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Investigation of Automated Sample Preparation Techniques for Gas Chromatography-Mass Spectrometry Analysis of Volatile Organic Compounds in Thai Green Curry Paste

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Abstract

Different automated headspace microextraction techniques were employed prior to analysis with gas chromatography-mass spectrometry (GC-MS) for profiling volatile organic compounds (VOCs) in Thai green curry paste: static headspace (SHS), headspace solid-phase microextraction (HS-SPME), and dynamic headspace (DHS) adsorption of VOCs onto Tenax materials with thermal desorption. By applying these methods, the number of VOCs identified by comparison with the MS and retention index (RI) database was in the order of DHS > SPME > SHS, with 36, 22, and 6 VOCs identified, respectively. This led to a total of 46 identified VOCs. In general, limonene and γ-terpinene were observed with all three extraction techniques. The major VOCs in the green curry were terpinen-4-ol, limonene, cuminaldehyde, and α-terpineol. These findings provide an improved understanding of automated extraction processes for the analysis of VOCs in curry pastes and other food samples in the future.

Keywords

Static headspace, Headspace solid-phase microextraction, Dynamic headspace, Gas chromatography-mass spectrometry, Green curry

Introduction

Green curry is one of the most famous Thai dishes which is well known and authentic [1, 2]. Thai green curry paste has gained popularity worldwide for its distinctive flavor and versatility in creating delicious Thai dishes. Some observations on its impact and adaptation in different culinary contexts international appeal: Thai cuisine [2].

Adaptation in fusion cuisine: In many countries, chefs and home cooks have embraced Thai green curry paste in fusion dishes. It's used to add a unique twist to traditional recipes or combine with local ingredients to create innovative culinary creations [3, 4]. Availability and accessibility of green curry paste is now commonly available in supermarkets and specialty stores worldwide, making it easier for people to experiment with Thai cooking at home.

Health consciousness: The trend towards healthier eating has also contributed to the popularity of Thai green curry paste. It typically contains natural ingredients and can be adapted to suit various dietary preferences, such as vegetarian or gluten-free diets Thai cuisine is regarded for its medicinal properties, with its array of vegetables, herbs, and spices collectively possessing various biological and pharmacological effects [5, 6].

Cultural influence: Beyond its culinary appeal, Thai green curry paste represents cultural exchange and appreciation. Its use in international kitchens fosters a deeper understanding and appreciation of Thai culinary traditions.

Its specific flavor and taste are derived from the chemical components of the ingredients used, including fresh green chili (CL), garlic (GL), pepper, cumin seeds (CM), lemongrass (LG), kaffir lime peel and/or kaffir lime leaves (KL), coconut milk, and basil leaves [7]. The CL paste typically contains the first eight ingredients, while coconut milk and basil leaves are added consecutively to the well-cooked green curry. Some of these ingredients also provide health benefits. Each raw material possesses a unique chemical profile [8-10]. Due to the large number of ingredients, Thai green curry is a complex sample containing both non-volatile compounds (such as proteins, peptides, amino acids, carbohydrates, sugars, fats, fatty acids, organic acids, phenolic acids, and alkaloids) and VOCs with a wide range of molecular weights, including aldehydes, ketones, furans, furanones, pyrazines, and sulfur compounds. Therefore, chemical profiling of Thai green curry presents a significant challenge.

Among different techniques, GC-MS is commonly used to analyze the volatiles in food. This technique has the capability to separate VOCs from a complex matrix prior to detection with MS, allowing for the identification of molecular weights and chemical formulas of the separated compounds [11-13]. GC-MS based technologies offer rapid analysis with a small amount of samples and provide high accuracy and sensitivity, leading to reliable data analysis of food volatiles [14-15].

Over the last decade, various sample preparation approaches have been developed for food analysis using GC-MS [16-19]. Analysis of the sample headspace is crucial for the determination of VOCs and can be performed without the use of solvents. Automation of these technologies enables highly confident and repeatable results, avoiding errors associated with manual methods. SHS extraction is a simple and solventless technique that samples the headspace of a sample using a relatively large syringe (e.g., 2.5 ml) and directly injects the headspace into the GC-MS instrument. This technique has been successfully applied in the field of food analysis [20-22].

