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AUTHENTICATION OF CAPSICUM USING CHROMATOGRAPHIC TECHNIQUES AND CHEMOMETRIC METHODS

Miss Worraluck Meemak

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
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By	Miss Worraluck Meemak
Field of Study	Chemistry
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วรลักษณ์ มีมาก : การยืนยันเอกลักษณ์ของพริกโดยเทคนิคโครมาโทกราฟีและวิธีทางเคโมเมตริก.(Authentication of capsicum using chromatographic techniques and chemometric methods) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร.ณัฐชนัญ ลีพิพัฒน์ไพบูลย์, 114 หน้า.

การจำแนกเอกลักษณ์ของพริกจากแหล่งที่มาและสายพันธุ์ที่แตกต่างกันโดยใช้ข้อมูลทางโครมาโทกราฟีและสเปกโตเมตรีในการตรวจวัดกลุ่มสารให้ความเผ็ดกลุ่มสารระเหยและสารประกอบฟีนอลลิกร่วมกับนำเทคนิคเคโมเมตริกได้แก่เทคนิคการวิเคราะห์องค์ประกอบหลักการวิเคราะห์กลุ่มและการวิเคราะห์จำแนกกลุ่มในการวิเคราะห์จัดกลุ่มข้อมูลสร้างรูปแบบเฉพาะของข้อมูลเพื่อช่วยระบุเอกลักษณ์แหล่งที่มาและสายพันธุ์ตัวอย่างพริก 3 กลุ่ม คือพริกขี้หนูเม็ดใหญ่พริกขี้หนูเม็ดเล็กและพริกชี้ฟ้าตามสายพันธุ์และแหล่งที่มาผลจากการศึกษาพบว่าพริกขี้หนูเม็ดเล็กมีปริมาณสารแคปไซซินและไดไฮโดรแคปไซซินมากที่สุด (2.51- 5.31 และ 0.74 -1.50 กรัมต่อกิโลกรัมน้ำหนักแห้ง) พริกขี้หนูเม็ดใหญ่ (1.35 - 3.10 และ 0.52 - 1.06 กรัมต่อกิโลกรัมน้ำหนักแห้ง) และพริกชี้ฟ้าน้อยที่สุด (0.11-0.26 และ 0.01 -0.04 กรัมต่อกิโลกรัมน้ำหนักแห้ง) การวิเคราะห์กลุ่มสารระเหยพบว่าพริกแต่ละพันธุ์มีชนิดของสารระเหยแตกต่างกันและในพริกสายพันธุ์เดียวกันในแต่ละจังหวัดจะมีกลุ่มสารระเหยเช่นเดียวกันแต่มีปริมาณแตกต่างกันการวิเคราะห์ปริมาณสารประกอบฟีนอลลิกพบว่ากลุ่มพริกขี้หนูเม็ดใหญ่มีปริมาณสารฟีนอลลิกมากที่สุด (2.25 – 2.75 มิลลิกรัมกรดแกลลิคต่อ100 กรัมน้ำหนักสด) พริกขี้หนูเม็ดเล็ก (2.00 – 2.27 มิลลิกรัมกรดแกลลิคต่อ100 กรัมน้ำหนักสด) และพริกชี้ฟ้ามีน้อยที่สุด (1.10 – 1.52 มิลลิกรัมกรดแกลลิคต่อ100 กรัมน้ำหนักสด) ในการจำแนกเอกลักษณ์ของพริกตามสายพันธุ์และแหล่งที่มาด้วยวิธีการวิเคราะห์กลุ่มสามารถแบ่งกลุ่มตามสายพันธุ์ที่ระยะห่าง 0.4 และตามแหล่งที่มาที่ระยะห่างช่วง 0.2-0.3 การวิเคราะห์ด้วยองค์ประกอบหลักสามารถจัดกลุ่มพริกตามสายพันธุ์และตามแหล่งที่มาได้อย่างชัดเจนโดยในแต่ละสายพันธุ์และแหล่งที่มาจะมีสารประกอบอินทรีย์ที่เป็นเอกลักษณ์เฉพาะตัวและจากการทำนายกลุ่มตัวอย่างตามสายพันธุ์และแหล่งที่มาด้วยวิธีการจำแนกกลุ่มแบบการตรวจสอบไขว้พบว่ามีความถูกต้อง 100 เปอร์เซ็นต์

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The discrimination of the geographical origins and varieties of chili peppers by using chromatography and spectroscopy to separate and analyze capsaicinoids, volatile compounds, and TPC components of chili peppers were studied. The combination of multivariate techniques such as principal component analysis (PCA), cluster analysis (CA), and linear discriminant analysis (LDA) were applied to the analytical data to monitor the chili pepper profiles and construct a mathematical model to aid in the identification of the origins and varieties of the chili pepper samples. The chili samples were classified into 3 major groups include the hot chili peppers (*Capsicum annum Linn*.), the bird chili (*Capsicum frutescens Linn*.), and the chili spur pepper (*Capsicum annuum Linn. Varacuminatum Fingerh*.), based on their varieties and geographical origins. The result show that the content of capsaicin and dihydrocapsaicin of bird chili peppers has the greatest content (2.51-5.31 g kg^{-1} and 0.74 -1.50 g kg^{-1} dry weight respectively), followed in order of decreasing concentration by hot chili peppers (1.35 - 3.10 g kg^{-1} and 0.52 - 1.06 g kg^{-1} dry weight, respectively), chili spurs pepper (0.11- 0.26 g kg^{-1} and 0.01 - 0.04 g kg^{-1} dry weight, respectively). The VOCs analysis showed that the volatile compounds of the three varieties of chili pepper samples have different qualities and quantities according to their varieties. In chili peppers from various origins have similar VOCs but different quantities. The total phenolic content analysis showed that the total phenolic content of the hot chili peppers (2.25 – 2.75 mg gallic acid/100 g FW) was higher than those of the others, whereas the bird chili peppers and the chili spur peppers were 2.00 – 2.27 mg gallic acid/100 g FW and 1.10 – 1.52 mg gallic acid/100 g FW, respectively. The discrimination of geographical origins and varieties of chili peppers samples indicated that CA can be clearly differentiated the varieties of chili peppers at distance 0.4 and the origins of chili peppers at distance 0.2-0.3. In PCA showed that all chili peppers sample clustered according to their variety and origins. Each of the varieties and origins of chili peppers have organic composition markers that are unique to each species. Original group of samples can be predicted with 100% correction by cross-validating LDA.

Department : Chemistry Student's Signature .....

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LIST OF ABBREVIATIONS

%	Percentage
TPC	Total phenolic content
PCA	Principal component analysis
CA	Cluster analysis
LDA	Linear discriminant analysis
UV-Vis	Ultra violet -Visible Spectrophotometer
g/kg	Gram per kilograms
FW	Fresh weight
PCs	Principal Components
RP-HPLC	Reverse phase-High performance Liquid Chromatography
NLM	Nonlinear mapping technique
SPM	Spectral mapping
v/v	Volume by volume
AOX	Antioxidant activity
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
mg/g	Milligram per gram
C18	Octadecyl
°C	Celsius
HS-SPME	Headspace-solid phase microextraction
As	Arsenic
Ca.	Calcium
Cd	Cadmium

Co	Cobalt
Cr	Chromium
Cu	Copper
Fe	Iron
Mg	Magnesium
Mn,	Manganese
Ni	Nickel
Sr	Strontium
Zn	Zinc
He	Helium
Ar	Argon
N_2	Nitrogen
CO_2	Carbon dioxide
H_2	Hydrogen
m	Meter
WHO	World Health Organization
EC	European Commission
NIR	Near infrared
MIR	Mid infrared
cm	Centimeter
mm	Millimeter
w/w	Weight by weight
MHz.	Megahertz
SNIF	Site-specific nuclear isotopic fractionation

IRMS	Isotopic ratio mass spectroscopy
GMOs	Genetically modified organisms
mL/min	Milliliter per minute
psi	Pound per square inch
WCOT	Wall-coated open tubular
PLOT	Porous layer open tubular
EI	Electron ionization
CI	Chemical ionization
RF	Radio frequency
DC	Direct current
AC	Alternating current
TOF	Time-of-flight
EMT	Electron multipliers
A	Absorbance
c	Concentration
l	Pathlength.
λ	Wavelength
d	Distance
ED	Euclidean distance
µL	Microliter
mL	Milliliter
Na_2CO_3	Anhydrous sodium carbonate
mg/L	Milligram per liter
µm	Micrometer

eV	Electron volt
GAE/100 g FW	Milligrams of gallic acid equivalent / 100 g FW
ANOVA	Analysis of variance
R^2	Correlative coefficient
SD	Standard deviation
VOCs	Volatile compounds
HS-GC-MS	Headspace- gas chromatography-mass spectrometry
μg/L	Microgram per liter
I.D.	Internal diameter

CHAPTER I

INTRODUCTION

1.1 Problem definitions

Today, consumers are paying more attention to food safety caused by contamination and chemical residues. This causes consumers to require information and be confident in the origin of the ingredients in foods. Thus manufacturers must give information to show product authentication, prove that no contaminant has been added or any counterfeiting has taken place with relatively low quality raw materials, or the original source of the product has been tampered with. Hence the technique for analyzing the qualitative and quantitative of chemicals is an important source of information. This includes a combination of chemometric techniques to evaluate the information. This will help to confirm the origin of products and clarify verifications clearly.

In Thailand, chili peppers (*Capsicum annuum L.*) are commonly grown in almost every region of the country and is also regarded as a high-value commodity in Thailand. Chili peppers (*Capsicum annuum L.*) are a popular and important ingredient of Thai and worldwide cooking because of their unique aroma and spiciness *(1, 2)*. Chili peppers are commonly used for cooking in households and as a feedstock in industry because they are an excellent source of vitamin A, C, E and contain a range of essential minerals *(3)*. Furthermore, chili peppers used extensively in disease prevention and health promotion. The unique aroma of the chili pepper arises from its volatile components, and its characteristic pungency is mainly the result of its capsaicinoid content, of which capsaicin and dihydrocapsaicin are the major components *(4)*. The total phenolic content (TPC) represents a group of potent antioxidants that are present in chili pepper *(5)*.

Due to the large variety and regions of origin, the traceability of chili peppers has been a challenge. Food traceability is important for the protection of consumer rights and to prevent fraudulent and deceptive practices. For food traceability and authenticity issues, it is necessary to characterize chili products to confirm their

original source. This is important for ensuring food safety, and for protecting consumers from food fraud. Thus, the objective of this study was to discriminate the variety and geographical origin of chili peppers by using chromatography andspectroscopic methods to separate and analyze capsaicinoids, volatile compounds, and TPC components in chili peppers. Multivariate techniques such as PCA, CA, and LDA were applied to the analytical data to monitor the profile of the chili peppers and also construct a mathematical model to aid in the identification of the varieties and origins of the chili pepper samples.

1.2 Literature review

To study the chemical composition of chili peppers, 5 major characteristics were chemically studied: pungency, color pigment, antioxidants, volatile compounds and other chemical compositions.

1.2.1 The literature review for the analysis of the pungency

The pungency is caused by capsaicinoids and the most abundant of these compositions are capsaicin and dihydrocapsaicin. There have been several capsaicinoid researches completed in both qualitative and quantitative analysis in order to identify the different geographical locations and species of chili pepper. In 2006, Choi et al. *(6)* used HPLC and LC-MS methods for the determination of capsaicinoids in 11 fresh Korean red peppers and in 12 commercial pepper-containing foods. In 2007, Topuz et al. *(7)* determined the carotenoids, capsaicinoid and ascorbic acid contents of ripe fruit of five *Capsicum annuum* cultivars. The result showed that the cultivars of 730 F1 and 1245 F1 had higher carotenoids, capsaicin, vitamin A and vitamin C content, without any significant difference among each of them. In 2010, Ornelas-Paz et al. *(8)* studied the influence of boiling (96 ºC) and grilling (210 ºC) the capsaicinoids and total phenols in the genotypes of peppers (Poblano, Bell, Chilaca, Caribe, Jalapeno, Serrano, Habanero and Manzano) most commonly found in Mexico. It was found that boiling moderately reduced the content of capsaicinoids in pepper but grilling largely increased such content, while boiling and grilling increased the

total phenolic content in pungent peppers. In non-pungent peppers the content of capsaicinoids and total phenolic content was reduced by boiling and grilling.

1.2.2 The literature review for the analysis of the color pigment

The color pigment of chili peppers is generated by carotinoid pigments such as capsanthin, capsorubin, zeaxanthin, luitein, betacarotene, and neoxanthin. Many previous studies on the qualitative and quantitative analysis of color pigments and the difference in color pigments in chili peppers have been conducted. In 1998, Rodrigues et al. *(9)* studied the influence of environmental conditions (time of storage and concentration of seeds added to the powder) on the stability of the color pigments of paprika (*Capsicum annuum*) powder. The color pigments were analyzed using RP-HPLC. The effect of these environmental conditions was performed by multivariate methods such as spectral mapping (SPM) and the two-dimensional nonlinear mapping technique (NLM). It was found that the length of storage exerts the highest influence of decomposition rate, whilethe selectivity of decomposition was dependent on both the concentration of the added seeds and the length of storage. In 2000, Cserhati et al. *(10)* studied the best separation procedure for the color pigments of chili powder in order to analyze the pigment composition of chili powders by the TLC method. The results indicated that the best separation of color pigments was obtained on the impregnated diatomaceous earth layer using acetone-water 17:13 v/v elutent. In this paper, PCA was used for the classification of the chili pepper according to pigment composition and showed that these differences could be used for classification between similar and dissimilar chili powders. In 2001, Kosa et al. *(11)* studied the determination of color pigments in chili (*Capsicum frutescenes*) powders from five different origins using RP-HPLC and used PCA for the discrimination of the origin of chili. The result indicated that the color pigment of chili powders from Malaysia, China, India and Pakistan showed similarities while the chili powder of Thailand was different.

1.2.3 The literature review for the analysis of antioxidants

Chili pepper has a good source of antioxidant content, mainly because they have an excellent source of phenolic compounds, flavonoid and ascorbic acid. The previous researches studies antioxidants in chili peppers both quantitatively and qualitatively and the different antioxidant contents during the growth stages in different parts of chili peppers.

In 2007, Deepa et al. *(12)* studied the variation during the three maturity stages (green, intermediate and red or yellow) in 10 genotypes of sweet pepper (*Capsicum annuum L.*) Total phenolics, antioxidant activity (AOX), carotenoid, capsaicin and ascorbic acid were analyzed in order to study the changes in the content of antioxidants and the total AOX as influenced by the different maturity stages and genotypes. The result indicated that sweet red peppersat the red stage were rich sources of AOX, total phenolic, carotenoids, ascorbic acid and capsaicin. In 2011, Ghasemnezhad et al.*(13)* studied theinfluence of harvest time based on the maturity stage of ascorbic acid, flovanoids, total phenolic content and antioxidant activity of five color bell pepper (*Capsicum annum*) genotypes. It was found that the content of ascorbic acid, flavonoids, total phenolic content and antioxidant activity differed in color bell pepper cultivar. Therefore, the harvest time and ripening state are one of the factors that affect the amount of ascorbic acid, flavonoids, the total phenolic content and the antioxidant activity.

1.2.4 The literature review for the analysis of volatile compounds

The main volatile compounds in chili peppers consist of alcohols, aldehyde, ketone, ester, terpene, pyrazines, and lactones. Previous research studies the qualitative and quantitative analysis of volatile compounds in different varieties of chili peppers and the difference in maturation. In 2006, Sousa et al. *(14)* analyzed volatile compounds in red, yellow and purple varieties of *Capsicum chinense sp*. peppers by HS-SPME/GC-MS. Multivariate chemometric techniques such as experimental design were applied in order to optimize the SPME extraction procedure. From the data obtained by the analysis of volatile compounds, 34

compounds were classified by PCA in order to group different varieties of C. chinense sp. peppers. In 2007, Pino et al.*(15)* determined the total capsaicinoids, color and volatile compounds of 10 Habanero chili pepper (*Capsicum chinense Jack.*) cultivars (four red, five orange and one brown) grown in Yucatan. The result indicated that the content of total capsaicinoids of the Habanero chili pepper samples varied between 41.8 and 65.9 mg/g in dry fruit. The composition of volatile compounds and color differed clearly in the different cultivars of the chili pepper samples. In 2011, Pino et al. *(16)* isolated and identified the volatile compounds of three cultivars of Cachucha mature peppers (*Capsicum chinense Jacq.*) grown in Cuba by GC and GC-MS. The major volatile compounds of Cachucha peppers included hexyl isopentanoate, hexyl pentanoate, hexyl 2-methylbutanoate, 3,3-dimethylcyclohexanol, γ-himachalene and germacrene. The result showed that the volatile compounds of the three cultivated varieties of Cachucha peppers differed clearly in different cultivars.

1.2.5 The literaturereview for the analysis of other chemical compositions

The other chemical compositions in chili peppers include starch, pectin and minerals. Several researches have been carried out to study the chemical compositions of chili peppers and quantitative analysis of other chemical compositions. In 1996, Hernandez et al. *(17)* determined the water, neutral-detergent fibre, D-glucose, D-fructose, sucrose, vitamin C, malic acid, starch, pectin and major pigment contents of *Capsicum annuum L. var. Longum* grown in Galicia. It was found that water and insoluble fibre was the most abundant component of these peppers (NDF content 2.2 g/ 100 g of fresh fruit). Among the D-glucose was 0.85 g/ 100 g, while sucrose was not detected. Malic acid was 208 mg/ l00 g and the vitamin C content was rather low (24 mg/ l00 g). Among the pigments, all-trans-lutein (1.6 mg/ 100 g) was the predominant carotenoid, and chlorophyll A was 7.9 mg/ l00 g. In 2006, Ekholm.*(18)* analyzed the contents of calcium, potassium, magnesium, phosphorous, aluminum, cobalt, copper, iron, manganese, nickel, selenium, zinc, cadmium and lead in samples of 18 cereal products, 10 root vegetables, 16 vegetables and 16 fruits. The data which were obtained from analysis were compared with those obtained 30 years earlier in food samples from Finland. It was found that in sweet pepper (*Capsicum annuum*), the element density in vegetable foods had decreased over the past three decades.

The application of chemometric technique for the discrimination of origin and quality control of samples has recently become a very active research tool. In 2010, Chun et al. *(19)* classified 19 *Schizonepeta tenuifolia Briq*. samples according to different regions by PCA. These samples were collected from various regions in Korea and China, and the main composition of several volatile compounds had been analyzed by HS-SPME- GC/MS. The result showed that PCA can visualize the group of origins of *Schizonepeta tenuifolia Briq.*samples into 2 groups, which can be analyzed to obtain the profiling data of volatile compounds. In 2011, Youssel, et al. *(20)* applied HS-SPME to determine the volatile compounds of virgin olive oils from the Oueslati variety and cultivated in 7 different geographical areas at the mature stage. In addition, PCA was used for classification of these samples from different origins by applying the 27 volatile compounds obtained from HS-SPME-GC/MS. The results showed three groups. The first group included Oueslati oil from Sfax, the second group composed Oueslati oil from Jebel Rihan, the third group included Oueslati oil from Haffouz, Ala, Ain Jloula Khitel Oued and Menzel Raiss areas. In 2012, Karadas et al. *(21)* employed the chemometric techniques which included a principal component analysis (PCA), a linear discriminant analysis (LDA) and a cluster analysis (CA) to classify herbs (mint, thyme and rosemary) and spices (black pepper, chili pepper, cinnamon, cumin, sweet red pepper and turmeric) using trace metallic analyte concentrations (As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Sr and Zn) which were analyzed by atomic spectrometry. The result showed that the herbs and spices were classified into five groups by PCA and CA. These groups are correctly classified as 100 % by using LDA. In 2012, Sarbu et al. *(22)* studied the fingerprint of four subspecies of kiwi and three subspecies of pomelo using HPLC and UV-Vis spectroscopy. The analyzed data were discriminated by PCA and LDA according to fruit species and subspecies. The result showed that Bidan kiwi subspecies differed the most, while Daeheung kiwi and Hayward kiwi presented close similarities. The Tha Knoi and Thong Dee subspecies of pomelo are closely related. In the same year, Wongsa et al. *(23)* studied phenolic compounds, main hydrocinnamic acid, antioxidant activity, and in vitro potential inhibition against α-amylase and α-glucosidase in thirty herbs commonly used in Thailand and combined PCA to provide insights into the relationships of these compounds. The results

indicated that there were differences in these compounds of thirty herbs that belong to the Cucurbitaceae and Solanaceae effectively inhibited both α-amylase and α-glucosidase.

1.3 Purpose of the study

According to concerns in the safety of food and the importance of product authentication, as well as traceability to product origin, this research includes the study in the authentication of Thai local crops, of which the chosen one is the Thai chili pepper. Chili peppers are widely consumed around the world. They have a pungent smell and colour depending on their variety and origin. From the proposed literature reviews, the aspect of species and growth period of Chili peppers were studied, as these topics are very distinct. At the same time, a qualitative and quantitative analysis of chemical components in different varieties of chili peppers and the difference levels of maturation was also carried out. It was found that there are only a few reports on the characterization of various commodities which have described the combination of analytical techniques with chemometric methods, such as the principal component analysis (PCA), the cluster analysis (CA), and the linear discriminant analysis (LDA) to statistically classify the sources of large amounts of quantities in samples. The commodities investigated by these studies are all economically important crops for their countries of origin.

Thus the objective of this study was to discriminate the geographical origin and varieties of chili peppers by using chromatography such as HPLC-DAD and GC-MS and spectroscopic methods, UV-visible spectroscopy to separate and analyze capsaicinoids, volatile compounds, and TPC of chili peppers. Multivariate techniques such as PCA, CA, and LDA were applied to the analytical data to monitor chili pepper profiles and construct a mathematical model to aid in the identification of the origins and varieties of the chili pepper samples.

CHAPTER II

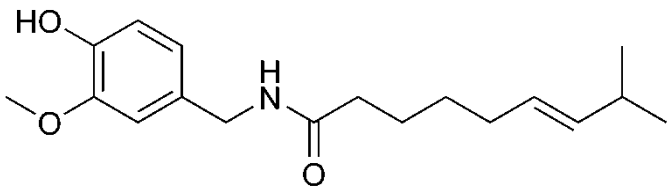
THEORY

2.1 Chili pepper *(24, 25, 26, 27)*

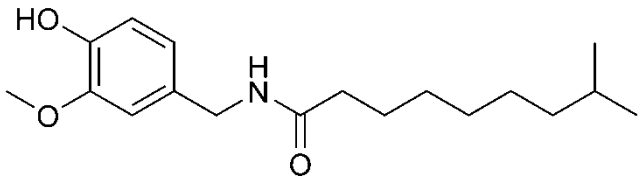
Chili peppers are the fruit of plants from the genus Capsicum, members of the nightshade family, Solanaceae, and are closely related to the tomato, potato, eggplant, and tobacco. It has many common names, such as Anaheims, Bell or Sweet, Jalapenos, Cayennes, chili, Poblanos, and Serranos, Chili peppers originated in the Americas. Chili peppers are one of the most popular and highly regarded food products in all parts of the world due to its large quantities and diverse varieties. Chili peppers spread across the world due to export and are grown everywhere in the tropics as well as in many temperate regions of the globe. It is used as condiments or spice for seasoning, in curry powder or Tabasco sauce due to its unique aroma and pungency. As a medicinal resource, the Chili pepper has been used in pharmaceutical industries, which use them for their anti-bacterial, anti-carcinogenic, analgesic and anti-diabetic properties.

Chili peppers in a fresh state are a rich source of vitamin A, C, E and contain a range of essential minerals. The main chemical compositions of chili peppers include pungent principles, volatile compounds and total phenolic content.The pungency of the chili pepper is produced by the group of alkaloid compounds known as capsaicinoids that are found only in this plant genus, Capsicum. Capsaicin (tran-8-ethyl-N-vanillynonanamide) and dihydrocapsaicin (8-mehtyl-N-vanilly-6-nonenamide) are the major components of capsaicinoids. Nornordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, are considered minor capsaicinoids. The chemical structures of capsaicin, dihydrocapsaicin, nornordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin are shown in Figure 2.1.

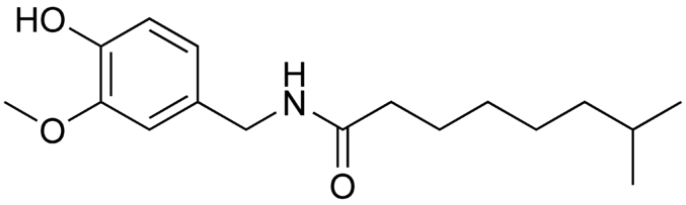
a) Capsaicin



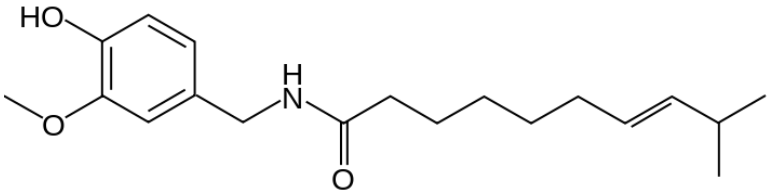
b) Dihydrocapsaicin



c) Nornordihydrocapsaicin



d) Homocapsaicin



e) Homodihydrocapsaicin

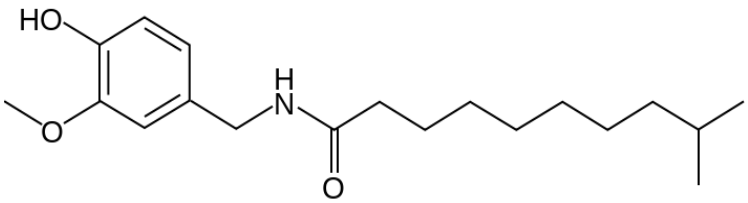


Figure 2.1 Chemical structures of a) capsaicin, b) dihydrocapsaicin, c) nornordihydrocapsaicin, d) homocapsaicin, and e) homodihydrocapsaicin

The capsaicinoids are produced in glands on the placenta of the fruit. While the seeds are not the source of pungency, they occasionally absorb capsaicin because of their proximity to the placenta. No other part of the plant produces capsaicinoids.The internal structure of a chili pepper is shown in Figure 2.2

Figure 2.2 The internal structure of a chili pepper

There are two ways of classifying chili peppers; by their pungency and shape. The genus Capsicum consists of approximately 22 species and five domestic species: *C. annuum L.*, *C. baccatum L.*, *C. chinense Jacq*, *C. frutescens L.*, and *C. pubescens R. &P.C. annuum L.* and *C. frutescens L.*, are economically important chili pepper species in Thailand.

2.1.1 *Capsicum annuum L.*

*Capsicum annuum L.*is the best known domesticated species in the world. It has spread to every part of globe, *C. annuum L.*is a catch crop. The plant has an upright shrub usually less than 1 m tall. *C. annuum L.* has a solitary flower, a solitary fruit, and white or cream; dark or purple colour petals are rarely found. Each species has varied shapes, and different amounts of “heat” and spiciness. The immature fruit is green or yellow, it will then become red and yellow or brown after it matures. There are 31 varieties of *C. annuum L.*, which are named locally: Cayenne pepper, big pepper, Jinda Chili, Red Chili, Pumpkin chili, bird’s eye chili, bird’s eye paprika, Jinda bird’s eye chili, sweet chili and bell pepper , and so on.

(a) **(b)**

Figure 2.3 a) flower and b) fruit of *C. annuum L.*

2.1.2 *Capsicum frutescens L.*

C.frutescenes L. is widely grown in tropical and warm climate zones.*C.frutescenes L.* is a short-lived perennial with plants which average 0.9 to 1.5 m tall. *C. frutescenes L.* has a shrubby growth pattern with a central stem and many twiggy branches. *C. frutescenes L.* is small and cream to greenish-white in color. Its cone or round shape of unripe fruit is also green or yellow, turning red as it matures and yellow or brown later. The *C. frutescenes L.* species are Tabasco pepper, hot cherry pepper, bird pepper, Chor Chili, and so on.

