วิธีวิเคราะห์เชิงโครมาโทกราฟีสำหรับการวิเคราะห์รสชาติและสารตกค้างและการพิสูจน์เอกลักษณ์ในตัวอย่างอาหาร

นางสาวโสภารัตน์ ยุทธวรศิษย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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CHROMATOGRAPHIC ANALYTICAL METHODS FOR TASTE AND RESIDUE ANALYSES AND FOR AUTHENTICATION IN FOOD SAMPLES

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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โสภารัตน์ ยุทธวรศิษย์ : วิธีวิเคราะห์เชิงโครมาโทกราฟีสำหรับการวิเคราะห์รสชาติและสารตกค้างและการพิสูจน์เอกลักษณ์ในตัวอย่างอาหาร (CHROMATOGRAPHIC ANALYTICAL METHODS FOR TASTE AND RESIDUE ANALYSES AND FOR AUTHENTICATION IN FOOD SAMPLES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.ธรรมนูญ หนูจักร, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร.ณัฐชนัญ ลีพิพัฒน์ไพบูลย์, 200 หน้า.

เทคนิคทางโครมาโทกราฟีหลากหลายเทคนิคเป็นส่วนสำคัญในงานวิเคราะห์ด้านต่างๆโดยเฉพาะการวิเคราะห์อาหาร ซึ่งประเด็นคุณภาพและความปลอดภัยทางอาหารเป็นประเด็นสำคัญเกี่ยวกับปัญหาทางด้านอาหารที่ทั่วโลกให้ความสนใจ งานวิจัยนี้มีจุดประสงค์เพื่อใช้วิธีวิเคราะห์เชิงโครมาโทกราฟีร่วมกับเทคนิคทางเคมีวิเคราะห์อื่นๆในการวิเคราะห์รสชาติและสารตกค้างรวมทั้งการพิสูจน์เอกลักษณ์ในตัวอย่างอาหาร โดยมีการใช้เทคนิค LC-DAD และเทคนิค LC-MS/MS ร่วมกับวิธีทางเคโมเมทริกซ์ในการระบุถึงเอกลักษณ์แหล่งที่มาของขิงจากหลากหลายพื้นที่ ข้อมูลที่ได้จากวิธีวิเคราะห์เชิงโครมาโทกราฟีของสารเก้าชนิดในตัวอย่างขิง 152 ตัวอย่างจากห้าจังหวัดและจากห้าประเทศนำไปประมวลผลด้วยวิธี similarity analysis, HCA, PCA และ LDA เป็นประโยชน์อย่างมากในการแปลผลและทำนายผล สำหรับการวิเคราะห์ด้านรสชาติ ใช้วิธี sensomics ในการศึกษาหาข้อมูลเชิงโมเลกุลที่มีผลต่อรสชาติของเนื้อวัวที่มีการเตรียมด้วยวิธีบ่มแบบแห้งจากบริษัท LUMA ในสวิตเซอร์แลนด์ โดยทำการวิเคราะห์ทั้งแบบ targeted analysis และ non-targeted analysis ร่วมกับการประเมินทางประสาทสัมผัสและวิธีการทางสถิติเพื่อเพิ่มความความรู้เข้าใจเกี่ยวกับสารที่มีผลต่อรสชาติ มีการศึกษาเปรียบเทียบองค์ประกอบทางเคมีต่างๆ (กรดอะมิโน กรดไขมัน กรดอินทรีย์ ประจุบวกและประจุลบ) ระหว่างเนื้อวัวที่เตรียมด้วยวิธีการธรรมดาและวิธีการเตรียมชนิดบ่มแบบแห้งด้วยเทคนิค LC-MS/MS และเทคนิค IC รวมทั้งใช้เทคนิค LC-TOF-MS การสังเคราะห์สารที่มีความเป็นไปได้ว่าจะมีผลต่อรสชาติ และเทคนิค GPC ร่วมกันกับการประเมินทางประสาทสัมผัสในการระบุสารที่มีผลต่อรสชาติเฉพาะตัวของเนื้อวัวจากบริษัท LUMA สำหรับการวิเคราะห์สารตกค้าง ศึกษาการใช้สาร analyte protectant (AP) ในวิธีใหม่ที่เรียกว่า AP priming ในการชดเชยปัญหาจากเมทริกซ์ที่ส่งผลทำให้เกิดความคลาดเคลื่อนต่อการทำปริมาณวิเคราะห์สารเคมีตกค้างในอาหารระดับต่ำมากด้วยเทคนิค GC ทดสอบโดยการวิเคราะห์สารฆ่าศัตรูพืช 100 ชนิดด้วยเทคนิค GC-MS/MS ในพริกแดงที่นับว่าเป็นตัวอย่างเมทริกซ์ที่มีสารรงควัตถุในปริมาณสูง ทำการประเมินประสิทธิภาพของผลการใช้วิธี AP priming เป็นระยะเวลานานด้วยการวิเคราะห์สารต่อเนื่อง 50 ครั้ง จากผลการวิเคราะห์พิสูจน์ว่าวิธี AP priming ให้ผลเช่นเดียวกับการใช้งาน AP วิธีปกติ แต่เป็นวิธีที่ รวดเร็ว ประหยัด และเหมาะสมกับงานวิเคราะห์ประจำมากกว่า ทั้งยังคงข้อดีของเมทริกซ์ในการช่วยเพิ่มสัญญาณการตรวจวัด ในขณะที่ช่วยลดปัญหาความไม่คงที่ของสัญญาณการตรวจวัดจากเมทริกซ์ งานวิจัยนี้เป็นประโยชน์และสามารถนำไปใช้ต่อยอดในการนำเทคนิคทางโครมาโทกราฟีไปใช้เพื่องานวิเคราะห์อาหารในประเด็นอื่นๆ

ภาควิชา เคมี	ลายมือชื่อนิสิต .....
สาขาวิชา เคมี	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
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SOPARAT YUDTHAVORASIT: CHROMATOGRAPHIC ANALYTICAL METHODS FOR TASTE AND RESIDUE ANALYSES AND FOR AUTHENTICATION IN FOOD SAMPLES. ADVISOR: ASSOC. PROF. THUMNOON NHUJAK, Ph.D., CO-ADVISOR: ASST. PROF. NATCHANUN LEEPIPATPIBOON, Dr.rer.nat., 200 pp.

Several chromatographic techniques predominantly impart in many analytical fields especially for food analysis. Among food-related concerns, the quality and safety of food are common issues in worldwide interests. This work aims to employ chromatography and other analytical methods for the purpose of food authentication, taste and residue analyses. The origin labelling was performed by using LC-DAD and LC-MS/MS in combination of chemometrics methods to define the fingerprint of ginger from various production areas. Nine chromatographic profiles of 152 gingers from five cities and five countries were successfully processed with similarity analysis, HCA, PCA and LDA for interpretation and prediction. For taste analysis, sensomics approach was selected to explore molecular taste information of dry-aged beef from LUMA company, Switzerland. Targeted analysis and non-targeted analysis with sensory evaluation and statistical methods help to better understand about taste constributors. Normal treated and dry-aged LUMA treated beefs were studied and compared for their chemical compositions (amino acids, fatty acids, organic acids, cations, and anions) by LC-MS/MS and IC. LC-TOF-MS, the synthesis of tentative compounds and GPC were jointly occupied with the sensory evaluation for indicating the impacted compounds on the characteristic taste of LUMA beef. In case of residue analysis, analyte protectant (AP) was studied in a new approach called AP priming to compensate the matrix effect problem which led to quantitative error in GC determination of chemical residue in food at trace level. One hundred pesticides from various classes at residual level were determined with GC-MS/MS in red chili, a high pigmented matrix. Long-term analysis (50 injections) was investigated to assess the application of AP priming. The results proved that the use of AP priming offers the similar performance with classical AP method, but achieves more cost-effective, time-saving and practical suitability for routine laboratory. Moreover, AP priming maintains the matrix benefit of signal enhancement while discards the drawback of signal inconsistency from matrix effect. The proposed studies are beneficial and could be used to extend the applications of chromatography in other field of food analyses.

Department:	Chemistry	Student's Signature	.....
Field of Study:	Chemistry	Advisor's Signature	.....
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CHAPTER I
INTRODUCTION

1.1 Problem Definition

Nowadays, analytical techniques are involved in many worldwide applications by dealing with all aspects of chemical research in industry, academics, and government. With recent technological advancement, the performance of analytical tools and instruments were greatly improved and able to successfully fulfill the global needs by enhancing the efficiency in measurement while reducing time, labor or cost. This is a very important for both product development and quality assurance in modern food industry. Since trends of food analysis nowadays have been moved from demand-driven forces to more focusing on food quality and safety, the analytical chemists have been developing a wide variety of qualitative and quantitative methods in order to solve food analysis difficulties and meet the food trade criteria. Several traditional methods are coupled with powerful instrumental techniques to extend their applications in order to cover the growing variety and complexity of available foods.

Recently, food authentication has been gaining more attention because consumers continuously demand the reassurance of origin and content of their food. Manufacturers must be able to confirm the authenticity of their products components in order to comply with the government legislation. Determining the authenticity of food can prevent false description, substitution of cheaper ingredients, and adulteration, as well as incorrect origin labeling [1]. European Union (EU) has enacted the regulation regarding food safety and traceability using the recent developments in the determination of food authenticity in order to encourage diverse agricultural production; to protect product names from misuse and imitation; and to help consumers by giving them information concerning the specific character of the products. In 1992, EU introduced the terms ‘Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Specialty Guaranteed (TSG)’ in the regulation to encourage diverse agricultural production and to protect product names from misuse and imitation [2]. The term ‘geographical indication’ is used to

describe an agricultural product or a foodstuff originating in that region; specific place or country and which possesses a specific quality; reputation or other characteristics attributable to that geographical origin; and the production and/or processing and/or preparation of which take place in the defined geographical area [3]. Since authenticity and quality parameters are often associated with particular information of food such as geographical origin, production area, brand or chemical composition, the indication of food profile has become an essential issue for global requirement. Due to the fact that food quality and price may vary in different geographical regions, the origin of a food commodity has become a significant factor in determining its quality, applicability and price. The data of its origin is necessary for improvement of the traceability systems in global food regulation. Also, consumers nowadays tend to purchase more food products with a certified genuineness and geographical origin in order to have good quality, pure, safe, authentic food.

Taste is a fundamental food profile related with chemical compositions contained in each food. Food taste is originated from the natural products or chemicals formed during treatment, storage, or distribution processes. Chemical compounds in food result as characteristic taste and a certain mixture of compounds may yield a new taste. The perception of human taste is a result of the interaction between chemical molecules and the receptors on a tongue or in an oral cavity. There are five basic tastes of food which are sweet, salty, sour, bitter and umami. Each taste has its own established chemical reference substances. The definition of each basic taste will vary with the chemical identity of the reference substance. This means that each chemical compound has the identical taste recognition chemistry attributes [4]. Taste profile is needed to characterize food tastes identified by the chemical compositions.

The profiles of geographical origin and taste are often used as recognition for product quality. Hence, the technologies that can distinguish the geographical origin or taste of food are an important in terms of protecting the value and establishing consumer trust. To verify the food profile, the selection of efficient analytical methods and tools is a critical factor for reliable results. The common analytical techniques available for testing food authenticity include chromatographic and spectroscopic

techniques, stable isotope analysis, enzymatic and immunological, and DNA methods and/or in combination with chemometrics technique for compositional analyses.

Food safety is one of food concern in global public health because food can promote health benefits or risks in human. Food normally consists of many bioactive components which provide desirable health-promoting effects and prevent some critical diseases. However, food may transfer microorganisms and serve as a growth medium for bacteria, which lead to short or long term health-risk effects to human body. To prevent foodborne illness and avoid potentially severe health hazards, several food safety considerations were legislated as a regulation in case of the origins of food and the practices relating to food labeling, food hygiene, food additives and pesticide residues. The extensive use of pesticides in modern farming on fruit and vegetables to control a wide range of pests and diseases has posed risks to public health. The inappropriate application on crops can result in unacceptably high residual levels of these compounds in agricultural products, environment and food chain. The U.S. Environmental Protection Agency (EPA) and EU have set the maximum residue limits (MRLs), for pesticides that can be used on various food and feed commodities [5, 6]. Due to those international regulations, the efficient analytical methods are needed to monitor the residues and ensure that the food is safe for consumption. Wide variety of food matrices and the low residue level of pesticide are the major complications in an analysis because large quantities of other food components could affect the determination of the analytes.

Chromatography is a technique in which the separation of molecules based on molecular structure and molecular composition. Chromatography is now used more often in many research fields, especially food science and technology, because of its high separation capacity. It can also separate molecules which have extremely similar chemical characteristics, and in complex matrices. With the combination of a powerful detector, chromatographic method becomes an extremely versatile technique to solve many recent analytical problems.

However, those developed analytical methods still does not completely cover the various types of food and fields of study. To deal with the mentioned concerns, new analytical methods for food quality and food safety are needed.

Thailand is one of the world's top ten food producers and exporters. The value of Thai food exports has been increasing every year, thus the quality and safety of food play an important role in the exportation business [7]. Therefore, Thai producer should find a suitable practice to implement global food standards as well as for domestic consumption. In scientific perspective, the application of analytical technique could be effectively used to support the expansion of various food projects in Thailand's food sectors in the case of product development, food processing, and agriculture.

1.2 Food quality and food safety

1.2.1 Food quality: The authentication of ginger origin

1.2.1.1 Food authentication

Food authentication is a process by which a food is verified as complying with its label description. Labeling and specification of food composition is necessary information for the whole food chain sectors (from producer to consumer). To assure the quality of product and follow regulations, food manufacturers must provide and confirm consumer about the authenticity of their foods. The legislation on food authentication may differ from country to country, but all the requirements are based on the determination or application of the appropriate method by using different technologies for a particular issue [1]. Labeling legislation has been set to ensure that the description of food is properly described. This is to protect consumer, distributors and honest traders from unfair competitions among the food industry. Food fraudulent and adulteration are serious economic problems because foods or ingredients are most likely to be adulterated targets especially high-value or fashionable foods. Each commodity has its own specific problems; therefore, the sufficient database should be continuously established. This process is related not only with testing, but also with the thorough understanding about authentic product [3]. There are three main difficulties in developing authenticity methodology which are (i) that the interpretation of the results has to be made with the presence of analytical uncertainty, natural variation, and any tolerance permitted by the requirements, (ii) finding a marker that characterizes the food with high accuracy and an acceptable variation, and (iii) having authentic samples available for the development and the evaluation of the method [8].

The authenticity issues of food can be mainly classified into 5 topics which are species/variety, region of origin, commercial process, industry frauds, and brands [1, 3].

1) Species/variety: The common problem is that food was mislabeled or a substitution of one species for another. It usually involves the mixing of authentic product with less expensive non-authentic food such as the mixing of olive oil with other vegetable oils. This type of adulteration is relatively easy to detect because the natural markers are always presents in the adulterated products. Also, the introduction of genetically modified organisms (GMOs) into food industry may produce a special case for the authenticity testing.

2) Region of origin: The geographical origin or cultivation area sometimes is used to determine food quality. In particular, wines are associated with a specific region. Wines from specific place may attract consumer by its production area more than similar products from another area. It is difficult to discriminate the geographical origin of a product without performing analytical test because of the similarity in physical appearance.

3) Commercial process: The special treatment process may lead to a premium price, and the quality of product or some processes are considered desirable by the consumer. For example, pasteurization is a preferable process for consumer, although this does not add value to the product. Moreover, cold pressed virgin olive oil represents a high quality process by using no heat in the extraction of oil from the olives in order to preserve the delicate flavors and aromas. The information of treatment process should be accurately labelled on products following the legal restriction.

4) Industry frauds: Even though the blend of other commodities in food products may not harm the consumer and help producer to reduce the production cost, it is illegal and called adulteration. Water and sugar solutions are frequently used for the dilution of products. Usually, the liquid food products suffer from this authentication case.

5) Brands: Protecting the authenticity of brands is an important issue for most businesses. This type of fraudulent is more related to the manufactured products than to commodities. Each company has the specific ways to protect its own products from being counterfeited by other producers. Normally, this case happen with high quality and famous products e.g. whisky, wine, and beer.

Considering all authentication issues, the development of analytical methods to indicate the food authenticity is necessary to cover all aspects of food analysis as much as possible. The determination of the food authentication can prevent false description, substitution of cheaper ingredients, and adulteration, as well as incorrect origin labeling.

1.2.1.2 Ginger

Ginger (*Zingiber officinale Roscoe*) is one of the most important and well-known traditional herbs. It is a valued spice and a classical medicine in Asia for centuries. Ginger rhizome in fresh and dried form has been used extensively for its flavor and pungency. It is a perennial and herbaceous plant which has many fibrous roots, aerial shoots with leaves, and branch rhizomes [9]. The rhizomes are aromatic, thick lobed, branched, and scaly structures with a spicy lemon-like scent. Ginger is believed to be originated in Southeast Asia, and is grown only under cultivation. It is a seasonal crop which is cultivated only once a year in tropical and subtropical regions such as China, India, Japan, Indonesia, Australia, Nigeria and Southeast Asia, all of which are major producers of ginger [9].

Nowadays, ginger has beneficial uses in both traditional and modern medicinal treatments of nausea, vomiting, motion sickness, diarrhea, and digestive and respiratory disorders. Furthermore, ginger also possesses numerous pharmacological properties such as anti-inflammatory, antimicrobial, anti-mutagenic, anticarcinogenic, analgesic and antioxidant activities [10]. Ginger shows the significant pharmacological effects on the inflammatory process and shares pharmacological properties with non-steroidal anti-inflammatory drugs by the inhibition of PG synthesis which related to the presence of the phenolic hydroxy group adjacent to the methoxy group in the main compositions. It inhibits the function of several genes such as the ones encoding cytokines, chemokines, and enzyme cyclooxygenase-2 which are all involved in the inflammatory response [11]. Also, it is believed to help the common cold, flu-like symptoms, and even menstrual pains. The chemo-preventive activity of ginger is highlighted because the presence of phenolic constituents are believed to suppress the transformative, hyperproliferative, and

inflammatory processes that initiate carcinogenesis, as well as the later steps of carcinogenesis, namely angiogenesis and metastasis [12]. From many pharmacology activities, ginger may exhibit some superior effects for consumer to conventional drugs such as intake dosage, health safety studies, health activity and price.

Ginger is widely consumed for both medicinal and culinary purposes in fresh and dried forms because it has been recognized for the characteristic pungency from oleoresin, aroma from essential oils, and bioactive compounds such as phenolic compounds. The biological activities of ginger arise from its active chemical components, which are gingerols, the pungent principles of ginger and gingerol-related compounds, which exist as a series of homologues with a range of unbranched alkyl chains. Other constituents are vitamins, carbohydrates, lipids, carboxylic acids, amino acids, and minerals. Due to its health benefits, ginger is being exported around the world and is extensively consumed in fresh form. It is also used in many kinds of food additives, dietary supplements, and traditional medicines. Majority of the fresh ginger consumption is located in Asia, and its demand in U.S. and Europe is increasing. The U.S. Food and Drug Administration (FDA) regards ginger as ‘‘generally recognized as safe’’ (GRAS) food additive [13]. The essential oil and oleoresins extracted from ginger rhizomes are very valuable due to its unique ginger flavor and pungency. Oils and oleoresins of ginger are preferred over the dried spices as flavoring by the food industry, because they are more stable, cleaner, free from contaminations, and can be standardized by blending oils from different sources. Its essential oils are imparted in the manufacture of soft drinks, ginger beer, in food preparation, and also many types of pharmaceutical formulations.

1.2.1.3 Literature review

Various analytical techniques have been used to qualitatively and quantitatively analyze essential oils and pungent compounds in ginger. A comprehensive review of current developments for ginger was reported in 2011 [13]. The processing, chemistry, biological activities, and medicinal uses of ginger including the available information on post-harvest technological treatments, recent

chemical constituents reported, extraction methodologies, and analysis of major chemical constituents along with their latest biological aspects have been discussed. Chromatographic techniques are typically utilized as a straightforward method to determine the non-volatile chemical composition of ginger and various ginger products with different detection methods. LC-DAD was employed to analyze 6-gingerol, a phenolic antioxidant compound, in different ginger cultivars [14]. The quantities of 6-gingerol ranged from 0.1% to 0.2% and the cultivars with high 6-gingerol content had the strongest antioxidant activities. Also, a Jamaican ginger was analyzed with LC-DAD and HPTLC for the chemo-profiling of 5 gingerol and shogaol compounds at various maturation and storage times [15, 16]. HPTLC results showed the chemical homogeneity and slight differences in the intensities of the gingerol and shogaol zones. Quantification by LC-DAD revealed significant differences in total pungency contents. Blue varieties of Jamaican gingers were found to be the most pungent and have the highest essential oil yields. The appropriate time to harvest ginger in Jamaica varies with locality and depends on the demand of oleoresin yields and quality of ginger. LC-MS is well-known as a promising tool to determine ginger compositions. The method to identify the individual pungent constituents of ginger extract was developed with LC-MS [17]. Seven compounds were identified as the major pungent constituents of ginger based on their UV spectra, mass data, and comparison to data of the available purified standards. The seven pungent compounds were [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-shogaol, [8]-shogaol, [10]-shogaol and [6]-gingediol. The other eight compounds were tentatively identified as gingerol analogues. With LC-MS/MS, 31 known and unknown gingerol-related compounds in fresh ginger rhizome with structural differences between the alkyl chain and the aromatic ring were characterized from the methanolic crude extracts [18]. This work suggested that negative and positive mode of LC-ESI-MS/MS analysis coupled to diode array detection was a powerful and fast on-line tool for the identification of ginger chemical profiles. Moreover, LC-MS/MS was employed for identification and quantification of gingerols and related compounds in ginger dietary supplements [19] and dried ginger [20]. LC-NMR [21] and GC-MS [22] were also applied for the analysis of the component in powdered, dried and fresh ginger. Since the gingerols and shogaols are only available commercial standards of

ginger compositions, the analysis of pungent constituents in most studies focused on the determination of gingerols and shogaols. Among those reports, the effects of various varieties [14, 15] and maturity stages [16] on the content of gingerols and related compounds were investigated and compared. Even though the physical appearance of ginger might sometimes be used to roughly discriminate the different varieties or ages of ginger, it is not suitable for accurate labeling of origin. Since the pungent principle is a well-known characteristic of ginger which arises from its chemical profile, the determination of those data would help to clarify the geographical origin of ginger.

There are several origin labeling studies based on food chemical profile of foods. Those researches require statistical methods to support data processing and create a fingerprint or a specific pattern for recognition of food. Chemometric is a mathematical method which can greatly assist and improve the quality of the fingerprint data obtained from complex chromatographic or spectroscopic profiles. The combination of analytical technique and chemometric can be found in many recent food analyses.

For traditional herb, fingerprint analysis is needed to understand the chemical composition for quality control because traditional Chinese medicine (TCM) has recently gained popularity in many countries. Each TCM contains many compounds that may be relevant to the medicine's pharmacological activity. Nowadays, several new types of herb have been newly discovered; therefore, the compositional analysis method for authentication will lead to more understanding about herb profiles and their benefits for product developments and to meet the regulatory requirements. Chromatographic fingerprint methods were combined with chemometric techniques in various studies. *Herba cistanche*, a famous Chinese herbal medicine was studied to comparatively analyze the crude herbs of four species with HPLC–DAD–MS fingerprint method [23]. Eighteen characteristic peaks in the fingerprints were identified and used to establish a standard pattern in order to compare the similarities with the other species by means of similarity and principle component analysis (PCA) methods. Chemical fingerprints produced by HPLC-DAD-ELSD and PCA of *Polygala japonica*, a TCM which is well-known as anti-inflammatory, antibacterial,

and antidepressant agent, were investigated from different cultivation locations in China [24]. Flavonol derivatives were found with DAD chromatogram while triterpenoid saponins were characterized by ELSD chromatogram. The PCA of the fingerprint data from the complementary information between the DAD and ELSD results led to an accurate classification of samples from different locations. In addition, several types of TCM have been analyzed for fingerprinting study or sample authentication with chromatographic techniques such as *Schisandra sphenanthera* profile by HPLC-DAD [25], *Panax quinquefolium.* L profile by HPLC-DAD [26], *Ganoderma lucidum* profile by HPLC-DAD and LC–MS [27], and *Bupleuri radix* profile by HPLC-DAD and HPTLC [28]. From previous studies, chromatographic fingerprint analysis was proved to be rational and practical to assess the authenticity, quality consistency, and stability of many herbal medicines with the combination of chemometrics.

1.2.2 Food quality: Molecular taste profile in beef

1.2.2.1 Beef

Beef is the culinary name for meat from bovines, especially cattle. It is one of the most widely consumed meats in the world. In the process of slaughtering, beef meat is divided into large sections called primal cuts as shown in Figure 1.1. Then, the primal cuts are further divided into subprimals or foodservice cuts, and then into individual steaks and other retail cuts [29]. Different cuts require different appropriate cooking methods to obtain the best taste. From US primal cuts, beef carcasses are split into two parts. Forequarters (front parts) contain chuck, rib, brisket, shank, and plate while hindquarters (back parts) consist of loin, round and flank [30]. Among those cuts, the loin and rib sections are preferable to be cooked as steak because they have the highest tenderness, thus they are the most expensive.

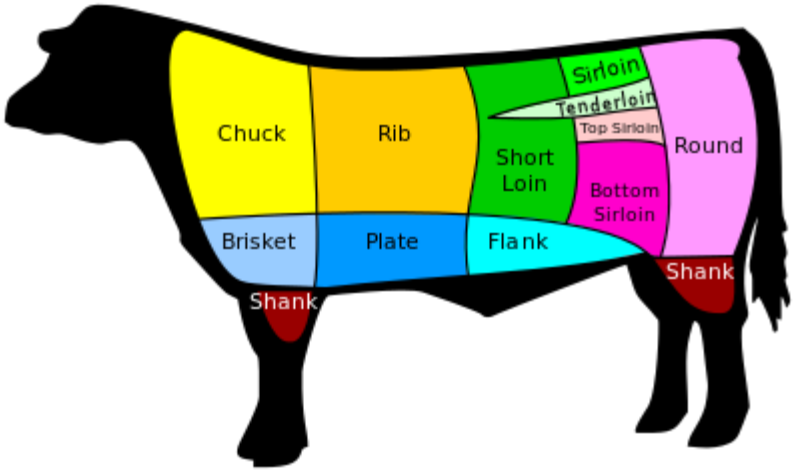


Figure 1.1 US Beef cuts
[31]

The retail cuts of beef are assigned into several grades for consumer's selection. Beef grading was classified by factors that affect palatability of meat (tenderness, juiciness, and flavor) to define meat quality. The U.S. department of agriculture (USDA) has established the standards for beef into two grades: quality grades and yield grades. The quality grade refers to the expected taste characteristics (tenderness, juiciness and flavor) of the cooked product. In the quality grade classification, the most desirable beef is the 'prime beef' and the least desirable is the 'canner beef'. The evaluation of beef quality was performed by physiological maturity and marbling indication. Yield grades are defined as the combined yield of closely trimmed, boneless retail cuts from the round, loin, rib and chuck to estimate beef carcass cutability. This is an estimation of the relative amount of lean, edible meat from a carcass. The lower value of the USDA yield grade refers to the higher the yield of closely trimmed, boneless retail cuts. The yield grade is determined by evaluating external fat thickness over the ribeye, ribeye area, estimated percentage of kidney, pelvic and heart fat, and carcass weight [32].

1.2.2.2 Beef Aging

In meat industry, meat aging is a widely used treatment to improve tenderness and flavor of beef. This process is important for meat products to meet the consumers' demands and expectations for high quality food. The aging process involves storing fresh meat at refrigerated temperatures and humidity for suitable time for the meat to develop palatability characteristics such as tenderness, juiciness, and flavor until the meat was ready to be sold or consumed. There are two common forms of postmortem aging methods which are wet aging and dry aging [33]. Wet aging is the more common process which allows the meat to age in a sealed package at refrigerated temperatures. In contrast, dry aging refers to the process of storing beef carcasses or wholesale cuts without any type of protective packaging in controlled temperatures and humidity [34]. Whereas the brown/roasted flavor of dry-aged beef is considered desirable and distinctive which differs significantly from bloody/serumy and metallic flavors of wet-aged beef [35]. The outstanding flavor of dry-aged beef was produced from natural enzymatic and biochemical processes. Normally, the selected primal cuts are prepared with wet aging process with improvements in yield and the capability of longer storage times when compared to the primal cuts which are usually treated with dry aging. Due to the enhanced flavor and remarkable palatability improvements, dry-aged beef is recognized as a premium product that costs higher in the market and are sold in upscale hotels, restaurants, or some retailers in gourmet market [35]. The price of dry-aged beef is high also because the special environmental control is required, and the production yield is low because of the evaporation of moisture from the meat.

In dry aging treatment, the primary factors involves days of aging, storage temperature, and relative humidity. These parameters relate to flavor development, product shrinkage, shelf life, and microbial spoilage. Dry aging periods of 14 to 35 days were reported as a suitable producing time for dry-aged beef. The storage temperature in the enzymatic processes and the elevated temperatures may promote pathogen growth. The optimal storage temperature is around 0 to 4°C. As for relative humidity, if the value is too high, the spoilage bacteria can grow and result in off-odors and possible off-flavors. However, if the relative humidity is too low, the product may shrink and lead to loss of product yield [36]. Other antibacterial

strategies such as ultraviolet (UV) lighting and air filtration systems, have also been used in the process.

1.2.2.3 Literature review

Dry-aged beef has been reviewed in case of parameters and the sensory properties effect on the quality of meat [37]. Flavor and texture are two main factors affecting palatability and mouth feel. After animal is slaughtered, the metabolism in the cell is terminated, and then the enzymatic processes occur and induce the changes in protein. The large, flavorless molecules are dissociated into small, flavorful fragments. The proteins turn into savory amino acids and fats which convert to aromatic fatty acids. These fragments are involved in the specific and intense flavor of aged meat. Additionally, there are chemical reactions, during the cooking process of dry-aged beef that produce new flavorful molecules. The enzymes also develop the texture of aged meat by reducing the muscle toughness. Aging time, temperature, air flow, humidity and UV light have been mentioned as important parameters for drying process in order to obtain qualified dry-aged beef. Moreover, consumer taste preferences were studied for wet-aged and dry-aged beef steaks [38]. From the sensory test, the results showed that more consumers prefer wet-aged beef. The authors described that this is because the average consumers may not be accustomed to the unique flavor of dry-aged beef. However, the consumers who preferred dry-aged beef are willing to pay more for the dry-aged samples. Furthermore, there are other researchers who investigated the comparison between wet-aged and dry-aged flavors [39]. At this time, the sensory test was performed by the highly trained sensory panelists. The flavor of dry-aged beef was found more significant than that of wet-aged steaks. The 14 and 21 days of dry aging process were reported as optimal durations to produce greater flavor of steaks.

Two different dry-aging methods (unpackaged and in the bag) were compared for two loin-cut styles (bone-in shell loins and boneless strip loins) on the physical, chemical, sensory, and microbial properties of dry-aged beef [40]. For sensory attribute, sweet, sour, salty, bitter, astringent, metallic, bloody/serumy tastes and

brown/roasted were tested and compared. The authors found that dry aging in a bag produced similar flavor to but less weight loss than unpackaged aging did. There was no difference in taste between shell and strip loins using both dry aging methods. Moreover, the dry-aged beef in a bag produced slightly higher yield with no negative effects on product quality from microbial effects, and it also had the flexibility and control of the aging environment.

Recently, there were German researchers who established a new, versatile and unique methodology entitled ‘Sensomics’ for the analysis of taste profile in food [41]. Sensomics is a molecular sensory science approach to identify and quantify the active compounds in food. The process combines instrumental analysis with sensory evaluation. Also, sensomics approach has been used to characterize the previously unknown taste compounds in food with several analytical methods. The experiments helped to uncover the taste modulator compounds, clarify the origin of the food taste, and define the profile of food qualitatively and quantitatively. In food industry, sensomics approach benefits product development, quality control, and chemical process recognition in food and food products. Several analytical techniques processing via statistical methods were coupled with human perception test in order to explore the profile of several types of food.

β-alanyl dipeptides were identified as the key compound contributing to the thick-sour and white-meaty taste in chicken broth [42]. Different preparative and analytical chromatographic techniques were employed with sensory techniques. Chicken broth was prepared, extracted, and fractionated by molecular weight. The fractions were evaluated for the entire taste profile by preliminary sensory test, and the indicated fraction was then isolated by gel permeation chromatography (GPC). The impact fraction from taste dilution analysis (TDA) was separated by HPLC into subfractions. These subfractions were analyzed for basic taste compounds (amino acids, carbohydrates, nucleotides and nucleosides, organic acids, cations, and anions) and evaluated sensorially for thick-sour and white-meaty mouthfeel by LC-MS/MS and high performance ion chromatography (HPIC). LC-MS/MS and NMR were then used to identify the taste-modulating compounds. After β-alanyl dipeptides were recognized, quantitative analysis and taste recombination/omission experiments were

performed to confirm these compounds as the important contributors to the thick-sour mouthfeel and white-meaty character of chicken broth.

In stewed beef juice, sensomics using both preparative and analytical chromatography, combined with sensory techniques led to the identification of N-(1-methyl-4- oxoimidazolidin-2-ylidene)-α -amino acids as taste modulators to the thick-sour and mouth-drying orosensation [43]. Stewed beef juice was prepared, filtered and evaluated for taste descriptors' intensities (sweetness, saltiness, bitterness, umami taste, acidic taste, thick-sourness, mouthfulness, and mouthdryness) by a trained sensory panel. Solvent extraction, molecular weight fractionation and sensory test provided the first information of taste active compounds. Then LC-MS/MS and HPIC were performed to quantitatively analyze the basic taste compounds. Also, the subfractions were separated by GPC and evaluated by TDA. LC-MS/MS, UV-vis, LC-TOF-MS, and NMR were then used to characterize the taste-modulating compounds in each individual fraction. The impact of found β-alanyl dipeptides on the thick-sour taste of beef juice is similar to that on the chicken broth [42]. The identification of three N-(1-methyl-4- oxoimidazolidin-2-ylidene)-α -amino acids was initially reported as the natural occurrence taste-modulating compounds from Maillard reactions in thermally processed foods.