HS-SPME is a conventional method that extracts the headspace of samples onto a fiber coated with various materials, allowing for the adsorption of different compounds based on their polarity and/or molecular weight [23]. This is a fast and sensitive approach for preparing samples prior to GC-MS analysis of VOCs in food samples such as wine, coffee, perfume, spices, and other food products [14, 24-27]. DHS, which uses a sorbent material coupled with a thermal desorption unit (TDU), has also been reported for food analysis [28-30]. While the performance of HS-SPME and SHS is limited by the equilibrium concentration of VOCs in the gas phase, the DHS extraction process can result in a larger amount of adsorbed compounds because more compounds can be accumulated onto the material due to the influence of carrier gas flow, such as N_2. Automated applications of these extraction techniques are thus desirable, especially for the analysis of green curry samples.

In this study, different automated sample preparation technologies (HS-SPME, SHS, and DHS) were applied for the analysis of instant green curry paste. The extraction conditions were optimized for each technique, and their performance was compared and discussed based on the VOCs detected.

Material and Methods

Material and chemicals

An n-alkane solution mixture of C_8-C_{20} (CAS-No. 203-777-6), analytical grade standard, was purchased from Sigma-Aldrich (St. Louis, MO) and used as a reference for the calculation of the RI of compounds. SPME fibers (50/30 µm DVB/CAR/PDMS, grey) and holders were purchased from Supelco (Sigma-Aldrich, Bellefonte, PA, USA). The SPME fiber was conditioned at 270 °C for 1 h by insertion into the GC injection port. Prior to sample analysis, a blank fiber was injected to check for background signals. Thai green curry paste was purchased from a local market in Bangkok, Thailand. Its ingredients include CL (30%), LG (20%), GL (15%), salt (11%), shallot (8.5%), galangal (GG) (5.5%), shrimp paste (3%), KL (3%), coriander root (2.5%), spices of CM, and turmeric (1.5%).

GC-flame ionization detector (GC-FID)

GC-FID analysis of VOCs in green curry paste was performed on an Agilent 7890B GC system (Agilent Technologies, USA) equipped with an FID. The GC separation was carried out using an Agilent J&W HP-5 column (30 m × 0.25 mm inner diameter, 0.25 µm film thickness) with 99.999% high-purity helium gas as the carrier gas at a flow rate of 2.5 ml/min. The injection port temperature was set to 260 °C in spitless mode. The FID temperature was maintained at 300 °C with flow rates of 30 ml/min for H_2, 300 ml/min for air, and 20 ml/min for N_2. The GC oven temperature program started at 40 °C, held for 0.5 min, and then increased to 280 °C at a rate of 4.0 °C/min. The volume or amount of sample injected for each sample introduction technique is detailed in sections 2.4 and 2.5.

GC-MS

For GC-MS analysis of VOCs in green curry, an Agilent 7890B GC system coupled with a 5977B single quadrupole MS (Agilent Technologies, USA) was used. The sample was injected in spitless mode at an injection port temperature of 240 °C, with a helium (99.999% purity) flow rate of 2.5 ml/min. The components were directed through a cooling injection system (CIS) before the GC separation. The oven temperature was programmed to start at 40 °C, hold for 0.5 min, and then ramp up to 280 °C at 4.0 °C/min. Separation was achieved using an Agilent J&W HP-5MS column (30 m × 0.25 mm inner diameter, 0.25 µm film thickness). The electron ionization source was set to 230 °C, and the mass spectra were recorded at -70 eV. Data collection covered an m/z range of 35-300, with the quadrupole and transfer line temperatures maintained at 150 and 250 °C, respectively. The sample introduction volumes and techniques are detailed in the following three sections.

SHS GC-FID and SHS GC-MS

The SHS and HS-SPME extraction for GC-FID analysis of Thai green curry paste was performed using a Gerstel

multipurpose sampler (MPS) with a single head (GERSTEL, GmbH & Co. KG, Mülheim an der Ruhr, Germany) and the aforementioned GC-FID system. For SHS GC-FID, 1.0 g of Thai green curry paste was placed into a 20 ml headspace vial, which was then sealed with a headspace aluminum crimp cap (HACC) with a PTFE/Si septum. The sample was extracted for 10 min at temperatures of 40, 50, 60, 70, 80, and 90 °C with the agitator turned off. A 2.5 ml sample of the headspace was automatically taken using a 2.5 ml syringe by the MPS (for GC-MS, GERSTEL) at different temperatures of 30, 50, 100, or 140 °C, and then injected into the GC-FID injection port. To achieve the highest total peak area for GC-FID, the optimal conditions for SHS GC-FID were determined to be an incubation and extraction temperature of 80 °C and a syringe temperature of 50 °C.

