การพิสูจน์เอกลักษณ์ของสารระเหยง่ายที่ให้กลิ่นในต้มยำโดยแก๊สโครมาโทกราฟี-แมสสเปกโทรเมตรีร่วมกับเทคนิคการประเมินทางประสาทสัมผัส

นางสาวพรรณิภา จันทา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2560

CHARACTERIZATION OF AROMA-ACTIVE VOLATILE COMPOUNDS IN TOM YUM SOUP BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY COMBINED WITH SENSORY EVALUATION TECHNIQUES

Miss Pannipa Janta

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2017

Thesis Title	CHARACTERIZATION OF AROMA-ACTIVE VOLATILE COMPOUNDS IN TOM YUM SOUP BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY COMBINED WITH SENSORY EVALUATION TECHNIQUES
By	Miss Pannipa Janta
Field of Study	Chemistry
Thesis Advisor	Associate Professor Thumnoon Nhujak, Ph.D.
Thesis Co-Advisor	Chadin Kulsing, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

....Dean of the Faculty of Science

(Associate Professor Polkit Sangvanich, Ph.D.)

THESIS COMMITTEE

....Chairman

(Associate Professor Vudhichai Parasuk, Ph.D.)

....Thesis Advisor

(Associate Professor Thumnoon Nhujak, Ph.D.)

....Thesis Co-Advisor

(Chadin Kulsing, Ph.D.)

....Examiner

(Assistant Professor Puttaruksa Varanusupakul, Ph.D.)

....Examiner

(Panita Ngamchuachit, Ph.D.)

....External Examiner

(Natthida Sriboonvorakul, Ph.D.)

พรรณิภา จันทา : การพิสูจน์เอกลักษณ์ของสารระเหยง่ายที่ให้กลิ่นในต้มยำโดยแก๊สโครมาโทกราฟี-แมสสเปกโทรเมตรีร่วมกับเทคนิคการประเมินทางประสาทสัมผัส (CHARACTERIZATION OF AROMA-ACTIVE VOLATILE COMPOUNDS IN TOM YUM SOUP BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY COMBINED WITH SENSORY EVALUATION TECHNIQUES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.ธรรมนูญ หนูจักร, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. ดร.ชฎิล กุลสิงห์, 96 หน้า.

ได้ประยุกต์เทคนิคแก๊สโครมาโทกราฟี-แมสสเปกโทรเมตรี/โอแฟกโทเมทรีร่วมกับเฮดสเปซโซลิดเฟสไมโครเอกซ์แทรกชัน (HS-SPME-GC-O/MS) สำหรับพิสูจน์ทราบสารระเหยง่ายในต้มยำและเครื่องเทศที่เป็นส่วนประกอบพื้นฐาน ได้แก่ ตะไคร้ ใบมะกรูด พริกขี้หนู น้ำปลา และน้ำมะนาว จากการใช้เทคนิค HS-SPME ด้วยไฟเบอร์ชนิด 50/30 µm DVB/CAR/PDMS และอุณหภูมิการสกัดที่ 40 องศาเซลเซียส เป็นเวลา 50 นาที พร้อมด้วยคอลัมน์แคพิลลารีชนิด HP-5MS ที่โปรแกรมอุณหภูมิจาก 50 ถึง 200 องศาเซลเซียส ที่อัตรา 3 องศาเซลเซียสต่อนาที และการไอออไนซ์แบบการชนด้วยอิเล็กตรอนของแมสสเปกโทรเมตรีที่ -70 eV พบว่ามีการตรวจพบ 101 พีคที่ปรากฏในโครมาโทแกรมของต้มยำ และสามารถระบุสารระเหยง่าย 96 ชนิด ได้แก่ แอลกอฮอล์ แอลดีไฮด์ เอสเทอร์ อีเทอร์ และเทอร์ปีน เป็นต้น การตรวจพบเหล่านี้ทำโดยการเปรียบเทียบแมสสเปกตรัมของสารกับระบบสืบค้น NIST พร้อมด้วยค่ารีเทนชันอินเด็กซ์จากการทดลองและค่าอ้างอิง จากการเปรียบเทียบโปรไฟล์สารระเหยง่ายของเครื่องเทศแต่ละชนิดของต้มยำทั้งก่อนและหลังปรุง พบว่ามีสารระเหยง่าย 5 ชนิดที่เกิดขึ้นหลังจากกระบวนการปรุง ได้แก่ *p*-mentha-3,8-diene, α-cyclocitral, iso-isopulegol, *p*-mentha-1,5-dien-8-ol และ decyl acetate ซึ่งอาจมาจากปฏิกิริยาทางเคมี เช่น การปิดวงของสารเคมีที่อยู่ในเครื่องเทศที่ผสมกัน นอกจากนี้ยังได้ตรวจพบและอธิบายสารให้กลิ่น 18 ชนิดที่ให้กลิ่นลักษณะของต้มยำ โดยเฉพาะอย่างยิ่งสารให้กลิ่นที่เด่น 7 ชนิด ได้แก่ β-citral, geranial, β-linalool, geraniol, nerol, 3-(methylthio)propanal and 2-isobutyl-3-methoxypyrazine โดยที่สาร 4 ชนิดแรกเป็นสารให้กลิ่นในต้มยำที่มาจากตะไคร้ ใบมะกรูด และน้ำมะนาว ส่วนสาร 3 ชนิดที่เหลือเป็นสารให้กลิ่นที่มาจากตะไคร้ น้ำปลา และพริกขี้หนู ตามลำดับ

ภาควิชา	เคมี	ลายมือชื่อนิสิต ....
สาขาวิชา	เคมี	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
ปีการศึกษา	2560	ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

5772074323 : MAJOR CHEMISTRY

KEYWORDS: GC-MS / SPME / TOM YUM SOUP / VOLATILE COMPOUNDS

PANNIPA JANTA: CHARACTERIZATION OF AROMA-ACTIVE VOLATILE COMPOUNDS IN TOM YUM SOUP BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY COMBINED WITH SENSORY EVALUATION TECHNIQUES. ADVISOR: ASSOC. PROF. THUMNOON NHUJAK, Ph.D., CO-ADVISOR: CHADIN KULSING, Ph.D., 96 pp.

Gas chromatography-mass spectrometry/olfactometry 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 including lemongrass, kaffir lime leaf, chili, fish sauce and lime juice. 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 programmed from 50 to 200 °C at 3 °C/min and an MS electron impact ionization at -70 eV, 101 peaks in the HS-SPME-GC-MS chromatogram of Tom Yum soup were detected, and 96 volatile 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 volatile compound profiles of each individual ingredient of Tom Yum soup (both before and after cooking), five extra volatile compounds including *p*-mentha-3,8-diene, α-cyclocitral, iso-isopulegol, *p*-mentha-1,5-dien-8-ol and decyl acetate were found after the cooking process, possibly due to chemical reactions (such as cyclization) among the compounds in the mixed ingredients. Furthermore, eighteen aroma compounds that contribute to the impressive aroma of Tom Yum soup were detected and described. Especially, the seven dominant aroma compounds found were β-citral, geranial, β-linalool, geraniol, nerol, 3-(methylthio)propanal and 2-isobutyl-3-methoxypyrazine. The first four aromas detected in Tom Yum soup are from the lemongrass, kaffir lime leaf, and lime juice; the last three aroma compounds are from lemongrass, fish sauce and chili, respectively.

Department:	Chemistry	Student's Signature	....
Field of Study:	Chemistry	Advisor's Signature	....
Academic Year:	2017	Co-Advisor's Signature	....

ACKNOWLEDGEMENTS

First of all, I am very grateful to my advisor, Associate Professor Dr. Thumnoon Nhujak, for his professional, helpful guidance, valuable advice, and proofreading throughout the research project. I am very grateful to my co-advisor, Dr. Chadin Kulsing for helpful guidance, valuable advice, and proofreading throughout the research project. I am also grateful to Professor Dr. Vudhichai Parasuk, Assistant Professor Dr. Puttaruksa Varanusupakul and Dr. Panita Ngamchuachit for their kindness, useful comments and suggestions. I wish to especially thank Assistant Professor Dr. Kanjana Mahattanatawee for her valuable guidance and suggestions for the research project.

I gratefully acknowledge the financial support from the Chulalongkorn University's Ratchadapisek Sompot Fund (GCURP_59_02_23_01) for the research project and the Chulalongkorn Academic Advancement into Its 2nd Century Project for instruments used in CMSS.

I am grateful to Mrs. Chanchana Siripanwattana and chef at Suan Dusit University for advice and helpfulness about the recipe of Tom Yum soup.

I would like to thank all member of Chromatography and Separation Reseach Unit (ChSRU) and special thanks to Mr. Poowadol Thammarat for providing a benefit suggestion as well as my friends for their friendship and encouragement.

Additionally, I would like to thank Department of Chemistry, Faculty of Science, Chulalongkorn University for supplying the instruments and all facilities in this research.

The last, I am grateful to my beloved family: my parents and my brother for their endless supporting, helpfulness and encouraging throughout my life.

CONTENTS

Page

Page

LIST OF FIGURES

Page

LIST OF TABLES

LIST OF ABBREVIATIONS

°C	degree celsius
ANOVA	analysis of variance
CAR	carboxen
Da	dalton
DVB	divinylbenzene
EI	electron ionization
eV	electron volts
g	gram
GC	gas chromatography
GC–O	gas chromatography–olfactometry
HP-5MS	(5%-Phenyl)-methylpolysiloxane
HS	headspace
I	retention index
min	minute
mL	milliliter
MS	mass spectrometry
ms	millisecond
NIST	national institute of standards and technology
ODP	olfactory detection port

PCA	principal component analysis
PDMS	polydimethylsiloxane
PTFE	polytetrafluoroethylene
RSD	relative standard deviation
RT	retention time
SD	standard deviation
SPME	solid-phase microextraction
TIC	total ion chromatogram

CHAPTER I
INTRODUCTION

1.1 Problem definition

Tom Yum, a spicy and sour soup, is one of the Thai dished that shows authentic flavor and has been well-known in many countries. Currently, the products of Tom Yum have various types that present in supermarket like instant noodles, snacks, frozen Tom Yum products [1] and dried seasoning [2, 3]. In addition Tom Yum is also several health benefits such as cancer prevention and antioxidant properties because of its ingredients [1]. Its main ingredients typically include lemongrass, kaffir lime leaf, chili, galangal and lime juice and the soup exhibits an impressive aroma and taste. Nowadays, several additional ingredients are also added into Tom Yum soup to enhance the flavor and colors (e.g. chili paste, coriander, and shallot). Aroma is especially resulted from volatile compounds in original and cooked ingredients. Because each ingredient releases of many complex mixture volatiles compounds such as terpene hydrocarbons, oxygenated compounds, monoterpene alcohols, sesquiterpene alcohols, aldehydes, ketones, esters, lactones, coumarins, ethers, and oxides [4], which those compounds lead to individual odor feature. For example, lemongrass contribute strong lemony aroma [5], galangal release sweet floral and camphoraceous aroma [6], or kaffir lime leaf show strong citrus aroma [7]. As indicating that various spices are released different aroma due to their various volatile compound profiles which it could show characteristic of each ingredient.

The study of constituents of volatile compounds to identify aroma compounds is widely used gas chromatography–mass spectrometry (GC–MS) technique which appropriate for volatile and semi-volatile organic compounds. Benefits of an MS detector are precisely identified volatile compounds in sample by compared their mass spectra with available libraries as well as accurate qualitative and quantitative analysis. Moreover volatile compounds are often identified according to retention index data [8] to double confirmation with MS library. Therefore GC–MS is popularly determined volatile compounds than other besides that in the analysis of aroma compounds is mostly used GC–olfactometry (GC–O), sensory evaluation technique, to correlate chemical compositions with the characteristic flavors of food [9].

Sample preparation techniques that are conventionally applied for the extraction of volatile compounds 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 lost and side reactions during the extraction. Alternatively, headspace solid phase micro extraction (HS–SPME) can be applied, offering simple and fast extraction process where volatile compounds in sample headspace can be adsorbed onto the SPME materials, e.g., divinylbenzene-based fibers for spice analysis [10] and directly injected into the GC inlet. It should be noted that the analytical method is an important part to identify both volatile and aroma compounds in various food and HS-SPME-GC─O/MS is one of the popularly analytical method used in various food research such as wine [11], green tea [12], honey [13] and fish sauce [14]. Therefore characterization of the constituents inside the complex matrix of Tom Yum and its individual ingredient by HS–SPME–GC─O/MS is still a challenge.

1.2 Literature Review

Special focus is on Tom Yum with its origin in Thailand which is a type of hot and sour soup usually cooked with shrimp. In recent years, Tom Yum is widely served in neighboring countries such as Cambodia, Brunei, Malaysia, Singapore, and Myanmar, and it has been popularized around the world [2]. Tom Yum ingredients typically include lemongrass, kaffir lime leaf, chili and lime juice [1] that show impressive aroma and taste. Aroma is especially resulted from volatile compounds in original and cooked ingredients because these ingredient contain various volatile profiles contributing to characteristic aroma of Tom Yum and individual ingredient. These volatile compounds also have a broad molar mass range, variable polarity, and a wide range of chemical abundance [4].

Apart from the chemical profiling, sensory analysis is also important in order to assess physical descriptors contributing to the aroma and tastes of Tom Yum. Due to complexity in food, such analysis can be effectively performed using GC with olfactometry (GC–O) [15] where compounds were separated prior to the sniffing analysis of the individual compound. This analysis can be combined with MS detection and can be named as GC–O/MS providing comprehensive information of both chemical components and odor description. To this end, the separated compounds will be splitted into two portions at the outlet of the analytical column. One portion is directed to the MS while the other portion is transferred through the hot transfer line and sniffed by trained panelists. The sniffing data are analyzed in order to identify odor description of the compound as well as rough quantification result. The data are used to confirm compound identity obtained with MS analysis for the same peaks eluting within the same times [15]. A lot of previous works that studied about volatile and aroma compounds of individual ingredient (lemongrass, kaffir lime leaf, chili and lime juice) will be discussed.

Lemongrass, *Cymbopogon citratus* (DC.) Stapf, is a tropical plant and its name come from the typical lemon-like odor [16]. Three species of lemongrass are *Cymbopogon flexuosus*, *Cymbopogon citratus* (DC.) Stapf and *Cymbopogon pendulus* (Nees ex Steud) Wats which three species knows as East Indian, West Indian and Jammu lemongrass, respectively [16]. In previous work [17] using HS–SPME–GC–MS with extraction temperature 35 °C and extraction time 60 min, 56 volatile compounds were found in powder of *Cymbopogon citratus* (DC.) with the major components such as being two isomers of citral: geranial (23.9%) and neral (19.7%), and the minor compounds such as *E*-caryophyllene (5.0%), citronellal (3.8%) and Linalool (3.0%). In addition, the extracted lemongrass oil was also found to contain two citral isomers as a major compound (65-80%) as well as other compounds such as limonene, citronellal, β-myrcene and geraniol [18]. Furthermore, aroma compounds of extracted lemongrass oil was also investigated by GC─O/MS [5], and lemony and floral odor were found to be dominant odor perception especially resulted from citral and geraniol, which are the major components in extracted lemongrass oil.

Kaffir lime leaf, *Citrus hystrix* (DC.), is one of the genus *Citrus* which indicates characteristic aromatic plant and its chemical compositions is unique and differs from other citrus leaf [19]. The main volatile compounds of extracted kaffir lime leaf essential oil were observed by some unpublished sources, monoterpenoids group: citronellal, β-myrcene, limonene, terpinen-4-ol, citronellol, citronellyl acetate, geranial, geranial acetate, β-pinene and neral [19]. Using an HS–SPME–GC–MS analysis of Kaffir lime leaf, a major compound includes citronellal (48.2%) as well as other compounds including citronellol, citronelly acetate and linalool (14.3, 7.78 and 5.13%, respectively) [19]. In addition, aroma active compounds of extracted Kaffir lime leaf were also identified by GC─O/MS using solvent extraction [7]. Citronellal and L-linalool were considered as key odorants because they showed the highest Log$_3$FD values (=4) and also corresponded to the top notes of citrus aroma. Otherwise β-citronellol and *trans*-geraniol were also considered as key odorants due to Log$_3$FD values equivalent of 3 [7].

Chili, *Capsicum frutescens* L., is popularly used in various food because it shows characteristic of color, pungency and aroma [20]. Using an HS─SPME─GC─MS analysis of Brazilian chili peppers, 83 compounds was found, mostly esters (40%) such as 2-methylpentyl hexanoate, hexyl-2,2-dimethyl propanoate and hexyl-3-methyl butanoate [21].

In addition, chili essential oil from Colombia mostly contains esters being isohexyl isohexanoate, isohexyl isovalerate, isohexyl 2-methylbutyrate and hexyl isovalerate [22]. In addition, sensory analysis of Capsicum fruits from the annuum-chinense-frutescens complex revealed that strong aroma was come from 3-isobutyl-2-methoxypyrazine (paprika, green, earthy) and 2-heptanethiol (paprika, green, kerosene-like) [23].

Lime, *Citrus aurantifolia* (Christm.) Swingle, well-known in citric fruit, is popularly ingredient such as alcoholic and nonalcoholic drinks, food additives and typical dishes [24]. The essential oil of lime was found to contain 32 compounds being mostly limonene (37%) and other major compounds including β-pinene (16%), γ-terpinene (9.5%), nerolidol (7.1%) and α-terpineol 6.7% with aldehydes (neral, geranial, dodecanal, tetradecanal) and esters as the minor components such as neryl acetate, geranyl acetate [25]. In addition, odor-active volatile compounds of extracted and distilled key lime oils indicated dominate compounds that contributed fresh, floral citrus-like odor: geranial, neral and linalool due to high odor spectrum values, and three compounds are also correlated for the characteristic fresh aroma of cold-pressed lime oil [26].

Other ingredients possibly added in Tom Yum includes galangal, *Alpinia galangal* (L.) and fish sauce, essential oil of galangal was found to contain the major components of 1,8-cineol (63.4%), α-terpineol (2.8%) and terpinen-4-ol (2.8%) with other compounds contributing to < 2% (α-pinene and β-pinene) [27]. Characteristic odor of this spice is a result from 1,8-cineol because its show the eucalyptus-like odor [28] and also important in pharmaceutical properties [27].

Fish sauce is another ingredient popularly used for enhance aroma and taste in worldwide. Fermenting fish with salt for several month, protein hydrolysate is occurred and produced flavor component [29]. Using GC─MS, SPME─Osme─GCO to characterize aroma-impact compounds of four fish sauce samples (2 Korean and 2 Thai fish sauce), perceived key aroma is a result from trimethylamine (fishy aroma), butanoic, pentanoic, hexanoic and heptanoic acids (pungent and dirty socks aroma), 2,6-dimethyl pyrazine (cooked rice and buttery popcorn aroma) and benzaldehyde (sweet and cotton candy aroma) [14]. In previous work [30] on HS─SPME identification of dominant aroma compounds in premium commercial Thai fish sauce, the following key aroma components contributed odor perception with OAVs > 500: methanethiol for rotten, 2-methylpropanal for dark chocolate, dimethyl trisulfide for sulfurous, 3-(methylthio)propanal for potato, and butanoic acid for cheesy aroma. In addition, other aroma compounds with odor-activity values (OAVs) >100 included dimethyl sulfide for corn, 2-methylbutanal for dark chocolate, acetic acid for sour, propanoic acid for cheesy, 2-methylpropanoic acid for Swiss cheese, and 3-methylbutanoic acid for sweaty.

1.3 Aim, scope and expected benefits of this work

As previous mentioned, chemical profiles of individual ingredient have been reported. A similar HS─SPME─GC─O/MS procedure was also used for supplementary data about GC─MS and HS─SPME conditions while characterization of their compositions inside the complex matrix of Tom Yum is still a challenge where generation of new compounds (e.g. caused by chemical interaction between the mixed ingredients) can be expected.

Firstly, GC─O/MS was optimized to obtain suitable separation and identification of volatile compounds in headspace extraction of Tom Yum soup. GC separation was also optimized to obtained suitable temperature program and injection mode (split ratio). In addition, MS detection was performed using the electron ionization voltage of -70 eV for a quadrupole mass analyzer with a scan mode. Secondly, the HS─SPME sample preparation of Tom Yum soup, using Polydimethysiloxane/carboxen/divinyl-benzene (PDMS/CAR/DVB) fiber, was optimized for GC─O/MS identification of volatile compounds: extraction temperature and time. Thirdly, the optimized HS─SPME─GC─O/MS was applied to identify and compare volatile compounds Tom Yum soup and individual raw and boiled ingredients, according to a comparison of their mass spectra with those from NIST library with match scores of >650, and also their experimental and literature values of linear retention index. Fourthly, aroma compounds were identified combined with sensory evaluation technique for the detection and description of the aroma compounds in the extracted Tom Yum soup. Finally, various commercial products of Tom Yum paste were compared for their volatile and aroma compounds.

Therefore, the objective of this work is to identify and compare the volatile compounds of Tom Yum soup and its individual ingredient as well as their odor descriptions. To our best knowledge, there have been no reports on 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.

The benefit of this work is to know aroma compounds that contribute to the impressive aroma of Tom Yum soup as well as extra volatile compounds from cooking process and can be applied for other Thai dishes.

CHAPTER II

THEORY

2.1 Tom Yum soup

Tom Yum soup is one of the traditional Thai dishes which has unique aroma, taste and color and provides health benefit such as cancer prevention, antioxidant properties and low calories [1]. The basic ingredients of Tom Yum soup are lemongrass, kaffir lime leaf, galangal and lime juice [3]. However, various kinds of herbs and spices were also added to enhance flavor such as shallots, mushroom, tomato and coriander [2]. The common seasonings of Tom Yum soup are fish sauce, sugar and chili paste. In Japan and Thailand, researchers have discovered that the ingredients of Tom Yum show inhibition of tumor growth in the digestive system [31]. The cooking process of Tom Yum started with boiling water (or soup stock) and addition of galangal root, lemongrass, kaffir lime leaf and chili. Meat is then added. Lime juice, tamarind pulp and dried garcinia slices were added in the last step [3].

Tom Yum becomes popular worldwide. A lot of Tom Yum soup products are thus available in the shelf, e.g. as instant noodles, snacks, frozen Tom Yum products [1] and dried seasoning [2, 3]. The other important product of Tom Yom is instant Tom Yum soup which contains flavor and color (as well as fresh Tom Yum soup) and is also ready to cook. However, aroma of instant Tom Yum soup may not the same as fresh Tom Yum soup since the aroma compounds could be lost during heating and drying process [1]. Instant Tom Yum soup can be divided into 3 types: soup, powder, and paste forms.

The impressive aroma of Tom Yum soup is especially resulted from volatile compounds in original and cooked ingredients including alcohols, phenols, aldehydes, esters, terpenes and alkaloids in various proportions. Food manufactures often try to synthesize ingredients instead of using natural products as the addition of instant Tom Yum soup [3]. For example, citric acid is used to enhance sour taste instead of lime juices. Synthetic ingredients also remain the physical and chemical properties after the cooking process preserving the characteristic of Tom Yum soup.

2.2 Gas Chromatography

Since gas chromatography (GC) has been established since the late-1940s. This technique is recognized as high resolution, good reliability and repeatability, and its ease of coupling with mass spectrometry (MS) to provide a capability to identify compounds. GC has been noted for excellent separation and identification of volatiles and some low-volatility compounds. Early applications of GC involved the analysis of hydrocarbons in oils, fatty acid methyl esters (FAME), short-chain alcohol by-product from wine fermentation [32], phenolic compounds, sugars and flavonoids (as silyl derivatives).

A schematic diagram for a conventional GC instrument is shown in Figure 2.1, including an inlet, a capillary column in an oven with controlled temperature program, and a detector.

