ascorbic acid and succinic acid were detected (Table 1). Citric acid was identified as the main acid contributing to sourness owing to its high DoT value (Table 2) as calculated from the ratio between the concentration of a non-volatile compound in a food sample and its taste detection threshold. Other organic acids including tartaric acid, lactic acid, and oxalic acid, which previous studies reported were present in limes [5], [16], were not detected in the Thai lime juice samples. Pan Puang and Pan Pijit showed the highest and lowest contents of total organic acids among the Thai lime juice samples, respectively. The differences in organic acid content among the juices from the different lime cultivars originate from genotypic variation. Aprile *et al.* [17] compared the genotypes of sweet (non-acid) and sour lemon fruits and suggested that the *Arabidopsis* P-type H^+-ATPase (AHA10), which is involved in the acidification of vacuoles, was not expressed in sweet lemons but was highly expressed in sour ones, which could result in a difference in metabolism, ultimately leading to a difference in organic acid content [4], [17], [18].

Table 1: Mean values for non-volatile compounds (organic acids, sugars, and bitter compounds) contents and physicochemical properties (pH, TA, TSS, TSS/TA) of lime juice from four Thai lime cultivars

	Cultivars			
	Pan Rumpai	**Pan Puang**	**Pan Pijit**	**Tahiti**
Organic acids (g/L)				
citric acid	73.7+5.9 a[a]	76.1+5.3 a	60.645.0 b	64.1+2.6 b
malic acid	4.3+0.4 b	5.3±0.1 a	2.6±0.6 c	4.7±0.4 ab
succinic acid	0.4±0.0 ab	0.5+0.0 a	0.3+0.0 c	0.4+0.1 b
ascorbic acid	0.4±0.0 a	0.4±0.0 a	0.2±0.0 c	0.3+0.0 b
Total organic acids	78.8 ab	82.2 a	63.7c	69.4 bc
Sugars (g/L)				
sucrose	1.8+0.2 b	2.3±0.3 b	0.6±0.3 c	4.4±0.9 a
fructose	5.0+0.4 bc	6.1+1.0 b	2.6±1.6 c	11.0±1.8 a
glucose	1.7+0.4 b	1.3+0.5 b	0.4±0.1 b	9.9±1.7 a
Total sugars	8.2 b	10.2 b	3.6 c	25.9 a
Bitter compounds (mg/L)				
limonin	18.4+2.6 b	17.3+2.5 b	9.8+ 2.5.c	26.0+2.2 a
naringin	2.0±0.8 a	1.5+1.1 a	0.6±0.1 a	2.3+1.4 a
Total bitter compounds	20.4 b	18.8 b	10.3 c	28.3 a
Physicochemical properties				
pH	2.34±0.02 ab	2.37±0.01 a	2.32±0.02 b	2.27±0.02 c
TA (%w/w)	6.7±0.4 a	7.1+0.0 a	6.4±0.0 b	6.1+0.0 b
TSS (%w/w)	7.8±0.1 b	8.2±0.1 b	6.9±0.1 c	8.8±0.2 a
TSS/TA	1.2+0.1 b	1.5±0.0 a	1.2+0.0 b	1.1±0.0 c

[a] Within each row, values with the same letter are not significantly different across cultivar ($p < 0.05$).

In agreement with previous studies on citrus fruit [27], our results showed the presence of three main sugars in lime juice including sucrose, fructose, and glucose, among which fructose was the dominant sugar. However, in the present study, the relative sugar proportion of lime juice (Figure 4) was found to be only 5% in Pan Pijit and 10% in Pan Rumpai and Pan Puang, as compared to 25% in the other *C. aurantifolia* lime juice reported by Rouseff *et al*. [14]. The sugar contents of the tested lime juices in Pan Rumpai, Pan Puang, and Pan Pijit, which were lower than in *C. latifolia* reported by White and Widmer [28] However, the sugar content in Tahiti lime juice was comparable to that reported by White and Widmer [28]. In addition, Tahiti lime juice was rated as being significantly sweeter than other cultivars by trained sensory panelists, which might have resulted from its contents of fructose and glucose being 3.5 and 5.9 times higher than the sweet sensory threshold concentrations, respectively (Table 2).

