Article

Characterization of volatile compounds in Tom Yum soup by headspace-solid phase microextraction-gas chromatography-mass spectrometry combined with sensory evaluation techniques

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Abstract: Gas chromatography-olfactometry/mass spectrometry coupled with headspace-solid phase microextraction (HS-SPME/GC-O/MS) was applied for the characterization of volatile compounds in Tom Yum soup and its individual ingredients. Using HS-SPME with a 50/30 µm DVB/CAR/PDMS fiber and an extraction temperature of 40 °C for 50 min along with an HP-5MS capillary column, 101 peaks in the HS-SPME/GC-MS chromatogram of Tom Yum soup were detected, and 96 compounds were identified including alcohols, aldehydes, esters, ethers, and terpenes. These findings are based on the comparison of MS spectra with the NIST library as well as experimental and literature retention index data. In comparison with the compound profiles of each individual ingredient of Tom Yum soup (both before and after cooking), five extra volatile compounds in Tom Yum soup were found after the cooking process. Furthermore, odor descriptions of the eighteen aroma compounds in Tom Yum soup, along with the odor ingredient sources, were also obtained.

Keywords: GC-MS; SPME; Tom Yum soup; volatile compounds; extra volatile compounds; aroma compounds; sniffing analysis

1. Introduction

Tom Yum, a spicy and sour soup, is an authentic Thai dish that is well-known worldwide. Its main ingredients typically include lemongrass, kaffir lime leaf, chili, galangal and lime juice, and the soup exhibits an impressive aroma and flavor along with possessing known health benefits [1]. Currently, several additional ingredients are also added into Tom Yum soup to enhance the flavor and color [1]. The mix of main ingredients in cooked Tom Yum soup results in the release of many volatile compounds, such as alcohols, aldehydes, esters, ethers, ketones and terpenes [2], which are mostly due to the spices, such as lemongrass [3], galangal [4], or kaffir lime leaf [5], with the different aromas due to their various volatile compound profiles. The characteristic flavor of Tom Yum thus depends on the volatile profiles. Most volatiles may be found in the individual ingredients; whilst, a few of them may be produced from chemical reactions during Tom Yum cooking process.

Gas chromatography-mass spectrometry (GC-MS) has been widely used to obtain profiles of volatile and semi-volatile organic compounds in food samples. Compounds are often identified according to MS library match and retention index data [6]. GC-MS allows for the precise identification of compounds by comparison of their mass spectra with available libraries as well as accurate quantitative analysis. This technique is often applied together with sensory analysis, including GC-olfactometry (GC-O) [7], to correlate chemical compositions with the characteristic flavors of food.

Sample preparation techniques that are conventionally applied for the extraction of volatile compounds in spices include simultaneous distillation extraction and hydrodistillation, which extract volatile analytes into a liquid phase. Although these techniques are efficient, they can be lengthy and involve several preparation steps with the risk of sample loss and side reactions during the extraction. Alternatively, headspace solid phase microextraction (HS-SPME) can be applied, offering a simple and fast extraction process where volatile compounds in the sample headspace can be adsorbed onto the SPME materials, e.g., divinylbenzene-based fibers for spice analysis [8], and directly injected into the GC inlet.

Lemongrass, *Cymbopogon citratus* (DC.) Stapf, has a characteristic lemony smell [9]. The HS-SPME-GC-MS analysis revealed volatile compounds in this spice, and the major components are two isomers of citral, neral and geranial (19.7 and 23.9% area, respectively) [3]. The extracted lemongrass oil was also found to contain two citral isomers as the major compounds (65-80%) as well as other compounds such as limonene, citronellal, β-myrcene and geraniol [10, 11].

Kaffir lime leaf, *Citrus hystrix* (DC.), has a characteristic citrus odor [5]. Using an HS-SPME-GC-MS analysis, the major compounds include citronellal (48.20%) as well as citronellol, citronelly acetate and linalool (14.3, 7.8 and 5.13%, respectively) [12]. Citronellal is also the most abundant compound in the extracted oil from the kaffir lime leaf and other extracted compounds include α-pinene, camphene, β-pinene, sabinene, myrcene, limonene, trans-ocimene, γ-terpinene, *p*-cymeme, terpinolene, copaene, linalool, β-cubebene, isopulegol, caryophyllene, citronelly acetate, citronellol, geranyl acetate and δ-cadiene [5].

Using the HS-SPME-GC-MS analysis, chili, *Capsicum frutescens* L., was found to contain 83 compounds comprised of mostly esters (40%) such as 2-methylpentyl hexanoate, hexyl 2,2-dimethyl propanoate and hexyl 3-methyl butanoate [13]. In addition, chili essential oil primarily contains esters including isohexyl isohexanoate, isohexyl isovalerate, isohexyl 2-methylbutyrate and hexyl isovalerate [14].