(a) **(b)**

Figure 2.4a) flower and b) fruit of *C. frutescenes L.*

2.2 Authentication *(28, 29, 30, 31, 32)*

Food authenticity is the process of checking for food fraud using analytical methods, such as chemometrics. This can be done by false labeling which is an act of commercial fraud. There is a need to do so to "protect the honest trader" and consumers. Some food adulteration, such as using alum for bleach flour to make turnip bread for the poor, can be dangerous; food allergies for instance may occur from false labeling. "The Codex Alimentarius FAO/World Health Organization (WHO) and the European Commission proposed a list of allergens" suggesting that they be labeled on pre-packaged food products. 12 listed groups of ingredients that show the potential to cause allergic reactions are milk, eggs, fish, crustaceous, peanuts, soybean, walnuts, whey, and other cereals with gluten. Europe established systems for developing and protecting food products for three reasons: "one, to encourage diverse agricultural production; two, to protect product names from misuse and imitation; and three, to help consumers by giving them information concerning the specific character of the products". Unrepentant producers of these food frauds continuously lie or withhold the truth. In turn, sellers believe the producers that the food is healthy and is not adulterated. Information about the food product, such as labeling, stickers, and advertisements, may inform sellers and buyers that the food they are choosing is safe. Labeling claims of food fraud are "rarely supported by analytical data" while compliance monitoring is "solely on paper auditing procedures". It is essential to give correct information to consumers since certain food may be a "reflection of lifestyles, such as vegetarianism or religious practices, such as Jews and Muslims, where pork meat should be absent". D.C.E. Roberts comments that food adulteration is "straightforward theft. Both are frauds on the public and the honest trader… In order to keep their market share, or even stay in business, traders large and small have had to 'swim with the tide'; hence the problems grow".

Although European Commission regulations are concerned about consumer protection, there continues to be loop - holes for producers to get away with claims of food fraud. For instance, "some EC states do not allow sugar to be added to the wine, however, the EC Wine Regulations allows sugar to be added to grapes in a controlled manner under certain circumstances prior to fermentation". Furthermore, the Food

Safety Act 1990 states that consumers may make a case against food sellers and producers if "the food is less than the commercial quality". This means producers and sellers must find sly ways to commit food fraud. This can be seen in pizza television commercials that show how cheesy and delicious their pizza is. Notice the television commercial may not use the word "cheese" to avoid false food labeling since what appears as cheese may actually be "an artificial cheese-flavored vegetable fat product".

2.2.1 Techniques of food authenticity

Regarding the previously mentioned circumstances, food authentication is vital to show food adulteration. Reid, O'Donnell, and Downey explained the techniques of food authentication in 2001, four techniques for doing so are: one, spectroscopy, which can be branched into UV, NIR, MIR, visible, and Raman; two, isotopic analysis; three, chromatography; four, electronic nose.

2.2.1.1 Spectroscopic techniques

2.2.1.1.1 Fluorescent and UV-vis spectroscopy

There has been little research with fluorescent and UV-vis spectroscopy for food authentication. Recent work distinguishes virgin olive oil from other types of olive oil by detecting the "extraneous colourants in cochineal food coloring". UV spectroscopy showed potential for industrial use in its "recent development of a portable UV-spectroscopic testing device for the authentication of Scotch whiskey". Using a portable UV-spectroscopic testing device enables authentication of Scotch whiskey in the field and reduces the screening time to less than one minute.

2.2.1.1.2 IR spectroscopy

Infrared spectroscopy, abbreviated as IR spectroscopy, uses food samples for rapid non-destructive food authentication. Mid-infrared spectroscopy analyzes food samples to show information about molecular bonds and detailed information about the types of molecules in the authentic food. Near-infrared gives relatively complex structural data about the "vibrational behavior" of bonds combining. To do this, NIR requires a spectral range from 14000 to 4000 cm^{-1}. Spectroscopic techniques are easy

to use and require “relatively low financial costs for obtaining and running the equipment”. Chemometric analysis is applied to the following IR studies creating “powerful analytical techniques”, which are used for “classification studies for a wide variety of food products”.

NIR spectroscopy has been used for authenticating fruit purees and juices, maple syrup, milk powder, honey, and fishmeal. The correct classification of grape varieties in different wines was up to levels of 100 percent. As with NIR, there are low error limits for detecting adulteration of various food products, such as olive oil. For instance, there is an 80 percent level of correct classification of differentiating meat from different animals, such as pork, beef, and lamb.

MIR uses wine samplings to differentiate the geographical and varietal origin. The vintage year of red wine can be authenticated 100 percent correctly. Combining chemo metrics and MIR, the detection of adulteration of apple juice with beet syrup and cane syrup is correctly classified by 96.2 percent. MIR and PLS detected an adulteration of honey with sugar solution at levels of 14 percent w/w. Fourier-transform MIR spectroscopy and a type of chemometrics called, potential curves, was applied to classifying apple juice beverages to check the percentage amount of pure apple juice.

2.2.1.1.3 Raman spectroscopy

Raman spectroscopy measures the wavelength and intensity of Raman scatter, which are in elastically scattered light, from molecules. Some Raman scatter is at different wavelengths. The advantages of Raman spectroscopy for analyzing food is the high sensitivity to specific bonds. These bonds are C=C, C≡C, and C≡N. Raman spectroscopy has “low sensitivity to water and high selectivity to inorganic substances”, such as salt. Thus, this type of spectroscopy can be used to apply niches in the food industry. By combining Raman spectroscopy with chemometric analysis, wide adulterations were successfully performed on samplings of olive oil and hazelnut oil. Not only can adulteration be identified, the geographical origin can also be identified and differentiated. This was carried out on various types of honey and edible oils.

2.2.1.1.4 NMR spectroscopy

According to Reid, O'Donnell, and Downey, NMR spectroscopy can be defined as involving "the analysis of the energy absorption by atomic nuclei with non-zero spins in the presence of a magnetic". Since the interactions of an individual atomic nucleus are dependent on its surrounding atoms, NMR spectroscopy reveals thorough data about the molecular structure of a food sample.

There are two types of NMR spectroscopy: high-resolution NMR, and low-resolution NMR. HR-NMR uses frequencies above 100 MHz whereas LR-NMR uses frequencies of 10 to 40 MHz.

2.2.1.2 Site-specific nuclear isotopic fractionation NMR spectroscopy and isotopic ratio mass spectroscopy

The most refined and precise techniques for determining food authenticity are SNIF-NMR and IRMS. The exact proportion and location of certain isotopes within a food sample can be determined by using these two techniques. One of the disadvantages of using these techniques is the relatively high financial costs to operate such equipment. In addition, it is time-consuming to prepare samples before performing an analysis. SNIF-NMR is widely accepted for authenticating wine and has been adopted by the European Union since 1990 as "the official method for controlling chaptalization, which is the addition of sugars prior to fermentation". IRMS analysis combined with SNIF-NMR can detect the adulteration of orange juice and milk samples. Other juices, such as apple juice, have successfully been authenticated using IRMS analysis of two ratios: C12/C13 and H1/H2. By doing so, low-cost sugar syrups to juice samples can be detected. Identification of geographic origin can be identified. For instance, IRMS identified milk from "the Apulia region in southern Italy with correct classification levels of 100 percent".

2.2.1.3 Chromatographic techniques

The separation and identification of almost any type of molecule in a food sample is performed by chromatographic techniques, including liquid and gas chromatography. The difference between the two types of chromatographic techniques is what it can detect. Liquid chromatography, specifically high

performance liquid chromatography, identifies compounds. Such compounds include proteins, amino acids, phenolic compounds, and carbohydrates. Gas chromatography analyzes naturally volatile or semi-volatile molecules. However, both chromatographic techniques have high costs of time and labor.

2.2.1.3.1 High performance liquid chromatography

Over recent years, the development of HPLC applied to food authentication has been low. “HPLC analysis of selected polyphenol compounds combined with PCA and LDA of the concentration of these compounds” enabled 100 percent correct classification of wines from different areas in the Canary Islands. Using principal components analysis and linear discriminant analysis of the HPLC information correctly differentiated Spanish table wines at levels of 83 to 86 percent.

2.2.1.3.2 Gas chromatography

GC was conducted to compare fatty acid composition from olive oil samplings that were detected as adulterated. In addition, there was a combination of chemo metric analysis with GC for fatty acid contents. The triacylglycerol and fatty acid composition of French olive oil samples underwent chemometric analysis to determine their varietal and geographical authenticity. Italian olive oils were distinguished between Italian and Argentinean. According to a study of Sicilian olive oil samples, GC analysis with chemo metrics provides relatively clear separation between cultivars than NMR analysis with chemo metric analysis of the same samples.

2.2.1.4 Electronic nose

According to Reid, O’Donnell, and Downey, electronic nose technology is “based on the detection by an array of semi-selective gas sensors of the volatile compounds present in the headspace of a food sample”. Electronic nose technology can be advantageous as it requires a small amount of sampling and can be analyzed quickly. Adulteration, differentiation, and geographical origin can be detected using electronic nose technology. The geographical origin of olive oil was demonstrated along with the adulteration of sunflower oil or olive-pomace oil. The differentiation and geographic origin of wines and juices were performed by having electronic nose

technology combined with chemometric analysis. There is a 100 percent correct classification of Italian wine based on their geographical and varietal origin. In addition, the geographical origins of Valencia orange juices were determined by the conjunction of these two technologies.

2.3 High Performance Liquid Chromatography (HPLC) *(33, 34)*

High-performance liquid chromatography (HPLC) is a widely used analytical technique for quantitative analysis of organics and biomolecules. HPLC is a form of liquid chromatography used to separate and identify compounds in a solution, of which a sample dissolved in another solution is injected into a column with small particle. The analyte is carried through to a stationary phase by a mobile phase at high pressure and separated in the column by differential partitioning behavior and interaction between the mobile phase and the stationary phase. In general, the main components in an HPLC system include the solvent reservoir, a high-pressure pump, a column, injector system and the detector. The schematic diagram of HPLC is shown in Figure 2.5.

Figure 2.5 Schematic diagram of HPLC

2.3.1 Mobile phase system

The reservoir holds the solvent, which is referred to as the mobile phase because it moves. The mobile phase is chosen because it has the optimal eluting power for the HPLC mode being used, low viscosity, high purity and stability and it is compatible with detection system. The mobile phase must be degassed in order to eliminate the formation of bubbles, prior to use, either by filter under vacuum or degassing online.

2.3.2 Pump

The pump is an essential part of HPLC because it generates pressure for the flow of the mobile phase through the liquid chromatograph at a specific flow rate, expressed in mL/min depending on the type of pump purchased, i.e. standard or micro flow. Pumps are differentiated as piston type pumps (constant flow pumps) and gas pressure (constant pressure pumps). During experimentation, the pump can deliver a constant mobile phase composition (isocratic) or an increasing amount of organic solvent in the mobile phase composition (gradient).

2.3.3 Sample introduction system

The most commonly used type is a loop injector fixed near the pump. It has a range to inject 20 to 100 micro-liters volume. The liquid sample is introduced into a sample loop from an injector with a syringe. In the load position, the sample is injected with a syringe into the injector. In injection mode the sample is injected from the loop into the pump. The injector, or auto sampler, introduces the solvent into a mobile phase stream and carries the sample into the column, which contains specific packing material needed to effect separation.

2.3.4 Column (Stationary Phase)

The column is the most important and sensitive part of HPLC. The column contains the particles that contain the stationary phase which must have a homogeneous stationary phase of a small particle size. The columns are commercially available as micro porous, pellicular and bonded phase types based on the physical and chemical nature of the packed stationary phase in the column. They are able toresist a pressure

of 8000psi. The columns are available in lengths of 20 to 50cm and have an internal diameter of 1 to 4 mm. The particle size range is 5 to 30 micron but most often lower sizes are preferred for better separation. HPLC is divided into two types namely, normal phase chromatography and reverse phase chromatography. In the normal phase chromatography, non-polar to polar stationary phases and non-polar to polar mobile phases separate compounds by their increasing polarity. In the reverse phase chromatography, the stationary phase has been modified to be a non-polar substance (silica gel has been bonded with a long-chain non polar substance e.g. octadecylsilyl), and polar solvents are used as the mobile phase. The order of elution is from polar to non polar compounds. Reversed-phase chromatography has become a highly powerful technique because of its selectivity over a wide range of solutes that can be adjusted by varying the polarity of the mobile phase. In most cases, mobile phases consist of water: methanol or water: acetonitrile.

2.3.5 Detector

The detector helps in detection and identification of analytes in the sample that elute from the column. A large number of detectors are used based on the method or principle. The detectors measure the amount of those molecules so that the chemist can quantitatively analyze the sample components. The detectors are available as UV-Visible, Fluorescent, Electrochemical, Photo diode-array detectors.UV detectors are the most commonly used detector. They measure the ability of a sample to absorb light. This can be accomplished at one or several wavelengths. A variable wavelength UV detector, capable of monitoring from 190 to 600 nm is suitable for the detection of the majority of samples. There are two types of UV detectors; the fixed wavelength detector and the multi-wavelength detector.

Fixed wavelength detectors do not allow the changing of wavelength of the radiation. A low-pressure mercury lamp emits very intense light at 253.7 nm. By filtering out all other emitted wavelengths, utilizing only 254 nm line to provide stable, highly sensitive detectors capable of measuring subnanogram quantities of any components which contains an aromatic ring. The 254 nm was chosen because it emits a very intense line at 254 nm and most UV-absorbing compounds have

absorbance at this wavelength. A diagram of a Fixed Wavelength UV Detector is shown in Figure 2.6

Multi-wavelength detectors use a narrow range of wavelength to detect the solute. Most multi-wavelength detectors can provide a UV spectrum of the eluted solute using a stop/flow set up. There are two types of multi-wavelength detectors: the variable wavelength detector (dispersion detector) (Figure 2.7) monitors the eluent at one wavelength and the diode array detector monitors the eluted solute over a range of wavelengths simultaneously. A diagram of variable wavelength detector and the diode array detector is Figure 2.8.

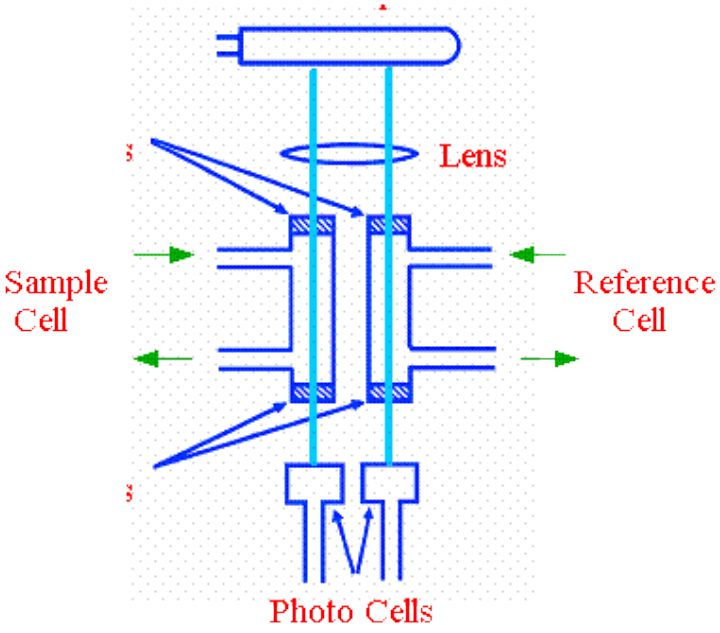


Figure 2.6 Schematic diagram of the fixed wavelength UV detector

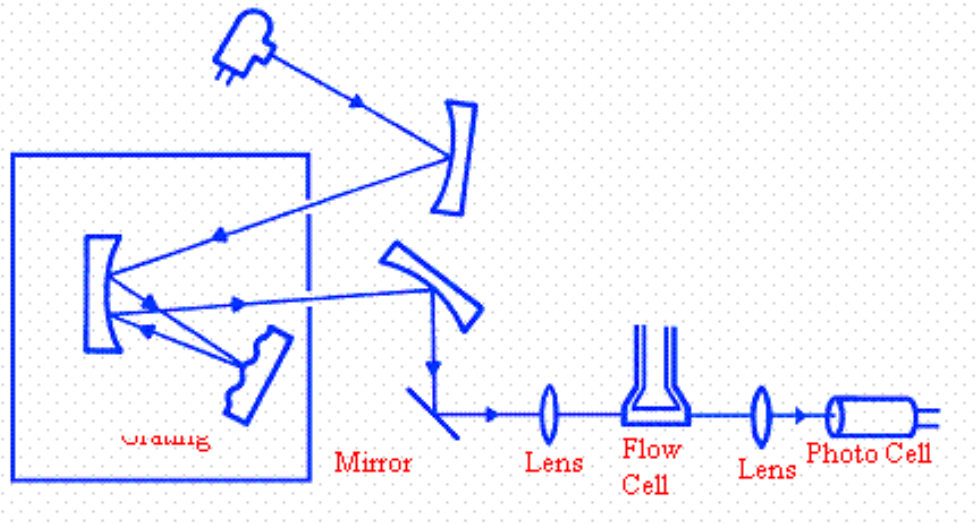


Figure 2.7 Schematic diagram of the variable wavelength detector

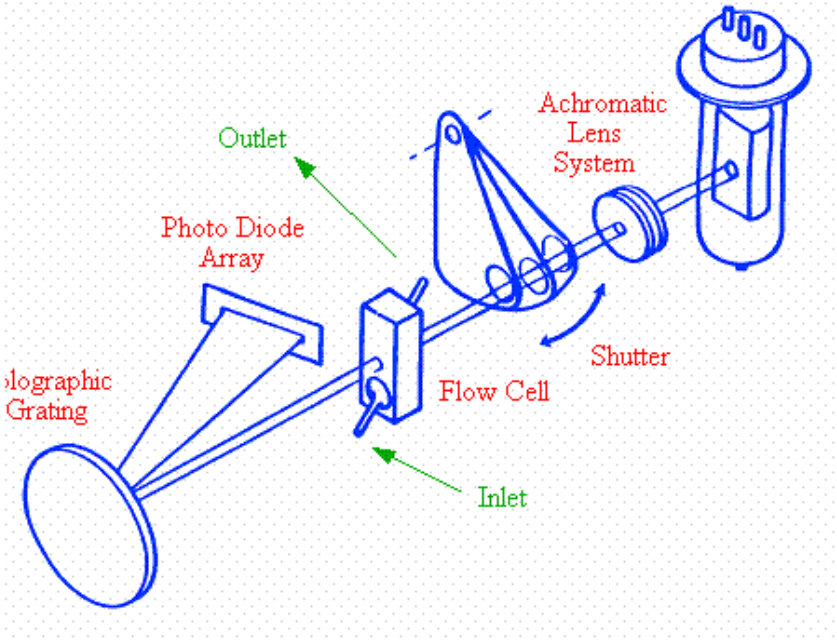


Figure 2.8 Schematic diagram of the diode array detector

2.4 Gas Chromatography Mass Spectrometry (GC-MS) *(35, 36, 37)*

Chromatography Mass Spectrometry (GC-MS)is one of the most important tools in analytical chemistry. GC-MS is a hyphenated technique, which combines Gas Chromatography(GC) and a mass spectrometer (MS). The sample is introduced into the GC inlet by syringe and volatized at a high temperature and mixed with the carrier gas to transport the sample into the column. After separation in the GC column, the analyte species flow into the column interface which creates a vacuum and transports it to an ion source to be ionized. The next component is a mass analyzer which separates ions according to their m/z valueor mass-to-charge ratio and passes them into the detector. The schematic diagram of GC-MS is shown in Figure 2.9.

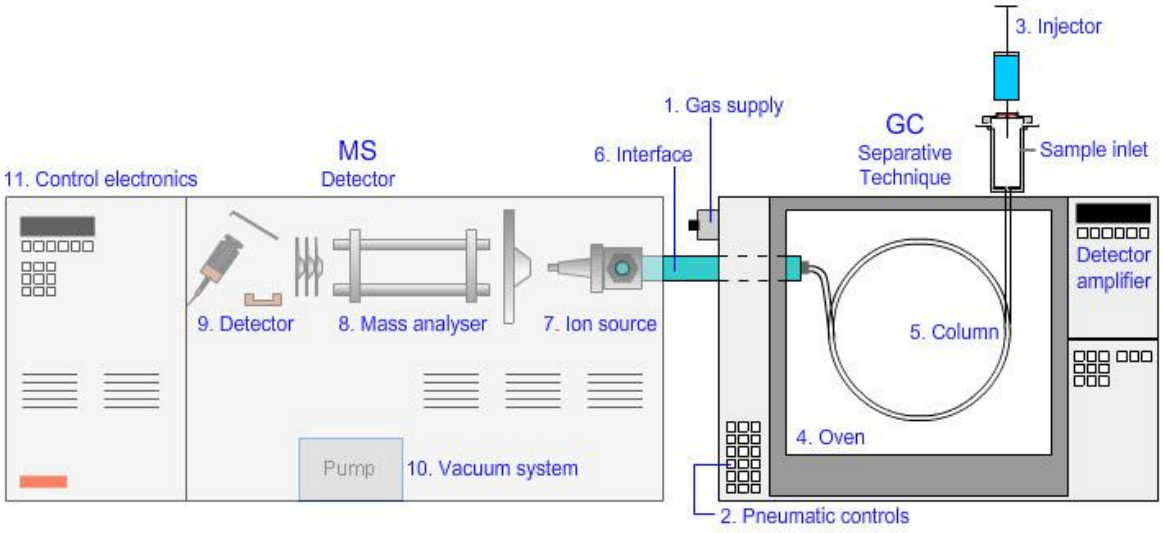


Figure 2.9 Schematic diagram of GC-MS system

2.4.1 Gas Chromatography (GC)

2.4.1.1 Carrier gas

A carrier gas is typically required in a GC systems to flow through the injector and push the gaseous components of the sample onto the GC column, which leads to the detector. The analytes have no interaction with the carrier gas. The choice of the carrier gas depends on the type of detector that is used and the components that are to be determined. The carrier gas must be dry, high in purity and chemically inert mobile-phase employed in GC, samples being, helium (He), argon (Ar), nitrogen (N_2), carbon dioxide (CO_2) or hydrogen (H_2). Helium is most commonly used because it is safer than, but comparable to hydrogen in efficiency, has a larger range of flow rates and is compatible with many detectors.

2.4.1.2 Injector

A syringe is used to inject a volume sample in the range of a few micro liters into the carrier gas through a rubber septum and volatilized in the high temperature injection port, entering the column as gas.The schematic of an injector is shown in Figure 2.10.

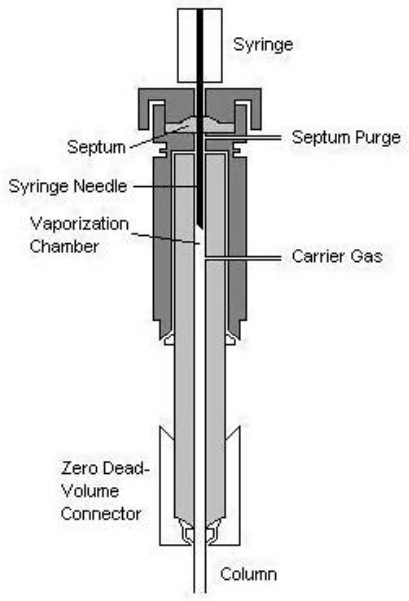


Figure 2.10 Schematic diagram of injector of GC

2.4.1.3 Column

In GC chromatography, the column is the most important component where the components of the sample are separated. Analyte is the interaction of the analyte with the stationary phase of the column that permits separation of the analytes in the sample. There are two types of columns in common use in GC. Packed columns are generally 1.5 – 10m lengths and 2 – 4mm internal diameter. The tubing is generally made of stainless steel or glass, and the inside of the column tube is densely packed with a solid support material (e.g., diatomaceous earth) onto which is coated a liquid stationary phase. The nature of the coating material determines what type of materials will be most strongly absorbed. Thus numerous columns are available that are designed to separate specific types of compounds. Capillary columns are typically 10-100 m in length and 250 μm inner diameters. Capillary columns are fabricated from stainless steel or quartz whereby the stationary phase is coated on the interior walls of a tubular column with inner surface. There are two general types of column; wall-coated open tubular (WCOT) columns and Porous Layer Open Tubular (PLOT) columns. The inner wall of WCOT columns has a liquid film of support material such as diatomaceous earth coated to the deactivated wall of column. In PLOT columns the stationary phase is a solid substance that is coated to the column wall.

2.4.1.4 Oven

The oven can be operated in two ways: isothermal programming or temperature programming. In isothermal programming, the temperature of the column is held constant throughout the entire separation. The optimum column temperature for isothermal operation is approximately the middle point of the boiling range of the sample. However, isothermal programming works best only if the boiling point range of the sample is narrow. If a low isothermal column temperature is used with a wide boiling point range, the low boiling fractions are well resolved but the high boiling fractions are slow to elute with extensive band broadening. If the temperature is increased closer to the boiling points of the higher boiling components, the higher boiling components elute as sharp peaks but the lower boiling components elute so quickly there is no separation. In the temperature programming method, the

column temperature is either increased continuously or in steps as the separation progresses. This method is well suited to separating a mixture with a broad boiling point range. The analysis begins at a low temperature to resolve the low boiling components and increases during the separation to resolve the less volatile, high boiling components of the sample.

2.4.1.5 Detector

The detector is the device located at the end of the column which provides a quantitative measurement of the components of the mixture as they elute in combination with the carrier gas.

2.4.2 Mass Spectrometry (MS) Detectors

MS detectors are the most powerful of the GC detectors. MS is useful for identification, quantitation and confirmation of compounds that explain chemical and structural information about molecules from their molecular weights and fragmentation patterns. The components of MS detectors include sample inlet system, ion source, mass analyzer and detector.

2.4.2.1 Sample inlet system

The sample inlet system can be as simple as a port through which the sample is injected into a chamber at high velocity and heated to achieve vaporization. This includes semi permeable membrane, open split interface and direct coupling interface. In GC-MS, direct coupling interface is the most common method where the tip of the GC capillary column is precisely inserted into the ion source by a length of fused capillary tubing (inlet) and a vacuum tight flange. The schematic of a GC is shown in Figure 2.11

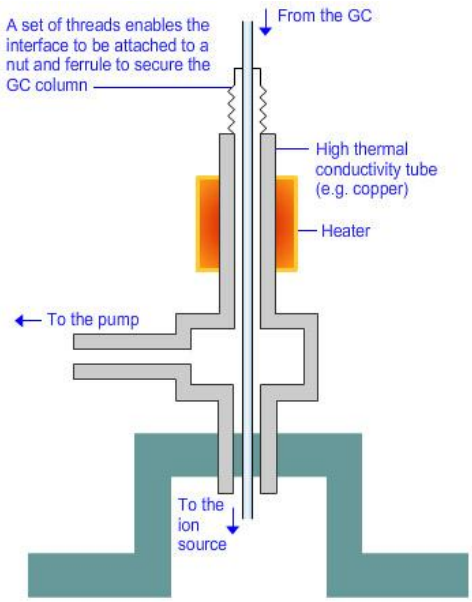


Figure 2.11 Schematic diagram of sample inlet systems

2.4.2.2 Ion source

2.4.2.2.1 Electron impact (EI)

The electron-impact ion source is the most commonly used ionization method. The gas sample is introduced into the electron impact chamber by inlet. Ions are formed during collision between the electron beam which is generated from a tungsten or rhenium filament and sample molecules

$$M + e^- \longrightarrow M^{+\cdot} + 2e^-$$

M represents the analyzed molecule and M+ is its molecular ion. The positive ions are accelerated by an electric field and passed into a magnetic field. By changing the accelerating voltage, i.e. the speed of the particle, or the magnetic field strength, ions of different mass-to-charge ratio can be collected and measured.