For the analysis of bitter compounds, Yusof *et al*. [29] reported that naringin was not detected in the Key lime. However, in our study, limonin and naringin were detected in trace amounts. The human taste system has evolved to detect bitter compounds at a very low concentration to avoid the ingestion of harmful substances that may cause illness or death [30]. Therefore, trace amounts of bitter compounds could affect the sensory qualities of lime juice if their concentrations were above the bitter threshold. In the present study, only limonin was identified as a contributor to perceived bitterness in all lime cultivars (dose-over-threshold > 1) (Table 2). Although Tahiti juice had the highest limonin content, it did not show a significantly higher perceived bitterness than the other studied lime cultivars. This might have been caused by the high sugar content of Tahiti juice raising the threshold concentration at which limonin could be perceived as a bitter taste. In support of this concept, an increased bitter threshold

Table 2: Flavor characteristics and dose-over-threshold of compounds in lime juice from four Thai lime cultivars

No.	Compound	Flavor Characteristics	Linear[c] Retention Index	Detection[d] Threshold (mg/L)	Dose Over Threshold			
					Pan Rumpai	Pan Puang	Pan Pijit	Tahiti
Non-volatile compounds[a]								
1.	citric acid	sourness		0.3[e]	245.7	253.7	202.0	213.6
2.	malic acid	sourness		0.3[e]	14.3	14.3	8.7	5.7
3.	succinic acid	sourness		0.1[e]	4.0	4.0	3.0	4.0
4.	ascorbic acid	sourness		1.0[f]	<1	<1	<1	<1
5.	sucrose	sweetness		6.85[g]	<1	<1	<1	<1
6.	fructose	sweetness		3.10[g]	1.6	2.0	<1	3.5
7.	glucose	sweetness		1.675[g]	1.0	<1	<1	5.9
8.	limonin	bitterness		1.0[h]	18.4	17.3	9.8	26.0
9.	naringin	bitterness		20.0[h]	<1	<1	<1	<1
Volatile compounds[b]								
1.	β-pinene	woody, pine[i]	992	1.5[i]	<1	1.1	<1	1.2
2.	β-myrcene	sweet, balsamic[i], pungent[j]	991	0.1[l]	19.6	<1	4.2	<1
3.	limonene	citrus	1029	1.2[l]	10.4	11.2	<1	10.5
4.	β-ocimene	terpenic, woody	1046	0.034[i]	<1	<1	2.4	<1
5.	γ-terpinene	terpenic, lime, herbal	1072	0.6[l]	26.7	24.9	<1	13.1
6.	terpinolene	floral, sweet[j]	1093	0.041[l]	62.9	59.5	4.1	28.3
7.	linalool	floral, woody[i]	1104	0.0074[i]	128.6	118.6	4.3	14.3
8.	terpinen-4-ol	floral, fresh[k]	1187	1.3[l]	1.5	1.4	<1	<1
9.	decanal	peel-like[i]	1209	0.03[l]	32.7	30.3	<1	<1
10.	caryophyllene	sweet, woody, spicy	1418	0.15[i]	<1	<1	<1	1.3
11.	β-farnesene	citrus, sweet	1475	0.087[i]	6.2	<1	<1	2.7

[a] Non-volatile compounds were identified by comparison with retention time of standard compounds detected by HPLC-DAD, [b] Volatile compounds were identified by comparison with mass spectra in the electron impact mode and linear retention index in mass libraries, [c] Linear retention index for volatile compounds, [d] Characteristic taste and aroma and detection threshold based on previous studies. [e] [19], [f] [20], [g] [21], [h] [22], [i] [23], [j] [24], [k] [25], [l] [26].

of limonin (6.5 mg/L) was detected in natural orange juice, as compared with the threshold of 1.0 mg/L in distilled water [22].

A total of 28 volatile compounds including monoterpene hydrocarbons, sesquiterpene hydrocarbons, monoterpene alcohols, aldehydes, monoterpene aldehydes, and monoterpene ester were detected by gas chromatography-mass spectrometry-olfactometry analysis (Supplementary Table S2). Among these volatile compounds, limonene and γ-terpinene were the most abundant components in the Thai lime juice samples. However, not all the detected volatile compounds were key aroma compounds. The potential odor-active compounds in food samples are often determined by the OAV, which is calculated from the ratio between the concentration of a volatile compound in a food sample and its odor detection threshold. The key odorant compounds are those with OAV ≥ 1 [31]. Terpinolene and linalool had OAV≥ 1 in all the Thai lime cultivars, which could imply that they might be key odorants in all Thai lime juices (Table 2).