Lime, *Citrus aurantifolia* (Christm.) Swingle, exhibits a characteristic citrus smell. The essential lime oil was found to contain 32 compounds, primarily limonene (37%) and other compounds such as β-pinene (16%), γ-terpinene (9.5%), nerolidol (7.1%) and α-terpineol (6.7%). Aldehydes (neral, geranial, dodecanal, tetradecanal) and esters constituted the minor components including neryl acetate and geranyl acetate [15, 16].

Another ingredient that may be added to Tom Yum soup is galangal, *Alpinia galangal* (L.). Its essential oil primarily contains 1,8-cineol (63.4%) and α-terpineol (2.8%) with various other compounds contributing less than 2% [4].

Although the chemical compositions of the individual ingredients have been profiled, characterization of their compositions inside the complex matrix of Tom Yum soup is still a challenge.

In this study, optimization of HS-SPME was performed, and suitable conditions were applied for the extraction of Tom Yum soup and related samples. The extracted samples were analyzed using GC-O/MS for the separation and identification of volatile compounds and their odor descriptions. The objective of this work is to identify the volatile compounds of Tom Yum soup and its individual ingredients as well as their odor descriptions. To the best of our knowledge, there have been no reports on the analysis of Tom Yum soup using HS-SPME-GC-O/MS. Therefore, the volatile compounds in Tom Yum soup and its individual ingredients, as well as their odor descriptions, were characterized using optimized HS-SPME-GC-O/MS.

2. Results and Discussion

2.1 Optimization of HS-SPME

SPME is an equilibrium process between the vapor and fiber phases [19]. The two main factors affecting the extraction performance, extraction temperature and extraction time were studied.

Extraction temperatures of 40, 60 and 80 °C were investigated with an extraction time of 45 min using the total peak area of all the volatile compounds detected [20] and the individual peak areas of selected aroma compounds [21], as shown in Figure S1 (Supplementary materials 1) and Figure 1. The results demonstrated that a suitable temperature was obtained in the range of 40-60 °C, as shown by the high peak areas. To avoid off-flavor effects from the high temperatures of HS-SPME [22], 40 °C was selected as the temperature for further analyses.

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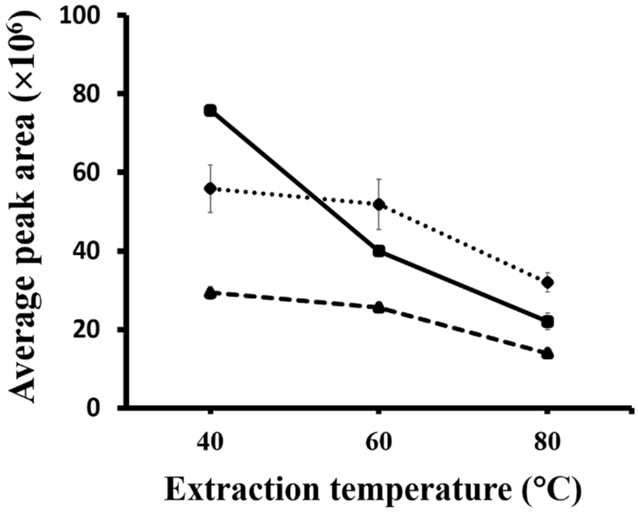

Figure 1. Average peak areas of selected aroma compounds, *D*-limonene (solid line), geranial (dotted line) and neral (dashed line), in the extracted Tom Yum soup at various HS-SPME extraction temperatures.

The effect of the extraction time (30, 45 and 60 min) on the extraction efficiency was determined at 40 °C. According to Figure S2 (Supplementary materials 1), which shows the total peak area of all the volatile compounds detected, and Figure 2, which shows the individual peak areas of selected aroma compounds, a longer extraction time of 45 to 60 min increases the extraction performance. Moving forward, an HS-SPME extraction time of 50 min was selected to best fit the total GC-MS separation time.

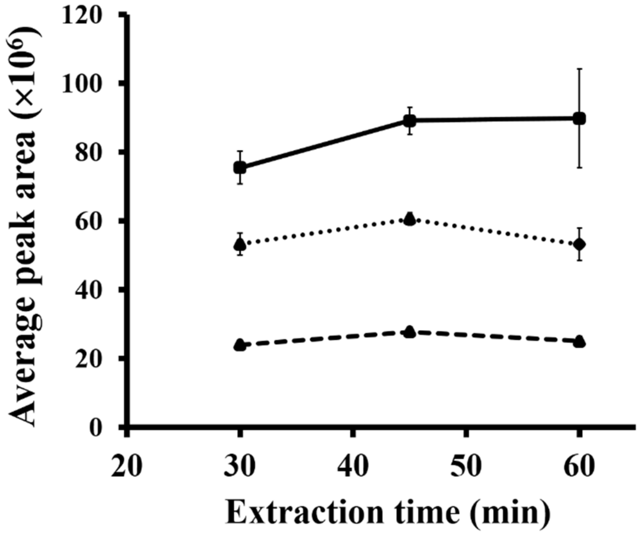

Figure 2. Average peak areas of selected aroma compounds, *D*-limonene (solid line), geranial (dotted line) and neral (dashed line), in the extracted Tom Yum soup at various HS-SPME extraction times.