SHS GC-MS analysis of Thai green curry paste was performed using a Gerstel MPS with dual heads (GERSTEL, GmbH & Co. KG, Mülheim an der Ruhr, Germany) and the aforementioned GC-MS system, using the optimal headspace conditions obtained from the SHS GC-FID analysis.

HS-SPME GC-FID and HS-SPME GC-MS

For HS-SPME GC-FID analysis, 1.0 g of Thai green curry paste was placed into a 20 ml headspace vial, which was then sealed with a HACC and PTFE/Silicone septa. The sample was incubated for 5 min, followed by headspace extraction for 15, 20, 30, and 45 min using an automated MPS (GERSTEL, GmbH & Co. KG, Mülheim an der Ruhr, Germany). The SPME fiber (1 cm length and 50/30 µm, Supelco, Steinheim, Germany), coated with DVB/CAR/PDMS on a flexible fused silica core, is specifically designed for food and beverage applications. The HS-SPME extraction was performed at different temperatures of 40, 50, 60, 70, 80, and 90 °C with the agitator turned off. To desorb the analytes from the SPME fiber, the fiber was removed from the vial and inserted into the GC injection port at 260 °C for 60 s prior to GC-FID analysis. The optimal conditions for HS-SPME GC-FID were determined to be an extraction temperature of 80 °C and an extraction time of 30 min.

HS-SPME GC-MS analysis of Thai green curry paste was performed using an MPS with dual heads (GERSTEL, GmbH & Co. KG, Mülheim an der Ruhr, Germany) and the aforementioned GC-MS system, utilizing the HS-SPME conditions optimized for GC-FID.

DHS GC-MS

HS-SPME GC-MS analysis was performed using a DHS system with a Gerstel MPS autosampler with dual heads. The DHS sorbent material used was Tenax TA (2,6-diphenylene oxide polymer, Gerstel). Thai green curry paste was placed into a 20 ml headspace vial and sealed with a HACC and PTFE/Silicone septa. The sample was incubated at 80 °C for 5 min, followed by DHS extraction with a trapping temperature of 25, 35, 50, 65, or 70 °C and a trapping volume of 50, 100, 200, 300, or 500 ml using the Gerstel MPS with the agitator turned off.

Desorption of analytes from the Tenax TA material was performed inside the Gerstel TDU. The TDU was heated to 250 °C for 10 min, and the desorbed volatiles were transferred into the cryotrapping system. A cooling injector system (CIS) was used with the following temperature program: 10 °C for 1 min, ramped to 240 °C at a rate of 10 °C/min, and held at 240 °C for 20 min prior to GC-MS analysis with an oven temperature increase rate of 4 °C/min.

The optimal conditions for DHS GC-FID were determined to be a trapping temperature of 70 °C and a trapping volume of 200 ml. DHS GC-FID was not utilized in this work due to the unavailability of a DHS system combined with a single-head Gerstel MPS autosampler for use with our GC-FID system. It should be noted that the Gerstel MPS hyphenated with GC-FID or GC-MS is controlled using GERSTEL MAESTRO Software.

Data analysis

The volatile compounds in Thai green curry paste were identified by comparing the experimental mass spectra with the NIST 14 Mass Spectral Library (NIST 14, Washington, DC, USA). The identification was based on matching the experimental spectra to the spectra in the library. Further confirmation was achieved by comparing experimental retention indices with literature values.

The relative amounts of volatile compounds were quantified based on their peak area percentages. This quantification process involved measuring the peak areas of the compounds in the GC-MS chromatograms and calculating each compound's peak area as a percentage of the total peak area of all detected volatiles.

Results and Discussion

SHS GC-FID and HS-SPME GC-FID

GC-FID analysis of Thai green curry paste was performed using two automated sample preparation techniques: SHS and HS-SPME. Examples of SHS GC-FID and HS-SPME GC-FID are shown in figure1a and 1b, respectively.

For SHS GC-FID with an extraction time of 10 min, extraction temperatures from 40 to 90 °C, and syringe temperatures from 30 to 140 °C were investigated. Figure 2 shows a plot of total peak area versus extraction temperature at different syringe temperatures. For HS-SPME GC-FID, extraction temperatures from 40 to 90 °C and extraction times from 15 to 45 min were investigated. Figure 3 shows a plot of total peak area versus extraction temperature at different extraction times. To obtain the highest total peak area for GC-FID, an extraction temperature of 80 °C was chosen for both SHS and HS-SPME, with a syringe temperature of 50 °C for SHS and an extraction time of 30 min.