In Pan Rumpai and Pan Puang, which were the lime cultivars preferred by the student chefs, the profiles of key odorants were quite similar (Table 2). Pan Rumpai and Pan Puang had eight and seven key aroma compounds, respectively. The majority of the odor-active compounds in these two cultivars were linalool, terpinolene, decanal, γ-terpinene, limonene, and terpinen-4-ol. However, Pan Puang had a low odor activity of β-myrcene (OAV < 1), which is a volatile compound that contributes sweet, balsamic, and pungent aroma notes, whereas a very high concentration of β-myrcene was found in Pan Rumpai (OAV = 19.6). This may have contributed to Pan Puang being the most preferred lime juice among the Thai lime cultivars in the chefs' preference test.

Tahiti had seven key aroma compounds (Table 2). Although the profile of odor-active compounds in Tahiti was similar to that in Pan Rumpai and Pan Puang, Tahiti had lower OAVs for all the odor-active compounds. This result agreed with the finding from the sensory analysis that Tahiti presented a moderate intensity for all aroma notes (Figure 2). The OAVs of linalool, terpinolene, and γ-terpinene in Tahiti were eight, two, and two times lower than those in Pan Puang. Moreover, the concentration of decanal, which was one of the major compounds that contributed to a pleasant aroma note in Pan Puang, was below the sensory threshold in Tahiti. This may have resulted in Tahiti having a lower aroma intensity and being less preferred by the master chefs and student chefs than Pan Rumpai and Pan Puang. Four key aroma compounds were detected in the Pan Pijit lime juice (Table 2). Among these compounds, the OAVs of linalool and terpinolene (the major compounds contributing to floral aroma notes in Thai lime juice) in Pan Pijit were 28 and 14 times lower than those in Pan Puang. These characteristics of the volatile compounds profile of the Pan Pijit lime juice may have result in its lack of a discernable floral aroma note. In addition, Pan Pijit was the only cultivar that contained β-ocimene (OAV = 2.35), which contributes undesirable terpenic and woody aroma notes. A high odor-activity (OAV = 4.2) of β-myrcene was also found. The presence of β-ocimene and β-myrcene might have contributed to the oxidized, pickled lime-like, and ripe aroma notes detected in the sensory descriptive analysis.

Figure 5 shows a heat map, a hierarchical cluster analysis based on Pearson's correlation coefficient with Ward's method, flavor descriptors, and the sensory descriptive attributes of 28 volatile and 9 non-volatile compounds of fresh-squeezed lime juice from the four lime cultivars. The hierarchical cluster analysis clustered the Thai lime cultivars into three groups based on their profiles of volatile and non-volatile compounds, as follows: Group 1, Pan Rumpai and Pan Puang; Group 2, Tahiti; Group 3, Pan Pijit. Most of the volatile compounds (β-myrcene, β-farnesene, α-thujene, limonene, γ-terpinene, terpinolene, linalool, fenchol, terpinene-4-ol, α-terpineol, decanal, neral, geranial, δ-eIemene, β-elemene, α-gurjunene, γ-elemene, α-bergamotene, germacrene D, cis-α-bisabolene, β-bisabolene, δ-cadinene, and germacrene B) associated with Pan Rumpai and Pan Puang correlated well with their perceived citrus, lemon, green, fruity, peely, and floral notes. Tahiti was associated with β-pinene, neryl acetate, caryophyllene, and sugars (sucrose, glucose, and fructose) as well as bitter compounds (limonin and naringin), whereas Pan Pijit was associated with β-ocimene and α-farnesene, which contribute oxidized, pickle lime-like, and ripe aroma notes.

4 Conclusions

Although limes are one of the most widely studied citrus fruits owing to their unique flavor, most studies