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The intraday and interday precision in the %area normalization were evaluated, using an HS-SPME extraction temperature of 40 °C and extraction time of 50 min, for the extracted Tom Yum soup on each day for three consecutive days. The following 13 aroma compounds were analyzed in triplicate: α-pinene, 6-methyl-5-hepten-2-one, β-linalool, unknown (I = 1165, MS of 152), nerol, β-citral, geraniol, geranial, 4-methylpentyl 4-methylpentanoate, citronellyl acetate, geranyl acetate and dodecanal. From ANOVA with a single factor analysis at a 95% confidence level, acceptable %RSD values for intraday and interday precision were less than 15% and 25%, respectively, for most of the aroma compounds with the exception of 4-methylpentyl 4-methylpentanoate, citronellyl acetate and geranyl acetate which had %RSD values for interday in a range of 35-65, possibly due to the small amount of the %area normalization, i.e., less than 0.2. In this work, the %RSD is calculated using equations from the literature [23].

2.2 GC-MS analysis of Tom Yum soup and compound identification

An example of the GC-MS results (total ion chromatogram, TIC) for Tom Yum soup is shown in Figure 3 with the corresponding results for the individual raw and boiled ingredients shown in Figure 4 and Table 1.

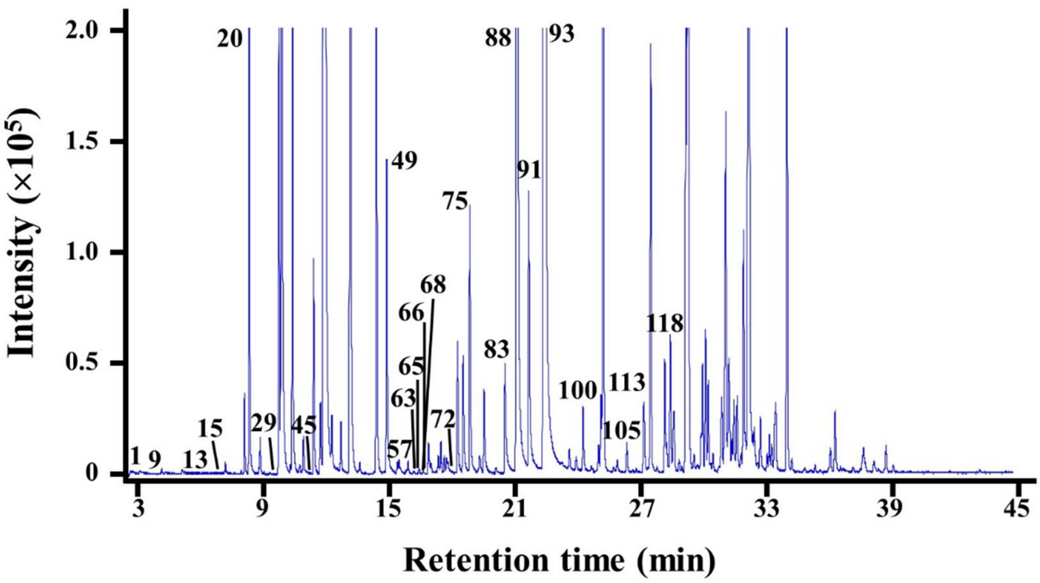

Figure 3. GC-MS chromatogram of Tom Yum soup.