2.4.2.2.2 Chemical impact

In chemical ionization, a sample molecule (M) is ionized by collision with ions produced by electron bombardment of the reagent gassuch as methane, oxygen, ammonia and hydrogen.Collision between the sample molecule (M) and highly reactive reaction products aroused from the reagent gas usually involves proton or hydride transfer leading to formation of either (M+1) + or (M-1) + ions.

2.4.2.3 Mass analysers

The mass analyzer separates the mass fragments produced by the ionization sources.Mass analyzers include quadrupole/quadrupole, ion trap, time-of-flight and magnetic sector.

2.4.2.3.1 Quadrupole/triple guadrupole

The most popular mass spectrometer that is used in combined GC-MS is the quadrapole mass spectrometer, either as a single quadrapole or as a triple quadrapole. Quadrupole mass analyzers use oscillating electrical fields to selectively stabilize or destabilize the paths of ions passing through a radio frequency (RF) quadrupole field. In a single quadrupole mass spectrometer, voltage comprising DC voltage and AC voltage (RF) is applied between adjacent rods, opposite rods being electrically connected. Ions are accelerated into the center, between the rods, with a relatively small potential ranging from 10 to 20 volts. Only a single m/z ratio is passed through the system at any time, but changes to the potentials on magnetic lenses which allow a wide range of m/z values to be swept rapidly. Thus a quadrupole mass analyzer acts as a mass-selective filter. ASingle quadrupole mass spectrometer consists of four parallel cylindrical rods that serve as electrodes, one pair being attached to positive side of a variable DC source and the other pair to the negative terminal. Triple quadrupole mass spectrometers have three quadrupoles arranged parallel to incoming ions. The first quadrupole (Q1) acts as a mass filter. The second quadrupole (Q2) acts as a collision cell where selected ions are broken into fragments. The resulting

fragments are scanned by the third quadrupole (Q3).A schematic of a quadrupole is given in Figure 2.12

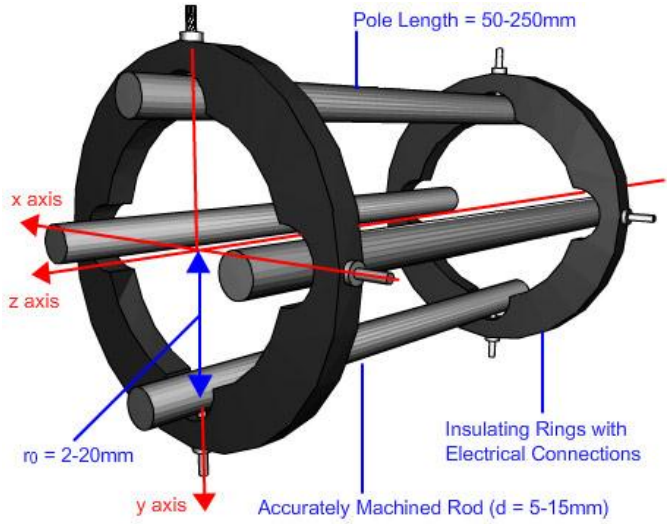


Figure 2.12 Schematic diagram of a quadrupole

2.4.2.3.2 Ion trap

A quadrupole ion-trap consists of a central doughnut-shaped ring electrode and a pair of end cap electrodes as seen in Figure 2.13. Ions are allowed into the cavity through a grid in the upper end cap. A radio-frequency is applied to the ring electrode and ions with an appropriate m/z value orbit around the cavity. As the radio-frequency is increased linearly, ions of a stable m/z value are ejected by mass-selective ejection in order of mass. Ions that are too heavy or too light are destabilized and their charge is neutralized upon collision with the ring electrode wall. Emitted ions then strike an electron multiplier which converts the detected ions into an electrical signal.

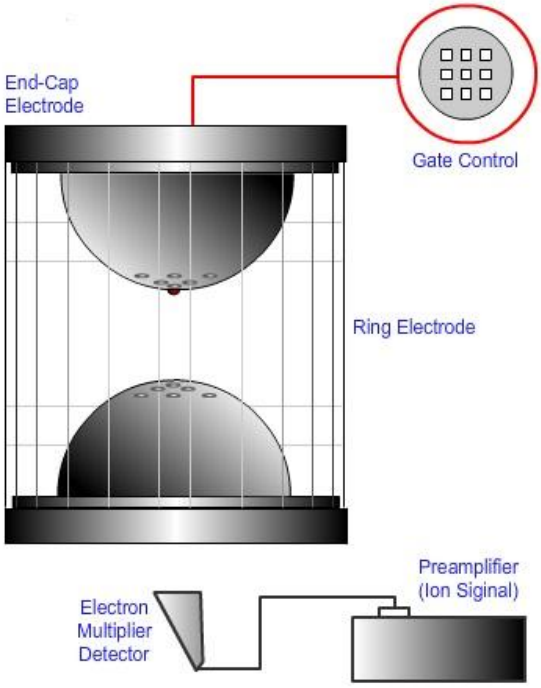


Figure 2.13 Schematic diagram of a quadrupole ion trap

2.4.2.3.3 Time-of-flight

The time-of-flight (TOF) analyzer uses an electric field to accelerate the ions from the ion source through the same potential. The accelerated particles pass into a field-free drift tube that is about a meter in length and then measures the time they take to reach the detector. A time-of-flight analyzer uses the differences in transit time through a drift region to separate ions of different masses. The particles all have the same charge, the kinetic energies will all be identical, and their velocities will depend only on their masses. Lighter ions will reach the detector first. A schematic of a TOF is given in Figure 2.14

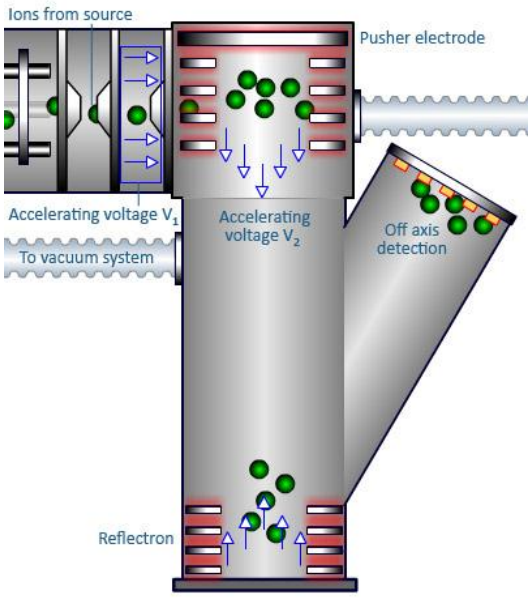


Figure 2.14 Schematic diagram of a TOF

2.4.2.3.4 Magnetic sector

The ions pass into the flight tube between the poles of a magnet and are deflected by the magnetic field. Only ions of a mass-to-charge ratio that have equal centrifugal and centripetal forces pass through the flight tube. Other ions collide against the magnet wall and will not be detected. By varying the acceleration voltage or the magnetic field strength, a complete spectrum can be obtained. A schematic of a magnetic sector is given in Figure 2.15

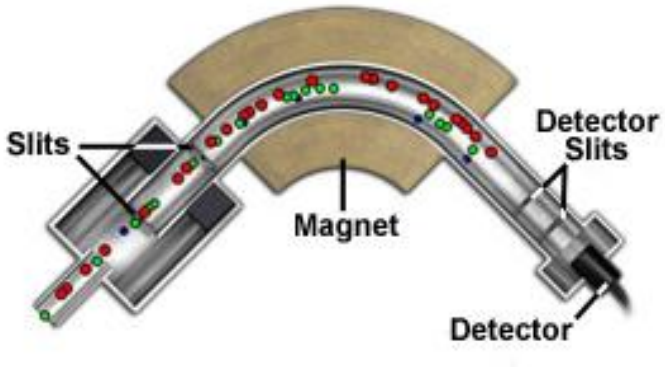


Figure 2.15 Schematic diagram of a magnetic sector

2.4.2.4 Mass detector

The fragments that survive the journey through the mass analyzer shoot into the detector of the GCMS. Detectors used in the mass spectrometer include electron multiplier detectors, photomultiplier detectors and Faraday cup collectors. Most detectors currently used to amplify the ion signal are the electron multiplier or photo multiplier tube. Electron multipliers (EMT) are used for the detection and measurements of electron charged particles. Electron multipliers have high gain and low noise, making them suitable for the detection of very small or low energy particles. Electron multipliers (EMT) consist of a hollow tube (channel) of either glass or a ceramic material with a semiconducting inner surface.A schematic of electron multipliers (EMT) is given in Figure 2.16. Electron multipliers use a process known as secondary electron emission. When the ions impact a surface, it causes the electrons in the outer most area of the atom to be released, which are known as secondary electrons. The number of secondary electrons released depends on several factors, such as the type of particle, the angle at which it hits the surface, and the energy and characteristics of the surface struck.Electron multiplier tubes are similar in design to photomultiplier tubes. They therefore multiply the ion current and can be used in analog or digital mode. A photomultiplier tube is a vacuum tube consisting of an input window, a photocathode and a series of dynodes. Figure 2.17 shows the schematic construction of a photomultiplier tube. When light passes through the input window, electrons are excited in the photocathode so that photoelectrons are emitted into the vacuum. Photoelectrons are accelerated and focused by the focusing electrode onto the first dynode where they are multiplied by means of secondary electron emission. This secondary emission is repeated at each of the successive dynodes. The multiplied secondary electrons emitted from the last dynode are finally collected by the anode.

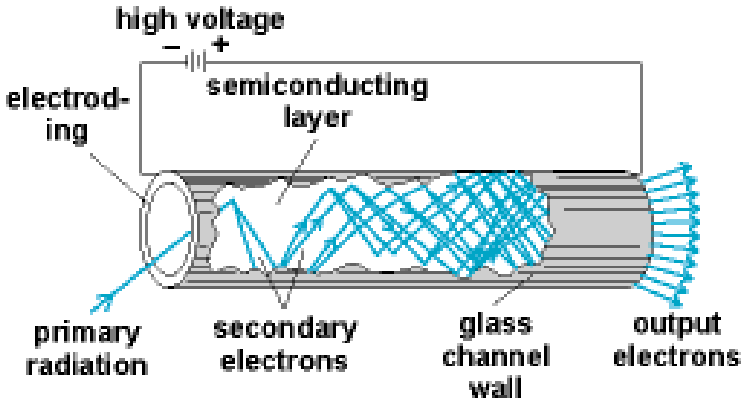


Figure 2.16 Schematic diagram of electron multipliers (EMT)

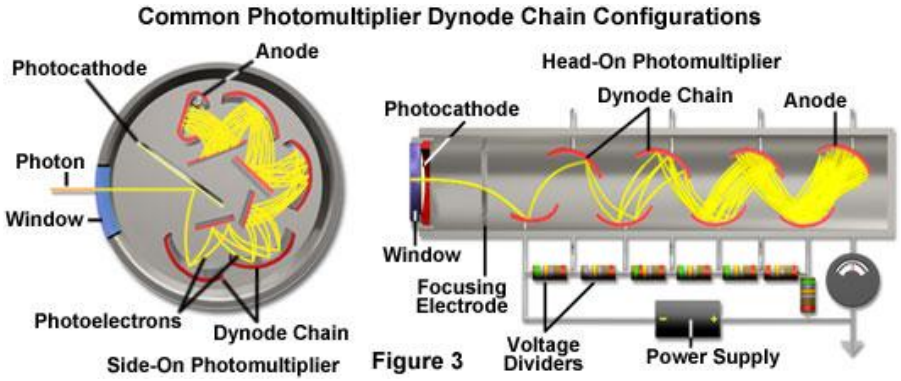


Figure 2.17 Schematic construction of a photomultiplier tube Light

2.5 UV-Vis Spectrophotometers

Regarding the electromagnetic spectrum, ultraviolet (UV) radiation covers the region from 190-350 nm and visible (Vis) radiation covers the region 350-800 nm. Moreover, the absorption of UV and/or Vis radiation corresponds to the excitation of outer electrons in the molecule. In UV-Vis absorption spectrometry, concentration of the species is related to absorbance by the Beer-Lambert Law

$$A_\lambda = \varepsilon_\lambda cl$$

where A_λ = absorbance at a particular wavelength (λ), ε_λ = extinction coefficient at a particular wavelength (λ), c = concentration and l = pathlength.

2.5.1 Instrument

Spectrophotometers are the measurement of the ratio of the radiant powers of two beams, a requirement to measure absorbance. Furthermore, spectrophotometers offer the considerable advantage that the wavelength can be varied continuously, consequently making it possible to record absorption spectra. Spectrophotometers can be either of single beam or double beam design.

2.5.1.1 Single-beam instruments

The simplicity of the instrumentation, low cost, and ease of maintenance offer distinct advantages compose several instrument manufacturers offer single-beam spectrophotometers of the single-wavelength type. Additionally, simple single beam multichannel instruments based on array detectors are widely available. Previously in single beam instruments, all of the light passes through the sample cell and consequently the sample must be replaced by a blank/reference sample to account for any matrix effects. A single-beam instrument will have a shutter which can be placed in front of the detector and as a result no light reaches it. Accordingly, through the shutter in position, a “dark current” adjusting knob is used to set the scale reading to zero percent transmittance (infinite absorbance). Therefore, the dark current is a small current that may flow in the absence of light, owing to thermal emission of electrons

from the cathode of the phototube. The advantage of zeroing the instrument with the blank which is that one reading, which always contains some experimental error, is eliminated.

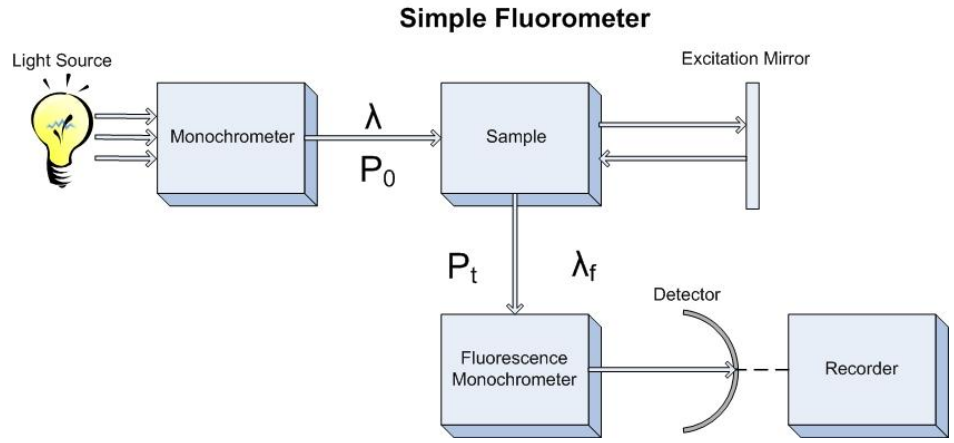


Figure 2.18 Schematic constructions of single-beam instruments

2.5.1.2 Double-beam instruments

Several modern spectrophotometers are based on a double-beam design. Double-beam instruments offer the advantage that they compensate for all but the most short-term fluctuations in the radiant output of the source. They also compensate for wide variations of source intensity with wavelength. Furthermore, the double-beam design is well suited for continuous recording of absorption spectra. A double-beam instrument, the light is split into two beams before it reaches the sample. One beam is used as areference beam while the other beam passes through the sample. However, some double-beam instruments have two detectors; consequently the sample and reference beam can be measured at the same time.

Double-beam instruments are used largely as recording instruments, whereby the instruments automatically vary the wavelength and record the absorbance as a function of thewavelength. Moreover, the instrument has two light paths, one for the sample and the other one for the blank or reference. In a typical setup, the beam from the source strikes a vibrating or rotating mirror that alternately passes the beam

through the reference cell and the sample cell then from each, to the detector. Accordingly in effect, the detector alternately sees the reference and the sample beam. Also, the output of the detector is proportional to the ratio of the intensities of the two beams (P/P_0).

The output is an alternating signal whose frequency is equal to that of the vibrating or rotating mirror. An ac amplifier is used to amplify this signal, and stray dc signals are not recorded. The wavelength is changed by a motor which drives the dispersing element at a constant rate, and the slit is continually adjusted by a servomotor to keep the energy from the reference beam at a constant value which refers to it automatically adjust to 100% transmittance through the reference cell (which usually contains the blank or the solvent). In the conclusion, this is a simplified discussion of a double-beam instrument. Furthermore, there are variations on this design and operation; however it illustrates the utility of these instruments. They are very useful for qualitative work in which the entire spectrum is required, and they automatically compensate for absorbance by the blank, as well as for drifts in source.

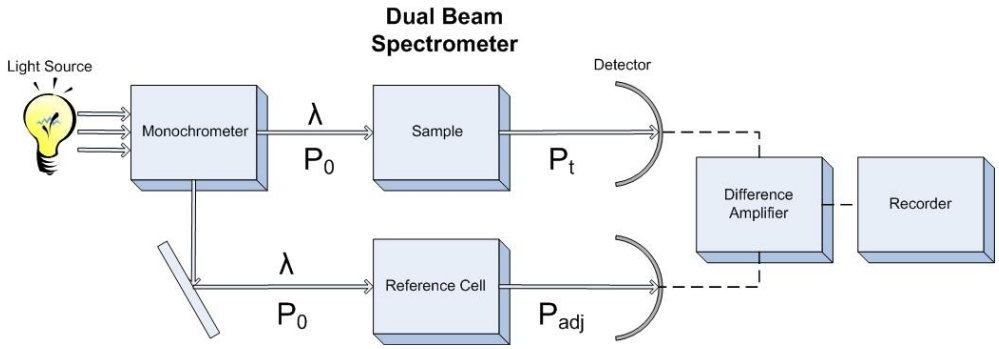


Figure 2.19 Schematic constructions of double-beam instrument

2.6 Chemometric methods*(38, 39, 40, 41)*

Chemometrics is a modern technique that uses the theory and methods from mathematics, statistics, computer science and other related disciplines to optimize the procedure of chemical measurement, and to extract chemical information as much as possible from an original chemical data (such as spectrum, chromatogram, elemental table etc.). Chemometrics could be defined as an interdisciplinary science for data treatment and data interpretation in order to evaluate the results and extract information from the complex system. It plays an important role providing a link between experimental data and statistical methods and complex chemical problems can be solved using chemometrics as a tool to produce some statistical results which are simple for chemists to interpret. Chemometrics has presented new insights for chemists and provided useful solutions to many chemical problems. Pattern recognition is one of the chemometricmethods most used in analytical chemistry. It can be divided into two disciplines: unsupervised pattern recognition and supervised pattern recognition. Unsupervised pattern recognition methods are often called "exploratory techniques" as the aim is to visualize the underlying information in the dataset with no further knowledge required. On the other hand, supervised pattern recognition is mainly used for prediction and classification based on a mathematical model called “classifier”.

2.6.1 Unsupervised pattern recognition

Unsupervised pattern recognition methods transfer raw data into a new group form for monitoring the large set of data conveniently. Nowadays, computer and scientific instruments are dramatically developed. They provide an opportunity to obtain a lot of detected sample and information about the given samples. Therefore, the spectroscopic, chromatographic and other experimental datasets are getting more complex. It is hard to interpret the underlying relationship of samples directly from these datasets. Some techniques are required in order to extract only the significant results in easier terms for interpretation. Principal Component Analysis (PCA) and Cluster Analysis (CA) are described in the section as they are concerned with enabling visualization of the relationship between samples and variables in the datasets.

2.6.1.1 Principal component analysis

Principal component analysis (PCA) is a multivariate technique that analyzes a data table in which observation are described by several inter-correlated quantitative dependent variables and used by almost all scientific disciplines. Principal component analysis (PCA) is a useful exploratory analysis used for reducing the number of variables comprising a dataset while retaining the variability in the data and identifying hidden patterns in the data.The size of data matrices is reduced by transform the multivariate profiles into a new coordinate system called "Principal Components" (*PCs*). *PCs* are calculated as linear combinations of the original variables that account for most of variance in the data.

The first component (*PC1*) is calculated in order to represent the major information, and then comes the second component (*PC2*) which represents the second major information, and vice versa.All *PCs* are completely uncorrelated (orthogonal). The number of *PCs* is limited to the number of variables in the data matrix. The procedure is calculated until the desired *PCs* are reached. By this way, the variation in the data can be described by minimum latent variables which allow easy in comparing between the samples. Result in mathematical transformation original data matrix, which takes the form

$$X = T.P + E$$

where X (n,k) are the data matrix with n samples and k variables, T are called the scores having n rows, P are the loading having k columns. The number of determined *PCs* equals the number of columns in T and the number of rows in P. In case of the number of *PCs* less than k, E is error matrix. For example, as shown in Figure 2.20, each example is explained by two variables, variable 1 and variables 2. The *PCs* are calculated in such a way that *PC1* explains the most variation in the sample and *PC2* will then carry the most remaining information. Each PC is the linear combination between variable 1 and variables 2. How the variable 1 and variables 2 are weighted in each *PC* is expressed by loadings which show the relationship among variables. Each sample coordinate referenced to the new axed (*PC1* and *PC2*) is

scores which emphasise relationships between samples. A highlight of the technique is the ability to represent simultaneously or separately the samples and variables in the space of initial components

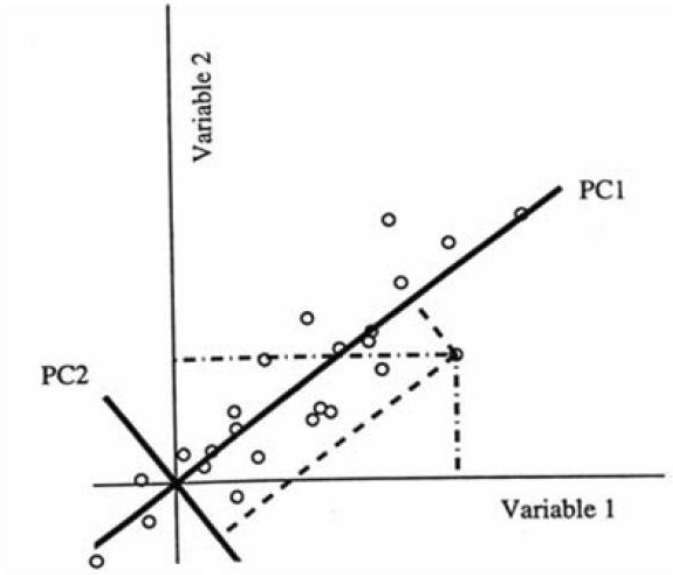


Figure 2.20 PC1 vs PC2 plot

2.6.1.2 Cluster analysis

Cluster analysis (CA) is another unsupervised pattern recognition method that rearranges the data into clusters. The distance, which represents the dissimilarity, between all pairs of objects is calculated. To be illustrated, the catagory of samples is visualized by dendrogram plot. The objects with smaller distances are clustered in the same group, whereas objects with larger distance are clustered in another group.

Cluster analysis is a method for dividing group of objects into classes so that similar objects are in the same class. Cluster analysis searches for objects which are close together in the variable space. The distance, d, between two points in n-dimensional space with coordinates (x_1, x_2, . . . , x_n) and (y_1, y_2, . . . , y_n) is usually taken as the Euclidean distance defined by

$$d = \sqrt{(x_1 - y_1)^2 + (x_2 - y_2)^2 + \ldots + (x_n - y_n)^2}$$

There are a number of methods for searching for clusters. One method starts by considering each object as forming a ‘cluster’ of size one, and compares the distances between these clusters. The two points which are closest together are joined to form a new cluster. The distances between the clusters are again compared and the two nearest clusters combined. This procedure is repeated and, if continued indefinitely, will group all the points together. There are a variety of ways of computing the distance between two clusters which contain more than one member. The simplest concept is to take the distance between two clusters as the distance between nearest neighbours. This is called the nearest linkage method.The successive stages of grouping can be shown on a dendrogram as in Figure 2.21. The vertical axis can show either the distance, d_{ij}, between two samples i and j when they are joined, or alternatively the similarity, s_{ij}, defined by $s_{ij} = 100(1 - d_{ij}/d_{max})$,where d_{max} is the maximum separation on between any two points. The resulting diagrams look the same but their vertical scales differ. The stage at which the grouping is stopped, which determines the number of clusters in the final classification, is a matter of judgement for the person carrying out the analysis.The method of cluster analysis described here is hierarchical, meaning that once an object has been assigned to a group the process cannot be reversed. For nonhierarchical methods the opposite is the case. One such method is the k-means method which is available, for example, in Minitab. This starts by either dividing the points into k clusters or alternatively choosing k ‘seed points’. Then each individual isassigned to the cluster whose centroid is nearest. When a cluster loses or gains a point the position of the centroid is recalculated. The process is continued until each point is in the cluster whose centroid is nearest.

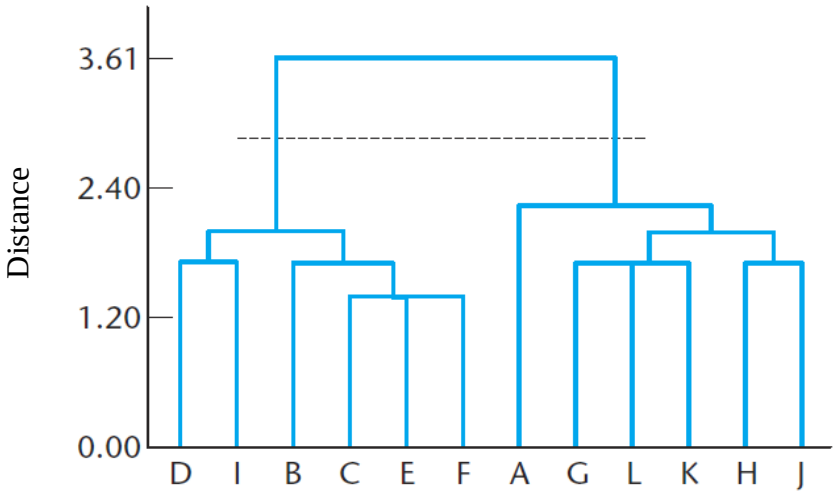


Figure 2.21 Dendrogram

2.6.2 Supervised pattern recognition

Supervised pattern recognition is used to predict the result of experiments from classification model based on similarities and dissimilarities of samples in experiments.

2.6.2.1 Linear discrimination analysis

Linear discrimination analysis (LDA) is a supervised pattern recognition that constructs the classification model to predict the origin of an unknown sample. LDA performs dimensional reduction while preserving as much of the class discriminatory information as possible. Group of the sample is determined by seeking the classes with the smallest LDA distance. In the study, the leave-one-out cross validation is performed to validate the model performance. This technique is very useful when many samples and several groups are involved. LDA constructed a classification model for predicting the group membership of new observation from raw data and leave-one-out cross validation was performed in order to validate the generated model.

CHAPTER III

EXPERIMENTAL

3.1 Instrument and apparatus

3.1.1 High-performance liquid chromatography (HPLC): model Agilent series 1100 HPLC system, Agilent technologies, CA, USA.

