DEVELOPMENT OF HEADSPACE-SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY/OLFACTOMETRY FOR ANALYSIS OF VOLATILE COMPOUNDS FROM CHICKEN PRODUCTS

Miss Wongkanok Yoosong

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การพัฒนาการสกัดระดับจุลภาคด้วยวัฏภาคของแข็งแบบเฮดสเปซและแก๊สโครมาโทกราฟี-แมสสเปกโทรเมตรี/โอลแฟคโทเมตรีสำหรับการวิเคราะห์สารระเหยง่ายจากผลิตภัณฑ์ไก่

น.ส.วงศ์กนก อยู่สงค์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	DEVELOPMENT OF HEADSPACE-SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY/OLFACTOMETRY FOR ANALYSIS OF VOLATILE COMPOUNDS FROM CHICKEN PRODUCTS
By	Miss Wongkanok Yoosong
Field of Study	Chemistry
Thesis Advisor	Dr. Chadin Kulsing

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

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วงศ์กนก อยู่สงค์ : การพัฒนาการสกัดระดับจุลภาคด้วยวัฏภาคของแข็งแบบเฮดสเปซและแก๊สโครมาโทกราฟี-แมสสเปกโทรเมตรี/โอลแฟคโทเมตรีสำหรับการวิเคราะห์สารระเหยง่ายจากผลิตภัณฑ์ไก่. (DEVELOPMENT OF HEADSPACE-SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY/OLFACTOMETRY FOR ANALYSIS OF VOLATILE COMPOUNDS FROM CHICKEN PRODUCTS) อ.ที่ปรึกษาหลัก : ดร.ชฎิล กุลสิงห์

งานวิจัยนี้ได้ทำการประยุกต์ใช้เทคนิคแก๊สโครมาโตกราฟี-แมสสเปกโทรเมตรีโอลแฟคโทเมทรีร่วมกับเฮดเสปซโซลิดเฟสไมโครเอกซ์แทรกชัน (HS-SPME-GC-MS/O) สำหรับพิสูจน์ทราบสารระเหยในไก่ต้มสุกและไก่ดิบ ด้วยเทคนิค HS-SPME โดยใช้ไฟเบอร์ชนิด 50/30 mm DVB/CAR/PDMS อุณหภูมิการสกัดที่ 50 องศาเซลเซียสสำหรับไก่สุก และที่อุณหภูมิห้องสำหรับตัวอย่างไก่ดิบ ทำการแยกสารโดยใช้คอลัมน์ชนิด HP-5MS ที่โปรแกรมอุณหภูมิจาก 40 ถึง 310 องศาเซลเซียส ด้วยอัตรา 4 องศาเซลเซียสต่อนาที จากการตรวจวัดสารระเหยง่ายสำหรับตัวอย่างไก่ต้มสุกพบว่าสามารถระบุสารระเหยง่ายได้ 16 ชนิด แบ่งเป็น 4 กลุ่ม ได้แก่ แอลดีไฮด์ อะโรมาติกไฮโดรคาร์บอน แอลกอฮอล์ และคีโตน สำหรับตัวอย่างไก่ดิบพบว่าสามารถระบุสารระเหยง่ายได้ 20 ชนิด แบ่งเป็น 3 กลุ่ม ได้แก่ แอลดีไฮด์ แอลกอฮอล์ และเอสเตอร์ โดยสารระเหยง่ายหลักที่พบในตัวอย่างไก่ทั้งสองชนิดเป็นสารประเภทแอลดีไฮด์ซึ่งมาจากกระบวนการออกซิเดชั่นของไขมัน แอลดีไฮด์ที่ตรวจพบทั้งตัวอย่างไก่สุกและไก่ดิบได้แก่ เฮกซะนาล โดยในตัวอย่างไก่สุกพบว่ามีปริมาณเฮกซะนาลสูงกว่าไก่ดิบ การระบุสารระเหยง่ายในผลิตภัณฑ์ไก่นี้ทำโดยการเปรียบเทียบแมสสเปกตรัมของสารกับฐานข้อมูลของ NIST พร้อมด้วยค่ารีเทนชันอินเด็กซ์จากการทดลองและค่าอ้างอิง จากการทดลองพบว่า ไก่ต้มสุกนั้นมีปริมาณของสารระเหยง่ายที่มากกว่าไก่ดิบ เนื่องจากไก่ดิบมีกลิ่นที่อ่อนมากทำให้การวิเคราะห์ด้วยวิธี HS-SPME ไม่สามารถวิเคราะห์ตัวอย่างไก่ดิบได้อย่างมีประสิทธิภาพ จึงได้มีการพัฒนาวิธีวิเคราะห์เพื่อเพิ่มประสิทธิภาพการตรวจวัดให้ดียิ่งขึ้นโดยการทำ Multiple HS-SPME ผลที่ได้แสดงให้เห็นว่า สามารถตรวจวัดชนิดของสารระเหยง่ายในไก่ดิบได้เพิ่มขึ้น

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Wongkanok Yoosong : DEVELOPMENT OF HEADSPACE-SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY/OLFACTOMETRY FOR ANALYSIS OF VOLATILE COMPOUNDS FROM CHICKEN PRODUCTS. Advisor: Dr. Chadin Kulsing

Gas chromatography-mass spectrometry/olfactometry coupled headspace solid phase microextraction (HS-SPME-GC-MS/O) was applied to analysis of volatile compounds from raw chickens and boiled chickens. The HS-SPME was 50/30 mm DVB/CAR/PDMS. The extraction temperature of boiled chicken was 50 °C for 30 min and raw chicken were extracted at room temperature. The SPME Probes were analyzed by GC-MS with a HP-5MS capillary column. 16 volatile compounds were identified in the boiled chickens in four chemical classes including aldehydes, aromatic hydrocarbon, alcohols and ketones and 20 volatile compounds were identified in raw chickens in three chemical classes including aldehydes, alcohols and esters. The identification of volatile compounds performed by comparison of experimental mass spectra and retention indices (*I*) with those from the NIST library and literatures. Comparison of results between boiled and raw chicken from the same source revealed that the boiled chicken had more volatile compounds than the raw chicken. HS-SPME approach was succesfully used to identify volatiles compounds from boiled samples. However, this method could detect only a few substances (e.g. hexanal) from the raw samples. In order to enhance extraction performance, multiple HS-SPME was performed to analyze volatile compound from the raw samples.

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Wongkanok Yoosong

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATION

β	phase ratio
C_G	gas phase concentration
CID	collision induced dissociation
C_S	sample phase concentration °C degree delsius
EI	Electron ionization
eV	electron volt
Exp	experimental
g	gram
GC	gas chromatography
GC-O	gas chromatography/olfactometry
HS	headspace
IS	internal standard
k	mass ratio
K	partition coefficient
Lit.	literature
I	linear retention index
mg	milligram
min	minute
mL	milliliter
mm	millimeter
MS	mass spectrometry
m/z	mass to charge ratios
QMS	quadrupole mass spectrometer
QqQ	triple-quadrupole
RSD	relative standard deviation
SIM	Selected Ion Monitoring
SPME	solid phase microextraction
TIC	total ion chromatograms
t_r	retention time
μg	microgram
μL	microliter
μm	micrometer

V_G	volume of gas phase
V_S	volume of liquid phase
Multiple injection HS-SPME	cumulative HS-SPME

CHAPTER I
INTRODUCTION

1.1 Problem definition

Chicken meat is the most popular protein-rich foods in the human diet (1). It is a great source of low-fat protein, which supports a healthy body weight, aids in weight loss and regulates cholesterol and blood pressure. The amounts of saturated fat and cholesterol found in red meat are much higher than that found in chicken, fish, and vegetables. According to the office of Agricultural Economics: chicken meat production rate in 2012 increased up to 40% of that in 2002 according to the needs of consumers (2). The report from USDA has shown that chicken 100 g contains protein 18 g, fat 15 g, saturated fat 4 g, cholesterol 75 mg, calcium 11 mg, iron 0.9 mg, magnesium 20 mg, phosphorus 147 mg, potassium 189 mg, sodium 70 mg, and zinc 1.3 mg all of which contribute to 215 kcal (3). It also contains multi vitamins. Therefore, the American Heart Association has suggested people to have chicken or fish instead of red meat for a lowered risk of cholesterol and subsequent heart disease development (4).

Since chicken is inexpensive and easy to cook, there are many ways to prepare it such as salads, sandwiches or soup in any meal. In cooking process, the optimum temperature should be controlled to get rid of all bacteria and produce great texture of meat by using a meat thermometer to measure temperature inside the chicken by placing the thermometer in a thick part of the meat and avoiding touching the bone. The internal temperature of chicken breast meat should reach an internal temperature of 74 °C. Chicken should be kept in a sealed container and stored in a refrigerator. It can also be frozen and kept for up to 9 months (5).

The raw meat has little aroma, more volatile compounds are generated during thermal processing enhancing the aroma and flavor (6-8). Flavor is the combination of senses of taste and aroma which governs the sensory quality of meat products. Chicken meat contains higher levels of unsaturated fatty acids than red meat so the former is more sensitive to quality deterioration mainly due to lipid

oxidation and results in off-flavor which is the main problems of the chicken meat quality. As a result, the producers and processors in chicken meat sector try to avoid off-flavor development through various prevention processing. To this end, the measurement of volatile compounds in fresh and cooked chicken meat can be used as an index for chicken quality control, which is necessary in order to produce flavorful and consistent products.

Headspace-solid phase microextraction (HS-SPME) is an analytical technique that is widely used for sample preparation of volatile compounds. This is because it is commercially available, inexpensive and portable analytical technique enabling volatile organic compounds (VOCs) sampling in the headspace (HS) of a sample and can directly extract volatile compounds from either liquid or solid samples without use of solvent. In addition, HS-SPME can be applied prior to analysis with gas chromatography-mass spectrometry (GC-MS) in order to measure and identify volatile compounds by comparing their mass spectra with those in library databases.

1.2 Literature review

Aroma compound characterization is important in analysis of foodstuffs (9). More than 350 volatile compounds have been found in different chicken meats. Major classes of volatiles identified in chicken include aldehydes, hydrocarbons, ketones, sulfur containing compounds and heterocyclic compounds (pyrroles, pyrazines, pyridines) (10-12). Carbonyl group containing compounds are a major class of flavor components identified in the cooked chicken meat and important to produce the “chicken-like” aroma. Without these compounds, the “chicken odor” is lost and the meaty odor becomes dominant (13). The composition of volatile compounds in the finished product is influenced by various factors: sex, breed, diet and age of an animal; type of muscle; conditions of meat storage and cooking process (roasting, smoking) (14).

The carbonyl compounds are formed by oxidation of unsaturated acyl lipids which is well known as the cause of rancidity development but it can also contribute to desirable food flavors. The oxidation process produces a wide range of aliphatic

compounds, including both saturated and unsaturated hydrocarbons such as aldehydes, alcohols, ketones and some cyclic compounds, such as furans, lactones and cyclic ketones (15, 16). Saturated and unsaturated aldehydes bearing 6–10 carbons are the major volatile compounds found in cooked meat and hence they probably play an important role in meat aroma. The odor threshold values of aldehydes are generally lower than those of volatile compounds, thus they have an important potential effect on total flavor of chicken meat (17, 18).

A useful analytical technique used for the analysis of flavor compounds in meat is the electronic nose. However, the result from this technique does not provide information about the compounds causing generation of the investigated aroma, which is reported by aroma pattern (19-23). This information can be obtained using GC-MS and GC-O that have been used for measurement of odor quality and the identification of both odor-active and -inactive compounds (24). Whilst, most studies investigate the volatile profiles of cooked meat (25-27) only a few research reported volatiles in raw meat (28-31). Several techniques have been developed to enhance the performance of volatile analysis in meat products, such as static headspace (SHS), dynamic headspace (DSH) or purge and trap (P&T), simultaneous distillation extraction (SDE) and headspace-solid phase microextraction (HS-SPME). Most research have used SDE for analysis of volatile compounds in meat products (29, 32-34). Comparison of the results between SDE and HS-SPME for the analysis of volatile compounds in heated beef fats and sheep fats showed higher abundances of volatile compounds which were extracted by HS-SPME compared to the semi-volatiles in the fat samples (35).

Moreover, HS-SPME has been used for identification of volatile compounds in meat products (36-38) as it can directly introduce a sample into GC system, as well as pre-concentration of the analytes. GC is often coupled with a mass spectrometry (MS) detector with the MS fingerprint database of various types of compounds, *e.g.* from Wiley or NIST library. Therefore, this makes it possible to quickly identify compounds in the sample by comparison with the database and further confirmation with the retention index data.

1.3 Aim, scope and expected benefits of this work

Most studies in the literature reported the volatile compounds of cooked (24-27) meat products, and only few research studies have been focused on volatile compounds present in raw meat (28-31) with very few of them investigating raw chicken meat (29).

In this research, GC-O/MS experiment was initially investigated to select a suitable condition to separate and identify volatile compounds in HS-SPME of boiled and raw chicken meat. Moreover, MS detection was performed using the electron ionization voltage of -70 eV with triple quadrupole mass analyzer operated as single quadrupole using a scan mode. Secondly, the HS-SPME conditions including extraction type, extraction temperature and extraction time using Polydimethylsiloxane/carboxen/divinylbenzene (PDMS/CAR/DVB) fiber were optimized for preparation of the boiled and raw chicken meat prior to the GC-O/MS analysis. The initial results reveal that the boiled samples had more volatile compounds and can be extracted using single HS-SPME. Due to the raw chicken meat containing the small volatile signal, these samples were extracted using multiple (cumulative) HS-SPME. Thirdly, the developed HS-SPME-GC-O/MS methods were applied to identify the volatile compounds in several boiled and raw chicken meat samples according to comparison of their experimental mass spectra and retention indices (*I*) with those from NIST library with the match scores of >650 and *I* difference $\pm$20 units.

Therefore, the aim of this research is to develop approaches to identify and compare the volatile compounds of boiled and raw chicken meat samples. According to our literature review, there have been no reports on analysis of raw chicken meat using multiple HS-SPME (accumulated HS-SPME).

The benefit of this work is the effective method to obtain volatile compound profiles that contribute to the characteristic aroma of boiled and raw chicken meat leading to the information of the volatile compound releasing from boiling process.

CHAPTER II
THEORY

2.1 Headspace (HS)

Headspace sampling is applicable with the quantitative or qualitative quantitative analysis of volatile species in samples where the volatiles evaporate into gas volume of the sample headspace from either a liquid or solid matrix. It is a good technique for injection of analytes into GC instrument without being in contact with the entire sample. Nonvolatile contaminants remain behind in the headspace vial and do not accumulate in the inlet or the column. Volatiles may be extracted from heavy or more polar sample matrices (39). Many kinds of samples can be prepared in HS vial and extraction in the headspace of the samples isolated from non-volatile components in the sample vials, and the volatile components can then be transferred into a GC system for analysis. HS sampling is also particularly amenable to the trace analysis. This technique is considerably easy and fast.

The theory of HS sampling can be explained with the example of liquid sample in a closed vial as shown in Figure 1 the volatile components diffused from the solid sample into the gas phase. In equilibrium, the concentrations in both phases remain constant. The ratio of analyte concentration in each phase is captured by a partition coefficient (K value). This parameter is the product of two terms including mass ratio of analyte in the solid to that in gas phase (k) and phase ratio of the solid to the gas volume (ß value) (40). The K value directly affects the efficiency of headspace analysis. Lower the K value by increasing sample temperature or by adding inorganic salts to the sample matrix increases the volatile amount in HS leading to improved sensitivity of the analysis. The sample volume can also be increased which increase the analyte yield in HS (41).

$$K = \frac{C_S}{C_M}$$

$$\beta = \frac{V_S}{V_G}$$

C_s = Concentration of analyte in solid phase

C_G = Concentration in gas phase

V_s = Volume of solid phase

V_G = Volume of gas phase

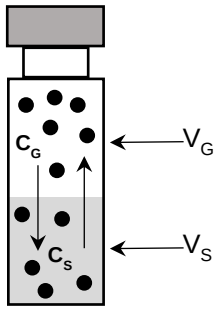

Figure 1 vial containing a solid sample with a volatile compound in the headspace

2.1.1 Static headspace (SHS)

Static headspace gas chromatography (SHS-GC) is a technique used for the sample preparation and separation of volatile organic compounds. This technique is easy to operate and applicable to a variety of sample matrices. It can provide good sensitivity especially for highly volatile compounds. This technique directly samplings the sample HS from the container in which the volatiles are taken by using of a gas-tight syringe auto-sampler to transfer the HS into the GC system. The gas-tight syringe technique operates by initially using a thermostat to control temperature of the sample in an incubation oven for a given time until it reaches a state of equilibrium. In equilibrium, the HS aliquot was taken by the gas-tight syringe (Figure 2) and injected into the GC system.