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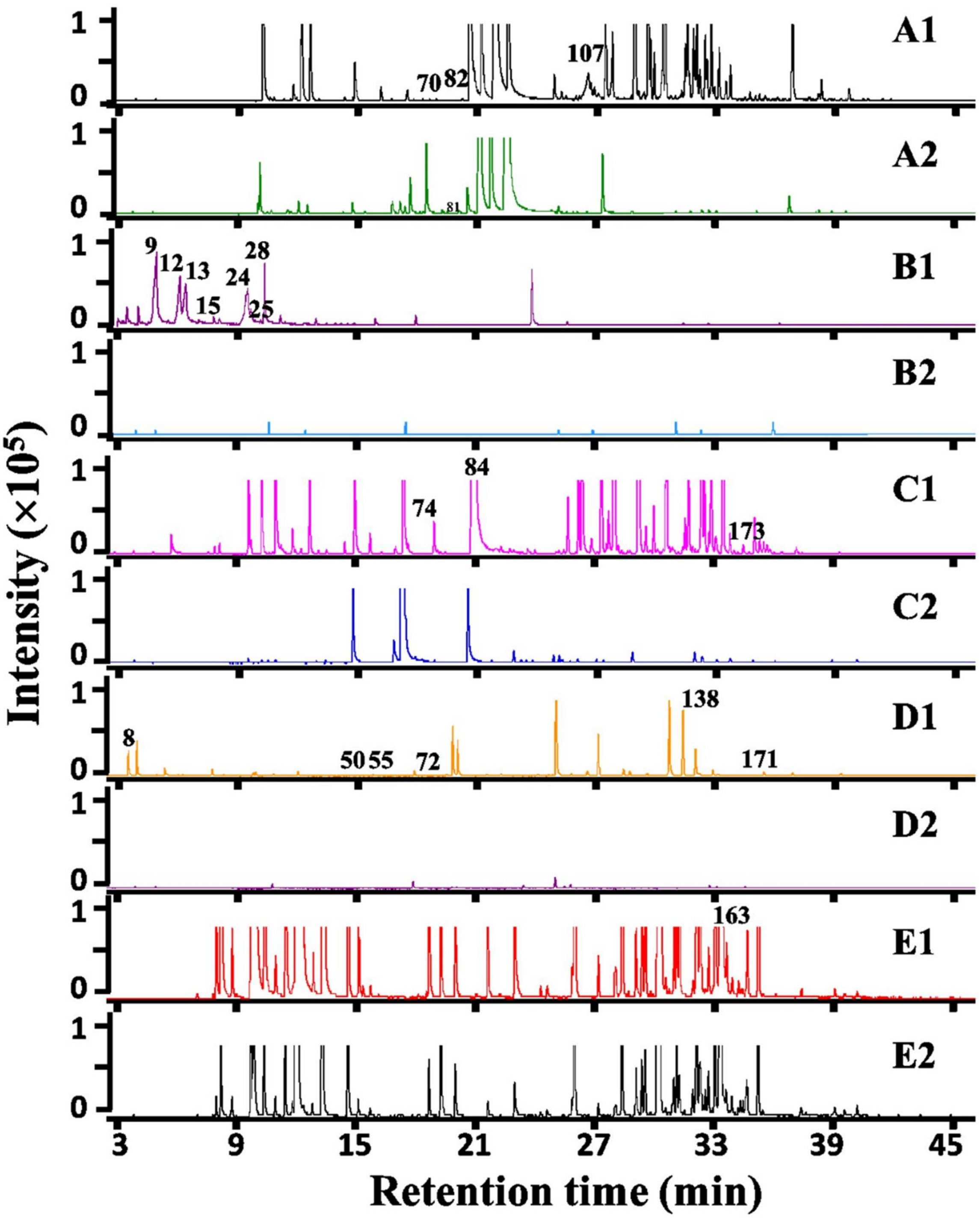

Figure 4. GC-MS chromatograms of volatile compounds in individual ingredients of Tom Yum soup: lemongrass (A), fish sauce (B), kaffir lime leaves (C), chili (D) and lime juice (E), where 1 and 2 refer to raw or boiled ingredients, respectively.

Table 1. Tentative volatile compounds in Tom Yum soup and its individual ingredients.

Peak no.	RT (min)	Tentative compound	Tom Yum soup	% Average area normalization (n = 3)									
				Lemongrass		Fish sauce		Kaffir lime leaf		Chili		Lime juice	
				Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled
8	3.70	1-Methylpyrrole	-	-	-	-	-	-	-	1.66 ±0.90	0.81 ±0.03	-	-
9	4.49	Butanoic acid	-	-	-	26.13 ±0.56	-	-	-	-	-	-	-
12	5.93	2-Methylbutanoic acid	-	-	-	14.83 ±0.90	-	-	-	-	-	-	-
13	6.26	3-Methylbutanoic acid	-	-	-	14.72 ±0.24	-	-	-	-	-	-	-
15	7.32	3-(Methylthio)propanal	-	-	-	1.27 ±0.09	3.9 ±1.3	-	-	-	-	-	-
24	9.00	4-Methylpentanoic acid	-	-	-	13.1 ±1.3	-	-	-	-	-	-	-
25	9.16	Benzaldehyde	-	-	-	3.40 ±0.60	5.95 ±0.57	-	-	-	-	-	-
28	9.84	Phenol	-	-	-	8.50 ±0.38	-	-	-	-	-	-	-
45	13.50	*p*-Mentha-3,8-diene	0.021 ±0.004	-	-	-	-	-	-	-	-	-	-
50	14.85	Isopentyl 2-methylbutanoate	-	-	-	-	-	-	-	0.082 ±0.025	2.28 ±0.34	-	-
55	15.37	2-Methylpentyl isobutyrate	-	-	-	-	-	-	-	0.61 ±0.21	0.91 ±0.16	-	-

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57	15.80	α-Cyclocitral	0.024 ±0.002	-	-	-	-	-	-	-	-	-	-
63	17.23	Iso-isopulegol	0.06 ±0.02	-	-	-	-	-	-	-	-	-	-
65	17.52	Unknown 1	0.034 ±0.006	-	-	-	-	-	-	-	-	-	-
68	17.70	*p*-Mentha-1,5-dien-8-ol	0.032 ±0.010	-	-	-	-	-	-	-	-	-	-
70	18.10	Rose furan oxide	-	0.026 ±0.002	0.014 ±0.002	-	-	-	-	-	-	-	-
72	18.32	2-Isobutyl-3-methoxypyrazine	-	-	-	-	-	-	-	0.049 ±0.014	0.76 ±0.03	-	-
74	18.60	(Z)-3-Hexenyl butanoate	-	-	-	-	-	0.15 ±0.04	-	-	-	-	-
81	19.97	Carveol	0.012 ±0.003	-	0.031 ±0.002	-	-	-	-	-	-	-	-
82	20.01	γ-Isogeraniol	-	0.019 ±0.005	-	-	-	-	-	-	-	-	-
84	20.51	β-Citronellol	-	-	-	-	-	47.7 ±1.1	2.65 ±0.21	-	-	-	-
107	26.17	Neric acid	-	0.32 ±0.20	-	-	-	-	-	-	-	-	-
112	26.92	(Z)-3-Hexenyl hexanoate	-	-	-	-	-	0.042 ±0.013	-	-	-	-	-
138	30.19	2-Methyltetradecane	-	-	-	-	-	-	-	7.40 ±2.63	3.09 ±0.21	-	-