There is another research dealing with the analysis of beef broth with sensomics approach [44]. The taste enhancer for sweetness was identified by ultrafiltration, GPC, and HPLC in combination with TDA. The chromatographic, spectroscopic, and sensory data with the synthetic reference compound led to the identification of the sweetness-enhancing N-(1-carboxyethyl)-6-(hydroxymethyl)pyridinium-3-ol inner salt, named alapyridaine. Since the sweetness and umami character significantly increase only in presence of alapyridaine, it could be indicated that alapyridaine exhibit a pronounced effect on the overall taste quality of beef broth especially on the sweetness and umami taste.

Even there are only few previous researches examined the taste of meat products with the combination of chemical composition analysis and sensory evaluation, there is no study apply sensomics procedure for dry-aged beef which is well-known for the remarkable taste.

1.2.3 Food safety: Matrix effect in pesticide residue analysis

1.2.3.1 Pesticide residue

Pesticides are natural, synthetic, or mixture of both substances intended for preventing, destroying, repelling, or mitigating any agricultural pests that can damage crops, cause nuisance, spread disease, or reduce farm productivity. The most commonly applied pesticides are insecticides (to kill insects), herbicides (to kill weeds), rodenticides (to kill rodents), and fungicides (to control fungi, mold, and mildew), defined by the targeted organism [45]. Pesticides are extensively used not only as plant-protecting products but also non-agricultural products. Pesticides pose a potential short and long term health risks to human. Even though pesticides can benefit farmers by preventing crop losses to pests, pesticides are directly related to numerous adverse health effects including neurologic and endocrine (hormone) system disorders, birth defects, cancer, and other acute and chronic diseases. Moreover, the use of pesticide increases the number of environmental concerns such as contamination in air, soil, and water resources. Also, pests have developed more resistance towards pesticide, resulting in the need for increased application of pesticides, or alternative formulation of pesticides. The major chemical classes of pesticides are organochlorines (OCs), organophosphates (OPs), carbamates, and pyrethroids. Each class has different modes of action [46].

Most OPs are insecticides. Its main mechanism is blocking the enzyme acetylcholinesterase, causing nervous and respiratory damages that result in the insects' death. These effects are similar to the effects on humans. OPs can be absorbed by all routes, including inhalation, ingestion, and dermal absorption. This class of pesticide consists of organic phosphorus(V)-containing compounds [47]. The significant OPs are carbofuran, chlorpyrifos and malathion. OCs are chlorinated hydrocarbon pesticides that contain carbon, chlorine, and hydrogen. This class is among the oldest, most toxic, and most environmentally destructive synthetic pesticides. DDT is the notable OCs which is used on a large scale worldwide. Many OCs are extremely persistent in the environment and in people's bodies; therefore, this class has been removed from the market. OCs has an acute effect on the central nervous system, where these compounds induce a hyperexcitable state in the brain

leading to convulsions which may cause death by interfering with pulmonary gas exchange and by generating severe metabolic acidosis. OCs also acts as an endocrine disruptor and possible carcinogen [48]. Carbamates are derivatives of carbamic acid. This class has killed insects in a similar way as OPs do. The mode of action is the inhibition of cholinesterase enzymes, affecting nerve impulse transmission. For OPs, the signs and symptoms are based on excessive cholinergic stimulation, but carbamate poisonings tend to have shorter duration because the inhibition of nervous tissue acetylcholinesterase is reversible, and carbamates are more rapidly metabolized [49]. Highlighted carbamates are aldicarb, ethiocarb, and methomyl. Pyrethroids are a group of synthetic pesticides similar to the natural pesticide pyrethrum, which is produced by chrysanthemum flowers. This class decomposes quickly, especially when exposed to natural sunlight. Pyrethroids have irritant and/or sensitizing properties and also act as dermal and respiratory allergens. Many compounds have also been linked to disruption of the endocrine system by mimicking the female hormone, estrogen, thus causing an excessive estrogen levels in females. This process can adversely affect reproduction and sexual development, interfere with the immune system, and increase chances of breast cancer [50]. Permethrin, bifenthrin, and cypermethrin are example compounds in this pesticide class.

EPA is a department of the U.S. which establishes tolerances or maximum residue levels that describe the amount of a given pesticide that can safely remain in a food. A long-term exposure of pesticides can cause problems that might not be observed in a short-term study. Pesticide levels on fruits and vegetables is mentioned by the US Food and Drug Administration (FDA) and the task of surveying pesticide residues in meat, eggs, and dairy products is in charge of the US Department of Agriculture (USDA) [51]. In Europe, EU has set the maximum residue limit (MRLs) for pesticides in foodstuffs to guarantee consumer safety and to regulate international trade by the estimation of the residue level and of the suitable total daily intake. For EU framework, the authorization of pesticides required directive 91/414/EEC concerning the placing of plant protection products on the market and the regulation 396/2005/EC is related to the MRLs of pesticides in food and feed of plant and animal origin [52]. In 2009, EU has laid down the new directive 2009/128/EC as a framework for community action to achieve the sustainable use of pesticides for

member states replaces directive 91/414/EEC [53]. This regulation is designated to reach target by reducing the risks and impacts of pesticide use on human health and the environment, and to promote the use of integrated pest management and of alternative approaches or techniques such as non-chemical alternatives to pesticides.

All MRLs for pesticides in the EU have been established, and the average MRLs is about 0.01 mg/kg. To check the compliance with the EU regulation of MRLs, pesticides in food are inspected in correspondence with SANCO document which describes the method validation and analytical quality control requirements [54]. From sampling to data analysis steps, this document was legislated for laboratories that are involved in official control of pesticide residues in food and feed in EU to ensure the quality of whole analytical processes. Chromatographic methods coupled with mass spectrometry (MS) is recommended for the identification and quantification of pesticides in food samples.

1.2.3.2 Matrix effect

Matrix refers to the components of a sample other than the analyte of interest such as lipids, pigments, and other soluble high molecular mass components. The effect of matrix has been highlighted as one of critical analytical problem especially in trace level analysis of organic contaminants [55]. Matrix effect (ME) complicates the GC and/or LC determinative step which led to inaccuracy or low quality of the generated data by inducing greater random and systematic errors. ME also causes more frequent instrument maintenance needs. The general strategy for residue analysis consists of (i) isolation of analytes from sample matrix, (ii) removing of co-extracts from extract, (iii) identification and quantification of target analytes and/or (iv) confirmation of target analytes. Even though the instrumental configurations of recent separation techniques by chromatography have been greatly improved in case of the detection level by advanced technologies, the selection of an appropriate sample preparation technique is still required.

Multiresidue method is widely applied in many routine laboratories because it reduces cost, time, and labor. This method involves the determination of multiple

compounds within a single run in a relatively short period [56]. However, the broad range of physico-chemical properties of the target compounds leads to the difficulty of removing the co-extract matrices which may interfere the chromatographic process. ME can occur in both GC- and LC-based methods, but its existence is different depending on the separation mechanism and instrumental design. Most pesticides are volatile and thermally stable compounds, therefore GC coupled with MS is preferable for the pesticide residue analysis. A common problem in GC analysis for pesticides is the loss and peak tailing of susceptible analytes from binding with active sites in the inlet and column. This may lead to wrong identification and quantification of the targeted pesticides.

The term ‘matrix-induced response enhancement effect’ was first described to explain the phenomenon in which the analyte concentration was overestimated in a sample matrix compared to the calibration standards in pure solvent (matrix-free solution) at the same concentration [57]. The different detected intensities lead to the problem of identification of compounds and the establishment of calibration curve for quantification. Figure 1.2 illustrated the schematic diagram of matrix-enhancement effect. When sample extract was injected into GC, sample matrix enhanced the transfer of analytes to the analytical column by masking the active sites (silanols, metal ions, and other active sites produced by thermally decomposed components of samples) in GC injector and column leading to a greater signal. This phenomenon is called ‘masking phenomena’ and is used to explain the recovery rates of some pesticides that exceed 100% in GC analysis, and to improve peak intensity and shape when the sample matrices are presented. When there is no sample matrix, the detected signal is lower because susceptible analytes tends to be retained or decomposed on the active sites. The important factors affecting MEs in GC include the number and types of active sites, analyte structure (hydrogen bonding capability) and concentration, amount and type of matrix, and instrument conditions [58]. The presence of adsorption sites inside GC system comes from silanol groups or metal ions on glass surfaces contacted by the sample during injection, separation and detection, or were created by deposition of nonvolatile and possibly thermally modified matrix components contaminating the injector and column from previous analyses.

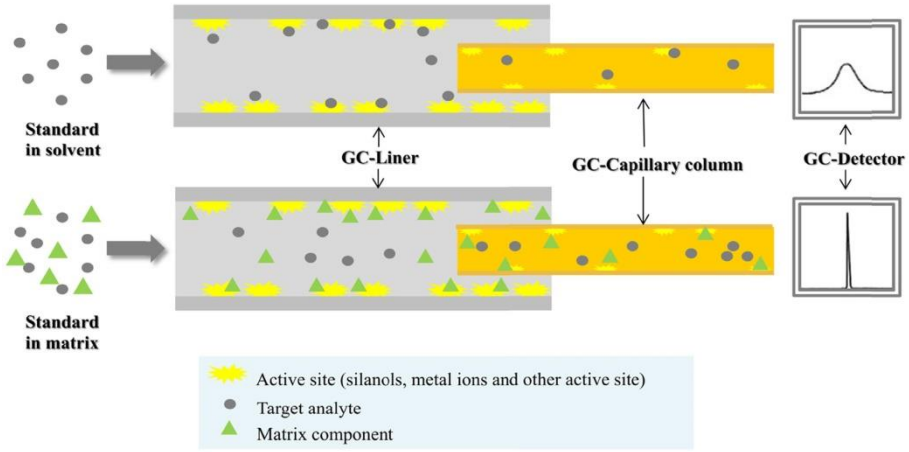


Figure 1.2 Schematic diagram of matrix-induced response enhancement effect [59]

Despite the commercial claims of inert materials for use in GC, new actives sites are formed during long analytical sequences by the deposited non-volatile matrix components. However, it is not uncommon in routine analysis to 'prime' the system by injecting several matrix blank extracts at the beginning of sequences to provide a masking effect [60]. One of the most common practical solutions to the matrix-induced response enhancement problem is to compensate the difference of the analyte response in calibration and sample by performing matrix-matched calibration. Because the matrix-induced response enhancement depends on the matrix type, the most accurate results using matrix calibration require the exact matching of the matrix for each sample type. In this approach, calibration is constructed by adding standard solutions to residue-free matrix extracts.

However, the limitations of this approach include frequent unavailability of residue-free sample, commodity-dependent behavior of different matrices, and difficulties when several matrices are analyzed in the same sequence. Moreover, the needs for several matrix injections lead to greater instability of results due to the contamination of matrix in the GC system. Some routine laboratories selected a

reference or representative matrix for calibration. To use a single representative matrix instead of several matrices, a suitable criteria for the selection of a representative matrix should be agree with the slopes for the matrix calibration solutions. This method is matched with the routine applications where a small uncertainty may not be critical [60].

Besides the matrix-matched standards approach, there are several approaches to overcome matrix-induced response enhancement effect such as standard addition method, isotopically labeled internal standards, on-column or other injection method in GC, and calculation of correction function [61]. Nevertheless, these methods are still impractical for routine applications of complex matrices. Although standard addition method is cheap and useful when there is no blank matrix to carry out the calibration, this approach requires much labor and leads to inaccuracy, because the matrix effect is concentration dependent. The use of isotopically labeled internal standards is very expensive and some standards are not commercially available. In addition, on-column injection techniques lead to more instrumental maintenance and are not suitable for routine work. The calculations of correction function are sometimes not acceptable because the effect is too variable and complicates the data analysis step [58]. One of the promising approaches to compensate this matrix complication is to use 'analyte protectants (APs)'. APs are introduced as masking agents at active sites instead of matrix and found to improve peak shapes and chromatographic signal, especially for susceptible analytes [58, 62]. APs compete with the analytes at the active sites and compensate response enhancement from matrices. Generally, compounds which could be used as APs should have strong hydrogen-bonding capability and similar volatility to that of the analytes. APs offer easier peak integration, lower detection limits, less maintenance of the GC system, simpler procedures, and lower operating costs.

1.2.3.3 Literature review

The problems of ME in pesticide residue analysis in GC are mentioned in several studies. Comprehensive reviews of matrix enhancement effect in GC reported

about the advantages, drawbacks, and solutions [59, 60]. Usually, the residue of pesticides is presented in a very low level. Therefore, a suitable solution should be carefully selected to match the real sample analysis of pesticide. Some compounds are not susceptible to matrix-induced response enhancement effect because they are thermally stable; or they have limited potential for adsorption interactions in GC injector; or the matrix is unable to provide a significant masking effect. The susceptible compounds should be polar and/or strong hydrogen-bond acids and/or bases exemplified by the presence of phosphate, hydroxyl, amino, imidazole, benzimidazole, carbamate, and urea functional groups [60]. The effect of matrix-induced response enhancement in pesticide residue analysis have been extensively studied in honey [63], vegetable oils [64], meat [65], fruit juices [66], and drinking water [67]. Among those studies, matrix-matched calibration was used for the quantification of target compounds in specific food matrices. On the other hand, this method is quite impractical for routine laboratory in which a wide variety of samples are analyzed consecutively. Also, US EPA and FDA did not allow the use of matrix-matched calibration due to the concerns over falsification of results.

Considering the routine applications of pesticide residues in food, APs have been widely employed as a potential approach to compensate the matrix-induced response enhancement effect. The first report of APs was performed by an addition of a single compound to act as masking agent. Six individual compounds were selected, and none of them was found to effectively eliminate ME for polar OPS pesticides in milk [62]. In 2003, several possible APs with a wide variety of chemical compounds for practical use were studied [58]. Since the hydrogen bonding and volatility are the key factors in the masking phenomena, 93 compounds with strong hydrogen bonding ability (various polyols and their derivatives, amino acids, carboxylic acids, basic derivatives of nitrogen containing heterocyclic groups, sugars, sugar alcohols, sugar derivatives, poly-ethers, and others) have been tested for optimal APs. Other parameters such as mass spectral interference, volatility, and concentration were also observed in the analysis of various GC-amenable pesticide classes. In this work, the authors summarized the advantages of APs application which are (i) improvement of intensity and shape of analyte peaks, (ii) easier and more accurate peak identification and integration, (iii) greater selectivity, lower detection limits, and greater confidence

in the results, (iv) elimination of the errors from matrix-induced enhancement effects, (v) very easy, fast, and inexpensive approach and (vi) less maintenance of the GC system. The chemical compounds which contain multiple hydroxy groups, such as sugars and sugar derivatives, and gulonolactone were found to be the suitable protecting agents for most of tested pesticides.

Due to a wide variety of physico-chemical properties of pesticides, the use of a single compound as an AP was not enough to cover the full multiresidue analysis. Thus, a combination of several APs was evaluated for early-, middle-, and late-eluting analytes to minimize the loss of susceptible pesticides and to improve the peak shapes, using GC–MS analysis. The reported optimal AP solution was the mixture of 3-ethoxy-1,2-propanediol (ethylglycerol), L-gulonic acid g-lactone (gulonolactone), and D-sorbitol (10:1:1) [68]. Ethylglycerol is used to protect volatile pesticides; gulonolactone is for pesticides of intermediate volatility; and sorbitol is for pesticides of low volatility. Several works then applied the proposed APs combination to compensate for MEs on pesticide analysis in fruit-based baby food [69], grapes and wines [70], leafy vegetables [71], and apples [72]. To improve the ability of the optimized APs combination, shikimic acid was added to the AP mixture to better protect base-sensitive compounds. Thirty-eight GC-amenable pesticides were quantitatively determined in spiked lemons, raisins, wheat flour, and cucumber with the minimization of matrix-effect-related errors [73]. The optimization of other AP combinations for pesticide analysis in various samples were also studied: the mixture of D-ribonic acid-g-lactone and D-sorbitol (2:1) for Chinese herbs [74], the mixture of triglycerol and D-ribonic acid-g-lactone (1:1) for teas [75], and the mixture of olive oil, L-gulonic acid g-lactone (1:1) for soil, juice, and honey [76]. Additionally, pepper leaf was applied as another choice of AP for analysis of thermolabile terbufos and its metabolites in pepper and plum [77]. For the long-term effect of APs, the study showed that the APs are still effectively used to compensate for the matrix-induced response enhancement even after more than 150 GC injections [68].

1.3 Purpose of the study

Since food quality and food safety are globally significant concerns, many researchers have been paying attention to develop several new and comprehensive methods to solve those concerns. For the food quality issues, the authentication and the assurance of food are required in the food supply chain to serve the related regulations and protect the consumers. Each food has the specific characteristic to identify itself, and such information is also beneficial for consumers' choice and producers' traceability. Due to the rapidly increasing number of food in the market from many sources and the advancement of technology, the available food should be guaranteed for its quality and safety. Therefore, the analytical methods should be developed to accommodate the recent aspects of food analysis.

In food quality issue, this work aims to characterize the origin and the taste of specific food by using chromatographic methods coupled with statistical techniques for data processing. Ginger was chosen as a sample to study the authentication of food in the case of origin labelling because many kinds of Thai food contain ginger, and Thailand is one of the largest ginger producer. Ginger samples from many geographical origins including the sources in Thailand and in neighboring countries were analyzed. The conditions of instrumental determination and sample preparation steps were optimized. The profiles of ginger samples from many origins were quantitatively and qualitatively identified with LC-DAD and LC-MS/MS to obtain their fingerprints. Furthermore, chemometric methods were applied to chromatographic data in order to establish a pattern for the recognition of ginger origin. The statistical methods for data visualization and data classification were performed to process all obtained data. Also, the chemical markers for each origin were investigated from the characteristic compounds for easier discrimination of ginger sources.

In the taste profile analysis for food quality investigation, a high quality dry-aged meat with a distinctive flavor and tenderness using a patented special noble mold culture method in the production from LUMA company in Switzerland was selected. For LUMA process, the pure and natural mold *Thamnidium elegans* was sprayed onto

the fresh meat during dry aging process. The unpackaged meat cut was hung in the air with bone under the optimized controlled conditions to allow meat maturing. The fungus slowly grows and permeates into the flesh inside and the mold enzymes employ their biochemical activity to create the unique intense flavor and increase tenderness and juiciness of LUMA dry-aged meat [78]. The LUMA beef has received approvals from many chefs for its tenderness and flavor. This dry-aged beef is sold only in upscale restaurants, hotels, and in a few supermarkets. In this work, dry-aged LUMA beefs with many steak cuts (rib eye, short loin, tenderloin, and sirloin) were studied for the chemical compositions which could effect on the characteristic taste. The normal and treated LUMA beef were compared and studied for their basic taste compounds with LC-MS/MS and IC. The quantitative data of organic acids, fatty acids, amino acids and other ions have been determined and processed with statistical methods to explore the significant information of taste compounds in LUMA beef. Moreover, LC-TOF-MS and GPC were employed with sensory evaluation to examine the possible taste modulator compounds in LUMA beef. The reaction between some available fatty acids and amino acids were also examined because the obtained products might be involved in the specific taste of LUMA beef.

In the food safety topic, the pesticide residue is a very critical problem for inspection in worldwide food business. Several regulations require a high performance method to effectively determine the trace level of chemical residue in food. Although an accurate analytical method is needed, there are some complications in the analysis. The interference of food matrices in the determination process of pesticide residue led to ME and inaccuracy results. In the multiresidue method with GC-MS/MS for pesticide residue, a combination of APs were previously used as a potential solution to compensate the ME problem. However, the use of APs in the classical approach did not match with the routine practice. Therefore, this work studied the use of APs in a new way called ‘AP priming’ which is more practical to routine laboratory, and reduces several difficulties. The ME and the use of the classical AP approach were observed in the analysis of 100 pesticides from various classes in red chili, a high pigmented matrix. Then, the performance of AP priming and its possibility on ME compensation was studied and evaluated in the analysis of 100 pesticides in red chili at a very low level. QuEChERS methodology was used as

sample preparation, and GC-MS/MS was employed for the instrumental determination. Long term performance of AP priming (50 injections) was also investigated, and the studies of using single and mixed APs in priming approach were compared.

CHAPTER II
THEORY

2.1 Sample preparation

2.1.1 Liquid-liquid extraction (LLE)

Liquid-liquid extraction (LLE) is the most common extraction technique for isolating an analyte from aqueous matrices. LLE based on the relative solubility of an analyte in two immiscible phases and is governed by the equilibrium distribution/partition coefficient. Extraction of an analyte is achieved by the differences in the solubilizing power (polarity) of the two immiscible liquid phases. Therefore, LLE has also been referred to as immiscible solvent extraction. The main advantages of this approach are the wide availability of pure, solvents and the use of low-cost apparatus. Drawbacks of this technique are that is labor intensive, non-environmental friendly and time-consuming. [79-81]

Typically, classical LLE operates manually by using a separatory funnel (Figure 2.1) to extract analytes from an aqueous solution into a non-polar or less polar organic solvent and the two immiscible phases are mixed by shaking and then allowed to separate. The relative position of each layer depends on the relative densities of the two immiscible phases.

Figure 2.1 Separatory funnel for use in LLE
[80]

Two terms are used to describe the distribution of an analyte between two immiscible solvents: partition coefficient and the distribution ratio. Equilibrium of solute in aqueous (S_{aq}) and in organic phase (S_{org}) can be written as an equation:

$$S_{aq} \leftrightarrow S_{org}$$

The partition coefficient (K) can be represented by:

$$K = \frac{[S_{org}]}{[S_{aq}]}$$

A large value for K indicates that the extraction of the solute (S) into the organic phase is favorable. The distribution ratio (D) is defined as the ratio of the solute's total concentration in each phase.

$$D = \frac{[S_{org}]_{tot}}{[S_{aq}]_{tot}}$$

When the solute exists in only one form in each phase, then the partition coefficient and the distribution ratio are identical. If the solute exists in more than one form in either phase, then K and D usually have different values.

Also, the n-octanol/water partition coefficient (K_{ow}) uses to describe the hydrophobicity of compounds based on the n-octanol reference system. K_{ow} refers to the amount of transfer of a solute from water into a particular immiscible solvent, usually n-octanol. The n-octanol/water reference system covers a wide scale of distribution coefficients, with K_{OW} values varying with organic molecular structure of chemical compounds.

$$K = K_{OW} = \frac{[S]_O}{[S]_W}$$

The larger the value of K_{OW}, the greater is the tendency of the solute to escape from water and transfer to a bulk hydrophobic phase. The compound with the higher number of K_{OW} is more hydrophobic than the others. Compounds with log K_{OW} less than 1 are recognized as highly hydrophilic, and compounds with a log K_{OW} above 3 to 4 are identified as highly hydrophobic.

The choice of two immiscible solvents in LLE critically influences on the selectivity and efficiency of the extraction process. Figure 2.2 shows a solvent miscibility chart which is useful for determining the immiscibility of solvent pairs in LLE. The more hydrophobic analytes prefer the organic solvent immiscible with water while the more hydrophilic compounds prefer the aqueous phase. Beside immiscibility, density and solubility are also two considerations when selecting an extraction solvent. Solvents with high density will form the lower layer below the pair solvent, while low density solvents will form the upper layer. In case of solubility, Table 2.1 presents the solubility of various solvents in water for solvent selection.

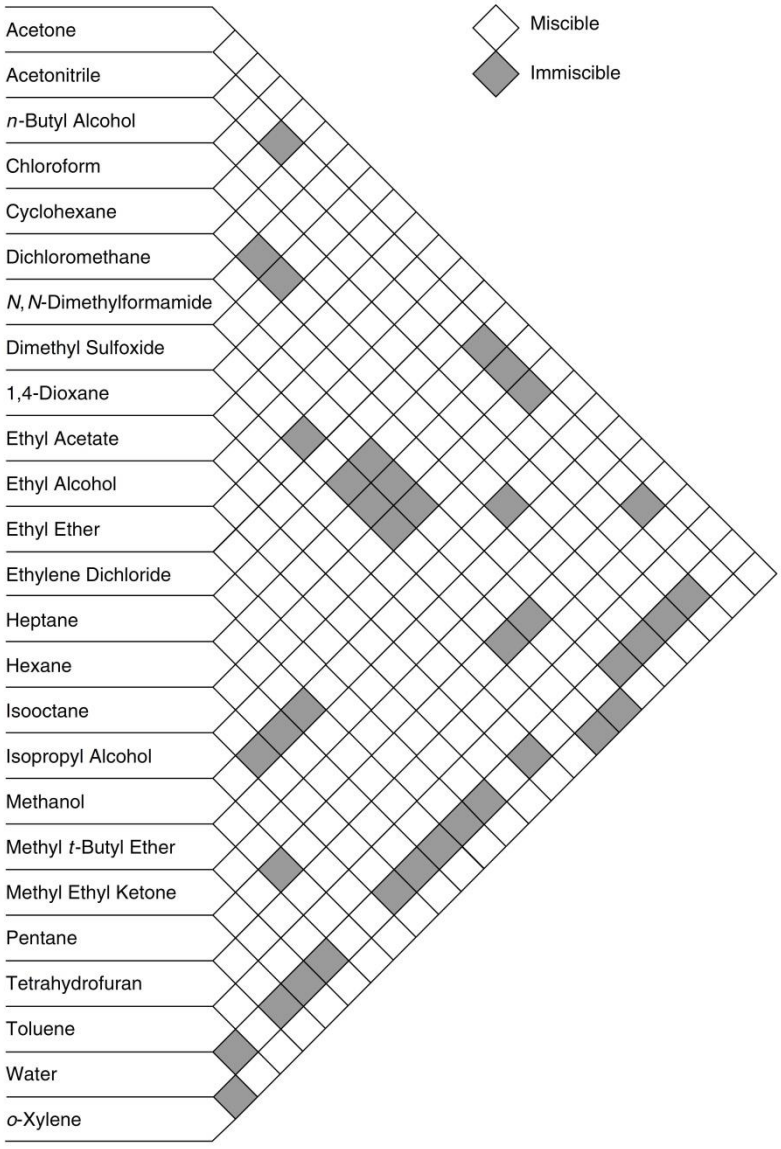


Figure 2.2 Solvent miscibility chart
[81]

Table 2.1 Solubility in Water
[81]

Solvent	Solubility (%)[a]
Isooctane	0.0002 (25°C)
Heptane	0.0003 (25°C)
1,2,4-Trichlorobenzene	0.0025
Cyclohexane	0.006 (25°C)
Cyclopentane	0.01
Hexane	0.014
o-Dichlorobenzene	0.016 (25°C)
1,1,2-Trichlorotrifluoroethane	0.017 (25°C)
o-Xylene	0.018 (25°C)
Pentane	0.04
Chlorobenzene	0.05
Toluene	0.052 (25°C)
n-Butyl chloride	0.11
Methyl isoamyl ketone	0.54
n-Butyl acetate	0.68
Ethylene dichloride	0.81
Chloroform	0.815
Dichloromethane	1.60
Methyl isobutyl ketone	1.7
Methyl *t*-butyl ether	4.8
Triethylamine	5.5
Methyl *n*-propyl ketone	5.95
Ethyl ether	6.89
n-Butyl alcohol	7.81
Isobutyl alcohol	8.5
Ethyl acetate	8.7
Propylene carbonate	17.5 (25°C)
Methyl ethyl ketone	24.0

Recovery (%R) is the percentage of analyte extracted into the organic solvent at equilibrium and can be calculated as following equation. This value used to determine the effectiveness in extraction process.

$$\%R = \frac{moles\ of\ analyte\ extracted\ into\ organic\ solvent}{moles\ of\ analyte\ in\ original\ sample} \times 100$$

LLE recovery is an equilibrium procedure in which exhaustive extraction is driven by the principle of repeated extractions. Typically, two or three replicates of

extraction are required with fresh organic solvent to achieve quantitative recoveries. The percent recovery obtained with three sequential extractions of 50 mL of organic solvent is greater than a single extraction of 150 mL solvent. For the recovery of n repeated extractions ($\%R_X$), the calculation is shown below.

$$\%R_X = \left\{1 - \left[\frac{1}{1 + K(V_E/V_O)}\right]^n\right\} \times 100$$

where V_O is the volume of the original sample and V_E is the extraction solvent volume. Repeated extractions may be required to recover the analyte sufficiently from the aqueous phase. The net amount of analyte extracted depends on the value of the distribution coefficient and the ratio of the volumes of the two phases used. Recovery is independent of the concentration of the original aqueous sample.

There are some precautions in LLE which are the formation of emulsions, salting out or the addition of a small amount of a different organic solvent, additional solvent evaporation step after extraction, different extraction rate for the same analyte in different sample matrices, or contamination from low purity solvent or glassware used. To preconcentrate analytes, rotary evaporator or gas blow-down may be needed to remove excess organic solvent before clean up step or instrumental determination. In rotary evaporator, the solvent is removed under reduced pressure by mechanically rotating the flask containing the sample in a controlled temperature water bath. The solvent is condensed and collected for disposal. For small scale evaporation, a high purity purge gas can be used by passing gas over the surface of the extract. The purge gas is directed towards the side of the vessel, and not directly onto the top of the extract to induce a swirling action. This approach may effectively works with low boiling point solvent and may be left the extract in a small volume.

2.1.2 QuEChERS methodology

QuEChERS (pronounced “catchers”) is an acronym for Quick, Easy, Cheap, Effective, Rugged and Safe, covers a variety of sample preparation and clean-up

techniques for the analysis of multiple pesticide residues in agricultural matrices. The technique is very simple, involves a minimum of steps, and is effective for the cleanup of complex samples by combining microscale extraction using acetonitrile (ACN) and purifying the extract using dispersive solid-phase extraction (d-SPE). QuEChERS was firstly developed by Anastassiades et al. for the multiclass, multiresidue analysis of pesticides in fruits and vegetables [82]. Since its first introduction, the method has been modified into many versions to improve recovery for specific types of pesticides or types of food. Due to some certain pH-dependent pesticides, the extraction step of original approach was added up with the buffering conditions to achieve high recovery of those pesticides. While Lehotay et al. used the acetate buffering conditions which obtain relatively stronger effect [83]; Anastassiades et al. suggested the citrate buffering conditions which have weaker ionic strength [84]. These methods have been extensively investigated to certify and ensure the accuracy of their analytical results, and then become the official standard methods for food control regulation in U.S. and EU. The acetate-buffering version was established as AOAC Official Method 2007.01and the citrate-buffering version was authorized as European Committee for Standardization (CEN) Standard Method EN 15662.

Before QuEChERS methodology, sample comminution is required by blender or homogenizer to achieve good sample homogeneity and to ensure representative of subsample for the analysis. The method is designed for samples with high moisture; therefore, water might be added to hydrate the dry samples which allow the extraction solvent to be accessed [82]. In QuEChERS, the practical approach consists of two major steps which are extraction and clean up [85], and the diagram for QuEChERS procedure presents in Figure 2.3.

1) Extraction: The partitioning of analytes, via salting-out extraction involving equilibrium between an aqueous and an organic layer, occurs from the addition of 10 mL ACN, selected buffers, $MgSO_4$ and NaCl. ACN was selected as suitable organic solvent providing the best characteristics for extracting the broad range of pesticides with low co-extractables and also amenable for both LC and GC analysis. Buffered version of QuEChERS is selected based on the studied analytes. Buffer prevents

degradation of pH sensitive analytes by maintaining optimal pH. Moreover, $MgSO_4$ facilitates solvent partitioning and improves recovery of polar analytes and NaCl helps in reducing the amount of polar interferences. Internal standard (ISTD) is typically used to correct the variations from water content differences in commodity and volume fluctuations. It is necessary for calibration establishment in quantitative analysis.

2) Clean up: A d-SPE step involves further cleanup using various combinations of salts and porous sorbents to remove interfering substances. $MgSO_4$ and d-SPE sorbents were added into the extract. The function of $MgSO_4$ is the same as in extraction step, and the selection of sorbent depends on types of analytes and co-extract matrices in samples. Primary and secondary amine (PSA) is the base sorbent used for QuEChERS d-SPE cleanup of fruit and vegetable. After clean up, the sample extracts in ACN can be directly analyzed by LC and GC combined with MS to determine a wide range of pesticide residues.

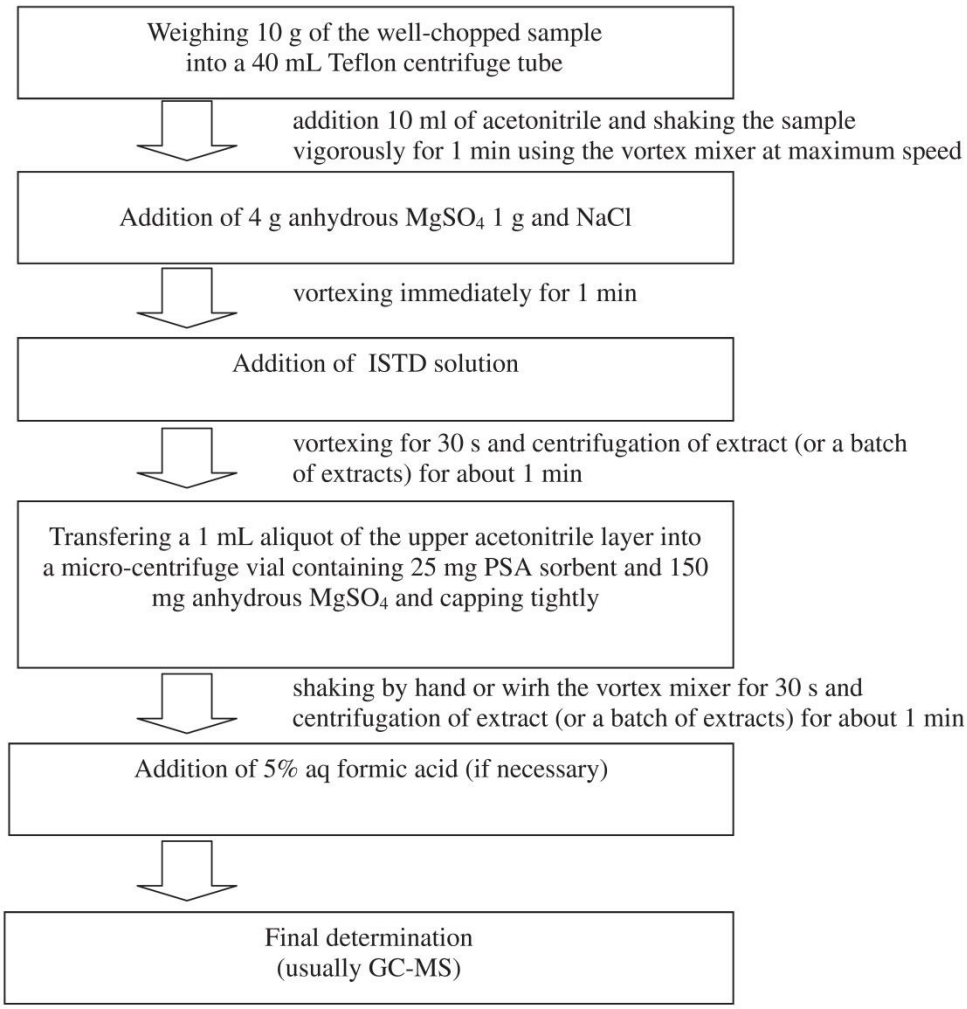


Figure 2.3 The main steps of QuEChERS analytical procedure for determining pesticides in food matrices

[86]

According to its acronym, the QuEChERS method has several advantages over most traditional extraction methods e.g., high recovery in multiresidue analysis of pesticides, high accuracy analytical results, low solvent usage and waste generation, high sample throughput, no chlorinated solvents used, no technical skill required, using inexpensive reagents, few equipment needed, safety for user, high efficiency and less time-consuming [87].