3.1.2 HPLC Column: Eclipse plus C18 column (4.6×100 mm, 3.5 µm), Agilent technologies, CA, USA.

3.1.3 Gas Chromatography (GC): Agilent HP 6890 Series GC system with 5973 Mass selective Detector.

3.1.4 Liquid Nitrogen, pressure 100 psi from TIG, Bangplee, Samutplakarn, Thailand.

3.1.5 Spectrophotometer: model HP/Agilent 8453 UV-Vis spectrophotometer, Agilent technologies, CA, USA.

3.1.6 Centrifuge: modeSorvall ST 16 centrifugel, Thermo Fisher Scientific, Inc.,

3.1.7 Blender:Scientific Industries, Inc., Bohemia, NY, USA.

3.1.8 Vortex: model vortex - genie 2, Scientific Industries, Inc., Bohemia, NY, USA.

3.1.9 Balance: model XS, Mettler-Toledo, Inc., OH, USA.

3.1.10 Ultrasonicate: model crest 575d, Crest Ultrasonic corporation, NY, USA.

3.1.11 Nylon syringe filter 0.2 µm from Chrom Tech Inc., AppleValley, MN, U.S.A.

3.1.12 HPLC Vial, 2 mL, Agilent Technologies, CA, USA.

3.1.13 GC Headspace vial, 10 mL, Agilent Technologies, CA, USA.

3.1.14 20 mm Aluminium Crimp caps and grey butyl septa, Agilent technologies, USA.

3.1.15 Crimper 20 mm from Agilent technologies, CA, USA.

3.1.16 Micropipetts : volume 0.1-10, 10-100, 25-200, 100-1000 µl and 1-5 ml with tips from Eppendorf, Hamburg, Germany.

3.1.17 Micropipette tips, 2-20 µL, and 200-1000 µL, Gilson, Inc., Middleton, USA.

3.1.18 Volumetric flask volume class A 10, 50 mL

3.1.19 Beaker 10, 50, 100 mL

3.1.20 Graduated cylinder 10,50 and 100 mL

3.1.21 Test tube

3.1.22 Cuvette

3.1.23 Spatulas

3.1.24 Droppers

3.1.25 Mili-Q, Ultrapure Water System, with Millipak@ 40 Filter unit 0.22 um, model Millipore ZMQS 5 V00Y, Millipore, USA

All experimental glassware was cleaned with detergents, dried in an oven and rinsed with deionized water before use

3.2 Chemical

3.2.1 Standard compounds

Natural capsaicinoids standard (60% capsaicin, 30% dihydrocapsaicin) and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

3.2.2 Organic solvents and other chemicals

Folin&Ciocalteu's phenol reagent was purchased from Sigma-Aldrich (St. Louis, MO, USA). Anhydrous sodium carbonate (Na_2CO_3) was purchased from Riedel-de Haën (Hanover, Germany). High purity water (Milli Q gradient), Methanol (HPLC-grade) was purchased from Merck (Darmstadt, Germany).

3.2.2.1 Folin&Ciocalteu's phenol reagent was prepared by diluting Folin&Ciocalteu's phenol reagent 5.00 mL with Milli Q water 50.00 mL in a glass bottle. The Folin&Ciocalteu's phenol reagent was kept in closed dark conditions but diluted solution was only kept for few days before analysis.

3.2.2.2 Sodium carbonate solution was prepared by dissolving an accurate weight of approximately 7.5 g of anhydrous sodium carbonate in a bottle with Milli Q water 100mL. This solutions was kept at room temperature before analysis

3.2.3 Preparation of standard solutions

3.2.3.1 Preparation of standard solutions for capsaicin and dihydrocapsaicin analysis

3.2.3.1.1 Preparation of stock standard solutions

Stock standard solution of 1,000 mg/L of capsaicin was prepared by dissolving an accurate weight of approximately 15.38 g of natural capsaicinoids standard in methanol and made up to 10.00 mL in a volumetric flask. This stock standard solution was kept in amber glass bottles with Teflon screw caps and stored in a refrigerator at 4 ºC, until required for analysis.

Stock standard solution of 1,000 mg/L of dihydrocapsaicin was prepared by dissolving an accurate weight of approximately 28.57 g of natural capsaicinoids standard in methanol and made up to 10.00 mL in a volumetric flask. This stock standard solution were kept in amber glass bottles with teflon screw cap and stored in a refrigerator at 4 ºC, until required for analysis.

3.2.3.1.2 Preparation of standard solutions for calibration curve

The standard solution of capsaicin at five concentrations for calibration curve were prepared by pipetting the volumes of the stock standard solution of 1,000 mg/L of capsaicin as shown in Table 3.1, Table 3.2 and Table 3.3 which were used for the measuring volume of standard capsaicin of hot chili pepper (*Capsicum frutescens Linn*.), Bird chili (*Capsicum frutescens Linn*.), and Chili spur pepper (*Capsicum annuum Linn. VaracuminatumFingerh.*), respectively and diluting them to 1.00 mL with milli Q water into a HPLC vial.

The standard solution of dihydrocapsaicin at five concentrations for calibration curve were prepared by pipetting the volumes of the stock standard solution of 1,000 mg/L of dihydrocapsaicin as shown in Table 3.1, Table 3.2 and Table 3.3 which were used for the measuring volumes of standard capsaicin of hot chili pepper (*Capsicum frutescens Linn*.), bird chili (*Capsicum frutescens Linn*.), and chili spur pepper (*Capsicum annuum Linn. VaracuminatumFingerh.*), respectively and diluting them to 1.00 mL with milli Q water into a HPLC vial.

Table 3.1 Preparation of standard capsaicin and dihydrocapsaicin of hot chili pepper (*Capsicum frutescens Linn*.)

Standard capsaicin		**Standard dihydrocapsaicin**	
Concentration (mg/L)	**Volume (µL)**	**Concentration of (mg/L)**	**Volume (µL)**
50	50	10	10
100	100	30	30
150	150	50	50
200	200	70	70
250	250	90	90

Table 3.2 Preparation of standard capsaicin and dihydrocapsaicin of bird chili (*Capsicum frutescens Linn*.)

Standard capsaicin		**Standard dihydrocapsaicin**	
Concentration (mg/L)	**Volume (µL)**	**Concentration of (mg/L)**	**Volume (µL)**
100	100	10	10
200	200	30	30
300	300	50	50
400	400	70	70
500	500	90	90

Table 3.3 Preparation of standard capsaicin and dihydrocapsaicin of chili spur pepper (*Capsicum annuum Linn. VaracuminatumFingerh*.)

Standard capsaicin		**Standard dihydrocapsaicin**	
Concentration (mg/L)	**Volume (µL)**	**Concentration of (mg/L)**	**Volume (µL)**
5	1	5	1
10	2	10	2
15	3	15	3
20	4	20	4
25	5	25	5

3.2.3.2 Preparation of standard solutions for total phenolic content analysis

3.2.3.2.1 Preparation of stock standard solutions

Stock standard solution of 1,000 mg/L of gallic acid was prepared by dissolving an accurate weight of 10 g of gallic acid in Milli Q water and made up to 10.00 mL in a volumetric flask with milli Q water. This stock standard solution was kept in amber glass bottles with Teflon screw cap and stored in a refrigerator at 4 ºC before analysis.

3.2.3.2.2 Preparation of intermediate standard solutions

The intermediate standard solutions of 200 mg/L of gallic acid was prepared by pipetting 1 mL of 1,000 mg/L of stock standard solution into a 5.00 mL volumetric flask and made up to 5.00 mL in a volumetric flask with milli Q water. This intermediate standard solution was kept in amber glass bottles with Teflon screw cap, prepared daily and stored at 4 ºC in a refrigerator, before analysis.

3.2.3.2.3 Preparation of standard solutions for calibration curve

The standard gallic acid solutions of each concentration for calibration curve were prepared by pipetting the volumes of the intermediate standard solution of 200 mg/L of gallic acid, methanol and Milli Q water into a glass test tube as shown in Table 3.4.

Table 3.4 Preparation of standard gallic acid solutions

Concentration of gallic acid (mg/L)	Volume (mL)		
	Standard gallic acid solution	**Methanol**	**Milli Q water**
10	0.025	0.125	0.350
30	0.075	0.125	0.300
50	0.125	0.125	0.250
70	0.175	0.125	0.200
90	0.225	0.125	0.150

3.3 Instrumental condition

3.3.1 Determination of capsaicin and dihydrocapsaicin

In this research, the analysis of capsaicin and dihydrocapsaicin was performed using HPLC (Agilent series 1100 HPLC system). The HPLC conditions are summarized in Table 3.5. The wavelength of 280 nm is the maximum absorption of capsaicin and dihydrocapsaicin.

Table 3.5 The HPLC condition for capsaicin and dihydrocapsaicin analysis

HPLC parameter	**Condition**
Analytical column	Column Eclipse XDB (C18 4.6 × 150 mm, 5μm) Eclipse plus C18 column (4.6×100 mm, 3.5 μm)
Injection volume	5 μL
Flow	1 mL/min
Stop time	20 min
Post time	4 min
Column temperature	27 °C
B % (methanol)	70 %
A % (water)	30 %
Detector	UV-Vis spectrophotometer
Wavelength	280 nm.

3.3.2 Determination of volatile compounds

In this study, the volatile compounds (VOCs) in chili pepper samples were analyzed using Headspace-GC-MS system (Agilent G1888 Headspace autosampler with Agilent HP 6890 Series GC system with 5973 Mass selective Detector). VOCs were identified by comparing the MS spectrum with the NIST library database. GC condition is shown in Table 3.6. The mass spectrometer parameters are shown in Table3.7.

Table 3.6 The gas chromatographic conditions using a headspace mode for the study of chili pepper samples

GC parameters	GC condition
Analytical column	GC capillary column (DB-624, 25m × 0.2 mm id × 1.12 µm)
Temperature program	40 ºC (3 min), 40 ºC /min to 240 ºC (15 min)
Oven temperature	60 °C
Transferline temperature	100 °C
Injection mode	Split mode
Injection volume	1 µL
Injection temperature	150 °C
Flow rate of carrier gas (He)	1mL/min
Detector	MS

Table 3.7 The mass spectrometer parameters for the study of chili pepper samples

MS parameters	**MS condition**
Solvent delay time	1.50 min
Transfer line temperature	280 ºC
Ionization source temperature	230 ºC
Quadrupole temperature	150 ºC
Electron impact ionization voltage	70 eV
Ionization type	EI
Acquisition mode	Full scan analysis (35-350 m/z)

3.3.3 Determination of total phenolic content

The total phenolic content was analyzed using the method modified from Ghasemnezhad et al, 2011 *(13)*. The total phenolic content was measured by the Folin-Ciocalteu method. Gallic acid was used as the reference standard. The total phenolic content was calculated in milligrams of gallic acid equivalent / 100 g FW (GAE/100 g FW). The wavelength of 760 nm is the maximum absorption of gallic acid, which was used for quantitative analysis of total phenolic in chili pepper samples. The total phenolic content was performed using a Spectrophotometer (HP/Agilent 8453 UV-Vis spectrophotometer).

3.4 Sample

In this study, all chili pepper samples were divided into 3 groups namely the hot chili pepper (*Capsicumfrutescens Linn.)* (n=6), the bird chili (*Capsicum frutescens Linn*.) (n=5), and the chili spur pepper (*Capsicum annuum Linn. Varacuminatum Fingerh*.) (n=4). The geographical origin and details of the samples are listed in Table 3.8.The discrimination of chili peppers was divided into two parts. The first part, three varieties of chili pepper samples were classified according to different geographical

origins. In the second part, samples were grouped according to varieties of chili peppers. Fresh chili peppers of each variety from each province in a mature state were purchased from Taladthai market in Thailand as shown in Table 3.8. Samples were kept in polyethylene bags and stored at 4°C in a refrigerator, before use.

Table 3.8 shows the geographical origin and varieties of chili pepper

Varities	**Provinces**	**Period**
Hot chili pepper (*Capsicum frutescens Linn*.)	Sisaket, Prachuapkhirikhan, Nakhonsawan,Chiangmai, Ubonratchathani, and Kamphaengphet	August, 2012
Bird chili (*Capsicum frutescens Linn*.)	Chumphon, Chanthaburi, Pathumthani, Chiangmai and Tak	September, 2012
Chili spurs pepper (*Capsicum annuum Linn. Varacuminatum Fingerh*.)	Suphanburi, Chiangmai, Phisanulok, Tak	August, 2012

3.4.1 Preparation of sample

3.4.1.1 Preparation of sample for capsaicin and dihydrocapsaicin analysis

Fresh chilli peppers were dried in an oven at 60°C for 2 days, and then homogenized into a fine powder using a blender. The powder was put into a glass bottle and kept in a desiccator. For extraction, a sample (0.5 g) was weighed in a test tube and then extracted by sonication with methanol (10 mL) for 1 hour at room temperature. The sample solution was then filtered through a 0.45 μm PTFE membrane filter before HPLC analysis. HPLC analysis of chili peppers extract was analyzed according to the HPLC conditions in Table 3.5.

3.4.1.2 Preparation of sample for volatile compounds analysis

Fresh chili peppers were homogenized using a blender.Homogenized fresh chilli pepper samples (3.0 g) were placed into a headspace vial for headspace-GC-MS analysis. Determination of volatile compounds was performed on a headspace-GC-MS system (Agilent HP 6890 Series gas chromatography (GC) system with 5973 Mass selective Detector) as described in section 3.4.2. Chemical constituents were identified by comparison of their mass spectra with the NIST library database.

3.4.1.3 Preparation of sample for total phenolic content analysis

Fresh chilli peppers were homogenized using a blender. Samples (1 g) were extracted with methanol (10 mL) and then centrifuged at 5000 rpm at room temperature for 15 minutes. The methanolic extract (125 µL) was diluted with Milli Q water (375 µL) in a test tube, and 10 % Folin- Ciocalteu reagent (2.5 mL) was then added. After 6 minutes mixing using a vortex, 2 ml of 7.5% Na_2CO_3 was added, and the mixture stood for 90 minutes at room temperature in darkness. The total phenolic content was determined as described in section 3.4.3.

3.5 Statistic analysis

3.5.1 F-test

All data from capsaicin and dihydrocapsaicin analysis, volatile compounds analysis and total phenolic content analysis of all chili peppers samples in Section 4.1.1.2, 4.1.1.3 and 4.1.1.4, respectively were analyzed by observing the significant difference of geographical origin data of chili peppers using one-way analysis of variance (one-way ANOVA) method.

3.5.2 Chemometric methods

In this work, the discrimination of chili peppers was divided into two parts;the discrimination of the geographical origin of chilli peppers; and the discrimination of the varieties of chilli peppers.

3.6.2.1 Discrimination of the geographical origin of chilli peppers by chemometric technique

The raw data obtained from capsaicin and dihydrocapsaicin analysis, volatile compounds analysis and total phenolic content analysis of all chili pepper samples in Section 4.1.1.2, 4.1.1.3 and 4.1.1.4, respectively. This data was normalized before discrimination by PCA, LDA and CA. Discriminations of the geographical origins were performed using chemometric techniques that were carried out by MATLAB Starter Application (version 2009). The results are shown in section 4.2.1.

3.6.2.2 Discrimination of the varieties of chilli peppers by chemometric technique

The raw data obtained from capsaicin and dihydrocapsaicin analysis, volatile compounds analysis and total phenolic content analysis of all chili pepper samples in Section 4.1.1.2, 4.1.1.3 and 4.1.1.4, respectively. This data was normalized before discrimination by PCA, LDA and CA. Discriminations of the varieties were performed using chemometric techniques and were carried out by MATLAB Starter Application (version 2009). The results are shown in section 4.2.2.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Determination of chili peppers

In this study, chili peppers samples were divided into 3 groups namely the hot chili pepper (Capsicum *frutescens Linn).* (n=6), the Bird chili (*Capsicum frutescens Linn*.) (n=5), and the chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh*.) (n=4). All chili samples were randomly purchased from Talaadthai market in Pathumthani. The geographical origin and details of the samples are listed in Table 3.8. This study was carried out using three methods in order to determine various chili peppers from different geographical origins. First, capsaicin and dihydrocapsaicin in chili pepper samples were analyzed by HPLC-DAD in section 4.1.1. Second, the volatile compounds of chili peppers were qualitatively and quantitatively analyzed using GC-MS in section 4.1.2. Third, the total phenolic content (TPC) was determined by UV-visible spectrophotometer in section 4.1.3.

4.1.1 Capsaicin and dihydrocapsaicin analysis

4.1.1.1 The result of standard calibration curves

The standard calibration curve was used to determine the reference concentration in the chili pepper samples.

4.1.1.1.1Hot chili pepper (*Capsicum frutescens Linn*.)

The standard capsaicin solutions were prepared as described in Section 3.2.3.1 (Table 3.1). The chromatogram obtained with standard capsaicin solution under HPLC conditions (Table 3.5) are shown in Figure 4.1. The standard capsaicin solutions covered the concentration range of 50-250 mg/L. The calibration curve of 5 points was plotted by peak area (mAU) versus concentration of standard capsaicin solutions (mg/L).The calibration curve can be acceptable for quantitation because this method provided good determination correlation coefficient (R^2) of 0.9996. The linear equation is y=2.7269x-3.4198. This linear equation was used for quantitative analyses

of capsaicin. The 5 point calibration curve, slope value, y-intercept and correlation coefficient are shown in Figure 4.2

The standard dihydrocapsaicin solutions were prepared as described in Section 3.2.3.1 (Table 3.1). The chromatogram obtained with standard dihydrocapsaicin solution under the HPLC conditions (Table 3.5) are shown in Figure 4.1. The standard dihydrocapsaicin solutions covered the concentration range of 10-90 mg/L. The calibration curve of5 point was plotted by peak area (mAU) versus concentration of standard dihydrocapsaicin solutions (mg/L). The calibration curve can be acceptable for quantitation because this method provided gooddetermination correlation coefficient (R^2) of 0.9996.The linear equation is y=3.5953x-3.4198. This linear equation was used for quantitative analyses of dihydrocapsain. Thecalibration curve slope value, y-intercept and determination correlation coefficient (R^2) are shown in Figure 4.3

The chromatogram of standard capsaicin and dihydrocapsaicin (Figure 4.1) indicated that capsaicin and dihydrocapsaicin were separated with retention time of 2.996 and 3.492 minutes under the HPLC conditions (Table 3.5). Norhydrocapsaicin cannot be detected at low concentration of natural capsaicinoids but can be detected at high concentration of natural capsaicinoids.

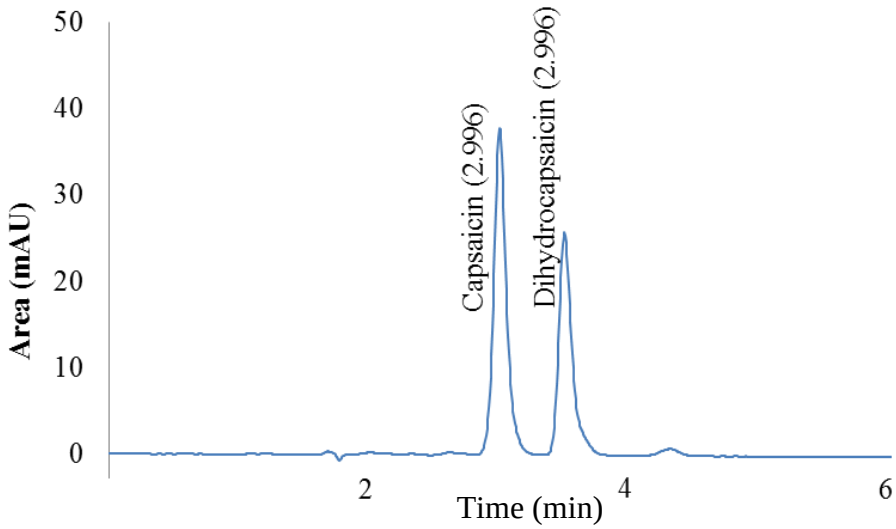


Figure 4.1 Chromatogram of standard capsaicin and dihydrocapsaicin at 100 and 30 mg/L, respectively for hot chili pepper (*Capsicum frutescens Linn.*) using Column Eclipse XDB (C18 4.6 × 150 mm, 5µm) by HPLC condition in Table 3.5

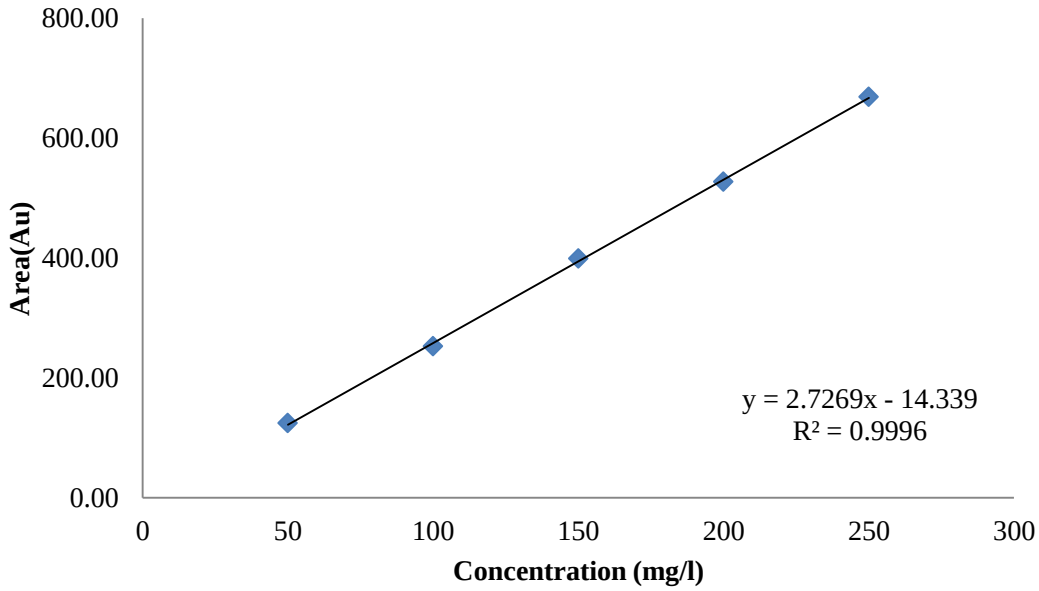


Figure 4.2 The calibration curve of standard capsaicin for hot chili pepper (*Capsicum frutescens Linn*.) using Column Eclipse XDB (C18 4.6 × 150 mm, 5 ×mm) by HPLC condition in Table 3.5

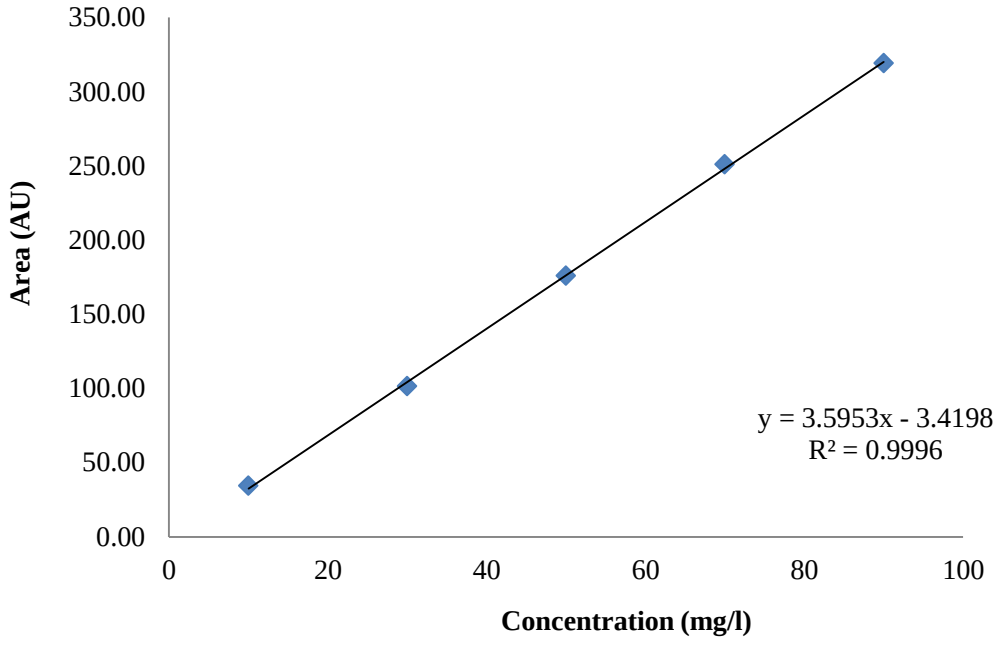


Figure 4.3 The calibration curve of standard dihydrocapsaicin for hot chili pepper (*Capsicum frutescens Linn*.) using Column Eclipse XDB (C18 4.6 × 150 mm, 5 mm) by HPLC condition in Table 3.5

4.1.1.1.2 Bird chili (*Capsicum frutescens Linn*.)

The standard capsaicin solutions were prepared as described in Section 3.2.3.1 (Table 3.2). The chromatogram obtained with standard capsaicin solution under HPLC conditions (Table 3.6) are shown in Figure 4.4.The standard capsaicin solutions covered the concentration range of 100-300 mg/L. The calibration curve of 5 points was plotted showing peak area (mAU) versus concentration of standard capsaicin solutions (mg/L). The calibration curve was acceptable for quantitation because this method showed determination correlative coefficient (R^2) of 0.9990.The linear equation is y=2.2572x-5.7667. This linear equation was used for quantitative analyses of capsaicin. The calibration curve, slope value, y-intercept and correlation coefficient are shown in Figure 4.5.

The standard dihydrocapsaicin solutions were prepared as described in Section 3.2.3.1 (Table 3.2). The chromatogram obtained with standard dihydrocapsaicin solution under the HPLC conditions (Table 3.5) are shown in Figure 4.4.The standard dihydrocapsaicin solutions covered the concentration range of 10-90 mg/L. The calibration curve of 5 points was plotted by peak area (mAU) versus concentrationof standard dihydrocapsaicin solutions (mg/L). The calibration curve can be acceptable for quantitation because this method showed decisively that the determination correlative coefficient (R^2) of 0.9986.The linear equation is used for quantitative analyses of dihydrocapsain. The calibration curve, slope value, y-intercept and correlation coefficient are shown in Figure 4.6.

The chromatogram of standard capsaicin and dihydrocapsaicin (Figure 4.4) indicated that capsaicin and dihydrocapsaicin were separated with a retention time of 4.012 and 5.649 min under the HPLC conditions (Table 3.5). Norhydrocapsaicin cannot be detected at low concentration of natural capsaicinoids but can be detected at high concentration of natural capsaicinoids.