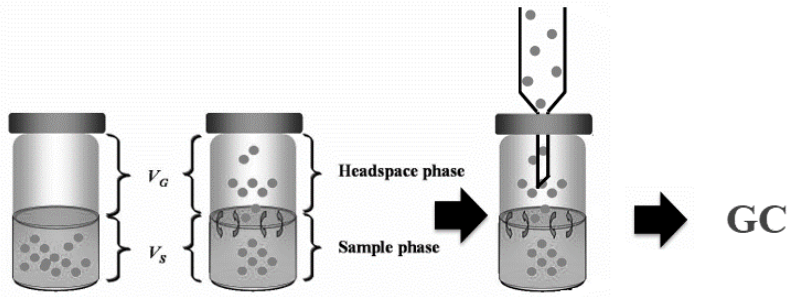

Figure 2 Static headspace using the gas-tight syringe (42)

2.1.2 Dynamic headspace (DSH)

Dynamic headspace (DHS) continually sweeps sample HS, collects and concentrates analytes onto a trap until desorption occurs at the GC inlet prior separation and detection. The DHS is used to extract and concentrate VOCs from liquid or solid samples by purging with an inert gas such as N_2 to concentrate VOCs. Analytes are subsequently introduced into a gas chromatographic system by thermal desorption. Advantages of Dynamic are lower detectability of analytes (PPB or lower), fully desorb analytes with less temperature dependent.

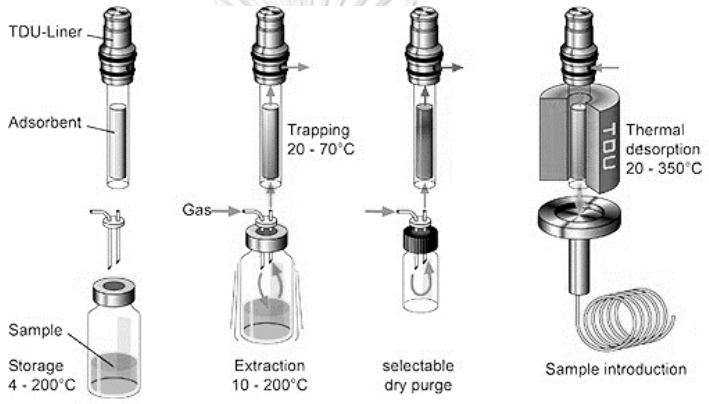

Figure 3 Dynamic headspace Process (43)

2.1.3 Headspace-solid phase microextraction (HS-SPME)

Solid phase microextraction is a technique that is used to extract and preconcentrate compounds from headspace vapor. It simplifies the extraction technique without gases or plumbing which is performed by insertion of a fiber into the headspace phase inside the vial [45]. The fiber was placed into the headspace of vial containing sample and starts to adsorb compounds. The HS vapor phase will be accumulated onto the SPME fiber. The fiber is then drawn back into the syringe needle and inserted into a heated GC inlet. The fiber is thermally desorbed at the injector liner and the carrier gas transfers analytes to the GC column for analysis as shown in Figure 4 It provides a good degree of analyte pre-concentration for a wide range of analytes and is very effective to eliminate the solvent effects which may enter the trap and column with other techniques (44).

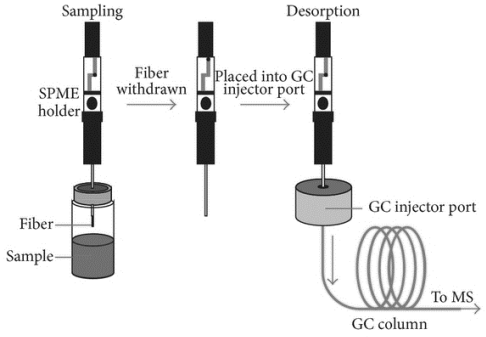

Figure 4 Schematic view of manual SPME approach (45)

2.1.3.1 Sorbent

The fiber is normally made of a fuse silica fiber coated with a film of an immobilized stationary phase connected to the plunger of a special syringe. The phases are not bonded to the silica fiber core except the polydimethylsiloxane coating. Other coatings are cross-linked to improve stability (prevent swelling effect) in organic solvents. Multi-component phases were developed to broaden selectivity in SPME such as divinylbenzene (DVB) and/or Carboxen particles suspended in either PDMS, a nonpolar phase, or Carbowax (CW), a moderately polar phase. They were developed to exploit adsorbent processes for SPME.

PDMS-DVB is a multiple-component bipolar sorbent coating, whilst DVB/Carboxen-PDMS is a multiple-component bipolar phase that contains a combination of DVB-PDMS (50 mm) layered over Carboxen-PDMS (30 mm) for. This handling expands the analyte molecular weight range, since larger analytes are retained in the meso- and macropores of the outer DVB layer, while the micropores in the inner layer of Carboxen retain smaller analytes. The dual-layered phase is used for extraction of odor compounds and volatile and semivolatile flavor compounds (46).

2.1.3.2 Extraction procedure of SPME

There are two steps in SPME analysis Figure 5 (a) Adsorption: the fiber is initially exposed to a sample HS (or directly into a liquid sample) for the certain period of time by insertion of the needle the sample vial containing the sample (S) moving the fiber (F) outside the needle into the sampling region (extraction) (step 3), then moving the fiber back into the syringe needle and transfer to the GC injector port (I) and capillary head (C). Next step (b) Desorption: the sorbent is transferred into a device interfacing with analytical instrument for thermal desorption such as GC for gas desorption or LC for solvent desorption by penetration of the septum with syringe needle (step 4), moving the fiber outside the needle (desorption) (step 5) and withdrawing the fiber back into the syringe needle (step 6) (47).

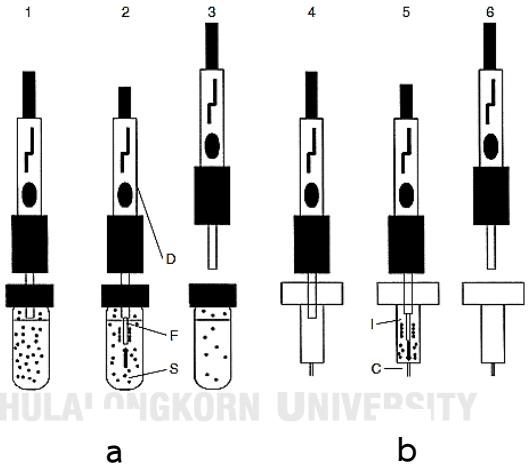

Figure 5 Diagram showing principle of SPME (48)

2.2 Gas chromatography-Mass spectrometry

Gas chromatography mass spectrometry (GC-MS) is a hyphenated method, combining the benefits of two analytical techniques: GC to separate volatile organic compounds in a complex sample, and MS to detect, identification and/or quantification. This technique is in dispensable in the field of environmental science, forensic, flavor and fragrance industry, food safety, packaging and many others. In order to be analyzed by GC-MS, a compound must be sufficiently volatile and

thermally stable. This technique provides separation of components in a complex mixture which results in mass spectra of individual compounds. This is useful for qualitative and quantitative proposes. The sample solution was injected into the GC inlet where it is vaporized and flows through the chromatographic column by the carrier gas. The mixture of compounds are separated according to their relative interaction with the stationary phase of the column and the carrier gas. The latter part of the column passes through a heated transfer line and ends at the entrance to an ion source as shown in Figure 6 where compounds eluting from the column are converted to ions.

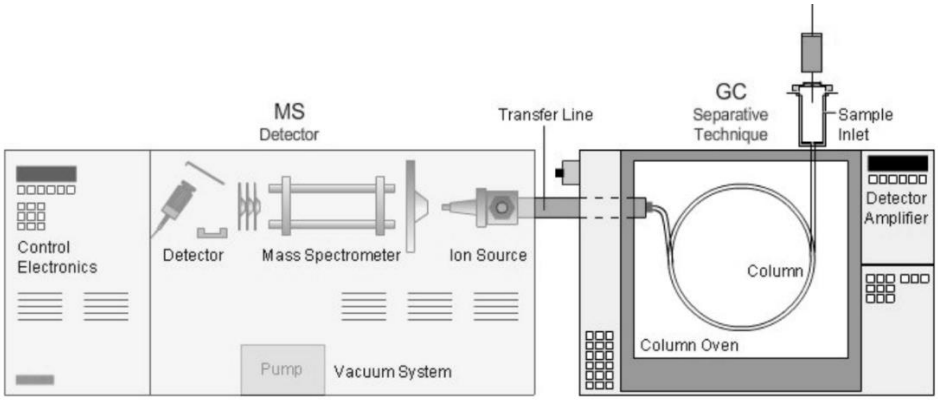

Figure 6 Schematic diagram of GC-MS instrument (49)

2.2.1 Gas chromatography (GC)

Gas chromatography (GC) is an analytical technique used to separate and analyze samples relies on differences in boiling points and/or the interaction with stationary phase, which is inert and should not react with any components of the mixture. The column is typically coiled to keep the size of the chromatograph manageable. The longer column generally allows better separation of components. At the end of the column, there is a detector, which records the amount of sample being in contact with it. The signals from the detector are used to produce a chromatogram which shows the amount and types of volatiles in the sample.

The chromatogram shows a series of peaks. The area of the peaks is directly relative to the amount of each component, although it cannot be directly used to

quantify the number of molecules in a sample. Usually, the first peak is from the unretained gas and the next peak is the solvent peak, followed by peaks representing compounds in a mixture. In order to identify the peaks in a chromatogram, the graph needs to be compared with a chromatogram from a standard solution mixture, to tentatively identify the peaks or they can be identified based on comparison with MS database (50).

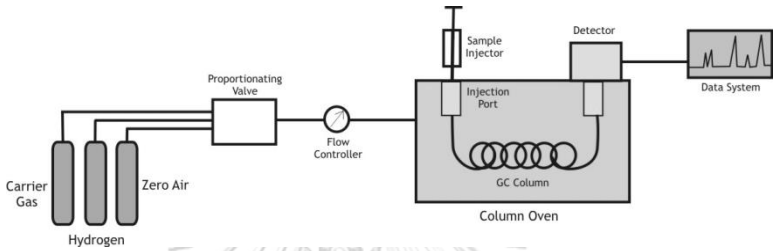

Figure 7 Schematic diagram of gas chromatography (51)

2.2.1.1 Carrier gas

Carrier gas is an inert gas used as mobile phase in GC. Helium (He), nitrogen (N_2), hydrogen (H_2), and argon (Ar) are often used. As carrier gas constantly flows into the detector, high-purity gas of at least 99.995 % needs to be used to reduced baseline noise and instrument contamination. Common sources for nitrogen or helium are the pressurized cylinders, which are steel tanks with two-stage pressure regulators. Alternatively, a commercially available nitrogen gas generator can be applied since it has advantages of safety and long term economic. Lifetimes of most GC columns can be shortened with the presence of moisture and oxygen by contaminate the gas line and instrument, cause column degradation and affect the accuracy of analysis results. Thus, the carrier gas should be filtered over molecular sieve beds in order to reduce moisture. In addition, specialized traps can be incorporated into the carrier gas line system to reduce or remove hydrocarbons and oxygen from the carrier gas (52).

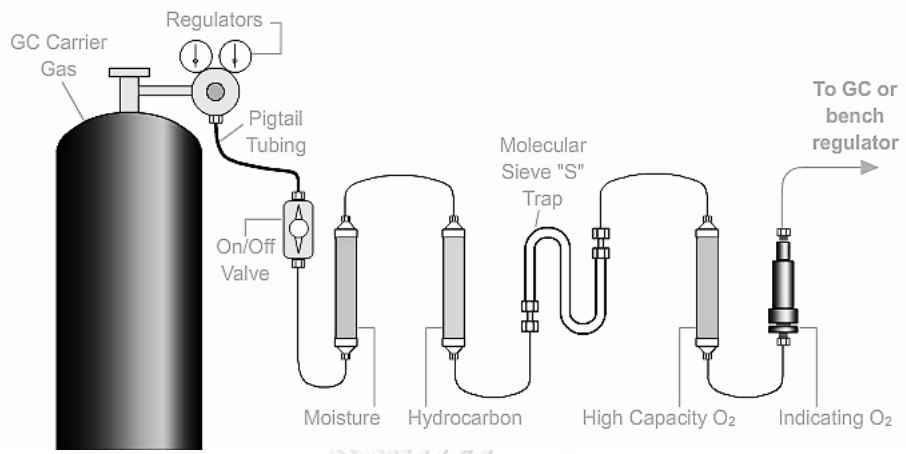

Figure 8 Component of carrier gas (53)

2.2.1.2 Injection

The GC analysis starts with the sample introduction process to transfer sample onto the column. Injection system in GC should fulfill the following two requirements: the amount injected should not overload the column and the width of the injected plug should be small. The most commonly used type applied with capillary columns is the heated split/splitless injector. Figure 9 is a schematic diagram representing of a typical split/splitless injector. Both split and splitless injection modes are performed at sufficiently high injection temperature to vaporize the solvent and the analytes in samples at the chamber, which is connected to the separation column. This injection temperature is constant throughout the GC run. Split injection is used for high concentration analyte are split so that only a few percent of the injected amount enters the column. This is in order to avoid column overloading and excessive band broadening. The splitless mode is applied for samples with very low analyte concentrations which are transferred to the column almost in their entirety by keeping the split vent closed for roughly one minute during the splitless injection. The vent is eventually opened to purge the liner of any remaining sample, which otherwise would continuously flow into the column (52).

- Split mode

In the split mode, the sample is injected and vaporized into the carrier gas stream, then gas flow passes through the septum purge and exits though the split vent. That lost sample will split away to waste. Only a small portion of the sample and solvent is transferred onto the inlet of the GC column. The remainder of the sample is vented going to waste (Figure 9 A). Typical split ratios can range from 10:1 to 400:1 (with the less amount of sample at higher split ratio) and can be calculated according to

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

For example, the split ratio of 1/49 means that 49 parts are split to waste and 1 part goes onto the column.

- Splitless mode

In the splitless mode, the sample is injected with the splitter vent closed during the injection, all the analyte sample vaporized in the injector goes onto the column. This technique is recommended for trace analysis with the sample containing very small amounts of analytes. Splitless mode is not suitable for injection of thermally labile compounds (54).

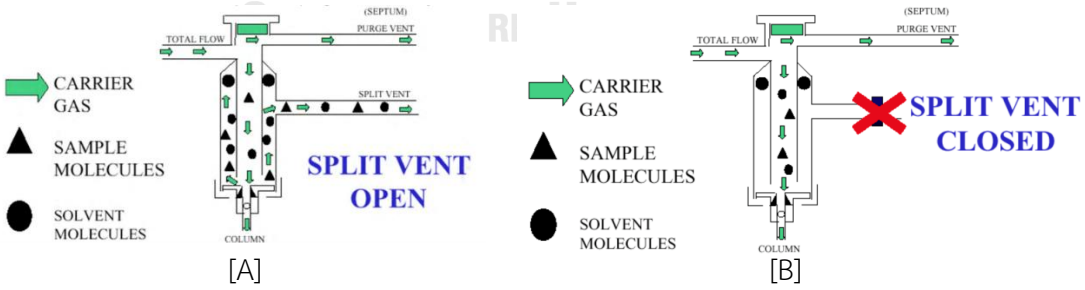

Figure 9 Diagram showing the flow path of the carrier gas in [A] the split mode and [B] the splitless mode (54)

2.2.1.3 Column

A sample is vaporized and injected into the separation column. The components are separated depending on physical characteristics of the stationary

phase inside the column with the flow of an inert gas employed as the mobile phase. For separation of most hydrocarbons, “boiling point” or “nonpolar” columns are applied to separate the components according to their individual boiling points or vapor pressures.