2.1.2.1 Extraction Solvents in QuEChERS

Many considerations for solvent selection in QuEChERS include extraction ability for desire analytes, selectivity during extraction, partitioning, and cleanup, ability in separation from water, amenability to chromatographic separation techniques, cost, safety, and environmental concerns and handling aspects [82]. ACN, acetone, and ethyl acetate have been studied for pesticide residue analysis. ACN and acetone can miscible with water led to single-phase solvent extraction, but the use of acetone as extraction solvent needs a nonpolar co-solvent to induce a well-defined phase separation with water, while ACN can be used without co-solvent, but the additional salts. After the partitioning step, the residual water can be removed better in ACN extracts by drying agents [88]. Even though ACN is not only compatible with GC applications, but because of its low viscosity and intermediate polarity, it is very useful in reversed-phase liquid chromatography (LC). Neither ethyl acetate nor acetone is useful in common LC applications. ACN, acetone, and ethyl acetate have been studied for pesticide residue in mixed fruit and vegetable and compared by means of the amounts of co-extractives [82]. The results showed that ACN had the fewest possible interfering peaks in GC/MS both before and after cleanup. Therefore, ACN has been recognized as the most advantageous solvent in QuEChERS methodology.

2.1.2.2 Various salts in QuEChERS

To induce phase separation in ACN extracts, $MgSO_4$ and NaCl were added. NaCl led to salting-out effect and increased recoveries of polar compounds. Fructose, $MgSO_4$, $MgCl_2$, $NaNO_3$, Na_2SO_4, LiCl, and NaCl have been previously investigated for the phase separation ability after analytes partitioning in ACN [82]. The best results obtained with $MgSO_4$ by providing highest recovery of polar GC-amenable pesticides, larger volume of the upper layer, and lowest concentrations of ACN in the lower phase. Too much $MgSO_4$ creates complication in vortexing, while too low amount did not yield satisfactory recovery of analytes. For NaCl, the high amount of salt used led to better phase separation and less water in ACN phase. Consequently,

the combination of $MgSO_4$ and NaCl were recommended to cooperatively work. The amount of 4 g $MgSO_4$ and 1g NaCl were considered as suitable amount in extraction/partitioning for 10 g sample. This ratio avoids co-extraction of polar matrix components but still achieve high recoveries of polar pesticides.

2.1.2.3 pH effect in QuEChERS

Due to the wide variety of acidity in fruits and vegetables, natural pH values are varied. Common pesticides used in agriculture are more stable at lower pH and degrade rapidly at higher pH such as chlorothalonil, captan, captafol, folpet, and dichlofluanid [82]. The degradation products of pesticide are not considered as residue in the definition of legal regulations, but they are still often monitored in GC/MS methods. Also, the degradation led to the inaccuracy analytical results. Therefore, the adjustment of pH and maintain the pH in commodities at the preferable pH value for certain pesticides are necessary to obtain better overall recovery for a large number of residues, especially base-sensitive pesticides. The addition of 0.1% acetic acid to ACN solutions was studied and the results proved that it helped prolong the stability of the problematic pesticides prior to analysis [89]. Maintaining pH of extract is not only important to base-sensitive pesticides, it was also critical for acid-sensitive pesticides (pymetrozine). The compromising of pH is favorable for sufficient stability of specific pesticides. Buffering of the extracts was a reasonable approach to control pH. Citrate buffering has weaker strength and slightly higher pH of 5.5 than strong acetate buffering at pH 4.8 [84]. The comparison study of recoveries using the 3 different QuEChERS methods for a diverse range of pesticides (including chlorothalonil, tolylfluanid and pymetrozine) in a variety of commodities reported that acetate buffering version (AOAC method) gave higher and more consistent recoveries for the pH-dependent pesticides in fruit and vegetable matrices than the citrate buffering version (CEN method). On the other hand, citrate-buffered version provides more compatible pH for utilizing d-SPE sorbents.

2.1.2.4 d-SPE sorbents in QuEChERS

Sample clean-up is a necessary step to reduce interferences which can damage analytical instrumentation and complicate analyte identification and quantification. A d-SPE clean-up was selected for QuEChERS method because it's quicker, easier, and less expensive than using traditional SPE cartridges. A principle of d-SPE is similar in some respects to matrix solid-phase dispersion (MSPD), but the sorbent is added to an aliquot of the extract rather than to the original sample as in MSPD [82]. Compared to traditional SPE, the d-SPE is used to remove matrix components, not the analytes. The d-SPE tubes can be prepared in the laboratory and also available commercially. Usually, $MgSO_4$ is added simultaneously as the SPE sorbent to remove excess water and to provide better cleanup. The amount of 150 mg is being used per 1 ml of extracts in all QuEChERS versions. The quantity and type of sorbent are critical parameters affecting on the effectiveness of interference removal. Sorbents can be adjusted for different matrix interferences and various analytes. Several types of sorbents in individual and combination forms have been investigated for cleanup efficiency of extracts. PSA has been suggested as typical QuEChERS d-SPE sorbent because it helps to remove common interfering substances such as fatty acids, sugars, polar organic acids, lipids and some polar pigments [86, 89]. A 50 mg PSA is recommended amount in AOAC method, while CEN method applied 25 mg of PSA. The use of PSA sorbent in d-SPE retains pesticides containing carboxylic acid groups, such as daminozide and 2,4-D; therefore, results for these pesticides are more variable depending on the matrix and conditions used.

Other d-SPE sorbents being commonly used are graphitized carbon black (GCB) and C_{18}. GCB is suitable for high pigmented matrices because its effective function is to remove pigments (e.g., chlorophyll, carotenoids), also polyphenols and other polar compounds. However, there is a loss of certain structurally planar pesticides from the use of GCB such as chlorothalonil, coumaphos, hexachlorobenzene, thiabendazole, terbufos, and quintozene [84, 86]. Therefore, GCB sorbent is only recommended when it is necessary, in a suitable amount. AOAC method suggested using 50 mg GCB per 1 mL of extract for enhanced matrix removal and CEN method defines the amount of GCB used by based on commodity types (2.5

mg for pigmented and 7.5 mg for highly pigmented commodities). The combination of PSA and GCB is often used in matrix removal of pigmented fruits and vegetables.

C_{18} is another d-SPE sorbent helps to remove long chain fatty compounds, sterols and other non-polar interferences [86]. There is no major adverse effect of pesticides causing by the addition of C_{18}. It was normally added to improve analyte detection in very complex sample matrices such as food containing biological matrix interferences, including hydrophobic substances (fats, lipids) and proteins. AOAC method recommends 50 mg C_{18} in addition to $MgSO_4$ and PSA sorbents and CEN method suggests 25 mg C_{18} instead. PSA and C_{18} were combined and food with fats and waxes.

The QuEChERS method is a useful extraction technique which now extensively applied to analyze several types of foods for pesticides with a wide range of polarity. Various extraction solvents and d-SPE sorbents were optimized. The approach is adaptable and can be easily modified to analyze the new matrices through the selection of alternative sorbents.

2.2 Instrumental Determination

2.2.1 Liquid Chromatography

2.2.1.1 Basic principle

Liquid chromatography (LC) is a separation technique based on different interactions of chemical components between a stationary and a liquid mobile phase. Separation is determined by solute/stationary-phase interactions, including liquid–solid adsorption, liquid–liquid partitioning, ion exchange and size exclusion, and by solute/mobile-phase interactions [80, 90-92]. These interactions can be controlled through different choices of both stationary and mobile phases. A schematic diagram of a typical HPLC instrument is shown in Figure 2.4.

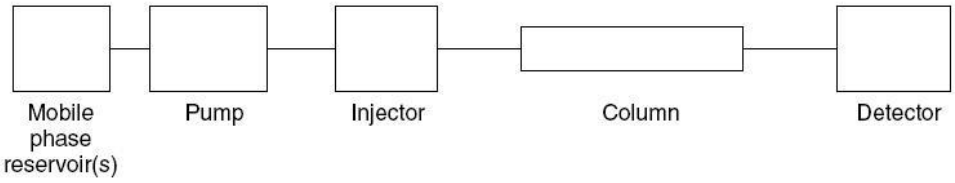


Figure 2.4 Schematic diagram of a typical HPLC instrument
[92]

In HPLC, the chromatographic process begins when the solute is injected into the injector, then the mobile phase, which is forced by a pumping system, carries the solute and flows through a chromatographic column. In the column, the mixture is separated into its components by the individual interaction of each component with mobile and stationary phase; and then the components are determined at the detector by certain physicochemical properties (UV absorption, refractive index, fluorescence, molecular mass and fragmentation in a mass spectrometer, or others). The result of separation is shown in forms of chromatogram. The separated components of a mixture eluting at different times (known as retention times, t_R) are displayed as peaks in the chromatogram. Different peaks on the chromatogram belong to different components of the separated mixture. The peaks in the chromatogram may have different heights and areas depending on a number of factors such as the amount of compound in the mixture, amount of sample injected, and sensitivity of the detection procedure. Since peak areas are dependent on the amount of the compound, HPLC can be used for quantitation after a proper calibration. From the HPLC diagram, the instrument consists of five parts (i.e., mobile phase, pump, injector, column, and detector).

2.2.1.1.1 Mobile phase and mobile phase reservoir

The most common type of mobile phase reservoir is a brown glass bottle. Most of the manufacturers supply these bottles with special caps, Teflon tubing, and filters to connect the bottles to the pumping system. The mobile phase reservoir

should be placed away from sunlight and temperature gradients should be avoided. Mobile phases in HPLC are usually mixtures of two or more individual solvents with or without additional additives or modifiers. The solvents chosen affect the elution of solute. In column HPLC, there are two elution types which are isocratic and gradient mode. The selection of elution mode depends on the polarities of analytes. In isocratic elution, the mobile phase is employed at constant composition, while change of mobile phase compositions during the separation is called gradient elution. Gradient elution mode reduces analysis time and increases resolution for complex mixtures. Solvents used must be high purity, most often HPLC grade because impurities in solvents or reagents can react with solute. Besides purity, there are other considerations to be made in solvent selection such as viscosity, polarity, toxicity, boiling point, vapor pressure and detector compatibility.

Mobile phases must be filtered and degassed before used because the dissolved gases in solvents can be collected in the columns, pumps, and detectors and, therefore, affect the reproducibility of the volume delivered. The connecting tubing between solvent reservoir and pump is usually made of polytetrafluoroethylene (PTFE) with an inner diameter of 2-4mm. The end of each pump-inlet tube should be fitted with an inlet-line filter, which filter contaminants and holds the inlet line at the bottom of the reservoir so that no air (bubbles) is drawn into the pump head. Bubbles may stop the pump from working. Buffers are often used as mobile phase in HPLC to control the degree of ionization of the analyte and thus the tailing of responses and the reproducibility of retention. A range of buffers is available but those most widely used are inorganic and non-volatile components, such as potassium or sodium phosphate.

2.2.1.1.2 Pump

The mobile-phase solvents are delivered from their reservoirs by pumping system. The purpose of pump is to ensure the delivery of precise, reproducible, constant, and pulse-free flow of mobile phase. High pressure pumps are needed to force solvents flow through column with a controlled flow rate because the particles in column are packed with high density. For HPLC, typical flow rates of 0.5-5.0 mL/min are produced by pumps operating at 300-6000 psi. The two major categories

of pumps applied are constant flow or volume and constant pressure. Constant flow pumps generate a certain flow rate of mobile phase, while constant pressure pumps apply a constant pressure to the mobile phase flowing through column. Most HPLC instruments use a reciprocating pump for both maintaining a constant flow rate up to several milliliters per minute and obtaining high output pressure to push the mobile phase through the chromatographic column. Reciprocating pump results in a pulsed flow that induces noise to the chromatogram. To eliminate this problem a pulse damper is placed at the outlet of the pump. New developments in using very fine particles in the chromatographic column require higher pressure and sometimes capability to produce flows at less than 0.1 mL/min. These systems called ultra performance liquid chromatography (UPLC or UHPLC) which can generate up to 8500 psi (about 600 bar) or higher.

2.2.1.1.3 Injector

The purpose of the injection system is to apply the sample extract onto the column in a narrow band. The role of the injector is to add a small, precisely measured volume of a solution containing the sample in the mobile phase. Conventional HPLC systems have injectors capable to inject between 1 µL up to 100 µL sample solution, typically between 2 µL and 20 µL. The three available techniques of injection are direct syringe injection, stop flow syringe injection, and injection valve. The sample injected should be in solution, so solid samples need to be dissolve in an appropriate solvent, which must not be the same type as mobile phase prior to injection. The injection valve is widely used as injection device for reproducibly introducing sample extracts into pressurized columns without flow interruption. The injector functions are crucial to the precision and accuracy of analytical results. After the valve is loaded with sample, it switches mode sample and mobile phase flow to the column.

2.2.1.1.4 Column

The chromatographic column is designed for performing the separation in HPLC. The two columns typically utilized are an analytical and a guard column. The guard column is placed before the analytical column to protect the analytical column from contamination while the analytical column is used to separate the sample.

Normally, the guard column is employed to eliminate two critical problems to the analytical column. Firstly, solutes binding irreversibly to the stationary phase will degrade the analytical column's performance by decreasing the available of the stationary phase. Secondly, particulate material injected with the sample may clog the analytical column. Guard column usually contains the same particulate packing material and stationary phase as the analytical column but are significantly shorter and less expensive and in some cases their stationary phase has larger particle size. It is placed in the path of the mobile phase before the column.

Typical analytical columns are constructed from stainless steel with 10, 15, or 25 cm in length and are fitted with extremely small diameter particles (3, 5, or 10 μm). The internal diameter of the columns is usually between 2.1 and 4.6 mm. Recent advances in column technology include use of 3-10 cm columns packed with 3-5 μm particles. The major advantages of these shorter columns are faster separations and improved sensitivity of detection. Factors important in producing efficient columns include narrow particle size distribution in the packing and minimal dead volume in the tubing, fittings, cells, and other components of the HPLC instrument.

The nature of the stationary phase in column is selected based on the type of chromatography utilized for the separation. The stationary phase usually consists of small, solid, rigid porous particles with high surface area and other special properties. The most widely used columns contain chemically modified silica stationary phase with the chemical modification determining the polarity of the column. Moreover, porous polymeric materials and monolithic materials were developed and used as the stationary phase. There are three major categories of stationary phase which are bonded phases, ion exchange, and size exclusion.

In bonded phase, reverse-phase HPLC (RP-HPLC) is the most utilized technique; the largest variety of columns is of RP type. These columns have a nonpolar stationary phase, for example, octadecyl groups (C18) and octyl groups (C8) bonded on silica, and normally employed with polar mobile phase. On the other hand, the combination of a polar stationary phase and a nonpolar or moderately polar mobile phase is called normal-phase HPLC (NP-HPLC). Examples of polar stationary phases include the compounds containing cyano, diol, or amino functional group. Beside NP-HPLC, there is the other type of stationary phase to analyze hydrophilic analytes named hydrophilic interaction liquid chromatography (HILIC). Due to the nonpolar solvent required as mobile phase in NP-HPLC, it is difficult to dissolve polar and hydrophilic compounds such as such as carbohydrates, amino acids, and peptides. In principle, HILIC has the similar separation as NP-HPLC, but HILIC can employ semi-aqueous mobile phases. Therefore, the hydrophilic analytes, which has a problem in NP-HPLC and cannot separate with RP-HPLC, have a strong retention on HILIC columns. The elution order in HILIC is likewise inverted to RP-HPLC. A HILIC separation system is essentially instrumentally identical to RP-HPLC systems because of the similarities to RP-HPLC in mobile phase conditions. This can be led to the same sample preparation and cleanup for RP-HPLC and HILIC. In HILIC stationary phases, the polar group is typically connected to the silica surface by a hydrocarbon chain. The common polar groups in HILIC stationary phases are amide and diol groups.

In ion-exchange chromatography or ion chromatography (IC), the stationary phase is a cross-linked polymer resin, usually divinylbenzene cross-linked polystyrene, with covalently attached ionic functional groups. This column can be used for high speed separations of large ionic molecules such as proteins and nucleic acids. IC separations are carried out on columns with ionized or ionizable groups attached to the stationary-phase surface. Commonly, there are commercial ion exchange materials contain sulfonate for strong cation exchange, carboxylate for weak cation exchange, tetraalkylammonium ion for strong anion exchange, and an amine for weak anion exchange. The capacity of exchangers is a function of the pH of the mobile phase. The pH controls retention by its effect on the ionic nature of both the analytes and the exchange sites. The exchange capacity is exhibited by different

exchangers at the following pH values: strong cation, above 3; weak cation, above 8; strong anion, below 9; and weak anion, below 6. A strong cation exchange mechanism is normally used for general analytical work. The most common detector measures the conductivity of the mobile phase as it elutes from the column.

Separations in the size exclusion chromatography (SEC) mode are based on molecular size and are controlled by the pore size of the packing material. Particle sizes in the 5-20 µm range are used to provide good column efficiency. Packing materials for SEC include semi-rigid organic gels, porous silica, and controlled pore glasses. The purpose of separation may be related to the purification of the polymer (e.g., of proteins) or analysis of polymers with the goal of assessing the molecular weight. The smaller analytes can enter the pores more easily and therefore spend more time in these pores, increasing their retention time. The resolution of the columns regarding the separation of two analytes with different molecular weight depends on pore size distribution. Columns with a wider range of pore diameters are able to separate a wider range of molecular weights. Typically, the resolution of these columns is lower, as compared to columns with a narrow pore-size distribution. The inertness of the SEC column is important in order to protect other interactions besides size exclusion influencing the retention process. If the mobile phase consists of or contains water, SEC is often called gel filtration chromatography (GFC). If the mobile phase is an organic solvent, the term gel permeation chromatography (GPC) is often used.

2.2.1.1.5 Detector

The function of an HPLC detector is to continuously and instantaneously monitor the components emerging from the column. The detection is based on the fact that the molecules of the sample have physicochemical properties different from those of the mobile phase. The selection of a specific detector is also correlated with the separation conditions used for the analysis. A good detector should have the following characteristics: high sensitivity and detection limit, good selectivity, fast response, wide range of linearity, no contribution to column band broadening, reliability and convenience. LC detectors can be universal detector, compound type selective,

specific compound selective, or have settings that make them compound selective. Some detectors can generate only quantitative information, while others offer both qualitative and quantitative information. Detector sensitivity is a very important factor in HPLC analysis. This sensitivity depends on several factors such as analyte properties, sample matrix, mobile phase properties, detector settings, and detector manufacturer. Parameters such as limit of detection (LOD) and limit of quantitation (LOQ) are normally reported to represent detector sensitivity in HPLC.

Type of detectors may be classified from their use into solute- or solvent-property detectors, selective or universal detectors and mass- or concentration-sensitive detectors. UV detector and the refractive index detector are solute- or solvent-property detectors by which the detector monitors a property of the solute (analyte) and a change in some property of the solvent (mobile phase) caused by the presence of an analyte, respectively. UV absorption can be both a specific or general detector, depending on practical application. The refractive index detector can also be classified as a general detector. It is usually recognized that general detectors are less sensitive than specific detectors. Mass spectrometer may be employed as either a general detector, when full-scan mass spectra are acquired, or as a specific detector, when selected-ion monitoring or tandem mass spectrometry (MS/MS) is being used. In the same way, mass spectrometer can function as a mass-sensitive detector, while in others, with LC–MS using electrospray ionization, it can behave as a concentration-sensitive detector.

The typical HPLC detectors are UV/Visible and fluorescence detectors. The analytical wavelength is selected in a modified spectrophotometer equipped with a flow cell. When using a UV/Visible detector, the resulting chromatogram is a plot of absorbance as a function of elution time. An instrument utilizing a diode array detector (DAD) is giving a three-dimensional chromatogram showing absorbance as a function of wavelength and elution time. One limitation in using absorbance is that the mobile phases must not have absorbance at the chosen wavelength. Fluorescence detectors provide additional selectivity when solutes can fluorescence. The resulting chromatogram is a plot of fluorescence intensity as a function of time. Nowadays, mass spectrometry (MS) is commonly used as one of main chromatographic detector.

MS determination can be definitive, providing information on analyte retention, and concentration, while simultaneously confirming analyte identity.

Evaporative light-scattering detector (ELSD) is a LC detector of choice in particular for compounds that do not have good light absorbance in UV, are not fluorescent, and may be difficult to ionize. The eluent from column is entered in the form of a spray from a nebulizer into a drift tube where a nebulizer gas is also introduced. The drift tube is heated and the solvent is evaporated, forming a fine mist from the nonvolatile molecules. This mist passes through a cell, where the scattered light from a beam that illuminates the cell is recorded. The intensity of the scattered light is dependent on the analyte concentration.

2.2.1.2 Medium-pressure liquid chromatography (MPLC)

Medium-pressure liquid chromatography (MPLC) is one of the preparative column chromatography techniques. Separation under medium pressure renders the use of smaller particle size supports possible and increases the diversity of usable stationary phases. The distinction between low pressure, medium pressure and high pressure LC is based on the pressure ranges applied. MPLC allows purification of large compound quantities and the faster and improved separations are obtained. Compared to HPLC, packing of material in MPLC is performed with lower particle size under pressure and the solid phase can be reused. The particle size of stationary phase ranges from 15-40 μm and the system pressures are between 5 and 20 bar allowing sample amount 0.05-100 g to be analyzed.

Selection of the stationary phase is probably the most crucial parameter affecting separation quality in MPLC. With regard to cost-effectiveness, the most frequently utilized stationary phase is silica gel. Beside its economic advantage, silica gel possesses other advantages such as a wide range of possible solvents as eluents, easy evaporation of the fractions and elution with high flow rates. Reversed phase or dihydroxypropylene-bonded silica gels are frequently used for MPLC separations. There are two most frequently used methods for column packing which are dry filling and the slurry method. Slurry filling is the preferred method for packing bonded

phases and dry filling is generally applied for silica gel. Recycling the stationary phase in MPLC is simple by only washing or repacking of the column. Automatic collection of fractions can be added by connecting a fraction collector to the column or detector outlet.

2.2.2 Gas Chromatography (GC)

2.2.2.1 Basic principle

The basic operating principle of GC involves volatilization of the sample in a heated inlet or injector of a gas chromatograph, followed by separation of the components of the mixture in a specially prepared column. The sample's components separation in GC based on their ability to distribute themselves between the mobile phase (gas) and stationary phases (solid or liquid) [80, 93-95]. A schematic diagram of a typical GC is shown in Figure 2.5.

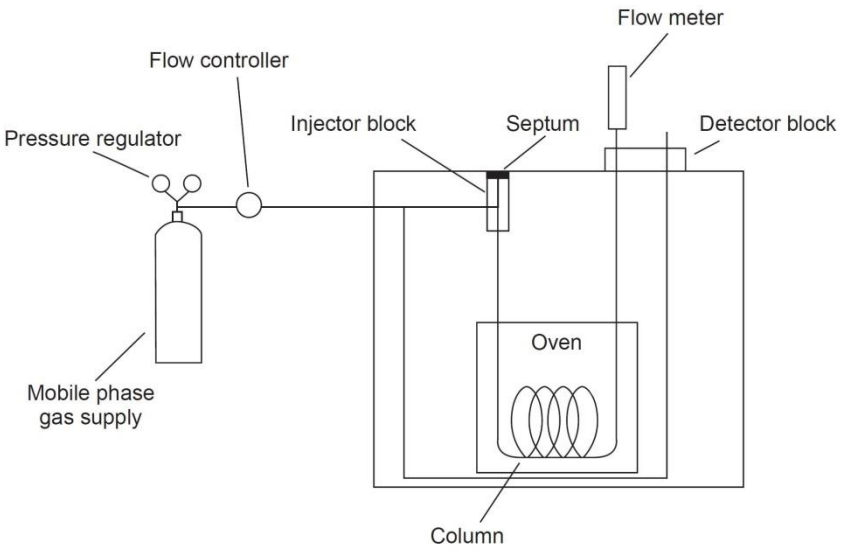


Figure 2.5 Schematic diagram of a typical GC instrument [80]

2.2.2.1.1 Carrier Gas

A carrier gas is used to transfer the sample from the injector, through the column, and into a detector. The most common carrier gases for GC are He, Ar, and N_2, which have the advantage of being chemically inert toward both the sample and the stationary phase. The choice of which carrier gas to use is often determined by the instrument's detector. High-purity gases should be used in GC. If lower quality carrier gases are used, purification through a molecular sieve trap to remove moisture and low molecular mass hydrocarbons and through an oxytrap to remove oxygen is recommended.

2.2.2.1.2 Injector

In GC, the sample is pulled into a syringe designed for use with gas chromatographs and typically about 1 μL is injected. There are several available types of GC injector. Split/splitless injector is the most commonly used injector for capillary columns. This injector can operate in two modes: split and splitless. Selection of either the split or the splitless mode depends on the concentration of the analytes in the sample. Split injection is used for samples where the analytes are dissolved in a solvent at relatively high concentrations. Only about 0.1–1% of the sample enters the column, with the remainder carried off as waste. On the other hand, splitless mode is used for samples containing analytes at trace levels. Both split and splitless injection modes are hot isothermal injection techniques; that is, the injector is set at a temperature that is hot enough to vaporize the solvent and the analytes in the sample, and this temperature is constant throughout the GC run. Programmable-temperature vaporizing injectors (PTV) are enclosed in an injector oven that is capable of cooling as well as heating the injector. The inlet is operated under cold temperature allowing the condensation of analyte inside the liner and the solvent is vented via split line. When the majority of the solvent is eliminated, the split valve is closed and the analytes transferred to the column in splitless mode. Large volume maybe injected in PTV at controlled speeds, namely large volume injection mode (LVI) in order to achieve very low detection limits. For samples that decompose

easily, an on-column injection may be necessary. In this method the sample is injected on the column without heating. The column temperature is then increased, volatilizing the sample with a low temperature. This injection technique gives precise and complete recovery of most analytes and is especially valuable for samples with compounds that have a wide-boiling range or that contain thermally labile analytes.

Several different glass inlet liners were used in GC injector. The role of liner is applied to make the connection between sample introduction and the GC column and to form a vessel into which the sample can be injected and heated. This should enable rapid, uniform vaporization of the sample and efficient transfer onto the head of the GC column as a tight band. All this should occur without secondary interactions between the analyte and the wall of the liner. Therefore, most commercially available glass liners have been deactivated to minimize decomposition of sensitive compounds and absorption of polar compounds on hot glass surfaces. The selection of a range of liners based on the chemical and physical properties of samples, injection techniques, injection volume, gas flow rates, and inlet temperature.

2.2.2.1.3 Column

Packed and capillary columns are two types of column used in GC. A packed column is constructed from glass, stainless steel, copper or aluminum and is typically 2–6 m in length, with an internal diameter of 2–4 mm. The column is filled with a particulate solid support (particle diameters ranging from 37–44 μm to 250–354 μm). A separation in gas-liquid chromatography is based on the partitioning of solutes between a gaseous mobile phase and a liquid stationary phase coated on the solid packing material. Nonbonded silanol groups on the silica wall can act as active sites to introduce tailing in eluting peaks. To avoid the adsorption of analyte molecules on exposed packing material, which degrades the quality of the separation, surface silanols are deactivated by silanizing with dimethyldichlorosilane and washing with an alcohol (typically methanol) before coating with stationary phase. Capillary or open tubular columns are constructed from fused silica coated with a protective polymer. Columns may be up to 100 m in length with an internal diameter of

approximately 150–300 μm. Wall-coated open tubular columns (WCOT) and support-coated open tubular columns (SCOT) are two types of capillary columns. A WCOT contains a thin layer of stationary phase, typically 0.25 μm thick, coated on the capillary's inner wall, while SCOT is a thin layer of a solid support coated with a liquid stationary phase is attached to the capillary's inner wall. Porous-layer open-tubular (PLOT) column is the SCOT column in which the porous layer consists of support particles coated with a liquid phase that was deposited from a suspension. Column length, column i.d., and thickness of stationary phase are critical parameters affecting on column efficiency. The selectivity in GC is influenced by the choice of stationary phase and the elution order is determined by the analyte's boiling point and the analyte's interaction with the stationary phase. Various polymers used for GC phases differ in their polarity. The degree to which the polarity of the analyte is similar to that of the phase is an indication of the strength with which it will dissolve into and be retained by the phase. The separation efficiency improves with thinner films. The most common film thickness is 0.25 μm. The thicker films are used for highly volatile analytes, such as gases, because they have a greater capacity and thinner films are used when separating analytes of low volatility. Dimethylsiloxanes and 5% phenyl/95% dimethylsiloxane are good general purpose phases for many applications. In the modern practice of GC, open tubular or capillary columns are almost always employed than packed column GC which is normally used for large-scale preparative GC at present.

Control of the column's temperature is essential to attaining a good separation. GC columns are installed in a column oven where the temperature must be accurately and precisely controlled, because column temperature has a pronounced influence on retention time. A column oven should be thermally insulated from heated injector and detector components. Ideally, the temperature of a column oven should remain constant and independent of environmental changes in the laboratory and any line voltage fluctuations. After column selection, the most critical step in developing a GC separation is selecting the column oven temperatures for the analysis. GC oven temperature modes include an isothermal run where the temperature remains constant, a temperature-programmed run where the temperature is increased at a constant rate, and a multilevel run where the temperature rate is increased at different rates at

different times during the GC run. When the sample contains many compounds of a wide boiling-point range, temperature programming is necessary to separate all of the components of the mixture. The initial temperature is set below that for the lowest boiling analyte. In addition, it is desirable to choose a solvent with a boiling point that is not too low compared to that of the first compound of interest. The ramp rate governs the tradeoff between analysis time and resolution. The final temperature would be after the last compound elutes from the column and there is no high-boiling impurities in the sample or in column.

2.2.2.1.4 Detector

Detector is one of GC main part producing an electrical signal which is proportional to either the concentration or the mass flow rate of the analyte molecules in the effluent stream. Retention times are automatically calculated, heights of peaks are measured, or they are automatically integrated to obtain their areas, and peaks can be identified by their elution within in a retention time window, and quantitated by comparison to the areas or heights of a quantitative standard. GC detectors are classified mainly on the basis of response or of detector selectivity.

Universal detectors respond to every component in the mobile phase; selective detectors respond only to a related group of substances; and specific detectors respond to a single sample component or to a limited number of components with similar chemical characteristics. Detectors which response is proportional to the concentration of a sample component in the mobile phase (g/mL) are called concentration-sensitive detectors whereas detectors which response is proportional to the amount of sample component reaching the detector in unit time (g/s) are called mass flow detectors. Another classification is destructive versus nondestructive detectors. Some detectors destroy the analyte as part of the process of their operation and the others leave the analyte in a state where it may be passed on to another type of detector for additional characterization.

Flame ionization detector (FID) is the most widely used and a nearly universal GC detector in which the solutes are combusted in an H_2-air flame, producing a

measurable current. FID is a destructive detector which responds to compounds with a carbon–hydrogen bond. Thermal conductivity detector (TCD) is a universal, non-destructive and concentration-sensitive detector in which the signal based on the mobile phase's thermal conductivity. When an analyte elutes from the column, the thermal conductivity of the mobile phase decreases and then the temperature of the wire filament and resistance increases. This resistance is measured and recorded. The electron capture detector (ECD) is an example of a selective detector. It has a very low detection limit for materials that readily capture electrons (electrophilic analytes) and gives a strong response to halogenated compounds. If the GC effluent contains a compound that can capture electrons, the current is reduced because the resulting negative ions move more slowly than electrons. The signal measured is the loss of electrical current. Nitrogen–Phosphorus Detector (NPD) is a selective detector which responds to compounds in a sample that contain nitrogen or phosphorus atom. Nitrogen and phosphorus compounds increase the current in the plasma of vaporized rubidium ions.

2.2.3 Mass Spectrometry (MS)

2.2.3.1 Basic principle

Mass spectrometry is one of the most important analytical tools, in order to obtain information about the chemical composition and abundance of isotopes. A mass spectrometer produces ions from the substance, separates them according to their mass to charge ratio (m/z), and records the relative abundance of each ionic species present. The three major components of a MS instrument are ion source, mass analyzer, and detector [80, 91, 92, 94]. Figure 2.6 shows a schematic diagram of the mass spectrometry process.