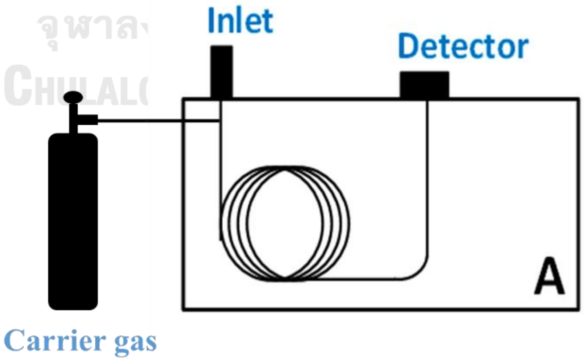


Figure 2.1 GC diagram. Adapted from [33].

2.2.1 Carrier gas

The gas lines deliver carrier gas from a gas tank through the inlet, column and detector with a constant gas flow or constant column head pressure. Samples can be injected into the column inlet. Use of an autoinjector is recommended to improve accuracy in repeated injections; high speed or suitably focused injection reduces peak broadening effects [34]. Analytes are separated through interaction with a stationary phase inside the column, and detected by the detector with the signal recorded, and finally observed as a chromatogram (a plot of signal vs time).

Different carrier gases can be applied depending on detector and performance requirements. Helium (He) is often used as a carrier gas, especially with MS detection. Separation in GC is based on the analyte boiling point difference and interactions of the analytes with the stationary phase. Analytes with lower boiling points are likely to elute earlier and the elution order can be altered by the effect of analyte/phase interactions.

2.2.2 Injector

The most common type of injector applied with capillary columns is the heated split/splitless injector [32] operating in split or splitless mode. Selection of the injection mode normally depends on compound concentrations in samples. Figure 2.2 is a schematic diagram representing of a typical split/splitless injector.

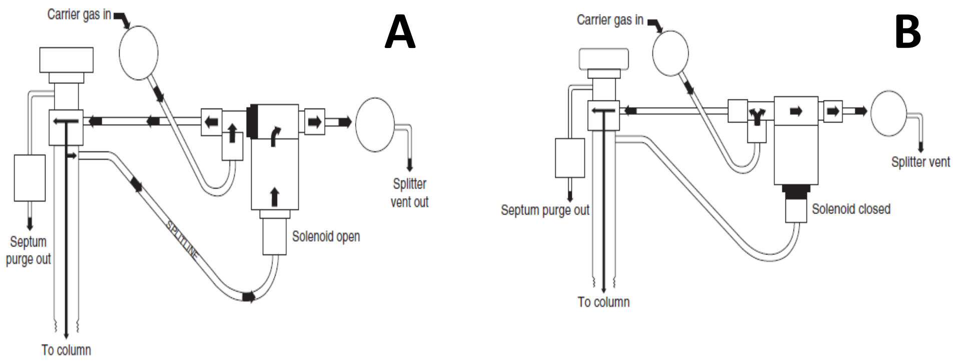


Figure 2.2 The flow path of the carrier gas in split (A) and splitless (B) modes. Reproduced from [32].

Both split and splitless injection modes are performed with the high injector temperature being sufficient to vaporize the solvent and the analytes of interest in samples. This injection temperature is normally constant throughout the GC analysis. Split injection is used for neat samples that cannot be dissolved in a solvent or samples with relatively high concentrations. The splitless mode is applied when samples contain analytes at trace levels.

- Split mode

The sample is injected and vaporized into the carrier gas stream. A small portion of the sample and solvent is then transferred onto the GC column inlet. The rest of the sample is vented to waste (Figure 2.2). Typical split ratios can range from 5:1 to 400:1 and can be calculated according to the equation.

$$\text{Split ratio}=\frac{\text{Column flow+Vent flow}}{\text{Column flow}}$$

- Splitless mode

In the splitless mode, the sample is injected with the splitter vent closed for a certain period (*e.g.* 0.5-1.0 min). Without sample discrimination during injection, this allows all the injected amount of the sample and solvent onto the GC column inlet. The rest of the sample goes to waste after the splitter vent is turned on.

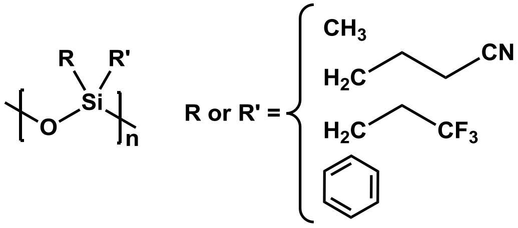
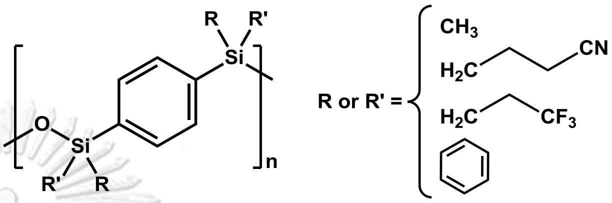
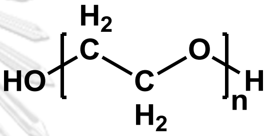
This technique is applied for trace analysis of compounds in samples or analysis of compounds with a fairly narrow boiling-point range. This mode is not suitable for injection of thermally labile compounds.

2.2.3 Column

A goal in GC is to obtain appropriate separation of analytes in chromatograms. Apart from difference in analyte boiling points, separation in GC can be optimized by changing experimental conditions, such as temperature, and stationary phase type; column dimensions and flow. Retentions of different analytes decrease at higher temperature. Therefore, variation of the temperature program in GC is a simple and straightforward method to tune separation result together with carrier gas flow optimization to improve theoretical plate number in separation. Change of stationary phase chemistry can redistribute analyte peak positions in chromatograms depending on different interactions between each analyte and the phase. Stationary phases can be referred to as the 'heart' of GC experiment as it critically affects the separation results. Several types of GC stationary phases have been developed, for different separation goals.

Common GC stationary phases providing separation based on polar/non-polar interactions and analyte boiling point differences are shown in Table 2.1. Their polarities depend on the number of phenyl (more polar with higher phenyl content), fluorinated alkyl, or other functional groups in each phase.

Table 2.1 Structure of stationary phase

Type of material	Structure
R,R'-polysiloxane	
Arylene (Low bleed)	
Poly(ethylene glycol) (PEG)	

2.3.4 Detector

Among different types of detectors, flame ionization detector (FID) is the most popular detection for hyphenation with GC due to its versatility, fast response, durability, ease of operation, low dead volume, low detection limit, and good linearity range. Some other common detectors include mass spectrometer (MS), electron-capture detector (ECD), thermal conductivity detector (TCD), flame photometric detector (FPD), nitrogen phosphorous detector (NPD), and atomic emission detector (AED).

2.3 Mass spectrometry

GC performance can be improved by coupling with mass spectrometry (MS). MS is a suitable and powerful tool for volatile analysis because it offers selective detection and qualitative characterization using MS information of the chromatographically separated compounds. GC─MS is also useful for efficient quantitative analysis [35].

2.3.1 Ion source: Electron ionization

Electron ionization (EI) is the most widely used ion source with GC─MS. Analyte molecules are shot with the electron beam resulting in molecular ions (M+•) with high internal energy. These ions will fragment into smaller ions resulting in multiple-peak MS spectra. EI relies on interaction of a low-pressure (~10^{-1} Pa) gas with electrons accelerated with the energy of -70 eV which is conventionally performed in routine analysis and establishment of library. As shown in Figure 2.3, EI source consists of a heated filament producing electrons accelerated towards an anode. These electrons collide with the analyte molecules in gas phase of the sample injected into the source. Compounds with low boiling points are introduced directly into the source.

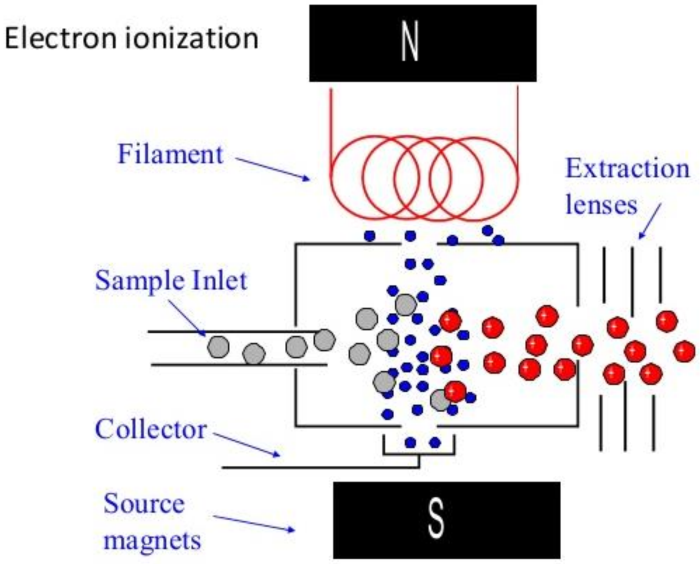


Figure 2.3 Diagram illustrating an electron ionization source. Reproduced from [36].

2.3.2 Mass analyzer

After ions are generated in the EI source, they are accelerated into a mass analyzer by application of an electric field. The mass analyzer differentiates these ions based on their *m/z* values. The selection of a mass analyzer depends on the analysis aims, *e.g.* focusing on MS resolution, mass range, scan rate or detection limits.

2.3.2.1 Quadrupole

One of the most common mass analyzer in GC–MS is quadrupole (Q) consisting of four rods or electrodes arranged across from (and in parallel to) each other, Figure 2.4. Ions travelling through the Q are filtered according to their m/z values. As a result in one moment, only a single m/z value ion can pass the Q and strike the detector. The selection of ions with certain m/z values is performed by application of the specific Radio Frequency (RF) and Direct Current (DC) voltages to the electrodes. This results in an oscillating electric field acting as a bandpass filter to transmit the ions with the selected m/z values.

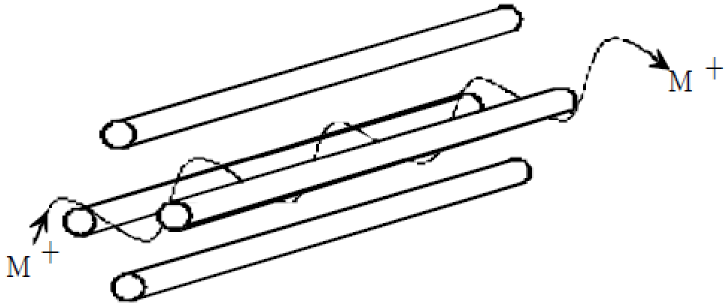


Figure 2.4 Diagram showing quadrupole mass analyzer. Reproduced from [37].

2.3.2.2 Triple quadrupole MS (QqQMS)

Highly efficient MS is important to achieve reliable identification performance. In this case, triple quadrupole MS (QqQMS) can be applied. This mass analyzer consists of 3 sets of quadrupole rods (Figure 2.4), having the 1st and 3rd sets of Q able to operate as mass analyzers (Q1, Q3) and the 2nd set as a collision cell (q2). Theory behind working principle is complex, involving various mathematical equations. A schematic diagram showing QqQMS instrument is illustrated in Figure 2.5. Briefly for tandem MS (MS/MS) analysis, Q1 is used to select a precursor ion. Collision induced dissociation (CID) occurs in q2. The structural information can then be obtained based on specific fragmentation pathways of analyte ions colliding with neutral molecules such as Ar or N_2 (collision gas). Application of voltages adds extra energy to the analyte ions, promoting collisionally induced fragmentation. Q3 generates a spectrum of the resulting product ions. Due to the MS/MS analysis performed according to specific fragmentation pathways of target analytes, this technique is especially useful for improved quantitative work.

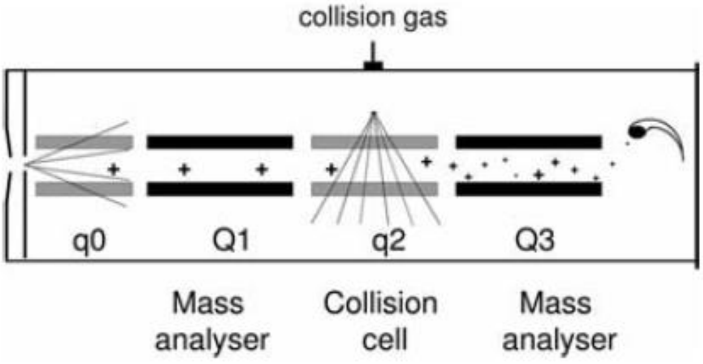


Figure 2.5 Triple quadrupole. Reproduced from [38].

2.3.2.3 Ion detector

Ions from mass analyzer are detected according to their charge or momentum which can be converted into current signal. A faraday cup is employed for large signal detection in order to collect ions and support measurement. Most detectors (including electron multipliers, channel electrons and multichannel plates) apply a collector to amplify the signals of ions. This is similar to the concept of a photomultiplier tube. The signal gain can be tuned by adjusting the voltage that is applied to the detector. Performance of a detector relies on its speed, dynamic range, gain, and geometry. With a highly sensitive detector, a single ion can be detected.

2.4 Gas chromatography─mass spectrometry/olfactometry

GC─O/MS is based on detection with MS supported by sensory evaluation which is widely used to identify the aroma compounds in food and beverage. The effluent from the analytical column outlet was divided by a T-junction to go to the MS and olfactory detection port (ODP). The instrument of ODP consist of [39]

1. Heated transfer line which is used to transfer the separated volatiles from the GC column to ODP.

2. Heated mixing chamber which is a heated transfer line.

3. Nose cone which is used to protect the nose of panelists not to get in contact with the high temperature.

4. Humidified air which is used to enhance moisture for improved sniffing performance.

5. Voice recorder which is used to record the response of odor perception.

The detector of GC─O can be a panelist, who is trained and expert in odor perception. The combination of olfactory and MS is critically important to obtain the reliable data for the aroma compounds. The MS is operated under vacuum conditions but olfactory is operated under atmospheric conditions. Together with the different detection mechanisms, the responses of the two detection methods are different.

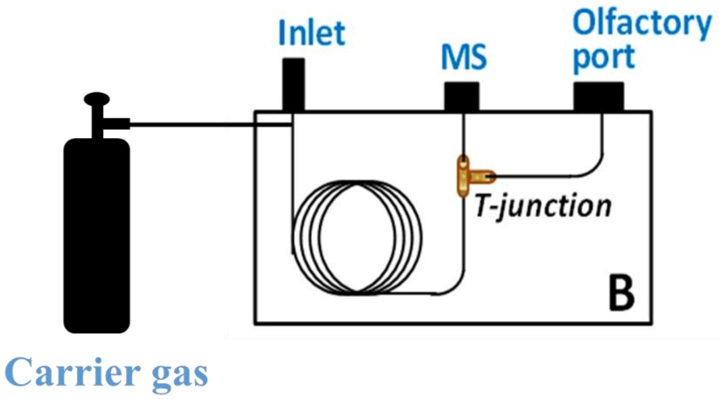


Figure 2.6 The diagram of GC─O/MS. Adapted from [39].

2.5 Sample preparation

Sample preparation is an important step to extract volatile compounds from the matrixes prior to the separation and detection. Widely used sample preparation techniques include simultaneous distillation extraction and hydrodistillation which extract volatile in liquid samples. Although these techniques are efficient, they can be lengthy and involve several preparation steps with the risk of sample lost and side reactions during the extraction.

2.5.1 Headspace─solid phase micro extraction

Alternatively, HS─SPME can be applied offering simple and fast extraction process where volatiles in sample headspace can be adsorbed (extracted) onto the SPME materials, *e.g.* divinylbenzene based fibers followed by direct injection into the GC inlet. The SPME consists of holder and fiber as shown in Figure 2.7.

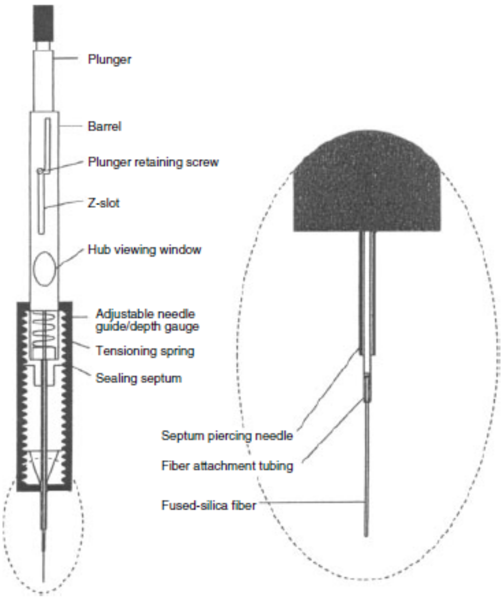


Figure 2.7 The compositions of SPME. Reproduced from [40].

The coated fiber is the most important part for extraction of volatiles. Therefore, the fiber material is optimized to obtain high performance of the extraction. Various types of fibers are shown in Table 2.2.

Table 2.2 Commercial SPME fiber. Reproduced from [40]

Fibre coating	Film thickness (μm)	Polarity	Coating method	Maximum operating temperature (°C)	Technique	Compounds to be analysed
Polydimethylsiloxane (PDMS)	100	Non-polar	Non-bonded	280	GC/HPLC	Volatiles
PDMS	30	Non-polar	Non-bonded	280	GC/HPLC	Non-polar semivolatiles
PDMS	7	Non-polar	Bonded	340	GC/HPLC	Medium- to non-polar semivolatiles
PDMS–divinylbenzene (DVB)	65	Bipolar	Cross-linked	270	GC	Polar volatiles
PDMS–DVB	60	Bipolar	Cross-linked	270	HPLC	General purpose
PDMS–DVB[a]	65	Bipolar	Cross-linked	270	GC	Polar volatiles
Polyacrylate (PA)	85	Polar	Cross-linked	320	GC/HPLC	Polar semivolatiles (phenols)
Carboxen–PDMS	75	Bipolar	Cross-linked	320	GC	Gases and volatiles
Carboxen–PDMS[a]	85	Bipolar	Cross-linked	320	GC	Gases and volatiles
Carbowax–DVB	65	Polar	Cross-linked	265	GC	Polar analytes (alcohols)
Carbowax–DVB[a]	70	Polar	Cross-linked	265	GC	Polar analytes (alcohols)
Carbowax-templated resin (TPR)	50	Polar	Cross-linked	240	HPLC	Surfactants
DVB–PDMS–Carboxen[a]	50/30	Bipolar	Cross-linked	270	GC	Odours and flavours

[a] Stableflex type is on a 2 cm length fibre.

The extraction procedure is shown in Figure 2.8. After sample loading into a SPME vial, the vial is initially heated for a certain time (called equilibration time) until the equilibrium between the analytes in gas and liquid (or solid) phase is ascertained. The coated fiber is then immersed into the sample headspace for a certain period of time (called adsorption time) until reaching equilibrium of the analytes between the headspace and the coated fiber phase. After adsorption, the coated fiber was withdrawn and desorbed in the GC injection port for a certain period of time (called desorption time). The advantages of HS—SPME are simple, solventless, reduced the time consumption in sample preparation, decrease in the cost of solvent and widely applicable in food research [40].

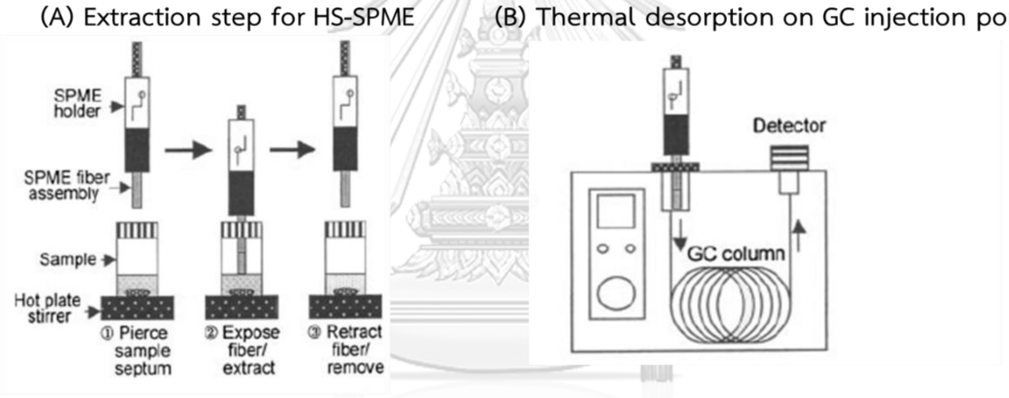

(A) Extraction step for HS-SPME (B) Thermal desorption on GC injection port

Figure 2.8 The extraction procedure of HS—SPME. Reproduced from [40].

CHAPTER III

EXPERIMENTAL

3.1 Instruments and apparatus

3.1.1 Gas chromatograph-Mass spectrometer (GC–MS), Agilent Technologies with GC Model 7890A and MS Model 7000 (CA, USA), where GC consists of autosampler and column oven, and MS consists of triple quadrupoles mass analyzer, electron ionization (EI) interface and MassHunter software processing

3.1.2 An olfactory detection port (ODP), Gerstel Model ODP3 consists of heated transfer line, heated mixing chamber, nose cone, humidified air, olfactory intensity device and voice recorder

3.1.3 An HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, USA)

3.1.4 SPME 50/30 μm DVB/CAR/PDMS fiber, Supelco (Sigma-Aldrich, Bellefonte, PA)

3.1.5 SPME holder, Supelco (Sigma-Aldrich, Bellefonte, PA)

3.1.6 Ultra-high purity helium (99.999%), Linde

3.1.7 Micropipette 100-1000 μL

3.1.8 Pot, Seagull

3.1.9 Hotplate

3.1.10 Balance (4 digits), Satorius Model AC211S-00MS (Germany)

3.1.11 Water bath

3.1.12 Thermometer

3.1.13 Glass vial HS 20- mL, Agilent technologies (USA)

3.1.14 Aluminum cap with a sealed PTFE/silicone septum, Agilent technologies (USA)

3.1.15 Crimper

3.2 Chemicals

3.2.1 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.

3.2.2 Series of *n*-alkanes

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

3.3 Sample preparation

All the raw ingredients were cleaned with deionized water and dried with air under atmospheric conditions. According to the recipe of Tom Yum soup, lemongrass was chopped into thin slices (15 g), the kaffir lime leaf was torn into medium pieces (2 g), chili was crushed (3 g), and lime was squeezed to collect the juice (21 g). The raw ingredients were progressively added into boiled water (300 mL) at 100 °C. The lemongrass was cooked for 1 min; fish sauce (19 g) was cooked for a few seconds, 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.

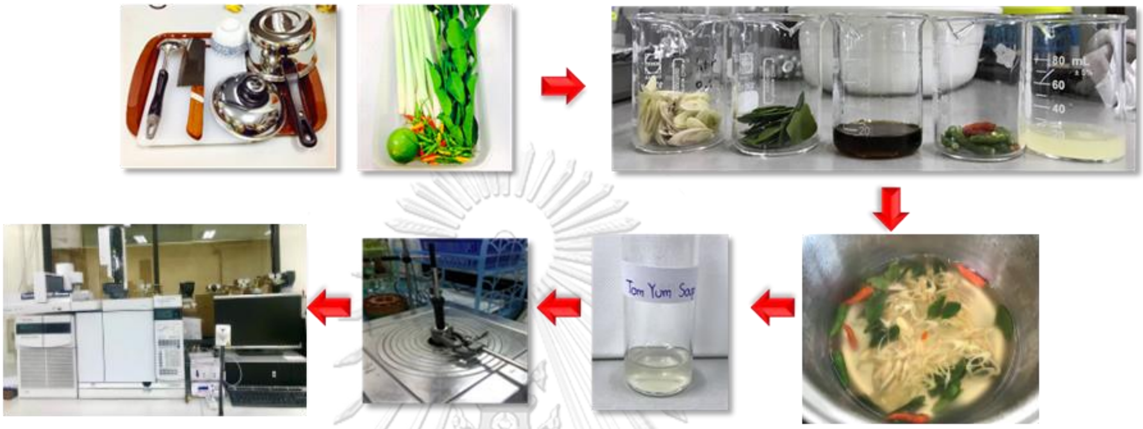


Figure 3.1 Flow diagram of sample analysis.