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163	32.12	β-Bisabolene	-	-	-	-	-	-	-	-	-	1.70 ±0.14	5.62 ±0.24
171	33.18	Hexyl benzoate	-	-	-	-	-	-	-	0.091 ±0.030	4.03 ±0.63	-	-
173	33.53	Hedycaryol	-	-	-	-	-	0.057 ±0.010	-	-	-	-	-

All compounds detected in the GC-MS chromatograms were identified according to a comparison of their mass spectra with those from the NIST library with match scores of >650 as well as experimental and literature values of the linear retention index. The tentative volatile compound profiles with their normalized peak areas for various samples are summarized in Supplementary materials 2.

As shown in Supplementary materials 2, the three major volatile compounds found in raw lemongrass (with the %area normalization in parentheses) are geraniol (39.7%), nerol (13.1%) and β-myrcene (8.15%). Other compounds found include geranial (7.15%) and β-citral (2.81%). However, the three major volatile compounds found in boiled lemongrass (Figure. 4A2) are geranial (69.1%), β-citral (24.8%), geraniol (2.89%), β-myrcene (0.40%) and nerol (0.31%). It should be noted that these five compounds are also bioactive marker compounds in the essential oil of lemongrass [11, 24] and exhibit strong lemony and floral perceptions [9]. In addition, we also observed carveol (0.03%) in boiled lemongrass, but not in raw lemongrass, in this work. This may be caused by a *D*-limonene transformation via a reaction with water molecules induced by heating [25]. As a result, *D*-limonene could be oxidized into its oxide forms including *p*-mentha-2,8-dienols, hydroperoxides, carveols, *L*-carvone and carvone oxide.

In fish sauce (Figure 4B1), organic acids such as butanoic acid (26.1%), 3-methylbutanoic acid (14.7%), 2-methylbutanoic acid (14.8%) and 4-methylpentanoic acid (13.1%) are the main volatile compounds present while the minor compounds are acetic acid (1.81%), 1-dodecanol (0.67%), and 3-methylbutanal (0.59%). In contrast, 1-dodecanol (34.6%), acetic acid (17.6%) and 3-methylbutanal (13.0%) are the main compounds found in boiled fish sauce (Figure 4B2), while 3-methylbutanoic acid, 2-methylbutanoic acid and 4-methylpentanoic acid were not detected under the HS-SPME-GC-MS conditions used in this work. 3-Methylbutanoic acid and 4-methylpentanoic acid exhibit cheesy and sweaty aromas [26].

The major volatile compounds found in raw kaffir lime leaf (Figure 4C1) are β-citronellol (47.7%) and caryophyllene (16.9%), while the minor compounds are copaene (4.66%), β-citronellal (3.90%), citronellyl acetate (2.49%), and β-linalool (1.09%). The major volatile compounds of boiled kaffir lime leaf (Figure 4C2) are β-citronellal (93.9%) and β-citronellol (2.65%), while the minor compounds are β-linalool (1.80%), caryophyllene (0.15%), citronellyl acetate (0.09%) and copaene (0.04%). Among these compounds, β-citronellal is considered the key odorant of kaffir lime leaf because of its high flavor dilution factor [5].

The dominant volatile compounds in raw chili (Figure 4D1) are 4-methylpentyl 4-methylpentanoate (45.3%), 4-methylpentyl 2-methylbutanoate (14.7%), 4-methylpentyl 3-methylbutanoate (11.0%) and δ-guaiene (0.12%). However, in boiled chili (Figure 4D2), 4-methylpentyl 4-methylpentanoate (53.4%) is the dominant volatile compound along with δ-guaiene (9.1%), 4-methylpentyl 3-methylbutanoate (5.59%) and 4-methylpentyl 2-methylbutanoate (3.96%). It should be noted that 4-methylpentyl 4-methylpentanoate exhibits soapy and weak fruity aromas [27], and 4-methylpentyl 3-methylbutanoate exhibits fruity and peach aromas [27].

In lime juice (Figure 4E1), the major volatile compounds are *D*-limonene (49.9%), β-pinene (19.9%) and γ-terpinene (9.21%). D-limonene (42.9%) is also a main compound in boiled lime juice (Figure 4E2) followed by γ-terpinene (10.0%) and β-pinene (6.92%). *D*-limonene is usually found in many essential oils of aromatic plants and herbs [25].