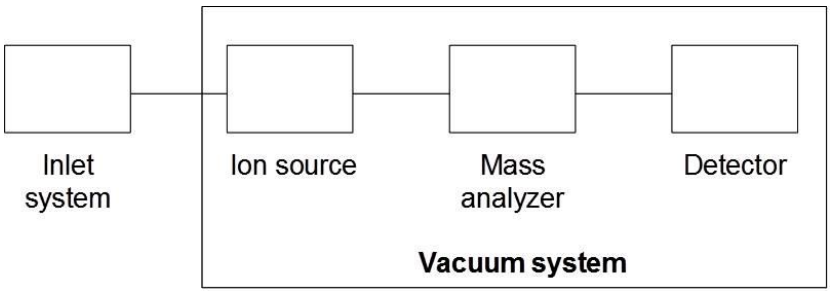


Figure 2.6 Schematic diagram of MS system
[96]

In MS, samples are transferred through the introduction system into the vacuum area of the mass spectrometer. In the ion source region, sample molecules are ionized to gas phase ions and accelerated into mass analyzer, where all ions are separated according to their mass to charge ratio. Finally, separated ions are determined with a detector and signals are delivered to data system analysis. All MS instruments have a high vacuum system to minimize the collision between ions, prevent the loss of ions, and increase the mean free path of ions.

2.2.3.2 Ion source

2.2.3.2.1 Ion source for LC-MS

In hyphenated systems of LC and MS, the ionization appears on the interface area of LC and MS, where the separated components from LC are introduced. The LC-MS interface is utilized to eliminate the mobile phase from LC and produce gas phase ions of analytes for further separation and detection in the MS system. Extensive ionization techniques in LC-MS are atmospheric pressure electrospray ionization (AP-ESI) and atmospheric pressure chemical ionization (APCI). The ionization technique is selected based on analyte properties. Most mass spectrometers use positive ions, which are easily created. However, sometimes negative ions are required.

2.2.3.2.1.1 Atmospheric pressure electrospray ionization (AP-ESI)

AP-ESI is a useful ionization technique to analyze samples that become single or multiple charged depending on their molecular structures. It can be used to create either positive or negative ions, and it also ionizes high molecular weight components. AP-ESI ionization process is followed by evaporation. The three basic steps of AP-ESI are nebulization and charging, desolvation, and ion evaporation. These steps are shown in Figure 2.7.

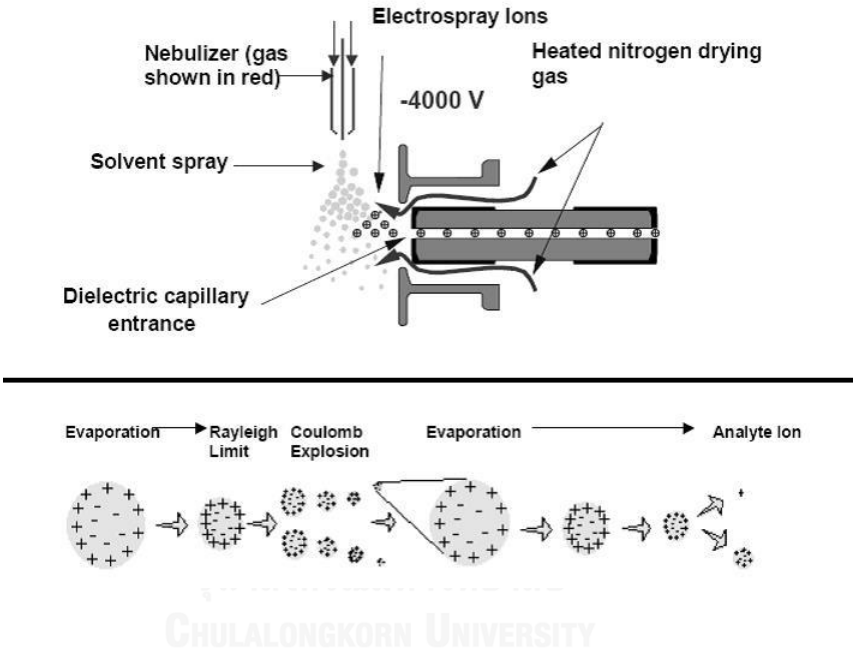


Figure 2.7 Atmospheric pressure electrospray ionization process [80]

Firstly, the HPLC effluent is pumped through a nebulizing needle, which is set at ground potential. The spray passes an electrode, which is held at high potential. The potential difference between the needle and the electrode produces a strong electrical field. This field charges the surface of the liquid and forms a spray of charged droplets. During the desolvation step, the droplets are attracted to the capillary and dried with a heated nitrogen gas flow and uncharged species are eliminated. After the charged droplet size is reduced, the repulsive force within charges overcomes the cohesive force of surface tension and creates coulombic explosion. This process is

repeated until the analyte ions are desorbed into the gas phase. These gas-phase ions are then continuously passed to the mass analyzer. AP-ESI is a concentration dependent technique and has many advantages such as high sensitivity to polar compounds; it produces multiply charged ions, and is suitable to reverse phase solvents.

2.2.3.2.1.2 Atmospheric pressure chemical ionization (APCI)

APCI is an ionization technique that is applicable to a wide range of polar and nonpolar analytes of moderate molecular weight. APCI differs from AP-ESI as evaporation process occurs and is followed by ionization. APCI also has three basic steps; nebulization, desolvation, and ionization. These steps are shown in Figure 2.8.

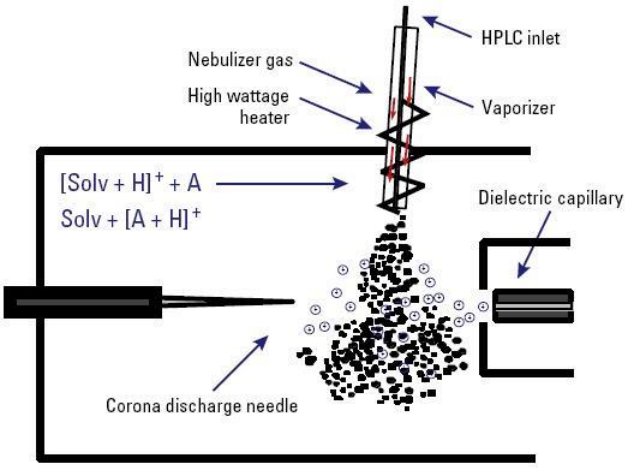


Figure 2.8 Atmospheric pressure chemical ionization process [80]

APCI nebulization is similar to API-ES, but APCI nebulization occurs in a hot vaporizer chamber (typically 250°C–400°C). The effluents from HPLC are evaporated to spray droplets of solvent and analytes in gas phase. The gas-phase solvent molecules are ionized by a corona needle discharge. Then, the charge is transfered from the ionized solvent species to the analyte molecules, and the charged

analytes are delivered to the mass analyzer. APCI can handle HPLC flow rates up to 2 mL/min, efficiently works with many compounds, especially non-polar, and produces only single charged ions. Nevertheless, possible thermal degradation is of concern in APCI; furthermore, compounds require a certain vapor pressure.

2.2.3.2.2 Ion source for GC-MS

2.2.3.2.2.1 Electron Ionization (EI)

In EI, the analyte of interest, in the vapor phase, is bombarded with high-energy electrons (usually 70 eV) emitted from a tungsten filament. Analyte molecules absorb some of this energy (typically around 20 eV) and this causes the ionization. The analyte is ionized by the removal of a single electron and yields a radical cation, termed the molecular ion [M]+•, the m/z of which corresponds to the molecular weight of the analyte. The molecular ion with sufficient amount of energy accumulated in its bonds tends to dissociate into typical fragment ions, radicals, and neutral species. The amount of internal energy retained by the [M]+• cation is very high that the [M]+• cation fragments completely. Thus, the [M]+• cation is sometimes not observed in the EI spectrum. The ionization efficiency and production of fragment ions in EI mode depends strongly on the chemistry of the analyte and the energy of the electrons.

2.2.3.2.2.2 Chemical Ionization (CI)

Chemical ionization is a technique that has been developed specifically to enhance the production of molecular species, i.e. to reduce the fragmentation associated with ionization. CI is recognized as soft ionization technique. In the approach, analyte molecules in the vapour phase are introduced into a mass spectrometer source containing a reagent gas. The reagent gas is ionized by an electron beam produced by the acceleration of electrons from a filament similar to EI. The potential used to accelerate the electrons in CI is much higher than that used in EI (~200 V) to assure that the electrons pass through the dense cloud of reagent gas. The most commonly used reagent gases are methane, isobutane and ammonia. The most

often-used reaction to ionize analytes is a protonation reaction resulting in a protonated molecule (MH+ or [M + H]+). It is possible to produce deprotonated molecules ([M − H]−) through reactions with negative-charge reagent ions, ions resulting from a hydride abstraction ([M − H]+). CI is very useful to elucidate the molecular ion of unknowns and is often the ionization method of choice for quantitative analysis.

2.3.3.3 Mass analyzer

The mass Analyzer separates ions by their mass to charge ratio (m/z) in space or in time. After ions are formed in the ion source region, they are accelerated into the mass analyzer. The mechanism is performed with electric and magnetic fields, sometimes including RF fields. There should have some ion focusing device to prevent the spread of ions from ion source. The selection of mass analyzer depends on the resolution, mass range, scan rate, and detection limits required for the application. Each analyzer has different operating characteristics, and an additional instrument. In hyphenated LC-MS, quadrupole and time-of-flight (TOF) are widely used mass analyzers. Both techniques are considered as ion transmission system.

2.3.3.3.1 Quadrupole mass analyzer

The quadrupole mass spectrometer is the most common mass analyzer because of its compact size, fast scan rate, high transmission efficiency, and moderate vacuum requirements. In the mass spectrometer, the quadrupole analyzer consists of four parallel metal rods or electrodes. Two parallel rods are connected to direct current (DC), while the others are connected to radio frequency (RF). Both DC and RF are chosen to filtered ions. When the ions travel through the quadrupole, they are selected by DC and RF according to their m/z, only ion of selected m/z or resonance ion pass through quadrupole analyzer. A quadrupole mass analyzer is schematically shown in Figure 2.9.

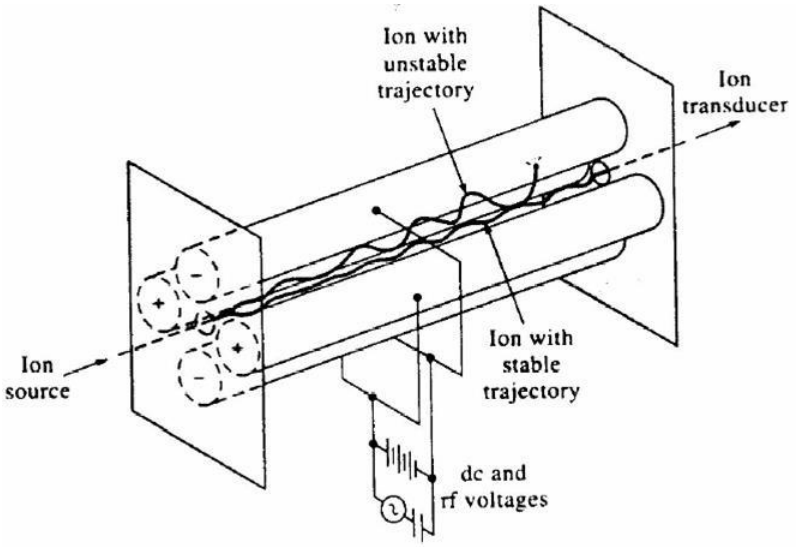


Figure 2.9 Quadrupole mass analyzer
[97]

2.3.3.3.2 Time-of-flight mass analyzer (TOF)

The time-of-flight mass analyzer (TOF) is the simplest configuration of the mass separation devices. The selection of ions is based on the movement of ion through the flight tube (Figure 2.10). TOF is usually applied to separate macromolecules with large m/z. The separation is based on the principle that ion of different masses experience individual velocities in the flight tube, and, in conclusion, have different flying time to the end of the tube, where transferred to the detector.

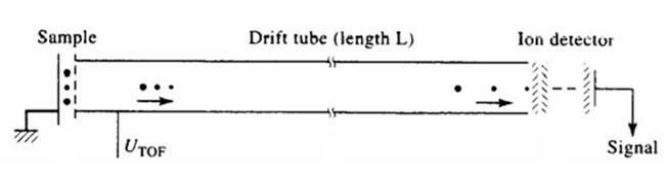


Figure 2.10 Time-of-flight mass analyzer
[97]

2.3.3.3.3 Tandem Mass spectrometry (MS/MS)

Tandem mass spectrometry uses two or more sequential mass spectrometers. MS/MS is a powerful technique that provides both the molecular weight of an analyte and information about the structure of the molecule involved. Therefore, MS/MS has been applied for many qualitative and quantitative applications. MS/MS is used to isolate an ion of interest in first mass analyzer (MS1) and then chemically or energetically modifies these ions with second mass analyzer (MS2). MS/MS process involves the determination of mass relationship between a precursor or parent ion in MS1 and a product or fragmented ion in MS2 (Figure 2.11).

The most commonly used MS/MS is the triple quadrupole (QqQ). The configuration of QqQ consists of three sets of quadrupole rods in a series. Both the first and third sets of quadrupoles are used for mass separation, while the second set acts as a collision cell. The selected precursor ions pass from first quadrupole, are then fragmented and focused in the second quadrupole before transmitted into third quadrupole, where the fragmented ions of analytes are separated and subsequently detected. With this mechanism, MS/MS separates components of same molecular masses but different product ions with high specificity.

In quadrupole–time-of-flight instrument (Q-TOF), the final stage of the triple quadrupole is replaced by an orthogonal time-of-flight (TOF) mass analyzer. The latest generation of TOF instruments has more than one reflectron to increase the flight path of the ions and thus increase the resolution. The Q-TOF analyser detects all of the MS/MS product ions that enter it at a specific time and provides full scan product ion spectra with high resolution and accurate mass.

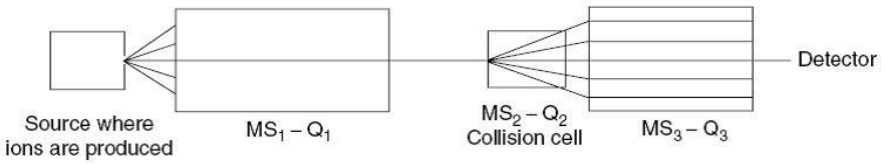


Figure 2.11 Schematic diagram of tandem mass spectrometry
[92]

2.3.3.4 Detector

The detector in MS is used to measure the ions leaving from the mass analyzer by converting ions into an electrical current or other forms of signal, processing and recording into mass spectrum. A detector is selected by speed, dynamic range, gain, and geometry. Most detectors currently used to amplify the ion signal are electron multiplier tube and photo multiplier tube. Electron multiplier tube offers electron from surface of tube for analyte ions. The entrance of tube is held with potential charge opposite to the analyte ions. Analyte ions are attracted to the entrance of tube and collide with tube surface, and then the inner surface coated with electron-emissive material releases electrons. These electrons are accelerated to hit another portion of tube by electrostatic force and the surface loses more electrons in every collision. Amplified electrons are counted by an electrical circuit and displayed as signal intensity. The photo multiplier tube comprises a photocathode and a series of dynodes. In the high voltage tube, incident photon strikes the photo cathode and emits electrons due to the photoelectric effect. These electrons are accelerated towards a series of additional electrodes called dynodes. At the dynodes, the amount of electrons is increased at every collision. This creates an amplified signal that is finally collected and measured at the anode.

2.2.3 Nuclear magnetic resonance (NMR)

At present, NMR is a very powerful analytical method not only because both liquid and solid state sample can be studied but also because it is a nondestructive method. This technique probably is the most important technique for structure elucidation, material characterization and studying molecular motion. NMR offers high potential for the analysis of multicomponent systems, such as food matrices. A NMR schematic diagram is shown in Figure 2.12. The effect of NMR initiates with the absorption of magnetic field absorb by magnetic nuclei which then re-emit electromagnetic (EM) energy. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and other factors. This allows the observation of specific quantum mechanical magnetic properties of an atomic

nucleus. Certain nuclei possess a property known as spin which refers to the nuclear spin angular momentum which is purely quantum mechanical propert [91, 98]. A spinning nucleus orients along the spin rotation axis and if high magnetic field is put nearby, there will be one most probable orientation of nucleus which has low-energy state. Generally, energy of nucleus has a “ground state” with spin of +1/2, and the excitation causes the poles to swap and the spin changes to the higher energy state of -1/2, a process referred to as resonance. The energy difference between +1/2 and -1/2 nuclear spin states is proportional to the strength of the magnetic field at the nucleus. The applied energy is proportional to electromagnetic radiation frequency, and the resonance frequencies are plotted on an NMR spectrum. Less electron density means less shielding and therefore a stronger magnetic field at the nucleus and a higher frequency required for resonance. A nucleus that is surrounded by greater electron density will generally resonate at lower frequency than a nucleus surrounded by less electron density.

In NMR spectrum, the frequency of resonance is plotted as a chemical shift. A chemical shift is the resonance frequency of a particular nucleus compared to that of a standard molecule and reported as parts per million (ppm). The units are parts per million because the chemical shift changes associated with electron density differences are about one millionth as large as the external magnetic field used in an NMR spectrometer.

In a 1H-NMR spectrum, a signal with a chemical shift that is larger, for example 4.8 ppm, corresponds to an H atom that either has relatively little electron density around it (it is adjacent to electronegative atoms or electron withdrawing groups) or is one that is attached to a carbon atom taking part in a pi bond. A signal with a smaller chemical shift, for example 1.8 ppm, is due to a H atom that has greater electron density around it, indicating it is not adjacent to any electronegative atoms, electron withdrawing groups, or pi bonds. NMR signals can split into several peaks by other H atoms that are not more than 3 bonds away. The signal splitting is caused by spin-spin coupling between adjacent nuclei. Signal splitting allows the determination of how different functional groups are connected in a molecule, because atoms of only adjacent functional groups can split each other. Different functional groups have

different characteristic chemical shifts, so comparing a given signal in an NMR spectrum to a reference table of chemical shifts for the identification of functional groups in a molecule. The introduction of two-dimensional NMR (2D NMR) techniques allows the interpretation and/or simplification of complex spectra, sensitivity gain, structural and conformational information of medium-sized and large molecules and study of dynamic NMR phenomena. A 2D NMR give data plotted in a space defined by two frequency axes rather than one. Each frequency axis is associated with one of the two time variables, which are the length of the evolution period (the evolution time) and the time elapsed during the detection period (the detection time). They are each converted from a time series to a frequency series through a two-dimensional Fourier transform. A single two-dimensional experiment is generated as a series of one-dimensional experiments, with a different specific evolution time in successive experiments, with the entire duration of the detection period recorded in each experiment.

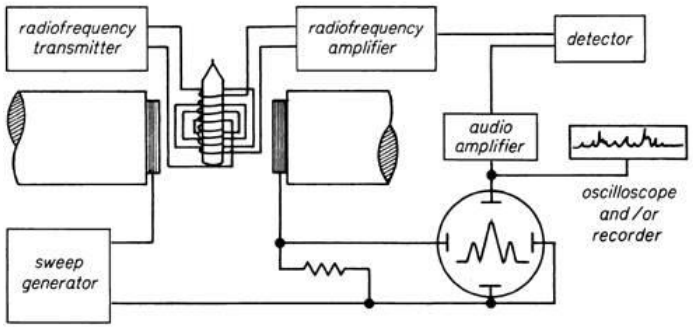


Figure 2.12 Schematic diagram of NMR system
[99]

2.3 Statistical Techniques

2.3.1 Chemometrics

Chemometrics is the chemical discipline that uses mathematical and statistical methods, to design or select optimal measurement procedures and experiments, and to provide maximum chemical information by analyzing chemical data. Chemometrics often involves using linear algebra methods to make qualitative or quantitative measurements of chemical data. At present, the use of modern hyphenated analytical methods producing huge amounts of data with increasing complexity of structure has become a driving force for algorithms in multimodal statistics. Therefore, multivariate statistical data analysis is a powerful tool for analyzing and structuring data sets that have been obtained from such systems, and for making empirical mathematical models that are for instance capable to predict the values of important properties not directly measurable [91, 100-103].

Several analytical methods have been constructed with the use of chemometrics to create the pattern of food compositions in the form of a mathematical model. The application of chemometric techniques can greatly improve the quality of the fingerprint obtained from complex chromatographic or spectroscopic profiles. Pattern recognition by means of multivariate statistical analysis can be divided into two categories: unsupervised and supervised. Unsupervised pattern recognition is utilized for data visualization by observing the relationship between samples and variables with no predetermined class. It is generally used as a primary step in order to monitor group of samples. These kinds of techniques are called exploratory analysis methods. On the other hand, supervised pattern recognition is a statistical method applied for data classification by attempting to create a model to predict the class of an unknown sample.

2.3.1.1 Unsupervised Pattern Recognition

2.3.1.1.1 Similarity Analysis

An important task of multivariate data analysis is classification of objects and variables. The subdivision of the whole data set is arranged into homogeneous groups of similar objects or variables. Similarity of variables is usually measured by their correlation coefficient, whereas similarity of objects is expressed in terms of the geometric distance. Similarity analysis was used to determine the degree of similarity or dissimilarity of samples from each other. A correlation coefficient is employed as a mathematical quantity to identify the statistical relationship among data objects. If there is no relationship between pair-wise samples, the correlation coefficient is 0 or very low. On the other hand, a perfect similarity gives a coefficient of 1.0. A value of the correlation coefficient close to 1.0 indicates the higher degree of similarity of the samples.

2.3.1.1.2 Hierarchical cluster analysis (HCA)

The main tool of unsupervised classification is cluster analysis, which produces a partition of the set of objects into several homogeneous subgroups. HCA is an exploratory analytical tool to assign a set of samples into groups by converting the observed data into statistical structures. The method allows gaining more insight into the relations between the objects by determining the degree of association among sample objects, and is expressed as distance. At the beginning, each object forms its own cluster during the mathematical process. The process is started by combining the two most similar single-object clusters in one larger cluster. Using a similarity measure between clusters, the clusters with the smallest similarity can be determined and combined into a new larger cluster. This process is repeated until all objects end up in only one big cluster. These clusters are linked at increasing levels of dissimilarity. The distance pattern allows the observation of sample profiles through simple interpretation. The smallest distance indicates the highest degree of relationship; therefore, those objects are considered to belong to the same group. Indeed, sample objects in HCA are ordered in a one-dimensional sequence called a

hierarchical tree diagram or dendrogram (Figure 2.13). In dendrogram, the vertical axis represents the dissimilarity measure at which each successive object joins a group. The height of each connecting data points in dendrogram represents the distance between the two data points being connected.

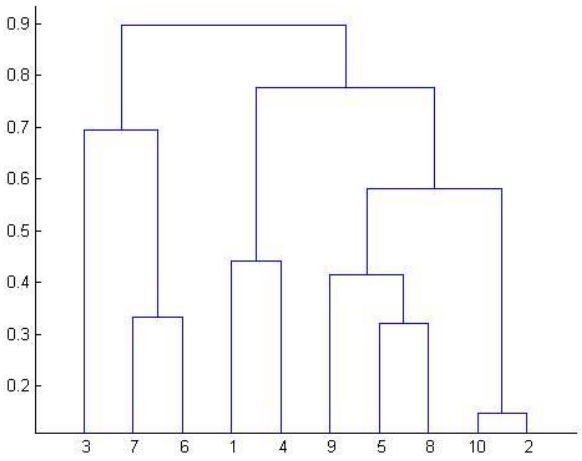


Figure 2.13 Dendrogram
[104]

2.3.1.1.3 Principal Component Analysis (PCA)

PCA is a well-known conventional exploratory data analysis technique which is used as a primary step in many fingerprint studies. PCA is applied to determine underlying information from multivariate raw data by transforming and reducing the dimensions of the original data (X_{nxm}) matrix for n samples and m variables into a product of two matrices, scores (T) and loadings (P), while containing the same information as of the original data. The matrix scheme of PCA is presented in Figure 2.14.

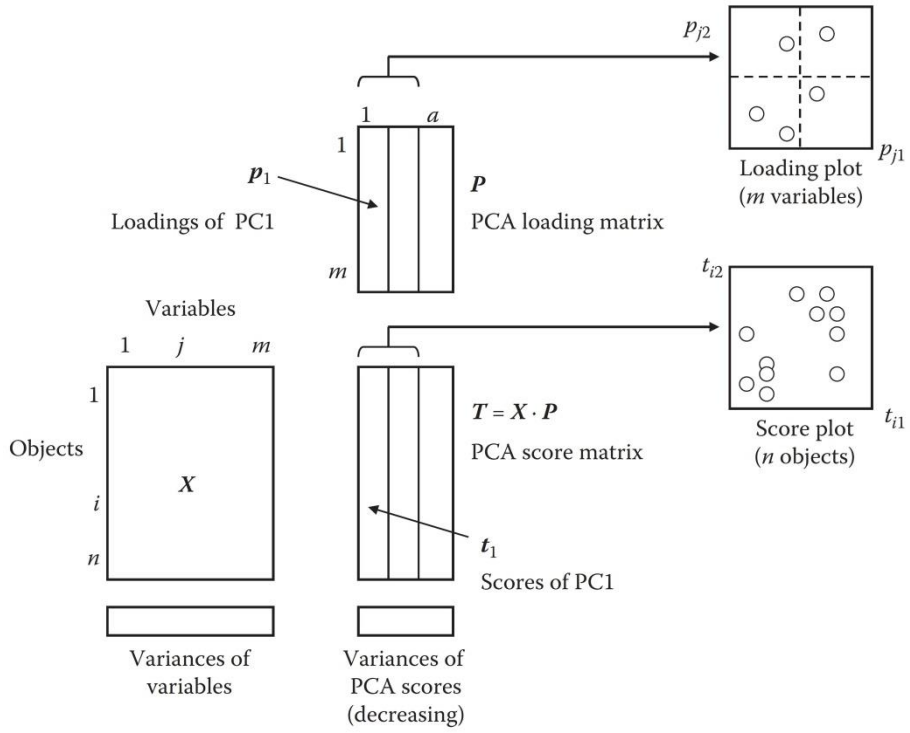


Figure 2.14 Matrix scheme of PCA
[101]

In PCA plots, the information about the samples is presented in the form of scores (Figure 2.15), while loadings (Figure 2.16) focus on the variables that have the most influence over the difference between groups of samples. This new, smaller set contains the uncorrelated components called principal components (PCs), the linear combinations of the original variables with optimal features. PC1 accounts for the greatest variance among all possible linear combinations in the data. A second principal component (PC2) that is orthogonal to the PC 1 is fit and captures the next greatest variance, and other PCs indicate smaller variability of data. Different PCs are always uncorrelated. The sum of percentage described by PC must be close 100%. The number of significant PCs is ideally equal to the number of significant components and should be appropriately chosen for the clear visualization of data. A few PCs (generally much fewer than the number of original variables) will employ to represent the greatest part of the total data variation. Using the coordinate system defined by the first two PCs is a very popular approach for visualizing data structure

in a diagram. The size of each PC is reported as eigenvalue which is the sum of squares of the scores and this value is frequently presented as percentage in PCA plot. The cumulative percentage eigenvalue is often used to determine what proportion of the data has been modelled using PCA. The closer to 100 % refers to the more faithful of the model.

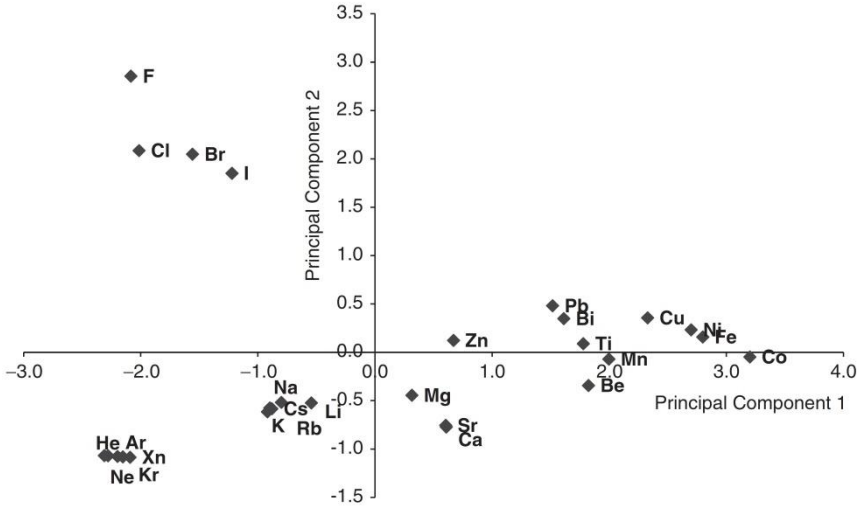


Figure 2.15 Scores plot example of the first two PCs [100]

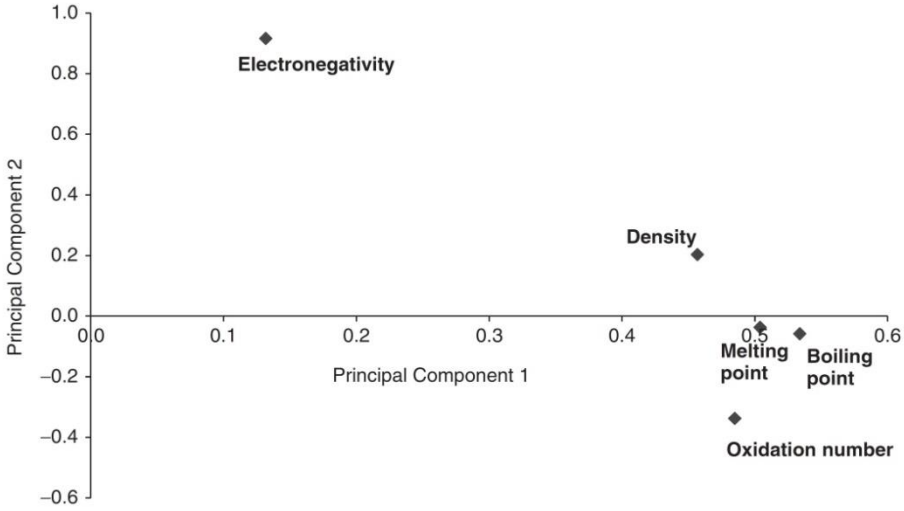


Figure 2.16 Loadings plot example of the first two PCs [100]

The horizontal axis is the first PC and the vertical axis for the second PC. If more than 90% of the total variance is preserved, the two-dimensional representation is excellent, and most distances between object points will reflect well the distances in the high-dimensional variable space. Samples that are similar to each other will have scores that cluster together in PC space. Samples that are different from each other will have scores that are further apart in PC space. In the same way for loadings, one PC can be plotted against that at the other PC. The loadings plots provide detailed information about the similarity of variable. If the variables are closely clustered, this suggests that those parameters have a similar trend in measurement. By comparing the score and loading plot, the relationships between samples and variables are identified that which variables are most associated with which sample.

2.3.1.2 Supervised Pattern Recognition

2.3.1.2.1 Linear Discriminant Analysis

Linear Discriminant Analysis (LDA) is a well-known method for dimensionality reduction and classification. It is a supervised pattern recognition technique which is applied to construct a classifier model from a data matrix and known class information. LDA is used for class prediction purposes by creating a model linear boundary (classifier) between classes using linear discriminant functions in order to define the directions in which the classes are best separated (Figure 2.17). Linear discriminant functions are generated by maximizing the ratio of between-class variance to within-class variance to ensure the highest efficiency of the model for class separation. After a model has been generated, the predictive ability of the developed model is evaluated by ‘‘leave-one-out’’ cross-validation (LOOCV). This validation procedure is performed by removing one chromatogram at a time, to be used as a test set, while the remaining chromatograms are formed as a training set. After repeating the procedure for all samples, a contingency table can then be constructed to express the performance and stability of the developed classifier. For an acceptable model, a high percentage of correct classification should be obtained.

LDA shares some similar characteristics with PCA which are a number of linear functions produced to provide data reduction through rearrangement of information. On the other hand, the major difference between LDA and PCA is that LDA is being used to maximize class discrimination by with no change of data location but only tries to draw a decision region between the given classes, whereas the objective of PCA is to reduce variance into as few components as possible with change of the shape and location of original data sets when transformed to a different space. In addition, LDA produces exactly as many linear functions as there are classes, whereas PCA produces as many linear functions as there are original variables.

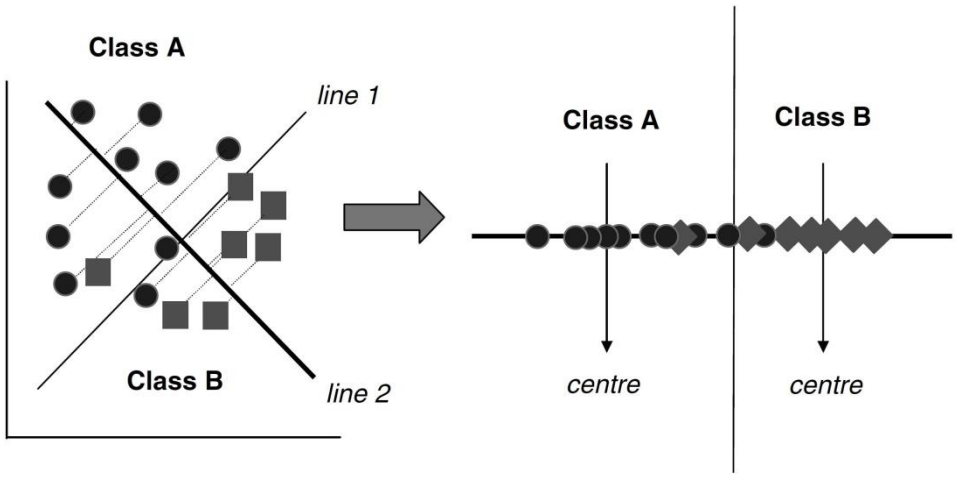


Figure 2.17 Discrimination of LDA between two classes, and projections [103]

Line 1 draws for discrimination between the two classes. If above the line 1, an object belongs to class A, otherwise to class B. The projections of all objects in two classes are drawn at right angles on to a line 2 as a new axis which passes through the two group centroids. Unlike x- and y-axis distribution, the data of two groups do not overlap on the new axis. This new axis represents a new variable which is a linear

combination of x and y, which is called a discriminant function. The projection can now be converted to a position along line 2. The distance can be converted to a number, analogous to a ‘score’. Objects with lower values belong to class A, whereas those with higher values belong to class B.