3.4 Optimization of GC separation

Tom Yum soup was prepared and used for optimization of GC separation. GC—O/MS was performed by using an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, USA) and extracted Tom Yum soup was injected into GC injection port at 250 °C. Linear temperature program from 50 to 200 °C with ramp 3 °C/min, otherwise stated, was assigned for separation volatile compounds and split ratio of 1:30, 1:10 and 1:5 were investigated to select a condition resulting in improved peak shapes with sufficient compound detectability. The linear temperature program and split ratio was optimized and results are given in Section 4.1. The suitable linear temperature program and split ratio was chosen for studying in the following section.

3.5 Optimization of HS–SPME sample preparation

To obtain high peak area of volatile compounds in Tom Yum soup, the following factor of HS–SPME sample preparation were optimized: extraction temperature and time. An SPME 50/30 µm DVB/CAR/PDMS fiber and the holder were purchased from Supelco (Sigma-Aldrich, Bellefonte, PA). The fiber was conditioned at 270 °C for 1 hour via insertion to the GC injection port. Prior to the real sample analysis, the blank fiber was injected to check background signal from the fiber. Each of the extracted raw ingredients and the Tom Yum soup sample (2 mL) were transferred into a 20 mL glass vial closed with an aluminum cap with a sealed PTFE/silicone septum. The vials were heated in a water bath at temperatures of 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 and the results are shown in Section 4.2.

3.6 Method validation

3.6.1 Precision

Using optimized HS–SPME–GC–O/MS conditions in Sections 3.3-3.5, method precision was evaluated for intraday and interday using the %area normalization of selected aroma volatiles for 3 days. For each day, triplicate cooking process (3 pots) and three replicate for each cooking process were used for estimated the precision. The results are presented in Section 4.3.

3.7 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 trained panelists (aged 25-35, 2 male and 4 female) were assigned for the detection and description of the aroma compounds in the extracted Tom Yum soup (triplicate per person). The trained panelists recorded their responses by pressing an olfactory intensity device (scoring 0 to 4) when they perceived the aroma compounds. The average odor intensity was evaluated by 18 analyses with six trained panelists in triplicate for each sample. Zero is also considered the average value [41, 42]. The results are presented in Section 4.4 and 4.5.

3.8 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 >650 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 [10].

$$I = 100n + 100\left(\frac{t_{R(i)} - t_{R(n)}}{t_{R(n+1)} - t_{R(n)}}\right)$$

Figure 3.2 The formula of the retention index. Reproduced from [10].

where t_R is retention time of peak *i*. *n* and *n*+1 are the carbon numbers of alkane standards bracketing the peak *i*.

3.9 Application to Tom Yum pastes

The HS─SPME─GC─O/MS method was also applied to identify and compare volatile compounds in Tom Yum pastes. Three commercial products of Tom Yum pastes were purchased from supermarket in Bangkok, Thailand. Tom Yum soup from these pastes were prepared by separately adding 50 g of each pastes in 300 ml of 100 °C boiled water for 4 min. After that, HS─SPME─GC─O/MS analysis was preformed according to Sections 3.3-3.5. The results are presented in Section 4.6.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Optimization of GC separation

From Section 3.4, the GC separation of volatile compounds extracted from Tom Yum soup was optimized to improve separation efficiency and peak shapes for qualitative and quantitative analysis. Using an HP-5MS capillary GC column and other GC conditions mentioned in Section 3.7, the GC column temperature was linearly programed from 50 to 200 °C with various ramp rates of 3, 5 and 7 °C/min and sample introduction with various split ratios of 1:30, 1:10 and 1:5. The results are shown in Figures 4.1-4.3.

Due to several volatile compounds extracted from the Tom Yum soup, the ramp rates of 5 (32.2 min) and 7 °C/min (23.6 min) provided the faster separation time, but poor separation of peaks. The ramp rate of 3 °C/min gave longer separation time within 52.2 min, however separation efficiency was suitable to separate and detect volatile compounds in Tom Yum soup. Therefore, the ramp rate of 3 °C/min was chosen for future analysis.

As shown in Figure 4.2 and 4.3, the split ratio of 1:5 provided the highest peak area but several asymmetric peaks, such as peak fronting and co-eluting, appeared due to compound overloading onto the GC column. On the other hand, the ratio of 1:30 showed the lowest total peak areas and several minor peaks could not be detected. Therefore, the 1:10 split ratio was selected for further analysis providing good peak symmetry and sufficient detection responds.

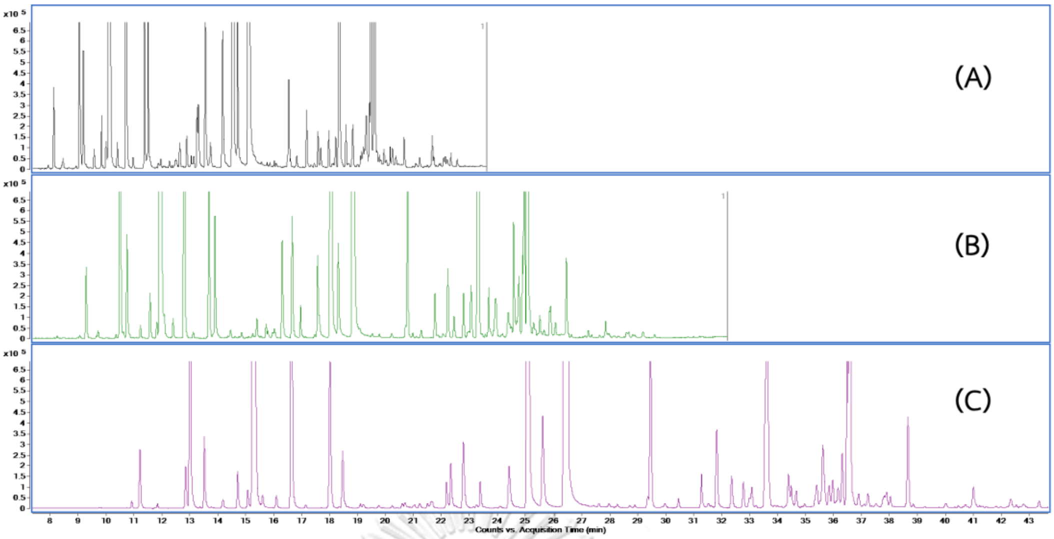


Figure 4.1 The effect of ramp rate 7 (A), 5 (B) and 3 °C/min (C).

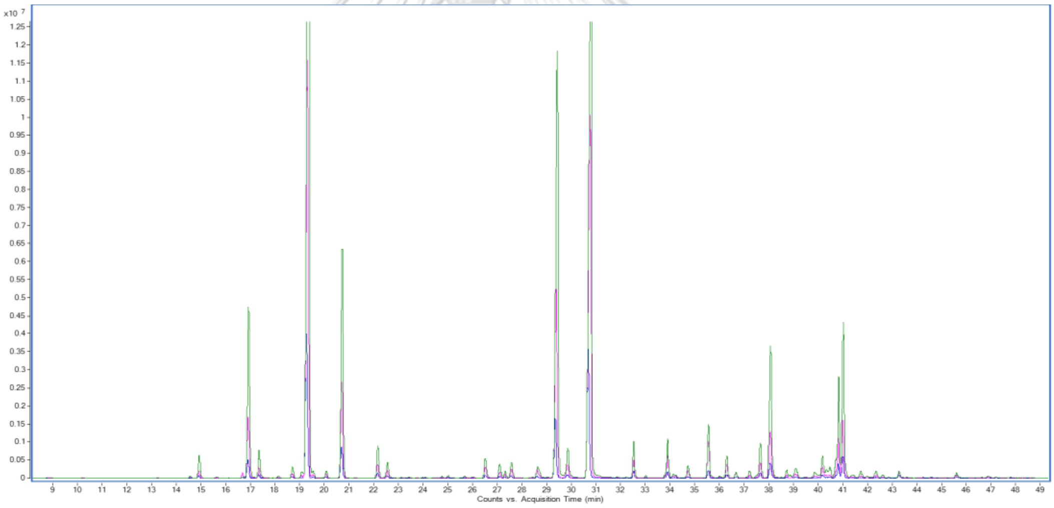


Figure 4.2 GC–MS chromatogram of overlaid split ratios.

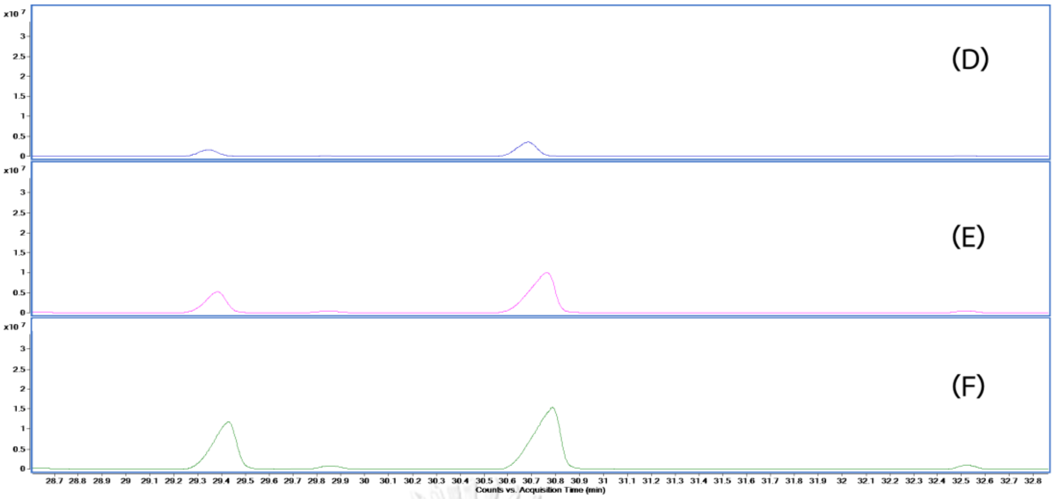


Figure 4.3 GC─MS chromatogram of separated split ratios 1:30 (D), 1:10 (E) and 1:5 (F).

4.2 Optimization of HS─SPME sample preparation

SPME is an equilibrium process between the vapor and the fiber phases [43]. The two main factors affecting the extraction performance which are extraction temperature and extraction time were studied according to Section 3.5. Using single factor optimization, extraction temperatures of 40, 60 and 80 °C were investigated with an extraction time of 45 min using the total peak area of all volatile compounds detected and the individual peak areas of selected aroma compounds: *D*-limonene, geranial and neral as shown in Figure 4.4 and Figure 4.5. The results demonstrated that a suitable temperature was obtained in the range of 40-60 °C, as shown by the high peak areas. Temperature increase results in

1. More amount of volatile compounds in the HS. Thus, the peak areas are expected to increase.
2. The desorption rate increases at high temperature. Therefore, the peak areas are expected to decrease.

In this case, vapor pressure of *D*-limonene is the highest, therefore the amount of this compound in HS at 40 °C was slightly lower than 80 °C. On the other hand desorption effect at 40 °C was much less than 80 °C. As a result total peak area at 40 °C was much higher at 80 °C. Moreover, to avoid off-flavor effects from the high temperature of HS—SPME [44], 40 °C was selected as the temperature for further analyses.

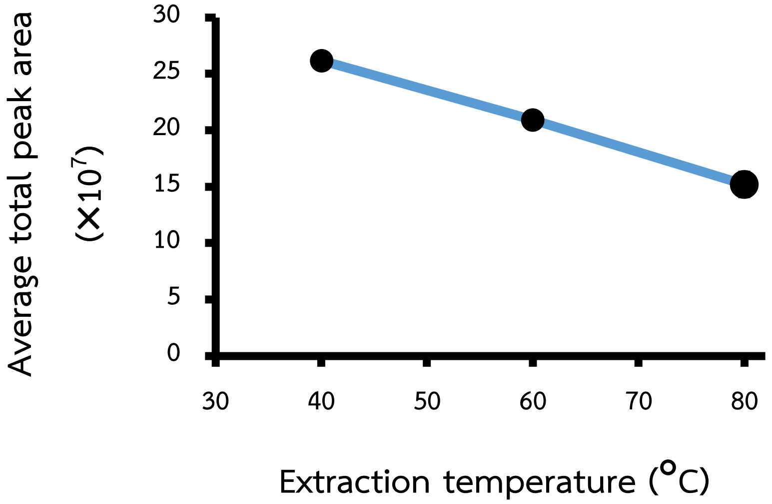


Figure 4.4 Average total peak areas of total volatile compounds in the extracted Tom Yum soup at various HS—SPME extraction temperatures.

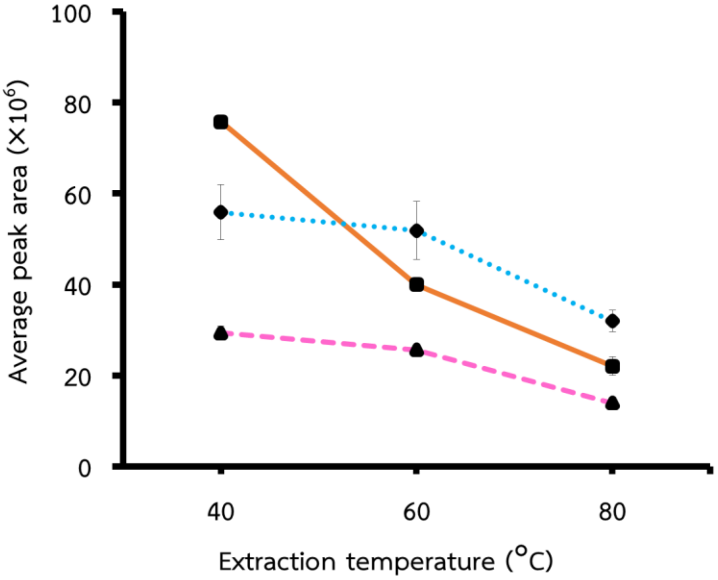


Figure 4.5 Average peak areas of selected aroma compounds: *D*-limonene (solid line), geranial (dotted line) and neral (dashed line), in extracted Tom Yum soup at various HS—SPME extraction temperatures.

The effect of the extraction time (30, 45 and 60 min) on the extraction efficiency was determined at 40 °C. According to Figure 4.6, which shows the total peak area of all volatile compounds detected, and Figure 4.7, which shows the individual peak areas of selected aroma compounds: *D*-limonene, geranial and neral, 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.

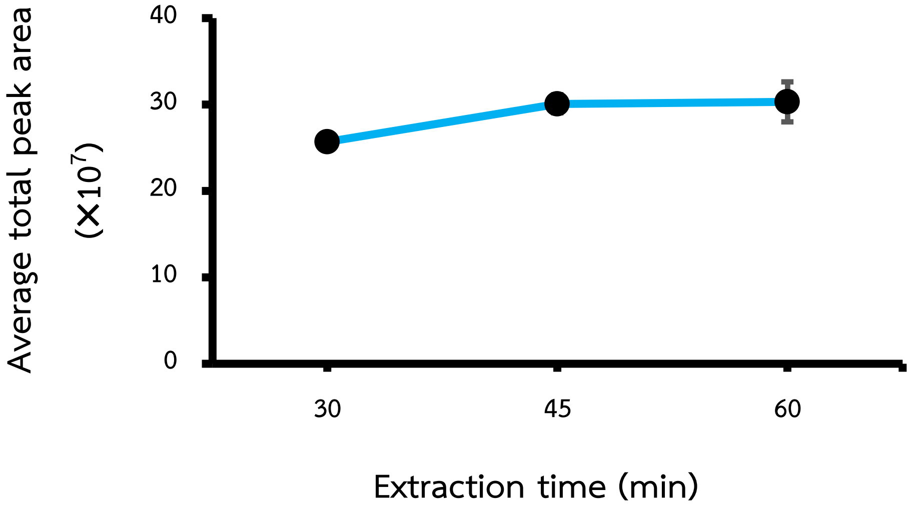


Figure 4.6 Average total peak areas of total volatile compounds in the extracted Tom Yum soup at various extraction time.

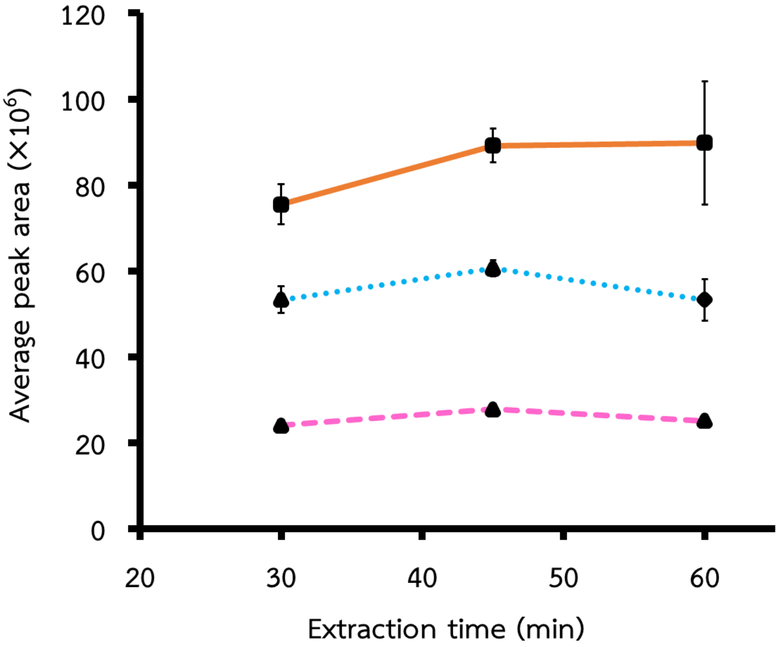


Figure 4.7 Average total peak areas of selected aroma compounds: *D*-limonene (solid line), geranial (dotted line) and neral (dashed line),

volatile compounds in the extracted Tom Yum soup at various extraction time.

4.3 Method validation

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 as shown in Table A.1 (Appendix A) was evaluated using triplicate batches of the extracted Tom Yum soup on each day for three consecutive days. Using statistical ANOVA with a single factor analysis at 95% confidence level [45], non-significant difference in standard deviation or relative standard deviation for intraday (P-value $\geq$ 0.05) was obtained for the following compounds: **α**-pinene, 6-methyl-5-hepten-2-one, **β**-citral, geraniol and geranial. On the other hand, significant difference in standard deviation or relative standard deviation (RSD) for intraday (P-value < 0.05) was obtained for the following compounds: **β**-linalool, unknown (I = 1165, MS of 152), **α**-terpineol, nerol, 4-methylpentyl 4-methylpentanoate, citronellyl acetate, geranyl acetate and dodecanal. Therefore, %RSD for intraday precision the former case is calculated using a single dataset (n = 9), and the %RSD for interday precision is equal to the %RSD for intraday precision. For the latter case, %RSD for intraday precision is calculated using %RSD = $100S_r/\bar{x}$, where $\bar{x}$ is the average %area normalization and S_r is the square root of within group mean square value obtained from the ANOVA data, and the %RSD for interday precision is calculated using the equations [46] as given below Table B.14 (Appendix B).

%RSD values for intraday and interday precision were obtained to be less than 15% and 25%, respectively, for most of the aroma compounds with the exception of 4-methylpentyl 4-methylpentanoate and citronellyl acetate with %RSD in a range of 25-40 for intraday and 35-65 for interday, and geranyl acetate with %RSD of 35 for interday. Poor %RSD values for the exception case is possibly due to small amount of the %area normalization, i.e., less than 0.2.

Table 4.1 Intraday and interday precision in %area normalization (three days with three batches for each day)

Selected aroma compounds	%Area normalization (%RSD) except for *P*-value				Overall		
	Day 1	Day 2	Day 3	*P*-value	%Area normalization	%RSD for Intraday	%RSD for Interday
α-Pinene	0.478 (1.3)	0.395 (3.9)	0.458 (15)	0.102	0.444	12	12
6-Methyl-5-hepten-2-one	0.011 (8.8)	0.013 (7.5)	0.013 (15)	0.204	0.013	13	13
β-Linalool	0.543 (6.2)	0.737 (7.5)	0.644 (2.4)	0.003	0.641	15	19
Unknown	0.073 (4.5)	0.089 (4.3)	0.081 (11)	0.039	0.081	17	18
α-Terpineol	0.695 (5.2)	1.016 (10)	0.841 (3.2)	0.003	0.851	18	23
Nerol	0.927 (4.0)	1.017 (2.4)	0.681 (9.8)	<0.001	0.875	13	20
β-Citral	9.50 (6.9)	11.1 (12)	11.6 (14)	0.172	10.8	13	13
Geraniol	1.028 (3.5)	0.899 (1.8)	0.992 (9.6)	0.089	0.973	7.9	7.9
Geranial	20.7 (5.6)	23.8 (7.9)	25.3 (19)	0.254	23.3	14	14
4-Methylpentyl 4-methylpentanoate	0.100 (12)	0.051 (17)	0.024 (3.5)	<0.001	0.059	36	64
Citronellyl acetate	0.168 (15)	0.196 (2.4)	0.039 (2.8)	< 0.001	0.134	27	57
Geranyl acetate	0.162 (4.5)	0.190 (0.5)	0.076 (4.5)	< 0.001	0.142	8	35
Dodecanal	0.285 (5.4)	0.222 (3.7)	0.220 (1.7)	0.0004	0.242	10	16

4.4 GC–MS separation 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 4.14 with the corresponding results for the individual raw and boiled ingredients shown in Figure 4.15 and Table 4.2.

All compounds detected in the GC–MS chromatograms were identified according to a comparison of their mass spectra with those from NIST library with math 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 the Table C.1 (Appendix C).

As shown in Table C.1 (Appendix C), three major volatile compounds found in raw lemongrass (Figure 4.15A1), 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 4.15A2) are geranial (69.1%), β-citral (24.8%) and geraniol (2.89%), while other compounds are 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 [18, 47] and exhibit strong lemony and floral perceptions [5]. In addition, we also observed carveol (0.03%) in boiled lemongrass is an extra compound observed 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 [48]. 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.

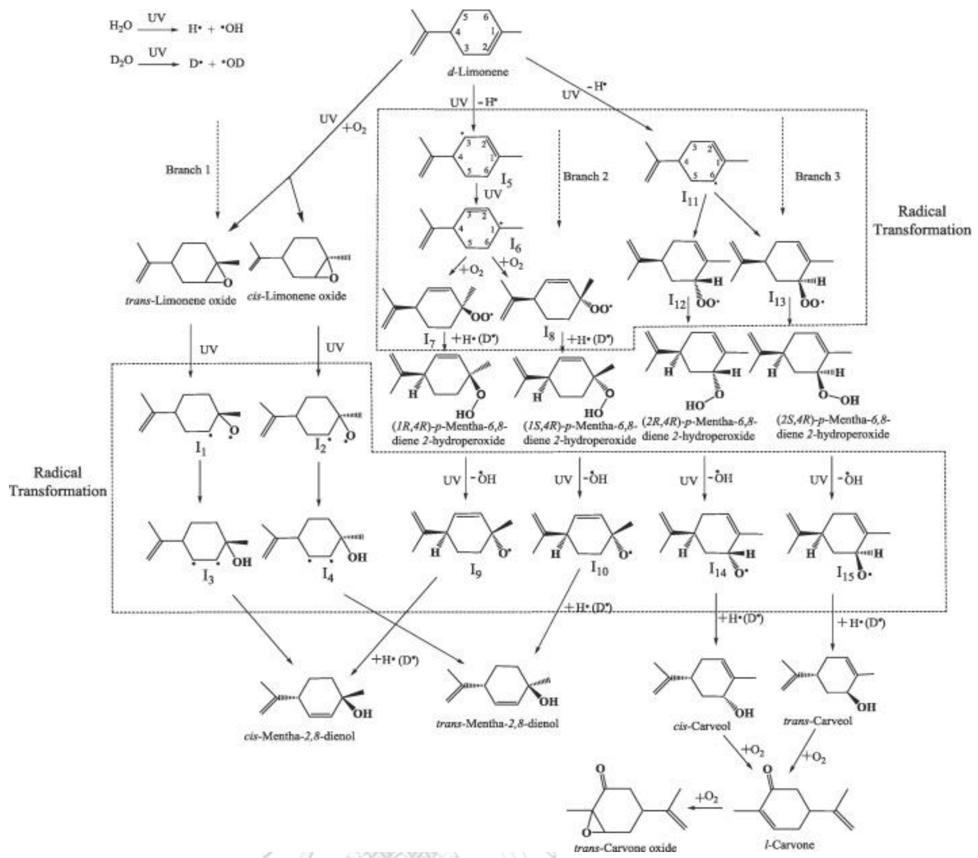


Figure 4.8 The transformation of *D*-limonene. Reproduced from [48].