As seen in Figure 3 and Supplementary materials 2, a total of 96 volatile compounds were identified from various volatile classes in Tom Yum soup. The major components are *D*-limonene (26.6%) and geranial (25.4%) from both the lime juice and lemongrass. Other compounds are α-muurolene (6.27%), β-pinene (4.79%) and γ-terpinene (4.46%), which are from the lime juice. In comparison with the individual raw and boiled ingredients, Tom Yum soup contains the following five extra volatile compounds: *p*-mentha-3,8-diene, *α*-cyclocitral, iso-isopulegol, *p*-mentha-1,5-dien-8-ol and decyl acetate. This implies that significant chemical reactions between the ingredient components generate volatile compounds in the Tom Yum soup during the cooking process.

According to an explanation in a previous work [28], p-mentha-3,8-diene may be a product of β-citronellal since the latter compound can be cyclized to result in isopulegol with byproducts including menthone, pulegol, and other cyclic hydrocarbons such as *α*-terpinene, *p*-mentha-3,8-diene and terpinolene.

Citrals are acyclic terpenes without an asymmetric center that are generally converted to cyclic terpenes including *α*-cyclocitral [29]. Moreover, *p*-mentha-1,5-dien-8-ol may come from citral under acidic conditions, and the mechanism of *p*-mentha-1,5-dien-8-ol is described in the literature [30].

Iso-isopulegol may occur as a result of cyclization of β-citronellal with three asymmetrical centers, which can result in four stereoisomers of isopulegol, and each isomer occurs as a pair of enantiomers: (±)-isopulegol, (±)-neoisopulegol, (±)-iso-isopulegol and (±)-neoiso-isopulegol [31].

Interestingly, decyl acetate, a long-chain ester, has a floral (orange-rose) odor and a characteristic flavor. This compound has been found in orange, lemon, melon, apple, citrus peel oils, orange juice, strawberry fruit, blue cheese, cognac, plums and cardamom [32].

2.3 Correlation with the olfactory analysis of Tom Yum soup

According to the HS-SPME-GC-O/MS analysis of Tom Yum soup detailed in Section 3.5, the odor descriptions for the aroma compounds from our experiment were compared with literature sources and summarized in Table 2.

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Table 2. Aroma compounds in Tom Yum soup detected by GC-O.

	RT (min)	LRI		Aroma compound	Ingredient source		Sensory evaluation				
		Exp[a]	Lit[b]		Exp[c]	Ref.[d]	Odor description	Detecting compound		Average odor intensity (n = 18, Maximum value = 4)	Ref.
								No. of panelist	Trial		
1	2.61	<700	624±23 (n = 3)	Acetic acid	Fish sauce	[5]	Sour	3	5	0.50	[5]
9	4.49	789	789±10 (n = 13)	Butanoic acid	Fish sauce	[5]	Cheesy	2	4	0.28	[5]
13	6.26	866	859±19 (n = 15)	3-Methylbutanoic acid	Fish sauce	[3]	Vomit-like, cheesy, sweaty	4	8	0.78	[3]
15	7.32	906	909±5 (n = 12)	3-(Methylthio)propanal	Fish sauce	[5]	Potato	5	13	1.5	[5]
20	8.25	933	935±6 (n = 26)	α-Pinene	Lime juice	[6]	Pine, woody	2	4	0.22	[6]
29	10.19	988	983±7 (n = 18)	6-Methyl-5-hepten-2-one	Lemongrass	[7]	Lemon leaf-like, green, citrusy	2	3	0.17	[7]
49	14.78	1101	1099±5 (n = 26)	β-Linalool	Lemongrass, kaffir lime leaf, lime juice	[4, 6, 7]	Flower, lavender	5	13	1.72	[1]
66	17.63	1165	-	Unknown 2 (MS of 152)	Lemongrass	-	Green	3	5	0.44	

72	18.32	1181	1183±6 (n = 4)	2-Isobutyl-3-methoxypyrazine	Chili	[2]	Paprika, green, earthy	5	13	1.4	[2]
75	18.73	1191	1189±5 (n = 29)	α-Terpineol	Lime juice, lemongrass, kaffir lime leaf	[4, 6, 9]	Piney/floral	3	6	0.39	[4]
83	20.40	1230	1227±3 (n = 8)	Nerol	Lemongrass,	[9]	Sweet	5	11	0.94	[1]
88	20.99	1242	1239±3 (n = 8)	β-Citral	Lemongrass, lime juice	[4, 6, 9]	Citrus	5	13	2.0	[8]
91	21.53	1258	1254±4 (n = 10)	Geraniol	Lemongrass, kaffir lime leaf, lime juice	[4, 9, 10]	Floral	4	12	1.3	[4]
93	22.33	1272	1273±13 (n = 2)	Geranial	Lemongrass, kaffir lime leaf, lime juice	[6, 9, 11]	Floral/citrus	5	11	1.8	[4]
100	24.12	1317	1315 (n = 1)	4-Methylpentyl 4-methylpentanoate	Chili	[2]	Soapy, weak fruity	2	3	0.28	[2]
105	25.75	1355	1352±3 (n = 5)	Citronellyl acetate	Kaffir lime leaf, Lime juice	[4, 6]	Berry/fragrant	2	3	0.28	[4]