2.3.1.2.2 Orthogonal partial least squares discriminant analysis (OPLS-DA)

OPLS-DA is a recent modification of the PLS-DA method to discriminate two or more groups (classes) using multivariate data. In comparison with PLS-DA, OPLS-DA produces models which are more transparent and therefore easier to interpret. The objective of OPLS-DA is to divide the systematic variation into two model parts (Figure 2.18), one part which models the co-variation between x and y, and another part which expresses the x-variation that is not related (orthogonal) to y. The OPLS model comprises two modeled variations, the Y-predictive ($T_pP_p^T$) and the Y-orthogonal ($T_oP_o^T$) components [105-107]. In OPLS-DA, a regression model is calculated between the multivariate data and a response variable that only contains class information.

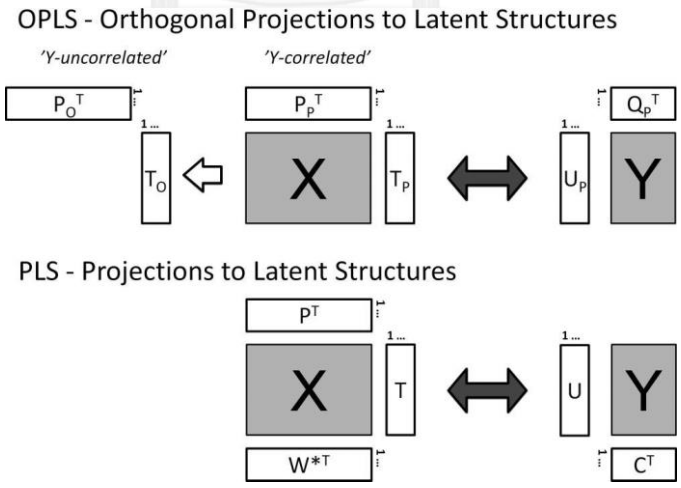


Figure 2.18 Schematic overview of both the OPLS-DA model structure and the PLS-DA model structure.

OPLS separates the modeled variation of X into two parts: (a) target correlating variation (Y-correlated) and (b) target uncorrelating variation (Y-uncorrelated).

[105]

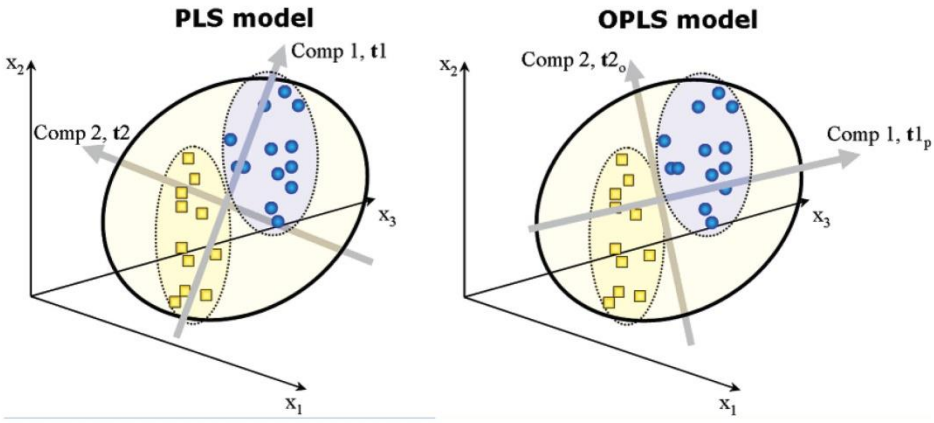


Figure 2.19 The PLS-DA and OPLS-DA models
[106]

In Figure 2.19, the PLS components cannot separate the between-class variation from the within-class variation, and the resulting PLS component loadings mixes both types of variations. On the other hand, the OPLS components are able to separate these two different variations. Component 1 ($t1_p$) is the predictive component and displays the between-class variation of the samples. The corresponding loading profile can be used for identifying variables important for the class separation. Component 2 ($t2_o$) is the Y-orthogonal component and models the within group (within-class) variation. OPLS-DA provides a more straight-forward and realistic model interpretation as it is possible to focus on the variation in x that really correlates with y. Also, OPLS-DA gives an opportunity to analyze the orthogonal variation in the x data that does not correlate with y, and understand its sources. The advantage of OPLS-DA compared to PLS-DA is that a single component is used as a predictor for the class, while the other components describe the variation orthogonal to the first predictive component or OPLS-DA can use to separate predictive from non-predictive (orthogonal) variation. The remarkable ability of OPLS-DA for separation of between-class variation and the within-class variation helps to promote a better class-resolution in a discriminant problem by generating less biased classification results in terms of sensitivity and specificity of the class predictions.

This method provides more understanding of profiles from the same group and different groups.

2.3.2 T-statistics

T-statistics is a frequently used method for significance testing to determine whether two groups differ from one another for a tested variable. It serves as a hypothesis test by which a null hypothesis (H_0) is proved. H_0 refers to a situation where there is no significant differences between two groups. Hence, these two groups cannot be notably differentiated from each other. T-statistics determines the results whether to reject or not reject H_0 by comparing the calculated t-value to the critical t-value. T-value was calculated via ‘‘one vs. all’’ criteria to show whether the one data group was well-separated from the others. The data of a studied group was assigned as a first group while the whole data of the other groups was classified as a second group in t-value calculation. If the calculated t-value is larger than the critical t-value or $P < 0.05$, the null hypothesis is rejected. This suggests the two groups are significantly different; the level of difference is indicated by observing how far the calculated t-value is from the critical t-value.

CHAPTER III
EXPERIMENTAL

3.1 Food quality: The authentication of ginger origin

3.1.1 Instrumental and Apparatus

3.1.1.1 LC-DAD system: Agilent 1100 series (Agilent Technologies, Santa Clara, CA, USA) equipped with a G1322A vacuum degasser, a G1312A binary pump, a G1313A autosampler, a G1316A column compartment, and a G1315A diode array detector using Chemstation software (Agilent Technologies, Santa Clara, CA, USA)

3.1.1.2 LC-DAD column: Waters Symmetry C18 column (150 x 3.9 mm, 5 µm) (Waters, Milford, MA, USA)

3.1.1.3 LC-MS/MS system: UPLC coupled to a Xevo™ TQ-S triple quadrupole mass spectrometer using an atmospheric pressure electrospray (AP-ESI) interface and Micromass Masslynx 4.1 software processing (Waters, Milford, MA, USA)

3.1.1.4 LC-MS/MS column: C_{18} Acquity UPLC HSS T3 (100 x 2.1 mm, 1.8 µm) (Waters, Milford, MA, USA)

3.1.1.5 Milli-Q ultra-pure water system: model Millipore ZMQS5V00 (Millipore, Billerica, MA, USA)

3.1.1.6 Balance: model XS (Mettler-Toledo, Inc., Columbus, OH, USA)

3.1.1.7 Ultrasonicate: model crest575d (Crest Ultrasonic corporation, Ewing Township, NJ, USA)

3.1.1.8 LC vial: 2 ml amber vials with PTFE cap (Agilent Technologies, Santa Clara, CA, USA)

3.1.1.9 Filter: 0.45 µm nylon membrane (Sigma-Aldrich, St. Louis, MO, USA)

3.1.1.10 Micropipettes: 2-20 µL, 50-200 µL, and 200-1000 µL micropipettes (Gilson Inc., Middleton, WI, USA)

3.1.1.11 Glasswares: volumetric flasks, solvent bottles, beakers and cylinders in various sizes (Schott, Elmsford, NY, USA)

3.1.2 Chemicals

3.1.2.1 Standard compounds

[6]-gingerol, [8]-gingerol, and [10]-gingerol were obtained from ChromaDex (Irvine, CA, USA) with purity of 91.4%, 88.2%, and 95.1 %, respectively.

3.1.2.2 Organic solvents

HPLC gradient grade ACN was supplied by J.T. Baker Chemical Co. (Phillipsburg, NJ, USA) and analytical grade methanol was purchased from Merck (Darmstadt, Germany).

3.1.2.3 Samples

Samples of fresh ginger (Z. officinale) from China (18 samples), India (18 samples), Malaysia (8 samples), Vietnam (18 samples) and Thailand (Chiangmai 18 samples, Chiangrai 18 samples, Leoy 18 samples, Nakonpatom 18 samples, Petchaboon 18 samples) were provided and authenticated by the Horticulture Research Institute (HRI), Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand, and by Gold Ginger Thai Co. (Petchaboon, Thailand). All samples were randomized by origin and collected in spring, 2012. The raw herbs were labeled according to their sources and then kept in a refrigerator at 4 °C until analysis.

3.1.3 Preparation of standard solutions

Standard solutions (1000 mg L^{-1}) of [6]-gingerol, [8]-gingerol, and [10]-gingerol for HPLC and LC–MS/MS identification were prepared individually by dissolving each compound in methanol; and then were stored at 0 °C in a refrigerator until use. A 100 mg L^{-1} mixture standard solution was prepared by pipetting 1 mL of 1000 mg L^{-1} [6]-gingerol, [8]-gingerol, and [10]-gingerol stock solution into a 10 mL volumetric flask and diluting with methanol. The mixture standard solution was kept in closed vials with Teflon screw cap and prepared daily.

3.1.4 HPLC system

3.1.4.1 LC-DAD

HPLC-DAD system (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector. All separations were carried out on a Waters Symmetry C18 column (150 x 3.9 mm, 5 μm). A binary gradient elution system composed of water (A) and ACN (B) was applied as follows: 0.0–2.0 min, 10–55% B; 2.0–8.5 min, 55% B; 8.6–12.5 min, 65% B; 12.6–19.0 min, 100% B. Each run was followed by equilibration time of 10 min. The injection volume was 3 μL per sample, the flow rate was 1 ml min^{-1} and the column temperature was maintained at 27 °C. The DAD detector was set at 230 nm for acquiring chromatograms.

3.1.4.2 LC-MS/MS

A Waters Acquity UPLC system coupled to a Xevo™ TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA, USA). Chromatographic separation was performed in a C_{18} Acquity UPLC HSS T3 column (100 x 2.1 mm, 1.8μm) with binary mobile phase in a gradient elution mode. Mobile phase A was an aqueous solution of 0.1% (v/v) formic acid, while mobile phase B was ACN. A binary gradient elution system was applied as follows: 0.0–2.0 min, 10–55% B; 2.0–8.5 min, 55% B; 8.6–12.5 min, 65% B; 12.6–19.0 min, 100% B. The flow rate was set at 0.3 mL/min and column temperature was 40°C. The injection volume was 2 μL.

MS spectra were recorded in the range of m/z 100–1000 using electrospray ionization (ESI) as the ionization source in positive/ negative ion-switching mode. The mass spectrometer settings used were: capillary voltage 3 kV, source temperature 150 °C, desolvation temperature 500 °C, cone gas (nitrogen) flow 150 L h^{-1}, desolvation gas (nitrogen) flow 800 L h^{-1}, and collision gas (argon) flow 0.15 ml min^{-1}. Instrument control and data acquisition and evaluation were performed with the Micromass MassLynx 4.1 software package.

3.1.5 Sample preparation

Ginger rhizomes were washed to remove debris and dirt, peeled and then cut into small pieces. Single extraction for each ginger sample was performed by weighing 5.0 g of sample and then placing it in a flask together with 40 ml methanol. The mixture was ultrasonicated for 60 min and then allowed to stand for 60 min at room temperature to cool down. The extraction solution was subsequently filtered through a 0.45 lm nylon membrane filter into a HPLC vial prior to analysis. Ginger samples from the same origin were analyzed within one day of the experiment.

3.1.6 Data analysis

The profiles of gingers from chromatographic determination were considered in two categories (city and country) on the basis of the scale of production area. The data sets of 80 x 9 matrix and a 90 x 9 matrix were achieved from LC-DAD measurement for five different ginger-producing cities (Chiangmai, Chiangrai, Leoy, Nakonpatom, and Petchaboon) and five different ginger-producing countries (China, India Malaysia, Thailand, and Vietnam), respectively. In data set, a row represented ginger sample from different sources and columns described the nine chemical compounds (variables). The relative peak area (RPA) was used in this study as a normalized data instead of peak area in order to adjust measured values on different scales into a common scale. The chemometric techniques of similarity analysis, hierarchical cluster analysis (HCA), principal component analysis (PCA), and linear

discriminant analysis (LDA) were performed in-house using MATLAB version 7.11 software (MathWorks, Natick, MA, USA) for statistical analysis.

3.2 Food quality: Molecular taste profile in beef

3.2.1 Instrumental and Apparatus

3.2.1.1 LC-MS/MS system: Dionex UHPLC UltiMate 3000 (Thermo Scientific, Dreieich, Germany) coupled to a 4000 QTRAP LC/MS/MS (AB Sciex, Darmstadt, Germany) using an atmospheric pressure electrospray (AP-ESI) interface and Analyst 1.6.1 software (AB Sciex, Darmstadt, Germany)

3.2.1.2 LC-MS/MS column 1: TSKgel Amide-80 (300 x 7.8 mm, 5 μm) (Tosoh Bioscience, Tokyo, Japan)

3.2.1.3 LC-MS/MS column 2: ZIC-pHILIC polymeric column (150 x 2.1 mm, 5 μm) (Merck, Darmstadt, Germany)

3.2.1.4 LC-MS/MS column 3: Phenomenex Synergi Polar-RP 80A (150 x 2 mm, 4 μm) (Phenomenex, Torrance, California, USA)

3.2.1.5 IC system: ICS-2000 Ion Chromatography System (Thermo Scientific, Dreieich, Germany) coupled to AS Autosampler, CSRS300 suppressor, and DS 6 Heated Conductivity Cell detector (Thermo Scientific, Dreieich, Germany) using Chromeleon 7.1. software (Thermo Scientific, Dreieich, Germany)

3.2.1.6 IC column 1: IonPac CS 19 (250 x 2.0 mm) (Thermo Scientific, Dreieich, Germany)

3.2.1.7 IC column 2: IonPac AS11-HC analytical column (250 x 2mm) (Thermo Scientific, Dreieich, Germany)

3.2.1.8 LC-TOF/MS system: Waters Synapt G2 HDMS mass spectrometer (Waters, Manchester, U.K.) coupled to an Acquity UPLC core system (Waters, Manchester, U.K.) using Micromass Masslynx 4.1 software processing (Waters, Milford, MA, USA)

3.2.1.9 LC-TOF/MS column: C_{18} Acquity BEH (150 x 2.0 mm I.D., 1.7μm) (Waters, Milford, MA, USA)

3.2.1.10 GPC system: Ultrarac Fraction Collector 7000 (LKB Produkter, Bromma, Sweden) equipped with a L-7420-type UV-vis detector (Merck, Darmstadt, Germany)

3.2.1.11 GPC column: 100 x 5 cmXK 50/100 glass column (GE Healthcare, Munich, Germany) filled with a slurry of Sephadex G-15 (GE Healthcare)

3.2.1.12 LC-ELSD system: Jasco (Jasco, Gross-Umstadt, Germany) equipped with a DG-2080-53 vacuum degasser, a PU-2087 Plus binary pump, a AS-2055 Plus autosampler, a MD-2010 Plus diode array detector, and a Sedex LT-ELSD Model 85 (Sedere, Alfortville, France) using Chemstation software (Agilent Technologies, Santa Clara, CA, USA).

3.2.1.13 LC-ELSD column: Varian Microsorb-MV 100-5 C18 (250 x 4.6 mm, 5 μm) (Varian, Palo Alto, CA, USA)

3.2.1.14 MPLC system: Sepacore chromatography system (Buechi, Flawil, Switzerland) equipped with two C-605 pumps with C-615 pump manager, manual rheodyne injection port (20 mL loop), C-660 fraction collector, C-635 UV/vis detector, and Sedex LT-ELSD Model 85 ELSD detector (Sedere, Alfortville, France) using Buechi Sepacore Record 1.0 software (Buechi, Flawil, Switzerland)

3.2.1.15 MPLC column: Polypropylene cartridge (150x40 mm i.d.), filled with RP 18 material (Lichroprep, 25-40μm (Merck, Darmstadt, Germany)

3.2.1.16 NMR system: Bruker DPX400 spectrometer (Bruker, Rheinstetten, Germany) using MestReNova 5.1.0-2940 (Mestrelab Research, Santiago de Compostella, Spain)

3.2.1.17 Milli-Q ultra-pure water system: Milli-Q Advantage A10 system (Millipore, Schwalbach, Germany)

3.2.1.18 LC vial: 2 ml amber vials with PTFE cap (Agilent Technologies, Santa Clara, CA, USA)

3.2.1.19 Balance: model XS (Mettler-Toledo, Inc., Columbus, OH, USA)

3.2.1.20 Centrifuge: Beckman Coulter Avanti J-E centrifuge (Beckman Coulter Inc., Brea, CA, USA)

3.2.1.21 Rotary Evaporator: Buechi Rotavapor R-210 (Buechi, Flawil, Switzerland)

3.2.1.22 Homogenizer: Ultra-Turrax T-25 Digital Homogenizer (Ika-Work Inc., Wilmington, NC, USA)

3.2.1.23 Blender: Krups blender Power Xtreme Premium Touchpad Blender Type 577 (Krups, Solingen, Germany)

3.2.1.24 Shaker: GFL Orbital Shaker 3005 (GFL, Burgwedel, Germany)

3.2.1.25 Stirrer: Hot Plate Stirrer Heidolph MR Hei-standard (Heidolph, Schwabach, Germany)

3.2.1.26 Micropipettes: Eppendorf research micropipettes size 0.5–10 μL, 2–20 μL, 20–200 μL, 100–1,000 μL (Eppendorf, Hamburg, Germany)

3.2.1.27 Glasswares: volumetric flasks, solvent bottles, beakers and cylinders in various sizes (Schott, Elmsford, NY, USA)

3.2.2 Chemicals

3.2.2.1 Standard compounds

All standard chemicals (fatty acids, amino acids, organic acids and cations) with analytical standard grade were purchased from Sigma-Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany) and Fluka (Neu-Ulm, Germany).

3.2.2.2 Organic solvents

HPLC gradient grade solvents (ACN and methanol) were supplied by J.T. Baker (Phillipsburg, NJ, USA). Analytical grade ACN, methanol and n-pentane were purchased from J.T. Baker (Phillipsburg, NJ, USA).

3.2.2.3 Reagents

Potassium hydroxide (pellets), ammonium acetate (ACS, Reag. Ph Eur), ammonium hydroxide (ACS, Reag. Ph Eur), acetic acid (glacial 100%), formic acid (98-100%) and hydrochloric acid (fuming 37%) were purchased from Merck (Darmstadt, Germany). Palmitoyl chloride (98%) and methanesulfonic acid (99.5%) was supplied from Sigma-Aldrich (Steinheim, Germany). 1,4-dioxane (99.5%) was obtained from Carl Roth (Karsruhe, Germany).

3.2.2.4 Samples

Dry-aged beefs with normal treatment and with noble mold treatment (LUMA) in different cuts (rib eye, short loin, tenderloin, sirloin) were purchased from LUMA D.A.C. (Neuhausen, Switzerland). All beefs were cut into small pieces (each 300 g approx.), pan-fried for 2 min each side and heated in oven for homogeneous temperature distribution of meat at 55°C. Furthermore, the cooked beefs were cooled down at room temperature, chopped and stored at -20°C until use.

3.2.3 Preparation of standard solutions

Standard solutions (1000 mg L^{-1}) for quantifications and identification were prepared individually by dissolving each compound in suitable solvents; and then were stored at 0 °C in a refrigerator until use. A 100 mg L^{-1} mixture standard solution was prepared by pipetting standard stock solutions into a 10 mL volumetric flask and diluting with suitable solvents. The mixture standard solution was kept in closed vials with Teflon screw cap and prepared daily.

3.2.4 LC-MS/MS system

A Dionex UHPLC UltiMate 3000 (Thermo Scientific, Dreieich, Germany) coupled to a 4000 QTRAP LC/MS/MS (AB Sciex, Darmstadt, Germany).

Chromatographic separation for amino acid analysis was performed in a TSKgel Amide-80 (300 x 7.8 mm, 5 μm) (Tosoh Bioscience, Tokyo, Japan) with binary mobile phase in a gradient elution mode. A 95% ACN solution containing 5 mM ammonium acetate, adjusted to pH 3 with acetic acid was used as mobile phase A, while 5 mM ammonium acetate, adjusted to pH 3 with acetic acid was employed as solvent B. A binary gradient elution system was applied as follows: 0.0–3.0 min, 10–25% B; 3.0–6.0 min, 25-55% B; 6.0–9.0 min, 55-100% B; 9.0–13.0 min, 100% B. The flow rate was set at 0.25 mL min^{-1} and column temperature was 40°C. The injection volume was 3 μL.

MS spectra were recorded in the range of m/z 50–1000 using electrospray ionization (ESI) as the ionization source in positive/ negative ion-switching mode. The mass spectrometer settings used were: capillary voltage 5.5 kV, source temperature 425 °C, desolvation temperature 450 °C, cone gas (nitrogen) flow 30 L h^{-1}, desolvation gas (nitrogen) flow 850 L h^{-1}, and collision gas (nitrogen) flow 0.15 ml min^{-1}. Instrument control and data acquisition and evaluation were performed with the Analyst 1.6.1 software (AB Sciex, Darmstadt, Germany).

Chromatographic separation for fatty acid analysis was performed in a Phenomenex Synergi Polar-RP 80A (150 x 2 mm, 4 μm) (Phenomenex, Torrance, California, USA) with binary mobile phase in a gradient elution mode. A 95% ACN solution containing 5 mM ammonium acetate, adjusted to pH 3 with acetic acid was used as mobile phase A, while 5 mM ammonium acetate, adjusted to pH 3 with acetic acid was employed as solvent B. A binary gradient elution system was applied as follows: 0.0–2.0 min, 100–50% B; 2.0–5.0 min, 50% B; 5.0–10.0 min, 50-0% B; 10.0–14.0 min, 0% B. The flow rate was set at 0.4 mL min^{-1} and column temperature was 40°C. The injection volume was 2 μL.

MS spectra were recorded in the range of m/z 50–1000 using electrospray ionization (ESI) as the ionization source in positive/ negative ion-switching mode. The mass spectrometer settings used were: capillary voltage 5.5 kV, source temperature 425 °C, desolvation temperature 450 °C, cone gas (nitrogen) flow 30 L h^{-1}, desolvation gas (nitrogen) flow 850 L h^{-1}, and collision gas (nitrogen) flow 0.15 ml

min^{-1}. Instrument control and data acquisition and evaluation were performed with the Analyst 1.6.1 software (AB Sciex, Darmstadt, Germany).

Chromatographic separation for organic acid analysis was performed in a ZIC-pHILIC polymeric column (150 x 2.1 mm, 5 μm) (Merck, Darmstadt, Germany) with binary mobile phase in a gradient elution mode. A 95% ACN solution containing 5 mM ammonium acetate, adjusted to pH 9 with ammonium hydroxide was used as mobile phase A, while 5 mM ammonium acetate, adjusted to pH 9 with ammonium hydroxide was employed as solvent B. A binary gradient elution system was applied as follows: 0.0–4.0 min, 20% B; 4.0–12.0 min, 20-100% B; 12.0–18.0 min, 100% B. The flow rate was set at 0.5 mL min^{-1} and column temperature was 40°C. The injection volume was 5 μL.

MS spectra were recorded in the range of m/z 100–1000 using electrospray ionization (ESI) as the ionization source in positive/ negative ion-switching mode. The mass spectrometer settings used were: capillary voltage 5.5 kV, source temperature 700 °C, desolvation temperature 450 °C, cone gas (nitrogen) flow 30 L h^{-1}, desolvation gas (nitrogen) flow 850 L h^{-1}, and collision gas (nitrogen) flow 0.15 ml min^{-1}. Instrument control and data acquisition and evaluation were performed with the Analyst 1.6.1 software (AB Sciex, Darmstadt, Germany).

3.2.5 IC system

ICS-2000 Ion Chromatography System (Thermo Scientific, Dreieich, Germany) coupled to AS Autosampler, CSRS300 suppressor, and DS 6 Heated Conductivity Cell detector (Thermo Scientific, Dreieich, Germany). Cations were analyzed on an IonPac CS16 column (250 x 3 mm) connected with an IonPac CG16 guard column (50 x 3 mm) and a self-regenerating cation suppressor CSRS 300 Ultra II (2 mm), which was installed between the column and the conductivity detector and operated in the autosuppression recycle mode at 37 mA. Chromatography was performed at 40 °C with isocratic elution using aqueous 5 mmol L^{-1} methane sulfonic acid as the eluent at a flow rate of 0.36 mL min^{-1}.

Anions were analyzed on an IonPac AS11-HC analytical column (250 x 2mm) connected with an IonPacAG11-HC guard column (50 x 2 mm) and a self-regenerating anion suppressor ASRS Ultra II (2mm), which was installed between the analytical column and the conductivity measuring cell and operated in the autosuppression recycle mode at 76 mA. Chromatography was performed at 30 °C with a flow rate of 0.38 mL min^{-1} using a gradient consisting of water (solvent A), an aqueous 5 mmol L^{-1} NaOH (solvent B), and an aqueous 100 mmol L^{-1} NaOH (solvent C). Starting with a mixture of 80% A and 20% B for 8 min, the NaOH concentration was increased successively to 70% A and 30% C within 10 min and, finally, to 40% A and 60% C within 10 min. The injection volume was 10 µL. System control and data processing were performed using Chromeleon software version 6.60 (Dionex, Sunnyvale, CA, USA).

3.2.6 LC-TOF-MS

Mass spectra of the compounds were measured on a Waters Synapt G2 HDMS mass spectrometer (Waters, Manchester, U.K.) coupled to an Acquity UPLC core system (Waters) consisting of a binary solvent manager, sample manager and column oven. The compounds were dissolved in 1 mL of methanol, and aliquots (1−5 µL) were injected into the UPLC-TOF-MS system equipped with a 150 × 2 mm, 1.7 µm, BEH C18 column (Waters). A UPLC operated with a flow rate of 0.4 mL/min at a temperature of 45 °C. A binary gradient elution system with water (A) and ACN (B) was applied as follows: 0.0–0.1 min, 97% B; 0.1–8.0 min, 97-40% B; 8.0–8.2 min, 10% B. Calibration of the Synapt G2 was performed with a solution of sodium formate (0.5 mmol L^{-1}) in 2-propanol/water (9:1 v/v).

MS spectra were recorded in the range of m/z 100–1000 using electrospray ionization (ESI) as the ionization source in positive and negative mode. The mass spectrometer settings used were: capillary voltage +2.5 or −3.0 kV, source temperature 150 °C, desolvation temperature 450 °C, cone gas (nitrogen) flow 10 L h^{-1}, desolvation gas (nitrogen) flow 850 L h^{-1}, and collision gas (nitrogen) flow 0.15 ml

min^{-1}. Instrument control and data acquisition and evaluation were performed with the MassLynx 4.1 software (Waters, Manchester, U.K.).

3.2.7 GPC

A 100 x 5 cmXK 50/100 glass column (GE Healthcare, Munich, Germany) filled with a slurry of Sephadex G-15 (GE Healthcare), which was conditioned with water adjusted to pH4.0 with aqueous formic acid (1 g/100 g). Chromatographic separation was performed using the same mobile phase at a flow rate of 3 mL/min for 22 h. Monitoring the effluent at 220 nm by means of an L-7420-type UV-vis detector (Merck, Darmstadt, Germany) allowed individual fractions to be collected every 6 min by means of an Ultrarac Fraction Collector 7000 (LKB Produkter, Bromma, Sweden).

3.2.8 LC-ELSD

A HPLC Jasco (Jasco, Gross-Umstadt, Germany) was equipped with a DG-2080-53 vacuum degasser, a PU-2087 Plus binary pump, a AS-2055 Plus autosampler, a MD-2010 Plus diode array detector, and a Sedex LT-ELSD Model 85 (Sedere, Alfortville, France) using Chemstation software (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was performed in a Varian Microsorb-MV 100-5 C18 (250 x 4.6 mm, 5 μm) (Varian, Palo Alto, CA, USA) with binary mobile phase in a gradient elution mode. A 0.1% formic acid was used as mobile phase A, while ACN was employed as solvent B. A binary gradient elution system was applied as follows: 0.0–2.0 min, 2% B; 2.0–8.0 min, 100% B; 8.0–18.0 min, 100% B. The flow rate was set at 1 mL min^{-1} and column temperature was operated at room temperature. The injection volume was 10 μL.

3.2.9 MPLC

MPLC was performed on a preparative Sepacore chromatography system (Buechi, Flawil, Switzerland) equipped with two C-605 pumps with C-615 pump

manager, manual rheodyne injection port (20 mL loop), C-620 control unit, C-660 fraction collector, C-635 UV/vis detector, and Sedex LT-ELSD Model 85 ELSD detector (Sedere, Alfortville, France) using Buechi Sepacore Record 1.0 software (Buechi, Flawil, Switzerland). Chromatography was performed on Polypropylene cartridge (150x40 mm i.d.), filled with RP 18 material (Lichroprep, 25-40µm (Merck, Darmstadt, Germany))

3.2.10 NMR

1H, 13C, COSY, and HSQC experiments were performed on a Bruker DPX400 spectrometer (Bruker, Rheinstetten, Germany). Data processing was performed by using MestReNova 5.1.0-2940 (Mestrelab Research, Santiago de Compostella, Spain). MeOD was used as solvent and tetramethylsilane as the internal standard.

3.2.11 Sample preparation

A 100g cooked beef was weighed, mixed and extracted three times (150 mL each) with methanol/water (70/30, v/v). The obtained extract solutions were then centrifuged at 9000 rpm for 20 min and the aqueous layer was collected with further methanol evaporation. An aqueous extract was added with 150 mL water, defatted by extraction with n-pentane (3 x150mL) and then filtered. The combined aqueous filtrates were lyophilized to give the insoluble materials which was made up to 100 mL with water and kept at -20°C until employed in sensory evaluation and chemical analysis.

3.2.12 Synthesis of N-acylamino acid

The amino acid and fatty acid chloride with the ratio of 2:1 were dissolved in 40 ml dioxane. The solution was stirred overnight at room temperature and subsequently added with 20 ml ACN. After that, a rotary evaporator was employed to dryness the solvent **[108]**.

3.2.13 Sensory evaluation

Twelve subjects (ages 23−35 years) have been participated in the sensory tests and had no history of known taste disorders, participated for at least two years in weekly training sessions and were recruited from the Chair of Food Chemistry and Molecular Sensory Science (Freising, Germany).

For the training of the taste quality, aqueous solutions (2 mL each) of the following standard taste compounds in bottled water (pH 6.0) were used: sucrose (50 mmol L^{-1}) for sweet taste, lactic acid (20 mmol L^{-1}) for sour taste, NaCl (20 mmol L^{-1}) for salty taste, caffeine (1 mmol L^{-1}) for bitter taste, monosodium L-glutamate (3 mmol L^{-1}) for umami taste, and reduced glutathione (5 mmol L^{-1}) for kokumi taste [42, 109].

For sensory studies, the taste recombinant solution (Rec) was prepared by dissolving the natural concentrations of amino acids, cations, and chloride in LUMA short loin (obtained from the results of targeted analysis in Topic 4.2.1) in 100 mL bottled water and, after solubilizing gelatin (5000 mg L^{-1}), the pH value of this solution was adjusted to 5.6 by the addition of trace amounts of an aqueous formic acid solution (1 mol L^{-1}). A gelatin solution was used for adjusting on viscosity. For comparative taste profile analysis, LUMA extract (short loin) and each GPC fractions (20 mL) were dissolved in an aqueous Rec (20 mL). These solutions were then presented to the trained sensory panel, and the intensities of the taste descriptors bitter, sweet, sour, salty, umami, and kokumi were rated on a scale from 0 (not detectable) to 5 (intensely perceived) in comparison to an aqueous Rec (reference).

3.3 Food safety: Matrix effect in pesticide residue analysis

3.3.1 Instrumental and Apparatus

3.3.1.1 GC-MS/MS system: Bruker Scion TQ triple quadrupole mass spectrometer equipped with a Bruker 436 GC (Bruker, Billerica, MA, USA) using an electron ionization mode and Bruker MS Workstation software (version 8) (Bruker, Billerica, MA, USA)

3.3.1.2 GC-MS/MS column: Rtx-5MS capillary column (30 m x 0.25 mm, 0.25 mm film thickness) with 5 m Integra-Guard column from Restek (Bellefonte, PA, USA).

3.3.1.3 Milli-Q ultra-pure water system: model Millipore ZMQS5VF01 (Millipore, Billerica, MA, USA)

3.3.1.4 Balance: model XS (Mettler-Toledo, Inc., Columbus, OH, USA)

3.3.1.5 Centrifuge1: Thermo Fisher Scientific Heraeus Megafuge 1.0R centrifuge (Thermo Fisher Scientific, Waltham, MA, USA)

3.3.1.6 Centrifuge2: Thermo Fisher Scientific Jouan B4i centrifuge (Thermo Fisher Scientific, Waltham, MA, USA)

3.3.1.7 Vortex: Vortex-Genie2 (Scientific Industries Inc., Bohemia, NY, USA)

3.3.1.8 QuEChERS extraction kit: Bond Elut QuEChERS extract packets (CEN method) (Agilent Technologies, Santa Clara, CA, USA) containing 50 mL polypropylene centrifuge tubes with 4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate, and 0.5 g sodium hydrogen citrate sesquihydrate

3.3.1.9 Bond Elut dispersive SPE kits consisting of 2 mL polypropylene centrifuge tubes with 25 mg primary secondary amine (PSA), 2.5 mg graphitized carbon black, and 150 mg magnesium sulfate (Agilent Technologies, Santa Clara, CA, USA)

3.2.1.11 Micropipettes: Eppendorf research micropipettes size 0.5–10 μL, 2–20 μL, 20–200 μL, 100–1,000 μL (Eppendorf, Hamburg, Germany)

3.2.1.12 Glasswares: volumetric flasks, solvent bottles, beakers and cylinders in various sizes (Schott, Elmsford, NY, USA)

3.3.2 Chemicals

3.3.2.1 Standard compounds

Pesticide standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and were of purity >95%.

3.3.2.2 Organic solvents

Pesticide residue grade ACN, toluene and acetone and HPLC grade formic acid were purchased from Sigma (St. Louis, MO, USA).