Fish sauce (Figure 4.15B1), 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 4.15B2) while 3-methylbutanoic acid, 2-methylbutanoic acid and 4-methylpentanoic acid were not detected under HS─SPME─GC─MS conditions used in this work. 3-methylbutanoic acid and 4-methylpentanoic acid exist cheesy and sweaty aroma [29].

The major volatile compounds found in raw kaffir lime leaf (Figure 4.15C1) 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 4.15C2) 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 and the other compounds: linalool, hexanal, sabinene and β-citronellol were also determined as key odorant in kaffir lime leaf, where **β**-citronellal shows strong citrus, green, kaffir lime leaf, citrus, linalool exists floral, sweet and β-citronellol exists fresh kaffir lime leaf, citrus [7].

The dominant volatile compounds in raw chili (Figure 4.15D1) are 4-methylpentyl 4-methylpentanoate (45.3%), 4-methylpentyl 2-methylbutanoate (14.7%) and 4-methylpentyl 3-methylbutanoate (11.0%) and **δ**-guaiene (0.12%). However, in boiled chili (Figure 4.15D2), 4-methylpentyl 4-methylpentanoate (53.4%) is the dominant volatile compounds 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 [23], and 4-methylpentyl 3-methylbutanoate exhibits fruity and peach aromas [23].

In lime juice (Figure 4.15E1), the major volatile compounds found 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 4.15E2) 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 as well as **β**-pinene and **γ**-terpinene were also main compounds identified in lime oil [24]. However, the most odor-active volatiles found in extracted and distilled lime oil were geranial, neral and linalool [26].

As seen in Figure 4.14 and Table C.1 (Appendix C), 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 lime juice and lemongrass. Other compounds are **α**-muurolene (6.27%), **β**-pinene (4.79%) and **γ**-terpinene (4.46%) which are from 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. Note that these compounds were not observed in the individual boiled sample. This indicates that there may be some effect (Na^+ from fish sauce, lower pH from lime juice) generating these compounds in Tom Yum soup.

According to an explanation in previous work [49], *p*-mentha-3,8-diene may be the a product of **β**-citronellal since the later compound can be cyclized to result in isopulegol with the byproducts including menthone, pulegol, and other cyclic hydrocarbons such as **α**-terpinene, *p*-mentha-3,8-diene and terpinolene. The reaction is presented in Figure 4.9.

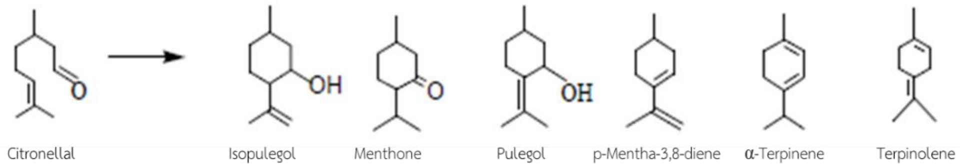


Figure 4.9 The cyclization of citronellal. Reproduced from [49].

Citrals are acyclic terpenes without asymmetric center that are generally converted to cyclic terpenes including **α**-cyclocitral (Figure 4.10) [50]. Moreover, *p*-mentha-1,5-dien-8-ol may come from citral under acidic conditions (Figure 4.11), and the mechanism of *p*-mentha-1,5-dien-8-ol is described in the literature [51].

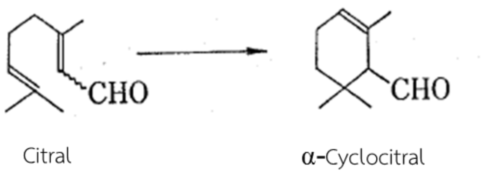


Figure 4.10 Cyclization of citral to α-cyclocitral. Reproduced from [50].

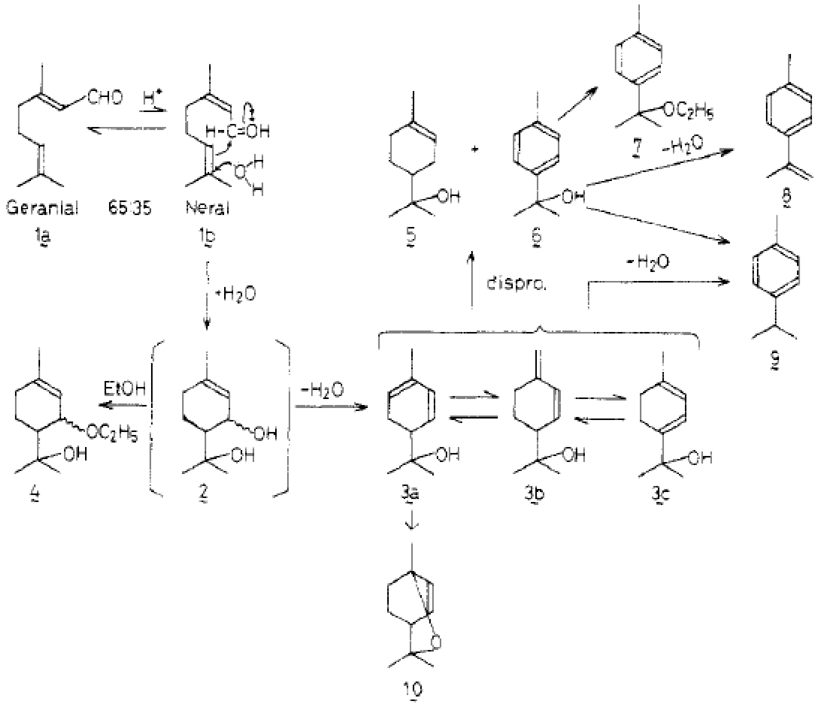


Figure 4.11 The mechanism of citral in an aqueous solution. Reproduced from [51].

Iso-isopulegol may occur as a result of cyclization of β-citronellal with three asymmetrical centers as shown in Figure 4.12, which can result in four stereoisomers of isopulegol, and each isomer occurs as a pair of enantiomers: (±)-isopulegol, (±)-neoisopulegol, (±)-iso-isopulegol and (±)-neoiso-isopulegol [52].

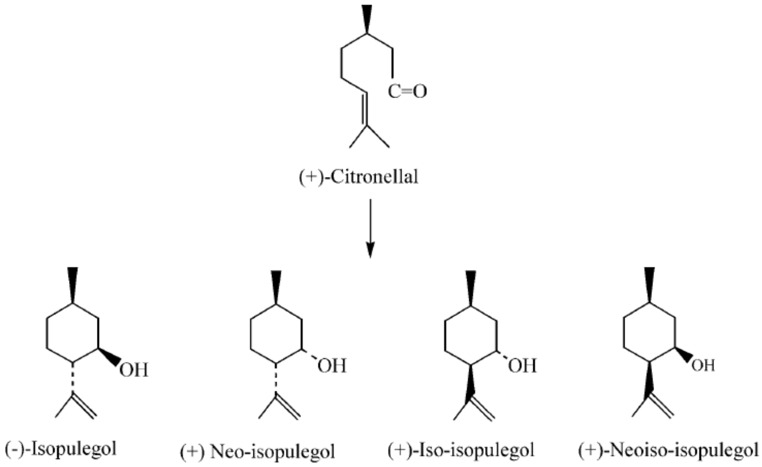


Figure 4.12 The cyclization of citronellal. Reproduced from [52].

Interestingly, decyl acetate (Figure 4.13), 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 [53].

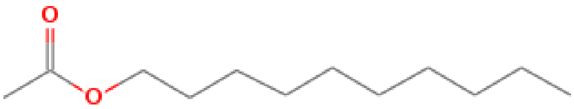


Figure 4.13 Structure of decyl acetate. Reproduced from [54].

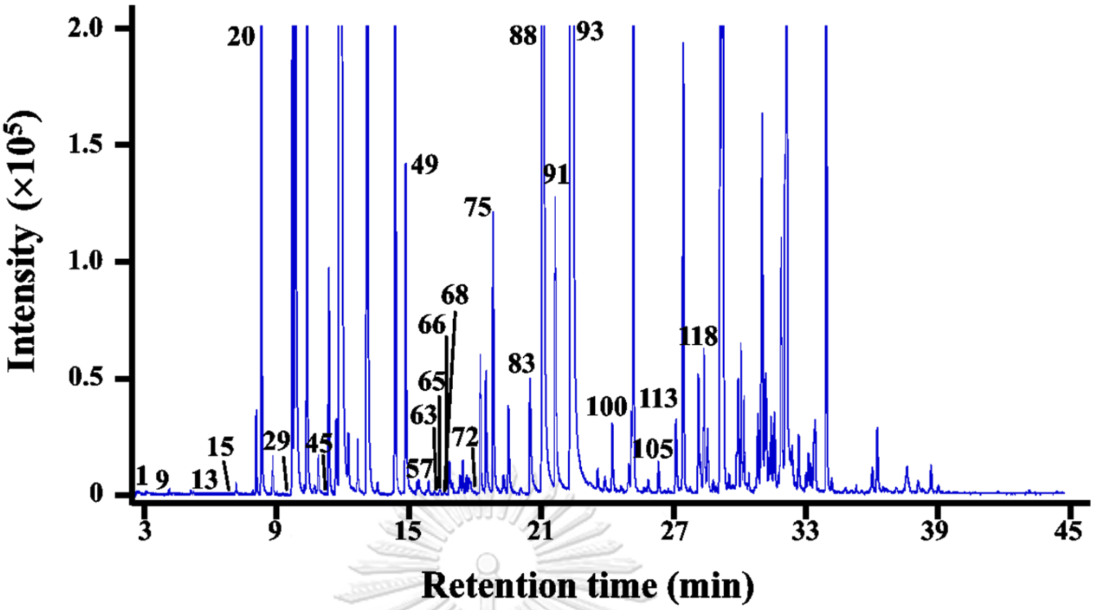


Figure 4.14 GC–MS chromatogram of Tom Yum soup.

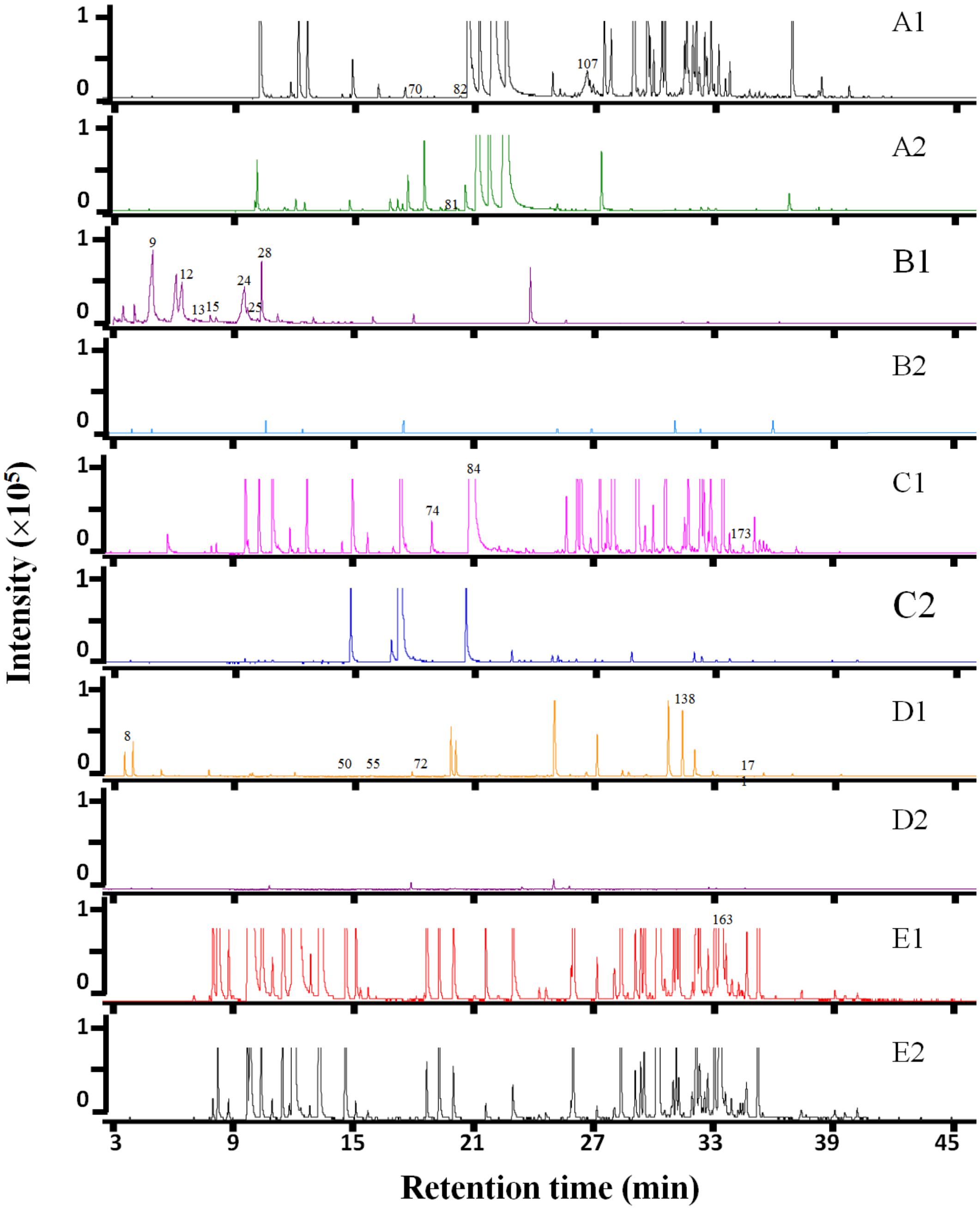


Figure 4.15 GC–MS chromatograms of volatile compounds in individual ingredient of Tom Yum soup: lemongrass (A), fish sauce (B), kaffir lime leaf (C), chili (D) and lime juice (E), where 1 and 2 refer to raw and boiled ingredient, respectively.

Table 4.2 Selected tentative volatile compounds in Tom Yum soup and individual ingredient

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	-	-	-	-	-	-	-

Table 4.2 (continued)

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
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	-	-
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	-	-	-	-	-	-	-	-

Table 4.2 (continued)

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
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	-	-

Table 4.2 (continued)

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
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	-	-	-	-	-

4.5 Correlation with olfactory analysis of Tom Yum soup

According to HS─SPME─GC─O/MS analysis of Tom Yum soup detailed in Section 3.7, the odor descriptions for the aroma compounds from our experiment were compared with literature sources and summarized in Table 4.3.

From the triplicate evaluations of the six trained panelists (*n* = 18), eighteen aroma compounds in Tom Yum soup were detected and described by at least two of the trained 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 [41], 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.

Table 4.3 Aroma compounds in Tom Yum soup detected by GC–O

Peak No.	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]

Table 4.3 (continued)

Peak No.	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		
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]

Table 4.3 (continued)

Peak	RT	LRI			Ingredient source		Sensory evaluation				
No.	(min)	Exp[a]	Lit[b]	Aroma compound	Exp[c]	Ref.[d]	Odor description	Detecting compound		Average odor intensity (n = 18, Maximum value = 4)	Ref.
								No. of panelist	Trial		
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 [54]

[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] = [23], [3] = [29], [4] = [26], [5] = [30], [6] = [7], [7] = [5], [8] = [55], [9] = [17], [10] = [56] and [11] = [25]

4.6 Application to Tom Yum pastes

As mentioned in Section 3.9, HS─SPME─GC─O/MS was also applied to identify and compare volatile compounds in three commercial products of Tom Yum paste with our Tom Yum soup. The results are summarized in Table 4.5.

Seventy volatile compounds and the major volatile compounds found in Paste I, with the %area normalization in parentheses, are iso-isopulegol (12.2%), *D*-limonene (10.8%), β-myrcene (9.97%), Isopulegol (9.01%) and geranial (5.88%), while the minor compounds are caryophyllene (4.72%), *p*-mentha-3,8-diene (4.54%) and β-Citral (4.38%).

Sixty-six volatile compounds were identified and four major volatile compounds found in Paste II are geranial (30.1%), β-citral (18.9%), caryophyllene (6.82%) and geranyl acetate (5.81%), while other compounds are γ-gurjunene (1.95%), β-myrcene (1.86%) and (Z,E)-α-farnesene (1.80%).

In Paste III, seventy-four volatile compounds and the dominant volatile compounds are iso-isopulegol (11.5%), isopulegol (11.3%), β-myrcene (10.8%) and *D*-limonene (10.8%), while geranial (5.99%), β-citral (5.41%) and β-linalool (5.02%) are the minor volatile compounds.

In comparison of volatile compounds in three commercial products of Tom Yum paste with our Tom Yum soup, most of the volatile compounds of three commercial products of Tom Yum paste were also found in our Tom Yum soup, except for thirty volatile compounds such as dimethyl disulfide, hexanal, furfural, 2-acetylfuran, methyl 1-propenyl disulfide, benzaldehyde, dimethyl trisulfide, diallyl disulphide, 3-carene, (E,E)-2,4-heptadienal, eucalyptol , benzeneacetaldehyde and β-citronellal and other as given in Table 4.5.

In addition, three of the five extra volatile compounds in our Tom Yum soup are also found in three commercial products of Tom Yum paste, for example, *p*-

mentha-3,8-diene for Paste I (4.54%), II (0.45%) and III (3.78%), α-cyclocitral for Paste I (0.05%), and iso-isopulegol for Paste I (12.2%), II (0.81%) and III (11.5%).

Table 4.6 shows the odor description of three commercial products of Tom Yum paste. Aroma compounds was considered from at least two from three panelists (*n*=3). In Paste I, the three dominant aroma compounds include 2-isobutyl-3-methoxypyrazine, 3-(methylthio)propanal, and dimethyl trisulfide because it shows high average odor intensity and moreover β-citronellol (rose aroma) was found only in Paste I. Two major aroma compounds found in Paste II include isopinocamphone and geranyl acetate due to high average odor intensity. *D*-limonene (citrus and mint aroma) and β-citral (citrus aroma) were found especially in Paste II. In Paste II, 3-(methylthio)propanal, isopinocamphone, 2-isobutyl-3-methoxypyrazine and β-linalool are considered aroma compounds because of high average odor intensity. Furthermore, 3-methylbutanoic acid (vomit-like, cheesy and sweaty aroma) are only detected for aroma in Paste III.

In comparison with our Tom Yum soup, the following aroma compounds were also found in three commercial products of Tom Yum paste: 3-methylbutanoic acid, 3-(methylthio)propanal from fish sauce, 6-methyl-5-hepten-2-one from lemongrass, β-linalool, geraniol and geranyl acetate from lemongrass, kaffir lime leaf and lime juice, α-terpineol from kaffir lime leaf and lime juice, 2-isobutyl-3-methoxypyrazine from chili and β-citral from lemongrass and lime juice. Moreover six aroma compounds differs from our Tom Yum soup such as dimethyl trisulfide (sulfur, fish and cabbage), β-pinene (pine, resin and turpentine), *D*-limonene (citrus and mint), nonanal (fat, citrus and green), isopinocamphone (cedar camphoreous) and β-citronellol (rose). Dimethyl trisulfide may be from garlic [57] and onion[58], β-pinene, nonanal and isopinocamphone from lime juice, *D*-limonene from lemongrass, kaffir lime leaf and lime juice and β-citronellol from kaffir lime leaf.

Principal component analysis (PCA) was applied to determine the difference of our Tom Yum soup and three commercial products of Tom Yum paste. The results show the correlation of scores plot (Figure 4.16), loadings plot (Figure 4.17) and biplot (Figure 4.18). From Figure 4.16, four clearly separated groups are seen in the scores plot, indicating that our Tom Yum soup, Paste I, Paste II and Paste III are difference. From Figure 4.17, loadings plot represented the key volatile compounds that correlate with the samples. From figure 4.18, biplot shows the correlation of scores and variables.

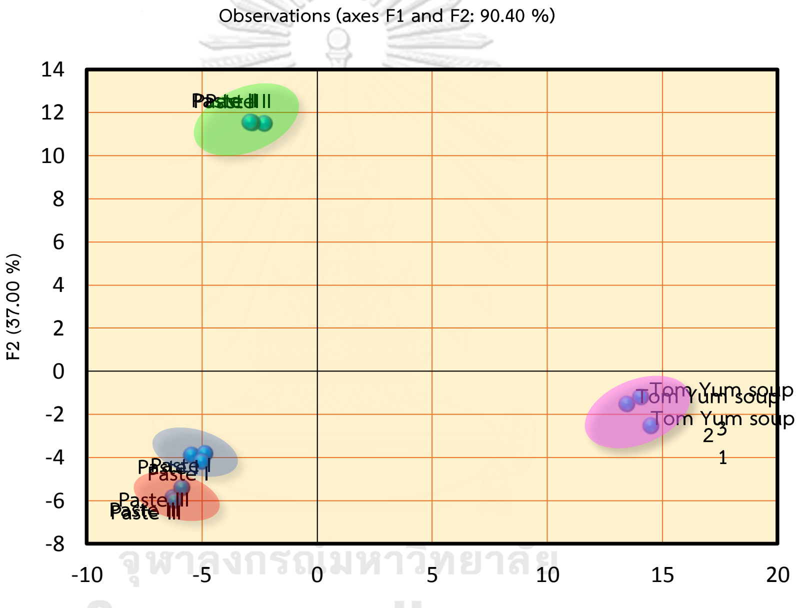


Figure 4.16 PCA scores plot shows the correlation of our Tom Yum soup and three commercial products of Tom Yum paste (I,II,III).

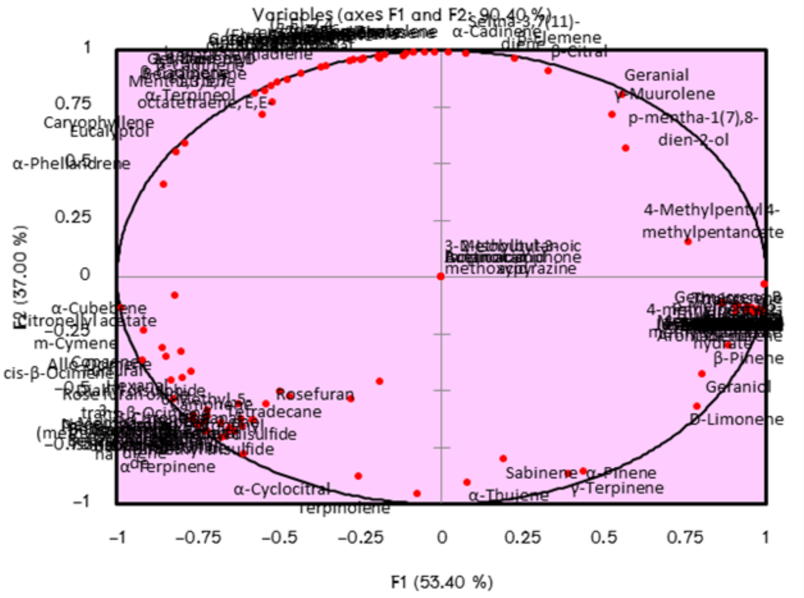


Figure 4.17 PCA loadings plot shows the correlation of volatile compounds (variables).