The separation in GC can be optimized by changing experimental conditions, such as temperature, stationary phase type; column dimension and flow. Beside the boiling point effects, the peak positions in chromatograms depend on different interactions between analytes and phases which are considered the “heart” or “brain” of the GC experiment as it critically affects the separation results. So, several types of GC stationary phases have been developed for different goals in separation (55).

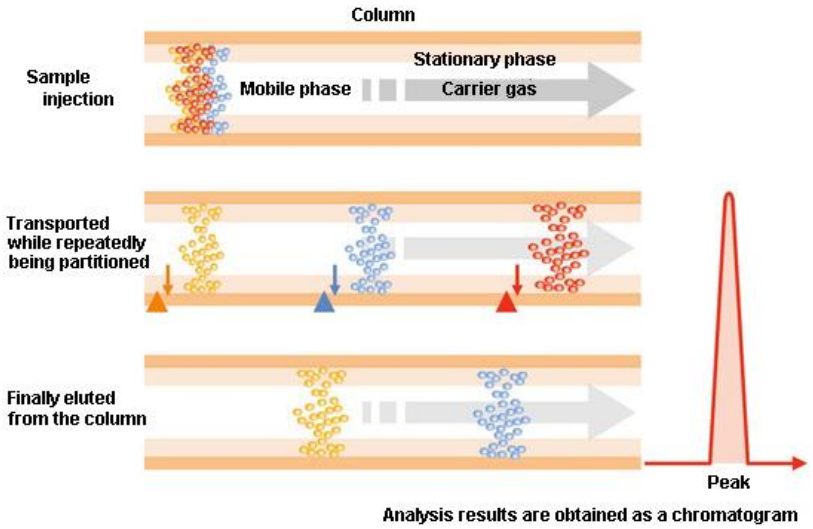

Figure 10 The separation process in GC (56)

The sample components are separated in the column as shown in Figure 11. The capillary column, especially an open tubular column is widely used for GC applications. Open tubular capillary columns are long hollow silica tubes with the inner walls of the capillaries coated with immobilized liquid stationary phase materials. These columns are well known as wall coat open tubular (WCOT) which provide high efficiency in separation.

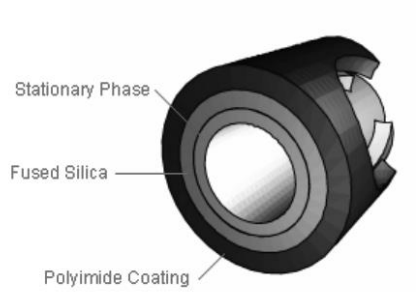

Figure 11 The tubular of capillary column (53)

Common GC stationary phases provide separation based on polar/non-polar interactions and analyte boiling point differences. Their polarities depend on types and amount of functionalities such as phenyl (more polar with higher phenyl content), fluorinated alkyl, or other functional groups in each phase.

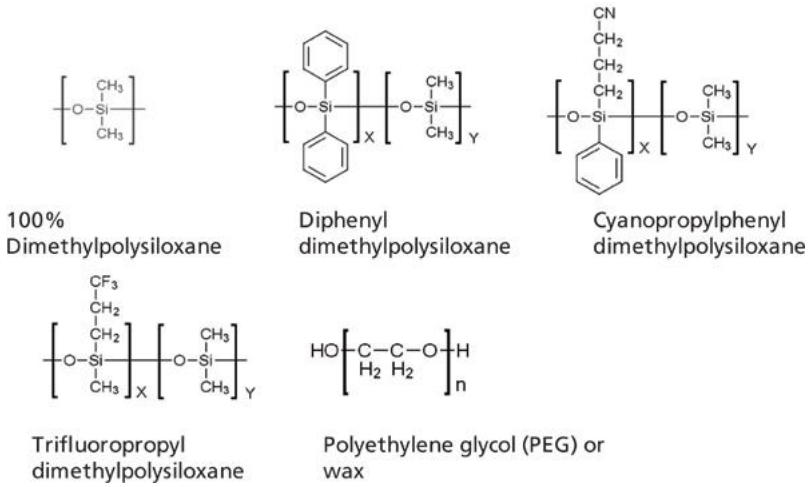

Figure 12 The five most common GC column stationary-phase chemistries (57)

Table 1 Stationary phases of GC

Stationary phase	Phase name
100% dimethyl polysiloxane	DB-1, HP-1, BP-1, OV-1, SPB-1, ZB-1, CP-Sil 5, RTX-1, SE-30, 007-1
5% diphenyl-dimethyl polysiloxane	BP-5, DB-5, HP-5, SPB-5, BPX-5, ZB-5, MND-5, CP-Sil 8CB, RTX-5, SE-54, 007-1
PolyethyleneGlycol (WAX or FFAP)	DB-WAX, CP-WAX, BP-20, 007-WAX

2.2.1.4 GC Oven

GC oven is used to control a suitable thermal environment for the elution of chromatographic peaks from GC columns using constant (isothermal) or temperature programmed by using rapidly circulating air inside an insulated enclosure to transfer heat to the column.

In isothermal GC separation, the temperature of GC oven remains constant during the whole analysis that provides an achieve separation of analyte with strong retention at long time. However, compounds high boiling points spend long time inside the column also occurs together with their diffusion inside the column. This leads to peak broadening and long analysis time. For temperature programming, the oven starts at low temperature to assist the separation of early eluting peak, the ramp and usually held for specific time at upper temperature. This technique is separated analytes with significantly different retention behavior. The temperature programming analysis allows an achieved separation of all analytes with reasonable time (53)

2.2.1.5 Detector

The detector generates an electronic signal which will be recorded with time resulting in a chromatogram. There are many types of detectors and their application depends on analyte chemistry as well as the analysis aim (quantitative or qualitative) and requires sensitivity. Examples of detectors applied in GC include flame ionization

detector (FID), electron capture detector (ECD), flame photometric detector (FPD), nitrogen phosphorous detector (NPD), thermal conductivity detector (TCD) and mass spectrometer (MS). FID is the most popular detector for hyphenation with GC due to its versatility, ease of operation, fast response, low detection limit and good linearity range (53).

2.2.2 Mass spectrometry

Mass spectrometry is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample by conversion of the sample into gaseous ions, conventionally by electron ionization with GC, with fragmentation and information concerning the nature and the structure of their precursor molecule, which are then separated according to their specific mass-to-charge ratio (*m/z*), and the relative abundance of each ion type is then recorded. A mass spectrum of the molecule is thus produced. Ions provide of a pure compound, the molecular ion, if present, appears at the highest value of *m/z* (except for the ions containing heavier isotopes) and gives the molecular *m/z* of the compound. From the Figure 13, The MS instrument consists of three major parts, including Ion source: producing ions in gas phase from the sample, Mass analyzer: resolving the ions into their characteristics *m/z* components and Detector system: detecting the ions and recording the relative abundance of resolved ionic species (58).

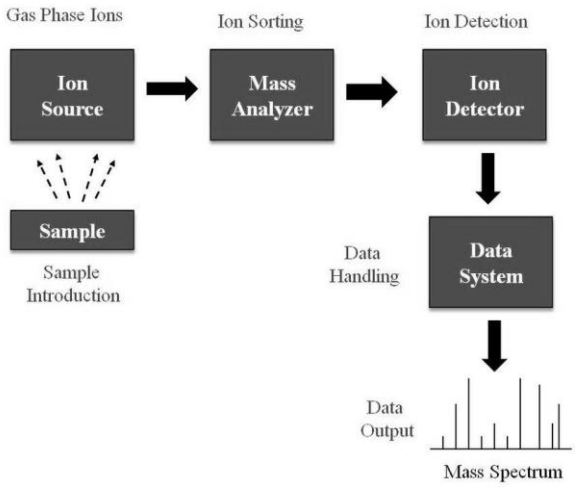

Figure 13 Diagram showing components of a Mass Spectrometer (59)

2.2.2.1 Ionization

Electron Ionization (EI) is the most common ionization technique used for GC-MS. An EI source consists of a heat filament that produces electron accelerated towards an anode. Analyte molecules produce molecular ions (M+•) with high amount of internal energy. Since the ionization is produced by a single electron that is accelerated to 70 V (corresponding to the energy of -70 eV), this is enough energy to cause extensive fragmentation and at this level small changes in the electron energy do not significantly affect the fragmentation patterns. The analyte molecules shot with the electron beam (Figure 14) are thus fragmented into smaller ions resulting in multiple-peak in a mass spectrum. The amount of energy transferred during this process depends upon how fast the electron is traveling and how close it reaches the molecule (60).

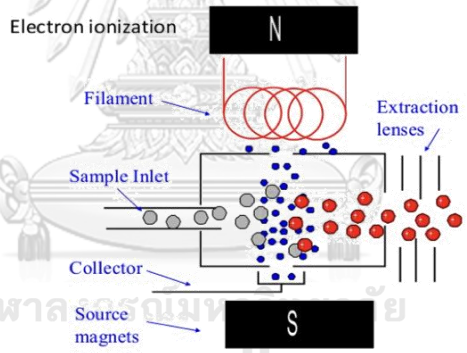

Figure 14 Diagram showing an electron ionization source (61)

2.2.2.2 Mass analyzer

The mass analyzer separates ions according to their *m/z* value. The selection of a mass analyzer depends upon the analysis goal with different focuses, *e.g.* on resolution, scan rate, mass range, dynamic range or detection limits. There are several types of mass analyzers, including magnetic sector, time of flight, quadrupole and ion trap.

- **Quadrupole**

Quadrupole mass spectrometer (QMS) is the most common mass analyzer in

GC-MS. A typical quadrupole mass analyzer consists of four rods with a hyperbolic cross section with a pair of positive and negative rods (Figure 15) with the application of fixed Direct Current (DC) and alternating Radio Frequency (RF) potentials. Ions produced in the source of the instrument then travel through the Q, and they are filtered according to their *m/z* value so that only ions with a single *m/z* value survive from the Q and strike the detector. The varied RF bring ions of different *m/z* into focus at the Q center and allow them to reach the detector resulting in a mass spectrum. Most quadrupole instruments are limited to unit *m/z* resolution and have a mass range up to *m/z* 1000. The most selective mode with a single quadrupole MS is called Selected Ion Monitoring (SIM) where a fixed set of DC and RF voltages is applied to the quadrupole allowing only ions with a single *m/z* to pass through. Ions with different *m/z* are filtered out.

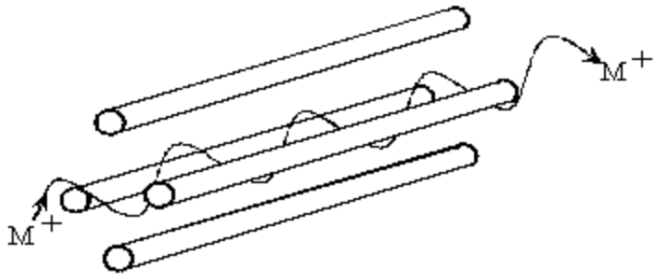

Figure 15 Quadrupole Mass Analyzer (62)

- **Triple-quadrupole**

Triple-quadrupole (QqQ) mass spectrometer operates under the same principle as the single quadrupole mass analyzer. Each of the two mass filters (Q1 and Q3) contains four parallel rods (Figure 16) which are used to select a precursor (parent) ion and to scan to generate a spectrum of the resulting product (daughter) ions, respectively. Both Q1 and Q3 are controlled by DC and RF potentials, while the collision induced dissociation (CID) occurs in the second quadrupole which is called the collision cell (q) is only subjected to RF potential to fragment the selected precursor/parent ions, and to generate fragment/daughter ions. The structural information can 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, enhancing collisionally induced fragmentation.

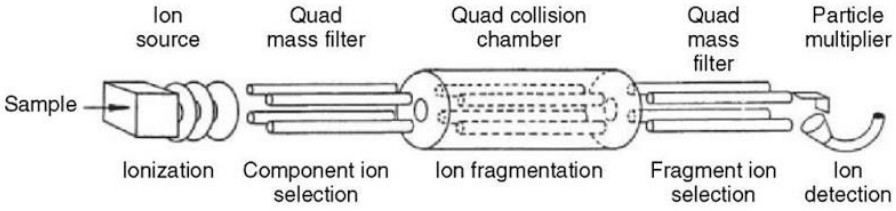

Figure 16 Triple quadrupole instrument (63)

2.2.2.3 Ion Detector

Ions from mass analyzer are detected based on their charge or momentum which is converted into current signal. For large signals, a faraday cup is used to collect ions and support measurement. Older instrumental approaches employ photographic plates to detect the ion abundance with different *m/z*. Most detectors currently apply a collector which is similar to a photomultiplier tube in order to amplify the ion signal. Such detectors include: electron multipliers, channeltrons and multichannel plates. The signal gain can be adjusted by changing the high voltage that is applied to the detector. Performance of a detector depends on its speed, dynamic range, gain, and geometry. Some highly sensitive detectors are able to detect a single ion (62).

2.3 Gas chromatography-mass spectrometry/olfactometry

GC-MS-O is based on detection with MS supported by sensory evaluation which is widely used to confirm the aroma compounds in food, beverage and fragrance. The effluent from the analytical column outlet was divided by a T-junction that is connected at the end of the analytical column to split the analyte flow prior to simultaneous detection with mass spectrometer and olfactory detection port (ODP). Odor active compounds are detected by properly trained human assessors that sniff the GC effluent by placing their nose at a specifically designed ODP connected in

parallel to a conventional detector such FID or MS. The assessors evaluate the duration of the odor stimulus (from start to end), provide a qualitative description of the perceived odor and give information about its relative intensity. GC-MS-O methodology can be a useful and reliable for the detection and identification of odor active VOCs, *e.g.* for detection of off-flavors coming from a wide range of indoor materials and consumer products (64).

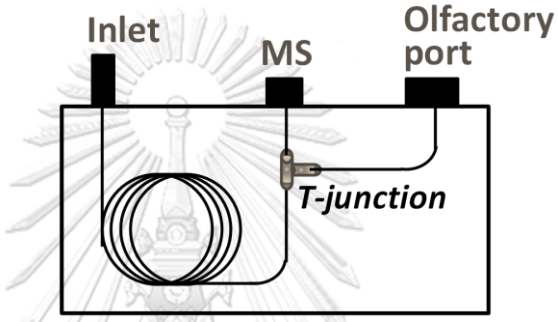

Figure 17 Schematic diagram of GC-MS/O

2.4 Principal component analysis

Principal component analysis (PCA) is widely used technique for analysis of data with the observations described by several inter-correlated (and quantitative) dependent variables to reduce the number of variables and to cluster samples into more parsimonious and manageable groups (65). The aims of a PCA are to identify correlated variables and reduce the dimensionality of the data by removing the noise and redundancy in the data.

PCA starts with differentiation of data matrix (X) of sample I and J variables with the dimension of I × J. After the analysis, X will be separated into two parts called scores (T) and loadings (P) according to the relationship, X = T P + E, see Figure 18.

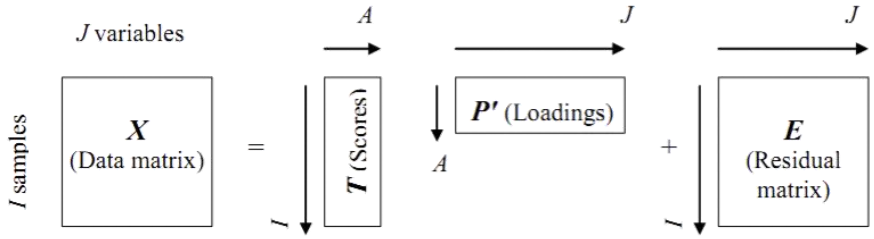

Figure 18 Diagram illustrating separation of data matrix (X) into Score (T) and loading (P) according to PCA (66)

PCA is applied to investigate variation of data compared with the origin. These new variables, called Principal Components (PC) which provide the most variation with the highest Eigenvalue and far fewer PCA variables are required than there were original variables. PCA is an alternative to Factor Analysis (both seek to find a simpler structure for a set of variables) but PC are linear combinations of variables whereas variables are linear combinations of Factors. Without comparison with the origin, the analysis can lead to false conclusion. A PCA is a multivariate ordination analysis. It pretends to order your samples in a plane typically defined by 2 axes (PC1 and PC2) according to their continuous variable values. In the figure below, the PC1 axis is the first principal direction along with the samples showing the greatest variation. The PC2 axis is the second most important direction and it is orthogonal to the PC1 axis. In this thesis, the PCA plots were applied to cluster samples into different groups similar to that applied in (66).