3.3.2.3 Reagents

Triphenylphosphate (TPP) was obtained from Dr. Ehrenstorfer (Augsburg, Germany) with 95% purity. 3-ethoxy-1,2- propanediol (98%), L-gulonic acid g-lactone (98%), D-sorbitol (99%), and shikimic acid (99%) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Chromadex (Irvine, CA, USA).

3.3.2.4 Samples

Chili samples were provided by Central Laboratory (Thailand) Co., Ltd., Bangkok, Thailand and were primarily confirmed as a blank sample before use in experiments. Samples from different sources were homogenized; and then samples were kept in the freezer at 20 °C and thawed at room temperature before analysis.

3.3.3 Preparation of standard solutions

Standard solutions (2000 mg L^{-1}) of each pesticide standard were prepared individually by dissolving each compound in acetone:toluene (1:1 v:v); and then were stored at -20 °C in a refrigerator until use. A 500 µg L^{-1} mixture standard solution was prepared by pipetting the appropriate quantities of pesticide standard stock solution into a 10 mL volumetric flask and diluting with 0.05% formic acid in ACN. The mixture standard solution was kept in closed vials with Teflon screw cap and prepared daily.

3.3.4 Preparation of AP solutions

Standard solutions of each AP were prepared individually. 3-ethoxy-1,2-propanediol was used in solid form and L-gulonic acid g-lactone, D-sorbitol, and shikimic acid were dissolved in ACN:H_2O (6:4), (1:1), and (6:4), respectively to yield 50 mg mL^{-1} stock solution. Individual stock AP solutions were stored at -20 °C in a refrigerator until use. A 500 µg L^{-1} mixture standard solution was prepared by pipetting the appropriate quantities of pesticide standard stock solution into a 10 mL volumetric flask and diluting with 0.05% formic acid in ACN. The mixture standard solution was kept in closed vials with Teflon screw cap and prepared daily. The AP mixture solution (40:2:1:1) for spiking in the final extracts contained 200 mg mL^{-1} 3-ethoxy-1,2-propanediol, 10 mg mL^{-1} L-gulonic acid g-lactone, 5 mg mL^{-1} D-sorbitol, and 5 mg mL^{-1} shikimic acid was prepared in ACN:water (7:3, v:v).

3.3.5 GC-MS/MS system

A Bruker Scion TQ triple quadrupole mass spectrometer was equipped with a Bruker 436 GC (Billerica, MA, USA). Chromatographic separations of 100 pesticides were done by using Rtx-5MS capillary column (30 m x 0.25 mm, 0.25 mm film thickness) with 5 m Integra-Guard column from Restek (Bellefonte, PA, USA). Helium (99.999%) was used as the carrier gas at a constant flow of 1 mL min^{-1}. An Agilent ultra-inert single taper splitless liner with wool (Part no.5190-2293) was used

(Santa Clara, CA, USA) in the injection port. A split/splitless injector was held at 250 °C and the injection volume was 1 mL. A venting was 20:1 until 0.01 min when the split vent was closed until 0.75 min, and then the split ratio was held at 50:1, and finally reduced to 20:1 after 41 min. The oven temperature program was as follows: 80 °C held for 2 min, ramped 10 °C min^{-1}-150 °C and held 1 min, 5 °C min^{-1}-220 °C, 10 °C min^{-1}-230 °C, and held 2 min, 15 °C min^{-1}-280 °C and held 4 min, followed by 20 °C min^{-1}-300 °C, and held 5.67 min. The total runtime was 36.5 min. After each injection, the syringe was washed with an acetone:water (1:1, v:v) followed by ACN. The liner was replaced in the injection port after each batch test or after 100 injections. The MS/MS detection was performed with electron ionization mode at -70 eV. The temperatures of the transfer line, ion source, and manifold were set at 280, 250, and 40 °C, respectively. Argon (99.999%) was used as collision gas for all MS/MS experiments, the pressure in the collision cell was set at 1.8 mTorr, and the solvent delay was at 5.5 min. The optimal ion transitions and collision energy in MRM for each pesticide were determined as shown in Table 3.1. The 100 studied pesticides were divided into 3 time ranges based on their elution time: range 1 was 8.7-19.4 min for 21early-eluting compounds; range 2 was 20.1-28.7 min for 50 middle-eluting compounds; and range 3 was 29.7-36.2 min for 29 late-eluting compounds. Bruker MS Workstation software (version 8), was used for instrument control and data acquisition and processing (Bruker, Billerica, MA, USA).

3.3.5 Sample preparation

The sample preparation was based on the QuEChERs citrate buffered version in which 10 g of chili sample was weighed in a 50 mL polypropylene centrifuge tube, 10 mL ACN was added, and the solution was mixed using a Vortex mixer for 1 min. Then, Bond Elut QuEChERS extract packets were transferred into the extracts, which were mixed again immediately for 1 min. The tubes were centrifuged for 10 min at 3293 rcf (4 °C). Cleanup was performed by transferring 1 mL of the upper layer (extract) to a 2 mL-polypropylene centrifuge tube for dispersive SPE. The extract was vortexed for 1 min and then centrifuged for 10 min at 13,500 rcf (4 °C). Lastly, 960 mL extract was fortified with 20 mL each of pesticide mixture and I.S. solutions.

Table 3.1 Average retention times (RTs), elution time range, and multiple reaction monitoring (MRM) transitions for each pesticide.

No.	Pesticide	RT (min)	Elution time range[a]	MRM transitions (*m/z*)			
				Quantifier	CE (V)	Qualifier	CE (V)
1	Dichlorvos	8.744	1	185>93	10	185>109	20
2	Dichlobenil	10.358	1	171>136	15	171>100	10
3	Etridiazole	12.229	1	211>183	10	211>140	25
4	Methacrifos	13.102	1	125>79	5	208>93	15
5	Heptenophos	14.583	1	124>89	30	215>89	15
6	Fenobucarb	15.188	1	150>121	16	150>103	24
7	Ethoprophos	15.665	1	158>97	18	158>114	10
8	Trifluralin	16.488	1	306>264	10	306>206	15
9	Dicrotophos	16.498	1	127>109	15	127>95	18
10	Monocrotophos	16.834	1	127>109	15	127>95	18
11	Phorate	16.857	1	231>129	25	231>175	15
12	alpha-BHC	17.069	1	181>145	15	219>183	10
13	Hexachlorobenzene	17.338	1	284>249	20	282>212	30
14	Gamma-BHC	18.341	1	181>145	15	219>183	10
15	beta-BHC	18.345	1	181>145	15	219>183	10
16	Terbufos	18.464	1	231>129	20	231>175	20
17	Fonofos	18.629	1	246>109	18	246>137	18
18	Pyrimethanil	18.879	1	198>118	35	198>156	25
19	Diazinon	18.958	1	304>179	10	179>137	20
20	delta-BHC	19.353	1	219>183	10	219>147	15
21	Tefluthrin	19.357	1	177>127	15	177>87	25
22	Pentachloraniline	20.113	2	265>194	25	265>230	15
23	Phosphamidon	20.463	2	264>127	15	227>127	15
24	Malaoxon	20.849	2	127>99	10	127>109	15
25	Tolclofos-methyl	20.939	2	265>250	15	265>220	20
26	Heptachlor	20.992	2	272>237	20	274>239	20
27	Metalaxyl	21.269	2	206>132	20	206>162	10
28	Fenchlorphos	21.309	2	285>270	10	285>240	25
29	Fenitrothion	21.834	2	277>260	5	277>109	20
30	Pentanochlor	21.924	2	141>106	15	141>77	30
31	Malathion	22.220	2	158>125	10	173>99	25
32	Aldrin	22.286	2	263>193	40	263>191	40
33	Metolachlor	22.423	2	162>133	15	238>162	15
34	Fenpropimorph	22.479	2	128>70	10	303>128	10
35	Fenthion	22.550	2	278>109	20	278>125	18
36	Dimethylvinphos	22.591	2	295>109	20	297>109	20

37	Chlorpyrifos	22.596	2	314>258	15	314>286	5
38	Parathion-ethyl	22.647	2	291>109	10	291>81	25
39	Chlorthal-dimethyl	22.793	2	332>301	10	301>223	18
40	Isobenzan	22.905	2	311>275	10	375>275	10
41	Pirimiphos-ethyl	23.414	2	290>125	25	290>151	25
42	Cyprodinil	23.608	2	224>208	18	224>118	40
43	Metazachlor	23.785	2	209>132	15	209>117	30
44	Penconazole	23.896	2	248>157	25	248>192	15
45	Chlorfenvinphos	24.160	2	267>159	20	323>267	25
46	Phenthoate	24.265	2	274>121	10	274>125	20
47	gamma-Chlordane	24.664	2	373>266	30	373>301	10
48	Methidathion	24.761	2	145>85	10	145>58	15
49	Bromophos-ethyl	24.794	2	359>303	28	359>331	10
50	o,p'-DDE	24.851	2	246>176	30	248>176	30
51	Tetrachlorvinphos	25.092	2	329>109	20	331>109	25
52	alpha-Chlordane	25.172	2	373>266	30	373>301	10
53	Iodofenphos	25.612	2	377>362	20	377>250	25
54	Prothiofos	25.677	2	267>239	10	309>239	10
55	Profenofos	25.825	2	337>267	15	339>269	10
56	p,p'-DDE	25.948	2	246>176	30	248>176	30
57	Oxadiazon	26.108	2	258>175	10	258>112	25
58	o,p'-DDD	26.296	2	235>165	25	237>165	20
59	Endrin	26.867	2	263>193	30	281>245	10
60	Chlorfenapyr	27.008	2	247>227	15	247>200	25
61	Chloropropylate	27.228	2	251>139	10	251>111	30
62	o,p'-DDT	27.591	2	235>165	15	237>165	15
63	p,p'-DDD	27.591	2	235>165	20	237>165	20
64	Ethion	27.719	2	231>129	25	231>203	10
65	Triazophos	28.196	2	257>162	10	257>119	25
66	Ofurace	28.416	2	232>158	20	281>232	5
67	Cyanofenphos	28.529	2	169>141	5	185>157	10
68	Quinoxyfen	28.558	2	307>237	20	307>272	20
69	Endosulfan sulfate	28.561	2	272>237	15	387>253	10
70	p,p'-DDT	28.704	2	235>165	20	237>165	20
71	Trifloxystrobin	28.718	2	116>89	15	190>130	10
72	Spiromesifen	29.654	3	272>254	5	272>209	10
73	Pyridaphenthion	29.825	3	340>199	10	340>109	20
74	Bromopropylate	29.922	3	183>155	15	341>183	10
75	Bifenthrin	29.950	3	181>166	10	181>165	20
76	Tetramethrin	29.977	3	164>77	35	164>107	28

77	Fenpropathrin	30.105	3	181>152	20	265>210	10
78	Terbufenpyrad	30.172	3	333>171	15	333>276	5
79	Fenazaquin	30.255	3	160>145	5	160>117	15
80	Tetradifon	30.528	3	159>111	25	229>166	20
81	Furathiocarb	30.536	3	194>161	10	194>179	13
82	lambda-Cyhalothrin	31.030	3	181>152	20	181>127	30
83	Fenarimol	31.332	3	139>111	10	139>75	35
84	Pyrazophos	31.447	3	221>193	10	232>204	10
85	Metrafenone	31.721	3	393>363	15	379>349	20
86	Permethrin I	32.080	3	183>168	15	183>128	20
87	Permethrin II	32.254	3	183>168	15	183>128	20
88	Coumaphos	32.483	3	362>109	15	362>226	15
89	Prochloraz	32.792	3	180>138	15	308>70	10
90	Fenbuconazole	33.089	3	198>129	10	198>102	30
91	Cyfluthrin (sum)	33.303	3	163>127	5	206>151	20
92	Flucythrinate I	33.850	3	157>107	15	199>107	30
93	Cypermethrin (sum)	33.857	3	181>152	20	181>127	25
94	Pyridalyl	34.051	3	204>148	20	164>146	10
95	Flucythrinate II	34.203	3	157>107	15	199>107	30
96	Fenvalerate I	35.110	3	167>125	12	225>119	15
97	tau-Fluvalinate I	35.344	3	250>200	15	250>55	15
98	tau-Fluvalinate II	35.344	3	250>200	15	250>55	15
99	Fenvalerate II	35.396	3	167>125	12	225>119	15
100	Deltamethrin	36.207	3	181>152	20	253>93	15

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Food quality: The authentication of ginger origin

4.1.1 HPLC fingerprint study

In this work, the chemical profiles of ginger samples based on chromatographic data were used to classify the geographical origin for authentication purpose. Reversed-phase HPLC was performed for ginger fingerprint study and the instrumental conditions of HPLC were optimized to obtain the best chromatographic separation of chemical profiles in ginger. Several parameters related mobile phase, column and detector were varied to provide the narrow and better-resolved peaks in proper elution time because peak resolution, baseline, retention times and number of characteristic peaks in each chromatogram are primary consideration of chromatographic profile. On the basis of several trials using different mobile phase, water-ACN binary system was selected as the most appropriate eluent under the optimized linear gradient mode. Due to large number of analyzed sample in fingerprint study, the time for analysis in each sample is an important factor; the total runtime in this work was 19 min which is considerably suitable when compare to other ginger researches [17, 20]. UV absorption at a wavelength of 230 nm was chosen because it generated the highest responses for the detection of all peaks.

All 170 ginger samples from five different countries (China (18 samples), India (18 samples), Malaysia (8 samples), Vietnam (18 samples) and Thailand (Chiangmai 18 samples, Chiangrai 18 samples, Leoy 18 samples, Nakonpatom 18 samples, Petchaboon 18 samples)) were selected. Sufficient numbers of sample should be provided and carefully labelled to represent the authentic profile of sample from each source. To monitor the ginger chromatographic profiles in preliminary investigation, ginger samples from nine different producing origins (China, India, Malaysia, Vietnam, Chiangmai, Chiangrai, Leoy, Nakonpatom, Petchaboon) were tested and their chromatograms were presented in Figure 4.1.

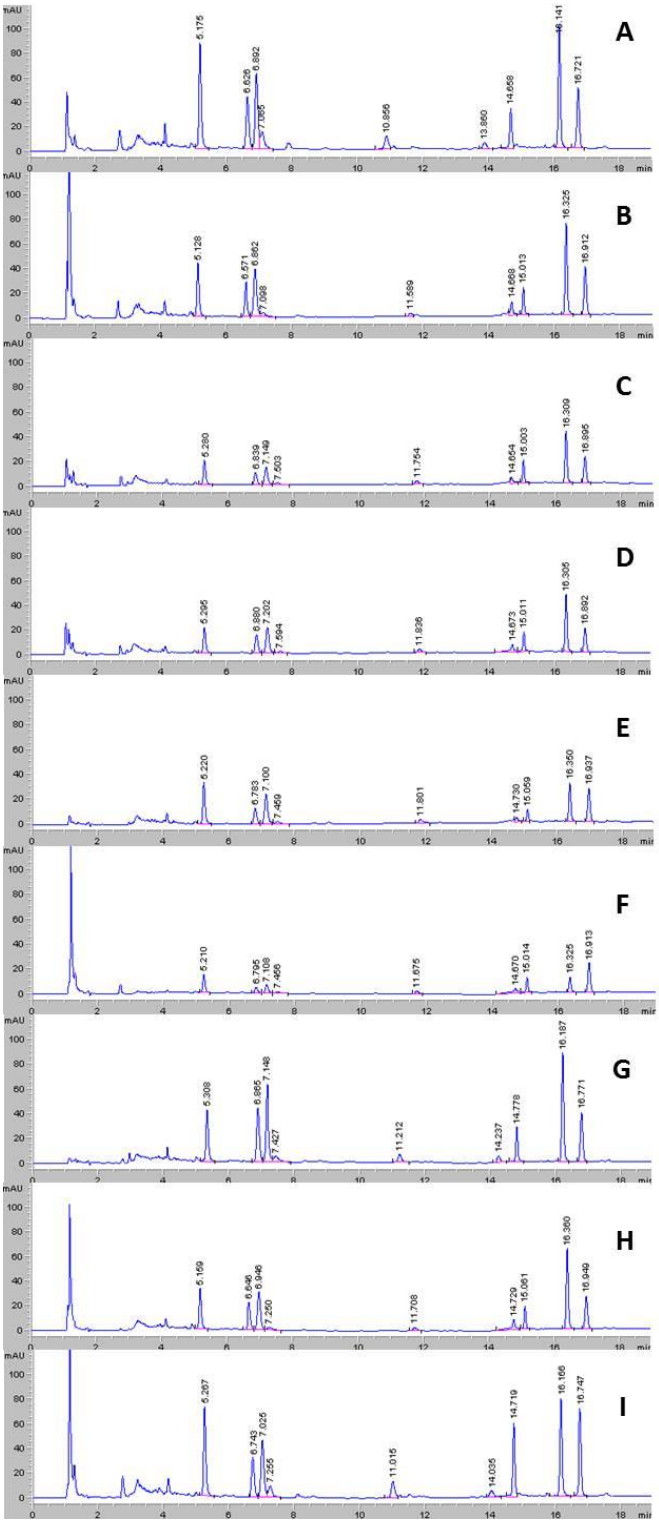


Figure 4.1 Representative chromatographic fingerprints of ginger samples from nine producing origins
(A) Chiangmai, (B) Chiangrai, (C) Leoy, (D) Nakonpatom, (E) Petchaboon, (F) China, (G) India, (H) Malaysia, and (I) Vietnam

From the chromatogram, there could be indicated that no obvious qualitative differentiation of ginger samples founded in each origin. Thus, this work chose the variation of quantitative data (peak intensity) to discriminate the fingerprint of each origin. Nine peaks which have peak area higher than 0.5% of the total area and exist in all chromatograms were selected as ‘‘common peaks’’ to express the characteristics of ginger extracts. The other peaks in early elution time were not included because those peaks tend to have errors from peak integration and LC system.

4.1.2 Optimization of extraction methods

Besides an effective chromatographic measurement, a practical sample preparation is also needed to serve a large number of analyzed samples in fingerprint study. Additionally, the selection of pathway should concern about the highest extraction efficiency and well-separated chemical profiles with the lowest background signal from the matrices. From the literature search of composition analysis, LLE is a traditional technique to prepare ginger sample. Therefore, this work selected a methanol-based LLE method with ultrasonication procedure because of its technical simplicity and optimal performance, suitable for fingerprint analysis. The volume of organic solvent and extraction time were optimized. Commercial standard solutions ([6]-gingerol, [8]-gingerol and [10]-gingerol) were analyzed and used to identify their peak position in the chromatogram of ginger profiles. Owing to the responsibility for ginger pungent principles, the contents of these three gingerol compounds were employed to evaluate the extraction conditions with the optimized LC conditions. Five grams of ginger samples were extracted with different volumes of methanol (10–80 mL) and each level was tested three times. The best results were obtained with 40 ml methanol with the similar tendency of three gingerol compounds as seen in Figure 4.2.

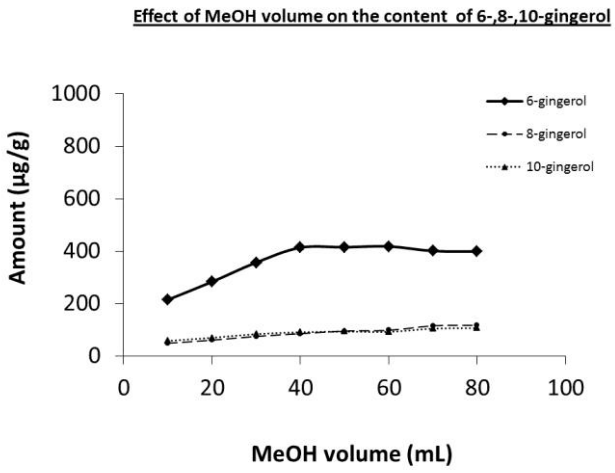


Figure 4.2 Effect of MeOH volume on the content of gingerols

Different extraction times (15, 30, 45, 60 and 75 min) were investigated to observe the dependence of the yield on the duration time. The best results were obtained with 60 min extraction time with the similar tendency of three gingerol compounds as seen in Figure 4.3.

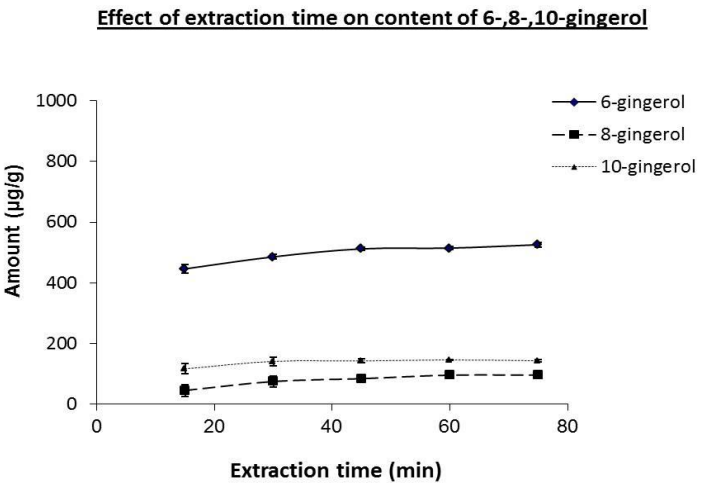


Figure 4.3 Effect of extraction time on the content of gingerols

The optimum pretreatment conditions presented in detail in Topic 3.1.5 allowed the fingerprint analysis of ginger to be simple, fast and suitable with the selected analytical instrument, while demonstrating high extraction efficiency of ginger compositions.

4.1.3 Method validation

The optimized method was validated to ensure the suitability of method in this fingerprint study. Six replicates of the determination were conducted for ginger extracts on the same day and on three consecutive days for intra-day and inter-day precision. Traditionally, RSD value refers to the variation of data, Table 4.1 presented the RSDs of intra-day and inter-day precision by considering the peak area data of nine common peaks. The data peak area is found in Appendix.

Table 4.1 RSD values (%) of intra-day and inter-day precision

	peak1	peak2	peak3	peak4	peak5	peak6	peak7	peak8	peak9
Day 1	0.93	0.41	0.85	2.19	2.25	1.41	0.86	0.76	0.75
Day 2	2.54	1.21	1.13	2.65	3.75	3.12	1.82	0.84	1.48
Day 3	1.41	1.47	1.58	3.00	2.70	2.40	2.06	0.98	1.15
Overall	2.03	1.25	1.40	2.98	2.79	2.73	1.70	1.00	1.74

The RSDs of intra-day and inter-day variability were below 3.00% indicating that the method has high precision. Furthermore, the stability test was conducted because a large sample size is analyzed for fingerprint study. In that case, a single sample solution was extracted, analyzed and stored at room temperature. Then this sample solution has been re-tested after 12hr. The RSD values were shown in Table 4.2.

Table 4.2 Peak area data and RSD values (%) for sample stability test

Duration	peak1	peak2	peak3	peak4	peak5	peak6	peak7	peak8	peak9
0	322.33	186.15	331.41	61.50	87.75	97.08	348.90	390.44	395.19
12	300.62	180.36	315.71	57.52	82.21	89.61	326.26	371.25	386.56
average	311.48	183.25	323.56	59.51	84.98	93.35	337.58	380.84	390.88
RSD	4.93	2.23	3.43	4.73	4.61	5.66	4.74	3.56	1.56

After 12 hr of storage time, the signals of all peaks decreased obviously, but with RSDs lower than 5.66%. These validation results suggested that the method is applicable and the extraction and determination of ginger composition should be performed within the same day.

4.1.4 LC–MS/MS identification and confirmation

To identify the type of chemical compounds from all peaks obtained, it is not practical with only LC-DAD analysis because there are only some commercially available authentic standards. Therefore, MS detector was additionally employed to define the molecular mass of compound which is the characteristic property of each chemical. The ginger profiles were thoroughly recognized and confirmed with and without the reference standards. The positive/negative ion-switching mode in MS analysis allows for the acquisition of MRM transition mass spectra in both ionization modes from a single LC–MS/MS analysis. The MS identification results of nine common peaks in ginger extracts were presented in Table 4.3 and the chemical structure of each compound was illustrated in Figure 4.4. A series of homologous phenolic ketones known as gingerols consisting of [6]-gingerol (peak 1), [8]-gingerol (peak 3), and [10]-gingerol (peak 5), the three major gingerols, were identified by comparing UV spectra, HPLC retention time and mass spectra with the authentic reference standards. In case of other six compounds with no available commercial authentic standards, the tentative identification based on the MS references to literature data [18, 19] were used. Methyl [6]-gingerol, diacetoxy-[6]-gingerdiol, acetoxy-[8]-gingerol, diacetoxy-[8]-gingerdiol, 1-dehydro-[8]-gingerdione and methyl diacetoxy-[8]-gingerdiol were defined as peak 2, 4, 6, 7, 8, and 9, respectively.

Table 4.3 MS/MS identification of ginger chemical components

Common peak no.	RT (min)	Mass data	Mode	MW	Compound
1	5.2	293>193 293>275	negative	294	[6]-gingerol
2	6.8	326>291	positive	308	methyl [6]-gingerol
3	7.1	321>178 321>303	negative	322	[8]-gingerol
4	7.5	321>137 321>261	positive	380	diacetoxy-[6]-gingerdiol
5	11.7	349>178 349>331	negative	350	[10]-gingerol
6	14.7	382>287 382>305	positive	364	acetoxy-[8]-gingerol
7	15.0	426>289 426>349	positive	408	diacetoxy-[8]-gingerdiol
8	16.3	317>134 317>167	negative	318	1-dehydro-[8]-gingerdione
9	16.9	440>177 440>303	positive	422	methyl diacetoxy-[8]-gingerdiol

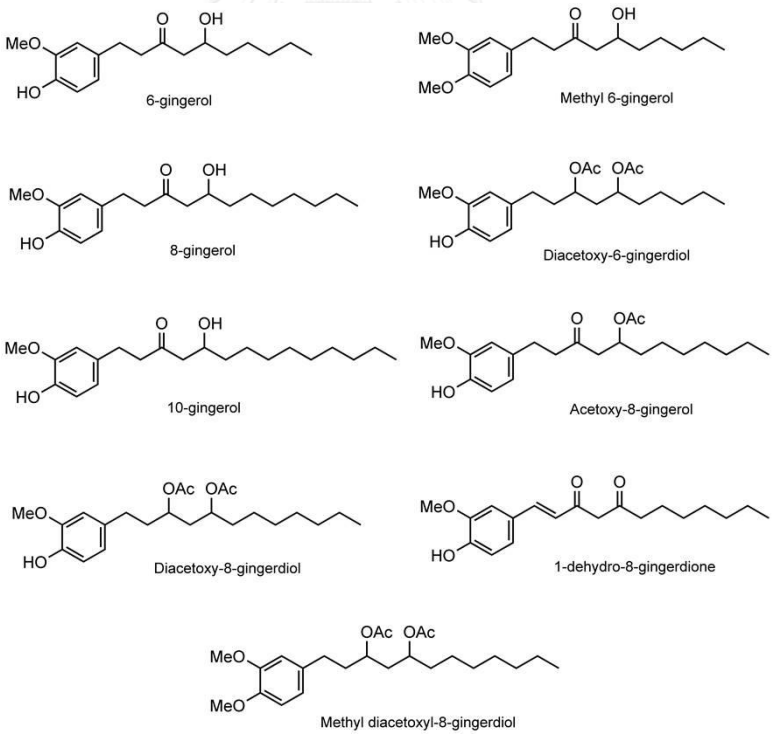


Figure 4.4 Chemical structure of 9 gingerol-related compositions for fingerprint study

4.1.5 Sample analysis

All 152 ginger samples from nine different origins (China (18 samples), India (18 samples), Malaysia (8 samples), Vietnam (18 samples) and Thailand (Chiangmai 18 samples, Chiangrai 18 samples, Leoy 18 samples, Nakonpatom 18 samples, Petchaboon 18 samples)) were prepared and analyzed using LC-DAD. Sufficient numbers of sample should be provided and carefully labelled to represent the authentic profile of sample from each source. The chemical profiles of ginger samples based on chromatographic data from LC-DAD were used to classify the geographical origin for authentication purpose. The optimized sample preparation and chromatographic conditions were applied to all samples. After analysis, the peak area of nine common peaks were integrated and presented in Appendix. This work selected RPA of all data to process with chemometrics, because RPA is usually utilized to establish pattern recognition in herbal fingerprint analysis [27, 110, 111]. Table 4.4 presented the RPA of nine characteristic peaks of ginger samples from nine different origins.

Table 4.4 The relative peak area (RPA) of characteristic peaks of ginger samples from different origins

Country of origin	Sample no.	Relative peak area								
		Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9
China	1	1.268	0.397	1.092	0.263	0.302	0.366	0.877	1.000	2.222
	2	1.212	0.471	0.817	0.230	0.416	0.366	1.681	1.000	2.320
	3	1.346	0.536	0.996	0.211	0.291	0.332	0.872	1.000	1.752
	4	1.756	0.489	1.179	0.190	0.220	0.395	0.528	1.000	2.055
	5	1.496	0.359	0.782	0.209	0.297	0.328	0.668	1.000	1.963
	6	1.827	0.300	0.756	0.228	0.291	0.268	0.548	1.000	1.901
	7	1.308	0.488	0.851	0.216	0.338	0.365	1.085	1.000	1.894
	8	1.339	0.533	1.014	0.224	0.299	0.349	0.873	1.000	1.842
	9	2.147	0.403	0.701	0.265	0.416	0.454	0.703	1.000	2.151
	10	1.560	0.399	0.650	0.242	0.422	0.395	1.212	1.000	2.292
	11	2.070	0.419	0.651	0.362	0.257	0.455	0.415	1.000	1.928
	12	1.739	0.397	0.653	0.210	0.303	0.411	0.540	1.000	1.671
	13	1.856	0.689	1.200	0.499	0.224	0.431	0.685	1.000	1.967
	14	1.388	0.594	0.996	0.221	0.357	0.381	1.444	1.000	2.302
	15	1.398	0.786	1.228	0.390	0.225	0.452	0.601	1.000	1.684
	16	1.256	0.687	1.030	0.185	0.282	0.376	1.237	1.000	2.057
	17	1.029	0.781	1.212	0.177	0.296	0.307	1.225	1.000	1.694
	18	1.624	0.721	1.168	0.206	0.333	0.411	0.914	1.000	2.220
India	19	0.598	0.423	1.632	0.037	0.102	0.134	0.429	1.000	0.528
	20	0.679	0.361	1.458	0.062	0.138	0.114	0.411	1.000	0.518
	21	0.572	0.433	1.301	0.041	0.122	0.100	0.405	1.000	0.508
	22	0.624	0.370	1.175	0.051	0.132	0.110	0.381	1.000	0.491
	23	0.644	0.531	1.494	0.051	0.118	0.134	0.476	1.000	0.536
	24	0.688	0.593	1.144	0.034	0.147	0.153	0.536	1.000	0.592
	25	0.944	0.476	1.634	0.030	0.174	0.187	0.414	1.000	0.610
	26	0.646	0.423	1.305	0.034	0.146	0.149	0.335	1.000	0.490
	27	0.818	0.387	1.443	0.096	0.164	0.303	0.354	1.000	0.695
	28	0.722	0.475	1.458	0.093	0.133	0.291	0.505	1.000	0.678
	29	0.676	0.408	0.995	0.083	0.108	0.215	0.358	1.000	0.561
	30	0.518	0.409	1.134	0.085	0.107	0.173	0.414	1.000	0.483
	31	0.731	0.599	1.296	0.094	0.111	0.234	0.469	1.000	0.610
	32	0.534	0.546	1.506	0.111	0.140	0.235	0.448	1.000	0.491
	33	0.654	0.585	1.222	0.092	0.098	0.224	0.325	1.000	0.552
	34	0.648	0.337	1.480	0.075	0.105	0.241	0.373	1.000	0.655
	35	0.628	0.742	1.307	0.113	0.143	0.246	0.610	1.000	0.534
	36	0.518	0.532	1.045	0.079	0.098	0.199	0.400	1.000	0.532
Malaysia	37	0.668	0.456	0.841	0.034	0.084	0.140	0.281	1.000	0.478
	38	0.592	0.464	0.892	0.044	0.075	0.136	0.277	1.000	0.477