The SPME conditions were optimized as shown in figure 3. However, the fiber type was fixed with DVB/CAR/PDMS (grey) since this contains mixed materials offering a wider range of compounds such as the smaller molecules extracted by CAR, hydrophobic molecules extracted by PDMS or the additional interaction with aromatic compounds with DVB.

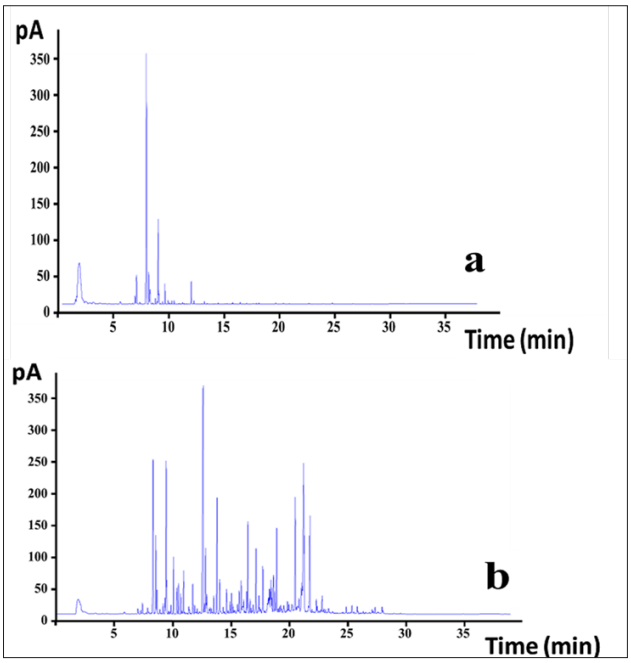

Figure 1: GC-FID chromatogram of Thai green curry paste prepared by **(a)** SHS, and **(b)** SPME under the optimized conditions of each method.

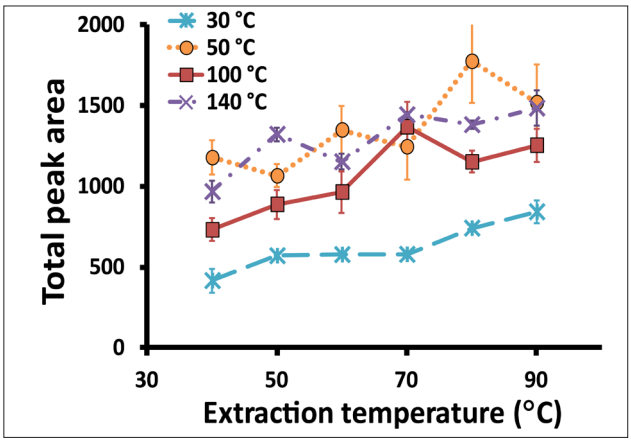

Figure 2: Plots of SHS extraction temperature and total peak area obtained from GC-FID analysis of Thai green curry paste prepared at different syringe temperatures: 30, 50, 100, and 140 ˚C (long dashed, dotted, solid, and dashed dotted lines, respectively).

SHS GC-MS, HS-SPME GC-MS and DHS GC-MS

GC-FID analysis of Thai green curry paste was performed using SHS, HS-SPME, and DHS automated sample preparation techniques. SHS and HS-SPME conditions used for GC-MS analysis were obtained from the GC-FID analysis, while the DHS trapping temperature and DHS extraction volume, which ranged from 50 to 300 ml, were varied. Figure 4 shows a plot of the total peak area versus extraction temperature at different extraction volumes. To obtain the highest total peak area for GC-MS, a trapping temperature of 80 ˚C and a trapping volume of 50 ml were determined as optimal for DHS GC-MS. An example of SHS, HS-SPME, and DHS under their optimized conditions for GC-MS analysis of volatile compounds in Thai green curry paste is shown in figure 5.