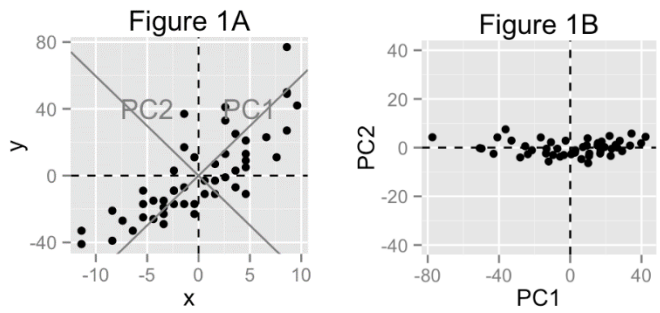

Figure 19 left) normal data (right) after using PCA plot (67)

Chapter III
Experimental

3.1 Instruments and apparatus

3.1.1 Gas chromatograph-mass spectrometer (GC-MS) Agilent technologies with 7890A equipped with a mass spectrometer Agilent 7000 Triple Quadrupole, where GC consists of autosampler and column oven, electron ionization (EI) interface and MassHunter software processing

3.1.2 Olfactory detection port, Gerstel model ODP3 consist of heat transfer line, heat mixing chamber, nose cone, humidified air, olfactory intensity device and voice recorder

3.1.3 Capillary column HP-5MS (30m x 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 GC Headspace Vial, 10 mL, Agilent Technologies, USA

3.1.7 Screw cap vial HS 10 mL, Agilent technologies

3.1.8 Ultra-high purity helium (99.999%), Lind

3.1.9 Balance (4 digits), Satorius Model AC2115-00MS (Germany)

3.1.10 Water bath

3.1.11 Thermometer

3.1.12 Micropipettes 100-1000 Eppendorf (Germany)

3.1.13 Volumetric flask 10 mL

3.1.14 Blender

3.1.15 Spatula

3.1.16 Droppers

3.2 Chemicals

3.2.1 Series of *n*-alkanes

A mixture of *n*-alkanes (C_8-C_{20}), sigma Aldrich (St. Louis, MO) was used as a reference to calculate retention index (*I*) of the compounds

3.2.2 Tetradecane internal standard (IS) 99% was obtained from sigma Aldrich (St. Louis, MO), AR grade

3.3 Chicken meat sample

Raw chicken breast meats obtained from a local supermarket were kept in odor-proof bags and stored at 4 °C until analysis. Boiled meat portions (500 g) were prepared using water bath at 85°C until an internal temperature of chicken increased to about 70°C. After boiling, the samples were rapidly cooled and kept at 4°C prior to further sample separation. The stored raw chicken meat was placed at room temperature before analysis and then cut into small pieces. The slices were finely minced and homogenized during 1 min in a household blender.

3.4 Optimization of sample preparation technique

In this study, boiled chicken meat was used to select suitable GC-MS conditions. Two studied techniques that were HS-SPME: 50 °C extraction temperature/ 50 min extraction time and SHS: 2.5 mL injection/ 50 °C extraction temperature. The suitable sample preparation technique was chosen for studying in GC-MS.m

3.5 Optimization of HS-SPME sample preparation

The main HS-SPME parameters were investigated: extraction temperature and time, the suitable conditions were that providing high total peak area of volatile compounds in chicken meat. Extraction of headspace volatile compounds was then performed using a HS-SPME device (Supelco, Bellefonte, PA) with a 50/30 µm Divinylbenzene/Carboxen/Polydimethyl-siloxane fiber (DVB/CAR/PDMS). The fiber was conditioned at 270°C for 1 hour prior to use and then injected into the GC-MS system

to check background signal from fiber. 4 g of minced sample was transfered into 20 mL SPME vial closed with aluminum cap with sealed PTFE/silicone septum. In extraction process, boiled chicken meat samples were heated in a water bath at 50 °C and the fiber of SPME was exposed to the sample headspace for 30 min in order to extract the volatile organic compounds (VOCs) onto the fiber. Similar process was applied for the raw chicken meat analysis but with the extraction performed at room temperature.

3.6 Optimization of HS-SPME type

Single HS-SPME was performed to analyze raw chicken meat sample resulting in relatively low signal of VOCs. In order to improve the sample signals, multiple (4x) HS-SPME (cumulative SPME analysis) were also performed prior to the GC-MS analysis. This approach applied with four consecutive extractions and desorptions using the same fiber with a 10 min interval between each injection (four cycles of single HS-SPME for one injection). The results in all the analysis above were expressed as the areas of peaks in total ion chromatograms (TIC), which was obtained using the scan mode of the MS for quantification.

3.7 Preparation of internal standard solution (IS)

3.7.1 Preparation of stock internal standard solution

Stock internal standard solution of 1,000 mg/L was prepared by dissolving 100 mg of tetradecane in ethanol and making up the volume to 100 mL in a volumetric flask. This solution was stored in a refrigerator at 4 ^{o}C, until required for analysis.

3.7.2 Preparation of working internal standard solution

The working internal standard solution of 10.0 μg/mL was prepared by pipetting 100 μL of the stock solution into a 10 mL volumetric flask, then making up the volume with ethanol.

3.8 Gas Chromatography Hyphenated with Mass Spectrometry (GC-MS)

For the identification and confirmation of the volatile compounds, a gas chromatograph Agilent technologies 7890A equipped with an Agilent 7000 mass spectrometer was employed. The compounds were thermally desorbed by inserting the fiber into the injection port of the GC for 5 min at 240°C under splitless mode. The compounds were separated on a HP-5 MS column (J&W Scientific (Agilent Technologies, USA), 30 m, 0.25 mm i.d., 0.25 µm film thickness) using ultra-high purity helium (99.999%) as the carrier gas with a flow rate of 1 mL/min. The GC oven temperature program began at 40 °C, ramped to 310 °C at 4 °C/min. C. Mass spectra were obtained under EI condition at -70 eV with the 35-300 of *m/z* range and the scan time of 100 ms (scan mode).

3.9 Precision study

The suitable conditions in sections 3.5-3.7 were selected to evaluate inter-day and intraday precision of the method according to peak area and peak area ratio of selected volatile compounds for 3 days. In each day, triplicate extractions were applied for the precision evaluation. In order to evaluate intra-day and inter-day precision for the developed method, 10 µL of tetradecane (10 µg/ mL), used as internal standards (IS), were spiked into a HS-SPME vial containing both types of chicken meat samples prior to the HS-SPME extraction. The ratios of peak areas of the selected volatile compounds to that of tetradecane (IS) were calculated. The intra-day and inter-day precisions were evaluated by considering the percentage of relative standard deviation (%RSD) of the peak areas of the selected compounds. Each sample was analyzed three times in the same day to determine the intra-day precision. The same procedure was repeated for three days and compared by calculating the %RSD for the average values for each day to determine the inter-day precision.

3.10 Data processing

The chromatographic peaks of each raw chicken meat and boiled chicken meat sample were identified using Agilent MassHunter software. The data processing and presentation were further performed using Microsoft Excel. The compounds were tentatively identified by comparison with MS spectra with those obtained from the library database (NIST) 2017 version. 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 literatures for the same (or a similar) stationary phase. *I* of a peak of interest was identified relative to the retention time of a homologous series of alkanes C8 to C20 (Sigma Aldrich, St. Louis, MO) which was obtained by injection of an alkane mixture under the same experimental conditions used for separation of the sample. *I* values for the temperature programmed separation were calculated according to the van den Dool and Kratz formula,

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

where

$t_{r(i)}$ is the retention time of a compound of interest

$t_{r(n)}$ is the alkane eluting immediately before the unknown compound with the carbon number of *n*.

$t_{r(n+1)}$ is the alkane eluting immediately after the compound with the carbon number of *n*+1.

The odor descriptors of compounds were obtained from the olfactometry database from literatures.

3.11 Statistic analysis

In this study, Principal component analysis (PCA) was used to present and visualize the data in two dimension. The multivariate statistical analyses were performed with XLSTAT.

Chapter IV
Results & Discussion

4.1 Optimization of sample preparation technique

The analysis of VOCs in sample HS is widely performed with three common sample preparation techniques which are static HS (SHS), dynamic HS (DHS) and HS-SPME. SHS is a method direct sampling analytes in HS that is dependent upon the formation of equilibrium conditions in a closed system. DHS continually sweeps the HS of the sample concentrating the analytes onto a trap material. Analytes are retained on the trap until desorption into GC-MS for separation and detection. HS-SPME technique has been introduced as an alternative to the DHS technique as a sample extraction and pre-concentration method. HS-SPME is a rapid, sensitive and solvent-free sampling technique.

Boiled sample was prepared by HS-SPME and SHS as shown in Figure 20 A and B, respectively. The GC-FID result shows more peaks obtained using HS-SPME even at lower extraction temperature, especially those with the retention times of >5 min. This is mainly due to the process of analyte pre-concentration onto the HS-SPME fiber, which is not expected with SHS without solid phase material.

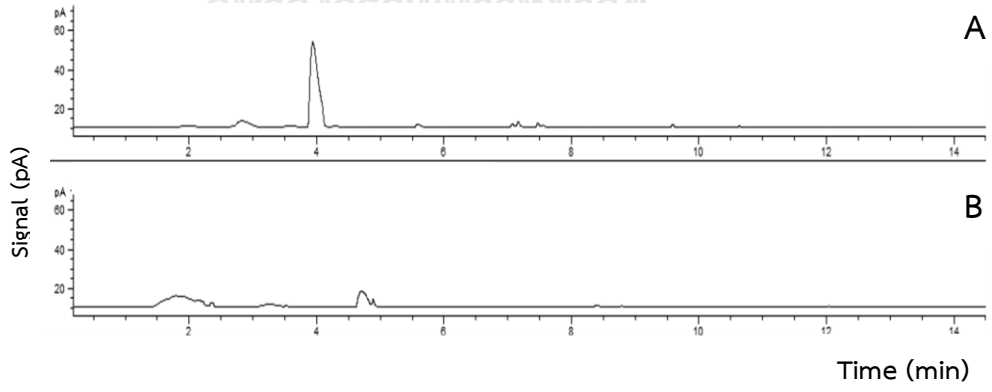

Figure 20 GC-FID chromatograms of HS-SPME: 35 °C extraction temperature and 10 min extraction time (A), and SHS: 2.5 mL injection and 50 °C extraction temperature (B)

4.1.2 Optimization of HS-SPME

In HS-SPME, the fiber is exposed to HS of liquid or solid sample. Critical factors in this technique are the extraction temperature and extraction time [44]. In general, the trapped compounds are thermally desorbed by introducing the fiber into the injection port of a gas chromatograph. The partition of analytes between the sample HS and the fiber is influenced by the type of sorbent and the extraction conditions. It is necessary to identify suitable HS-SPME conditions. The uptake of the analytes and therefore the sensitivity of the method can be improved by optimizing parameters including time and temperature of extraction which were described in section 3.5. Sample was prepared by 50 °C extraction temperature and 30 min extraction time (Figure 21 A) and 35 °C extraction temperature and 10 min extraction time (Figure 21 B). The demonstrated that suitable condition higher temperature and longer extraction time as shown in Figure 21 A gave the higher overall peak areas. So, this condition was selected as the separation condition for further analysis. The effect of extraction time (10, 30 and 50 min at 50 °C extraction temperature) on the total peak area of volatile compounds with GC-FID is shown in Figure 22.

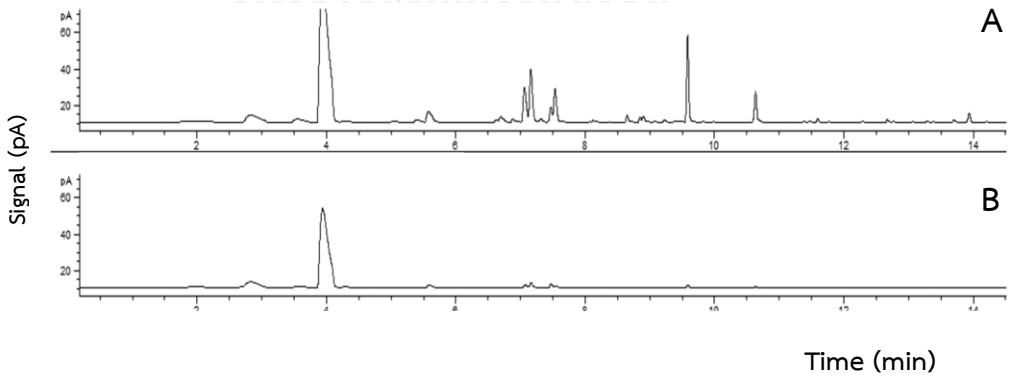

Figure 21 GC-FID chromatograms of 50 °C extraction temperature/ 30 min extraction time (A) and 35 °C extraction temperature/ 10 min extraction time (B) in bolied samples

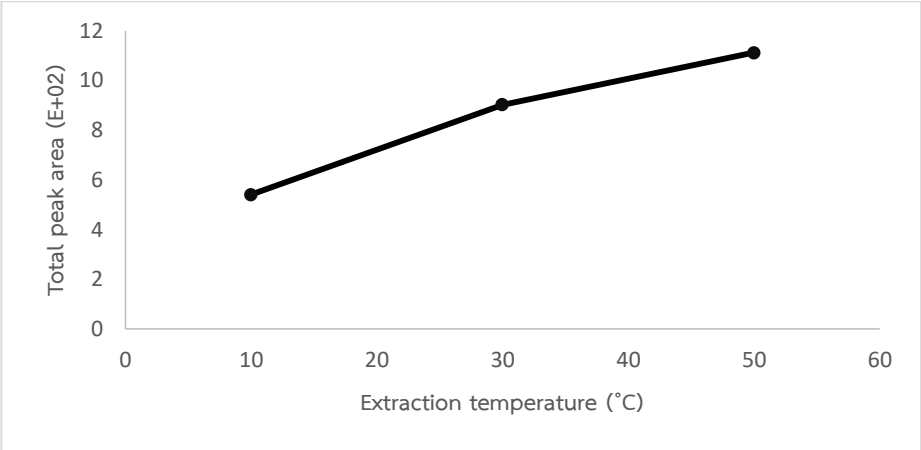

Figure 22 Effect of extraction time on total peak areas of volatile compounds with GC-FID from boiled chicken sample

4.1.3 Optimization of HS-SPME type

Raw chicken meat has a little or no aroma. So, the suitable condition from previous section cannot be sufficiently used to identify VOCs in raw chicken meat sample. This method resulted in detection of only a few substances in HS of the raw sample. In order to enhance the sensitivity of the method, multiple HS-SPME were applied. Sample was prepared by HS-SPME: 50 °C extraction temperature and 30 min extraction time multiple injection HS-SPME (4 times) and SHS: 50 °C extraction temperature/ multiple injection SHS (4 times). The comparison of the total ion chromatogram is shown in Figure 23. This indicated that multiple HS-SPME is more suitable for analysis of volatile compounds in chicken meat sample than multiple SHS. Effect of the number of injections on the extraction performance was then investigated. The 2, 3 and 4 times injections (extraction cycles) were performed with the result shown in Figure 24. To this end, 4x multiple injection approach was selected to analyze raw chicken meat samples since this resulted in high peak areas improving detectability of the odor active compounds within the reasonable analysis time.