	39	0.523	0.343	0.969	0.068	0.095	0.192	0.318	1.000	0.616
	40	0.610	0.362	0.625	0.058	0.083	0.194	0.313	1.000	0.665
	41	0.465	0.348	0.606	0.071	0.070	0.148	0.247	1.000	0.421
	42	0.530	0.411	0.739	0.053	0.065	0.183	0.321	1.000	0.592
	43	0.494	0.245	1.024	0.074	0.073	0.186	0.331	1.000	0.486
	44	0.473	0.323	0.687	0.073	0.075	0.151	0.324	1.000	0.422
Vietnam	45	0.826	0.477	0.849	0.158	0.225	0.253	0.894	1.000	1.012
	46	0.689	0.409	0.713	0.110	0.184	0.239	1.001	1.000	0.939
	47	0.985	0.502	0.889	0.092	0.109	0.301	0.522	1.000	1.144
	48	0.769	0.345	0.591	0.134	0.158	0.202	0.570	1.000	1.044
	49	1.017	0.649	1.363	0.128	0.109	0.354	0.636	1.000	1.300
	50	0.684	0.488	0.818	0.105	0.127	0.232	0.582	1.000	0.978
	51	0.891	0.484	0.828	0.120	0.163	0.263	0.700	1.000	1.234
	52	0.987	0.628	1.128	0.118	0.112	0.339	0.584	1.000	1.232
	53	1.068	0.386	0.595	0.093	0.118	0.209	0.490	1.000	1.359
	54	0.825	0.325	0.951	0.131	0.105	0.268	0.303	1.000	0.697
	55	0.947	0.456	0.820	0.149	0.143	0.348	0.292	1.000	1.011
	56	0.893	0.331	0.622	0.146	0.171	0.287	0.274	1.000	0.872
	57	0.974	0.353	0.637	0.110	0.127	0.224	0.527	1.000	1.304
	58	0.957	0.429	0.748	0.190	0.272	0.207	0.611	1.000	1.434
	59	0.692	0.410	0.675	0.149	0.123	0.271	0.281	1.000	0.758
	60	1.049	0.400	0.676	0.139	0.153	0.270	0.262	1.000	0.873
	61	1.185	0.318	0.810	0.171	0.315	0.397	0.490	1.000	1.580
	62	0.889	0.423	0.635	0.096	0.114	0.217	0.440	1.000	1.127
Chiangmai	63	0.919	0.552	1.197	0.079	0.146	0.132	0.381	1.000	0.669
	64	1.416	0.382	1.472	0.154	0.154	0.158	0.365	1.000	1.145
	65	0.813	0.462	1.475	0.024	0.200	0.122	0.600	1.000	0.694
	66	1.127	0.431	1.961	0.075	0.129	0.149	0.326	1.000	0.778
	67	1.070	0.743	1.574	0.096	0.125	0.131	0.462	1.000	0.866
	68	1.378	0.574	1.983	0.063	0.191	0.129	0.452	1.000	0.887
	69	1.511	0.712	2.007	0.092	0.189	0.171	0.403	1.000	0.869
	70	0.870	0.336	1.802	0.046	0.135	0.136	0.433	1.000	0.709
	71	1.463	0.544	2.050	0.070	0.238	0.175	0.469	1.000	1.071
	72	1.154	0.635	1.427	0.042	0.241	0.157	0.592	1.000	1.021
	73	1.291	0.821	1.680	0.056	0.262	0.153	0.558	1.000	0.990
	74	1.179	0.704	1.776	0.104	0.152	0.164	0.507	1.000	0.888
	75	0.972	0.592	1.387	0.113	0.134	0.129	0.473	1.000	0.558
	76	1.291	0.685	1.490	0.039	0.332	0.160	0.616	1.000	0.995
	77	0.919	0.535	1.251	0.036	0.188	0.141	0.684	1.000	0.903
	78	0.938	0.549	1.343	0.106	0.138	0.133	0.385	1.000	0.528
	79	0.942	0.565	1.339	0.046	0.201	0.145	0.579	1.000	0.942
	80	0.983	0.505	2.037	0.096	0.171	0.139	0.514	1.000	0.756
Chiangrai	81	0.705	0.692	1.163	0.021	0.073	0.129	0.312	1.000	0.604
	82	0.959	1.015	1.678	0.040	0.057	0.194	0.469	1.000	0.838

	83	0.805	0.839	1.411	0.026	0.073	0.157	0.365	1.000	0.646
	84	1.256	0.784	1.386	0.039	0.102	0.170	0.306	1.000	0.856
	85	0.775	0.621	1.077	0.020	0.066	0.122	0.293	1.000	0.626
	86	0.974	0.932	1.594	0.030	0.084	0.204	0.465	1.000	0.876
	87	0.976	1.021	1.722	0.048	0.072	0.197	0.427	1.000	0.774
	88	1.005	0.811	1.389	0.029	0.062	0.163	0.419	1.000	0.669
	89	1.110	0.915	1.525	0.057	0.051	0.194	0.430	1.000	0.819
	90	0.896	0.901	1.531	0.025	0.059	0.197	0.415	1.000	0.805
	91	1.061	0.860	1.435	0.030	0.078	0.140	0.400	1.000	0.873
	92	0.794	0.749	1.302	0.016	0.069	0.140	0.291	1.000	0.603
	93	0.779	0.773	1.325	0.020	0.059	0.144	0.305	1.000	0.643
	94	0.919	0.787	1.336	0.034	0.054	0.148	0.306	1.000	0.683
	95	1.022	0.737	1.255	0.026	0.067	0.144	0.291	1.000	0.695
	96	0.742	0.646	1.118	0.028	0.070	0.131	0.352	1.000	0.617
	97	0.743	0.692	1.204	0.025	0.049	0.140	0.282	1.000	0.576
	98	0.957	0.706	1.227	0.037	0.052	0.157	0.283	1.000	0.656
Leoy	99	1.249	0.450	1.636	0.086	0.197	0.081	0.410	1.000	0.975
	100	1.306	0.710	1.226	0.080	0.139	0.079	0.367	1.000	0.754
	101	1.111	0.489	0.926	0.072	0.166	0.073	0.345	1.000	0.595
	102	1.165	0.450	1.030	0.107	0.173	0.170	0.395	1.000	0.849
	103	1.054	0.516	1.058	0.059	0.131	0.115	0.401	1.000	0.718
	104	1.243	0.730	1.332	0.122	0.139	0.082	0.404	1.000	0.969
	105	1.603	0.808	1.510	0.099	0.288	0.089	0.427	1.000	0.524
	106	1.348	0.581	1.208	0.089	0.201	0.078	0.358	1.000	0.824
	107	1.216	0.720	1.303	0.093	0.178	0.074	0.342	1.000	0.742
	108	1.279	0.728	1.299	0.061	0.203	0.089	0.412	1.000	0.724
	109	1.221	0.719	1.384	0.079	0.205	0.159	0.391	1.000	0.767
	110	1.071	0.512	1.153	0.109	0.183	0.190	0.402	1.000	0.828
	111	0.918	0.423	0.671	0.052	0.172	0.221	0.406	1.000	0.629
	112	0.988	0.493	0.759	0.119	0.205	0.216	0.496	1.000	0.694
	113	0.880	0.466	0.668	0.121	0.200	0.191	0.410	1.000	0.578
	114	0.755	0.634	0.943	0.086	0.137	0.201	0.344	1.000	0.661
	115	0.981	0.604	0.870	0.093	0.158	0.217	0.383	1.000	0.780
	116	0.979	0.563	0.873	0.148	0.170	0.218	0.369	1.000	0.663
Nakonpatom	117	0.534	0.487	0.950	0.049	0.104	0.241	0.378	1.000	0.474
	118	0.492	0.402	0.704	0.061	0.055	0.491	0.462	1.000	0.582
	119	0.397	0.475	0.901	0.061	0.068	0.207	0.351	1.000	0.423
	120	0.567	0.385	0.823	0.088	0.111	0.175	0.408	1.000	0.586
	121	0.456	0.320	0.576	0.070	0.094	0.167	0.329	1.000	0.518
	122	0.431	0.496	0.819	0.068	0.093	0.202	0.486	1.000	0.513
	123	0.524	0.568	0.873	0.070	0.099	0.205	0.454	1.000	0.474
	124	0.617	0.431	0.637	0.067	0.090	0.218	0.440	1.000	0.611
	125	0.607	0.454	1.021	0.104	0.115	0.237	0.431	1.000	0.619
	126	0.474	0.447	0.772	0.057	0.096	0.179	0.275	1.000	0.473

	127	0.488	0.393	0.706	0.078	0.101	0.199	0.550	1.000	0.552
	128	0.491	0.458	0.699	0.069	0.092	0.190	0.412	1.000	0.504
	129	0.390	0.380	0.561	0.056	0.079	0.168	0.293	1.000	0.357
	130	0.646	0.548	0.821	0.071	0.104	0.230	0.534	1.000	0.662
	131	0.659	0.552	0.821	0.075	0.116	0.209	0.517	1.000	0.570
	132	0.642	0.514	0.757	0.082	0.104	0.232	0.347	1.000	0.636
	133	0.453	0.471	0.708	0.085	0.091	0.184	0.376	1.000	0.381
	134	0.468	0.475	0.714	0.082	0.086	0.185	0.467	1.000	0.400
Petchaboon	135	0.491	0.326	0.599	0.057	0.101	0.182	0.489	1.000	0.582
	136	0.507	0.390	0.618	0.059	0.098	0.248	0.493	1.000	0.600
	137	0.526	0.268	0.430	0.061	0.093	0.155	0.364	1.000	0.471
	138	0.536	0.367	0.843	0.079	0.102	0.193	0.389	1.000	0.457
	139	0.560	0.435	0.657	0.065	0.097	0.182	0.373	1.000	0.448
	140	0.719	0.471	0.764	0.071	0.100	0.195	0.481	1.000	0.519
	141	0.622	0.453	0.701	0.073	0.109	0.194	0.492	1.000	0.597
	142	0.572	0.462	0.737	0.072	0.113	0.220	0.481	1.000	0.583
	143	0.529	0.488	0.738	0.081	0.117	0.228	0.629	1.000	0.444
	144	0.520	0.431	0.669	0.055	0.084	0.181	0.409	1.000	0.526
	145	0.712	0.410	0.654	0.085	0.100	0.226	0.477	1.000	0.667
	146	0.826	0.398	0.682	0.070	0.116	0.229	0.377	1.000	0.712
	147	0.525	0.397	0.680	0.034	0.129	0.113	0.403	1.000	0.494
	148	0.539	0.461	0.759	0.070	0.102	0.166	0.408	1.000	0.489
	149	0.656	0.429	0.659	0.080	0.120	0.199	0.504	1.000	0.644
	150	0.670	0.491	0.749	0.073	0.125	0.158	0.362	1.000	0.543
	151	0.596	0.473	0.706	0.057	0.123	0.171	0.410	1.000	0.563
	152	0.462	0.356	0.558	0.102	0.121	0.135	0.432	1.000	0.517

4.1.6 Chemometric methods

To monitor the overall data from nine sources, the fingerprint study of the ginger samples were divided based on the scale of production area into two categories; city (Chiangmai, Chiangrai, Leoy, Nakonpatom, and Petchaboon) and country (China, India, Malaysia, and Vietnam).

4.1.6.1 Similarity analysis

For preliminary assessment of ginger profiles from different cities and countries, the mean chromatogram of samples was calculated and used as representative object from each origin. In this similarity analysis, the values of RPA were employed to compute the correlation coefficient of ginger profiles from different sources. The calculation results of ginger data from various cities and countries were presented in Table 4.5 and Table 4.6, respectively.

Table 4.5 Similarity comparison of HPLC fingerprint of ginger from five cities

	Chiangmai	Chiangrai	Leoy	Nakonpatom	Petchaboon
Chiangmai	1.000	0.962	0.955	0.834	0.815
Chiangrai		1.000	0.948	0.886	0.853
Leoy			1.000	0.866	0.879
Nakonpatom				1.000	0.988
Petchaboon					1.000

The correlation coefficient values of gingers from five cities were in a narrow range of 0.815–0.988, indicating that gingers in Thailand tend to have quite similar profiles. In order to define the internal qualities of the samples, the relationship of profiles from each origin is monitored. The higher correlation coefficient value refers to the closer relationship of origins. Due to a highest correlation coefficient value (0.988), it could be indicated that gingers from Nakonpatom were closer to Petchaboon gingers than the other three cities. Also, ginger samples from Chiangmai were closely related to samples of Chiangrai and Leoy with high value of correlation coefficient (0.962 and 0.955). Interestingly, the closeness of some data seemed to be in accordance with the closeness of geographical location of each city, when using a representative profile. For more information, other statistical methods should be performed.

Table 4.6 Similarity comparison of HPLC fingerprint of ginger from five countries

	China	India	Malaysia	Thailand	Vietnam
China	1.000	0.472	0.560	0.693	0.908
India		1.000	0.930	0.893	0.745
Malaysia			1.000	0.898	0.852
Thailand				1.000	0.870
Vietnam					1.000

With the same consideration used in gingers from five cities, the correlation coefficients were calculated for five ginger-producing countries and the values ranged from 0.483–0.964. The ginger samples from India and Malaysia were found to closely relate as seen from the highest correlation coefficient value (0.964). In contrast, Indian ginger was clearly differentiated from Chinese ginger, based on the low correlation coefficient (0.483). When compared to ginger profiles of five cities, the obtained results were in wider range of similarity analysis. This could be implied that it was possible to basically discriminate the ginger samples from each country based on their greater dissimilarity.

4.1.6.2 Hierarchical cluster analysis (HCA)

Although the correlation coefficient from similarity analysis can roughly represent the relationship of gingers from each origin, there is no information about clusters or groups of related samples by their origin. HCA is a technique applied for determination of association degree of data. This work selected the RPAs of random three samples as a representative actual data from each source to avoid the complication from large numbers of samples. The HCA results were presented as dendrogram in form of pair-wise distances among the same and different origins. Figure 4.5 and 4.6 illustrated the clusters of samples from different cities and countries, respectively.

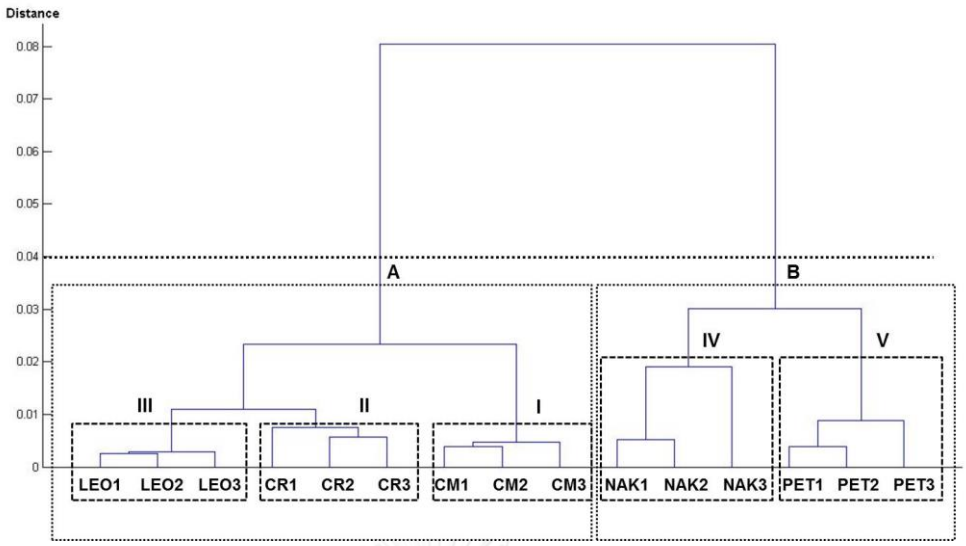


Figure 4.5 Dendrogram of HCA of ginger samples from 5 cities. Three representative ginger samples were used for each origin:
CM= Chiangmai (Cluster I); CR= Chiangrai (Cluster II); LEO= Leoy (Cluster III); NAK= Nakonpatom (Cluster IV); and PET= Petchaboon (Cluster V)

Five main clusters were observed in the dendrogram by a plot of distance (y-axis) and the selected representative samples (x-axis). The profiles of gingers from the same origin were correctly grouped as clusters. At a distance threshold of 0.04, two large clusters (cluster A and B) were clearly separated. Cluster A contained samples from Chiangmai, Chiangrai and Leoy, reflects a higher degree of sample association from these three cities. On the other hand, ginger profiles of Nakonpatom and Petchaboon formed cluster B, implies that the quality of gingers from these two cities are quite similar. From the illustrated dendrogram, a single distance value could not be used to discriminate five clusters of gingers from five cities because the small scale of production area produced the high similarity of profiles.

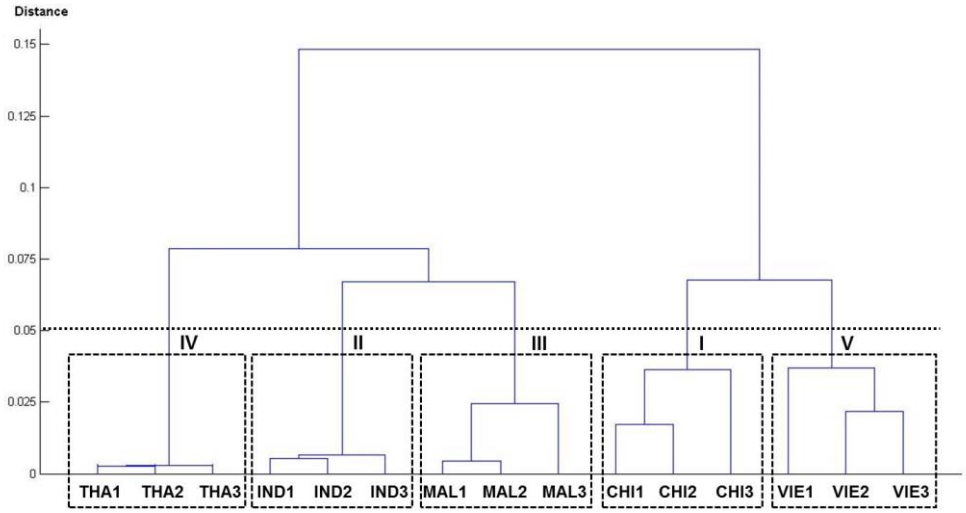


Figure 4.6 Dendrogram of HCA of ginger samples from 5 countries. Three representative ginger samples were used for each origin
CHI= China (Cluster I); IND= India (Cluster II); MAL = Malaysia (Cluster III); THA = Thailand (Cluster IV); and VIE = Vietnam (Cluster V)

From dendrogram, five clusters were grouped on the basis of ginger-producing country. Ginger samples from identical origins are correctly clustered, while the ginger profiles from different five areas are greatly separated at a single distance threshold of 0.05. The closeness of cluster II and cluster III implies that the profiles of Indian ginger are more similar to Malaysian ginger than other countries. Furthermore, gingers from China and Vietnam are closely related, as indicated by the adjacent location of clusters IV and V. Based on cluster distances, Thai gingers (cluster I) are quite similar to gingers from India and Malaysia. The application of the HCA method could be used to support the results from similarity analysis for gingers of both categories.

4.1.6.3 Principal component analysis (PCA)

PCA is a typical exploratory analysis to monitor the outline of all data in multivariate analyses. The application of PCA in this work is to explore the overall profiles of gingers from each origin. PCA was computed by using every obtained

chromatographic profile. PCA works on the principle of dimension reduction by replacing a large number of variables with a new, smaller number of variables, namely principal components (PCs). This work used RPA values (90 samples from five cities and 80 samples from five countries) as input data for PCA calculation. The results from PCA were shown in Figure 4.7 and 4.8 for the ginger profiles from different cities and countries, respectively.

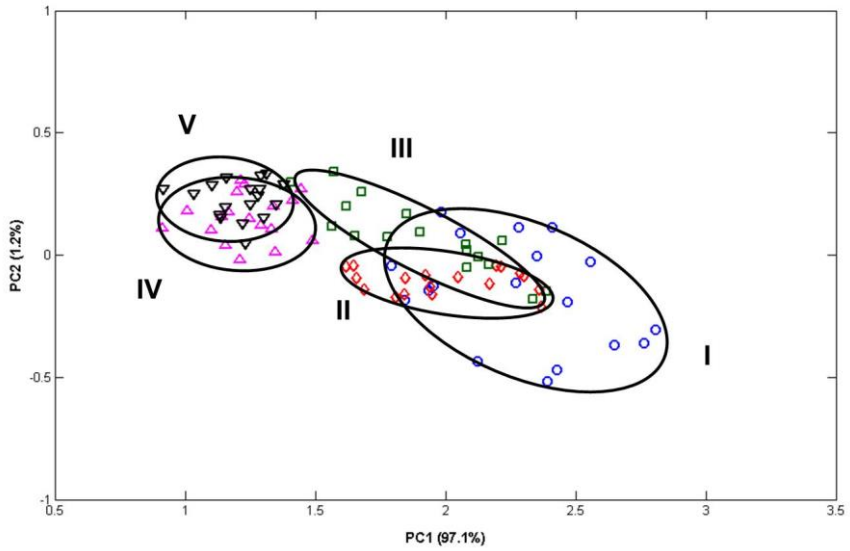


Figure 4.7 PCA score plot of the 90 ginger samples from five cities with 8 variables (RPAs).

Five groups (Groups I–V) were formed according to geographical origin: I = Chiangmai; II = Chiangrai; III = Leoy; IV = Nakonpatom; and V = Petchaboon

In PCA methodology, the variations of ginger profiles from five origins produced from the difference in their HPLC fingerprints. The visualization and differentiation were conveniently monitored in the PCA score plot of PC1 (x-axis) and PC2 (y-axis). From Figure 4.7, the two PCs (PC1 and PC2) provided the highest variation of data objects (97.1% and 1.2% of the variation). Owing to ginger origins in small scale of production area, five groups from different cities were not significantly separated. Only two large groups were assigned for ginger profiles from five cities. The profiles of gingers from Chiangmai, Chiangrai, and Leoy (group I, II,

and III were almost at the same position in score plot. Therefore, it could be assumed that the variations of gingers from these three cities were quite similar. In the same way, the profiles of samples from Nakonpatom (group IV) were close to and Petchaboon samples (group V). This classification is in agreement with the results from cluster analysis.

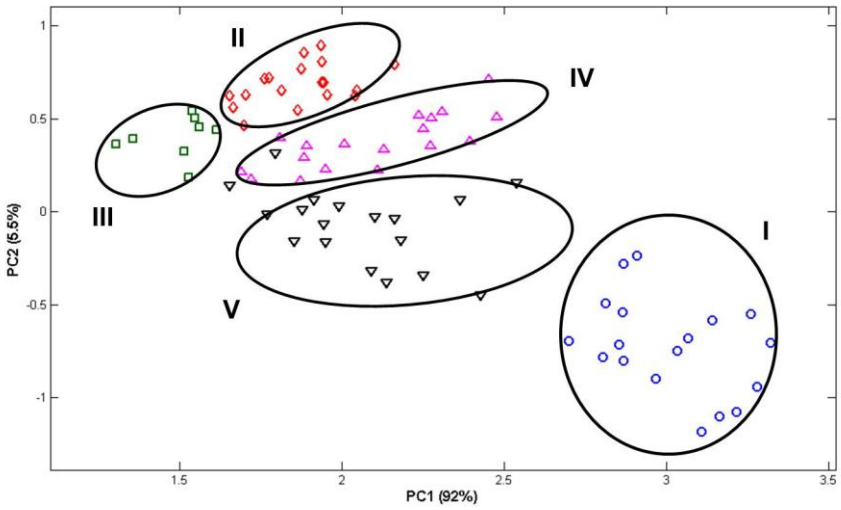


Figure 4.8 PCA score plot of the 80 ginger samples from five countries with 8 variables (RPAs).
Five groups (Groups I–V) were formed according to geographical origin: I = China; II = India; III = Malaysia; IV = Thailand (Leoy); and V = Vietnam

From Figure 4.8, the two PCs (PC1 and PC2) which referred to 92% and 5.5% of the variation provided the highest variation of data objects. The classification of the five groups based on their RPA profile clearly shows that the discrimination of gingers from five producing countries. In PCA score plot, Thai gingers (group IV) were found next to ginger groups of India (group II) and Malaysia (group III), while the ginger samples from Vietnam (group V) are close to Chinese ginger (group I). The obtained PCA differentiation is in good agreement with the relationship of each country derived from similarity analysis and HCA. The superior advantage of PCA over the other two techniques is that every sample data is utilized in visualization for

the variation of chemical profiles among the same and different source. This work selected three exploratory analyses to guarantee the quality evaluation of the ginger fingerprints.

4.1.6.4 Linear discriminant analysis (LDA)

Since the use of various unsupervised pattern recognition techniques did not provide any information about the prediction of the origin for an unknown sample, the application of LDA as supervised pattern recognition was investigated. This method helps to implement the comprehensive establishment of ginger fingerprints from different regions by creating a mathematical model for classification of ginger samples according to their origins. Linear discriminant function is applied to assign a linear classifier or boundary on the data set and offer the benefit of allocating each sample, including an unknown sample, to its determined class. The LDA classification results of all sample based on their RPA profiles from those five groups are shown in Table 4.7 and 4.8.

Table 4.7 Classification results of ginger samples from different cities using LDA method

	Predicted group memberships					
Original data set	Chiangmai	Chiangrai	Leoy	Nakonpatom	Petchaboon	Correct classification (%)
Chiangmai	16	0	2	0	0	88.9
Chiangrai	0	18	0	0	0	100.0
Leoy	1	0	15	1	1	83.3
Nakonpatom	0	0	0	11	7	61.1
Petchaboon	0	0	0	2	16	88.9
Recognition ability (%)						84.4
Cross-validation	Chiangmai	Chiangrai	Leoy	Nakonpatom	Petchaboon	Correct classification (%)
Chiangmai	14	0	4	0	0	78
Chiangrai	0	18	0	0	0	100
Leoy	1	0	15	1	1	83
Nakonpatom	0	0	0	11	7	61
Petchaboon	0	0	0	3	15	83
Prediction ability (%)						81.1

When calculated recognition ability (% of the objects belonging to the training set correctly classified) on LDA method, 84.4% of the original data set was correctly classified by the discriminant functions. This means that the achieved linear classifier cannot completely separate gingers from five cities, which is in correspondence with the results from unsupervised pattern recognition techniques (similarity analysis, HCA and PCA). Few misclassifications of ginger origins were found due to the closeness of overall data from five cities. For example, two gingers from Chiangmai were incorrectly assigned as Leoy and two Leoy gingers were labelled as samples from Nakonpatom and Petchaboon. Among all profiles from five cities, the highest number of false classification was found for sample from Nakonpatom, in which seven gingers was incorrectly assigned as Petchaboon gingers. This could be assumed that the fingerprint of gingers from Nakonpatom and Petchaboon were quite similar.

To evaluate the predictive capacity, the generated model was then validated using the leave-one-out cross-validation method (LOOCV). The predictive ability of this model (% of the objects belonging to the testing set correctly classified using the developed model) was 81.1%. This value defines a good performance of this model in classification of gingers from five cities. A high predictive percentage of the validated set indicates the efficiency of model to classify the origin of an unknown sample based on the relative profiles of the eight common compounds. The value of predictive ability is normally lower than the recognition ability percentage because of the validation process of LOOCV. Before obtaining the predictive ability value, this method removes one chromatogram at a time, to be used as a test set, while the remaining chromatograms are formed as a training set. In contrast, all data were used as a training set and a test set to create the model and then yield the recognition ability percentage.

Table 4.8 Classification results of ginger samples from different countries using LDA method

	Predicted group memberships					
Original data set	China	India	Malaysia	Thailand	Vietnam	Correct classification (%)
China	18	0	0	0	0	100
India	0	17	1	0	0	94
Malaysia	0	0	8	0	0	100
Thailand	0	0	0	18	0	100
Vietnam	2	0	0	0	16	89
Recognition ability (%)						96.7
Cross-validation	China	India	Malaysia	Thailand	Vietnam	Correct classification (%)
China	18	0	0	0	0	100
India	0	15	2	0	1	83
Malaysia	0	1	7	0	0	88
Thailand	0	0	0	18	0	100
Vietnam	3	0	0	0	15	83
Prediction ability (%)						90.8

Considering the LDA application for classification, the constructed model from ginger profiles of five different countries produced 96.7% of recognition ability. A high percentage of correct classification refers to the efficiency of model in discrimination of origin. Only one sample from India was incorrectly assigned as Malaysia and two samples from Vietnam were incorrectly assigned as China. This classification pattern for gingers from different countries is also in good agreement with the results of the three exploratory analyses (similarity analysis, HCA and PCA). The gingers from China and Vietnam were more closely related, while the samples from India and Malaysia were more similar to each other.

The results of model validation using LOOCV presented 90.8% of model predictive ability. This high percentage value proved that the established model has a satisfactory performance for country prediction of an unknown ginger. The uses of both unsupervised and supervised pattern recognition methods accomplish the origin labelling study of ginger from different cities and countries by using the chromatographic profile.

4.1.6.5 Marker discovery

For identification of origin, T-statistics method was employed for marker selection from ginger profiles. The t-value is calculated from the mean and standard deviation of two populations using the ‘‘one vs. all’’ criterion [112]. The RPA data of eight compounds from the selected country was assigned as first population and the remaining data from the other countries as second population. Critical t-value at 95% confidence level (P = 0.05) was applied to decide whether the characteristic profiles of ginger could possibly be used as markers for indicating the origin. When the absolute value of calculated t is higher than the critical t-value, this refers to the significant difference of two selected populations. In this study, T- test was applied in the marker discovery for all profiles of ginger from different cities and countries. The results of selection were presented in Figure 4.9 and 4.10 for city and country marker, respectively.

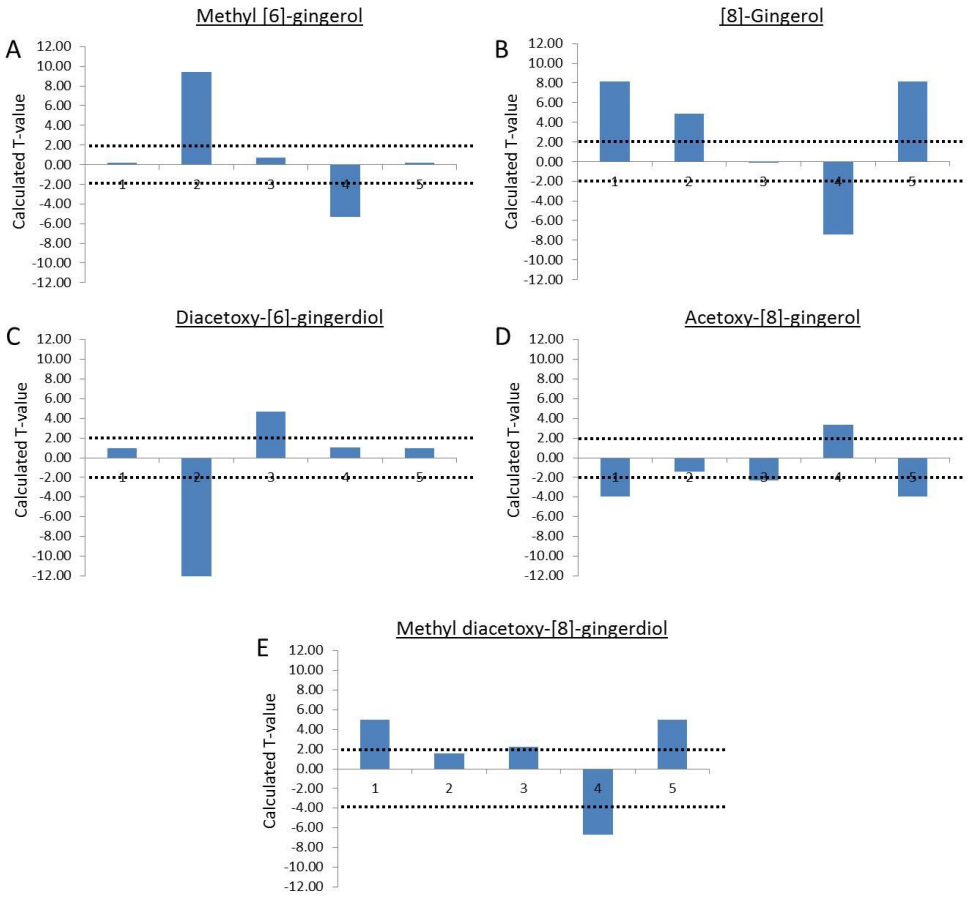


Figure 4.9 T-statistics of (A) methyl [6]-gingerol, (B) [8]-gingerol, (C) diacetoxy-[6]-gingerdiol, (D) acetoxy-[8]-gingerol and (E) methyl diacetoxy-[8]-gingerdiol in ginger from 5 cities
(1=Chiangmai, 2=Chiangrai, 3=Leoy, 4=Nakonpatom, 5=Petchaboon).
Dashed line represents the critical t-value.

Among the eight characteristic compounds, methyl [6]-gingerol (Figure 4.9A), [8]-gingerol (Figure 4.9B) and methyl diacetoxy-[8]-gingerdiol (Figure 4.9E) can be individually used as the low range marker of ginger from Nakonpatom. This is because their calculated t-values exceeded the critical t-value (dashed line) and possessed excessively negative sign, when compared to the t-value of other cities. With the same criteria, acetoxy-[8]-gingerol (Figure 4.9D) is a high range marker to discriminate the gingers of Nakonpatom from the other countries because of its excessively positive sign. To discriminate Chiangrai gingers from those of other

cities, methyl [6]-gingerol (Figure 4.9A) and diacetoxy-[6]-gingerdiol (Figure 4.9C) can be specifically used as a marker, as observed from their excessively positive and negative sign, respectively. Moreover, diacetoxy-[6]-gingerdiol (Figure 4.9C) is a high range marker for Leoy gingers according to the significant difference in positive sign of the t-values. However, there is no apparent single chemical marker for gingers from Chiangmai and Petchaboon was obtained from t-tests of the eight compounds.

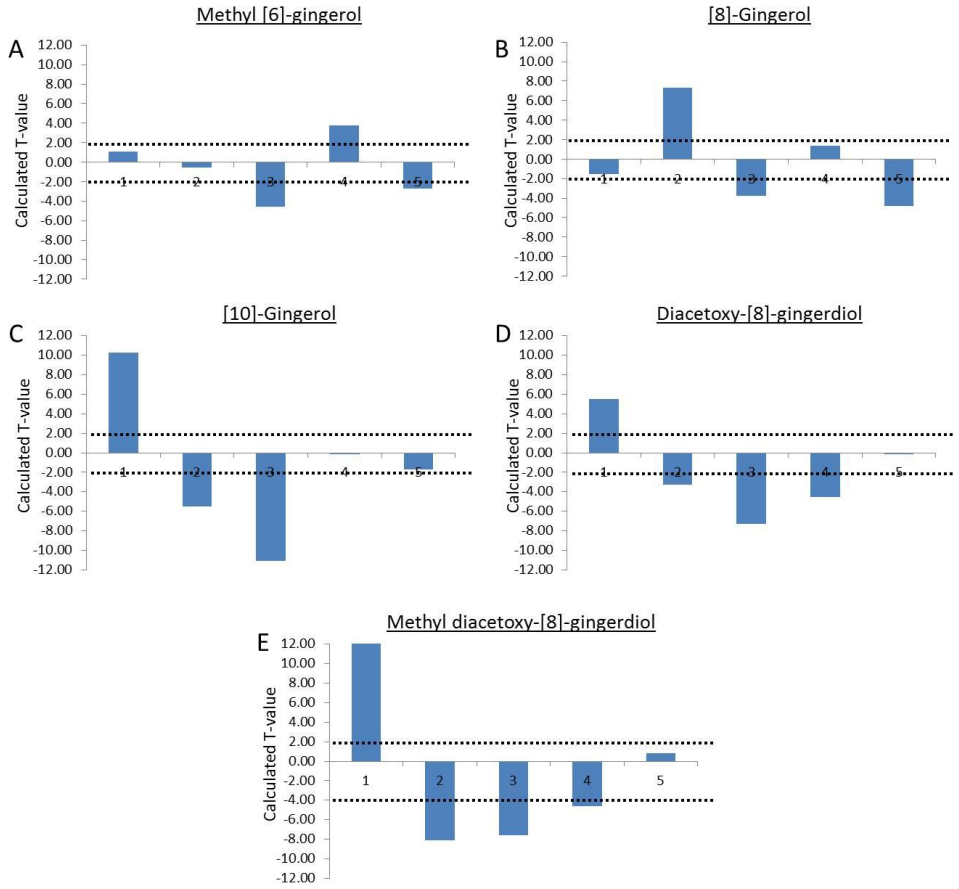


Figure 4.10 T-statistics of (A) methyl [6]-gingerol, (B) [8]-gingerol, (C) [10]-gingerol, (D) diacetoxy-[8]-gingerdiol and (E) methyl diacetoxy-[8]-gingerdiol in ginger from five countries of origin
(1 = China, 2 = India, 3 = Malaysia, 4 = Thailand, 5 = Vietnam).
Dashed line represents the critical t-value.