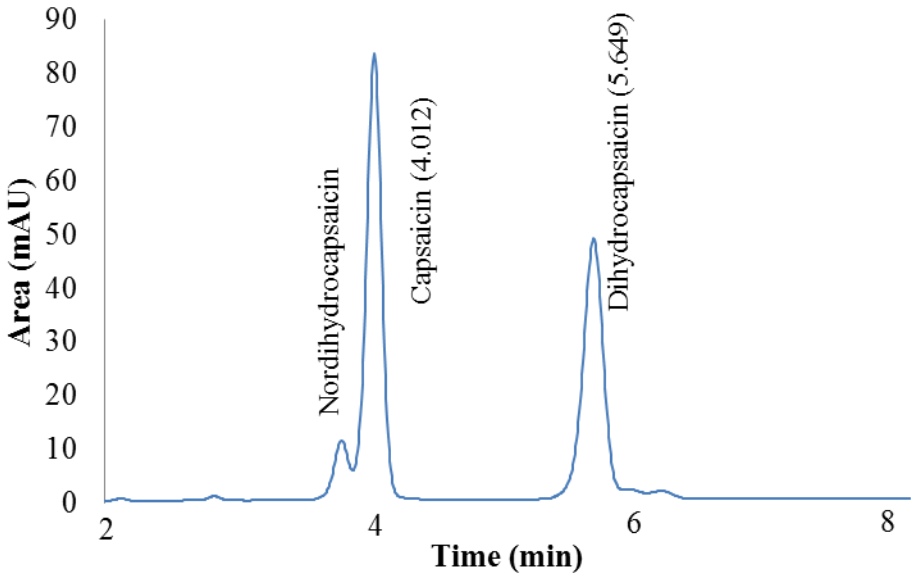


Figure 4.4 Chromatogram of standard capsaicin and dihydrocapsaicin at 300 and 70 mg/L, respectively for bird chili (*Capsicum frutescens Linn*.) using Eclipse plus C18 column (4.6×100 mm, 3.5 µm) by HPLC condition in Table 3.5

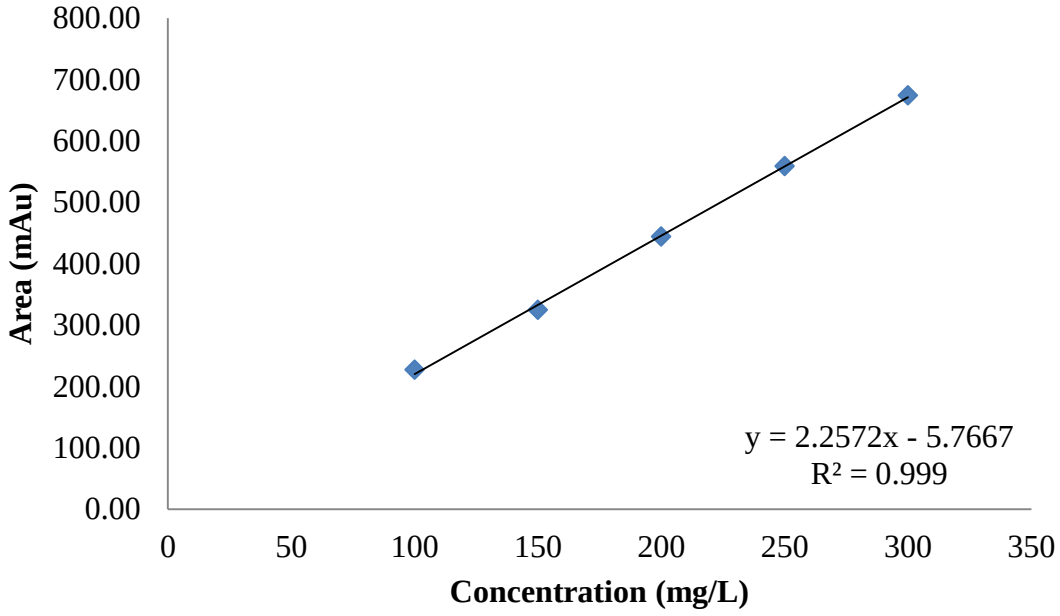


Figure 4.5 The calibration curve of standard capsaicin for bird chili (*Capsicum frutescens Linn*.) using Eclipse plus C18 column (4.6×100 mm, 3.5 µm) by HPLC condition in Table 3.5

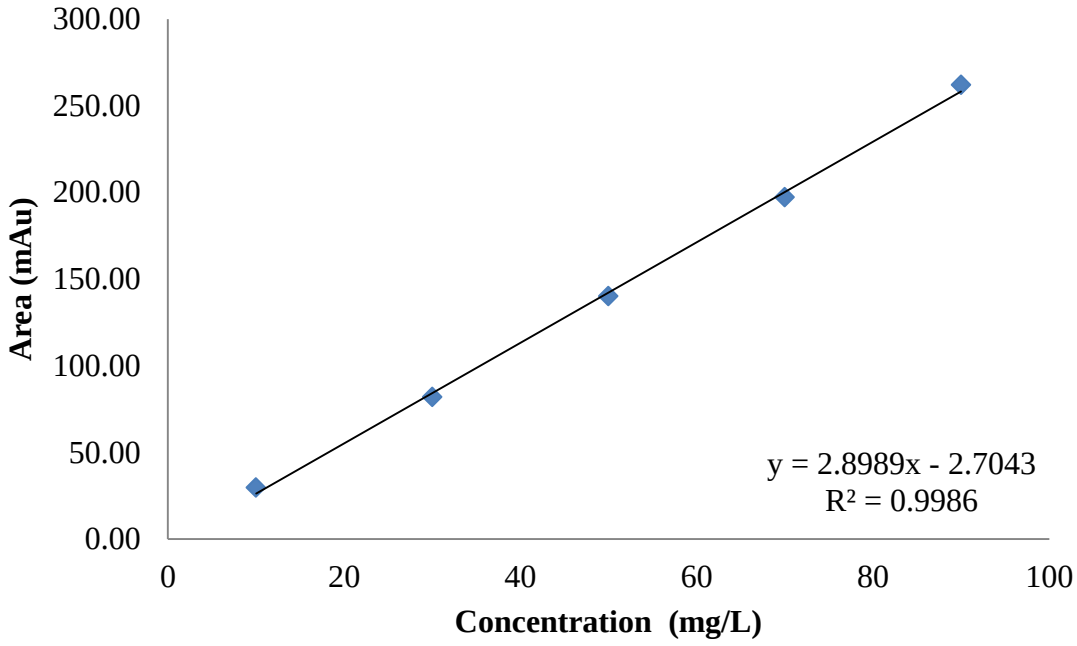


Figure 4.6 The calibration curve of standard dihydrocapsaicin for bird chili (*Capsicum frutescens Linn.*) using Eclipse plus C18 column (4.6×100 mm, 3.5 µm) by HPLC condition in Table 3.5

4.1.1.1.3 Chili spur pepper(*Capsicum annuum Linn. Var acuminatum Fingerh.*)

The standard capsaicin solutions were prepared as described in Section 3.2.3.1 (Table 3.3). The chromatogram obtained with standard capsaicin under HPLC conditions (Table 3.5) are shown in Figure 4.7. The standard capsaicin solutions covered the concentration range of 5-25 mg/L. The calibration curve of 5 points was plotted by peak area (mAU) versus concentration of standard capsaicin solutions (mg/L). The calibration curve can be acceptable for quantitation because this method showed the determination correlative coefficient (R^2) of 0.9987. The linear equation is y=2.0266x-2.7945. This linear equation was used for quantitative analyses of capsain. The calibration curve, slope value, y-intercept and determination correlation coefficient are shown in Figure 4.8.

The standard dihydrocapsaicin solutions were prepared as described in Section 3.2.3.1 (Table 3.3). The standard dihydrocapsaicin solutions covered the concentration range of 5-25 mg/L. The calibration curve of 5 point was plotted by peak area (mAU) versus concentration of standard dihydrocapsaicin solutions (mg/L). The chromatogram obtained with standard dihydrocapsaicin solutions under the HPLC conditions (Table 3.5) are shown in Figure 4.7. The calibration curve can be acceptable for quantitation because this method showed the determination correlative coefficient (R^2) of 0.9958.The linear equation is y=2.9885+1.2402. This linear equation was used for quantitative analyses of dihydrocapsain. The slope value, y-intercept and determination correlation coefficient are shown in Figure 4.9.

From chromatogram of standard capsaicin and dihydrocapsaicin (Figure 4.7) indicated that capsaicin and dihydrocapsaicin were separated with retention time of 3.944 and 5.557 min under the HPLC conditions (Table 3.5). Norhydrocapsaicin cannot be detected at low concentration of natural capsaicinoids but can be detected at high concentration of natural capsaicinoids.

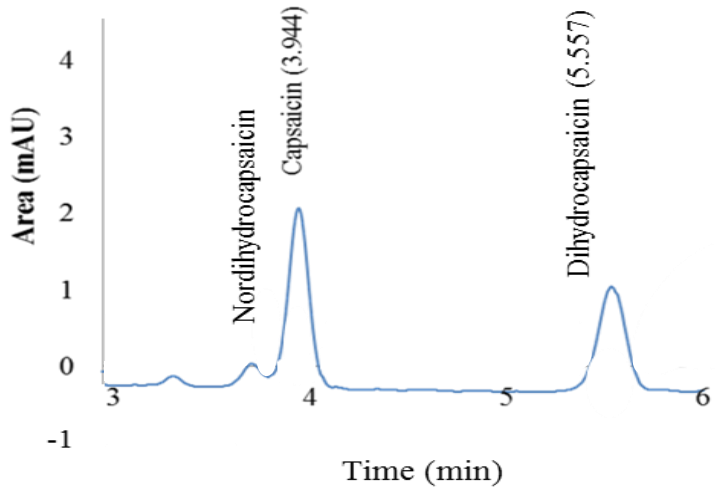


Figure 4.7 Chromatogram of standard capsaicin and dihydrocapsaicin at 15 and 5 mg/L, respectively for chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh.*) using Eclipse plus C18 column (4.6×100 mm, 3.5 µm) by HPLC condition in Table 3.5

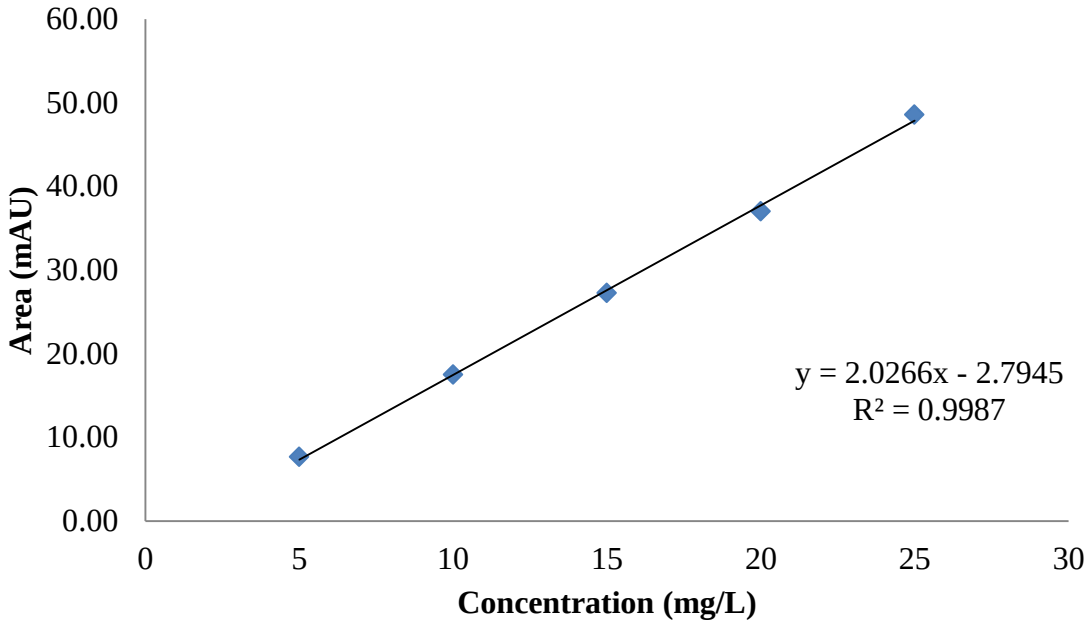


Figure 4.8 The calibration curve of standard capsaicin for chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh.*) using Eclipse plus C18 column (4.6×100 mm, 3.5 µm) by HPLC condition in Table 3.5

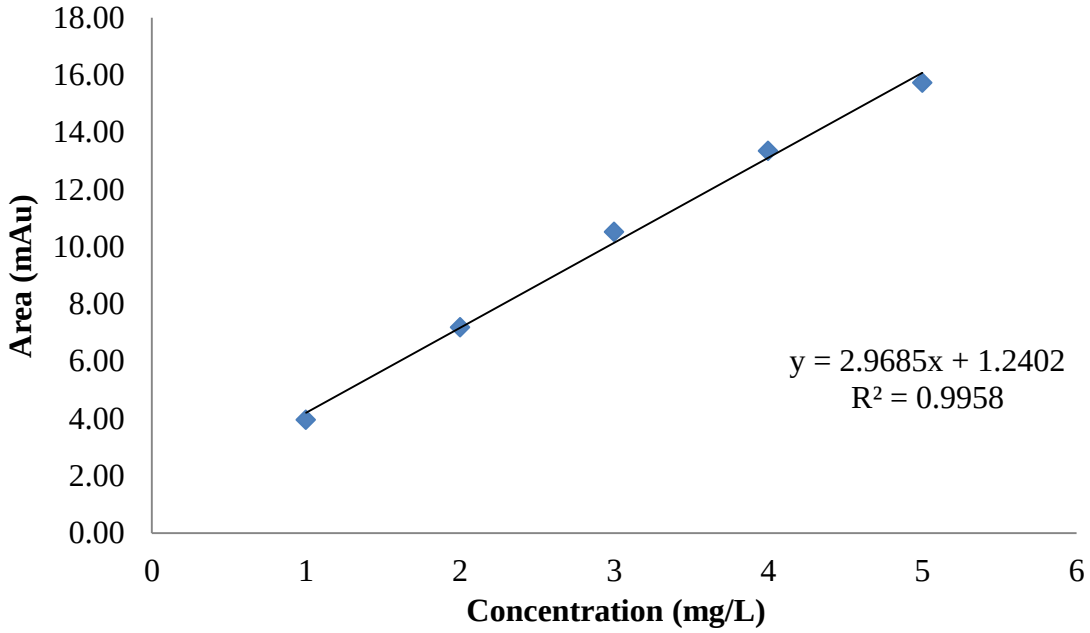


Figure 4.9 The calibration curve of standard dihydrocapsaicin for chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh*.) using Eclipse plus C18 column (4.6×100 mm, 3.5 µm)by HPLC condition in Table 3.5

4.1.1.2 The result of capsaicin and dihydrocapsaicin analysisof chili peppers samples

4.1.1.2.1 Hot chili peppers (*Capsicum annum Linn*.)

Capsaicin and dihydrocapsaicin content from six different geographical origins (Ubonratchathani, Kamphaengphet, Sisaket, Nakhonsawan, Prachuapkhirikhan, and Chiangmai) of hot chili peppers were analyzed using HPLC. The separation of capsaicin and dihydrocapsaicin were well separated within 10 minutes using Eclipse XDB C18 Column (4.6 × 150 mm, 5 ×μm) under HPLC conditions shown in Table 3.5. Both capsaicin and dihydrocapsicin effectively separated with retention times of 3.45 and 6.79 min respectively, which give strong, well-resolved peaks. Figure 4.10 shows the results in a comparison the amounts of capsaicin and dihydrocapsaicin in six different geographical origins of hot chili peppers. The final concentrations of capsaicin and dihydrocapsaicin were calculated using the linear regression equation shown in Figure 4.2 and 4.3, respectively. All analyses were performed in triplicate and the results were expressed as mean ± standard deviation (SD). Capsaicin and dihydrocapsaicin were clearly identified by comparing the retention time of the samples with the retention time of natural standard capaicin and dihydrocapsaicin. Thequantitative HPLC data of Capsaicin and dihydrocapsaicin content of six different geographical origins of hot chili peppers are presented in Table 4.1. The results show that among the various chili peppers samples, the Ubonratchathani sample has the most amounts of capsaicin and dihydrocapsaicin, followed in order of decreasing concentration by samples from Kamphaengphet, Sisaket, Nakhonsawan, Prachuapkhirikhan, and Chiangmai.

Data was analyzed by one-way analysis of variance (ANOVA) to examine the difference amongst their origins. The F-test paired six samples for means at 95% confident level was employed for the evaluation of significant difference as shown in Table 4.2 and 4.3. If the value of F critical is less than the value of F calculation, the evaluation indicated that there are significant differences of the six geographical origins of hot chili peppers.

Table 4.1 Thequantitative HPLC data of capsaicin and dihydrocapsaicin content in six geographical origins of hot chili peppers (*Capsicum annum Linn.)*

Origin	**Capsaicin (mg/kg dry weight)**	**Dihydrocapsaicin (mg/kg dry weight)**
Prachuapkhirikhan	2251.40±1.90	699.02±8.33
Sisaket	2785.06±1.30	852.43±2.84
Nakhonsawan	2621.01±1.10	821.86±2.41
Chiangmai	1357.39±1.82	527.36±3.93
Ubonratchathani	3099.23±2.76	1057.48±013.78
Kamphaengphet	2916.17±2.71	722.67±0.95

Each value in the table was obtained by calculating the average of three experiment ± standard deviation (SD)

Table 4.2 ANOVA table of capsaicin content in six geographical origins of hot chili peppers (*Capsicum annum Linn.)*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5986121.81	5.00	1197224.36	289851.62	4.7E-30	3.11
Within Groups	49.57	12.00	4.13			
Total	5986171.38	17.00				

Table 4.3ANOVA table of dihydrocapsaicin content in six geographical origins of hot chili peppers (*Capsicum annum Linn.)*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	472988.11	5.00	94597.62	1960.32	4.89E-17	3.11
Within Groups	579.07	12.00	48.26			
Total	473567.18	17.00				

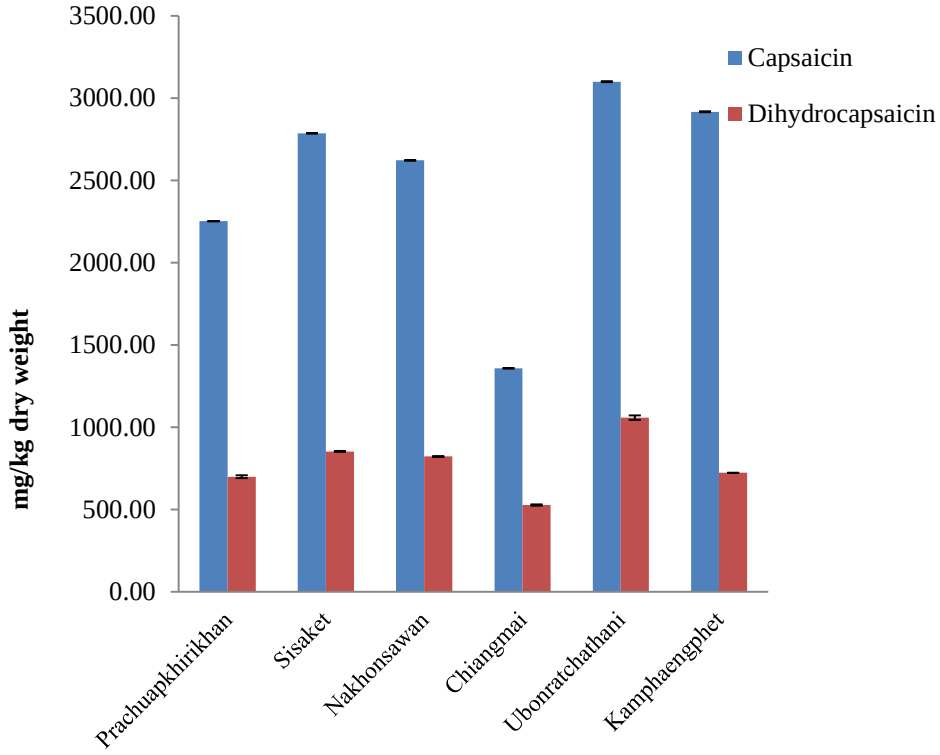


Figure 4.10 Capsaicin and dihydrocapsaicin of six geographical origins of hot chili peppers (*Capsicum annum Linn.*).using Column Eclipse XDB (C18 4.6 × 150 mm, 5 mm) by HPLC condition in Table 3.5

4.1.1.2.2 Bird chili peppers (*Capsicum frutescens Linn.*)

The amount of capsaicin and dihydrocapsaicin of five different geographical origins(Chumphon, Chanthaburi, Pathumthani, Suphanburi, Chiangmai and Tak) in bird chili peppers were analyzed using Eclipse plus C18 column (4.6×100 mm, 3.5 µm) under HPLC conditions in Table 3.5. The column was changed due to clogging from impurities in samples. The separation of capsaicin and dihydrocapsaicin were well separated within 10 minutes and effectively separated with retention times of 4.01 and 5.65 minutes respectively, which gave strong, well-resolved peaks as shown in Figure 4.11. The final concentrations of capsaicin and dihydrocapsaicin were calculated using the linear regression equation in Figure 4.5 and 4.6, respectively. It was found that among various chili pepper samples, the sample from Tak had the most amounts of capsaicin and dihydrocapsaicin, followed in order of decreasing

concentration by samples from Chanthaburi, Pathumthani, Chumphon, and Chiangmai. All analyses were analyzed in triplicate and the results were expressed as mean±standard deviation. Capsaicin and dihydrocapsaicin were clearly identified by comparing the retention time of the samples with the retention time of natural standard capaicin and dihydrocapsaicin. The quantitative HPLC data of capsaicin and dihydrocapsaicin content from six different geographical origins of bird chili peppers are presented in Table 4.4.

All data was analyzed using one-way analysis of variance (ANOVA) to examine the difference amongst their origins. The F-test paired five samples for means at 95% confidence level was employed for the evaluation of significant difference as shown in Table 4.5 and 4.6.The significant difference was considered by F value. If F critical is less than the F calculation, this showed that in the data there are significant differences of six geographical origins of hot chili peppers.

Table 4.4 The quantitative HPLC data of capsaicin and dihydrocapsaicin content of bird chili peppers (*Capsicum annum Linn.)*

Origin	**Capsaicin (mg/kg dry weight)**	**Dihydrocapsaicin (mg/kg dry weight)**
Chumphon	2665.12±0.87	743.40±1.05
Chanthaburi	3273.47±0.31	812.26±1.87
Pathumthani	3160.97±0.92	854.09±1.95
Chiangmai	2499.58±1.02	777.25±1.36
Tak	5306.12±1.49	1501.80±1.11

Each value in the table was obtained by calculating the average of three experiment ± standard deviation (SD)

Table 4.5 ANOVA table of capsaicin content in five geographical origins of bird chili peppers (*Capsicum annum Linn.)*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	15166303.50	4.00	3791575.87	3812859.81	7.27E-31	3.478
Within Groups	9.94	10.00	0.99			
Total	15166313.44	14.00				

Table 4.6 ANOVA table of dihydrocapsaicin content in five geographical origins of bird chili peppers (*Capsicum annum Linn.)*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1213310.49	4.00	303327.62	131732.34	1.48E-23	3.478
Within Groups	23.03	10.00	2.30			
Total	1213333.52	14.00				

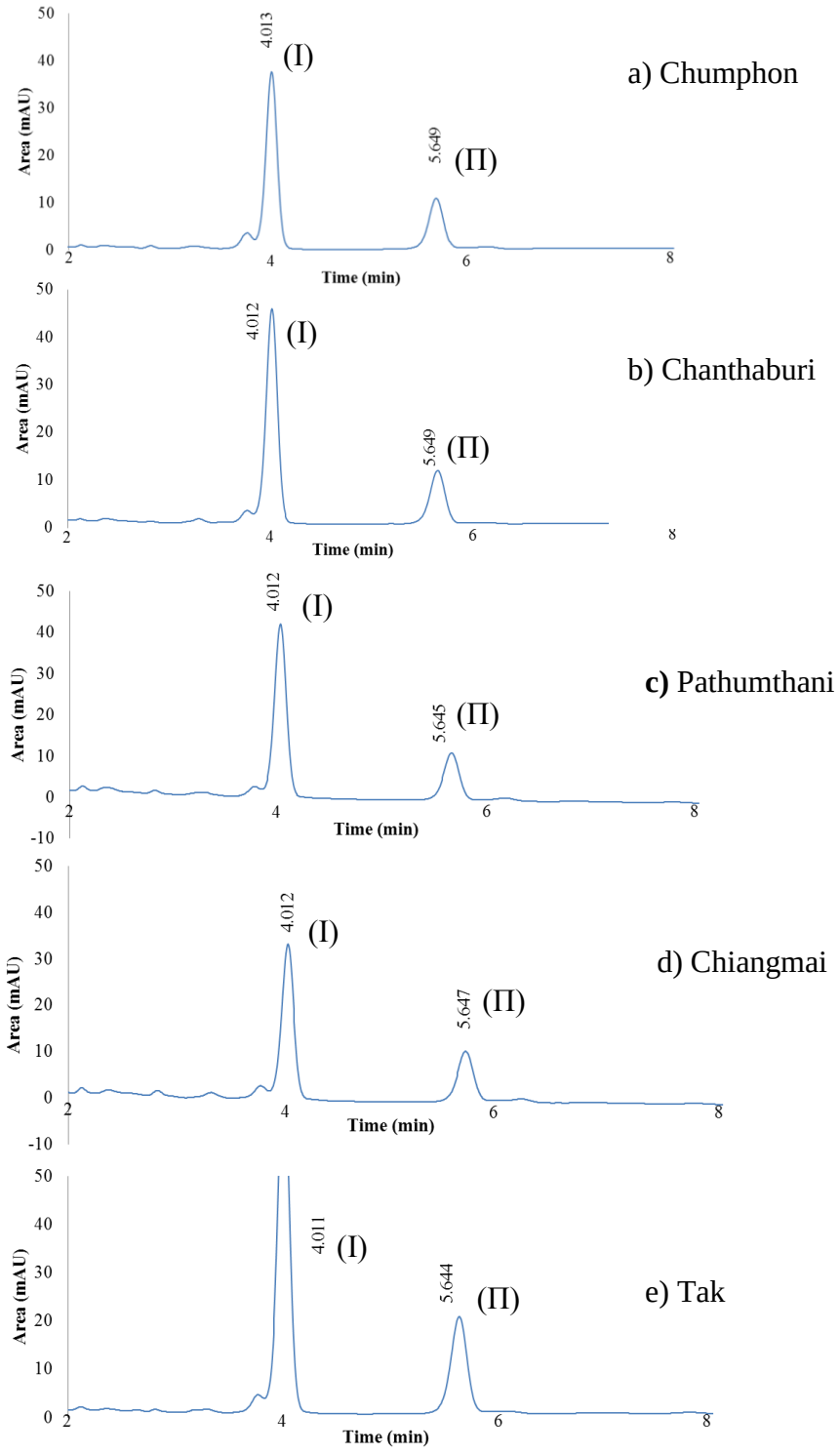


Figure 4.11 HPLC chromatogram of capsaicin (I) and hydrocapsaicin (II) in Bird chili peppers (*Capsicum annum Linn.)* using Eclipse plus C18 column (4.6×100 mm, 3.5 µm) by HPLC condition in Table 3.5

4.1.1.2.3 Chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh.*)

The content of capsaicin and dihydrocapsaicin of four different geographical origins (Tak, Phitsanulok, Chiangmai and Suphanburi) of chili spur peppers were estimated by HPLC analysis .The separation of capsaicin and dihydrocapsaicin were well separated within 10 minutes using Eclipse plus C18 column (4.6×100 mm, 3.5 μm) under HPLC condition in Table 3.5 and effectively separated with retention times of 3.94 and 5.56 min, respectively. The chromatograms of the four different geographical origins of chili spur peppers are shown in Figure 4.12. The final concentrations of capsaicin and dihydrocapsaicin were calculated using linear regression equation in Figure 4.8 and 4.9, respectively. The result indicated that the Phitsanulok sample had the greatest capsaicin and dihydrocapsaicin content, followed in order of decreasing concentration by samples from Suphanburi, Chaingmai, and Tak. All analyses were performed in triplicate and the results were expressed as mean±standard deviation (mean ± SD). Capsaicin and dihydrocapsaicin were clearly identified by comparing the retention time of the samples with the retention time of natural standard capaicin and dihydrocapsaicin. The quantitative HPLC data of Capsaicin and dihydrocapsaicin content of chili spur peppers is shown in Table 4.7.

All data was analyzed using one-way analysis of variance (ANOVA) to examine the difference in geographical origin. The F-test paired four samples for means at 95% confident level was employed for the evaluation of significant difference as shown in Table 4.8 and 4.9.The significant difference was considered by F value. If the value of F critical is less than the value of F calculation, the evaluation indicated that there are significant differences of the four geographical origins of chili spur peppers.