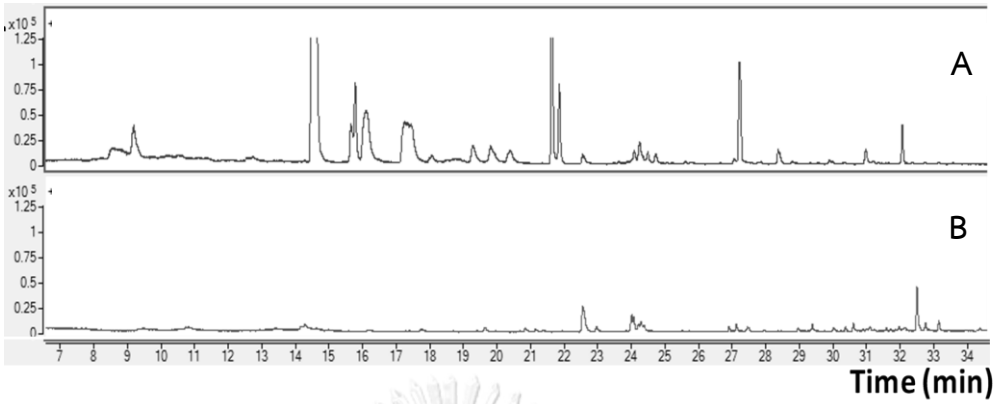

Figure 23 Total ion chromatograms (TIC) of a raw chicken sample obtained using 4x multiple injections with HS-SPME: 50 °C extraction temperature and 10 min extraction time for each injection (A), and SHS: 50 °C extraction temperature (B)

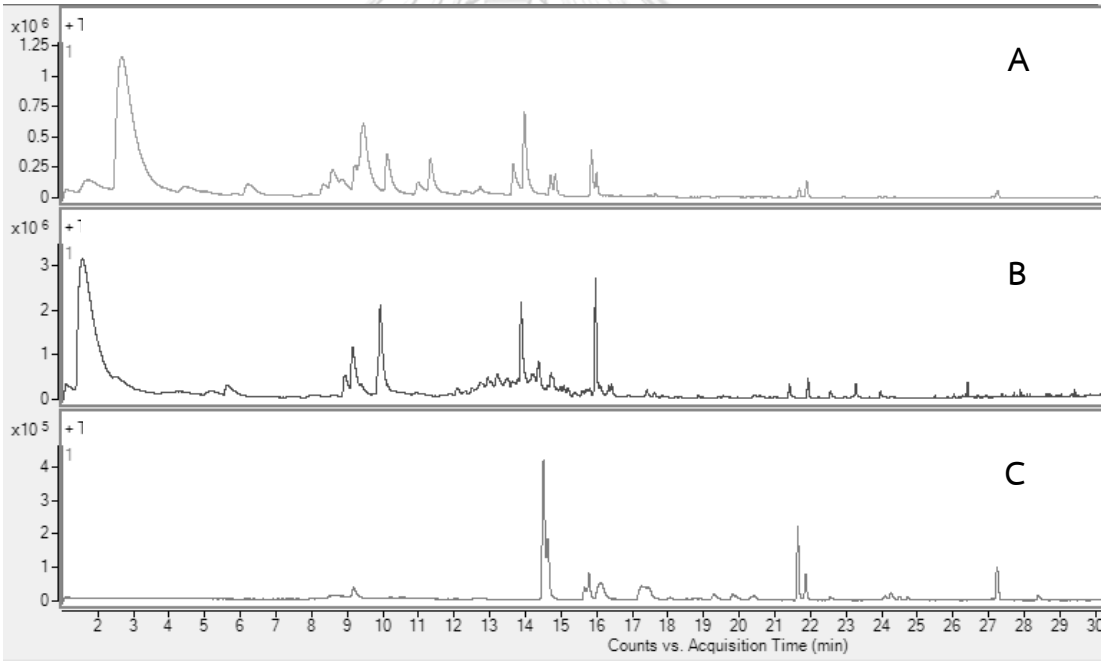

Figure 24 Total ion chromatogram (TIC) of a raw chicken sample obtained using multiple injection HS-SPME with different number of injections: 4x, 3x and 2x (A-C, respectively), and 10 min extraction time for each injection

4.2 Identification of volatile compounds

Peaks detected with the GC-MS chromatograms were identified by comparison of their experimental MS spectra with that from NIST 2017 version library. The criteria for compound identification required a mass spectrum matching score of > 650 and with *I* difference of ≤20 units between the calculated and the experimental values from the database with the equivalent stationary phase.

4.2.1 Identification of volatile compounds in boiled chicken meat

The selected single injection HS-SPME method in section 4.1- 4.2 was applied prior to GC-MS analysis for all boiled chicken samples. The volatile compounds were separated by using HP-5MS capillary column which is suitable for volatile compounds analysis. The total ion chromatogram (TIC) of boiled chicken samples is shown in Figure 25.

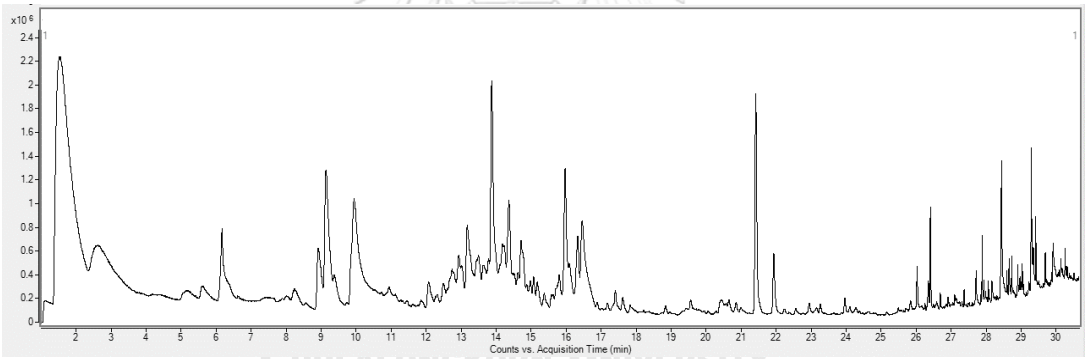

Figure 25 The total ion chromatogram (TIC) of boiled chicken samples obtained from HS-SPME-GC-MS analysis

Analysis of boiled chicken meat sample revealed 16 possible odor active compounds as shown in Table 2. It should be noted that some peaks are alkyl siloxanes from SPME fiber, which are typically observed with SPME analysis (68). Fours chemical classes were observed, including 7 aldehydes, 1 aromatic hydrocarbon, 6 alcohols and 2 ketones. These compounds were also found in cooked chicken meat samples as reported by several authors (24, 26, 27). The major compounds found in chicken meat sample were carbonyl compounds which could be formed by peroxidation of unsaturated acyl lipids (69, 70).

Table 2 Tentative volatile compounds of boiled chicken meat sample

No.	RT (min)	Compound	CAS No.	LRI		Odor Description
				Exp	Database	
		Aromatic hydrocarbon				
1	2.66	Toluene	108-88-3	<800	773	Sweet
		Aldehydes				
2	4.44	Hexanal	66-25-1	<800	800	Green
3	6.19	3-Methyl hexanal	19269-28-4	891	910	Green
4	7.78	Heptanal	111-71-7	858	903	Green
9	11.33	Octanal	124-13-0	1000	1003	Aldehydic
11	12.61	2-Octenal	2548-87-0	1056	1063	Green
14	13.97	Nonanal	124-19-6	1101	1102	Aldehydic
15	23.62	Decanal	112-31-2	1202	1206	Aldehydic
		Alcohols				
5	8.59	Heptanol	111-70-6	970	970	Green
7	9.45	1-Octen-3-ol	3391-86-4	978	976	Mushroom
10	12.25	2-Nonen-1-ol	22104-79-6	1051	1081	Fatty (E), Green (z)
12	12.72	2-Octen-1-ol	18409-17-1	1058	1069	Fatty
13	11.33	Octanol	111-87-5	1070	1068	Green
16	17.00	Decenol	3913-81-3	1258	1250	Waxy
		Ketones				
6	8.59	2,3-Octanedione	585-25-1	971	967	Dill type
8	9.45	2,5-Octanedione	3214-41-3	973	984	Milky and sweet

Exp = Experimental *I* calculated using *n*-alkanes standards on a HP-5MS Column.
Database = *I* values obtained on HP5 and HP5-ms capillary columns from NIST database.
Odor description obtained from http://www.thegoodscentscompany.com

4.2.2 Identification of volatile compounds in raw chicken meat

The multiple injection HS-SPME was applied prior to GC-MS analysis for the raw chicken samples. The total ion chromatograms (TIC) of raw chicken samples are shown in Figure 26. Overall 20 odor active compounds were identified in the raw samples as shown in Table 3. Three chemical classes were observed, including 10 aldehydes, 7 alcohols and 3 esters. The major compounds in the raw chicken breast meat samples in this research have also been reported elsewhere, which were aldehyde, alcohol, ketone, ester, volatile phenol, acid and terpenes (29).

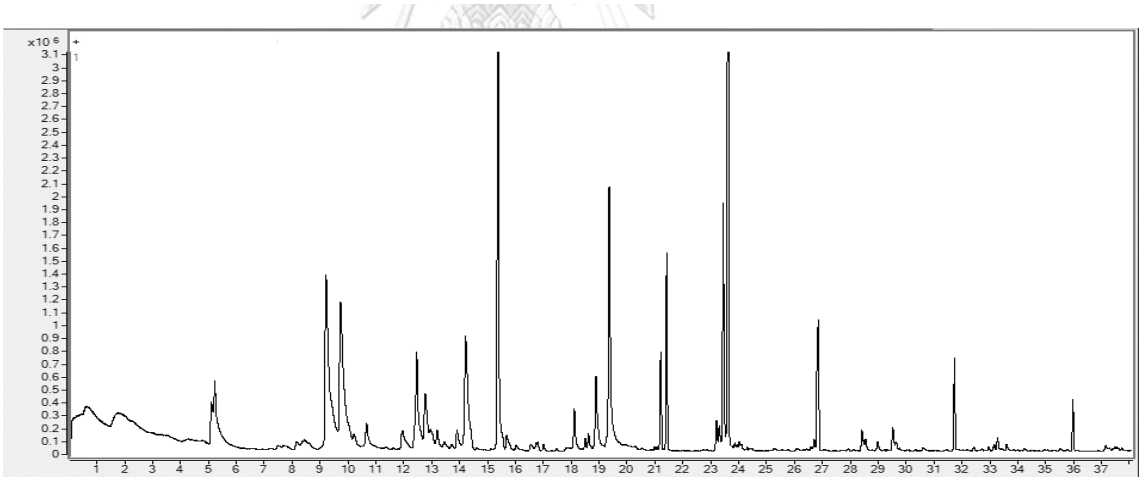

Figure 26 The total ion chromatogram (TIC) of raw chicken samples obtained from Multiple HS-SPME-GC-MS Analysis

Table 3 Tentative volatile compounds of raw chicken meat sample

No.	RT (min)	Compound	CAS No.	LRI		Odor Description
				Exp	Database	
		Aldehydes				
1	0.77	Hexanal	108-88-3	<800	773	Green
2	4.44	3-Methyl hexanal	19269-28-4	891	910	Green
3	7.12	2- Heptanal	2463-63-0	955	956	Green
7	11.35	2-Octenal	2548-87-0	1056	1063	Green
9	13.19	Nonanal	124-19-6	1102	1102	Aldehydic
10	17.00	Decanal	112-31-2	1202	1206	Aldehydic
11	18.99	2-Decenal	3913-81-3	1258	1250	Waxy
13	22.45	2-Undecenal	2463-77-6	1358	1364	Fruity
14	23.91	Dodecanal	112-54-9	1402	1407	Aldehydic
16	35.50	hexadecanal	629-80-1	1813	1817	Cardboard
		Alcohols				
4	7.77	1- Heptanol	111-70-6	970	970	Green
5	8.14	1-Octen_3-ol	3391-86-4	979	976	Mushroom
6	10.24	1-Hexanol-2-ethyl	104-76-7	1028	1031	Citrus, Floral
8	11.97	Octanol	111-87-5	1070	1068	Green
12	19.39	Decanol	112-30-1	1269	1273	Waxy, Green
20	41.94	Octadecanol	112-92-5	2083	2082	Bland
17	37.11	1-hexadecanol	36653-82-4	1878	1880	Waxy
		Esters				
15	29.54	Dodecanoic, ethyl ester	106-33-2	1589	1595	Sweet
18	39.89	Pentadecanoic, ethyl ester	41114-00-5	1894	1894	Honey sweet
19	40.62	Isopropyl palmitate	142-91-6	2025	2023	Bland

Exp = Experimental *I* calculated using n-alkanes standards on a HP-5MS Column.
Database = *I* obtained on HP5 and HP5-ms capillary columns from NIST database.
Odor description obtained from http://www.thegoodscentscompany.com

Previous work reported that most VOCs in chicken meat sample were potentially derived from the oxidative decomposition of unsaturated fatty acids. Nonanal may be the product from oleic acid oxidation; whilst, alcohols may be obtained from the lipoxygenase-initiated peroxidation (71), e.g. 1-octen-3-ol results from the enzymatic degradation of linoleic acid and lipid degradation (72). Hexanal and 2,4-decadienal are the most abundant aldehydes identified in chicken flavor which are known to be the primary oxidation products of linoleic acid (Figure 27) (73). Toluene may be obtained from degradation of amino acids with the phenyl group (74).

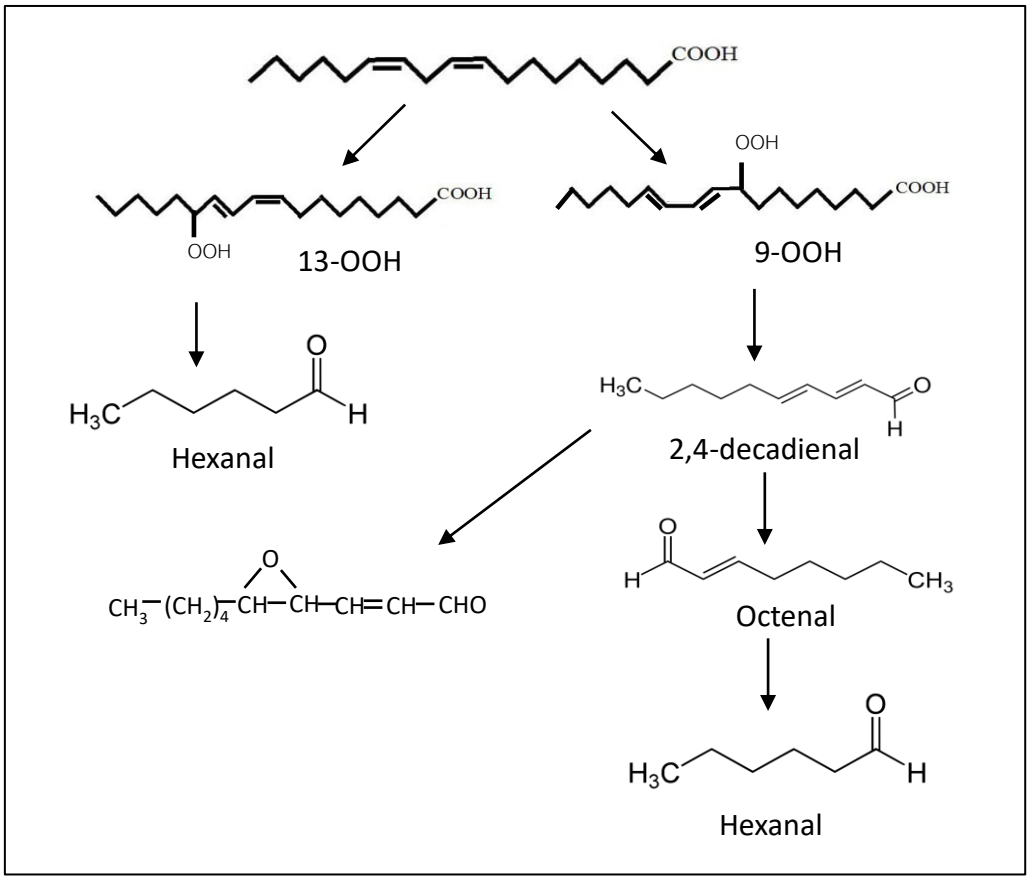

Figure 27 Oxidation and degradation products of linoleic acid (73)

4.3 Method precision

4.3.1 Boiled chicken meat analysis with single injection HS-SPME

Boiled chicken meat samples were extracted by HS-SPME at 50 °C for 30 min. The selected volatile compounds were hexanal, heptanal, 1-octen-3-ol, octanol and nonanal as shown in Table 8 (Appendix A). Figure 28 shows an example of TIC of the analysis in this section. The relative standard deviation (%RSD) values of the "peak areas" and "peak area ratios of the compounds to tetradecane (IS)" were shown in Table 9 (Appendix A). For intra-day precision the %RSD for the peak area analysis was found to be ≤20%; whilst, %RSD from the peak area ratio analysis was found to be ≤10%. Inter-day %RSD for the peak area analysis was ≤15% and in %RSD for the precision peak area ratio analysis was ≤10%. Intra-day and inter-day from the peak area ratio analysis were in the acceptance range as shown by the data in Table 4. This indicated that the internal standard can improve precision for single injection HS-SPME.