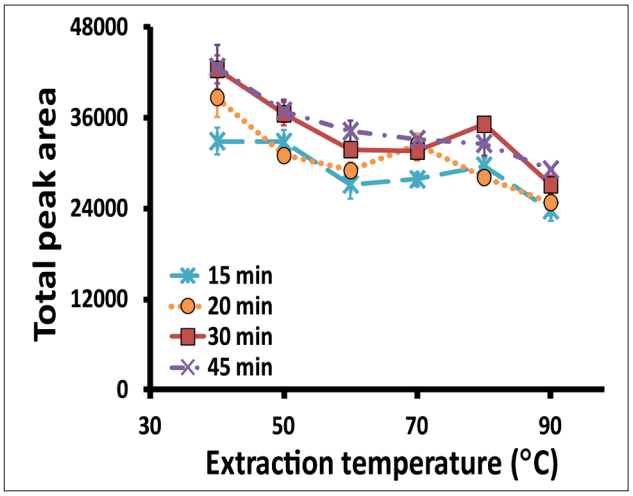

Figure 3: Plots of HS-SPME extraction temperature and total peak area obtained from GC-FID analysis of Thai green curry paste prepared at different extraction times: 15, 20, 30, and 45 min (long dashed, dotted, solid, and dashed dotted lines, respectively).

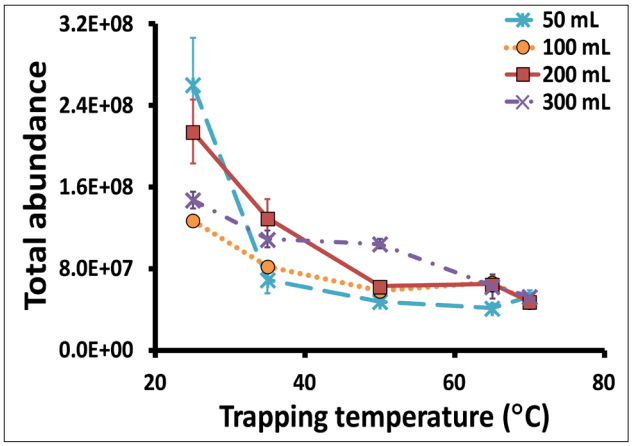

Figure 4: Plots of DHS trapping temperature and total peak area obtained from GC-MS analysis of green curry paste prepared at different extraction volumes: 50, 100, 200, and 300 ml (long dashed, dotted, solid, and dashed dotted lines, respectively).

Analysis of Thai green curry sample

SHS, HS-SPME, and DHS automated sample preparation techniques for GC-MS were applied for the analysis of Thai green curry paste in triplicate runs. The chemical composition of the sample was profiled by comparing the results with the NIST MS library and the RI database. The performance of the investigated sample preparation approaches was compared and discussed. Overall, all extraction analyses revealed more than 100 detected peaks, with 46 compounds identified by means of MS match scores more than 80% and a ± 20 RI deviation from literature values. All the identified compounds obtained from the headspace extraction methods under optimized conditions are documented in table 1.

The extraction performance for SHS, HS-SPME, and DHS can be expressed by the total peak area in GC-FID or GC-MS. As shown by the results of different headspace extraction techniques for the analysis of volatile compounds

Table 1: Comparison of SHS, HS-SPME, and DHS extraction.