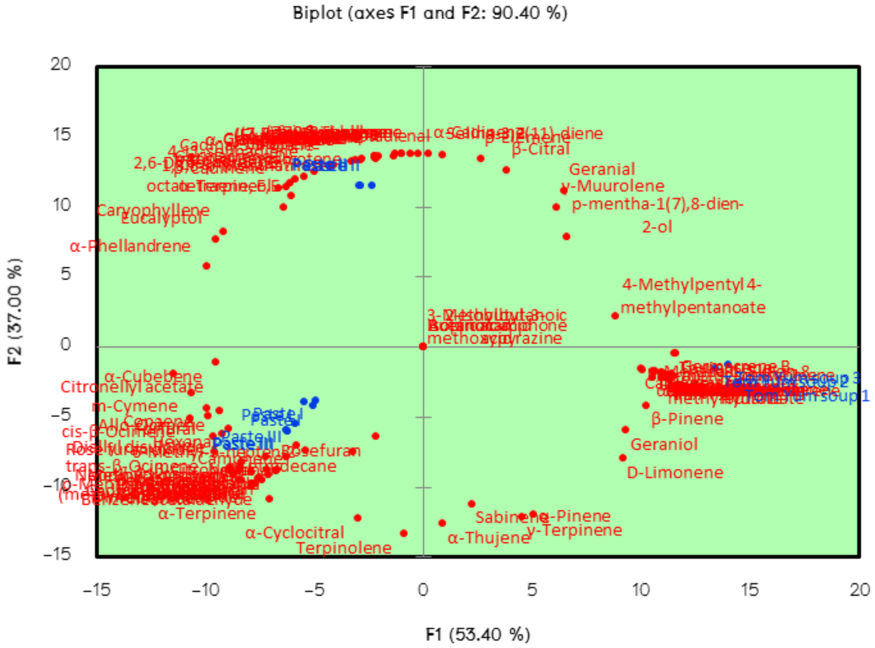


Figure 4.18 PCA biplot shows the correlation of scores and variables.

Table 4.4 The key volatile compounds that correlate with the group of each sample

Group	Sample	Key volatile compound
1	Tom Yum soup	*D*-limonene, Geraniol, β-pinene, 4-methylpentyl 2-methylbutanoate, Methyl geranate, Nerol, Terpinen-4-ol, Cedrene, α-Muurolene, Decanal, Tricyclene, α-Fenchene, γ-Elemene, Thuja-2,4(10)-diene, α-Elemene, Epicubebol, α-Bergamotene, (Z)-α-Bisabolene, Juniper camphor, α-Guaiene, β-Guaiene, Allo-Aromadendrene, α-Santalol, Unknown 1, *p*-Mentha-1,5-dien-8-ol, (E)-Nerolidol, α-Bisabolol, epi-γ-Eudesmol, Nonane, Carveol, Fenchol, τ-Muurolol, Tridecane, Unknown 2, α-Bisabolene, β-Gurjunene, δ-Elemene, Undecanal, trans-Sesquisabinene hydrate, Thujopsene, Germacrene B, Dodecanal, δ-Selinene, Decyl acetate
2	Paste I	α-Cubebene, Citronellyl acetate, m-Cymene, Copaene
3	Paste II	α-Terpineol, 2,6-Dimethyl-1,3,5,7-octatetraene, E,E-, β-Cadinene, 1,3,8-p-Menthatriene, δ-Cadinene, trans-α-Bergamotene, Germacrene D, 4,11-selinadiene, Cadina-3,9-diene, γ-Cadinene, α-Caryophyllene, γ-Eudesmol, (E)-γ-Bisabolene, α-Farnesene, Geranyl acetate, α-Chamigrene, (Z)-γ-Bisabolene, (E)-β-Farnesene, δ-Guaiene, (Z,E)-α-Farnesene, *p*-Cymenene, Cyclosativene, (E,E)-2,4-Heptadienal, γ-Gurjunene, Seychellene, (Z)-β-Farnesene, α-Phellandrene, Eucalyptol, Caryophyllene
4	Paste III	Hexanal, Rose furan oxide, Diallyl disulphide, Camphene, β-Citronellal, 6-Methyl-5-hepten-2-one, trans-β-Ocimene, β-Citronellol, Neodihydrocarveol, p-Mentha-3,8-diene, β-Linalool, Benzaldehyde, Cadina-1(2),4-diene, 3-(methylthio)propanal, Nonanal, β-Myrcene, Methyl 1-propenyl disulfide, 2-Acetylfuran, Dimethyl disulfide, 3-Methylpentanal, Elixene, iso-Isopulegol, Isopulegol, Dimethyl trisulfide, 3-Methylbutanal, 3-Carene, Benzeneacetaldehyde, α-Terpinene, Tetradecane, Rosefuran

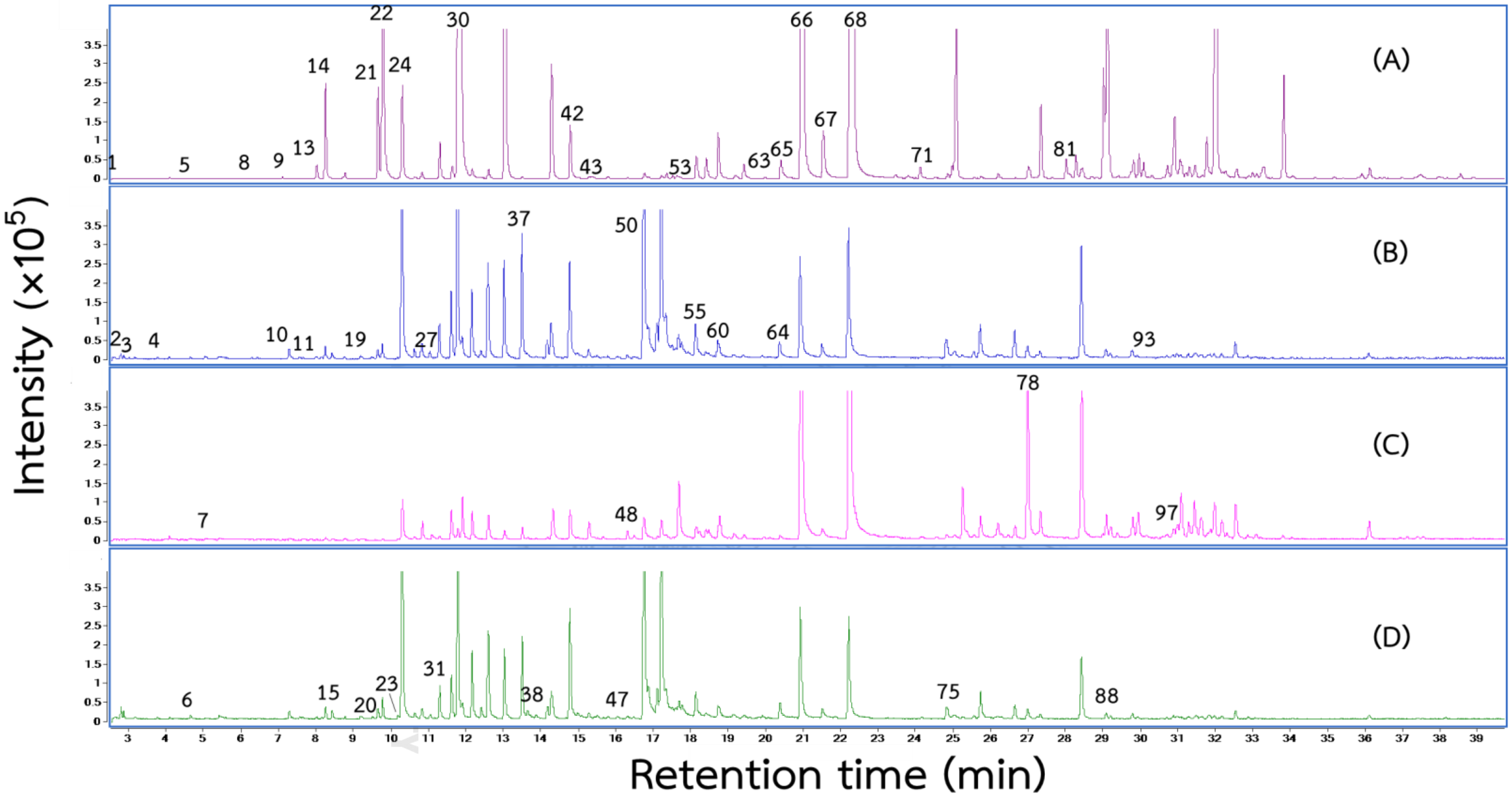


Figure 4.19 GC–MS chromatogram of our Tom Yum soup (A), Paste I (B), Paste II (C) and Paste III (D).

Table 4.5 GC–MS chromatogram of three commercial products of Tom Yum paste with our Tom Yum soup

Peak No.	RT (min)	Tentative compound	% Average area normalization (*n* = 3)			
			Tom Yum soup	Paste 1	Paste 2	Paste 3
1	2.61	Acetic acid	-	-	-	-
2	2.79	3-Methylbutanal	-	0.151±0.020	-	0.295±0.020
3	2.86	3-Methylpentanal	-	0.061±0.006	-	0.185±0.020
4	3.77	Dimethyl disulfide	-	0.027±0.004	-	0.076±0.007
5	4.49	Butanoic acid	-	-	-	-
6	4.64	Hexanal	-	0.049±0.006	0.032±0.002	0.108±0.004
7	5.40	Furfural	-	0.116±0.010	0.081±0.010	0.206±0.010
8	6.26	3-Methylbutanoic acid	-	-	-	-
9	7.11	Nonane	0.013±0.001	-	-	-
10	7.28	3-(methylthio)propanal	-	0.294±0.010	0.047±0.001	0.305±0.010
11	7.55	2-Acetylfuran	-	0.037±0.006	-	0.089±0.002
12	7.87	Tricyclene	0.003±0.001	-	-	-
13	8.02	α-Thujene	0.063±0.020	0.068±0.004	-	0.075±0.007
14	8.25	α-Pinene	0.574±0.070	0.421±0.008	0.062±0.010	0.462±0.030
15	8.42	Methyl 1-propenyl disulfide	-	0.196±0.005	0.026±0.010	0.392±0.010
16	8.73	α-Fenchene	0.008±0.001	-	-	-
17	8.77	Camphene	0.043±0.003	0.054±0.004	0.046±0.010	0.117±0.010
18	8.96	Thuja-2,4(10)-diene	0.002±0.001	-	-	-
19	9.18	Benzaldehyde	-	0.102±0.010	0.021±0.003	0.172±0.010
20	9.49	Dimethyl trisulfide	-	0.053±0.007	-	0.083±0.003
21	9.66	Sabinene	0.380±0.140	0.342±0.040	-	0.393±0.010
22	9.78	β-Pinene	4.790±0.960	0.571±0.020	-	0.874±0.030
23	10.19	6-Methyl-5-hepten-2-one	0.015±0.003	0.025±0.006	0.014±0.001	0.154±0.006
24	10.30	β-Myrcene	0.716±0.034	9.970±0.380	1.860±0.200	10.81±0.40

Table 4.5 (continued)

Peak No.	RT (min)	Tentative compound	% Average area normalization (*n* = 3)			
			Tom Yum soup	Paste 1	Paste 2	Paste 3
25	10.82	α-Phellandrene	0.071±0.005	0.662 ±0.009	0.810±0.100	0.569±0.040
26	11.04	3-Carene	-	0.256 ±0.010	-	0.201±0.030
27	11.08	(E,E)-2,4-Heptadienal	-	-	0.164±0.010	-
28	11.31	α-Terpinene	0.336±0.020	1.320±0.020	0.181±0.010	1.300±0.080
29	11.64	m-Cymene	0.125±0.003	2.510±0.040	1.380±0.200	1.960±0.100
30	11.86	*D*-Limonene	26.60±3.00	10.85±0.42	0.488±0.050	10.77±0.49
31	11.89	Eucalyptol	-	1.030±0.030	1.540±0.100	0.898±0.040
32	12.18	cis-β-Ocimene	0.087 ±0.010	2.72±0.06	1.390±0.160	2.790±0.060
33	12.39	Benzeneacetaldehyde	-	0.371±0.010	-	0.473±0.050
34	12.60	trans-β-Ocimene	0.093±0.010	3.940±0.100	1.120±0.100	3.710±0.100
35	13.05	γ-Terpinene	4.45±0.40	4.040±0.080	0.372±0.010	3.22±0.08
36	13.50	p-Mentha-3,8-diene	0.021±0.004	4.540±0.300	0.474±0.030	3.79±0.04
37	13.63	Neodihydrocarveol	-	0.239±0.010	0.062±0.020	0.448±0.030
38	13.87	Diallyl disulphide	-	0.075±0.010	0.040±0.010	0.134±0.010
39	14.28	Terpinolene	1.200±0.080	1.800±0.030	-	1.500±0.040
40	14.32	*p*-Cymenene	-	-	1.450±0.100	-
41	14.67	Rosefuran	0.006±0.002	-	-	0.025±0.010
42	14.78	β-Linalool	0.790±0.120	4.120±0.020	1.290±0.040	5.020±0.200
43	15.01	Nonanal	0.007±0.001	0.287±0.020	-	0.111±0.004
44	15.28	1,3,8-p-Menthatriene	0.022±0.004	0.332±0.020	0.773±0.050	0.230±0.016
45	15.35	Fenchol	0.045±0.010	-	-	-
46	15.80	α-Cyclocitral	0.024±0.002	0.050±0.004	-	0.040±0.005
47	16.03	Allo-Ocimene	-	0.047±0.004	0.039±0.005	0.104±0.003
48	16.30	(E)-2,6-dimethyl-1,3,5,7-octatetraene	-	0.067±0.007	0.302±0.016	0.131±0.032

Table 4.5 (continued)

Peak No.	RT (min)	Tentative compound	% Average area normalization (n = 3)			
			Tom Yum soup	Paste 1	Paste 2	Paste 3
49	16.76	Isopulegol	0.102±0.02	9.010±0.100	0.878±0.023	11.33±0.080
50	17.09	β-Citronellal	-	1.640±0.100	0.089±0.016	0.810±0.064
51	17.23	Iso-isopulegol	0.059±0.020	12.17 ±0.28	0.812±0.023	11.54±0.23
52	17.52	Unknown 1	0.034 ±0.006	-	-	-
53	17.63	Unknown 2	0.044±0.010			
54	17.70	*p*-Mentha-1,5-dien-8-ol	0.032±0.010	-	-	-
55	18.04	Isopinocamphone	-	-	-	-
56	18.12	Rose furan oxide	-	1.730±0.040	0.526±0.002	1.310±0.050
57	18.15	Terpinen-4-ol	0.284±0.020	-	-	-
58	18.32	2-Isobutyl-3-methoxypyrazine	-	-	-	-
59	18.41	*p*-mentha-1(7),8-dien-2-ol	0.345±0.056	0.241±0.008	0.328±0.027	0.070±0.007
60	18.73	α-Terpineol	0.660±0.080	0.800±0.050	1.27±0.03	0.971±0.040
61	19.20	4-methylpentyl 2-methylbutanoate	0.051±0.008	-	-	-
62	19.41	Decanal	0.154±0.008	-	-	-
63	19.97	Carveol	0.012 ±0.003	-	-	-
64	20.36	β-Citronellol	-	0.669±0.018	0.144±0.006	0.923±0.065
65	20.40	Nerol	0.444±0.067	-	-	-
66	20.99	β-Citral	12.40±1.70	4.380±0.230	18.91±0.32	5.410±0.080
67	21.53	Geraniol	0.906±0.010	0.469±0.048	0.286±0.018	0.483±0.048
68	22.33	Geranial	25.40±2.80	5.880±0.280	30.14±0.51	5.880±0.400
69	23.48	Tridecane	0.027±0.004	-	-	-
70	23.8	Undecanal	0.020±0.003	-	-	-

Table 4.5 (continued)

Peak No.	RT (min)	Tentative compound	% Average area normalization (*n* = 3)			
			Tom Yum soup	Paste 1	Paste 2	Paste 3
71	24.12	4-Methylpentyl 4-methylpentanoate	0.114±0.010	-	0.064±0.003	0.063±0.001
72	24.51	Methyl geranate	0.012±0.002	-	-	-
73	25.08	δ-Elemene	1.670±0.070	-	-	-
74	25.58	α-Cubebene	0.005±0.001	0.232±0.015	0.127±0.007	0.123±0.017
75	25.75	Citronellyl acetate	0.024±0.003	1.370±0.050	1.050±0.020	1.530±0.050
76	26.21	Cyclosativene	0.045±0.003	-	0.604±0.046	-
77	26.67	Copaene	0.004±0.001	1.040±0.040	0.423±0.016	0.690±0.023
78	27.01	Geranyl acetate	0.207±0.022	0.505±0.026	5.810±0.260	0.511±0.031
79	27.34	β-Elemene	0.683±0.036	0.294±0.016	1.320±0.050	0.202±0.011
80	27.63	Tetradecane	0.007±0.001	-	-	0.039±0.001
81	28.01	Dodecanal	0.137±0.025	-	-	-
82	28.10	Decyl acetate	0.043±0.008	-	-	-
83	28.28	α-Bergamotene	0.225±0.012	-	-	-
84	28.45	Caryophyllene	0.160±0.005	4.720±0.200	6.820±0.110	3.570±0.040
85	28.7	γ-Elemene	0.017±0.002	-	-	-
86	28.83	β-Gurjunene	0.008 ±0.001	-	-	-
87	29.02	α-Guaiene	0.946±0.053			
88	29.07	trans-α-Bergamotene	-	0.334±0.012	0.913±0.028	0.226±0.011
89	29.12	Thujopsene	3.710±0.100	0.167±0.008	0.455±0.004	-
90	29.41	Seychellene	0.022±0.003	-	0.198±0.002	-
91	29.74	Cedrene	0.048±0.003	-	-	-
92	29.81	α-Caryophyllene	0.168±0.005	0.278±0.010	0.913±0.025	0.206±0.007

Table 4.5 (continued)

Peak No.	RT		% Average area normalization (*n* = 3)			
	(min)	Tentative compound	Tom Yum soup	Paste 1	Paste 2	Paste 3
93	29.93	(E)-β-Farnesene	-	-	1.280±0.020	-
94	29.96	(Z)-β-Farnesene	0.222±0.013	-	-	-
95	30.09	Allo-Aromadendrene	0.151±0.007	-	-	-
96	30.72	γ-Muurolene	0.143±0.008	0.056±0.010	0.162±0.030	0.044±0.002
97	30.87	Germacrene D	-	0.115±0.008	0.439±0.012	0.122±0.017
98	30.91	β-Guaiene	0.715±0.024	-	-	-
99	30.97	4,11-selinadiene	-	0.158±0.011	0.597±0.015	0.125±0.020
100	31.06	γ-Gurjunene	0.290±0.013	0.102±0.008	1.950±0.040	0.077±0.017
101	31.21	α-Elemene	0.055±0.001	-	-	-
102	31.28	Cadina-3,9-diene	-	0.148±0.005	0.722±0.022	0.111±0.016
103	31.31	δ-Selinene	0.159±0.011	-	-	-
104	31.45	(Z,E)-α-Farnesene	0.152±0.010	-	1.800±0.020	-
105	31.48	Elixene	-	0.315±0.030	-	0.235±0.062
106	31.62	α-Chamigrene	-	-	1.140±0.030	-
107	31.64	Epicubebol	0.036±0.002	-	-	0.158±0.056
108	31.77	(Z)-α-Bisabolene	0.422±0.014	-	-	-
109	31.87	δ-Guaiene	-	-	0.370±0.030	-
110	31.95	α-Farnesene	-	0.138±0.008	1.620±0.010	0.123±0.036
111	32.01	α-Muurolene	6.270±0.180			
112	32.2	γ-Cadinene	0.010±0.001	0.179±0.011	0.811±0.010	0.111±0.001
113	32.27	(Z)-γ-Bisabolene	0.035±0.004	-	0.246±0.003	-
114	32.53	β-Cadinene	-	0.620±0.047	1.439±0.024	0.443±0.027
115	32.56	δ-Cadinene	0.092±0.002	-	-	-
116	32.86	Cadina-1(2),4-diene	-	0.081±0.008	0.159±0.006	0.064±0.010

Table 4.5 (continued)

Peak No.	RT (min)	Tentative compound	% Average area normalization (n = 3)			
			Tom Yum soup	Paste 1	Paste 2	Paste 3
117	32.88	(E)-γ-Bisabolene	0.026±0.001	-	-	-
118	32.99	Selina-3,7(11)-diene	0.042±0.003	-	-	-
119	33.1	α-Cadinene	0.040±0.002	-	0.186±0.007	-
120	33.3	α-Bisabolene	0.114±0.010	-	-	-
121	33.83	Germacrene B	1.000±0.040	-	0.127±0.004	-
122	34.06	(E)-Nerolidol	0.026±0.003	-	-	-
123	34.68	trans-Sesquisabinene hydrate	0.007±0.001	-	-	-
124	36.12	γ-Eudesmol	0.161±0.0120	0.193±0.010	0.807±0.031	0.205±0.010
125	36.97	δ-Cadinol	0.0120±0.001	-	-	-
126	37.47	τ-Muurolol	0.055±0.006	-	-	-
127	37.54	epi-γ-Eudesmol	0.019±0.001	-	-	-
128	37.97	α-Santalol	0.021±0.005	-	-	-
129	38.53	α-Bisabolol	0.035±0.006	-	-	-
130	38.89	Juniper camphor	0.013±0.001	-	-	-

Table 4.6 Aroma compounds in three commercial products of Tom Yum paste with our Tom Yum soup detected by GC─O

Peak No.	LRI_{exp}	Aroma compound	Average odor intensity (n = 3, Maximum value = 4)			
			Tom Yum soup	Paste I	Paste II	Paste III
1	<700	Acetic acid[b]	0.7	-	-	-
5	789	Butanoic acid[b]	0.3	-	-	-
8	866	3-Methylbutanoic acid[b]	1.3	-	-	0.7
10	906	3-(Methylthio)propanal[b]	3.3	2.7	1.0	3.3

Table 4.6 (continued)

Peak No.	LRI$_{exp}$	Aroma compound	Average odor intensity (*n* = 3, Maximum value = 4)			
			Tom Yum soup	Paste I	Paste II	Paste III
14	933	α-Pinene[b]	0.3	-	-	-
20	968	Dimethyl trisulfide[a]	-	2.0	-	1.0
22	976	β-Pinene[a]	-	1.0	1.0	-
23	988	6-Methyl-5-hepten-2-one[b]	0.3	1.7	1.7	1.3
30	1028	*D*-Limonene[a]	-	-	1.0	-
42	1101	β-linalool[b]	2.7	1.7	1.7	2.0
43	1105	Nonanal[a]	-	1.7	-	1.3
53	1165	Unknown 2 (MS of 152) [b]	0.7	-	-	-
55	1175	Isopinocamphone[a]	-	1.7	2.7	2.7
58	1181	2-Isobutyl-3-methoxypyrazine[b]	1.7	3.0	1.3	2.3
60	1191	α-Terpineol[b]	0.7	1.0	-	0.7
64	1228	β-Citronellol[a]	-	1.3	-	-
65	1230	Nerol[b]	0.3	-	-	-
66	1242	β-Citral[b]	3.0	-	1.3	-
67	1258	Geraniol[b]	0.7	-	1.0	1.0
68	1272	Geranial[b]	2.0	-	-	-
71	1317	4-Methylpentyl 4-methylpentanoate[b]	0.7	-	-	-
75	1355	Citronellyl acetate[b]	0.7	-	-	-
78	1385	Geranyl acetate[b]	0.7	-	2.0	1.7
81	1410	Dodecanal[b]	0.3	-	-	-

[a] Odor description refers http://www.flavornet.org/flavornet.html

Sulfur, fish and cabbage for dimethyl trisulfide

Pine, resin and turpentine for β-pinene

Citrus and mint for *D*-limonene

Fat, citrus and green for nonanal

Cedar camphoreous for isopinocamphone

Rose for β-citronellol

[b] Odor description are described as following Table 4.3

CHAPTER V
CONCLUSION

The chemical compositions of Tom Yum and the individual ingredient samples were profiled with HS–SPME–GC–O/MS. Volatile compounds were separated on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and the extracted sample was injected at 250 °C with split ratio of 1:10. The GC oven temperature was programed from 50 to 200 °C at 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 ion source in MS was set at 230 °C and the electron impact ionization voltage at -70 eV. Mass spectra were acquired over the mass range of 35–300 Da with the scan time of 100 ms. Data processing, volatile compounds were tentatively identified by a comparison of their MS spectra with those obtained from the NIST library as well as experimental and literature linear retention index (I) data for the same (or a similar) stationary phase. For sensory evaluation, six trained panelists were assigned for the detection and description of the aroma compounds in the extracted Tom Yum soup.