Table 4.7 The quantitative HPLC data of capsaicin and dihydrocapsaicin content of chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh.*)

Origin	**Capsaicin (mg/kg dry weight)**	**Dihydrocapsaicin (mg/kg dry weight)**
Tak	108.20±0.06	17.51±0.05
Phitsanulok	257.40±0.17	34.02±0.34
Chiangmai	126.70±0.26	12.43±0.23
Suphanburi	247.20±0.45	38.54±0.15

Each value in the table was obtained by calculating the average of three experiment ± standard deviation (SD)

Table 4.8 ANOVA table of capsaicin content in four geographical origins of chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh.*)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	55226.90	3.00	18408.97	239628.16	3.77E-20	4.07
Within Groups	0.61	8.00	0.08			
Total	55227.51	11.00				

Table 4.9 ANOVA table of dihydrocapsaicin content in four geographical origins of chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh.*)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1431.68	3.00	477.23	9907.18	1.29E-14	4.07
Within Groups	0.39	8.00	0.05			
Total	1432.06	11.00				

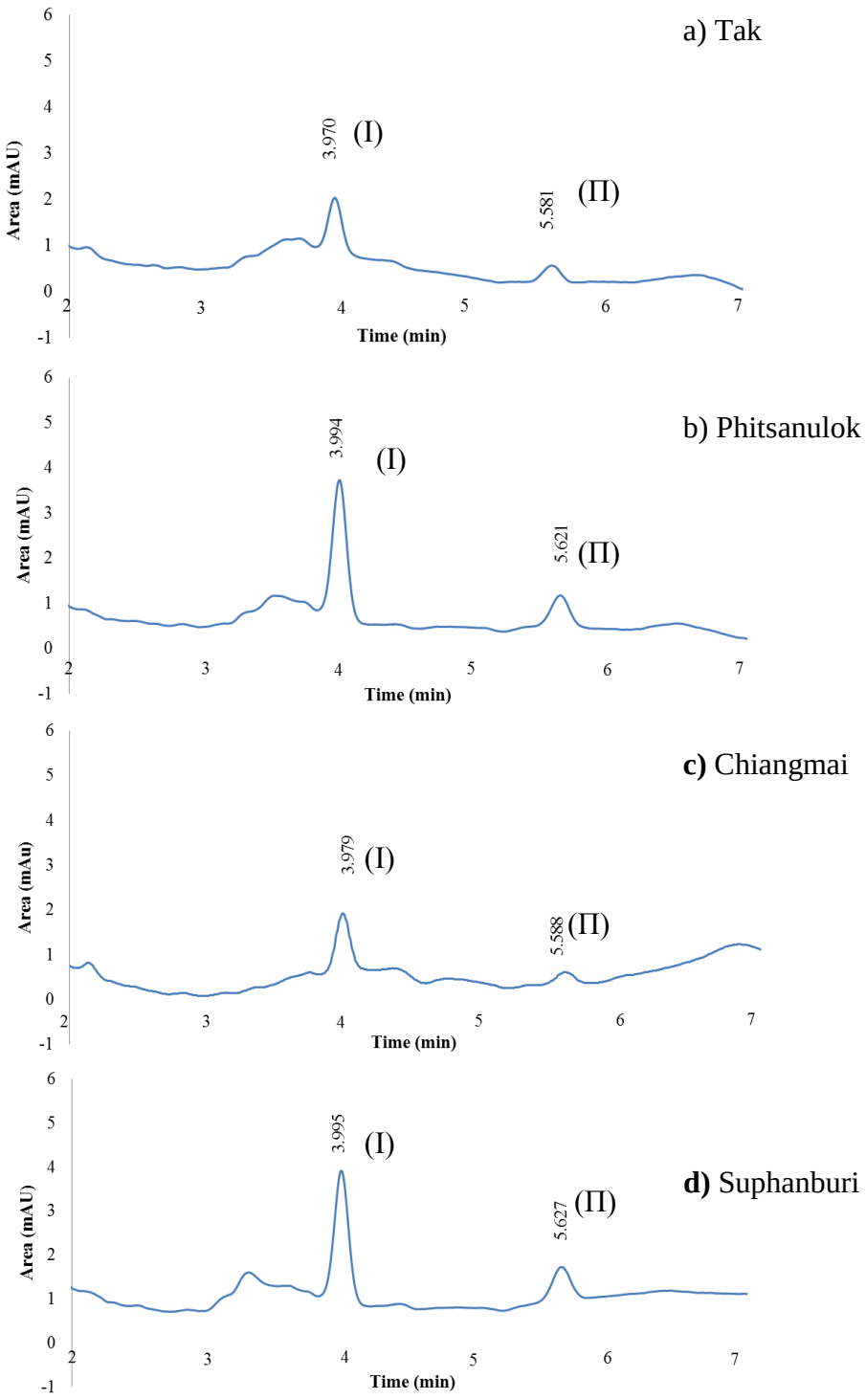


Figure 4.12 HPLC chromatogram of capsaicin (I) and hydrocapsaicin (II) in chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh.*) using Eclipse plus C18 column (4.6×100 mm, 3.5 µm) by HPLC condition in Table 3.5

4.1.2 Volatile compounds analysis

4.1.2.1 The result of volatile compounds analysis of chili peppers samples

4.1.2.1.1 Hot chili pepper (*Capsicum frutescens Linn*.)

Hot chili pepper samples were randomly purchased from Talaadthai market. These were harvested in September 2012 from six geographical origins and hot chili pepper samples were prepared as described in Section 3.5.2. The quantity and quality of VOCs of various bird chili peppers from six different origins which included Ubonratchathani, Kamphaengphet, Sisaket, Nakhonsawan, Prachuapkhirikhan, and Chiangmai were analyzed using HS-GC-MS conditions as shown in Section 3.4.2. VOCs were identified by comparing the MS spectrum with the NIST library database. Identified were sixteen VOCs as the main volatile components of the hot chili pepper samples. The GC-MS chromatogram of sixteen VOCs of hot chili peppers from Ubonratchathani, Kamphaengphet, Sisaket, Nakhonsawan, Prachuapkhirikhan, and Chiangmai were shown in Figure 4.13. Table 4.4 showed the quantitative and qualitative of sixteen VOCs of six different origins of hot chili peppers.

Table 4.10 The area of major organic compounds in hot chilli pepper of six different origins.

No.	Compounds	Peak area (mAU)					
		Prachuapkhirikhan	**Sisaket**	**Nakhonsawan**	**Chiangmai**	**Ubonratchathani**	**Kamphaengphet**
1	Ethanol	675019	1394509	2305220	2007400	2005598	3450797
2	Ethene	42923	60427	50033	55954	102612	24759
3	Pentanal	49744	69473	59610	56256	102754	37559
4	Prop-2-enyl hexanoate	19981	ND	26423	29814	51930	19360
5	Prop-2-yn-1-ol	30120	43308	43777	50700	46842	58202
6	Pentan-3-one	49309	48805	56504	53222	44029	86952
7	2-Methyl-3-vinyl-oxirane	18300	21854	16484	25038	66068	80158
8	Hexan-l-ol	182740	168802	53467	19740	46388	29749
9	4-Methyl-1-pentanol	61680	48257	27754	73080	135822	78822
10	(E)-hex-2-enal	1104601	729597	311634	121520	260183	167408
11	(E)-hex-2-en -1-ol	475138	234309	217342	224949	213016	375675
12	Hexyl 2-methylpropanoate	21853	33274	20512	11058	167359	16102
13	Hexyl 2-methylbutanoate	73143	125456	72861	31493	378456	85826
14	Hexyl 3-methylbutanoate	22375	34409	22258	65524	104310	25873
15	6-ethyl-2-methyldecane	29510	40760	29823	44720	64514	50061
16	1-(6-nitro-1,3-benzodioxol-5-yl)-N-phenethylmethanimine	ND	39552	25595	37343	71330	48706.67

Each value in the table was obtained by calculating the average of three experiment

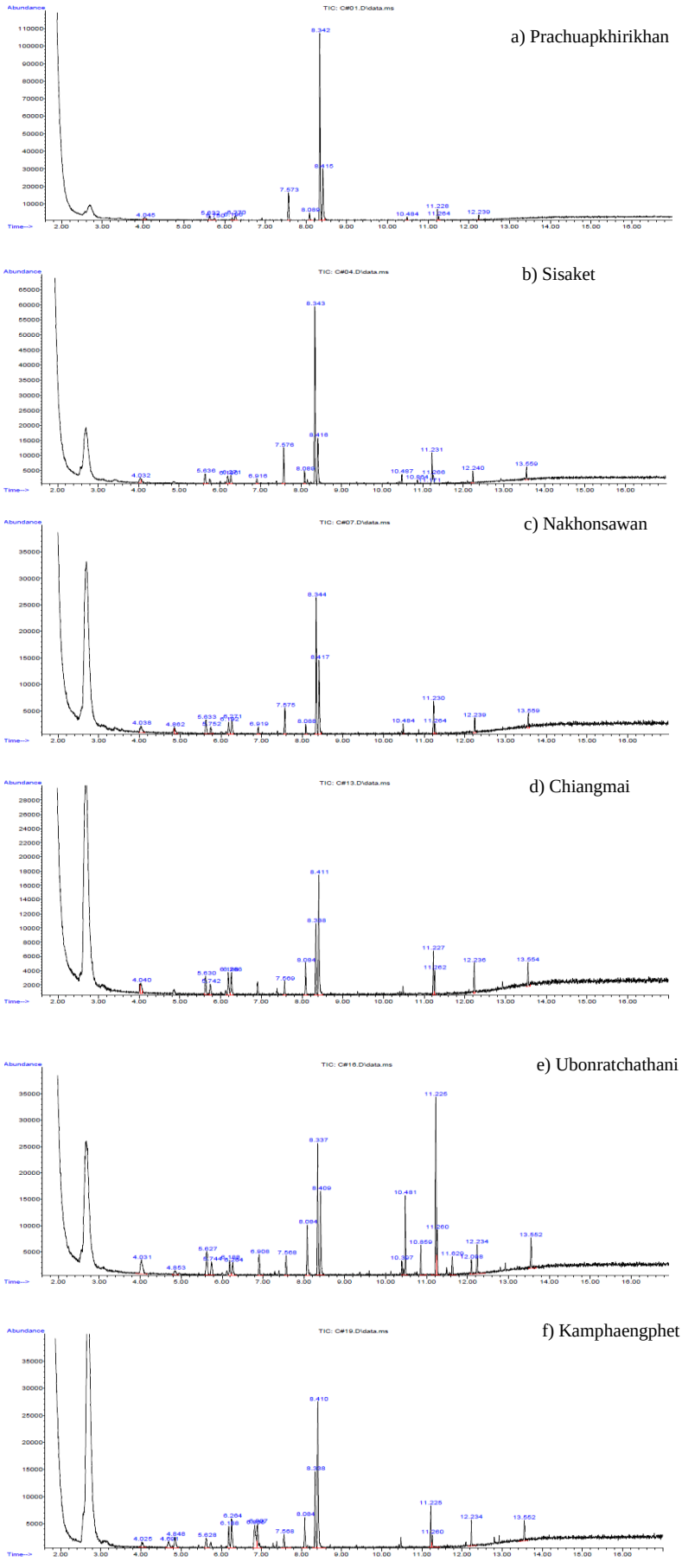


Figure 4.13 The chromatogram of hot chili pepper (*Capsicum frutescens Linn*.) sample using HS-GC-MS.

4.1.2.1.2Bird chili pepper (*Capsicum frutescens Linn*.)

Bird chili pepper samples were randomly purchased from Talaadthai market. These were harvested in August 2012 from five geographical origins and bird chili pepper samples were prepared as described in Section 3.5.2. The volatile compounds (VOCs) from five different origins in hot chili peppers which included Chumphon, Chanthaburi, Pathumthani, Chiangmai and Tak were analyzed using HS-GC-MS conditions as shown in Section 3.4.2. VOCs were identified by comparing the MS spectrum with the NIST library database. We identified nineteen VOCs as the main volatile components of the bird chili pepper samples. The GC-MS chromatogram of nineteen VOCs of bird chili peppers of Chumphon, Chanthaburi, Pathumthani, Chiangmai and Tak are shown in Figure 4.14. Table 4.5 shows the quantitative and qualitative of nineteen VOCs from five different origins of bird chili peppers.

Table 4.11 The area of major organic compounds in bird chilli pepper of five different origins.

No.	Compounds	Peak area (mAU)				
		Chumphon	**Chanthaburi**	**Pathumthani**	**Chiangmai**	**Tak**
1	Hexan-l-ol	1676528	1290475	2895858	1749168	85412
2	4-Methyl-1-pentanol	1395889	740042	981612	276071	832619
3	(E)-hex-2-enal	518490	551910	638393	537214	204214
4	(E)-hex-2-en -1-ol	1930388	2318346	2787561	2815249	1203803
5	(3Z)-3,7-dimethylocta-1,3,6-triene	ND	17915	352981	38397	27802
6	Hexyl 2-methylpropanoate	2706589	1009062	1503486	437366	833240
7	2-methylbutyl 2-methylpropanoate	350413	99695	432208	54176	97015
8	Hexyl butyrate	292239	43550	96444	97767	38468
9	Hexyl 2-methylbutanoate	3356630	2536395	2379537	1192268	2461013
10	Hexyl 3-methylbutanoate	2730526	1558970	1925265	1433591	1628672
11	3-methylbutyl hexanoate	394964	83220	73732	265919	40927

Each value in the table was obtained by calculating the average of three experiment ± standard deviation (SD)

ND : Not detected

Table 4.11 (continued) The area of major organic compounds in bird chilli pepper of five different origins.

No.	Compounds	Peak area (mAU)				
		Chumphon	**Chanthaburi**	**Pathumthani**	**Chiangmai**	**Tak**
12	Hexyl 2-ethylbutanoate	657183	410755	1034297	359128	451401
13	Hexyl-n-valerate	924919	248963	242465	316838	217219
14	Hexyl hexanoate	4358943	1412870	984819	2082829	1184121
15	3-Methyldodecane	154412	60720	39823	35089	59159
16	6-Ethyl-2-methyldecane	844032	227290	362186	460893	186616
17	(3Z)-2-Methyl-3-undecene	131153	129388	89715	48495	131108
18	Decane	75266	56132	45380	27402	72413
19	3,4-bis (methoxycarbonyl) benzoic acid	36773	14385	14108	15898	19038

Each value in the table was obtained by calculating the average of three experiment ± standard deviation (SD)

ND: Not detected

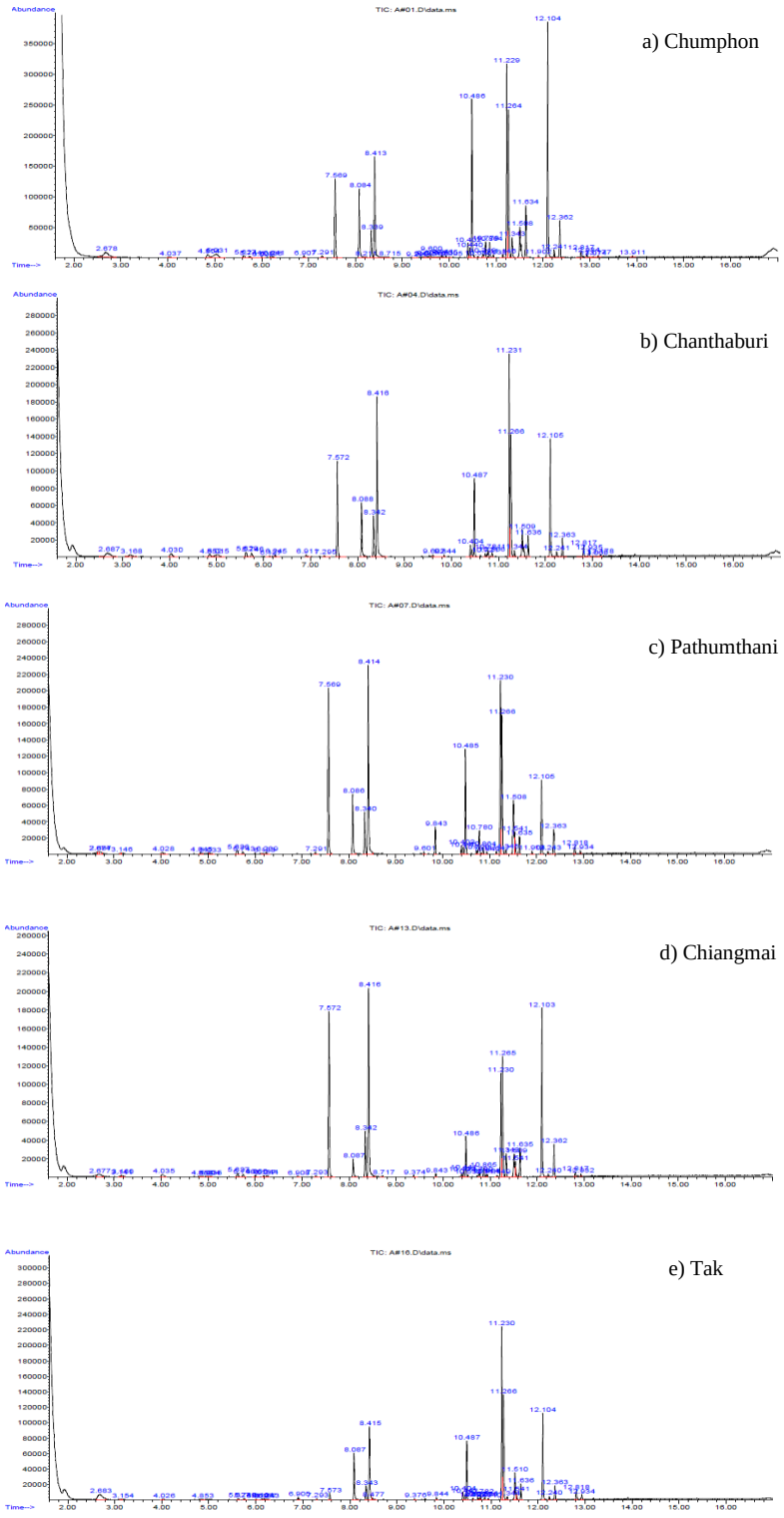


Figure 4.14 The chromatogram of bird chili pepper (*Capsicum frutescens Linn.*) samples using HS-GC-MS

4.1.2.1.3Chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh*.)

Four different origins of chili spur pepper samples were randomly purchased from Talaadthai market including Tak, Phitsanulok, Chiangmai and Suphanburi. These were harvested in September 2012 from four geographical origins and chili spur pepper samples were prepared as described in Section 3.5.2. The quantity of VOCs of various chili spur pepper samples from four different origins were analyzed using HS-GC-MS conditions as shown in Section 3.4.2. VOCs were identified by comparing the MS spectrum with the NIST library database. Identified were fourteen VOCs as the main volatile components of the chili spur peppersamples. The GC-MS chromatogram of fourteen VOCs of hot chili peppers from Tak, Phitsanulok, Chiangmai and Suphanburi are shown in Figure 4.15. Table 4.6 shows the quantitative and qualitative of fourteen VOCs from four different origins in chili spur pepper samples.

Table 4.12 The area of major organic compounds in chili spurs pepper (*Capsicum annuum Linn. Var acuminatum Fingerh*.) of four different origins.

No.	Compounds	Peak area (mAU)			
		Tak	**Phitsanulok**	**Chiangmai**	**Suphanburi**
1	Ethanal	27602	218632	202447	579452
2	o-Methylisourea hydrogen sulfate	27710	38735	71910	ND
3	Acetic acid, c yano	ND	ND	ND	209433
4	Ethoxyethene	ND	ND	ND	22698
5	N-[(E)-2-Nitrosoethenyl] hydroxylamine	15447	18663	30607	32526
6	Prop-2-enyl formate	ND	ND	ND	14608
7	2-Nitroethanol	93271	123021	154994	295496
8	Hexan-l-ol	69400	57920	109159	ND
9	(E)-hex-2-enal	25304	12782	78186	ND
10	(E)-hex-2-en -1-ol	46600	11849	78162	13499
11	Cyclopentene-1-carboxylic acid methyl ester	ND	ND	13847	ND
12	2,3,4,5,6-Pentafluorobenzylalcohol	10570	14456	14531	26220
13	2-Phenoxy-1-ethanol.	31162	4055	47481	28584
14	9H-fluorene-2-carboxylic acid	13011	ND	ND	ND

Each value in the table was obtained by calculating the average of three experiment ± standard deviation (SD)

ND : Not detected

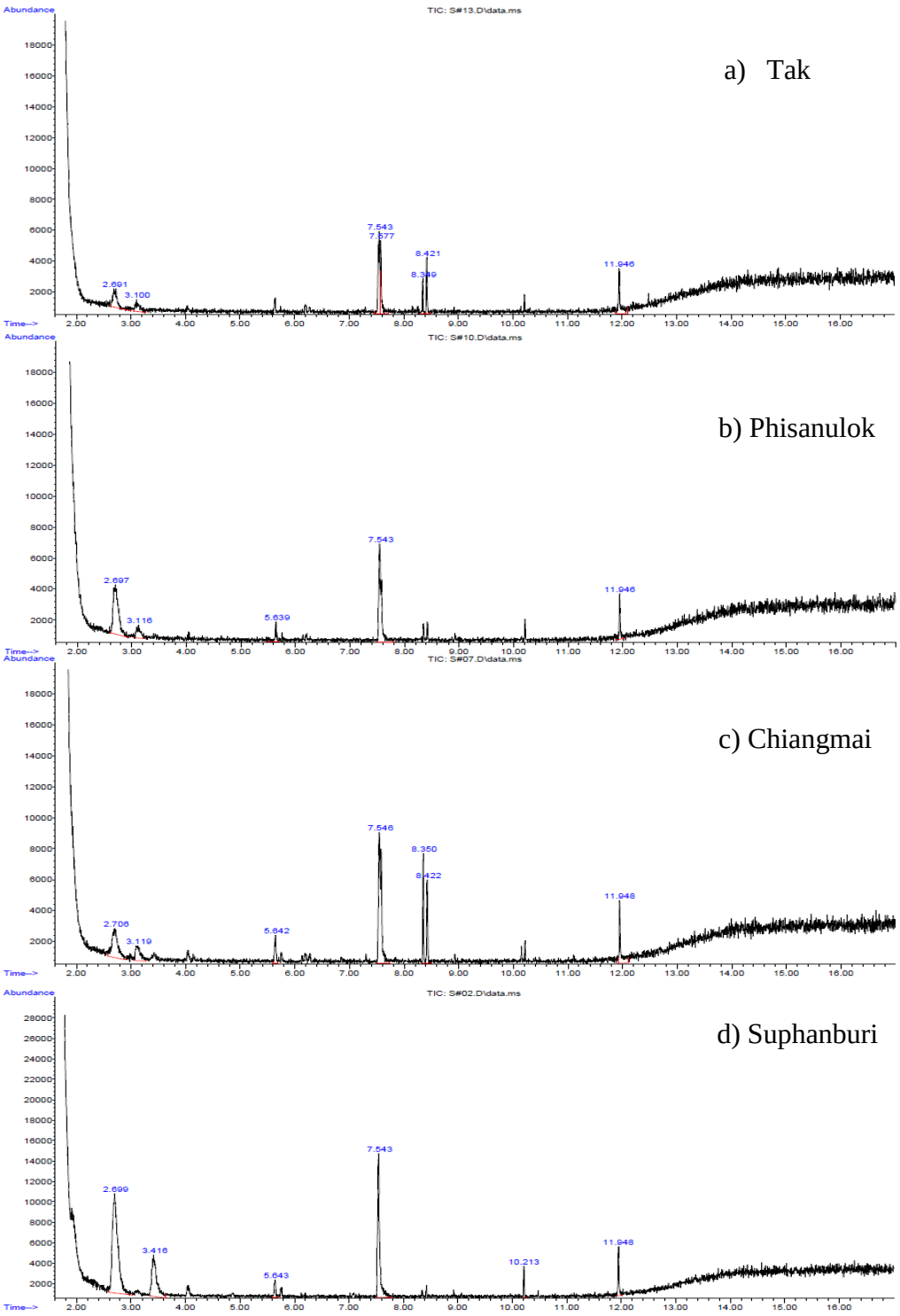


Figure 4.15 The chromatograms of chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh.*) samples using HS-GC-MS

For each of the varieties of the maturity state of hot chili pepper samples, bird chili pepper samples and chili spur peppers samples were randomly purchased from Talaadthai market. The occurrence (or not) of each variety is shown in Table 4.7.It was found that the main VOC in each variety were compounds of numbers 11 (hexan-l-ol), 13 ((E)-hex-2-enal) and 14 ((E)-hex-2-en -1-ol) as seen in Table 4.7. Compound numbers 4 (ethene), 5 (pentanal), 6 (prop-2-enyl formate), 7 (prop-2-yn-1-ol), 8 (pentan-3-one) and 9 (2-methyl-3-vinyl-oxirane) as seen in Table 4.8 were only found in hot chili pepper varieties, of which these VOCs can be used as markers of the hot chili pepper variety. Compounds of numbers 15 ((3Z)-3,7-dimethylocta-1,3,6-triene), 19 (2-methylbutyl 2-methylpropanoate), 20 (hexyl butyrate), 23 (3-methylbutyl hexanoate), 24 (hexyl 2-ethylbutanoate), 25(hexyl-n-valerate), 27 (hexyl hexanoate), 31(decane), 32 (3,4-bis (methoxycarbonyl) benzoic acid) and 33 (1-(6-nitro-1,3-benzodioxol-5-yl)-N-phenethylmethanimine) were found only in the bird chili pepper varietyas markers differentiate bird chili pepper variety from other varieties.Compounds of numbers 2 (o-methylisourea hydrogen sulfate), 10 (2-nitroethanol), 17 (2, 3, 4, 5, 6-pentafluorobenzyl alcohol) and 26 (2-phenoxy-1-ethanol) were found only inchili spur peppers, of which these VOCs can be used as markers of chili spur pepper variety.

Table 4.13 Comparison of organic composition of three varieties of chili pepper samples

No.	Compound name	Detected compounds		
		Hotchili peppers	**Bird chili peppers**	**Chili spur peppers**
1	Ethanol	+	+	+
2	o-Methylisourea hydrogen sulfate	-	-	+
3	Acetic acid, c yano	-	-	+
4	Ethene	+	-	-
5	Pentanal	+	-	+
6	Prop-2-enyl formate	+	-	-
7	Prop-2-yn-1-ol	+	-	-
8	Pentan-3-one	+	-	-
9	2-Methyl-3-vinyl-oxirane	+	-	-
10	2-Nitroethanol	-	-	+
11	Hexan-1-ol	+	+	+
12	4-Methyl-1-pentanol	+	+	-
13	(E)-Hex-2-enal	+	+	+
14	(E)-Hex-2-en -1-ol	+	+	+
15	(3Z)-3,7-Dimethylocta-1,3,6-triene	-	+	-
16	Cyclopentene-1-carboxylic acid methyl ester	-	-	+
17	2,3,4,5,6-Pentafluorobenzylalcohol	-	-	+
18	Hexyl 2-methylpropanoate	+	+	-
19	2-Methylbutyl 2-methylpropanoate	-	+	-

+ detected compound and – not detected compound

Table 4.13 (continued) Comparison of organic composition of three varieties of chili pepper samples

No.	Compound name	Detected compounds		
		Hot chili peppers	**Bird chili peppers**	**Chili spur peppers**
20	Hexyl butyrate	-	+	-
21	Hexyl 2-methylbutanoate	+	+	-
22	Hexyl 3-methylbutanoate	+	+	-
23	3-Methylbutyl hexanoate	-	+	-
24	Hexyl 2-ethylbutanoate	-	+	-
25	Hexyl-n-valerate	-	+	-
26	2-Phenoxy-1-ethanol	-	-	+
27	Hexyl hexanoate	-	+	-
28	6-Ethyl-2-methyldecane	+	+	-
29	9H-fluorene-2-carboxylic acid	-	-	+
30	(3Z)-2-Methyl-3-undecene	-	+	-
31	Decane	-	+	-
32	3,4-Bis (methoxycarbonyl) benzoic acid	-	+	-
33	1-(6-nitro-1,3-benzodioxol-5-yl)-N-phenethylmethanimine	+	-	-
34	Total phenolic compound	+	+	+
35	Capsaicin	+	+	+
36	Dihydrocapsaicin	+	+	+

+ detected composition, - not detected composition

4.1.3 Total phenolic content analysis

4.1.3.1 The result of standard calibration curves

In this study, the amount of total phenolic content was measured by the Folin-Ciocalteu method in section 3.4.3. The Folin-ciocalteu reagent is sensitive in reducing compounds, thereby producing a blue color upon reaction. The blue color is measured using UV-VIS spectrophotometer. Gallic acid was used as a standard compound. The wavelength of 760 nm is the maximum absorption of gallic acid, which is used for quantitative analysis of total phenolic in samples. The total phenolic content in chili peppers was calculated as milligrams of gallic acid equivalent / 100 g FW (GAE/100 g FW).