Peak	RI		Peak area (10^3 counts × s)			CAS number	Compound names	Odor descriptions	Compounds found in previous work
	RI_{exp}	RI_{lit}	DHS	SPME	SHS				
1	< 800	477	-	56	-	75-31-0	2-propanamine	Fishy ammoniacal	-
2	915	890	-	-	187	488-97-1	cyclofenchene	-	-
3	917	929	-	35	-	7785-70-8	α-pinene	Harsh herbal terpene aromatic minty	LG [31], CL [32], CC [33], and GG [34, 35]
4	920	952	-	-	2104	79-92-5	camphene	Camphoreous cooling pine woody terpenic citrus green minty green spicy	CC [33], GG [34, 35], CM [36], and KL [37]
5	965	979	-	-	2221	127-91-3	β-pinene	Cooling, woody, piney and turpentine-like with a fresh minty, eucalyptus, and camphoraceous note with spicy peppery, and nutmeg nuance	CC [33], GG [34, 35], CM [36], and KL [37, 38]
6	968	974	2155	862	-	3387-41-5	sabinene	Woody spicy citrus terpenic green oily camphoreous	CC [33], GG [34], and KL [37]
7	986	1028	48	-	-	99-84-3	β-terpinene	Citrus-like, terpeny, herbal, fruity, and sweet	-
8	991	991	4946	-	-	123-35-3	β-myrcene	Peppery terpenic spicy balsamic herbal woody rose celery carrot	LG [31], CM [36], and LG [37]
9	1008	986	726	-	-	110-93-0	sulcatone	Powerful, fatty, green, and citrus	-
10	1020	1023	446	-	-	586-62-9	4-methyl-3-(1-methylethylidene)- cyclohexene	Lime and citrus fruit	-
11	1028	1023	1175	-	-	535-77-3	ρ-cymene	Citrus, woody, sweet, and earthy	CC [33], and CM [36]
12	1019	1030	26851	552	814	138-86-3	limonene	Citrus orange fresh sweet peel	LG [31], CC [33], LG [37], and KL [37]
13	1051	1060	3072	116	105	99-85-4	γ-terpinene	Oily woody terpenic lemon lime tropical herbal	CC [33], CM [36], and KL [37]
14	1076	1074	3274	-	-	60047-17-8	linalyl oxide or linalool-oxide	Green floral fatty woody fermented herbal fruity berry	KL [38]
15	1092	1086	1612	-	-	34995-77-2	trans-linalool oxide (furanoid)	Woody floral cooling terpenic green herbal earthy	KL [38]
16	1101	1099	4058	-	-	78-70-6	linalool	Citrus floral sweet bois waxy rose woody green blueberry orange	CC [33], GG [34, 35], CM [36], LG [37], KL [37], and KL [38]
17	1130	1122	837	-	513	29803-82-5	cis-2-menthenol	-	-
18	1142	1140	1049	-	-	29803-81-4	trans-2-menthenol	Floral	-
19	1154	1153	1322	-	-	565-48-0	β-terpinol	Pungent earthy woody pungent	-
20	1165	1163	388	-	-	59905-53-2	isopulegol	Minty cooling medicinal woody	LG [31], and LG [37]
21	1176	1177	46057	1279	-	562-74-3	terpinen-4-ol	Peppery woody earthy musty sweet	CC [33], GG [34, 26], and KL [37]
22	1196	1189	11111	-	-	98-55-5	α-terpineol	Pine woody resinous cooling lemon lime citrus floral	CC [33], GG [34, 35], CM [36], LG [37], KL [38], and KL [39]
23	1241	1239	24941	1310	-	122-03-2	cumal or cuminaldehyde	Spicy cumin green herbal	CM [36]
24	1307	1297	2959	-	-	2050-87-5	diallyl trisulfide	Strong GL smell	GL [39]
25	1345	1343	1953	-	-	57709-95-2	2-oxabicyclo[2.2.2]octan-5-ol, 1,3,3-trimethyl-, acetate, (1α,4α,5β)-	Minty clean fresh eucalyptus	-
26	1374	1376	1456	100	-	3856-25-5	copaene	Woody spicy honey	CC [33], KL [37], and KL [38]
27	1415	1435	56	-	-	13474-59-4	trans-α-bergamotene	Woody warm tea	LG [31], CC [33], and GG [34, 35]
28	1420	1419	1049	184	-	87-44-5	caryophyllene	Sweet woody spicy clove dry	GG [34, 35], KL [37], and KL [38]
29	1431	1435	227	31	-	17699-05-7	α-bergamotene	Woody warm tea	LG [31], CC [33], and GG [34, 35]
30	1447	1433	-	68	-	29873-99-2	γ-elemene	Dry fruity	CC [33]
31	1477	1453	-	26	-	6831-16-9	(-)-aristolene	Weedy scent	-
32	1444	1457	83	-	-	18794-84-8	(E)-β-famesene or its isomer	Woody citrus herbal sweet	LG [31], and CL [32]

33	1457	1457	181	-	-	18794-84-8	(E)-β-famesene or its isomer	Woody citrus herbal sweet	LG [31], and CL [32]
34	1463	1454	448	-	-	6753-98-6	humulene	Woody	LG [31], CC [33], and GG [34]
35	1483	1485	923	-	-	483-75-0	naphthalene, 1,2,4a,5,6,8a-hexa-hydro-4,7-dimeth-yl-1-(1-methylethyl)-	Fresh woody herbal vetiver	-
36	1488	1475	166	-	-	28477-64-7	(±)-β-acoradiene	-	CC [33]
37	1487	1492	164	142	-	4630-07-3	valencene	Citrus-derived odorants	GG [34]
38	1506	1509	432	82	-	495-62-5	β-bisabolene	Balsamic woody	LG [31, 37], and GG [34]
39	1515	1513	193	37	-	1460-97-5	γ-cadinene	Herbal woody	CC [33], and GG [34, 35]
40	1522	1524	1242	202	-	483-76-1	δ-cadinene	Thyme herbal woody dry	LG [31], CC [33], GG [35], KL [37], and KL [38],
41	1526	1508	-	17	-	502-61-4	α-farnesene	Citrus herbal lavender bergamot myrrh neroli green woody vegetable	LG [31], CL [32], and GG [35]
42	1624	1631	1253	661	-	1209-71-8	γ-eudesmol	Woody sweet	CC [33], and GG [34]
43	1639	1645	-	46	-	36564-42-8	δ-cadinol	Spicy odor	-
44	1659	1664	-	1126	-	532-65-0	aR-turmerone	Dry turmeric aroma	CC [33]
45	1705	1712	301	1856	-	108645-4-1	(E)-γ-atlantone	Clearly Atlas cedarwood	-
46	1744	1744	144	42	-	72445-42-2	mintsulfide	Heavy woody earthy odor	-