In initial result, the two main factors affecting the extraction performance, extraction temperature and extraction time of the HS–SPME sample preparation were studied using single factor optimization. Suitable extraction temperature of 40˚C and extraction time of 50 min were obtained to give the high peak areas of total peak area of all the volatile compounds detected and selected aroma compounds as well as the achieved resolution of most peaks.

In method validation, using the following HS–SPME conditions were evaluated for intraday and interday precision in the %area normalization of 13 aroma compounds. From ANOVA with a single factor analysis at a 95% confidence level, %RSD values for intraday and interday precision were less than 20% and 30%, respectively, for most of the aroma compounds with the exception of 4-methylpentyl 4-methylpentanoate, citronellyl acetate and geranyl acetate which had %RSD values in a range of 35-65, possibly due to the small amount of the %area normalization, i.e., less than 0.2.

The optimum of HS─SPME was applied for the selection of the most suitable method to allow the detection of 101 peaks in the GC─MS chromatogram of Tom Yum soup headspace. However, 96 peaks or compounds were identified representing various volatile classes. In comparison with volatile profiles in individual raw and boiled ingredients, Tom Yum soup were 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 reaction (such as cyclization) among the compounds in the mixed ingredients in Tom Yum soup: for example, *p*-mentha-3,8-diene and iso-isopulegol from cyclization of β-citronellal found in kaffir lime leaf, α-cyclocitral and p-mentha-1,5-dien-8-ol from cyclization of citrals found in lemongrass.

The eighteen aroma compounds that contribute impressive aroma of Tom Yum soup were characterized by HS─SPME─GC─O/MS along with at least two of the 6 trained panelists, 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), chili (2-isobutyl-3-methoxypyrazine and 4-methylpentyl 4-methylpentanoate), while β-linalool, α-terpineol, geraniol, β-citral, geranial, citronellyl acetate and geranyl acetate are from three ingredients such as lime juice, lemongrass and kaffir lime leaf.

In addition, our HS─SPME─GC─O/MS method was also applied to identify and compare the volatile compounds in Tom Yum soup obtained from three commercial products of Tom Yum paste with those in our Tom Yum soup. Most of the volatile compounds of three commercial products of Tom Yum paste were also found in our

Tom Yum soup and three of the five extra volatile compounds in our Tom Yum soup are also found in three commercial products of Tom Yum paste. Moreover, the aromas detected in three commercial products of Tom Yum paste were also found in our Tom Yum soup.

In the future work, this optimized HS–SPME–GC–O/MS method may be applied for other Thai dishes such as Green curry, Red curry and Yellow curry, Pad Thai, Tom Kha soup etc., as well as their individual ingredient before and after a cooking process in order to investigate extra compounds obtained from cooking and volatile compounds that contribute impressive aroma of the particular dish.

REFERENCES

[1] Kitsawad, K. and Tuntisripreecha, N. Sensory characterization of instant Tom Yum soup. KMUTNB International Journal of Applied Science and Technology 9(2) (2016): 1-8.

[2] Phornphisutthimas, S. Pilot-scale development of dried seasoning with Tom Yam flavour using mushroom as adsorbent. Asian Journal of Food and Agro-Industry 3(3) (2010): 335-342.

[3] Siripongvutikorn, S., Thongraung, C., Usawakesmanee, W., Buatoom, T., and Thammarutwasik, P. Development of instant Garcinia (Garcinia atroviridis) Tom Yum mix as a high acid seasoning. Journal of Food Processing and Preservation 33(1) (2009): 74-86.

[4] Zachariah, T.J., Leela, N.K., and Shamina, A. Methods of analysis of herbs and spices. Handbook of Herbs and Spices (Second edition) (2012): 89-117.

[5] Kamath, A., Asha, M.R., Ravi, R., Narasimhan, S., and Rajalakshmi, D. Comparative study of odour and GC-olfactometric profiles of selected essential oils. Flavour and Fragrance Journal 16(6) (2001): 401-407.

[6] Kubota, K., Nakamura, K., Kobayashi, A., and Amaike, M. Acetoxy-1,8-cineoles as Aroma Constituents of Alpinia galanga Willd. Journal of Agricultural and Food Chemistry 46(12) (1998): 5244-5247.

[7] Jirapakkul, W., Tinchan, P., and Chaiseri, S. Effect of drying temperature on key odourants in kaffir lime (Citrus hystrixD.C., Rutaceae) leaves. International Journal of Food Science and Technology 48(1) (2013): 143-149.

[8] Nolvachai, Y., Kulsing, C., and Marriott, P.J. Multidimensional gas chromatography in food analysis. TrAC - Trends in Analytical Chemistry 96 (2017): 124-137.

[9] Parker, J.K. Introduction to aroma compounds in foods. (2015): 3-30.

[10] Jiang, M., Kulsing, C., Nolvachai, Y., and Marriott, P.J. Two-dimensional retention indices improve component identification in comprehensive two-dimensional gas chromatography of saffron. Analytical Chemistry 87(11) (2015): 5753-61.

[11] Azzi-Achkouty, S., Estephan, N., Ouaini, N., and Rutledge, D.N. Headspace solid-phase microextraction for wine volatile analysis. Critical Reviews in Food Science and Nutrition 57(10) (2017): 2009-2020.

[12] Liu, G., et al. Analysis of the volatile components of tea seed oil (Camellia sinensis O. Ktze) from China using HS-SPME-GC/MS. International Journal of Food Science and Technology 51(12) (2016): 2591-2602.

[13] Patrignani, M., Fagúndez, G.A., Tananaki, C., Thrasyvoulou, A., and Lupano, C.E. Volatile compounds of Argentinean honeys: Correlation with floral and geographical origin. Food Chemistry 246(Supplement C) (2018): 32-40.

[14] Pham, A.J., Schilling, M.W., Yoon, Y., Kamadia, V.V., and Marshall, D.L. Characterization of fish sauce aroma-impact compounds using GC-MS, SPME-Osme-GCO, and Stevens' power law exponents. Journal of Food Science 73(4) (2008): C268-74.

[15] Wardencki, W., Chmiel, T., and Dymerski, T. Gas chromatography-olfactometry (GC-O), electronic noses (e-noses) and electronic tongues (e-tongues) for in vivo food flavour measurement. Instrumental Assessment of Food Sensory Quality (2013): 195-229.

[16] Skaria, B.P., Joy, P.P., Mathew, G., Mathew, S., and Joseph, A. Lemongrass. International Journal of Pharmaceutical Sciences Review and Research 35(2) (2012): 348-370.

[17] Bossou, A.D., et al. Characterization of volatile compounds from three Cymbopogon species and Eucalyptus citriodora from Benin and their insecticidal activities against Tribolium castaneum. Industrial Crops and Products 76 (2015): 306-317.

[18] Schaneberg, B.T. and Khan, I.A. Comparison of extraction methods for marker compounds in the essential oil of lemon grass by GC. Journal of Agricultural and Food Chemistry 50(6) (2002): 1345-1349.

[19] Wongpornchai, S. Kaffir lime leaf. Handbook of Herbs and Spices (Second edition) (2012): 319-328.

[20] Bogusz Junior, S., et al. Analysis of volatile compounds in Capsicum spp. by headspace solid-phase microextraction and GC × GC-TOFMS. Analytical Methods 7(2) (2015): 521-529.

[21] Bogusz Junior, S., Tavares, A.M., Filho, J.T., Zini, C.A., and Godoy, H.T. Analysis of the volatile compounds of Brazilian chilli peppers (Capsicum spp.) at two stages of maturity by solid phase micro-extraction and gas chromatography-mass spectrometry. Food Research International 48(1) (2012): 98-107.

[22] Quijano, C.E. and Pino, J.A. Volatile compounds of Capsicum frutescensL. cultivars from Colombia. Journal of Essential Oil Research 22(6) (2010): 503-506.

[23] Rodriguez-Burruezo, A., Kollmannsberger, H., Gonzalez-Mas, M.C., Nitz, S., and Fernando, N. HS-SPME comparative analysis of genotypic diversity in the volatile fraction and aroma-contributing compounds of Capsicum fruits from the annuum-chinense-frutescens complex. Journal of Agricultural and Food Chemistry 58(7) (2010): 4388-400.

[24] Cruz-Valenzuela, M.R., Tapia-Rodriguez, M.R., Vazquez-Armenta, F.J., Silva-Espinoza, B.A., and Ayala-Zavala, J.F. Lime (Citrus aurantifolia) Oils. Essential Oils in Food Preservation, Flavor and Safety (2016): 531-537.

[25] Ramesh Yadav, A., Chauhan, A.S., Rekha, M.N., Rao, L.J.M., and Ramteke, R.S. Flavour quality of dehydrated lime [Citrus aurantifolia (Christm.) Swingle]. Food Chemistry 85(1) (2004): 59-62.

[26] Chisholm, M.G., Wilson, M.A., and Gaskey, G.M. Characterization of aroma volatiles in key lime essential oils (Citrus aurantifolia Swingle). Flavour and Fragrance Journal 18(2) (2003): 106-115.

[27] Raina, A.P., Verma, S.K., and Abraham, Z. Volatile constituents of essential oils isolated from Alpinia galanga Willd. (L.) and A. officinarum Hance rhizomes from North East India. Journal of Essential Oil Research 26(1) (2013): 24-28.

[28] Kirsch, F. and Buettner, A. Odor Qualities and Thresholds of Physiological Metabolites of 1,8-Cineole as an Example for Structure-Activity Relationships Considering Chirality Aspects. Chemistry and Biodiversity 10(9) (2013): 1683-1695.

[29] Wichaphon, J., Thongthai, C., Assavanig, A., and Lertsiri, S. Volatile aroma components of Thai fish sauce in relation to product categorization. Flavour and Fragrance Journal 27(2) (2012): 149-156.

[30] Lapsongphon, N., Yongsawatdigul, J., and Cadwallader, K.R. Identification and characterization of the aroma-impact components of Thai fish sauce. Journal of Agricultural and Food Chemistry 63(10) (2015): 2628-38.

[31] Siripongvutikorn, S., Thummaratwasik, P., and Huang, Y.-w. Antimicrobial and antioxidation effects of Thai seasoning, Tom-Yum. LWT - Food Science and Technology 38(4) (2005): 347-352.

[32] C.F. Poole, S.A.S. Contemporary Practice of Chromatography. 1984, Elsevier.

[33] Gas-chromatography[Online]. Available from: https://basicmedicalkey.com/gas-chromatography/

[34] Poole, C.F. and Schuette, S.A. Chapter 3 - Instrumental requirements for gas chromatography. in Contemporary Practice of Chromatography, pp. 145–212. Amsterdam: Elsevier, 1984.

[35] Fulton G. Kitson, B.S.L., Charles N. McEwen. Gas Chromatography and Mass Spectrometry: A Practical Guide. 1996.

[36] Kommineni.vidyachowdhary. Mass spectrometry [Online]. Available from: https://www.slideshare.net/vidyachowdary50/mass-spectrometry-49346011

[37] Martens, L. Mass spectrometry basics.

[38] Gallery images and information: Triple Quadrupole.

[39] GERSTEL. Olfactory Detection Port

[40] Vas, G. and Vekey, K. Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. Journal of Mass Spectrometry 39(3) (2004): 233-54.

[41] He, C., Guo, X., Yang, Y., Xie, Y., Ju, F., and Guo, W. Characterization of the aromatic profile in “zijuan” and “pu-erh” green teas by headspace solid-phase microextraction coupled with GC-O and GC-MS. Analytical Methods 8(23) (2016): 4727-4735.

[42] Fan, W. and Qian, M.C. Identification of aroma compounds in Chinese ‘Yanghe Daqu’ liquor by normal phase chromatography fractionation followed by gas

chromatography[sol]olfactometry. Flavour and Fragrance Journal 21(2) (2006): 333-342.

[43] Tankiewicz, M., Morrison, C., and Biziuk, M. Application and optimization of headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-flame-ionization detector (GC-FID) to determine products of the petroleum industry in aqueous samples. Microchemical Journal 108 (2013): 117-123.

[44] Zhu, J., et al. Comparison of aroma-active volatiles in Oolong tea infusions using GC-olfactometry, GC-FPD, and GC-MS. Journal of Agricultural and Food Chemistry 63(34) (2015): 7499-7510.

[45] Harwell, M.R., Rubinstein, E.N., Hayes, W.S., and Olds, C.C. Summarizing Monte Carlo Results in Methodological Research: The One- and Two-Factor Fixed Effects ANOVA Cases. Journal of Educational Statistics 17(4) (1992): 315-339.

[46] Ermer, J., et al. Precision from drug stability studies investigation of reliable repeatability and intermediate precision of HPLC assay procedures. Journal of Pharmaceutical and Biomedical Analysis 38(4) (2005): 653-63.

[47] Ranade, S.S. and Thiagarajan, P. Lemon grass. International Journal of Pharmaceutical Sciences Review and Research 35(2) (2015): 162-167.

[48] Li, L.J., et al. Water accelerated transformation of d-limonene induced by ultraviolet irradiation and air exposure. Food Chemistry 239 (2018): 434-441.

[49] Shieh, D.-L., Tsai, C.-C., Chen, C.-W., and Ko, A.-N. Vapor-phase reaction of citronellal over mesoporous molecular sieves MCM-41 and zeolites. Journal of the Chinese Chemical Society 50(4) (2003): 853-860.

[50] Shibasaki, M., Terashima, S., and Yamada, S. Stereochemical studies. XXXIV. A novel biogenetic-type cyclization of citral to α-Cyclocitral via an Enamine. Chemical and Pharmaceutical Bulletin 23(2) (1975): 272-278.

[51] Kimura, K., Iwata, I., Nishimura, H., and Mizutani, J. Deterioration mechansim of lemon flavor. 2. Formation mechanism of off-odor substances arising from citral. Journal of Agricultural and Food Chemistry 31(4) (1983): 801-804.

[52] Yongzhong, Z., Yuntong, N., Jaenicke, S., and Chuah, G. Cyclisation of citronellal over zirconium zeolite beta-a highly diastereoselective catalyst to (±)-isopulegol. Journal of Catalysis 229(2) (2005): 404-413.

[53] Oliveira, M.V., Rebocho, S.F., Ribeiro, A.S., Macedo, E.A., and Loureiro, J.M. Kinetic modelling of decyl acetate synthesis by immobilized lipase-catalysed transesterification of vinyl acetate with decanol in supercritical carbon dioxide. The Journal of Supercritical Fluids 50(2) (2009): 138-145.

[54] http://webbook.nist.gov/cgi/cbook.cgi?Name=decyl+acetate&Units=SI&cGC=on

[55] Marković, K., Vahčić, N., Ganić, K.K., and Banović, M. Aroma volatiles of tomatoes and tomato products evaluated by solid-phase microextraction. Flavour and Fragrance Journal 22(5) (2007): 395-400.

[56] Wongpornchai, S. Kaffir lime leaf. in Handbook of Herbs and Spices (Second edition), pp. 319-328: Woodhead Publishing, 2012.

[57] Kimbaris, A.C., Siatis, N.G., Daferera, D.J., Tarantilis, P.A., Pappas, C.S., and Polissiou, M.G. Comparison of distillation and ultrasound-assisted extraction methods for the isolation of sensitive aroma compounds from garlic (Allium sativum). Ultrasonics Sonochemistry 13(1) (2006): 54-60.

[58] Soto, V.C., Maldonado, I.B., Jofré, V.P., Galmarini, C.R., and Silva, M.F. Direct analysis of nectar and floral volatile organic compounds in hybrid onions by HS-SPME/GC–MS: Relationship with pollination and seed production. Microchemical Journal 122(Supplement C) (2015): 110-118.

APPENDICES

APPENDIX A

Table A.1 Intraday and interday precision of 13 selected aroma compounds using triplicate batches of the extracted Tom Yum soup on each day for three consecutive days

Day 1

RT	Selected aroma	Pot 1						Pot 2						Pot3					
(min)	compounds	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD
8.25	α-Pinene	0.395	0.500	0.518	0.471	0.066	14	0.506	0.464	0.470	0.480	0.023	4.7	0.491	0.474	0.483	0.483	0.008	1.7
10.2	6-Methyl-5-hepten-2-one	0.013	0.007	0.010	0.010	0.003	27	0.012	0.011	0.011	0.012	0.001	4.5	0.011	0.011	0.013	0.012	0.001	10
14.8	β-Linalool	0.564	0.491	0.522	0.526	0.036	6.9	0.611	0.561	0.573	0.582	0.026	4.5	0.486	0.512	0.565	0.521	0.041	7.8
17.6	Unknown	0.091	0.048	0.071	0.070	0.021	30	0.075	0.069	0.076	0.073	0.004	5.2	0.073	0.077	0.080	0.077	0.004	4.7
18.7	α-Terpineol	0.734	0.587	0.646	0.656	0.074	11	0.736	0.675	0.703	0.704	0.030	4.3	0.656	0.709	0.813	0.726	0.080	11
20.4	Nerol	0.978	0.841	0.884	0.901	0.070	7.8	1.010	0.926	0.975	0.970	0.042	4.3	0.845	0.903	0.984	0.911	0.070	7.7
21	β-Citral	10.8	7.96	9.29	9.35	1.414	15	9.32	8.55	8.94	8.94	0.386	4.3	9.36	10.2	11.2	10.2	0.898	8.8
21.5	Geraniol	1.18	0.961	1.04	1.058	0.111	11	1.08	0.99	1.04	1.04	0.045	4.3	0.902	0.927	1.14	0.988	0.128	13
22.3	Geranial	25.8	17.5	20.3	21.2	4.221	20	20.3	18.6	19.3	19.4	0.846	4.4	20.1	21.4	23.1	21.5	1.503	7.0
24.1	4-Methylpentyl 4-methylpentanoate	0.102	0.088	0.093	0.094	0.007	7.6	0.125	0.115	0.103	0.114	0.011	9.6	0.088	0.089	0.100	0.092	0.007	7.6
25.7	Citronellyl acetate	0.209	0.178	0.190	0.192	0.016	8.2	0.179	0.164	0.162	0.168	0.009	5.6	0.133	0.141	0.154	0.142	0.011	7.6
27	Geranyl acetate	0.182	0.164	0.164	0.170	0.010	6.1	0.166	0.152	0.149	0.156	0.009	5.9	0.162	0.156	0.163	0.160	0.004	2.3
28	Dodecanal	0.309	0.268	0.275	0.284	0.022	7.7	0.292	0.268	0.343	0.301	0.038	12.7	0.276	0.253	0.282	0.270	0.015	5.6

Day 2

RT (min)	Selected aroma compounds	Pot 1						Pot 2						Pot 3					
		1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD
8.25	α-Pinene	0.403	0.384	0.400	0.396	0.010	2.6	0.421	0.398	0.414	0.411	0.012	2.9	0.385	0.376	0.378	0.380	0.005	1.3
10.2	6-Methyl-5-hepten-2-one	0.014	0.015	0.014	0.014	0.001	6.1	0.012	0.014	0.015	0.014	0.001	8.8	0.011	0.012	0.014	0.012	0.001	11
14.8	β-Linalool	0.744	0.747	0.833	0.774	0.051	6.5	0.660	0.676	0.683	0.673	0.012	1.8	0.727	0.773	0.788	0.763	0.032	4.2
17.6	Unknown	0.083	0.093	0.102	0.093	0.010	11	0.089	0.080	0.086	0.085	0.005	5.7	0.087	0.093	0.090	0.090	0.003	3.2
18.7	α-Terpineol	1.05	1.122	1.21	1.125	0.079	7.0	0.933	0.900	0.942	0.925	0.022	2.4	0.927	1.015	1.051	0.998	0.064	6.4
20.4	Nerol	0.981	1.044	1.092	1.039	0.056	5.4	0.996	0.988	0.987	0.991	0.005	0.5	0.991	1.019	1.060	1.023	0.035	3.4
21	β-Citral	11.8	11.7	13.8	12.4	1.203	9.7	9.31	9.73	10.5	9.83	0.579	5.9	10.4	11.6	11.6	11.2	0.693	6.2
21.5	Geraniol	0.832	0.941	0.972	0.915	0.074	8.0	0.878	0.877	0.891	0.882	0.008	0.9	0.887	0.911	0.906	0.901	0.013	1.4
22.3	Geranial	22.4	25.7	26.2	24.8	2.046	8.3	21.1	21.4	22.4	21.6	0.692	3.2	24.0	25.6	25.5	25.0	0.913	3.7
24.1	4-Methylpentyl 4-methylpentanoate	0.057	0.061	0.066	0.061	0.004	6.9	0.046	0.043	0.043	0.044	0.002	5.0	0.050	0.049	0.049	0.049	0.001	1.4
25.7	Citronellyl acetate	0.191	0.199	0.196	0.195	0.004	2.0	0.205	0.184	0.185	0.191	0.012	6.2	0.210	0.192	0.200	0.201	0.009	4.4
27	Geranyl acetate	0.181	0.200	0.187	0.189	0.009	5.0	0.209	0.184	0.179	0.191	0.016	8.5	0.209	0.175	0.182	0.189	0.018	9.3
28	Dodecanal	0.206	0.234	0.197	0.212	0.019	9.1	0.241	0.213	0.230	0.228	0.014	6.3	0.239	0.221	0.216	0.225	0.012	5.5

Day 3

RT (min)	Selected aroma compounds	Pot 1						Pot 2						Pot 3					
		1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD
8.25	α-Pinene	0.406	0.437	0.426	0.423	0.016	3.7	0.494	0.518	0.597	0.536	0.0539	10	0.432	0.410	0.403	0.415	0.015	3.6
10.2	6-Methyl-5-hepten-2-one	0.012	0.012	0.011	0.011	0.001	4.5	0.012	0.011	0.014	0.012	0.0014	12	0.016	0.015	0.015	0.015	0.000	2.3
14.8	β-Linalool	0.545	0.705	0.713	0.654	0.095	14	0.572	0.557	0.749	0.626	0.1066	17	0.619	0.625	0.710	0.651	0.051	7.8
17.6	Unknown	0.083	0.091	0.098	0.091	0.007	8.0	0.075	0.071	0.086	0.078	0.0077	10	0.077	0.070	0.077	0.075	0.004	5.7
18.7	α-Terpineol	0.704	0.871	0.854	0.810	0.092	11	0.833	0.793	0.938	0.855	0.0749	8.8	0.873	0.834	0.864	0.857	0.020	2.4
20.4	Nerol	0.565	0.668	0.699	0.644	0.070	11	0.624	0.585	0.714	0.641	0.0662	10	0.763	0.729	0.780	0.758	0.026	3.4
21	β-Citral	11.6	14.1	14.5	13.4	1.524	11	9.673	9.883	11.572	10.376	1.0412	10	11.131	10.376	11.694	11.067	0.661	6.0
21.5	Geraniol	0.980	1.119	1.079	1.059	0.072	6.8	0.919	0.845	0.884	0.883	0.0371	4.2	1.071	1.014	1.015	1.034	0.033	3.2
22.3	Geranial	27.9	31.4	31.5	30.3	2.022	6.7	22.6	22.0	17.7	20.8	2.6384	13	25.4	23.1	25.5	24.7	1.357	5.5
24.1	4-Methylpentyl 4-methylpentanoate	0.024	0.022	0.024	0.024	0.001	4.6	0.025	0.021	0.025	0.023	0.0022	9.5	0.027	0.024	0.024	0.025	0.002	6.0
25.7	Citronellyl acetate	0.037	0.041	0.041	0.040	0.002	5.1	0.038	0.036	0.039	0.037	0.0016	4.2	0.042	0.038	0.037	0.039	0.002	5.7
27	Geranyl acetate	0.069	0.079	0.075	0.074	0.005	6.5	0.074	0.070	0.076	0.073	0.0032	4.4	0.083	0.077	0.079	0.080	0.003	4.3
28	Dodecanal	0.216	0.218	0.227	0.220	0.006	2.7	0.212	0.224	0.214	0.217	0.0065	3.0	0.225	0.218	0.230	0.224	0.006	2.8