The standard gallic acid solutions covered the concentration range of 10-90 mg/L. The calibration curve of 5 points was plotted by peak area versus concentration. The calibration curve of hot chili pepper, bird chili pepper and chili spur pepper can be acceptable for quantitation because this method provided well the correlative coefficient (R^2) that was 0.9994, 0.996 and 0.9996, respectively. The slope value, y-intercept and correlation coefficient of hot chili pepper, bird chili pepper and chili spur pepper are shown in Figure 4.16, 4. 17 and 4.18, respectively.

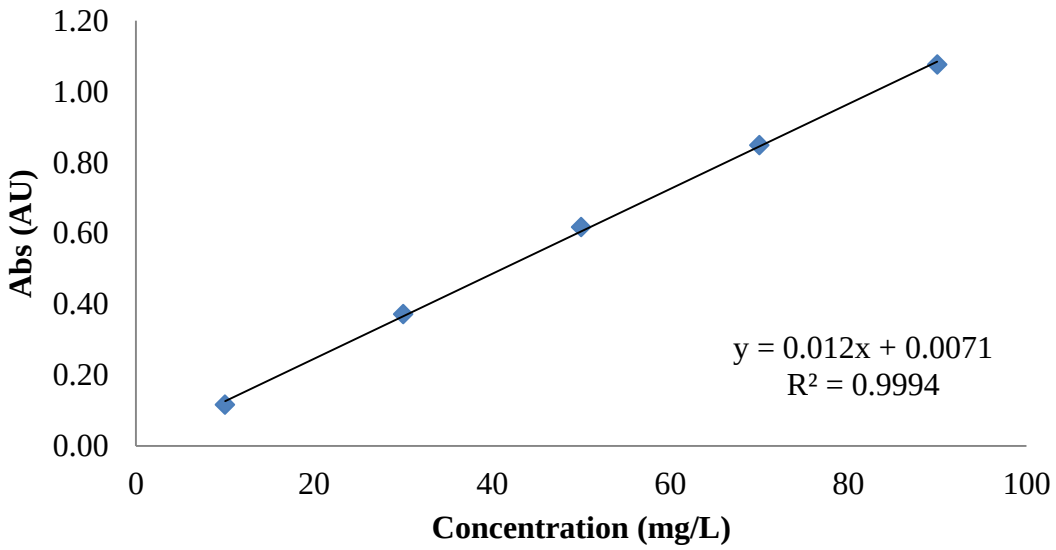


Figure 4.16 The calibration curve of standard gallic acid for hot chili pepper (*Capsicumfrutescens Linn*.)

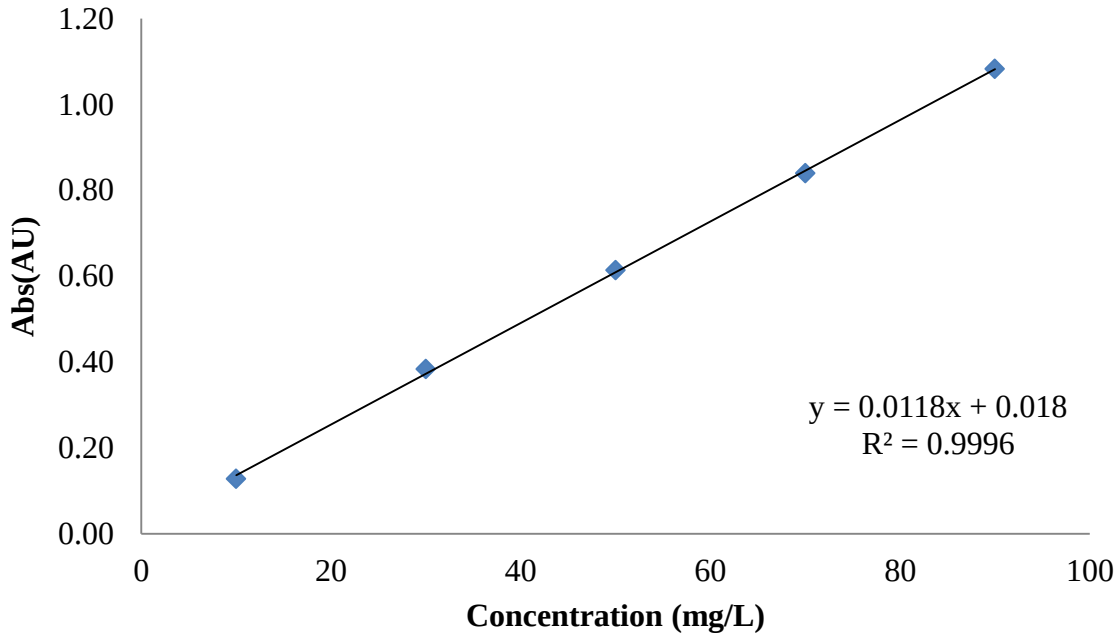


Figure 4.17 The calibration curve of standard gallic acid for bird chili pepper (*Capsicum frutescens Linn*.)

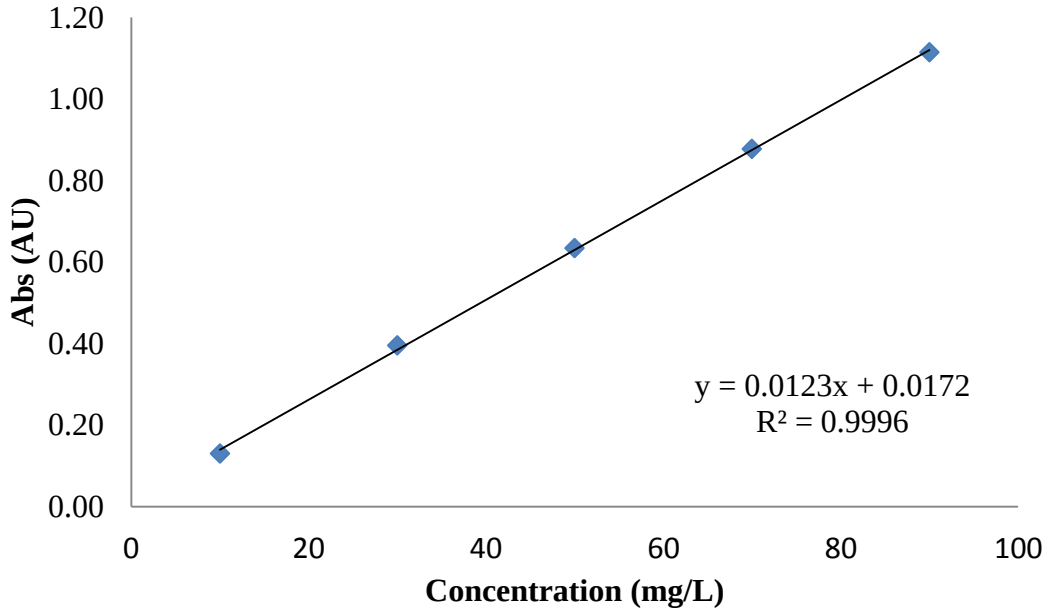


Figure 4. 18 The calibration curve of standard gallic acid for chili spurs pepper (*Capsicum annuum Linn. Var acuminatum Fingerh*.)

4.1.3.2 The result of total phenolic content analysis

All chili samples were randomly purchased from Talaadthai market. The geographical origin and details of these chili samples are listed in Table 3.8. All chili peppers were prepared as described in Section 3.5.3. The total phenolic content was determined by the Folin-Ciocalteu method as follows in Section 3.4.3. The final concentrations of hot chili peppers, bird chili peppers and chili spur peppers were calculated using linear regression equation in Figure 4.16, 4.17 and 4.18, respectively. The total phenolic content calculated as milligrams of gallic acid equivalent / 100 g FW (GAE/100 g FW).The UV-Vis spectrums at wavelength 760 nm of hot chili peppers, bird chili peppers and chili spur pepper are shown in Figure 4.19, 4.20 and 4.21, respectively. Table 4.8 is divided into 3 parts. The first part is for hot chili peppers showing that the

Chiangmai sample had the most total phenolic content, followed in order of decreasing content by Ubonratchathani, Sisaket, Nakhonsawan, Kamphaengphet, and Prachuapkhirikhan. The second part shows that the bird chili peppers from the Pathumthani sample have the most total phenolic content, followed in order of decreasing content by Chiangmai, Tak, Chumphon and Chanthaburi. The third part, chili spur pepper from the Suphanburi sample showed the greatest total phenolic content, followed in order of decreasing content by Chiangmai, Tak, and Phitsanulok. The measured total phenolic contents of 15 all chili samples are shown in Table 4.14. The results are reported as mean values for three replicates±standard deviation (mean ± SD).

The data were analyzed by one-way analysis of variance (ANOVA) to examine the difference among origins. The F-test paired each different origin from the varieties of chili samples for means at 95% confidence level was employed for the evaluation of significant difference as shown in Table 4.15, 4.16 and 4.17. If the value of F critical is less than the value of F calculation, the evaluation indicates that there are significant differences of each different the origins of each varieties of chili samples.

Table 4.14 Total phenolic content of all chili peppers samples.

Varieties	**Origin**	**Total phenolic content (mg gallic acid / 100 g FW)**
Hot chili peppers (*Capsicum frutescens Linn.*)	Prachuapkhirikhan	2.25±0.05
	Sisaket	2.36±0.02
	Nakhonsawan	2.32±0.16
	Chiangmai	2.75±0.02
	Ubonratchathani	2.69±0.01
	Kamphaengphet	2.32±0.03
Bird chili peppers (*Capsicum frutescens Linn.*)	Chumphon	2.01 ± 0.01
	Chanthaburi	2.00 ± 0.03
	Pathumthani	2.27 ± 0.02
	Chiangmai	2.26 ± 0.01
	Tak	2.24 ± 0.04
Chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh.*)	Tak	1.15 ± 0.01
	Phitsanulok	1.10 ± 0.03
	Chiangmai	1.44 ± 0.04
	Suphanburi	1.52 ± 0.01

Each value in the table was obtained by calculating the average of three experiment ± standard deviation (SD)

Table 4.15 ANOVA table of total phenolic content in six geographical origins of hot chili peppers (*Capsicum annum Linn.)*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.67	5.00	0.13	25.72	0.00	3.11
Within Groups	0.06	12.00	0.01			
Total	0.74	17.00				

Table 4.16 ANOVA table of total phenolic content in five geographical origins of bird chili peppers (*Capsicum annum Linn.)*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.22	4.00	0.06	103.40	0.00	3.48
Within Groups	0.01	10.00	0.00			
Total	0.23	14.00				

Table 4.17 ANOVA table of total phenolic content in four geographical origins of chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh*.)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.39	3.00	0.13	194.70	0.00	4.07
Within Groups	0.01	8.00	0.00			
Total	0.39	11.00				

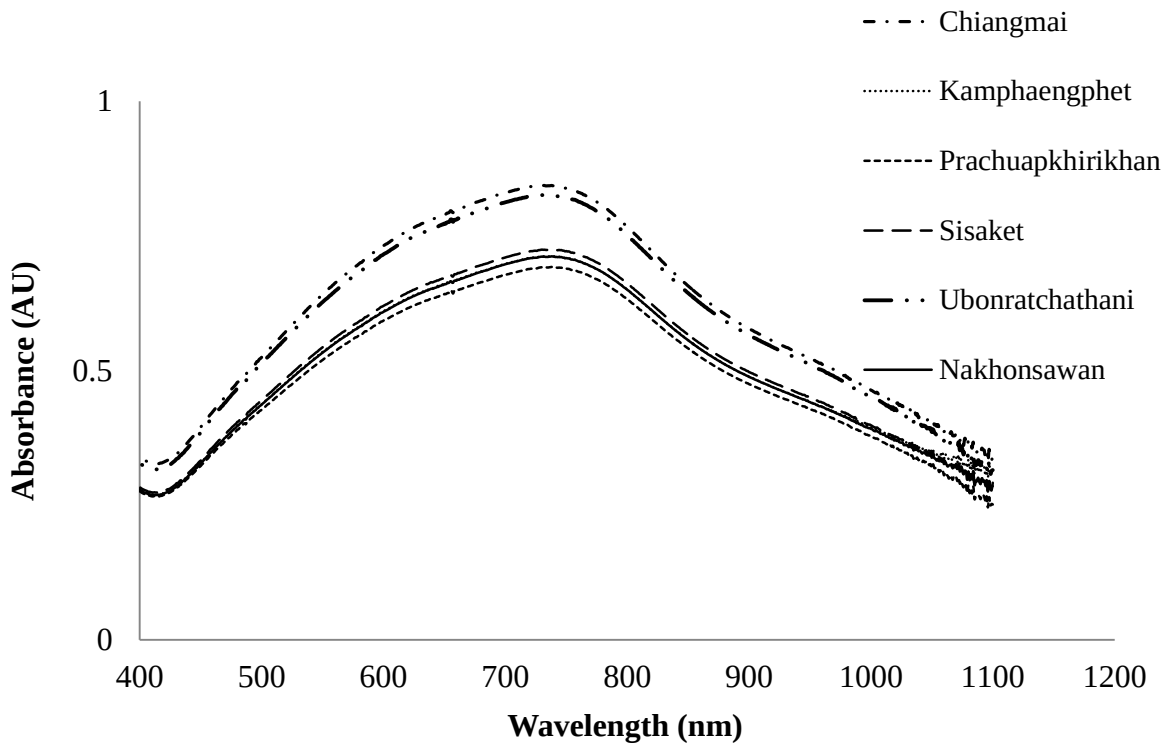


Figure 4.19 UV-VIS spectrums at 400-1100 nm of hot chili peppers (*Capsicum frutescens Linn.*)

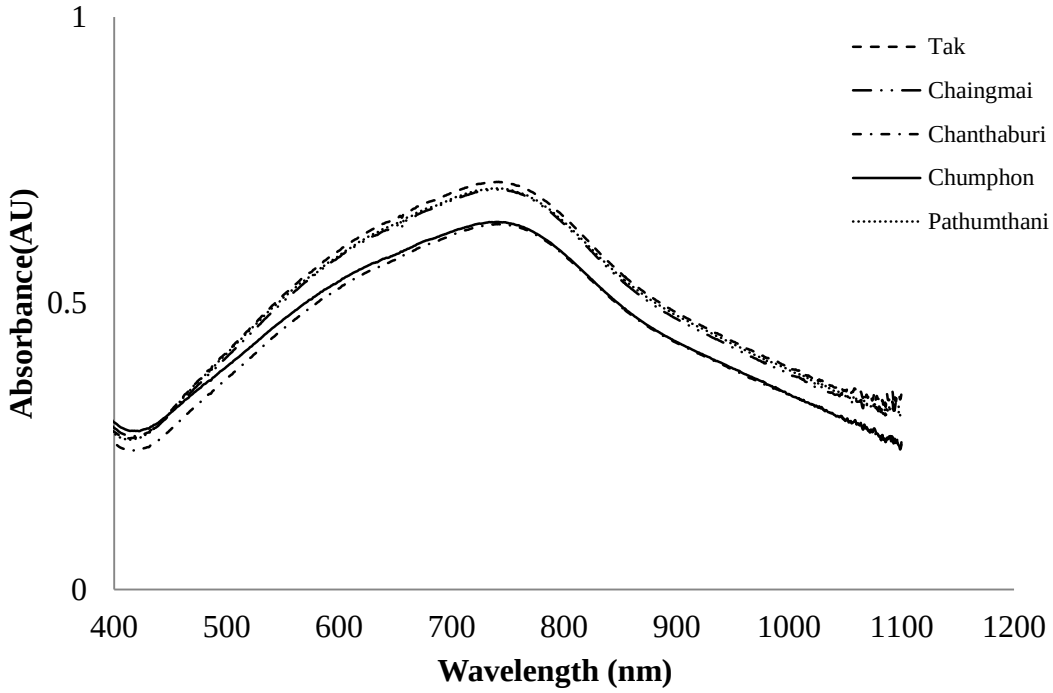


Figure 4.20 UV-VIS spectrums at 400-1100 nm of bird chili (*Capsicum frutescens Linn*.)

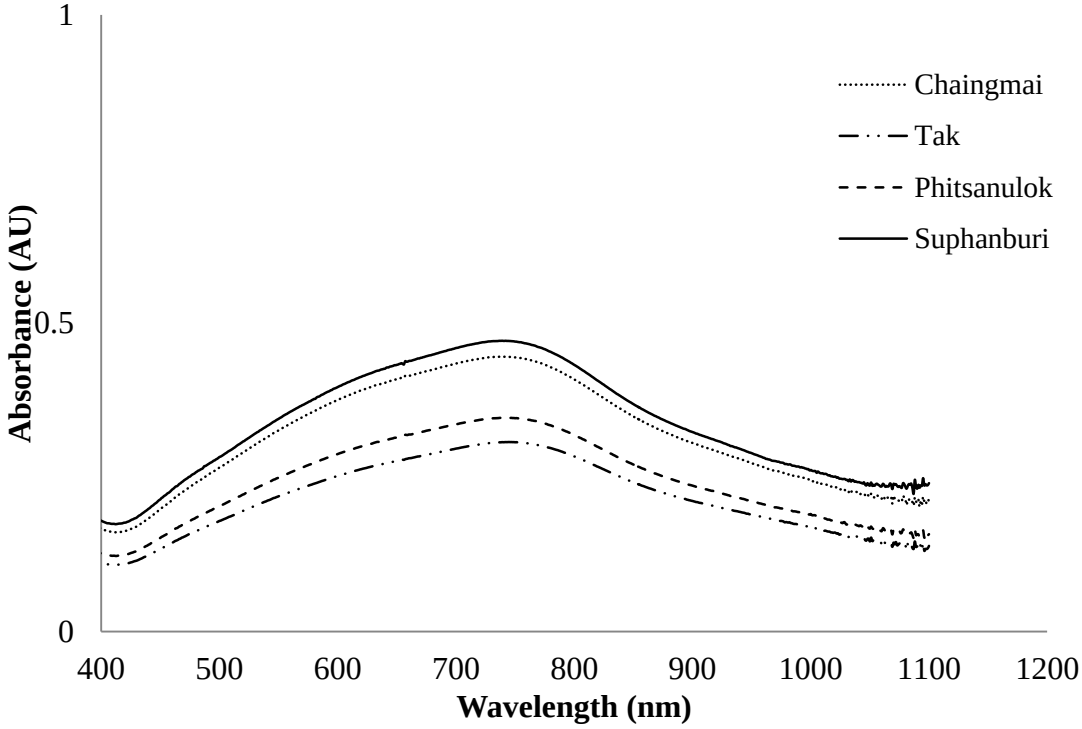


Figure 4.21 UV-VIS spectrums at 400-1100 nm of chili spur pepper (*Capsicum annuum Linn. Var acuminatum Fingerh*.)

4.2 Discrimination of Thai chili peppers

From the result in section 4.1, the raw data were standardized to present numerical data from many experiments on the same scale, and thus facilitate comparisons. The chemometric analysis was performed using the Matlab software program. In this study, the discrimination of chili peppers divided was into two parts. In first part, samples were classified according to different geographical origins of each variety of chili pepper. In the second part, samples were grouped according to varieties of chili peppers. Geographical origin and variety of chili samples were discriminated by chemometric method such as PCA, CA, and LDA.

4.2.1 Origins of chili peppers

4.2.1.1 Hot chili peppers (*Capsicum frutescens Linn*.)

The CA results show similarities in the data in the form of a dendrogram. In the figure 4.22, the X-axis represents the number of samples, while the Y-axis represents the Euclidean distance. The dendrogram illustrated that the samples could be categorized into 6 groups of origin at the distance of 0.3. Due to CA using less information than PCA, CA tended to show data which were not indicated in relationships between samples and compounds (variables). Hence PCA was used to classify different geographical according to organic composition (variables).

Pre-processing stage outputs of hot chili peppers are grouped by chemometric technique. The PCA results in Figure 4.23 are shown as score and loading plots. The score plot (Figure. 4.23a) emphasisesthe relationships between samples, while the loading plot shows the relationship amongst the variables. Two principle components were sufficient to account for 72.16% of the total variance for score and loading plot. PC1 and PC2 have a variance of 47.84% in the X-axis and 24.32%in the Y-axis, respectively. Score plot (Figure 4.23a) shows the clear differentiation of 6 origins by forming 6 groups of samples from Srisaket, Prachuapkhirikhan, Nakhonsawan, Chiangmai, Ubonratchathani, and Kamphaengphet which are in agreement with the result

of CA. The loading plot (Fig. 4.23b) expresses the relationships between nineteen variables. The association between variables and the chilli samples could be visualized by relating the data score and loading plot for each location. For example, Figure 4.23 shows how the Prachuapkhirikhan chili sample was effectively characterized by its high hexan-1-ol and (E)-hex-2enal contents, indicating that these compounds could be used as marker to identify Prachuapkhirikhan chilli peppers among samples from other regions.Moreover, the Kamphaengphet chilli samples exhibit a characteristically high level of Ethanol and Pentan-3-one that allowed their identification from the panel of six samples. The Ubonratchathanichili samples are characterized by high ethene, pentanal, hexyl 2-methylpropanoate, hexyl 2-methylbutanoateandhexyl 3-methylbutanoate. The analyte concentrations of chili peppers from Sisaket, Nakhonsawan and Chiangmai showed moderate associations to the samples regions of origin.

LDA constructed a classification model for predicting the origin of chilli peppers from raw data and leave-one-out cross validation was performed in order to validate the generated model. It was found that chili peppers from six origins were 100 % correctly classified.

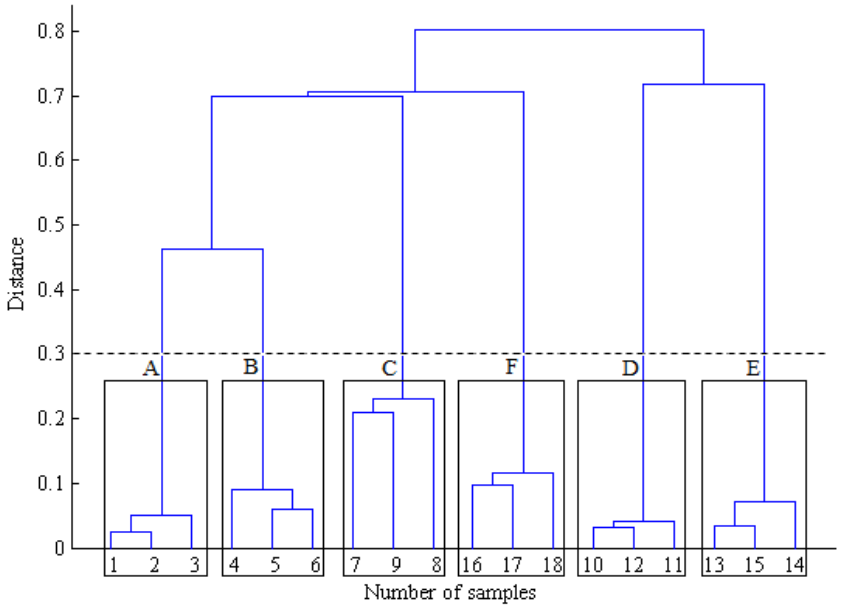
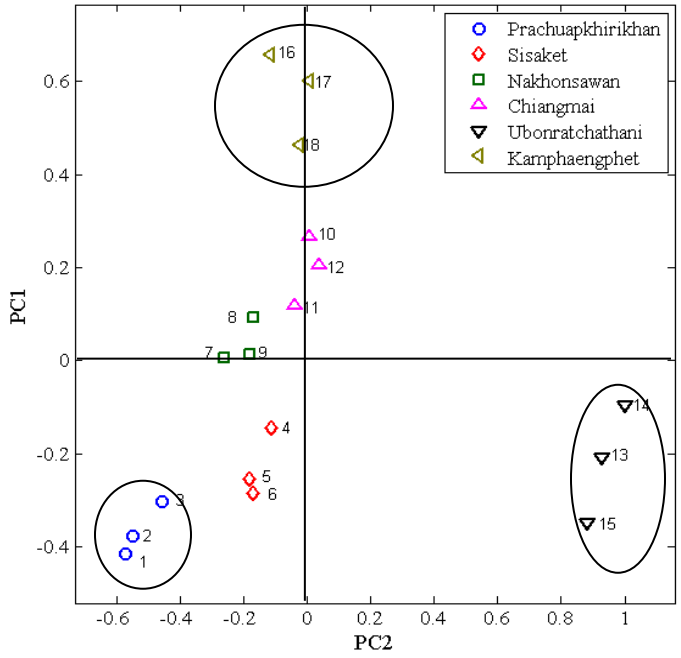


Figure 4.22 Dendrogram of cluster analysis of chilli peppers of six origins. (A: Prachuapkririkhan, B: Sisaket, C: Nakhonsawan, D: Chiangmai, E: Ubonratchathani, F: Kamphaengphet)

(a)



(b)

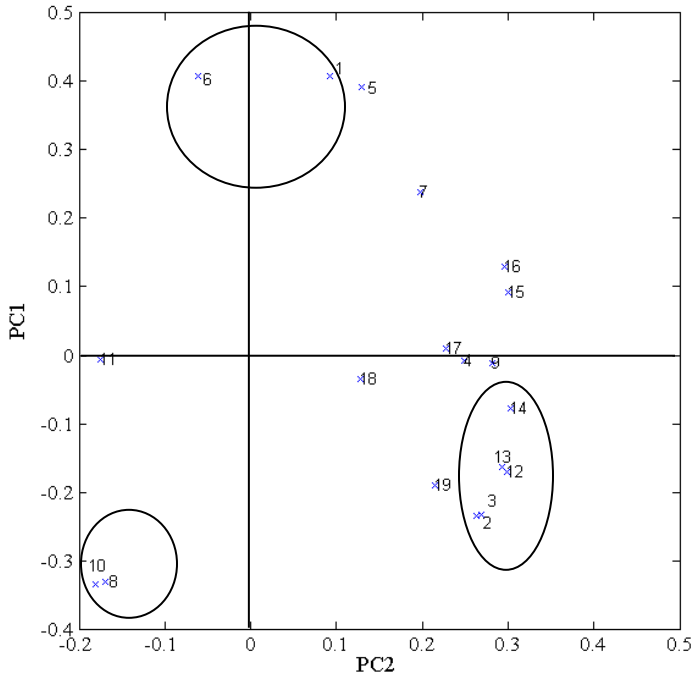


Figure 4.23 Principal component analysis (a) score plot and (b) loading plot of hot chili peppers samples (*Capsicum frutescens Linn.*).(1.Ethanol, 2. Ethene, 3. Pentanal, 6. Pentan-3-none, 8. Hexan-l-ol, 10.(E)-hex-2 enal, 12. Hexyl 2-methylpropanoate, 13. Hexyl 2-methylbutanoate, 14. Hexyl 3-methylbutanoate.

4.2.1.2 Bird chili (*Capsicum frutescens Linn*.)

The CA results show similarities in the data in the form of a dendrogram (Figure. 4.24). The dendrogram illustrates that the samples could be categorized into 5 groups of origin at the distance of 0.2.