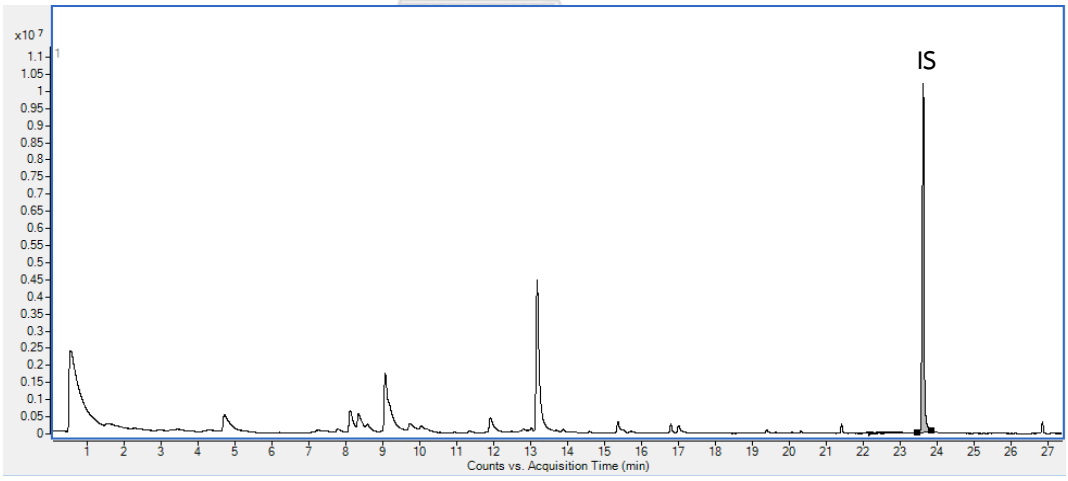

Figure 28 The total ion chromatogram (TIC) of boiled chicken meat sample spiked with the internal standard

Table 4 Inter-day and intra-day precision studies in boiled chicken meat sampl (n=3)

Selected volatile compounds	Peak area (No IS)			Peak area ratio of VOCs to IS		
	Mean	Intra-day precision (%RSD)	Inter-day precision (%RSD)	Mean	Intra-day precision (%RSD)	Inter-day precision (%RSD)
Hexanal	1706264	12.6	4.9	2.8	9.5	6.6
Heptanal	435845	18.4	2.5	0.7	5.5	2.7
1-Octen-3-ol	111275	9.5	1.5	0.2	7.4	3.7
Octanal	1101704	9.9	6.4	1.7	6.2	7.2
Nonanal	1185616	14.7	10.5	1.7	6.9	7.5
tetradecane	610866	14.2	3.5	1.0	0	0

4.3.2 Raw chicken meat analysis with 4x multiple injection HS-SPME

Raw chicken meat samples were extracted by 4x multiple HS-SPME extraction at room temperature with a 10 min interval for each extraction. The focused volatile compounds were hexanal, 1-octen-3-ol, 1-hexanol-2-ethyl, octanol, nonanal, decanal, decanol, dodecanoic, ethyl ester, 1-hexadecanol and pentadecanoic, ethyl ester as shown in Table 10 (Appendix A). Figure 29 shows an example TIC of the analysis in this section. The standard deviation (SD) values of the “peak areas” and “peak area ratios of the compounds to tetradecane (IS)” were shown in Table 11 (Appendix A) For intra-day precision, %RSD of the peak areas was ≤35%, and that of the peak area ratios was ≤40%. For inter-day precision, %RSD of the peak areas was ≤35% and that in the ratio of peak area was ≤30%. This indicated that application of the selected internal standard only improves the inter-day precision for the multiple injection HS-SPME.

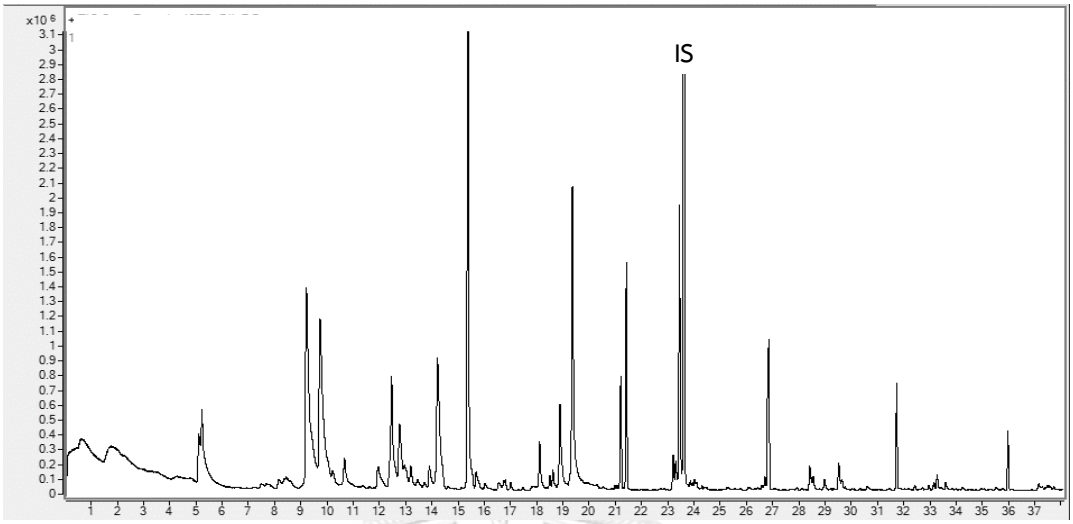

Figure 29 The total ion chromatogram (TIC) of raw chicken meat sample

Table 5 Inter-day and intra-day precision studies in raw chicken meat sample (n=3)

Selected volatile compounds	Peak area (No IS)			Peak area ratio of VOCs to IS		
	Mean	Intra-day precision (%RSD)	Inter-day precision (%RSD)	Mean	Intra-day precision (%RSD)	Inter-day precision (%RSD)
Hexanal	1496201	32.2	12.2	0.17	34.8	11.8
1-Octen-3-ol	468462	21.3	21.0	0.05	23.8	15.7
1-Hexanol-2-ethyl	317203	27.3	32.8	0.03	28.5	29.0
Octanol	364820	30.3	6.8	0.04	18.4	2.0
Nonanal	502947	17.2	25.4	0.07	16.1	22.2
Decanal	503739	21.5	17.2	0.02	29.4	11.6
Decanol	8589984	20.8	5.2	0.87	35.2	9.0
tetradecane	10315603	19.0	7.0	1.00	0.0	0.0
Dodecanoic, ethyl ester	821951	27.7	23.2	0.09	26.2	26.5
1-hexadecanol	3750833	26.7	8.3	0.42	15.2	11.9
Pentadecanoic, ethyl ester	190698	14.3	6.4	0.02	23.3	7.6

4.4 Sample analysis

The selected method was used for identification of volatile compounds in both types of chicken meat samples. From preliminary study in Section 4.2, different method was applied to investigate volatile compounds in five boiled and five raw chicken product samples. The results are summarized in Table 6 and Table 7.

4.4.1 Analysis of boiled chicken meat samples

Table 6 shows comparison of volatile compounds in five boiled chicken meat samples. Aldehydes observed in all the samples were hexanal, octanal and nonanal which can be produced from lipid oxidation process (73). According to all the boiled sample profiles, the total of sixteen volatile compounds was observed. The major volatile compounds found in Boiled_A are hexanal (77.17%area), toluene (5.66) and 2,5-octanedione (4.62%). Eleven volatile compounds were identified and the major volatiles in Boiled_B were hexanal (73.94), 3-methyl hexanal octanal (6.02) and 2,3-octanedione (4.73). Eleven volatile compounds were identified in Boiled_C and the major volatile compounds were hexanal (78.17), octanal (12.30) and 2-nonen-1-ol (3.49). Boiled_D contained twelve volatile compounds and the major volatile compounds were hexanal (30.57), 2,3-octanedione (17.78) and octanal (13.36). In Boild_E, nine volatile compounds were identified and the major volatile compounds were hexanal (50.82), 2,3-octanedione (15.65) and nonanal (7.59). The volatile compound profiles in the boiled samples are summarized in Figure 30, with hexanal as the major compounds. The characteristic odor of hexanal is cut glass which is in off-flavor odor class.

Table 6 Average %area of boiled chicken meat sample

RT (min)	Tentative volatile Compounds	Average % area (n=3)				
		Boiled_A	Boiled_B	Boiled_C	Boiled_D	Boiled_E
2.66	Toluene	5.66±1.65	1.02±0.10	0.97±0.30	-	7.40±1.30
4.434	Hexanal	77.17±16.60	73.94±4.71	78.17±1.19	30.57±3.21	50.82±0.27
6.19	3-Methyl hexanal	1.04±1.18	2.79±0.35	-	1.56±0.52	-
7.78	Heptanal	1.17±1.29	-	-	1.06±0.36	-
8.59	Heptanol	1.68±0.16	1.39±0.03	1.76±0.04	8.60±9.30	-
9.22	2,3-Octanedione	0.68±0.72	4.73±0.47	-	17.78±1.11	15.65±0.75
9.45	1-Octen-3-ol	0.36±0.40	-	0.26±0.04	6.50±1.81	7.37±1.50
10.12	2,5-Octanedione	4.62±4.84	-	0.65±0.12	-	1.19±0.12
11.33	Octanal	1.95±1.92	6.02±0.30	12.30±1.06	13.36±3.76	4.86±0.32
12.25	2-Nonen-1-ol	1.71±1.84	0.25±0.04	3.49±0.21	-	3.63±0.48
12.61	2-Octenal	0.12±0.13	-	0.44±0.14	1.23±0.12	-
12.72	2-Octen-1-ol	0.07±0.07	-	0.94±0.11	0.37±0.04	-
13.19	Octanol	0.15±0.16	0.11±0.03	-	0.43±0.06	1.49±0.48
13.97	Nonanal	1.03±1.14	2.27±0.48	0.33±0.04	6.46±2.52	7.59±0.55
17.00	Decenol	2.46±2.58	3.63±0.32	-	12.08±1.77	-
23.62	Decanal	0.07±0.07	0.12±0.01	0.69±0.50	-	-

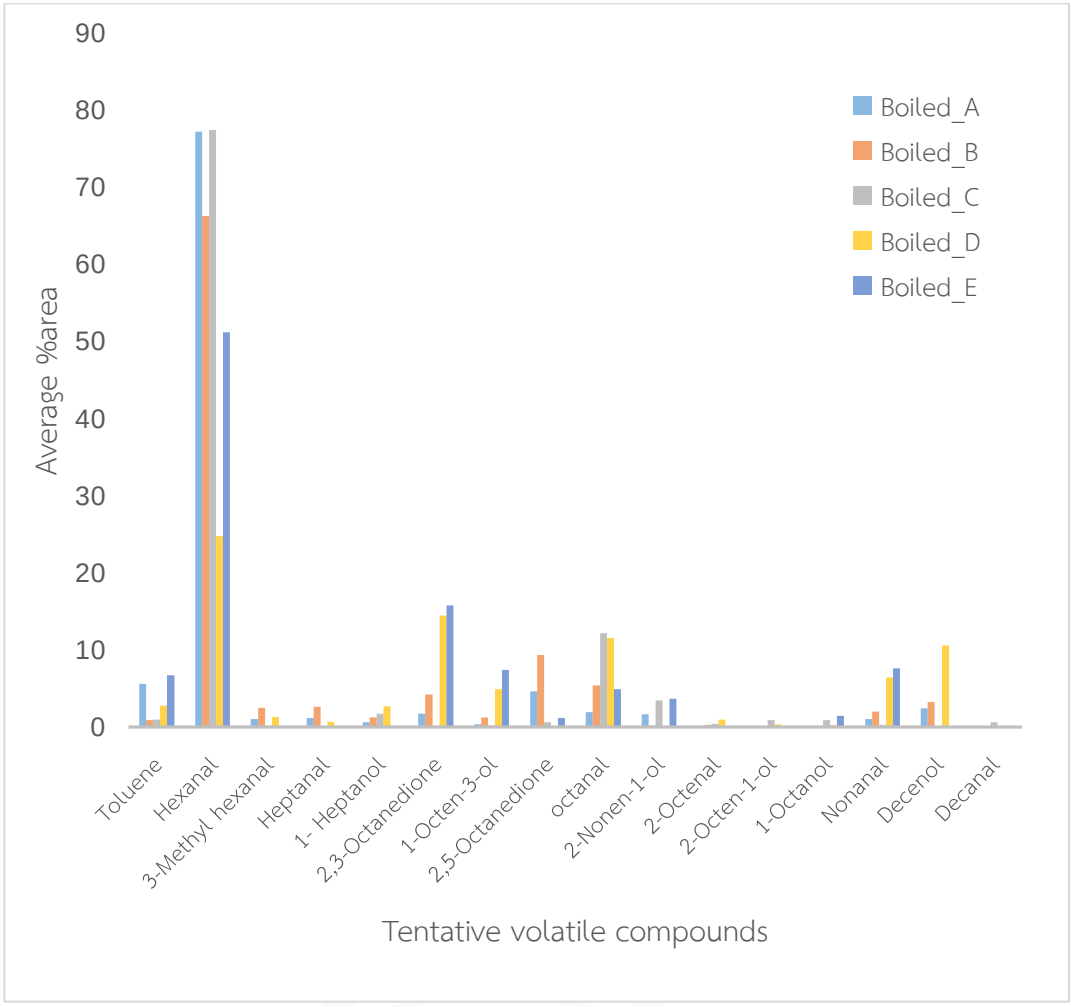

Figure 30 The Average %area of volatile compounds in boiled chicken meat sample

4.4.2 Analysis of raw chicken meat samples

Table 7 and Figure 31 show comparison of volatile compounds in five raw chicken meat samples. Eight volatile compounds were identified in Raw_A with the major volatiles of hexanal (33.48), 2-decenal (34.42) and hexadecanal (13.06). Five volatile compounds were identified, and the major volatile compounds found in Raw_B were nonanal (81.54), 3-methyl hexanal (11.05) and 1-Octen_3-ol (3.09). Twelve volatile compounds were identified in Raw_C and the major volatile compounds were decanal (46.86), 1-hexadecanol (19.04) and Hexanal (14.79). Raw_D contained twelve volatile compounds with the major volatiles of dodecanoic,

ethyl ester (21.10), decanol (20.28) and 1-hexadecanol (24.23). In Boild_E, fourteen volatile compounds were identified with decanol (32.73), dodecanal (30.18) and 1-Hexanol-2-ethyl (21.61) as the major volatile compounds. Figure 4.12 showed relative amount of odor active compounds in all the samples with hexanal as the major compound (4x injection at room temperature) but lower than that detected in boiled chicken meat (single injection at 50 °C). The highest %area of Raw_B was nonanal which is arranged in sweet odor group.