Table A.2 Summarized average %area normalization of each batch for 3 days

RT (min)	Selected aroma compounds	Day 1						Day 2						Day 3					
		1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD
8.25	α-Pinene	0.471	0.480	0.483	0.478	0.0063	1.3	0.396	0.411	0.380	0.395	0.016	3.9	0.423	0.536	0.415	0.458	0.0678	15
10.2	6-Methyl-5-hepten-2-one	0.010	0.012	0.012	0.011	0.001	8.8	0.014	0.014	0.012	0.013	0.001	7.5	0.011	0.012	0.015	0.013	0.002	16
14.8	β-Linalool	0.526	0.582	0.521	0.543	0.034	6.2	0.774	0.673	0.763	0.737	0.055	7.5	0.654	0.626	0.651	0.644	0.015	2.4
17.6	Unknown	0.070	0.073	0.077	0.073	0.003	4.5	0.093	0.085	0.090	0.089	0.004	4.3	0.091	0.078	0.075	0.081	0.009	11
18.7	α-Terpineol	0.656	0.704	0.726	0.695	0.036	5.2	1.125	0.925	0.998	1.016	0.101	10	0.810	0.855	0.857	0.841	0.027	3.2
20.4	Nerol	0.901	0.970	0.911	0.927	0.037	4.0	1.039	0.991	1.023	1.017	0.025	2.4	0.644	0.641	0.758	0.681	0.067	9.8
21	β-Citral	9.346	8.937	10.228	9.504	0.660	7	12.403	9.831	11.200	11.145	1.287	12	13.424	10.376	11.067	11.622	1.598	14
21.5	Geraniol	1.058	1.038	0.988	1.028	0.036	3.5	0.915	0.882	0.901	0.899	0.016	1.8	1.059	0.883	1.034	0.992	0.095	9.6
22.3	Geranial	21.23	19.39	21.517	20.716	1.151	5.6	24.765	21.643	25.017	23.808	1.880	7.9	30.292	20.766	24.692	25.250	4.787	19
24.1	4-Methylpentyl 4-methylpentanoate	0.094	0.114	0.092	0.100	0.012	12.0	0.061	0.044	0.049	0.051	0.009	17	0.024	0.023	0.025	0.024	0.001	3.5
25.7	Citronellyl acetate	0.192	0.168	0.142	0.168	0.025	15	0.195	0.191	0.201	0.196	0.005	2.4	0.040	0.037	0.039	0.039	0.001	2.8
27	Geranyl acetate	0.170	0.156	0.160	0.162	0.007	4.5	0.189	0.191	0.189	0.190	0.001	0.54	0.074	0.073	0.080	0.076	0.003	4.5
28	Dodecanal	0.284	0.301	0.270	0.285	0.015	5.4	0.212	0.228	0.225	0.222	0.008	3.7	0.220	0.217	0.224	0.220	0.004	1.7

APPENDIX B

Table B.1 Statistical ANOVA with a single factor analysis of α-Pinene for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	1.433632	0.477877	4.01E-05
Row 2	3	1.186093	0.395364	0.000243
Row 3	3	1.374682	0.458227	0.004596

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.011146	2	0.005573	3.426577	0.102	5.143253
Within Groups	0.009759	6	0.001626			
Total	0.020905	8				

Table B.2 Statistical ANOVA with a single factor analysis of 6-Methyl-5-hepten-2-one for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	0.033689	0.01123	9.7E-07
Row 2	3	0.040492	0.013497	1.03E-06
Row 3	3	0.038933	0.012978	4.05E-06

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	8.47E-06	2	4.23E-06	2.098761	0.204	5.143253
Within Groups	1.21E-05	6	2.02E-06			
Total	2.06E-05	8				

Table B.3 Statistical ANOVA with a single factor analysis of β-linalool for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	1.628614	0.542871	0.001134
Row 2	3	2.209678	0.736559	0.003082
Row 3	3	1.931434	0.643811	0.000245

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.056306	2	0.028153	18.92719	0.003	5.143253
Within Groups	0.008925	6	0.001487			
Total	0.065231	8				

Table B.4 Statistical ANOVA with a single factor analysis of unknown for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	0.21977	0.073257	1.09E-05
Row 2	3	0.267826	0.089275	1.49E-05
Row 3	3	0.243231	0.081077	7.35E-05

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000385	2	0.000192	5.811827	0.039	5.143253
Within Groups	0.000199	6	3.31E-05			
Total	0.000584	8				

Table B.5 Statistical ANOVA with a single factor analysis of α-terpineol for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	2.08616	0.695387	0.001294
Row 2	3	3.047339	1.01578	0.010286
Row 3	3	2.521658	0.840553	0.000705

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.154429	2	0.077215	18.85543	0.003	5.143253
Within Groups	0.024571	6	0.004095			
Total	0.179	8				

Table B.6 Statistical ANOVA with a single factor analysis of nerol for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	2.782035	0.927345	0.001399
Row 2	3	3.05237	1.017457	0.000602
Row 3	3	2.041978	0.680659	0.004432

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.182406	2	0.091203	42.53797	0.0003	5.143253
Within Groups	0.012864	6	0.002144			
Total	0.19527	8				

Table B.7 Statistical ANOVA with a single factor analysis of β-citral for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	28.51125	9.50375	0.43525
Row 2	3	33.4338	11.1446	1.656625
Row 3	3	34.86644	11.62215	2.553652

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.408039	2	3.70402	2.391991	0.172	5.143253
Within Groups	9.291054	6	1.548509			
Total	16.69909	8				

Table B.8 Statistical ANOVA with a single factor analysis of geraniol for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	3.084147	1.028049	0.001311
Row 2	3	2.698157	0.899386	0.000262
Row 3	3	2.975881	0.99196	0.0091

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.026427	2	0.013213	3.714044	0.089	5.143253
Within Groups	0.021346	6	0.003558			
Total	0.047773	8				

Table B.9 Statistical ANOVA with a single factor analysis of geranial for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	62.14759	20.71586	1.325888
Row 2	3	71.42473	23.80824	3.533577
Row 3	3	75.75069	25.25023	22.92016

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	32.20263	2	16.10132	1.738827	0.254	5.143253
Within Groups	55.55924	6	9.259873			
Total	87.76187	8				

Table B.10 Statistical ANOVA with a single factor analysis of 4-methylpentyl 4-methylpentanoate for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	0.300584	0.100195	0.000145
Row 2	3	0.154216	0.051405	7.91E-05
Row 3	3	0.071924	0.023975	6.86E-07

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.008942	2	0.004471	59.59271	0.0001	5.143253
Within Groups	0.00045	6	7.5E-05			
Total	0.009392	8				

Table B.11 Statistical ANOVA with a single factor analysis of citronellyl acetate for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	0.503073	0.167691	0.000619
Row 2	3	0.586931	0.195644	2.23E-05
Row 3	3	0.115993	0.038664	1.2E-06

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.042072	2	0.021036	98.28915	2.60E-05	5.143253
Within Groups	0.001284	6	0.000214			
Total	0.043356	8				

Table B.12 Statistical ANOVA with a single factor analysis of geranyl acetate for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	0.485691	0.161897	5.32E-05
Row 2	3	0.568806	0.189602	1.07E-06
Row 3	3	0.227557	0.075852	1.15E-05

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.02111	2	0.010555	481.9816	2.37E-07	5.143253
Within Groups	0.000131	6	2.19E-05			
Total	0.021242	8				

Table B.13 Statistical ANOVA with a single factor analysis of dodecanal for 3 days

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	0.854876	0.284959	0.000234
Row 2	3	0.665325	0.221775	6.84E-05
Row 3	3	0.661362	0.220454	1.44E-05

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.008155	2	0.004077	38.55425	0.0004	5.143253
Within Groups	0.000635	6	0.000106			
Total	0.008789	8				

Table B.14 Summarized AVOVA data for calculation intraday %RSD of selected aroma compounds (*P*-value <0.05)

Selected aroma compounds	Within group MS (SD$^2_{within}$)	Between group MS	SD$^2_{between}$	SD$_{within}$ (S$_r$)	SD$_{interday}$	Average %area normalization ($\bar{x}$) (*n* = 9)	Intraday %RSD = (100S$_r$/$\bar{x}$)×100	Interday %RSD = (SD$_{interday}$/$\bar{x}$)×100
β-Linalool	0.009	0.06	0.005	0.09	0.12	0.641	15	19
Unknown	0.0002	0.00039	2.0695E-05	0.014	0.015	0.081	17	18
α-Terpineol	0.025	0.15	0.014	0.16	0.20	0.851	18	23
Nerol	0.013	0.18	0.019	0.11	0.18	0.875	13	20
4-Methylpentyl 4-methylpentanoate	0.00045	0.0089	0.001	0.021	0.037	0.059	36	64
Citronellyl acetate	0.0013	0.042	0.0045	0.036	0.076	0.134	27	57
Geranyl acetate	0.00013	0.021	0.0023	0.011	0.050	0.142	8.0	35

Selected aroma compounds	Within group MS (SD^2_{within})	Between group MS	$SD^2_{between}$	SD_{within} (S_r)	$SD_{interday}$	Average %area normalization ($\overline{x}$) (n = 9)	Intraday %RSD = $(100S_r/\overline{x})\times100$	Interday %RSD = $(SD_{interday}/\overline{x})\times100$
Dodecanal	0.00064	0.0082	0.00084	0.025	0.038	0.242	10	16

$$\text{SDinterday} \quad = \quad \sqrt{\mathbf{SD^2within+SD^2between}}$$

$$\text{SD}^2\text{between} \quad = \quad \frac{\textbf{between group MS-within group MS}}{\mathbf{n}}$$

$$\text{SD}^2\text{within} \quad = \quad \text{within group MS}$$

Where the data of the within group MS and between group MS are obtain from the ANOVA data in Table B.3, 4, 5, 6, 10, 11, 12 and 13 and *n* is the number of replicate measurements (9).

APPENDIX C

Table C.1 Tentative volatile compounds in Tom Yum soup and individual ingredient

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
1	2.61	Acetic acid	-	-	-	1.81 ±0.11	17.6 ±0.6	-	-	-	-	-	-
2	2.79	3-Methylbutanal	-	-	-	0.59 ±0.10	13.0 ±0.5	-	-	-	-	-	-
3	2.86	3-Methylpentanal	-	-	-	1.04 ±0.07	10.9 ±0.4	-	-	-	-	-	-
4	3.03	Propanoic acid	-	-	-	2.75 ±0.04	9.05 ±1.01	-	-	-	-	-	-
5	3.17	3-Pentanone	-	-	-	-	-	0.012 ±0.002	-	-	-	-	-
6	3.57	3-Methyl-1-butanol	-	-	-	1.04 ±0.06	-	-	-	-	-	-	-
7	3.62	2-Methyl-1-butanol	-	-	-	0.50 ±0.02	-	-	-	-	-	-	-
8	3.70	1-Methylpyrrole	-	-	-	-	-	-	-	1.66 ±0.90	0.81 ±0.03	-	-
9	4.49	Butanoic acid	-	-	-	26.13±0.56	-	-	-	-	-	-	-
10	5.43	4-Methyl-1-pentanol	-	-	-	-	-	-	-	0.74 ±0.38	-	-	-
11	5.90	3-Hexen-1-ol	-	-	-	-	-	0.10 ±0.02	-	-	-	-	-
12	5.93	2-Methylbutanoic acid	-	-	-	14.83±0.90	-	-	-	-	-	-	-
13	6.26	3-Methylbutanoic acid	-	-	-	14.72 ±0.24	-	-	-	-	-	-	-
14	7.10	Nonane	0.013 ±0.001	-	-	-	-	-	-	-	-	0.007 ±0.001	0.007 ±0.001
15	7.32	3-(methylthio)propanal	-	-	-	1.27 ±0.09	3.94 ±1.25	-	-	-	-	-	-
16	7.59	2,6-Dimethylpyrazine	-	-	-	0.69 ±0.12	-	-	-	-	-	-	-
17	7.70	Anisole	-	-	-	-	-	0.007 ±0.002	-	0.56 ±0.31	-	-	-
18	7.87	Tricyclene	-	-	-	-	-	-	-	0.034 ±0.020	-	0.006 ±0.001	0.004 ±0.001
19	8.01	α-Thujene	0.063 ±0.020	-	-	-	-	0.037 ±0.005	-	0.023 ±0.014	-	0.25 ±0.01	0.078 ±0.006
20	8.25	α-Pinene	0.57 ±0.07	0.011 ±0.003	-	-	-	0.044 ±0.005	-	0.047 ±0.030	-	1.64 ±0.10	0.93 ±0.01
21	8.70	α-Fenchene	0.008 ±0.001	-	-	-	-	-	-	-	-	0.002 ±0.001	0.029 ±0.003
22	8.77	Camphene	0.043 ±0.003	0.011 ±0.007	-	-	-	-	-	0.028 ±0.015	-	0.088 ±0.005	0.11 ±0.01
23	8.96	Thuja-2,4(10)-diene	-	-	-	-	-	-	-	-	-	0.005 ±0.001	0.003 ±0.001
24	9.00	4-Methylpentanoic acid	-	-	-	13.1 ±1.3	-	-	-	-	-	-	-
25	9.16	Benzaldehyde	-	-	-	3.40 ±0.60	5.95 ±0.57	-	-	-	-	-	-
26	9.65	Sabinene	0.38 ±0.14	-	-	-	-	1.21 ±0.10	0.059 ±0.006	0.18 ±0.11	-	1.24 ±0.21	0.60 ±0.10
27	9.76	β-Pinene	4.79 ±0.96	-	-	-	-	0.081 ±0.007	-	0.25 ±0.16	-	19.99 ±0.31	6.92 ±0.54
28	9.84	Phenol	-	-	-	8.50 ±0.38	-	-	-	-	-	-	-
29	10.19	6-Methyl-5-hepten-2-one	0.015 ±0.003	0.009 ±0.001	0.072 ±0.003	-	-	-	-	-	-	-	-

Peak No.	RT (min)	Tentative compound	Tom Yum soup	% Average area normalization (n = 3)									
				Lemongras		Fish sauce		Kaffir lime leaf		Chili		Lime juice	
				Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled
30	10.30	β-Myrcene	0.72 ±0.03	8.15 ±1.67	0.40 ±0.02	-	-	1.30 ±0.18	0.034 ±0.003	0.038 ±0.02	-	1.11 ±0.03	1.03 ±0.02
31	10.83	α-Phellandrene	0.071 ±0.005	0.019 ±0.002	0.026 ±0.003	-	-	0.009 ±0.002	0.021 ±0.002	-	-	0.054 ±0.003	0.13 ±0.01
32	10.93	(Z)-3-hexenyl acetate	-	-	-	-	-	2.10 ±0.13	0.047 ±0.005	-	-	-	-
33	11.22	(E)-3-Hexenyl acetate	-	-	-	-	-	0.07 ±0.01	-	-	-	-	-
34	11.32	α-Terpinene	0.34 ±0.02	0.014±0.001	-	-	-	-	-	-	-	0.38 ±0.03	0.75 ±0.05
35	11.62	m-Cymene	0.125 ±0.003	0.013±0.003	0.044 ±0.002	-	-	-	-	0.066 ±0.030	-	0.023 ±0.004	0.11 ±0.01
36	11.79	D-Limonene	26.55 ±3.01	0.16 ±0.01	0.017 ±0.002	-	-	0.19 ±0.02	0.010 ±0.001	0.34 ±0.19	-	49.93 ±0.85	42.94 ±0.42
37	11.81	2-Ethyl-1-hexanol	-	-	-	0.24 ±0.01	2.98 ±0.85	-	-	-	-	-	-
38	11.93	Eucalyptol	-	0.028 ±0.003	0.011 ±0.002	-	-	0.025 ±0.002	0.013 ±0.001	0.038 ±0.025	-	-	-
39	12.17	cis-β-Ocimene	0.087 ±0.010	3.18 ±0.37	0.100 ±0.004	-	-	0.036 ±0.005	0.013 ±0.001	-	-	0.033 ±0.003	0.019 ±0.001
40	12.39	Benzeneacetaldehyde	-	-	-	0.81 ±0.19	-	-	-	-	-	-	-
41	12.60	trans-β-Ocimene	0.093 ±0.010	1.91 ±0.22	0.073 ±0.003	-	-	0.83 ±0.11	-	0.024 ±0.010	-	0.037 ±0.001	0.067 ±0.002
42	13.04	γ-Terpinene	4.46 ±0.41	0.009 ±0.001	-	-	-	0.022 ±0.002	-	-	-	9.21 ±0.09	10.01 ±0.13
43	13.33	Acetophenone	-	-	-	0.16 ±0.03	-	-	-	-	-	-	-
44	13.42	Isoterpinolene	-	-	-	-	-	0.021 ±0.003	0.027 ±0.004	-	-	-	-
45	13.50	*p*-Mentha-3,8-diene	0.021 ±0.004	-	-	-	-	-	-	-	-	-	-
46	13.95	3-Ethyl-2,5-dimethylpyrazine	-	-	-	0.17 ±0.03	-	-	-	-	-	-	-
47	14.28	Terpinolene	1.20 ±0.08	0.032 ±0.002	0.013 ±0.001	-	-	0.10 ±0.02	-	0.033 ±0.008	-	0.63 ±0.04	3.43 ±0.25
48	14.67	Rosefuran	0.006 ±0.002	0.026 ±0.002	-	-	-	-	-	-	-	-	-
49	14.79	β-Linalool	0.79 ±0.12	0.65 ±0.12	0.11 ±0.01	-	-	1.09 ±0.10	1.80 ±0.14	-	-	0.17 ±0.03	0.11 ±0.01
50	14.85	Isopentyl 2-methylbutanoate	-	-	-	-	-	-	-	0.082 ±0.02	2.28 ±0.34	-	-
51	15.00	Nonanal	0.007 ±0.001	-	-	-	-	-	-	-	-	0.017 ±0.002	0.012 ±0.001
52	15.07	Isopentyl isovalerate	-	-	-	-	-	-	-	0.077 ±0.023	-	-	-
53	15.30	1,3,8-*p*-Menthatriene	0.022 ±0.004	-	-	-	-	-	-	-	-	0.001 ±0.001	0.001 ±0.001
54	15.33	Phenylethyl Alcohol	-	-	-	0.78 ±0.11	-	-	-	-	-	-	-
55	15.36	2-Methylpentyl isobutyrate	-	-	-	-	-	-	-	0.61 ±0.21	0.91 ±0.16	-	-
56	15.37	Fenchol	0.045 ±0.010	-	-	-	-	-	-	-	-	0.023 ±0.007	0.068 ±0.007
57	15.80	α-Cyclocitral	0.024 ±0.002	-	-	-	-	-	-	-	-	-	-
58	16.05	Allo-Ocimene	-	0.16 ±0.02	0.006 ±0.001	-	-	-	-	-	-	-	-
59	16.52	Pentyl 2-methylbutanoate	-	-	-	-	-	-	-	0.038 ±0.012	-	-	-
60	16.75	Isopulegol	0.10 ±0.02	-	-	-	-	0.050 ±0.010	0.71 ±0.05	0.033 ±0.006	-	0.001 ±0.001	0.014 ±0.003
61	16.76	cis-*p*-Mentha-2,8-dien-1-ol	-	0.005 ±0.001	0.109 ±0.001	-	-	-	-	-	-	-	-
62	17.17	β-Citronellal	-	0.015 ±0.002	0.11 ±0.01	-	-	3.90 ±0.66	93.92 ±0.50	-	-	-	-
63	17.23	Iso-isopulegol	0.059 ±0.016	-	-	-	-	-	-	-	-	-	-

Peak No.	RT (min)	Tentative compound	Tom Yum soup	% Average area normalization (n = 3)									
				Lemongras		Fish sauce		Kaffir lime leaf		Chili		Lime juice	
				Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled
64	17.30	Hexyl isobutyrate	-	-	-	-	-	-	-	0.037 ±0.011	-	-	-
65	17.52	Unknown 1	0.034 ±0.006	-	-	-	-	-	-	-	-	-	-
66	17.63	Unknown 2	0.044 ±0.010	0.011 ±0.001	0.34 ±0.01	-	-	-	-	-	-	-	-
67	17.64	Isothujol	-	-	-	-	-	-	-	-	-	0.006 ±0.002	0.021 ±0.004
68	17.70	*p*-Mentha-1,5-dien-8-ol	0.032 ±0.010	-	-	-	-	-	-	-	-	-	-
69	18.04	Isopinocamphone	-	-	-	-	-	-	-	-	-	0.009 ±0.002	0.005 ±0.001
70	18.10	Rose furan oxide	-	0.026 ±0.002	0.014 ±0.002	-	-	-	-	-	-	-	-
71	18.16	Terpinen-4-ol	0.28 ±0.02	-	-	-	-	-	-	-	-	0.47 ±0.09	0.38 ±0.03
72	18.32	2-Isobutyl-3-methoxypyrazine	-	-	-	-	-	-	-	0.049 ±0.014	0.76 ±0.03	-	-
73	18.41	*p*-Mentha-1(7),8-dien-2-ol	0.34 ±0.06	0.014 ±0.002	0.62 ±0.02	-	-	-	-	-	-	0.001 ±0.001	-
74	18.60	(Z)-3-hexenyl butanoate	-	-	-	-	-	0.15 ±0.04	-	-	-	-	-
75	18.73	α-Terpineol	0.66 ±0.08	0.016 ±0.002	-	-	-	0.027 ±0.004	0.021 ±0.006	-	-	0.24 ±0.06	1.51 ±0.17
76	18.90	Methyl salicylate	-	-	-	-	-	-	-	0.28 ±0.11	-	-	-
77	19.14	Dodecane	-	-	-	-	-	0.030 ±0.005	-	-	-	0.002 ±0.001	0.003 ±0.001
78	19.20	4-methylpentyl 2-methylbutanoate	0.051 ±0.008	-	-	-	-	-	-	14.75 ±2.11	3.96 ±0.28	-	-
79	19.41	Decanal	0.15 ±0.01	-	-	-	-	-	-	-	-	0.24 ±0.01	0.32 ±0.01
80	19.43	4-Methylpentyl 3-methylbutanoate	-	-	-	-	-	-	-	11.04 ±1.59	5.59 ±0.18	-	-
81	19.97	Carveol	0.012 ±0.003	-	0.031 ±0.002	-	-	-	-	-	-	-	-
82	20.01	γ-Isogeraniol	-	0.019 ±0.005	-	-	-	-	-	-	-	-	-
83	20.40	Nerol	0.44 ±0.07	13.07 ±0.73	0.31 ±0.01	-	-	-	-	-	-	0.010 ±0.002	-
84	20.51	β-Citronellol	-	-	-	-	-	47.7 ±1.1	2.65 ±0.21	-	-	-	-
85	20.52	cis-3-Hexenyl isovalerate	-	-	-	-	-	-	-	0.033 ±0.006	-	-	-
86	20.59	cis-3-Hexenyl-α-methylbutyrate	-	-	-	-	-	-	-	0.037 ±0.013	-	-	-
87	20.79	Butanoic acid, 2-methyl-, hexyl ester	-	-	-	-	-	-	-	0.44 ±0.13	-	-	-
88	20.99	β-Citral	12.36 ±1.68	2.81 ±0.48	24.84 ±0.24	-	-	-	-	-	-	0.15 ±0.02	0.10 ±0.01
89	21.00	Butanoic acid, 3-methyl-, hexyl ester	-	-	-	-	-	-	-	0.10 ±0.03	-	-	-
90	21.49	Hexyl n-valerate	-	-	-	-	-	-	-	1.05 ±0.27	-	-	-
91	21.53	Geraniol	0.91 ±0.09	39.7 ±3.2	2.89 ±0.05	-	-	0.020 ±0.005	0.026 ±0.001	-	-	0.009 ±0.002	0.005 ±0.002
92	21.81	Methyl citronellate	-	-	-	-	-	0.036 ±0.001	-	-	-	-	-
93	22.33	Geranial	25.4 ±2.8	7.15 ±0.64	69.05 ±0.21	-	-	0.018 ±0.005	-	-	-	0.36 ±0.05	0.24 ±0.02
94	22.44	Citronellyl formate	-	-	-	-	-	0.010 ±0.001	-	-	-	-	-
95	23.13	Indole	-	-	-	6.70 ±0.93	1.98 ±0.93	0.036 ±0.004	-	-	-	-	-