Pre-processing stage outputs of Bird chili peppers are grouped by chemometric technique. The PCA results in Figure. 4.25 are shown as score and loading plots. Two principle components were sufficient to account for 76.32% of the total variance for score and loading plot. PC1 and PC2 have a variance of 55.63% in the X-axis and 20.69% in the Y-axis, respectively. The score plot (Figure 4.25a) shows the clear differentiation of 5 origins by forming 5 groups of samples from Chumphon, Chanthaburi, Pathumthani, Chiangmai and Tak which are in agreement with the result of PCA. The loading plot (Figure.4.25b) expresses the relationships between twenty-two variables. The association between variables and the chilli samples could be visualized by relating the data score and loading plot for each location. For example, Figure. 4.25 shows how the Pathumthani sample was effectively characterized by its high hexan-1-ol and (3Z)-3,7-dimethylocta-1,3,6-triene contents, indicating that these compounds could be used as marker to identify Pathumthani chilli peppers among samples from other regions. Moreover, the Chumphon samples exhibited characteristically high level of 4-methyl-1-pentanol, hexyl 2-methylpropanoate, hexyl-n-valerateandhexyl hexanoate allowed their identification from the panel of five samples. The Tak samples are characterized by high capsaicin and dihydrocapsaicin.The analyte concentrations of chili peppers from Chanthaburi and Chiangmai showed moderate associations to the samples regions of origin.

LDA constructed a classification model for predicting the origin of chilli peppers from raw data and leave-one-out cross validation was performed in order to validate the generated model. It was found that chili peppers from five origins were 100% correctly classified.

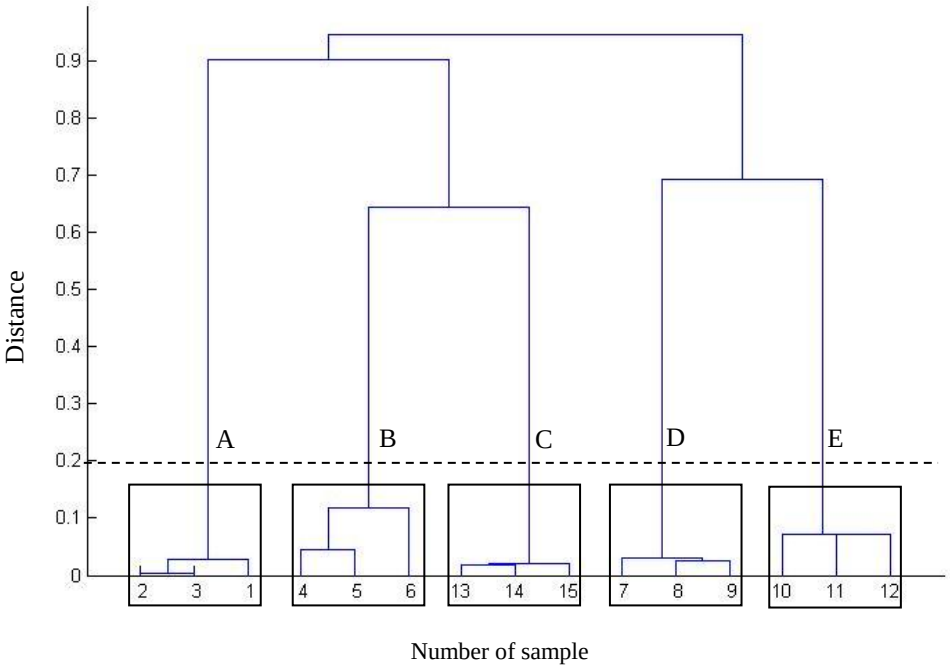
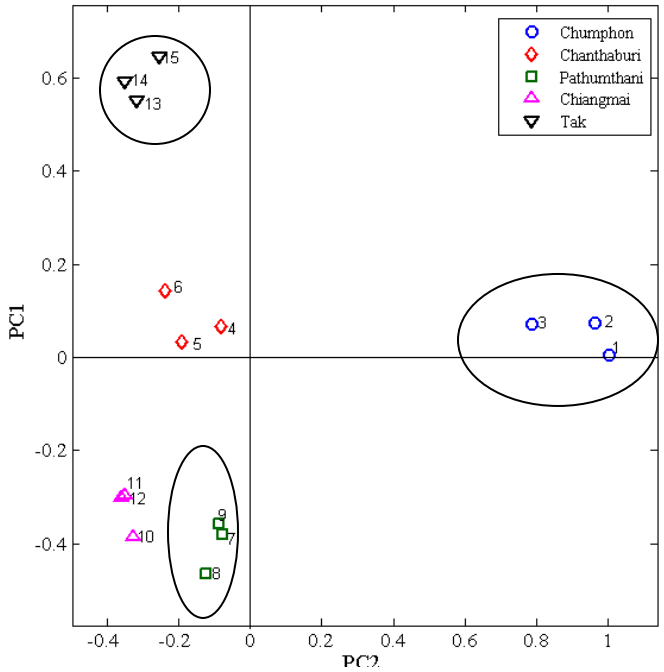


Figure 4.24 Dendrogram of cluster analysis of bird chilli peppers of five origins. (A: Prachuapkririkhan, B: Sisaket, C: Nakhonsawan, D: Chiangmai, E: Ubonratchathani)

(a)



(b)

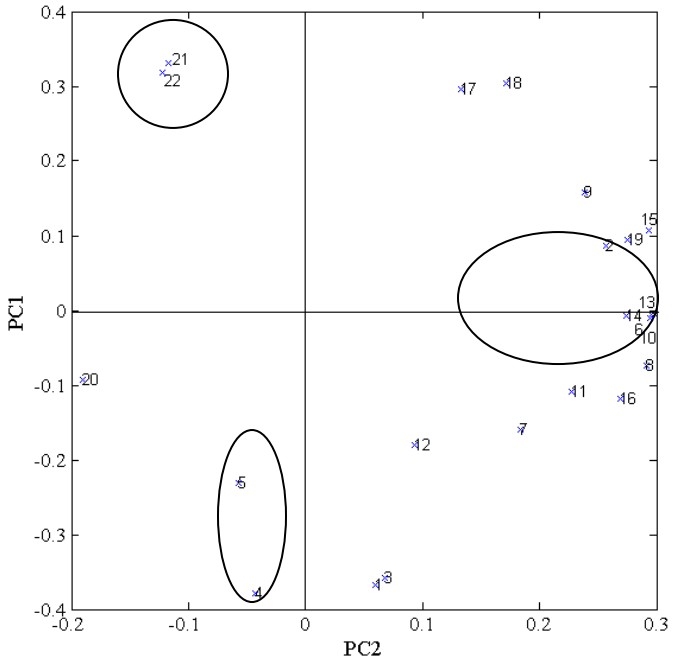


Figure 4.25 Principal component analysis (a) score plot and (b) loading plot of bird chili peppers samples (*Capsicum frutescens Linn*.).(2. 4-Methyl-1-pentanol, 4Hexan-1-ol, 5. (3Z)-3,7-dimethylocta-1,3,6-triene, 6. Hexyl 2-methylpropanoate, 13. Hexyl-n-valerate, 14. Hexyl hexanoate, 21. Capsaicin and 22. Dihydrocapsaicin)

4.2.1.3 Chili spur pepper(*Capsicum annuum Linn. Var acuminatum Fingerh.*)

The CA results show similarities in the data in the form of a dendrogram (Figure.4.26). The dendrogram illustrated that the samples could be categorized into four groups of origin at the distance of 0.2.

Pre-processing stage outputs of chili spur pepperare grouped by chemometric technique.The PCA results in Figure.4.27 are shown as score and loading plots. Two principle components were sufficient to account for 79.52% of the total variance for score and loading plot. PC1 and PC2 have a variance of 57.76% in the X-axis and 21.76% in the Y-axis, respectively. Score plot (Figure.4.27a) shows the clear differentiation of 4 origins by forming 4 groups of samples from Tak, Phitsanulok, Chiangmai and Suphanburi which are in agreement with the result of PCA. The loading plot (Figure. 4.27b) expresses the relationships between seventeen variables. The association between variables and the chilli samples could be visualized by relating the data score and loading plot for each location. For example, Figure. 4.27 shows how the Suphanburi sample was effectively characterized by its high acetic acid, cyano, ethoxy ethene, and prop-2-enyl formate contents, indicating that these compounds could be used as marker to identify Suphanburi chili peppers among samples from other regions. Moreover, the Chiangmai samples are characterized by high o-methylisourea hydrogen sulfate,(E)-hex-2-enal, (E)-hex-2-en -1-ol, cyclopentene-1-carboxylic acid methyl ester and 2-(phenoxy) ethalnol. The Tak chili samples exhibit characteristically high level 9H-fluorene-2-carboxylic acid allowed their identification from the panel of four samples. The analyte concentrations of chili peppers from Phitsanulok showed moderate associations to the samples' regions of origin.

LDA constructed a classification model for predicting the origin of chili peppers from raw data and leave-one-out cross validation was performed in order to validate the generated model. It was found that chili peppers from six origins were 100 %correctly classified.

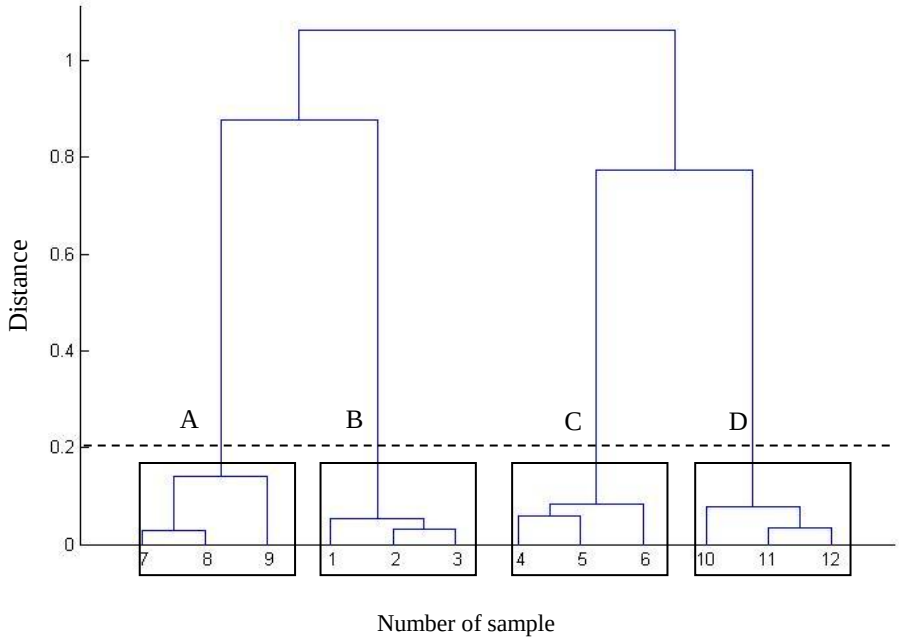
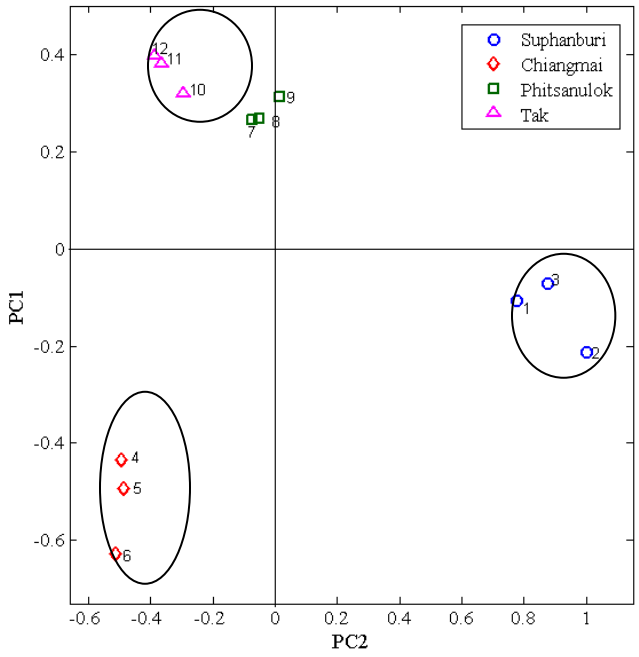


Figure 4.26 Dendrogram of cluster analysis of chili spur pepperof four origins. (A: Prachuapkririkhan, B: Sisaket, C: Nakhonsawan, D: Chiangmai)

(a)



(b)

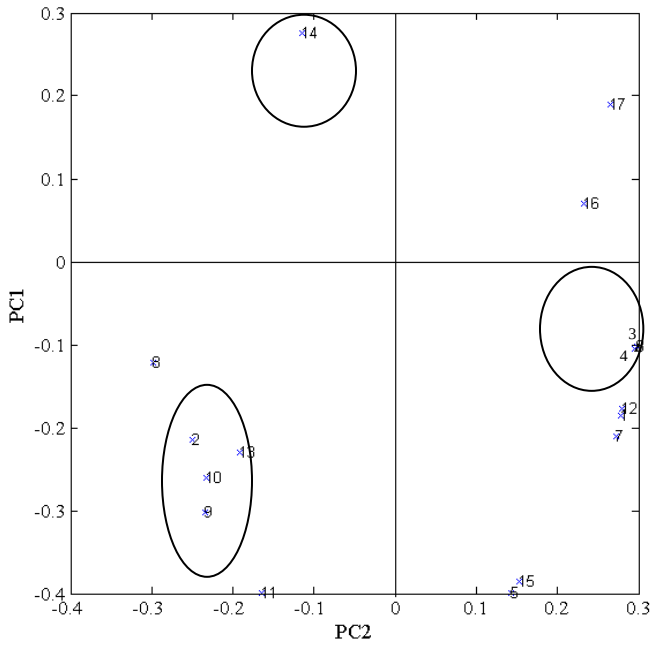


Figure 4.27 Principal component analysis (a) score plot and (b) loading plot of chili spur pepper(*Capsicum annuum Linn. Var acuminatum Fingerh.*)(2. o-Methylisourea hydrogen sulfate, 3. Acetic acid, c yano, 4. Ethoxy ethene 6. Prop-2-enyl formate 9. (E)-Hex-2-enal,10. 2-Hexen-1-ol-(E), 11. Cyclopentene-1-carboxylic acid methyl ester 13. 2-Phenoxy-1-ethanol and 14. 9H-fluorene-2-carboxylic acid)

4.2.2 Varieties of chili peppers

The CA results show similarities in the data in the form of a dendrogram. In the figure 4.28, the X-axis represents the number of samples, while the Y-axis represents the Euclidean distance. The dendrogram illustrates that the samples could be categorized into 3 groups of varieties of chili peppers at the distance of 0.4. The first cluster (labeled A) consists of 18 hot chili peppers. The second cluster (labeled B) includes15 bird chili peppers. The third cluster (labeled C) composes of 12 chili spur peppers.Due to CA using less information than PCA, CA tended to show data which were not indicated in relationships between samples and compounds (variables). Hence PCA was used for classified different varieties according to organic composition (variables).

Pre-processing stage outputs of three varieties of chili peppers which included hot chili peppers, bird chili pepper and chili spur peppers are grouped by chemometric technique; the PCA results in Figure 4.29 are shown as score and loading plots. Two principle components were sufficient to account for 83.03% of the total variance for score and loading plot. PC1 and PC2 have a variance of 58.70% in the X-axis and 24.33%in the Y-axis, respectively. Score plot (Figure 4.29a) shows the clear differentiation of three varieties of chili peppers by forming 3 groups of samples. The loading plot (Fig. 4.29b) expresses the relationships between thirty-six variables. The association between variables and the chili pepper samples could be visualized by relating the data score and loading plot for three varieties. The first group, all 18 hot chili pepper samples are located on the middle left hand side of the plot as shown in Figure. 4.29a.In Figure 4.29 shows how the hot chili peppers variety samples were effectively characterized by its high compounds of numbers 4, 5, 6, 7 8 and 9(ethene, pentanal, prop-2-enyl formate, prop-2-yn-1-ol, pentan-3-one and 2-methyl-3-vinyl-oxirane, respectively as seen in Table 4.13) contents, indicating that these compounds could be used as marker to identify the hot chili peppers variety samples from other variety.The second group, the location of 15 bird chili peppers in the upper right-hand quadrant of plot (Figure. 4.22a) explained their high values of compounds of numbers 15, 19, 20, 23, 24, 25, 27, 30, 31 and 32 ((3Z)-3,7-

dimethylocta-1,3,6-triene, 2-methylbutyl 2-methylpropanoate, hexyl butyrate, 3-methylbutyl hexanoate, hexyl 2-ethylbutanoate, hexyl-n-valerate, hexyl hexanoate, (3Z)-2-methyl-3-undecene, decane, 3,4-bis (methoxycarbonyl) benzoic acid, respectively as seen in Table 4.13), indicating that these compounds could be used as marker to identify the bird chili peppers variety samples. The third group, all 12 chili spur peppers which the lower right-hand quadrant of plot (Figure. 4.22a) were characterised by high level of compounds of numbers 2, 10, 17 and 26 (o-methylisourea hydrogen sulfate, 2-nitroethanol, 2,3,4,5,6-pentafluorobenzyl alcohol, 2-phenoxy-1-ethanol, respectivelyas seen in Table 4.13)

LDA constructed a classification model for predicting the varieties of chili peppers from raw data and leave-one-out cross validation was performed in order to validate the generated model. It was found that chili peppers from three variety were 100 % correctly classified.

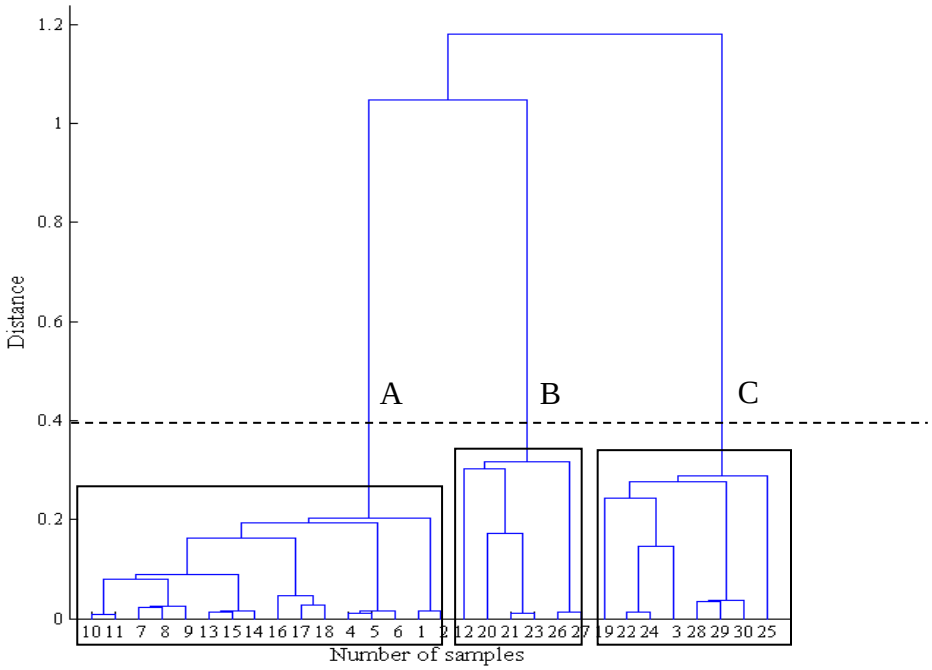


Figure 4.28 Dendrogram of cluster analysis of three varieties of chilli peppers. (A: Hot chili peppers, B: Bird chili peppers, C: Chili spurs peppers)

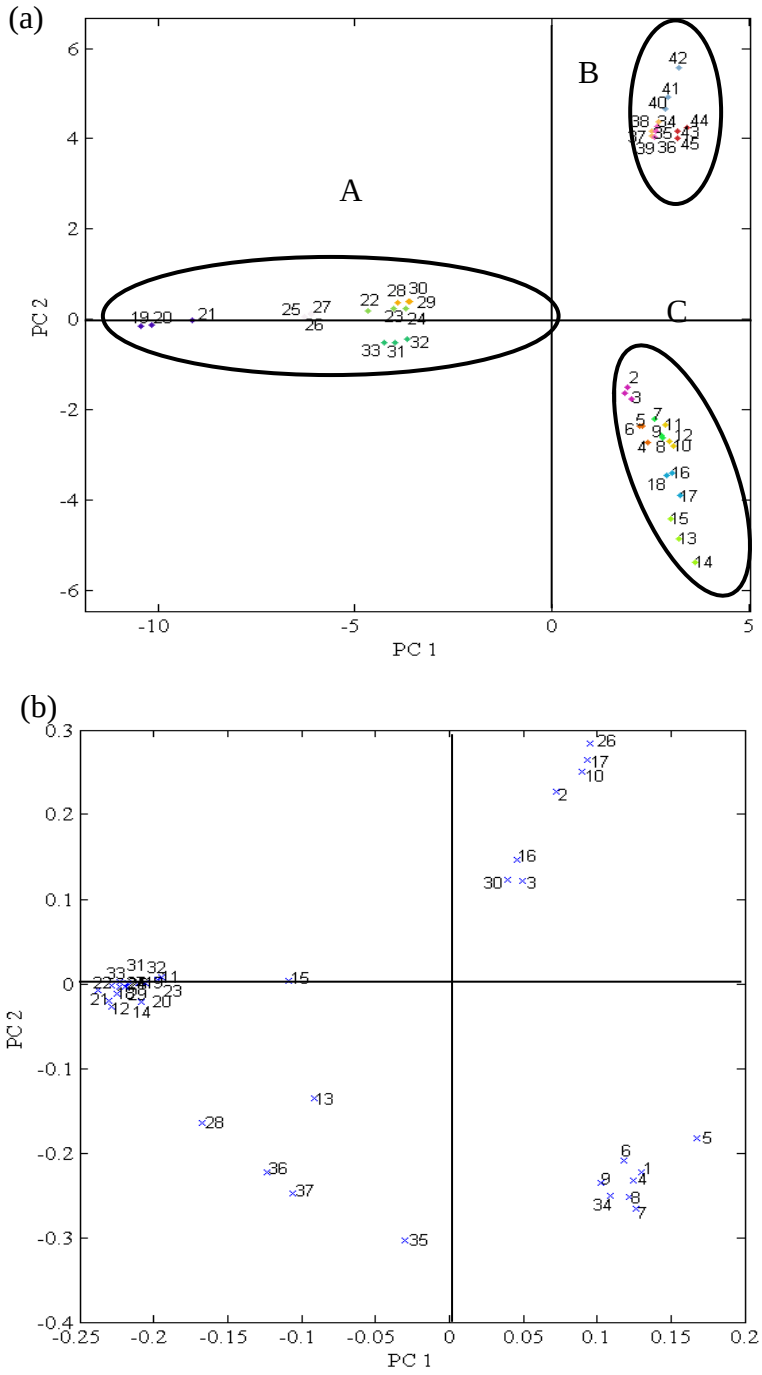


Figure 4.29 Principal component analysis (a) score plot and (b) loading plot of chili pepper samples. (A: hot chili peppers variety, B: bird chili peppers variety, C: chili spur peppers)

CHAPTER V

CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDY

In this study, the discrimination of various geographical origins in Thailand and three varieties of chili peppers were studied by using chromatography and spectroscopic methods to identify the organic composition of chili peppers. Chemometric methods were applied to the analytical data to monitor chili pepper profiles and construct a mathematical model to aid the classification of the origins and varieties of chili pepper samples.

This work was designated into two parts. Firstly, the total composition of chili peppers, capsaicinoids, volatile compounds and total phenolic content (TPC) which are a potent part of antioxidants agents. These were all qualitatively and quantitatively analyzed using HPLC-DAD, GC-MS and UV-visible spectroscopy, respectively. Secondly, the combination of PCA, CA, and LDA were subsequently utilizedin order to discriminate against chili peppers according to their origins and varieties.Three chemo metric methods were applied to the analytical data to monitor chili pepper profilesand construct a mathematical model to aid in the identification of the origins and varieties of chili pepper samples. We found that chili peppers from various origins have a unique organic composition as markers of the geographical origins which can be used to group the geographical origins of chilis as shown in Table 5.1. Classification of the varieties of chili peppers were put into 3 groups. Each of the varieties of chili peppers have organic composition markers that are unique to each species.

The first group, the hot chili pepper variety samples were effectively characterized by its high compounds of ethene, pentanal, prop-2-enyl formate, prop-2-yn-1-ol, pentan-3-one and 2-methyl-3-vinyl-oxirane contents, indicating that these compounds could be used as a marker to identify the hot chili peppers variety samples from other varieties.The second group, bird chili peppers explained their high values of (3Z)-3,7-dimethylocta-1,3,6-triene, 2-methylbutyl 2-methylpropanoate, hexyl butyrate, 3-methylbutyl hexanoate, hexyl 2-ethylbutanoate, hexyl-n-valerate, hexyl hexanoate, (3Z)-

2-methyl-3-undecene, decane, 3,4-bis (methoxycarbonyl) benzoic acid, indicating that these compounds could be used asa marker to identify the bird chili peppers variety samples. Moreover, The third group, chili spur peppers exhibitedcharacteristically high levels of o-methylisourea hydrogen sulfate, 2-nitroethanol, 2,3,4,5,6-pentafluorobenzyl alcohol, 2-phenoxy-1-ethanol allowed their identification from the panel of three variety.

The capsaicin, dihydrocapsaicin, volatile compounds, and total phenolic content of chili peppers varied according to the location of the plants growth. Thus, PCA, CA, and LDA chemometric techniques can be used to assign a chili pepper sample's profile for these compounds to a particular geographical origin. The proposed methods are highly efficient and produce reliable data.

All the presented analyses indicated that the combination of chromatography and spectroscopic methods with chemo metric methods lead to more powerful classification and discrimination of their geographical origins and varieties of chili pepper samples. The significance of this work is further developed and applied to other food produce, and as a tool for the investigation of the composition of their geographical origins and varieties of chili pepper samples.

Table 5.1 Comparison of organic composition markers, EDC distance and % LDA prediction in chili samples

Varieties	Origin	Markers*	EDC distance/group	LDA prediction
Hot chili pepper (*Capsicum frutescens Linn*.)	Prachuapkhirikhan	Hexan-1-ol and (E)-Hex-2enal	0.3	100%
	Sisaket	-		
	Nakhonsawan	-		
	Chiangmai	-		
	Ubonratchathani	Ethene, Pentanal, Hexyl 2-methylpropanoate, Hexyl 2-methylbutanoate, Hexyl 3-methylbutanoate		
	Kamphaengphet	Ethanol and Pentan-3-one		
Bird chili pepper (*Capsicum frutescens Linn*.)	Chumphon	4-Methyl-1-pentanol, Hexyl 2-methylpropanoate, Hexyl 3-Methylbutanoate, Hexyl-n-valerate, Hexyl butyrate	0.2	100%
	Chanthaburi	-		
	Pathumthani	Hexan-1-ol , (3Z)-3,7-dimethylocta-1,3,6-triene		

Table 5.1 (continued) Comparison of organic composition markers, EDC distance and % LDA prediction in chili samples

Varieties	Origin	Markers*	EDC distance/group	LDA prediction
Bird chili pepper (*Capsicum frutescens Linn.*)	Chaingmai	-	0.2	100%
	Tak	Capsaicin and Dihydrocapsaicin		
Chili spur pepper(*Capsicum annuum Linn. Var acuminatum Fingerh.*)	Tak	9H-fluorene-2-carboxylic acid	0.2	100%
	Phitsanulok	-		
	Chiangmai	o-Methylisourea hydrogen sulfate, 2-Hexen-1-ol-(E)-, Cyclopentene-1-carboxylic acid methyl ester and 2-Phenoxy-1-ethanol		
	Suphanburi	Acetic acid, c yano, Ethoxyethene and Prop-2-enyl formate		

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