Table 7 Average %area of raw chicken sample

RT (min)	Tentative volatile compounds	Average %area (n=3)				
		Raw_A	Raw_B	Raw_C	Raw_D	Raw_E
0.77	Hexanal	33.48±2.83	2.71±1.71	14.79±2.10	3.30±0.28	0.61±0.08
4.44	3-Methyl hexanal	4.56±0.61	11.05±2.80	2.04±0.19	-	-
7.12	2- Heptanal	-	-	-	-	1.81±0.17
7.77	1- Heptanol	-	-	-	-	1.71±0.12
8.14	1-Octen_3-ol	2.10±0.27	3.09±0.91	2.64±0.35	9.31±0.56	2.92±0.43
10.24	1-Hexanol-2-ethyl	-	-	1.30±0.10	3.70±0.18	21.61±3.80
11.35	2-Octenal	-	-	-	0.59±0.07	-
11.97	1-Octanol	1.64±0.73	1.61±0.37	2.14±0.64	6.86±0.16	1.02±0.13
13.19	Nonanal	-	81.54±3.60	2.40±0.08	4.12±0.29	0.62±0.07
17.00	Decanal	-	-	1.16±0.11	8.20±0.48	1.56±0.09
18.99	2-Decenal	34.42±1.70	-	0.94±0.14	-	-
19.39	Decanol	7.23±0.88	-	46.86±2.82	20.28±0.55	32.73±1.47
22.45	2-Undecenal	-	-	-	4.15±0.20	-
23.91	Dodecanal	-	-	-	-	30.18±1.53
29.54	Dodecanoic, ethyl ester	13.06±0.22	-	5.57±0.44	21.10±0.82	0.44±0.06
35.50	hexadecanal	-	-	-	-	0.45±0.05
37.10	1-hexadecanol	3.50±0.54	-	19.04±2.80	14.23±0.26	3.83±0.17
39.89	Pentadecanoic, ethyl ester	-	-	1.12±0.14	4.15±0.21	0.51±0.06

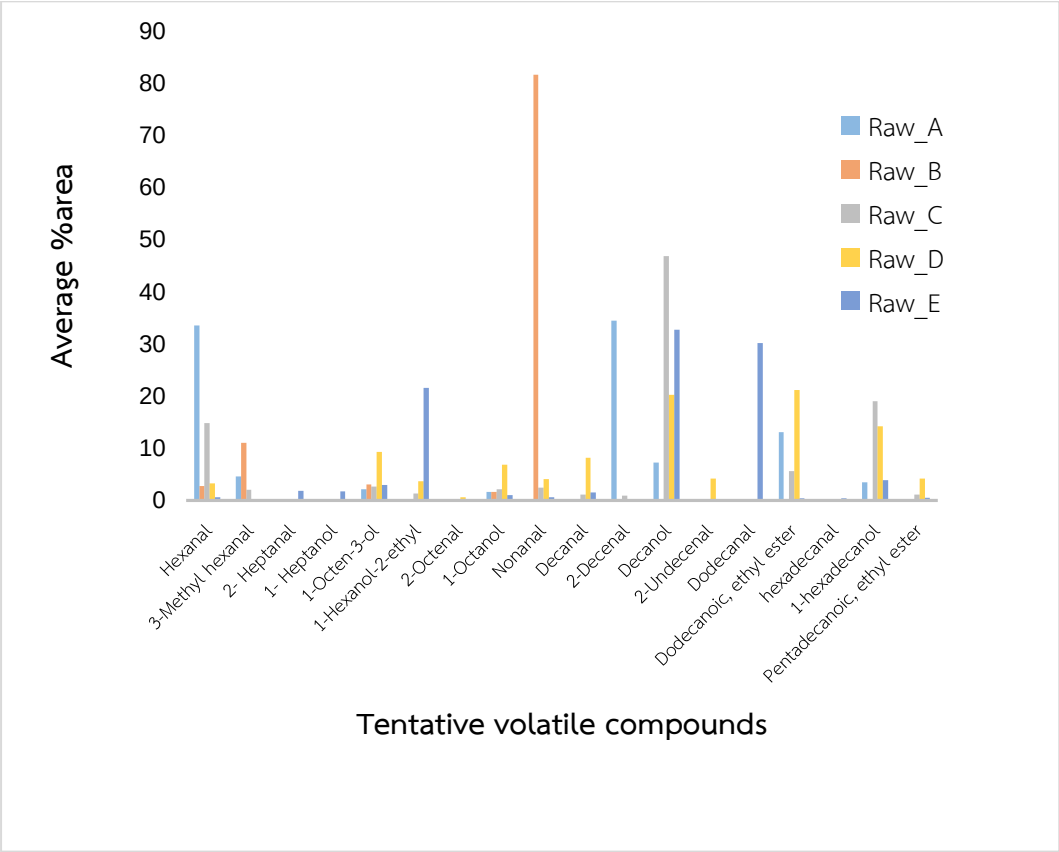

Figure 31 The Average %area of volatile compounds in raw chicken meat sample

4.5 Summary of PCA analysis of chicken meat samples

The principal component analysis (PCA) was further applied (separately with the boiled and raw samples) to determine the differences in the comparative amounts of volatile compounds in different chicken meat samples from the same source according to volatile compounds in Table 6 and Table 7 and experimental data of average %area. PCA results of boiled chicken meat samples are shown in Figure 32 with the PCA score plot (Figure 32 A) and biplot (Figure 32 B). The corresponding results for the raw chicken meat samples are shown in Figure 33 with the PCA score plot (Figure 33 A) and biplot (Figure 33 B).

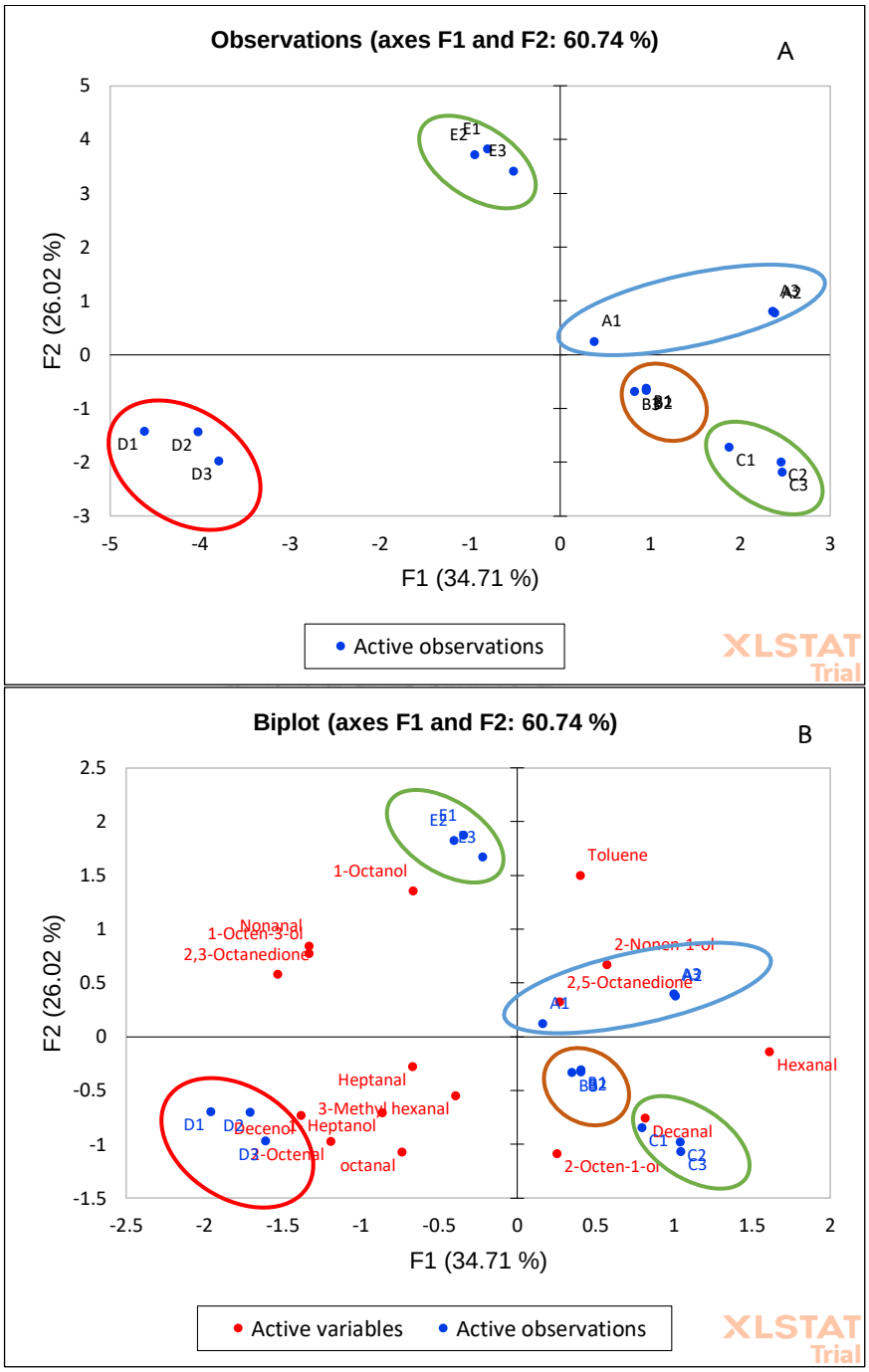

Figure 32 PCA Score plot and Biplot of the volatile compounds and the boiled chicken meat samples

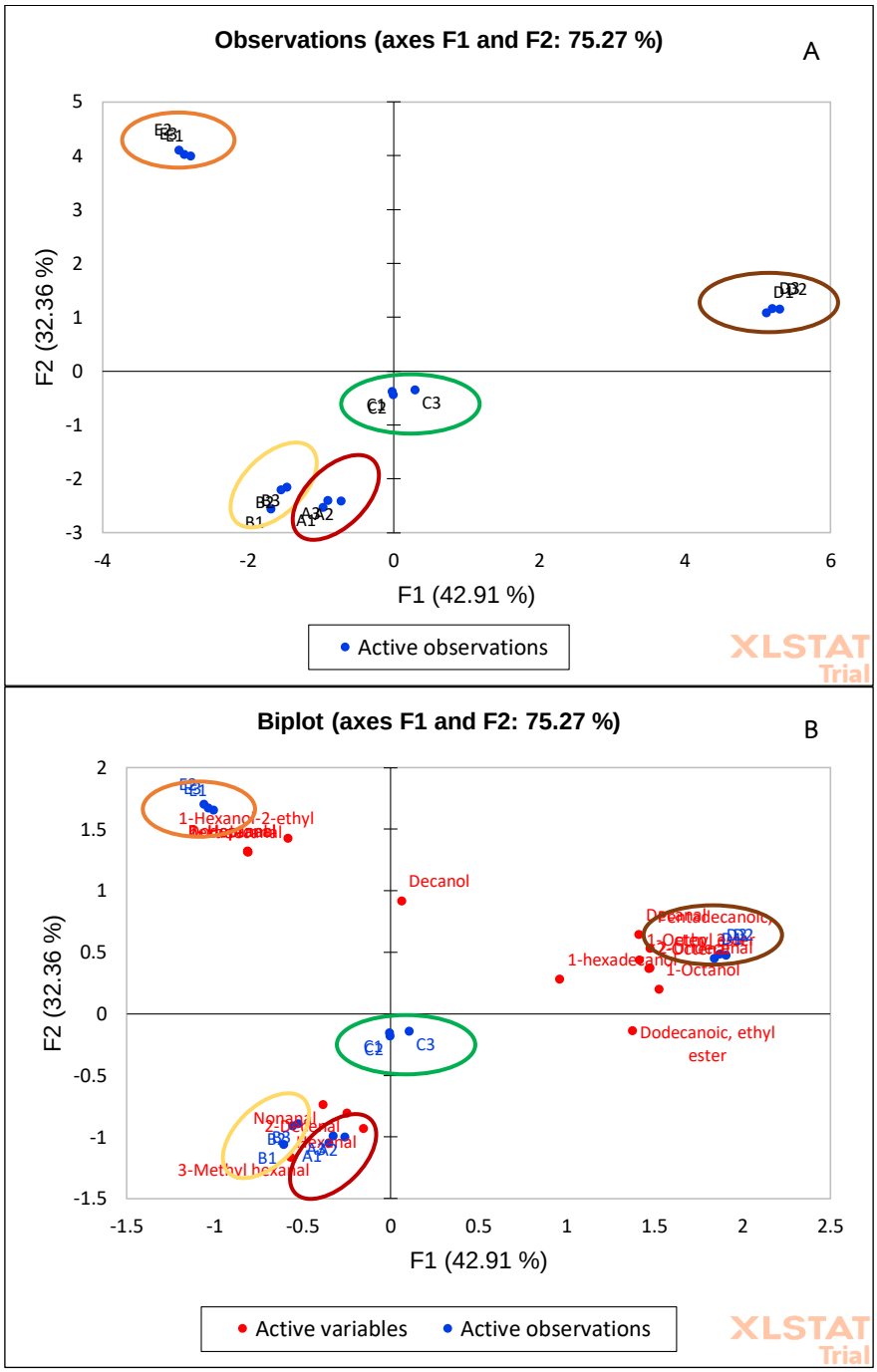

Figure 33 PCA Score plot and Biplot of the volatile compounds and the raw chicken meat samples

It should be pointed out that the PCA score plot is used to differentiate the samples, while the Biplot is used to correlate the samples with the volatile compounds. The result shown in Figures 32A and 33A clearly separate data of the five groups of the boiled and raw samples, respectively, according to the different sample origins. The Figures 32B and 33B showed biplot of the five groups sample overlaid with the odor active compounds which represent a relationship between the collated compounds and the sample groups. The marker compound of each group can be selected from the region within each group such as volatile compounds in sample A and B of the raw samples can be defined hexanal and nonanal, respectively (see the Biplot in Figure 33B).

CHAPTER V
CONCLUSION

Approaches to analyze odor active compound in chicken meat samples were demonstrated. The volatile compounds in boiled and raw chicken meat samples were extracted by single injection HS-SPME and multiple injection (cumulative) HS-SPME, respectively, prior to the analysis with GC-MS. With the expense of longer injection time and lower precision, significant improvement of signal intensity was clearly achieved by using 4x multiple HS-SPME. This enabled detection of the volatile compounds in the raw samples, the signals of which were too small to be detectable with the single injection approach. With the identification criteria of a match score of >650 and a difference of 20 units between the experimental retention index (*I*) and that from the literatures, totals of 16 and 20 odor active compounds were identified in boiled and raw samples, respectively. Intra-day and inter-day precision were further improved using tetradecane as the internal standards spiked into the samples prior to the HS-SPME-GC-MS analysis. %RSD values for intraday and inter-day precision were ≤10% in the boiled samples and ≤30% for most of the volatile compounds in the raw samples with the exception of hexanal and decanol with the %RSD of ≤40%. The multiple HS-SPME sample preparation showed lower precision due to loss of the volatile compounds during the several times extraction process.

Comparison between the volatile compounds in boiled chicken meat samples revealed hexanal as the major compound. The characteristic odor of hexanal is cut glass which contributes to the off-flavor of the chicken meats. This compound was also detected in the samples but with lower amount than that detected in the boiled samples. In addition, Dodecanoic, ethyl ester was observed with the highest %area in one of the investigated raw samples (Raw_D). This compound contributes to the sweet odor group.

The PCA score plot clearly separated either the boiled or the raw samples into different groups according to their origins. The biplots further revealed a

relationship between each clustered group and the related odor active compounds. This correlates to the markers compound within each group such as hexanal and nonanal that can be used as the potential markers for differentiation of the raw samples obtained from different sources.

Finally, the proposed analytical method and the obtained scientific data of the tentatively identified volatiles in the studied chicken meat samples are expected to be useful in the area of food quality control and authentication.

In the future work, the developed methods, especially for the multiple HS-SPME-GC-MS, may be applied for improved analysis of any other samples with low signals of volatile compounds.

APPENDICES

Appendix A Data from precision study of chicken meat sample

Table 8 Intraday and inter day precision of peak area of selected volatile compounds performed in boiled chicken meat samples using triplicate batches on each day for three consecutive day.

RT	Selected volatile	Day 1 (Peak area)						Day 2 (Peak area)						Day 3 (Peak area)					
(min)	Compound	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD
1.720	Toluene	-	-	2749827	-	-	-	-	3049827	-	-	-	-	-	-	-	-	-	-
2.660	Hexanal	1948475	1535629	1934687	1806263.667	234477.87	12.98	1770414	1876549	1576422	1741128.33	152191.63	8.74	1530373	1602057	1618222	1583550.67	46757.08	2.95
6.191	Heptanal	447145	523846	376543	449178	73672.54	16.40	430352	451710	447145	443069	11247.28	2.54	456748	460987	456321	458018.667	2579.50	0.56
7.778	1- Heptanol	-	-	-	-	-	-	795725	-	-	-	-	-	-	-	-	-	-	-
8.591	2,3-Octanedione	-	-	52836	-	-	-	-	-	-	-	-	-	-	54768	-	-	-	-
9.219	1-Octen-3-ol	118988	106544	108293	111275	6736.66	6.05	120318	110627	102898	111281	8728.40	7.84	101898	117865	102560	107441	9033.51	8.41
9.454	2,5-Octanedione	-	-	-	-	-	-	2442202	-	2256095	-	-	-	2442202	-	-	-	-	-
10.116	octanal	1126484	1105643	1003724	1078617	65691.00	6.09	1070676	1223452	1300984	1198370.67	117184.68	9.78	1226484	1256430	1256901	1246605	17426.89	1.40

RT	Selected volatile	Day 1 (Peak area)						Day 2 (Peak area)						Day 3 (Peak area)					
(min)	Compound	1	2	3	(min)	Compound	1	2	3	(min)	Compound	1	2	3	(min)	Compound	1	2	3
11.334	2-Nonen-1-ol	-	-	-	-	-	-	258883	-	-	-	-	-	-	-	-	-	-	-
12.246	2-Octenal	-	-	-	-	-	-	3147367	-	-	-	-	-	-	-	-	-	-	-
12.610	2-Octen-1-ol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12.722	1-Octanol	-	-	-	-	-	-	3147367	-	-	-	-	-	-	-	-	-	-	-
13.187	Nonanal	944316	1303261	947513	1065030	206320.29	19.37	1373714	1030189	1152945	1185616	174077.29	14.68	1144316	1289439	1294431	1242728.67	85264.41	6.86
13.971	Decenol	-	-	-	-	-	-	175159	-	-	-	-	-	-	-	-	-	-	-
17.001	Decanal	-	-	-	-	-	-	1064915	-	-	-	-	-	-	-	-	-	-	-
23.623	tetradecane	7042295	6007372	5295471	6115046	878375.65	14.36	6502843	6781788	6292453	6525694.67	245466.57	3.76	6246278	6226591	6866318	6446395.67	363796.60	5.64

Table 9 Intraday and inter day precision of peak area ratio of selected volatile compounds to IS performed in boiled chicken meat samples using triplicate batches on each day for three consecutive day.