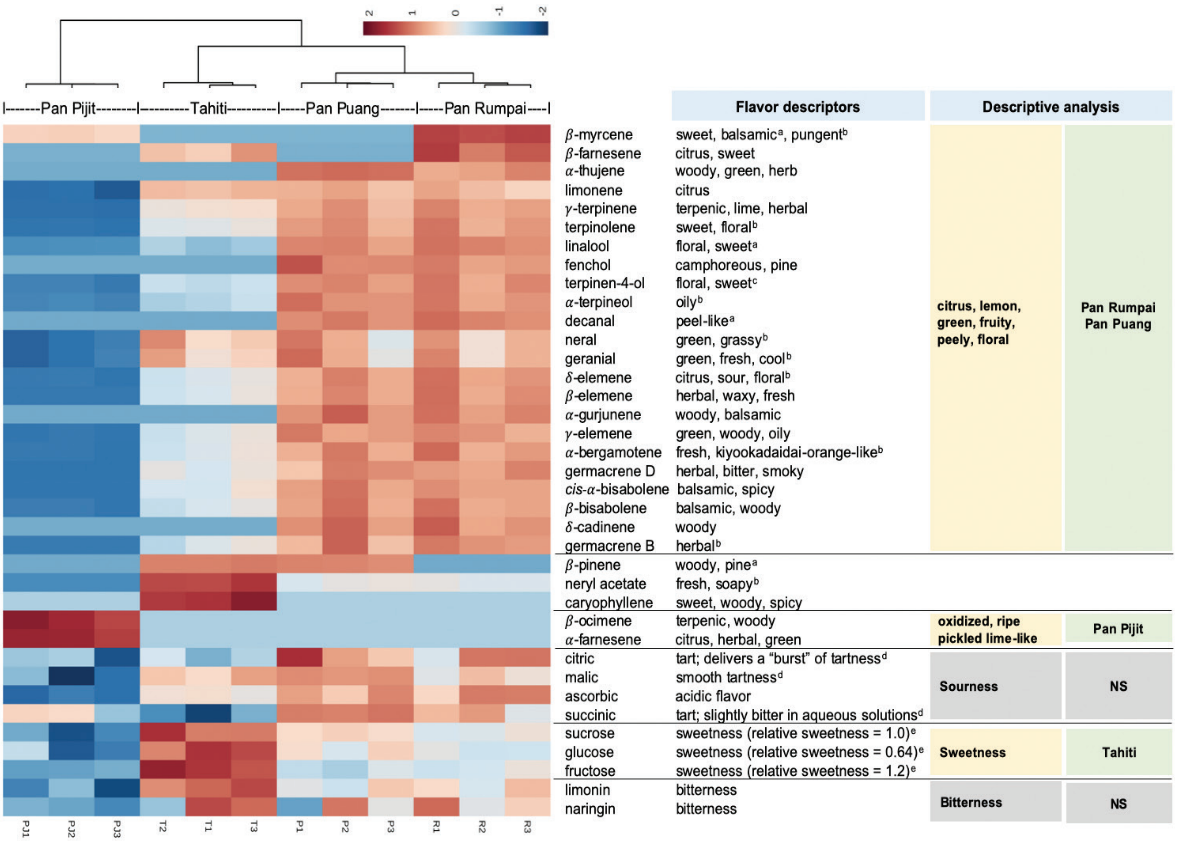

Figure 5: Heat map, hierarchical cluster analysis based on Pearson's correlation coefficient with Ward's method, flavor descriptors and sensory descriptive attributes of 28 volatile and 9 non-volatile compounds of fresh-squeezed lime juice from four lime cultivars. Each square of the heat map expressed normalized value shown in color scale (red color for a higher content and blue color for a lower content of the related compounds). [a] [23], [b] [24], [c] [25], [d] [32], [e] [4], NS = Not significant difference among Thai lime cultivars.

have focused only on their profiles of volatile and non-volatile compounds. Here, by investigating their chemical profiles, sensory characteristics, and selection by chefs, we discovered that desirable key odorants including terpinolene and linalool contributed floral aroma characters that influenced the chefs' selection. Moreover, we identified compounds resulting in undesirable flavors, such as β-ocimene and β-myrcene. Chemical differences among the four commercial Thai lime cultivars affected the flavor profiles of their juice samples. The flavor-influencing compounds identified in this study may be important contributors to the distinctive flavor characteristics of Thai lime fruit that influence chefs' selection of fruits for use in Thai cuisine.

Acknowledgements

This work was supported by Chulalongkorn Academic Advancement into its 2nd Century Project (CUAASC) (To P.N.), Chulalongkorn University's Ratchadapisek Sompot Fund (GCURP59022301 and CF58032301) (to P.N.), and the 90th Anniversary of Chulalongkorn University (Ratchadapisek Sompot Endowment Fund) (to J.K.). We would like to thank Dr. Punnida Techaruvichit and Dr. Chawin Ounkomol for comments on the manuscript. We thank Charnnarong's orchard in Petchaburi province and Wasa garden in Nakhon Nayok province, for providing lime fruits for this study. We are also thankful to Betagro Science Center (Bangkok, Thailand) for the assistance in sensory study and GERSTEL LLP (Singapore) for the assistance in volatile analysis.

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