Note: RI_{exp} is the average experimental values obtained from triplicate runs for each extraction techniques with RSD < 1%, while RI_{lit} is the literature value obtained from NIST MS library. Peak area is the average value from triplicate runs with RSD < 20%, except for the small peak area of less than 10×10^3 counts × s. The typical ingredients in green curry paste include CL, GL, LG, KL, CM, CC, and GG.

in Thai green curry paste, a higher headspace temperature resulted in better SHS extraction performance, as seen in figure 2, but worse HS-SPME and DHS extraction performance, as shown in figure 3 and figure 4, respectively. This is because increasing the temperature in SHS extraction enhances the volatile compound content in the headspace. In contrast, both HS-SPME and DHS extractions involve the adsorption of volatiles onto materials and the desorption of volatiles from materials. A decrease in HS-SPME extraction performance at higher temperatures is due to the dominant effect of the desorption process from the SPME fiber. Generally, DHS extraction performance depends on the amount of compounds in the sample and the headspace, as well as the interaction of compounds with the Tenax material. In figure 4, the DHS extraction performance decreases with increasing extraction temperature due to the enhanced desorption process, a result of direct heating of the Tenax material, referred to as the trapping temperature. Therefore, a suitable extraction temperature of 80 ˚C was chosen for SHS and HS-SPME, while a trapping temperature of 25 ˚C was selected for DHS.

In addition, the performance of SHS depends on both the extraction time and the syringe temperature used for headspace injection into the GC injection port. Figure 2 demonstrates that the smallest total peak areas were observed at a syringe temperature of 50 ˚C, and the performance of SHS extraction improved with increasing syringe temperatures from 30 to 50 ˚C. This occurs because a syringe temperature that is too low can lead to the condensation of volatile compounds, preventing their injection into the GC inlet and causing them to remain in the syringe. Conversely, a syringe temperature that is too high (e.g., 100 and 140 ˚C) results in fewer volatiles being present in the syringe for the same volume and pressure, as described by the ideal gas law (nT = constant, where n is the number of moles of gas and T is the syringe temperature). Thus, a syringe temperature of 30 ˚C was selected for SHS GC-FID and SHS GC-MS.

In the SHS extraction time study, 10 and 15 min yielded comparable total peak areas, so an extraction time of 10 min was chosen. For HS-SPME, longer extraction times increased the amount of adsorbed compounds, with significantly larger total peak areas observed at 30 min or more (Figure 4). However, excessively long extraction times prolong the overall analysis time without further enhancing peak areas. Thus, HS-SPME extraction times of 30 or 45 min were considered optimal, with 30 min selected for this study.

In the HS study, it was anticipated that increasing the N2 trapping volume would enhance the amount of volatiles adsorbed onto the Tenax material, similar to increasing extraction time, as shown by the larger total peak areas observed with a 300 ml extraction volume at trapping temperatures of 50 ˚C or higher (Figure 4). Nonetheless, a larger N_2 flow can also flush compounds from the Tenax material and cause heat convection from the sample chamber (at the 80 ˚C extraction) to the material. This effect reduced DHS extraction performance, particularly at lower trapping temperatures, resulting in the highest total peak areas being observed with a 50 ml trapping volume at a trapping temperature of 25 ˚C.