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
96	23.47	Tridecane	0.027 ±0.004	-	-	-	-	0.024 ±0.006	-	-	-	0.013 ±0.002	0.023 ±0.001
97	23.76	Hexyl 2-methylbutanoate	-	-	-	-	-	-	-	0.29 ±0.04	-	-	-
98	23.79	Undecanal	0.020 ±0.003	-	-	-	-	-	-	-	-	0.017 ±0.001	0.036 ±0.003
99	23.97	Hexyl 3-methylbutanoate	-	-	-	-	-	-	-	0.20 ±0.03	-	-	-
100	24.12	4-Methylpentyl 4-methylpentanoate	0.11 ±0.01	-	-	-	-	-	-	45.25 ±4.46	53.42 ±2.30	-	-
101	24.50	Methyl geranate	0.012 ±0.002	0.18 ±0.01	0.014 ±0.002	-	-	-	-	-	-	-	-
102	25.08	δ-Elemene	1.67 ±0.07	0.035 ±0.002	-	-	-	0.38 ±0.02	-	0.031 ±0.004	2.57 ±0.34	0.88 ±0.06	1.84 ±0.08
103	25.58	α-Cubebene	0.005 ±0.001	0.029 ±0.003	-	-	-	1.06 ±0.08	0.013 ±0.002	-	-	0.003 ±0.001	0.006 ±0.001
104	25.63	4-methylpentyl hexanoate	-	-	-	-	-	-	-	1.28 ±0.21	-	-	-
105	25.75	Citronellyl acetate	0.024 ±0.003	0.023 ±0.006	-	-	-	2.49 ±0.40	0.085 ±0.005	-	-	0.001 ±0.001	0.003 ±0.001
106	26.14	2-Methyltridecane	-	-	-	-	-	-	-	4.95 ±1.50	1.14 ±0.09	-	-
107	26.17	Neric acid	-	0.32 ±0.20	-	-	-	-	-	-	-	-	-
108	26.21	Cyclosativene	0.045 ±0.003	-	-	-	-	0.16 ±0.01	0.009 ±0.001	-	-	0.059 ±0.006	0.069 ±0.004
109	26.30	(+)-cycloisosativene	-	0.14 ±0.03	-	-	-	-	-	-	-	-	-
110	26.47	Ylangene	-	0.12 ±0.02	-	-	-	0.013 ±0.004	-	0.071 ±0.025	-	0.001 ±0.001	0.003 ±0.001
111	26.66	Copaene	-	0.056 ±0.016	-	-	-	4.66 ±0.32	0.038 ±0.010	0.027 ±0.006	-	0.001 ±0.001	0.003 ±0.001
112	26.92	(Z)-3-hexenyl hexanoate	-	-	-	-	-	0.042 ±0.013	-	-	-	-	-
113	27.01	Geranyl acetate	0.21 ±0.02	0.86 ±0.17	0.54 ±0.01	-	-	0.29 ±0.04	0.043 ±0.003	-	-	0.067 ±0.007	0.095 ±0.004
114	27.29	β-Cubebene	-	0.11 ±0.01	-	-	-	1.66 ±0.02	-	-	-	-	-
115	27.34	β-Elemene	0.68 ±0.04	0.60 ±0.11	-	-	-	0.67 ±0.03	-	0.51 ±0.29	2.67 ±0.11	0.47 ±0.04	0.98 ±0.04
116	27.49	(+)-Sativen	-	0.017 ±0.005	-	-	-	0.083 ±0.005	-	-	-	-	-
117	27.65	Tetradecane	0.007 ±0.001	-	-	-	-	0.040 ±0.006	-	0.56 ±0.15	-	0.005 ±0.001	0.007 ±0.001
118	28.01	Dodecanal	0.14 ±0.02	-	-	-	-	-	-	-	-	0.11 ±0.01	0.30 ±0.02
119	28.06	α-Gurjunene	-	-	-	-	-	0.034 ±0.002	-	-	-	-	-
120	28.10	Decyl acetate	0.043 ±0.008	-	-	-	-	-	-	-	-	-	-
121	28.28	α-Bergamotene	0.23 ±0.01	0.038 ±0.010	-	-	-	-	-	-	-	0.19 ±0.03	0.34 ±0.01
122	28.45	Caryophyllene	0.16 ±0.01	6.63 ±0.73	0.020 ±0.001	-	-	16.90 ±0.61	0.15 ±0.04	0.55 ±0.03	-	0.20 ±0.03	0.50 ±0.01
123	28.71	γ-Elemene	0.017 ±0.002	-	-	-	-	-	-	-	-	0.008 ±0.001	0.026 ±0.001
124	28.83	β-Gurjunene	0.008 ±0.001	0.029 ±0.010	-	-	-	0.25 ±0.01	-	-	-	0.004 ±0.001	0.014 ±0.001
125	29.02	α-Guaiene	0.95 ±0.05	0.56 ±0.12	-	-	-	0.36 ±0.01	-	-	-	0.42 ±0.03	1.15 ±0.04
126	29.10	trans-α-Bergamotene	-	2.57 ±0.14	-	-	-	-	-	-	-	-	-
127	29.20	Thujopsene	3.71 ±0.12	-	-	-	-	-	-	-	-	3.35 ±0.49	5.82 ±0.12
128	29.40	Seychellene	0.022 ±0.003	0.41 ±0.07	-	-	-	0.038 ±0.002	-	-	-	0.020 ±0.002	0.022 ±0.001

Peak No.	RT (min)	Tentative compound	Tom Yum soup	% Average area normalization (*n* = 3)									
				Lemongras		Fish sauce		Kaffir lime leaf		Chili		Lime juice	
				Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled
129	29.59	epi-β-Santalene	-	-	-	-	-	-	-	-	-	0.006 ±0.001	0.018 ±0.007
130	29.61	α-Himachalene	-	-	-	-	-	-	-	0.68 ±0.27	-	-	-
131	29.62	Aromadendrene	-	0.025 ±0.003	-	-	-	-	-	-	-	-	-
132	29.67	cis-muurola-3,5-diene	-	-	-	-	-	0.036 ±0.003	-	-	-	-	-
133	29.72	Cedrene	0.048 ±0.003	0.10 ±0.02	-	-	-	-	-	-	-	0.004 ±0.001	0.060 ±0.002
134	29.81	α-Caryophyllene	0.168 ±0.005	0.74 ±0.09	-	-	-	1.85 ±0.06	0.012 ±0.001	-	-	0.10 ±0.01	0.221 ±0.004
135	29.95	(E)-β-Farnesene	-	1.06 ±0.09	-	-	-	0.054 ±0.002	-	-	-	-	-
136	29.97	(Z)-β-Farnesene	0.22 ±0.01	-	-	-	-	-	-	-	-	0.24 ±0.03	0.44 ±0.01
137	30.09	Allo-Aromadendrene	0.15 ±0.01	-	-	-	-	0.065 ±0.002	-	-	-	0.13 ±0.02	0.273 ±0.003
138	30.19	2-Methyltetradecane	-	-	-	-	-	-	-	7.40 ±2.63	3.09 ±0.21	-	-
139	30.40	Acoradiene	-	0.034 ±0.004	-	-	-	0.006 ±0.001	-	-	-	-	-
140	30.58	1-Dodecanol	-	-	-	0.67 ±0.14	34.6 ±1.2	-	-	-	-	-	-
141	30.60	γ-Himachalene	-	0.027 ±0.010	0.009 ±0.002	-	-	0.056 ±0.003	0.023 ±0.001	-	-	-	-
142	30.72	γ-Muurolene	0.14 ±0.01	0.12 ±0.04	-	-	-	0.28 ±0.01	-	-	-	0.033 ±0.008	0.214 ±0.004
143	30.78	Longifolene-(V4)	-	-	-	-	-	-	-	3.18 ±1.29	-	-	-
144	30.90	Germacrene D	-	0.59 ±0.19	-	-	-	1.01 ±0.05	-	0.38 ±0.13	-	-	-
145	30.93	β-Guaiene	0.72 ±0.02	-	-	-	-	-	-	-	-	0.51 ±0.05	1.12 ±0.03
146	31.00	4,11-selinadiene	-	0.93 ±0.22	-	-	-	-	-	0.21 ±0.07	-	-	-
147	31.07	γ-Gurjunene	0.29 ±0.01	-	-	-	-	-	-	-	-	0.20 ±0.02	0.562 ±0.001
148	31.10	β-Selinene	-	0.23 ±0.05	-	-	-	0.056 ±0.001	-	0.043 ±0.014	-	-	-
149	31.23	α-Elemene	0.055 ±0.001	-	-	-	-	-	-	-	-	0.011 ±0.002	0.077 ±0.004
150	31.30	Cadina-3,9-diene	-	0.65 ±0.15	0.001 ±0.002	-	-	-	-	-	-	-	-
151	31.31	Bicyclosesquiphellandrene	-	-	-	-	-	0.030 ±0.001	-	-	-	-	-
152	31.32	δ-Selinene	0.16 ±0.01	-	-	-	-	-	-	-	-	0.017 ±0.004	0.19 ±0.01
153	31.38	Valencene	-	0.14 ±0.04	-	-	-	-	-	-	-	-	-
154	31.46	(Z,E)-α-Farnesene	0.15 ±0.01	0.87 ±0.02	-	-	-	-	-	-	-	0.079 ±0.012	0.358 ±0.003
155	31.51	Elixene	-	-	-	-	-	2.43 ±0.09	0.14 ±0.03	0.037 ±0.008	6.65 ±1.20	-	-
156	31.62	α-Chamigrene	-	0.38 ±0.09	-	-	-	-	-	-	-	-	-
157	31.63	Pentadecane	-	-	-	-	-	-	-	0.55 ±0.21	-	-	-
158	31.65	α-Muurolene	6.27 ±0.18	-	-	-	-	0.60 ±0.01	-	-	-	3.70 ±0.30	6.60 ±0.14
159	31.66	Epicubebol	0.037 ±0.003	-	-	-	-	-	-	-	-	0.004 ±0.001	0.020 ±0.001
160	31.80	(Z)-α-Bisabolene	0.42 ±0.01	-	-	-	-	-	-	-	-	0.34 ±0.03	0.76 ±0.01
161	31.88	δ-Guaiene	-	0.63 ±0.14	0.040 ±0.007	-	-	0.18 ±0.01	0.072 ±0.015	0.12 ±0.03	9.08 ±1.67	-	-
162	31.98	α-Farnesene	-	0.37 ±0.07	-	-	-	0.98 ±0.17	-	-	-	-	-

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
163	32.12	β-Bisabolene	-	-	-	-	-	-	-	-	-	1.70 ±0.14	5.62 ±0.24
164	32.19	γ-Cadinene	0.010 ±0.001	0.91 ±0.23	0.018 ±0.002	-	-	0.20 ±0.01	-	-	-	-	-
165	32.33	(Z)-γ-Bisabolene	0.035 ±0.004	0.12 ±0.03	-	-	-	-	-	-	-	0.076 ±0.014	0.083 ±0.020
166	32.56	δ-Cadinene	0.092 ±0.002	0.54 ±0.15	0.017 ±0.002	-	-	3.14 ±0.07	0.046 ±0.011	0.024 ±0.007	-	0.021 ±0.003	0.092 ±0.003
167	32.87	Cadina-1(2),4-diene	-	0.19 ±0.06	-	-	-	0.17 ±0.01	-	-	-	-	-
168	32.91	(E)-γ-Bisabolene	0.026 ±0.001	-	-	-	-	-	-	-	-	0.018 ±0.002	0.039 ±0.001
169	33.02	Selina-3,7(11)-diene	0.042 ±0.003	-	-	-	-	-	-	-	-	0.009 ±0.002	0.061 ±0.003
170	33.08	α-Cadinene	0.040 ±0.002	-	-	-	-	0.022 ±0.002	-	-	-	0.007 ±0.002	0.071 ±0.008
171	33.18	Hexyl benzoate	-	-	-	-	-	-	-	0.091 ±0.030	4.03 ±0.63	-	-
172	33.31	α-Bisabolene	0.11 ±0.01	-	-	-	-	-	-	-	-	0.11 ±0.01	0.352 ±0.004
173	33.53	Hedycaryol	-	-	-	-	-	0.057 ±0.010	-	-	-	-	-
174	33.82	Germacrene B	1.00 ±0.04	0.012 ±0.004	-	-	-	0.010 ±0.001	-	-	-	0.47 ±0.03	1.33 ±0.04
175	34.05	2-Methylpentadecane	-	-	-	-	-	-	-	0.37 ±0.14	-	-	-
176	34.06	(E)-Nerolidol	0.030 ±0.003	0.056 ±0.014	-	-	-	0.23 ±0.02	-	-	-	0.003 ±0.001	0.053 ±0.003
177	34.34	(Z)-3-Hexenyl benzoate	-	0.025 ±0.006	-	-	-	0.090 ±0.037	0.020 ±0.001	0.036 ±0.018	-	-	-
178	34.67	trans-Sesquisabinene hydrate	0.007 ±0.001	-	-	-	-	-	-	-	-	0.007 ±0.001	0.016 ±0.002
179	34.80	Viridiflorol	-	0.019 ±0.006	-	-	-	0.035 ±0.006	-	-	-	-	-
180	35.42	Hexadecane	-	-	-	-	-	-	-	0.25 ±0.10	1.94 ±0.35	-	-
181	36.09	Junenol	-	-	-	-	-	0.035 ±0.004	-	-	-	-	-
182	36.12	γ-Eudesmol	0.16 ±0.01	1.07 ±0.27	0.15 ±0.01	-	-	-	-	-	-	-	-
183	36.97	(-)-δ-Cadinol	0.011 ±0.001	0.032 ±0.008	-	-	-	-	-	-	-	-	-
184	37.42	Bulnesol	-	0.053 ±0.017	0.007 ±0.001	-	-	-	-	-	-	-	-
185	37.48	τ-Muurolol	0.055 ±0.01	-	-	-	-	-	-	-	-	0.021 ±0.004	0.073 ±0.004
186	37.56	epi-γ-Eudesmol	0.020 ±0.001	0.12 ±0.03	-	-	-	-	-	-	-	-	-
187	37.73	2-Methylhexadecane	-	-	-	-	-	-	-	0.20 ±0.09	1.10 ±0.11	-	-
188	37.96	α-Santalol	0.021 ±0.005	-	-	-	-	-	-	-	-	0.013 ±0.003	0.053 ±0.003
189	38.54	α-Bisabolol	0.035 ±0.006	-	-	-	-	-	-	-	-	0.016 ±0.004	0.060 ±0.006
190	38.89	Juniper camphor	0.013 ±0.001	0.062 ±0.026	0.007 ±0.001	-	-	-	-	-	-	-	0.008 ±0.001

APPENDIX D

Mass spectrum of extra volatile compounds

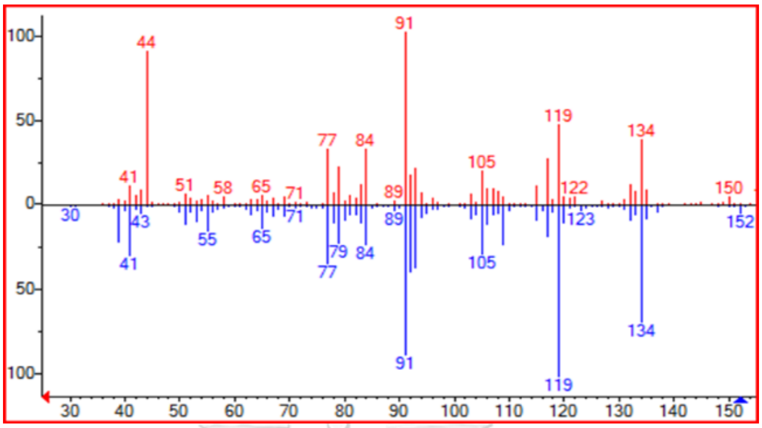


Figure D.1 Mass spectrum of carveol.

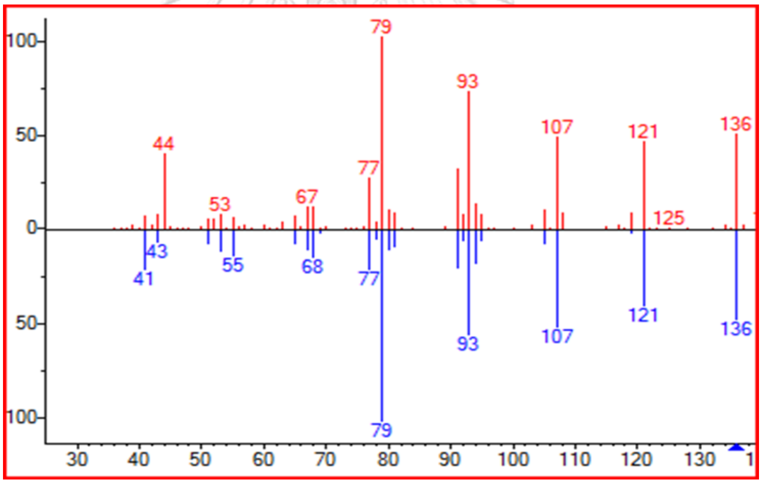


Figure D.2 Mass spectrum of *p*-mentha-3,8-diene.

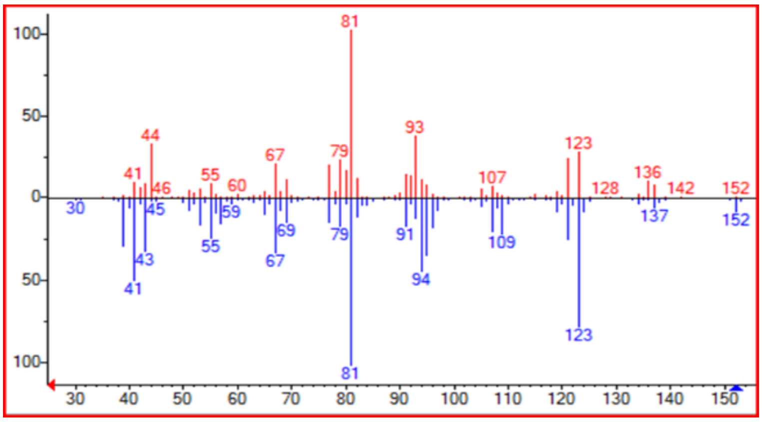


Figure D.3 Mass spectrum of α-cyclocitral.

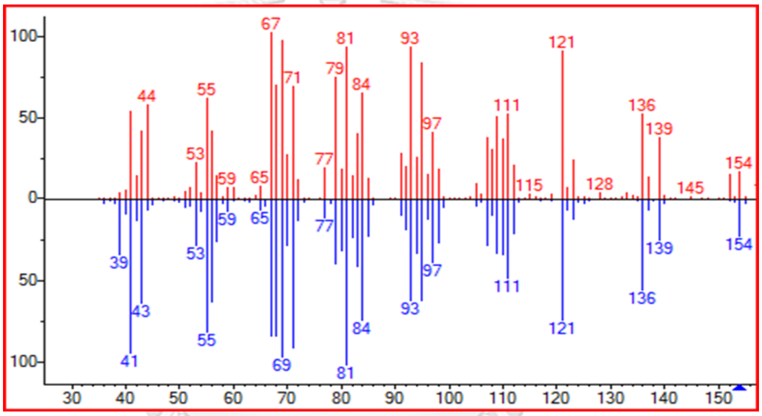


Figure D.4 Mass spectrum of iso-isopulegol.

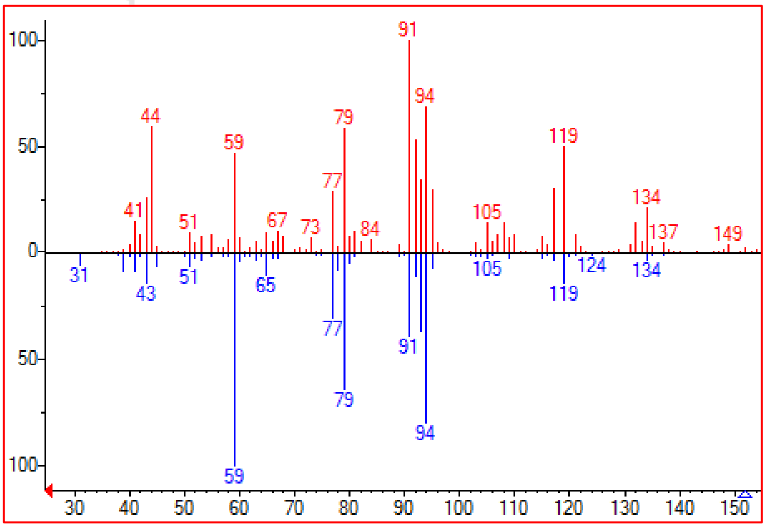


Figure D.5 Mass spectrum of *p*-mentha-1,5-dien-8-ol.

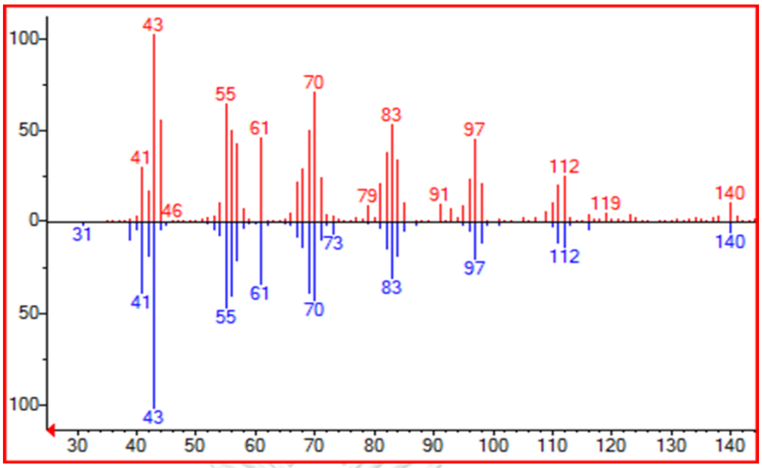


Figure D.6 Mass spectrum of decyl acetate.

VITA

Miss Pannipa Janta was born on 12th March 1992, in Chachoengsao, Thailand. She graduated a high school level from a Science division of Benchamaratrangsarit in Chachoengsao, Thailand. She graduated with Bachelor's degree of Science in Chemistry from Thammasat University in 2014. Afterwards, she continued her academic education for Master's degree at Department of Chemistry majoring in Analytical Chemistry, Faculty of Science, Chulalongkorn University. She completed her Master's degree of Science in Analytical Chemistry in 2017.