113	27.01	1385	1378±8 (n = 9)	Geranyl acetate	Lime juice, Kaffir lime leaf, Lemongrass	[4, 9,11]	Floral	3	4	0.22	[4]
118	28.01	1410	1404±3 (n = 3)	Dodecanal	Lime juice	[4,11]	Waxy	3	6	0.44	[4]

[a] Exp = linear retention indices are determined using n-alkanes (C_8-C_{20}) on an HP-5 column
[b] Ref = linear retention indices of reference compounds from http://webbook.nist.gov/
[c] Exp = aroma compounds found in the raw and boiled ingredients from the experiment
[d] Ref = aroma compounds found in the ingredients from the literature
Number of panelists detecting compound = 6 panelists
[1] = http://www.flavornet.org/flavornet.html, [2] = (Rodriguez-Burruezo et al., 2010), [3] = (Wichaphon et al., 2012), [4] = (Chisholm, Wilson, & Gaskey, 2003), [5] = (Lapsongphon, Yongsawatdigul, & Cadwallader, 2015), [6] = (Jirapakkul et al., 2013), [7] = (Kamath et al., 2001), [8] = (Marković, Vahčić, Ganić, & Banović, 2007), [9] = (Bossou et al., 2015), [10] = (Wongpornchai, 2012) and [11] = (Yadav et al., 2004)

From the triplicate evaluations of the six panelists (n = 18), eighteen aroma compounds in Tom Yum soup were detected and described by at least two of the panelists. Taking into account that aromas are only active if at least half of the total sniffing trials detected a similar odor quality and retention time [18], the seven dominant aroma compounds were β-citral (13), geranial (11), β-linalool (13), geraniol (12), nerol (11), 3-(methylthio)propanal (13) and 2-isobutyl-3-methoxypyrazine (13). According to the aroma compounds found in both the raw and boiled ingredients, the first four aromas detected in Tom Yum soup were from the lemongrass, kaffir lime leaf, and lime juice; the nerol is from lemongrass; the 3-(methylthio)propanal is from fish sauce and the 2-isobutyl-3-methoxypyrazine is from chili. There were eleven minor aroma compounds that had medium and small perception levels, including 3-methylbutanoic acid, acetic acid, dodecanal, unknown 2 (I of 1165, MS of 152), α-terpineol, butanoic acid, 4-methylpentyl 4-methylpentanoate, citronellyl acetate, geranyl acetate, α-pinene and 6-methyl-5-hepten-2-one. It should be noted that the seven active aromas found in Tom Yum soup also showed the stronger odor perceptions with an average odor intensity of > 0.9 compared to those of the latter eleven aromas. In addition, acetic acid and four other aroma compounds with I values near 789, 866, 906 and 1181 were perceived from sniffing GC-O, where the two latter aromas were particularly strong but were not detected by an MS detector. Using individual raw and boiled ingredients, along with a comparison of the I values and odor description, these four aroma compounds should be butanoic acid, 3-methylbutanoic acid and 3-(methylthio)propanal from fish sauce and 2-isobutyl-3-methoxypyrazine from chili.

3. Materials and Methods

3.1 Recipe and raw ingredients

The selected recipe for Tom Yum soup is from Suan Dusit University (Thailand). Lemongrass, kaffir lime leaf, chili and lime were purchased from a local supermarket in Bangkok, Thailand and then kept in a refrigerator at 4 °C prior to use. Fish sauce was purchased from a local supermarket in Bangkok, Thailand.

3.2 Chemicals

A mixture of n-alkanes (C_8-C_{20}) purchased from Sigma Aldrich (St. Louis, MO) was used as a reference to calculate the linear retention index (LRI) of the compounds.

3.3 Sample preparation

All raw ingredients were cleaned with deionized water and dried with air under atmospheric conditions. According to the recipe for Tom Yum soup, the lemongrass was chopped into thin slices (15.0 g), the kaffir lime leaf was torn into medium pieces (2.0 g), the chili was crushed (3.0 g), and the lime was squeezed to collect the juice (21.0 g). The raw ingredients were progressively added into boiled water (300 mL) at 100 °C. The lemongrass was cooked for 1 min; the fish sauce (19.0 g) was cooked for a few seconds; the kaffir lime leaf was cooked for 1 min; the crushed chili was cooked for 0.5 min; and the lime juice was cooked for 0.5 min. For the analyses of individual ingredients, each raw ingredient was divided into two portions. One was boiled in water (300 mL) at 100 °C while the other was prepared from raw ingredients without boiling to create the corresponding control samples.