Analysis of green curry sample

As illustrated in figure 5 and detailed in table 1, only six volatile compounds in Thai green curry paste were clearly identified using SHS GC-MS, while HS-SPME GC-MS identified a greater number of volatile compounds, totaling twenty-two. Conversely, DHS GC-MS identified thirty-six volatile

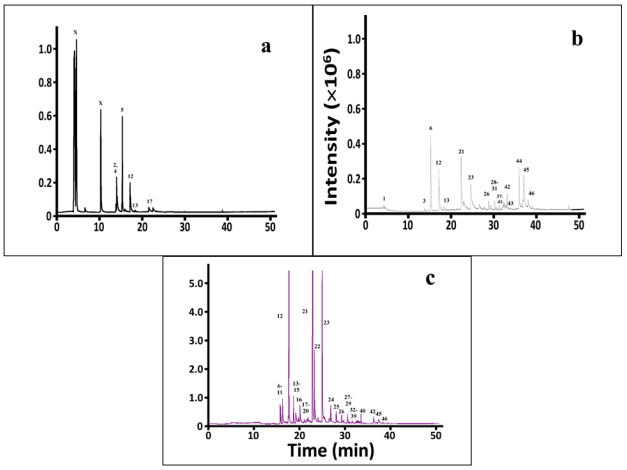

Figure 5: GC-MS chromatograms of Thai green curry paste prepared by **(a)** SHS, **(b)** SPME, and **(c)** DHS under the optimized conditions of each method.

compounds in the same sample. Overall, a total of forty-six distinct volatile compounds were identified using the combination of SHS GC-MS, HS-SPME GC-MS, and DHS GC-MS. The GC-MS analysis revealed that twenty-four volatile compounds with higher LRI values (> 1125) were detected using DHS extraction, while seventeen such compounds were identified using HS-SPME extraction, with none of these being detected using SHS. Conversely, three volatile compounds with lower LRI values (< 960) were observed with SHS extraction, and two with HS-SPME extraction, but these were not identified using DHS. This suggests that SHS is more suitable for detecting easily volatile compounds compared to DHS. Among the twenty-two volatile compounds identified by HS-SPME, seven were not detected using DHS. Conversely, of the thirty-six volatile compounds identified through DHS, twenty-one were not observed using HS-SPME.

In general, limonene and γ-terpinene were observed with all the extraction approaches. The major volatile compounds in the green curry include (1) terpinen-4-ol, limonene, cumal, α-terpineol and β-myrcene using DHS GC-MS, (2) cumal, terpinen-4-ol, aR-turmerone, sabinene and limonene using HS-SPME GC-MS, and (3) β-pinene, camphene, limonene, cis-2-menthenol and γ-terpinene using SHS GC-MS. Comparison with the other analytical method was also performed with the more advance instrument of comprehensive two-dimensional GC [40], and the identified compounds could be confirmed by the other studies using different techniques.

VOCs found in green curry paste may originate from individual raw ingredients as cited in Table 1 or derived from paste manufacturing. According to previous work, the key VOCs found in green curry paste may be obtained from the following ingredients: terpinene-4-ol from KL [28], curcuma (CC) [33], and GG [34, 35]; limonene from LG [31, 37], KL [37], and CC [33]; cumal from CM [36]; α-terpineol from CM [36], KL [37, 38], LG [37], CC [33], and GG [34, 35]; β-myrcene from LG [31, 37], and CM [36]; linalool from CM [36], KL [37, 38], CC [33], GG [34, 35], and LG [37]; linalyl oxide from KL [38]; γ-terpinene from CM [36], KL [37], and CC [33]; diallyl trisulfide from GL [39]; sabinene from KL [37], CC [33], and GG [34]; trans-linalool oxide from KL [38]; copaene from KL [37, 38], and CC [33]; δ-cadinene from LG [31], KL [37, 38], CC [33], and GG [35]; ρ-cymene from CM [36], and CC [33]; caryophyllene from KL [37, 38], and GG [34, 35].

Conclusion

Different automated headspace microextraction approaches were investigated prior to analysis with GC-FID or GC-MS. The study revealed that each method had distinct extraction mechanisms for green curry paste, as indicated by their unique optimization profiles. SHS extraction performance mainly depended on the amount of volatile compounds in the headspace, while SPME exhibited additional mechanisms, such as increased desorption of volatiles from the SPME fiber at higher extraction temperatures. DHS operation allowed for more adjustable parameters, with performance governed by the Tenax material temperature and N_2 flow volume. All the extraction approaches, under their optimized conditions, were applied to profile the chemical composition of Thai green curry paste. In total, 46 peaks were identified, representing various volatile classes, including alkanes, ketones, amines, terpenes, tetrahydrofuran's, organosulfur compounds, aromatic hydrocarbons, and sulfur-containing sesquiterpenes. The methods and materials described provided acceptable extraction and analytical performance, which is particularly useful in the field of food analysis.

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Conflict of Interest

None.

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