RT	Selected volatile	Day 1 (Peak area ratio)						Day 2 (Peak area ratio)						Day 3 (Peak area ratio)					
(min)	Compound	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD
1.719	Toluene	-	-	0.52	-	-	-	-	0.45	-	-	-	-	-	-	-	-	-	-
2.660	Hexanal	0.28	0.26	0.37	0.30	0.06	19.46	0.27	0.28	0.25	0.27	0.01	5.26	0.25	0.26	0.24	0.25	0.01	4.41
4.436	3-Methyl hexanal	-	-	-	-	-	-	-	-	1.36	-	-	-	-	-	-	-	-	-
6.191	Heptanal	0.06	0.09	0.07	0.07	0.01	16.37	0.07	0.07	0.07	0.07	0.00	3.98	0.07	0.07	0.07	0.07	0.00	5.81
7.778	1- Heptanol	-	-	-	-	-	-	0.12	-	-	-	-	-	-	-	-	-	-	-
8.591	2,3-Octanedione	-	-	0.01	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-
9.219	1-Octen-3-ol	0.02	0.02	0.02	0.02	0.00	10.12	0.02	0.02	0.02	0.02	0.00	7.35	0.02	0.02	0.01	0.02	0.00	12.13
9.454	2,5-Octanedione	-	-	-	-	-	-	0.38	-	0.36	-	-	-	0.39	-	-	-	-	-
10.116	octanal	0.16	0.18	0.19	0.18	0.02	8.85	0.16	0.18	0.21	0.18	0.02	11.57	0.20	0.20	0.18	0.19	0.01	4.97
11.334	2-Nonen-1-ol	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-
12.246	2-Octenal	-	-	-	-	-	-	0.48	-	-	-	-	-	-	-	-	-	-	-

RT	Selected volatile	Day 1 (Peak area ratio)						Day 2 (Peak area ratio)						Day 3 (Peak area ratio)					
(min)	Compound	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD
12.610	2-Octen-1-ol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12.722	1-Octanol	-	-	-	-	-	-	0.48	-	-	-	-	-	-	-	-	-	-	-
13.187	Nonanal	0.13	0.22	0.18	0.18	0.04	23.48	0.21	0.15	0.18	0.18	0.03	16.30	0.18	0.21	0.19	0.19	0.01	6.50
13.971	Decenol	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-
17.001	Decanal	-	-	-	-	-	-	0.16	-	-	-	-	-	-	-	-	-	-	-
23.623	tetradecane	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00

Table 10 Intraday and inter day precision of peak area of selected volatile compounds performed in raw chicken meat samples using triplicate batches on each day for three consecutive day

RT	Selected volatile	Day 1 (Peak area)						Day 2 (Peak area)						Day 3 (Peak area)					
(min)	Compound	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD
0.768	Hexanal	2292889	1562306	1829389	1894861.33	369665.86	19.51	1225478	1209869	2053257	1496201.33	482487.48	32.25	1431104	1506170	1939623	1625632.33	274501.97	16.89
7.118	2- Heptanal	-	-	-	-	-	-	-	-	559876	-	-	-	-	-	539858	-	-	-
7.773	1- Heptanol	-	-	-	-	-	-	-	-	657894	-	-	-	-	-	645319	-	-	-
8.139	1-Octen_3-ol	380182	456329	513467	449992.67	66868.04	14.86	534809	353789	516789	468462.33	99717.90	21.29	546790	622734	777174	648899.33	117399.60	18.09
10.242	1-Hexanol-2-ethyl	189330	213570	263477	222125.67	37806.67	17.02	356890	217823	376895	317202.67	86644.61	27.32	336778	349545	270204	318842.33	42602.99	13.36
11.347	2-Octenal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	601293	-	-	-
11.967	1-Octanol	278450	326549	489461	364820.00	110588.94	30.31	313682	324570	326748	321666.67	7000.15	2.18	356789	370104	351996	359629.67	9382.27	2.61
13.186	Nonanal	387644	416780	423789	409404.33	19168.09	4.68	406890	574062	527890	502947.33	86332.04	17.17	678956	657390	686875	674407.00	15259.80	2.26
17.002	Decanal	208845	193778	189065	197229.33	10331.79	5.24	180670	186765	150674	172703.00	19319.55	11.19	196532	298755	230609	241965.33	52049.11	21.51
18.99	2-Decenal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	351826	-	-	-
19.39	Decanol	8083428	8370187	7483423	7979012.67	452509.14	5.67	9209148	8110114	9154809	8824690.33	619437.39	7.02	9856420	9367901	6545632	8589984.33	1787231.11	20.81

RT	Selected volatile	Day 1 (Peak area)						Day 2 (Peak area)						Day 3 (Peak area)					
(min)	Compound	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD
22.453	2-Undecenal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	206715	-	-	-
23.514	tetradecane	8965318	9175363	11008384	9716355.00	1123847.82	11.57	8862230	9287999	8729204	8959811.00	291898.10	3.26	9027210	9352167	12567432	10315603.00	1956897.97	18.97
23.91	Dodecanal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	98896	-	-	-
29.543	Dodecanoic, ethyl ester	990345	950261	903467	948024.33	43482.17	4.59	960123	946754	558976	821951.00	227841.11	27.72	501843	609875	654839	588852.33	78634.65	13.35
35.501	hexadecanal	-	-	187129	-	-	-	-	-	-	-	-	-	256780	300865	356784	-	-	-
37.105	1-hexadecanol	2782015	3175909	3782015	3246646.33	503738.85	15.52	3555518	4560983	3205531	3774010.67	703645.38	18.64	2897065	3500764	4854670	3750833.00	1002474.54	26.73
39.894	Pentadecanoic, ethyl ester	194673	215670	161751	190698.00	27178.39	14.25	189065	234617	200959	208213.67	23626.65	11.35	199875	210986	237865	216242.00	19532.77	9.03
40.617	Isopropyl palmitate	-	-	96092	-	-	-	-	-	-	-	-	-	-	902046	923457	-	-	-
41.938	Octadecanol	-	-	18782179	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 11 Intraday and inter day precision of peak area ratio of selected volatile compounds to IS performed in raw chicken meat samples using triplicate batches on each day for three consecutive day

RT	Selected volatile	Day 1 (Peak area ratio)						Day 2 (Peak area ratio)						Day 3 (Peak area ratio)					
(min)	Compound	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD
0.768	Hexanal	0.26	0.17	0.17	0.20	0.05	25.62	0.14	0.13	0.24	0.17	0.06	34.79	0.16	0.16	0.15	0.16	0.00	2.15
7.118	2- Heptanal	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-	0.04	-	-	-
7.773	1- Heptanol	-	-	-	-	-	-	-	-	0.08	-	-	-	-	-	0.05	-	-	-
8.139	1-Octen_3-ol	0.04	0.05	0.05	0.05	0.00	7.95	0.06	0.04	0.06	0.05	0.01	23.85	0.06	0.07	0.06	0.06	0.00	5.03
10.242	1-Hexanol-2-ethyl	0.02	0.02	0.02	0.02	0.00	6.47	0.04	0.04	0.04	0.04	0.00	3.86	0.04	0.04	0.02	0.03	0.01	28.53
11.347	2-Octenal	-							-	-	-	-	-	-	-	0.05	-	-	-
11.967	1-Octanol	0.03	0.04	0.04	0.04	0.01	18.41	0.04	0.03	0.04	0.04	0.00	3.69	0.04	0.04	0.03	0.04	0.01	18.66
13.186	Nonanal	0.04	0.05	0.04	0.04	0.00	8.35	0.05	0.06	0.06	0.06	0.01	15.73	0.08	0.07	0.05	0.07	0.01	16.09
17.002	Decanal	0.02	0.02	0.02	0.02	0.00	15.11	0.02	0.02	0.02	0.02	0.00	8.99	0.02	0.03	0.02	0.02	0.01	29.44
18.990	2-Decenal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-
19.390	Decanol	0.90	0.91	0.68	0.83	0.13	15.79	1.04	0.87	1.05	0.99	0.10	10.00	1.09	1.00	0.52	0.87	0.31	35.23

RT	Selected volatile	Day 1 (Peak area ratio)						Day 2 (Peak area ratio)						Day 3 (Peak area ratio)					
(min)	Compound	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD	1	2	3	Avg	SD	%RSD
22.453	2-Undecenal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	-
23.514	tetradecane	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00
23.910	Dodecanal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-
29.543	Dodecanoic, ethyl ester	0.11	0.10	0.08	0.10	0.01	15.00	0.11	0.10	0.06	0.09	0.02	26.19	0.06	0.07	0.05	0.06	0.01	11.78
35.501	hexadecanal	-	-	0.02	-	-	-	-	-	-	-	-	-	0.03	0.03	0.03	-	-	-
37.105	1-hexadecanol	0.31	0.35	0.34	0.33	0.02	5.99	0.40	0.49	0.37	0.42	0.06	15.24	0.32	0.37	0.39	0.36	0.03	9.65
39.894	Pentadecanoic, ethyl ester	0.02	0.02	0.01	0.02	0.00	23.32	0.02	0.03	0.02	0.02	0.00	8.49	0.02	0.02	0.02	0.02	0.00	9.37
40.617	Isopropyl palmitate	-	-	0.01	-	-	-	-	-	-	-	-	-	-	0.10	0.07	-	-	-
41.938	Octadecanol	-	-	1.71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix B Identified volatile compounds in chicken meat sample

Table 12 Tentative volatile compounds in boiled chicken meat samples

Tentative volatile	sample_A			sample_B			sample_C			sample_D			sample_E		
Compounds	A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3	E1	E2	E3
Toluene	3.76	6.51	6.70	1.06	0.78	0.92	0.82	1.20	1.05	2.95	2.41	3.04	6.68	6.61	6.81
Hexanal	58.00	86.94	86.58	66.56	67.20	65.05	78.13	77.11	76.87	29.45	32.24	11.90	50.84	51.09	51.70
3-Methyl hexanal	2.40	0.35	0.36	2.38	2.40	2.71	-	-	-	1.97	1.59	0.49	-	-	-
Heptanal	2.66	0.41	0.45	2.60	2.58	2.87	-	-	-	0.89	0.76	0.45	-	-	-
1- Heptanol	1.51	0.26	0.26	1.32	1.20	1.22	1.72	1.71	1.81	3.97	2.32	1.81	-	-	-
2,3-Octanedione	3.92	0.62	0.66	4.07	4.22	4.39	-	-	-	16.79	18.54	8.11	16.49	15.41	15.40
1-Octen-3-ol	0.82	0.13	0.14	1.47	1.41	1.03	0.30	0.25	0.22	6.15	5.51	3.22	5.96	8.95	7.38
2,5-Octanedione	10.21	1.81	1.85	9.35	8.87	9.83	0.76	0.62	0.54	-	-	-	1.05	1.25	1.30
octanal	4.19	0.88	0.80	5.55	5.23	5.42	11.15	11.99	13.41	15.66	14.25	4.74	4.77	4.60	5.34
2-Nonen-1-ol	3.83	0.63	0.67	0.21	0.22	0.25	3.67	3.29	3.40	-	-	-	4.17	3.29	3.50
2-Octenal	0.27	0.04	0.05	0.27	0.26	0.29	0.60	0.36	0.35	1.05	1.27	0.63	-	-	-
2-Octen-1-ol	0.15	0.03	0.03	0.09	0.13	0.13	1.05	0.87	0.87	0.46	0.38	0.19	-	-	-
1-Octanol	0.33	0.05	0.06	0.07	0.11	0.11	1.33	1.41	0.10	0.39	0.37	0.24	1.84	1.69	0.96

Tentative volatile	sample_A					sample_B					sample_C				
Compounds	A1	A2	A3	B1	B2	A1	A2	A3	B1	B2	A1	A2	A3	B1	B2
Nonanal	2.36	0.36	0.38	1.72	2.01	2.35	0.37	0.30	0.32	7.54	7.76	4.11	8.19	7.13	7.61
Decenol	5.44	0.95	0.99	3.18	3.27	3.32	-	-	-	12.73	12.59	6.54	-	-	-
Decanal	0.15	0.03	0.03	0.11	0.10	0.11	0.11	0.90	1.04	-	-	-	-	-	-

Table 13 Tentative volatile compounds in raw chicken meat samples

Tentative	Sample_A			Sample_B			Sample_C			Sample_D			Sample_E		
volatile compounds	A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3	E1	E2	E3
Hexanal	34.25	35.85	30.35	4.01	2.39	1.74	14.09	17.14	13.12	3.62	3.21	3.09	0.69	0.52	0.63
3-Methyl hexanal	5.18	3.97	4.53	14.28	9.33	9.54	2.14	1.82	2.15	-	-	-	-	-	-
2- Heptanal	-	-	-	-	-	-	-	-	-	-	-	-	1.83	1.64	1.98
1- Heptanol	-	-	-	-	-	-	-	-	-	-	-	-	1.61	1.68	1.84
1-Octen_3-ol	1.78	2.26	2.26	2.39	2.77	4.13	2.34	2.55	3.02	9.90	8.78	9.24	3.36	2.50	2.89
1-Hexanol-2-ethyl	-	-	-	-	-	-	1.16	1.20	1.55	3.83	3.48	3.78	19.04	25.97	19.82
2-Octenal	-	-	-	-	-	-	-	-	-	0.51	0.65	0.63	-	-	-
1-Octanol	1.32	2.48	1.13	1.94	1.69	1.21	1.71	1.83	2.88	7.04	6.67	6.87	1.16	1.02	0.89
Nonanal	-	-	-	77.39	83.83	83.39	2.38	2.33	2.49	4.02	3.89	4.45	0.54	0.63	0.69
Decanal	-	-	-	-	-	-	1.28	1.08	1.11	7.76	8.13	8.72	1.51	1.51	1.67
2-Decenal	34.08	32.91	36.27	-	-	-	0.83	0.88	1.10	-	-	-	-	-	-
Decanol	6.65	6.80	8.24	-	-	-	49.68	46.86	44.04	19.65	20.64	20.56	33.93	31.08	33.17
2-Undecenal	-	-	-	-	-	-	-	-	-	4.07	4.37	4.00	-	-	-

Tentative	Sample_A			Sample_B		Sample_C				Sample_D			Sample_E		
volatile compounds	A1	A2	A3	B1	B2	A1	A2	A3	B1	B2	A1	A2	A3	B1	B2
Dodecanal	-	-	-	-	-	-	-	-	-	-	-	-	31.02	28.41	31.10
Dodecanoic, ethyl ester	13.14	12.81	13.23	-	-	-	6.09	5.32	5.32	21.41	21.73	20.17	0.52	0.39	0.42
hexadecanal	-	-	-	-	-	-	-	-	-	-	-	-	0.48	0.48	0.39
1-hexadecanol	3.61	2.92	3.98	-	-	-	17.10	17.78	22.26	14.09	14.08	14.54	3.89	3.64	3.96
Pentadecanoic, ethyl ester	-	-	-	-	-	-	1.20	1.21	0.95	4.11	4.38	3.95	0.44	0.54	0.55

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