3.4 HS-SPME

An SPME 50/30 μm DVB/CAR/PDMS fiber and holder were purchased from Supelco (Sigma-Aldrich, Bellefonte, PA). The fiber was conditioned at 270 °C for 1 hour via insertion into the GC injection port. Prior to the real sample analysis, the blank fiber was injected to check the background signal from the fiber. Each of the extracted raw ingredients and the Tom Yum soup sample (2 mL) were transferred into 20 mL glass vials closed with an aluminum cap with a sealed PTFE/silicone septum. The vials were heated in a water bath at 40 °C unless otherwise stated for an equilibrium time of 5 min. The SPME fiber was then exposed inside the vial to extract volatile compounds in the headspace of the sample with an extraction time of 50 min unless otherwise stated. All samples were performed in triplicate.

3.5 GC-O/MS

The determination of volatile compounds was performed using GC-MS (7890A-7000, Agilent technologies Inc.) combined with an olfactory detection port (ODP3; Gerstel). Volatile compounds were separated on an HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, USA) using ultra-high purity helium (99.999%) as the carrier gas with a flow rate of 2 mL/min. The extracted sample was injected at 250 °C (desorption temperature) with a split ratio of 1:10. The GC oven temperature was programmed to increase from 50 to 200 °C at a rate of 3 °C/min. At the analytical column outlet, the column effluent was divided by a T-junction with a ratio of 1:4 between the MS and ODP. The temperature of the ion source in the MS was set at 230 °C. The electron ionization voltage was -70 eV. The mass spectra were acquired over the mass range of 35–300 Da with a scan time of 100 ms. Six panelists (aged 25-35, 2 male and 4 female) were trained for at least 20 h over a period of one month were assigned for the olfactory analysis and description of the aroma compounds in the extracted Tom Yum soup (triplicate per person). The panelists recorded their responses by pressing an olfactory intensity device (with intensity scale scoring from 0 to 4 where "1" and "4" corresponded to "slight" and "extreme" aroma intensity, respectively) when they perceived the aroma compounds. During the sniffing analysis of the effluents from the sniffing mask, the panelists recorded the perceived aromas with the corresponding retention times, intensities and aroma descriptors. A peak was considered aroma active only if at least half of the total sniffing trials found a similar odor quality at the same retention time. The average odor intensity was evaluated by 18 analyses with six panelists in triplicate for each sample. When a panelist could not detect an aroma compound, the intensity was recorded as zero, and zero is also taken into account in calculation of the average values [17, 18].

3.6 Data processing

The chromatographic peak and MS data of each extracted raw ingredient, boiled ingredient and Tom Yum soup were identified using Agilent MassHunter software. The data processing and presentation were further performed using Microsoft Excel. Compounds were tentatively identified by the comparison of their MS spectra with those obtained from the NIST library. The identification criteria were selected with a match score of > 65 and a difference of 20 units between the calculated retention index (*I*) and the *I* data from the literature for the same (or a similar) stationary phase.

The experimental *I* value for each peak in the chromatograms relative to the alkane retention time data was obtained by injection of an alkane mixture under the same experimental conditions used for the sample separation. *I* values for the temperature-programmed separation were calculated according to the literature [8].

4. Conclusions

The chemical composition of Tom Yum soup and its individual ingredient samples were profiled with HS-SPME-GC-O/MS. The optimization of HS-SPME was applied for the selection of the most suitable method to allow the detection of 101 volatile compounds in the Tom Yum soup headspace. Ninety-six peaks were identified representing various volatile classes. In comparison with the volatile profiles of the individual raw and boiled ingredients, Tom Yum soup was found to produce five extra volatile compounds including *p*-mentha-3,8-diene, α-cyclocitral, iso-isopulegol, *p*-mentha-1,5-dien-8-ol and decyl acetate, possibly due to chemical reactions (such as cyclization) among the compounds in the mixed ingredients in Tom Yum soup. In addition, eighteen aroma compounds that contribute to the impressive aroma of Tom Yum soup were characterized by HS-SPME-GC-O/MS and originated from the following ingredients: fish sauce (acetic acid, butanoic acid, 3-methylbutanoic acid, and 3-(methylthio)propanal), lime juice (α-pinene and dodecanal), lemongrass (6-methyl-5-hepten-2-one, unknown with *I* of 1,165, MS of 152 and nerol), and chili (2-isobutyl-3-methoxypyrazine and 4-methylpentyl 4-methylpentanoate). β-linalool, α-terpineol, geraniol, β-citral, geranial, citronellyl acetate and geranyl acetate are derived from lime juice, lemongrass and kaffir lime leaf.

Supplementary Materials

Supplementary materials associated with this article can be found in the online version. Figure S1: Average total peak area and extraction temperature. Figure S2: Average total peak area and extraction time. Table S1: Tentative volatile compounds in Tom Yum soup and individual ingredient.

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Author Contributions

Author Pannipa Janta performed all the experiments, data curation, investigation, methodology and writing original draft. Author Chadin Kulsing provided formal analysis and writing original draft. Author Thumnoon Nhujak designed conceptualization, formal analysis, funding acquisition, project administration, supervision, validation and writing review & editing.

Conflicts of Interest

The authors declare no conflict of interest.

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