Among the eight fingerprint compounds, only high range markers were found for the origin labelling of gingers from five countries. Methyl [6]-gingerol (Figure 4.10A) is assigned as marker to discriminate the gingers of Thailand from the other countries since its calculated t-value exceeded the critical t-value and possessed excessively positive sign. In the same way, [8]-gingerol (Figure 4.10B) can be specifically used to discriminate Indian gingers from those of other countries owing to its calculated t-value over the critical t-level (dashed line) in a positive way. For the other three compounds ([10]-gingerol, diacetoxy-[8]-gingerdiol and methyl diacetoxy-[8]-gingerdiol), the sign and the calculated t-value in Figure 4.10C-E indicated that they can be individually used as a marker for discriminate ginger from China. No significant marker was found for gingers from Malaysia and Vietnam, thus the combination of more than one compound might be required. Additionally, all the calculated T-value data of gingers from different cities and countries were presented in Appendix.

4.2 Food quality: Molecular taste profile in beef

Chemical analysis of the major components in meat reflects to its quality of meat. Variations that can occur in the nature of these components are responsible for the sensory difference that shows in the flavor, tenderness, color, or nutritional quality of the meat. Besides main nutrients, there are some characteristic compounds jointly contributing to the specific taste of food. Metabolomics approach for food analysis can be classified into the targeted and non-targeted methodologies [113]. While targeted analysis aims to detect and accurately quantify a specific, pre-determined set of known compounds, non-targeted analysis focuses on the detection of chemical components in food as much as possible without specific identification in order to observe the pattern of food composition.

4.2.1 Targeted analysis

For targeted analysis of food flavor components, groups of known compounds which respond to the basic taste of food (bitter, sweet, sour, salty and umami) are commonly determined for their concentration. In this work, the dry-aged beefs with normal treatment and with noble mold treatment (LUMA) in different cuts (rib eye, short loin, tender loin, and sirloin) were studied to find the differences in taste profile of LUMA beef. The contents of the basic taste compounds (amino acids, fatty acid, organic acids, cations and anions) related to the ordinary taste of beef were examined. The LC-MS/MS technique was chosen to define the amount of amino acids, fatty acids, and organic acids. In addition, various cations and anions were determined by IC.

4.2.1.1 Quantitation of amino acids by LC-MS/MS

The largest constituent of muscle is protein. Muscle proteins are involved in maintaining the structure and organization of the muscle and muscle cells. Amino acids are the main components of the muscle protein structure. The amino acid

composition in meat influences the protein quality, meat taste and also indirectly effect aroma by interacting with other compounds. Among five basic tastes, amino acid takes a significant role as the contributor of sweet, bitter and umami tastes in meat [42]. Each amino acid relates to the different degrees of taste. This work analyzed twenty amino acid compounds of which eighteen amino acids are common nutrients and the other two compounds (carnosine and anserine) was included because they have been previously reported as a substantial amino acid contributing to the specific taste in meat [42]. The quantitative results of these components in two different treatments for four beef parts were presented in Table 4.9.

Table 4.9 Quantitative data (mmol/kg) of amino acids in beef cuts with normal and LUMA treatment

Compounds	Rib eye		Short loin		Tenderloin		Sirloin	
	Normal	LUMA	Normal	LUMA	Normal	LUMA	Normal	LUMA
Alanine	50.71	55.37	23.52	56.51	69.96	60.36	37.65	57.73
Arginine	9.29	12.73	3.23	6.72	4.99	8.66	6.92	14.16
Asparagine	10.22	6.17	2.52	10.47	7.01	7.88	5.47	10.96
Aspartic acid	2.34	51.04	0.23	17.07	2.11	1.20	0.47	31.65
Glutamine	57.15	33.17	31.82	11.12	77.08	85.39	66.20	35.27
Glutamic acid	46.59	79.76	12.34	64.09	42.07	50.39	24.59	54.01
Glycine	35.70	39.58	16.43	71.22	23.43	27.94	33.08	40.32
Histidine	80.28	76.36	105.59	79.52	30.95	30.13	64.57	109.84
Isoleucine	20.50	29.28	5.86	35.76	11.85	15.34	12.29	31.42
Leucine	23.34	32.30	9.10	37.52	16.64	23.21	16.71	32.84
Lysine	28.00	43.63	6.05	70.91	13.98	13.32	17.13	50.05
Methionine	21.16	36.31	5.09	56.25	10.77	7.92	11.19	33.63
Phenylalanine	17.84	25.48	5.95	26.65	10.55	15.55	10.65	25.87
Proline	28.41	33.76	7.21	64.10	11.16	15.51	24.11	43.28
Threonine	19.05	26.55	4.93	39.54	9.46	11.52	9.69	28.39
Tryptophan	3.01	4.86	0.78	4.99	1.71	1.75	1.81	4.78
Tyrosine	15.60	24.31	5.61	2.90	5.52	9.02	10.69	20.02
Valine	46.44	70.02	10.08	104.72	14.91	13.67	20.32	75.70
Carnosine	21.32	13.41	23.39	14.93	8.52	5.14	17.35	17.07
Anserine	9.42	7.19	12.63	7.55	13.84	9.27	6.49	10.19

Among these compounds, hydrophobic amino acids (arginine, histidine, isoleucine, leucine, lysine, phenylalanine, tyrosine, tryptophan, and valine) were reported to participate in the bitter taste of beef. Moreover, alanine, glycine, methionine, proline, and threonine are hydrophilic amino acids responsible for the sweet taste. Asparagine, aspartic acid, glutamine and glutamic acid are the taste compounds for umami [43, 114]. Additionally, the unique thick-sour and white-meaty characteristic in meat were enhanced by carnosine and anserine contents [42].

The results were discussed on the basis of taste quality. When compared to normal treatment, most of bitter-tasting amino acid compounds tend to have similar or higher level in LUMA. For sweet taste, the specific amino acids which are the taste contributor were found at the greater amount in treated beef with LUMA process. Also, LUMA beef seemed to occupy more umami taste as seen from the content of asparagine, aspartic acid, and glutamic acid. Furthermore, the higher amount of carnosine and anserine found in normal treatment indicated that LUMA beef tends to have less thick-sour than normal beef.

The higher levels of specific amino acids as a taste contributor were mostly found in beef cuts from LUMA than from normal preparation. This could be demonstrated the high possibility to have stronger bitter, sweet, and umami taste in LUMA beef. Among the four parts of beef, it is complicated to define the most intense amino acid cut and to discriminate beefs from two types of treatment with the obtained data set because the concentrations of all compounds have a wide variation. Therefore, statistical processing is required to explore and interpret the overall data. Moreover, a sensory test should be performed to support the discussion regarding taste quality and intensity.

4.2.1.2 Quantitation of fatty acids by LC-MS/MS

Besides protein, another main component of muscle is lipid. It plays roles in energy storage, membrane structure, and in various other processes in the organ, including immune responses and cellular recognition pathways. Lipid is a source of flavor constituents, both directly (unmodified) and indirectly (reaction products). The

lipids present in meat are composed of fatty acids that may be of saturated and/or unsaturated chemical structure [115, 116]. The fatty acid contents are important for the quality of meat and meat products in the case of fat tissue texture (tenderness and mouthfeel), shelf life (lipid and pigment oxidation), juiciness and flavor [117]. Its effect on the meat flavor is due to the production of volatile and lipid oxidation products during cooking. The involvement of fatty acids with the products from Maillard reaction (a chemical reaction between amino acids and reducing sugars that gives browned foods their desirable flavor during cooking) is to form other volatile components which affect aroma and flavor. For taste quality, eventhough fatty acid is not a direct basic taste contributor, the differences in fatty acid composition relate to specific flavor of meat. Hence, the information from fatty acid is required for more understanding about the profile of LUMA beef, which has been mentioned for its juiciness, flavorful, and tenderness [118]. This work determined the concentration of fifteen common fatty acid with various structures (saturated, monounsaturated, and polyunsaturated). The results of fatty acid concentration were presented in Table 4.10.

Table 4.10 Quantitative data (mmol/kg) of fatty acids in beef cuts with normal and LUMA treatment

Equivalent[a]	Compounds	Rib eye		Short loin		Tenderloin		Sirloin	
		Normal	LUMA	Normal	LUMA	Normal	LUMA	Normal	LUMA
Saturated fatty acid									
4:0	Butyric acid	2.37	0.08	1.29	0.10	0.21	0.11	0.07	2.18
6:0	Caproic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8:0	Caprylic acid	6.44	3.71	4.81	3.86	4.19	2.84	5.56	4.41
10:0	Capric acid	0.10	0.06	0.05	0.04	0.17	0.10	0.08	0.05
12:0	Lauric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14:0	Myristic acid	0.45	0.14	0.32	0.19	0.16	0.25	0.20	0.12
15:0	Pentadecanoic acid	0.89	0.41	0.75	0.25	0.67	0.73	0.40	0.35
16:0	Palmitic acid	11.27	3.95	13.72	1.91	5.17	18.63	1.95	1.14
18:0	Stearic acid	7.27	2.50	9.52	0.84	4.34	18.12	1.36	0.83
20:0	Arachidic acid	0.23	0.13	0.30	0.10	0.28	0.33	0.15	0.21
22:0	Behenic acid	0.27	0.14	0.27	0.08	0.18	0.26	0.14	0.22
24:0	Lignoceric acid	0.17	0.14	0.13	0.17	0.21	0.11	0.25	0.37
Monosaturated fatty acid									
16:1	Palmitoleic acid	6.83	4.73	3.37	6.42	3.38	2.47	5.36	11.52
18:1	Oleic acid	24.48	15.01	18.14	16.56	16.07	16.99	13.24	13.22
Polysaturated fatty acid									
18:2	Linoleic acid	2.70	0.89	1.19	0.37	1.40	3.10	0.67	0.52
18:3	Linolenic acid	0.76	0.51	0.12	0.07	0.16	0.09	0.31	0.32
20:4	Arachidonic acid	1.41	0.44	0.43	0.06	0.93	0.78	0.32	0.03

[a] equivalent name is the carbon number in the fatty acid chain followed by the number of double bonds

Lipid oxidation in cooking plays an important role in the development of the complex flavor profile of the meat (species-specific flavors) [115]. The saturation of fatty acid structure is related to the oxidation degree of meat. The higher number of double bond in the structure indicates the higher oxidation ability of fatty acid. However, the lipid auto-oxidation also leads to the production of strange and undesirable flavors (off-flavor) during long-term meat storage [114]. Thus, fatty acids lead to both desirable and undesirable flavors in meats. Additionally, lipids act as the solvent to accumulate the lipid-soluble, hydrophobic aroma compounds in meat.

Among the three types of studied fatty acid, the results showed that saturated (no double bond) and monosaturated (one double bond) fatty acids were found in higher amount than polysaturated (two or more double bonds) fatty acid. Beside oxidation capability, the more saturated fatty acid profile of beef also naturally induces the higher firmness [119]. In this work, oleic acid was the fatty acid which possessed the highest concentration in all studied beefs. This is corresponds to the previous observation, which claimed that oleic acid is the major fatty acid in all meats [117]. Moreover, palmitic acid was the saturated fatty acid with the highest concentration level in the four beef cuts. The variations of fatty acids are the result of the actions of enzymes present in the meat-producing animals and the cooking method.

Two main lipid classes of fatty acids in meat are neutral triacylglycerol and more polar phospholipid. The lower amount of polysaturated fatty acid occurs from the increasing ratio of triacylglycerol to phospholipid in animal after slaughter. From the previous report about beef taste, samples with high concentrations of polysaturated fatty acid had the highest oxidation and received the lowest taste panel scores for beef flavor. It also scored the highest for abnormal flavor and rancid [120]. Thus, the lower level of polysaturated fatty acid found in LUMA beef indicate that it tends to have higher pleasure score for the taste than normal beef. Nevertheless, it is still unclear to use this information of fatty acid to discriminate the beef from normal and LUMA treatment.

4.2.1.3 Quantitation of organic acids by LC-MS/MS

Organic acid is the common compound responsible for the development of the sour flavor in meat. The sourness of organic acid is produced from its hydrogen ion [4]. The nature of anion is important, and it partly contributes to aroma of food. The relative sourness of organic acid depends on its concentration and the type of compound. Lactic acid is one of the most important organic acids in meat. It is produced by the conversion of glycogen in the muscle of animal after it is slaughtered. Lactic acid is necessary to generate a more tasteful and tender meat, and

it is also required to maintain the meat quality and color. In addition, lactic acid in the muscle has the effect of retarding the growth of bacteria to protect spoilage of meat [116]. The type of acid affects the intensity and quality of the meat flavor. If the acid content is too high, the meat loses its water-binding ability and becomes pale and if the acid is too low, the meat will be tough and dry. The results of fatty acid concentration were presented in Table 4.11.

Table 4.11 Quantitative data (mmol/kg) of organic acids in beef cuts with normal and LUMA treatment

Compounds	Rib eye		Short loin		Tenderloin		Sirloin	
	Normal	LUMA	Normal	LUMA	Normal	LUMA	Normal	LUMA
Lactic acid	0.1289	0.1150	0.1996	0.0404	0.0682	0.0695	0.0735	0.1004
Citric acid	0.0018	0.0018	0.0018	0.0020	0.0019	0.0027	0.0011	0.0017
Succinic acid	0.2508	0.2362	0.2641	0.2357	0.2639	0.1784	0.1774	0.2463
Oxalic acid	0.0042	0.0043	0.0045	0.0047	0.0042	0.0031	0.0028	0.0042

From the results, the concentrations of lactic acid and succinic acid were found at the higher level than that of citric acid and oxalic acid. Studies reported that lactic acid and succinic acid are organic acid found to impart in meat sourness and to help providing more of the meaty taste [121, 122]. Most normal beef tends to have greater lactic acid and succinic acid levels than LUMA beef does. Therefore, normal beef cut should possess more of the sour taste. Due to the lower level of organic acids over other known compounds in normal and LUMA beef, it could be tentatively assumed that the sourness of studied beef is not an outstanding taste. However, the sensory evaluation is needed to confirm the beef taste profile.

4.2.1.4 Quantitation of cations and anions by IC

Various inorganic ions of animal tissue have been called mineral elements. The functions of ions are involved with the metabolisms of meat-producing animal. Calcium, potassium, sodium, chlorine, and magnesium are considered as the major mineral elements. Cells in living things require a specific balance of anions and

cations to function efficiently [123]. The positively charged magnesium and calcium ions play a vital role in muscle contraction, while sodium and potassium help in cell metabolism, activate some essential enzyme in the body, and act as electrolytes for the acid–base balance which regulate the body fluids. Also, ammonium ion deals with the maintenance of pH in tissues and has an essential role in protein synthesis of cellular tissues. Chloride is an important anion as it aids potassium absorption in the human body. It is a component of the digestive stomach acid and enhances the ability of blood to carry carbon dioxide from respiring tissues to the lungs. Phosphate is the only ion which could help to separate muscle fiber of meat after rigor mortis led to the solubility of muscular protein. The protein can then immobilize high levels of added water as well as emulsify a large amount of fat. Those activation of protein improves the texture of meat products. Phosphates can bind heavy-metal ions and therefore slow down the process of rancidity as heavy-metal ions are pro-oxidative materials. Besides all their functions, those mentioned cations and anions are strongly associated with the salty taste in meat.

The roles of cations and anions as taste contributor to saltiness were reported in meat [42, 43], this work studied five common cations and two main anions. Their concentrations were demonstrated in Table 4.12.

Table 4.12 Quantitative data (mmol/kg) of cations and anions in beef cuts with normal and LUMA treatment

Ion	Rib eye		Short loin		Tenderloin		Sirloin	
	Normal	LUMA	Normal	LUMA	Normal	LUMA	Normal	LUMA
Ammonium	17.65	18.94	16.23	19.26	22.34	20.94	18.27	15.16
Calcium	4.34	4.02	4.78	2.06	4.02	3.66	3.91	2.75
Magnesium	3.14	3.76	5.59	2.49	5.13	4.60	5.90	4.28
Potassium	49.08	44.2	58.79	26.72	41.56	63.74	67.30	41.67
Sodium	19.19	10.96	12.94	8.30	13.21	17.34	19.75	11.65
Chloride	218.27	309.63	276.02	198.50	332.31	520.54	433.56	411.47
Phosphate	507.29	756.74	730.77	380.44	686.35	1004.44	897.63	875.49

The salty taste of meat products containing sodium chloride originates predominantly from the negatively charged chloride ions and to a small degree from the positively charged sodium ions [124]. When the anion is the same, the increasing size of the cation from sodium to potassium progressively reduces saltiness and enhances bitterness [4]. Thus, potassium chloride is more related to bitter taste in the finished meat product than sodium chloride is. Moreover, the increasing size of anions with the same cation promotes greater bitterness. However, the level of saltiness in meat also depends on the concentration of the ions.

In this work, phosphate was an anion found in the highest amount. Due to its alkalinity, the slightly sour taste was obtained together with saltiness in meat. In addition, the salty taste with light bitterness should be exhibited in beefs owing to the high concentrations of ammonium, potassium and chloride ions. With the consideration of saltiness, rib eye and tenderloin cuts from LUMA treatment seem to have more salty taste because of their higher concentration of chloride ion. Nevertheless, the beefs from two treatment methods did not show an apparent differentiation with the cations and anions profile.

4.2.1.5 Data processing

Considering the results of all targeted analyses, the main composition in normal and LUMA beef was amino acid which was found in the highest level. Amino acid is well-known as a taste contributor for bitter, sweet, and umami. Also, amino acid is involved in many important chemical reactions to produce changed flavors such as Maillard reaction, one of the most important mechanisms for the formation of flavor compounds in meat [115]. Therefore, the contents of amino acid should mainly be associated with the major beef taste quality.

Owing to the data on Table 4.8, the investigation of amino acids results performed with statistical method to monitor the difference of beef profiles with two treatment methods in various beef cuts. A sensomics heatmap was produced in order to directly visualize and interpret the whole amino acid quantity. The heatmap was calculated using R software version 2.13.2 (R Development Core Team, Vienna,

Austria). The actual data was primarily employed for calculation. The established heatmap was presented in Figure 4.11. The variation of color refers to the concentration level of amino acid. The dendrograms were constructed to examine the relationship of data from two treatment methods with three different cuts and also the similarity of profiles from each amino acid.

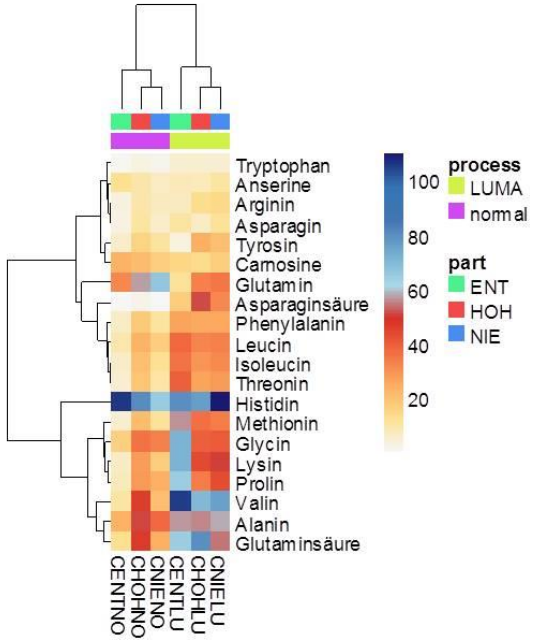


Figure 4.11 Sensomics heatmapping of the concentration of amino acid (from Table 4.9) in normal and LUMA treated beef with different cuts (ENT = short loin, HOH = rib eye, NIE = sirloin; NO = normal treatment, LU = LUMA treatment).

From the color arrangement in heatmap, the amino acids with low level were found on the top rows, while the high level amino acids were presented on the bottom rows. The darkest blue row of histidine means the highest level among other amino acids. In the same way, short loin with LUMA treatment have high amount of all amino acids over the others from the color observation in six columns.

According to the dendrograms, there is a separation between normal beef and LUMA beef, as seen in two clusters on the top dendrogram. The profiles of sirloin cut were similar to those of rib eye, indicating that their amino acid contents are more similar to each other than to short loin. From the left-side dendrogram, the amino acid which located at the adjacent row reflects a higher degree of data association.

However, the sensomics heatmap constructed from the actual data was not providing a clear discrimination of overall information. The color in heat map was quite a random distribution, which led to difficulty in interpretation. For better visualization, a data set of amino acid from Table 4.8 was scaled by mean centering approach for each compound. The average value of an individual compound was set at zero, and the deviation of each concentration from mean value was calculated and assigned as a new, representative value of the original concentration. The heatmap with the new scale was displayed in Figure 4.12.

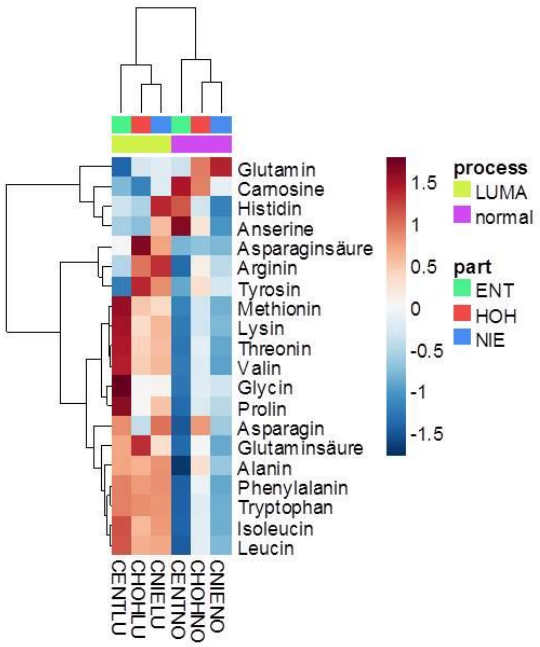


Figure 4.12 Sensomics heatmapping of the scaled concentration of amino acid in normal and LUMA treated beef with different cuts
(ENT = short loin, HOH = rib eye, NIE = sirloin; NO = normal treatment, LU = LUMA treatment).

The mean-centered heatmap presented the data in the new distribution scale. In each amino acid, the mean deviation was calculated for all beef samples. The darkest red color represents the highest deviation of data from the mean value in a positive way, implying the highest concentration of amino acid. On the other hand, the largest mean deviation on the negative side displayed by the darkest blue color. The concentration data which equals to the average value of each component is exhibited in white color. From the scaled heatmap, the discrimination of data between normal beef and LUMA beef. The significant difference in targeted compound content depends on the treatment method. The three columns on the right side, which are the profiles of normal beef, possessed the major area in blue color. On the other hand, most of the LUMA beef profile was colored red. This area with red color on the right side means that the most amino acid presented at the significantly higher level in the LUMA beef cuts. In the indicated red area on the left column, short loin cut occupied the darkest red; this refers to the highest level of amino acid in this part over the other LUMA beef cuts. When considered the intense red color on each row, the types of amino acid with the highest level in LUMA beef were methionine, lysine, threonine, valine, glycine, and proline which located in the middle row zones. The level of these taste contributors can be used to describe the possibility of LUMA beef having the more bitter and sweet taste. However, this is a tentative prediction, a sensory evaluation is still required because sometimes the concentration level is not related with the intensity of taste.

The dendrogram above the scaled heatmap presented the same trend as when using the original concentration of amino acid. Two clusters of normal and LUMA beef were differentiated. The closeness of sirloin and rib eye profiles explained the similarity in concentration trend when compared to those of short loin. From overall results, the short loin LUMA beef was selected for non-targeted analysis due to its remarkably high concentration of profiles over other cuts.

4.2.2 Non-targeted analysis

Non-targeted analysis is a comprehensive approach which aims to detect the pattern of a sample by involving the detection of the existing compounds as much as possible without identifying specific component [113]. The main advantage of this procedure is to explore the novel components responsible for specific properties of a sample without bias and to understand all chemical information in complex samples. The aim of non-targeted analysis in this work is to find the possibility to obtain key compound for the characteristic taste of LUMA. LC-TOF-MS, synthesis of tentative compounds, GPC, and sensory test were combined to fulfill the task.

4.2.2.1 Non-targeted screening by LC-TOF-MS

Among other MS instruments, TOF-MS provides highly accurate mass measurement, from the high resolution ability to full scan spectral sensitivity. Accurate mass measurement yields the elemental composition of parent and fragment ions, used to identify unknown species. This work applied non-targeted screening of an unlimited number of chemical components in LUMA short loin sample and compared it with normal short loin beef. Two ionization methods (positive and negative) in scan mode were used to uncover all possible chemical information. The chromatograms were illustrated in Figure 4.13.

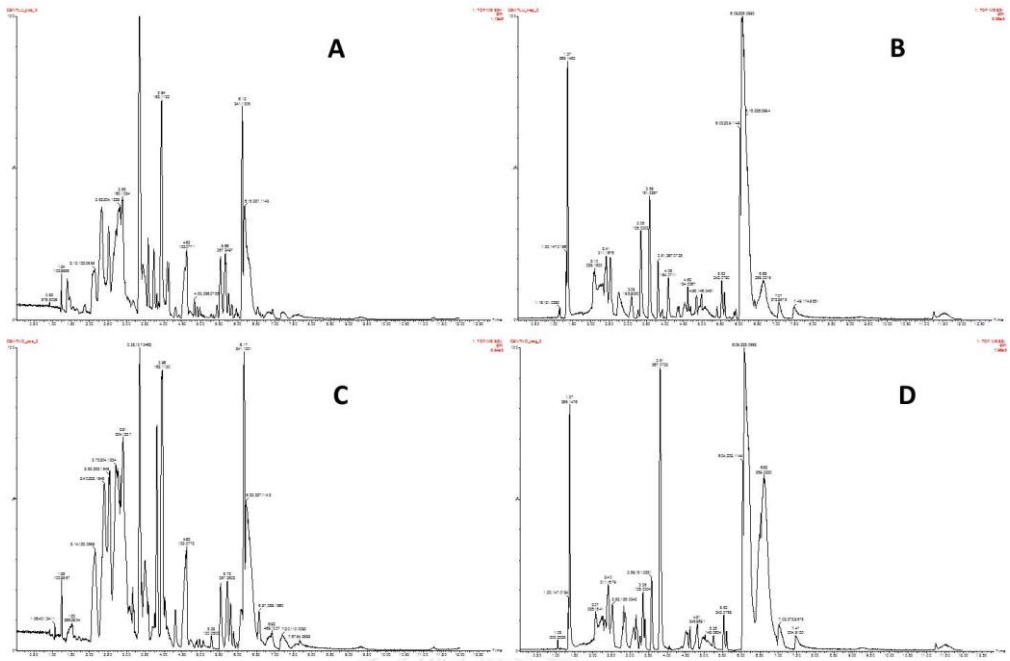


Figure 4.13 LC-TOF-MS chromatogram of short loin beefs
(A) LUMA in positive mode (B) LUMA in negative mode (C) normal in positive mode (D) normal in negative mode

The TOF-MS chromatograms of two ionization modes showed the variation of chemical patterns between LUMA beef (Figure 4.13A and 4.13B) and normal beef (Figure 4.13C and 4.13D). In order to obtain more details about the possibility of differentiation between two beefs, a data processing was performed in the next step.

The results of all the existing compounds in the beef samples from LC-TOF-MS measurements in both positive and negative modes were computed with OPLS-DA, since this statistical method provides clearer interpretation of graphs. OPLS-DA has a remarkable separating ability of between-class variation and the within-class variation. It facilitates a better class-resolution and understanding of the profiles from the same group and different groups. In data processing, the TOF-MS results of five replicates of short loin cuts with normal and LUMA treatment were employed. The OPLS-DA plot presented in Figure 4.14.

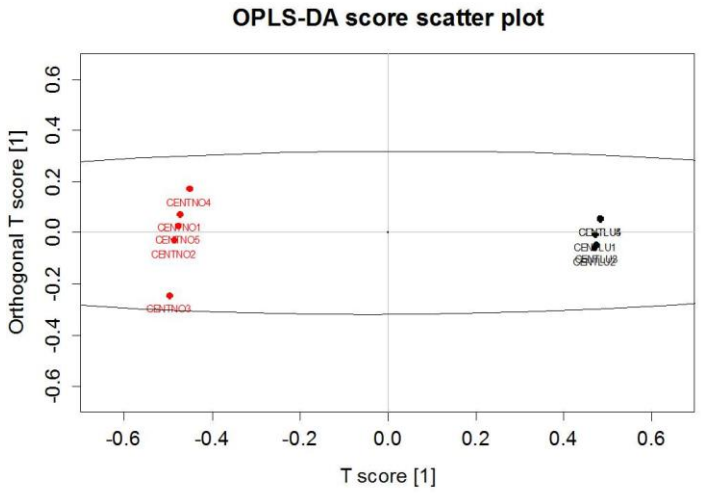


Figure 4.14 OPLS-DA score plot of the short loin beef with normal and LUMA treatment
(CENTNO = normal treatment beef, CENTLU = LUMA treatment beef, 1-5 = number of experiment)

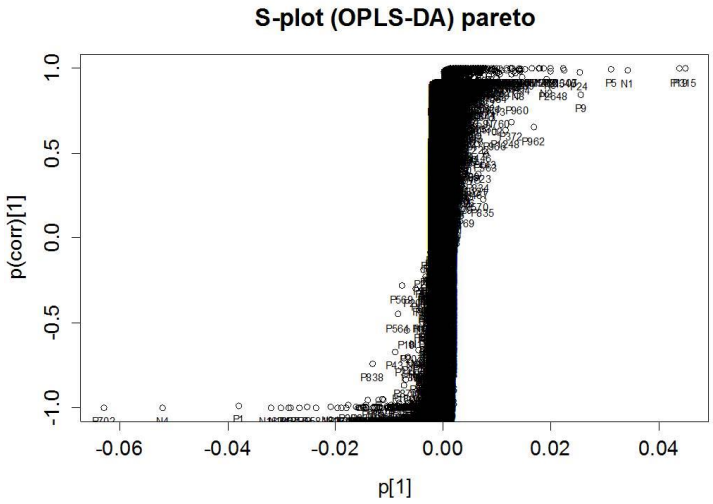


Figure 4.15 OPLS-DA loading S-plot of the short loin beef with normal and LUMA treatment.
The X-axis represents a measurement of the relative abundance of ions and the Y-axis represents a measurement of the correlation of each compound to the model. This

loading plot illustrated the relationship between variables (compounds) in relation to normal and LUMA profiles presented in the score plot (Figure 4.14).

The clear separation of normal beef and LUMA beef were observed in the OPLS-DA score plot (Figure 4.14). The profiles of the similar beef treatments were grouped, and the different treatment samples were distinguished by using the information of all existing compounds in the beef sample. This discrimination of LUMA beef from normal beef with the use of non-targeted analysis, agrees with the results from the targeted analysis. Therefore, it can be summarized that LUMA beef has a unique chemical pattern which could be discriminated from normal beef. This might be related to the distinctive flavor of LUMA beef.

To define the variables or ions associated with the group differentiation between the two types of beef in the OPLS-DA score plot, the S-plot was constructed. The S-plot is one form of the visualization of the OPLS-DA loadings and generally used for marker identification. It combines the covariance and correlation from the OPLS-DA model. The important feature of the S-plot is that the directions in the score plot correspond to directions in the loading plot. Therefore, the S-plot is convenient for identifying the variables (loadings) which separate different groups of objects (the scores). This is a powerful tool for understanding the underlying patterns in the data [125].

In general, the OPLS-DA loading plot will look like s-shape if the data profiles of the two groups have variation. From Figure 4.15, the s-shape of the plot means the LC-TOF-MS results of normal and LUMA samples have variation in peak intensities. From the data on the x-axis, the p1 describes the modelled co-variation or magnitude of each variable (compound), while the p(corr)1 on the y-axis represents the modelled correlation or reliability of each variable on the x-axis. The ideal marker should have high magnitude and high reliability. Therefore, the compounds on the top-right and bottom-left area in the S-plot are considered as top ranking for the marker of each group. From Figure 4.14 and 4.15, when considered the direction of LUMA profiles in score plot, the presence of few compounds on the top-right zone in s-plot revealed the potent marker for LUMA beef sample. The identification of

compound type and the ability to be as taste contributor of LUMA beef will be extended to further study.

4.2.2.2 Synthesis of possible taste contributor compounds

From the previous literature reports, N-acyl amino acids were identified as an important contributor to the characteristic taste of meat products [42, 43]. Also, the targeted analysis revealed that amino acid is the main composition in the LUMA beef. Therefore, this work attempted to synthesize the possible N-acyl amino acid to examine whether this compound is present in LUMA beef and become its taste contributor.

N-acyl amino acid is a carboxamide product from the acylation between carboxylic acid and an amino acid group via condensation [126]. The reaction occurs between a selected amino acid and a fatty acid to form N-acyl amino acid or fatty acid amide. From the results of amino acids in the targeted analysis, LUMA beef possesses more of sweet and umami taste than normal beef does. Thus, the three amino acids, contributing to sweetness (alanine, glycine) and umami taste (glutamic acid) with the highest content, were selected with the highest concentration saturated fatty acid (palmitic acid). The saturated type of fatty acid was chosen to protect unwanted lipid oxidation. The synthesis procedure was presented in Topic 3.2.12. The chemical structures of the three synthesized N-acyl amino acids were illustrated in Figure 4.16.

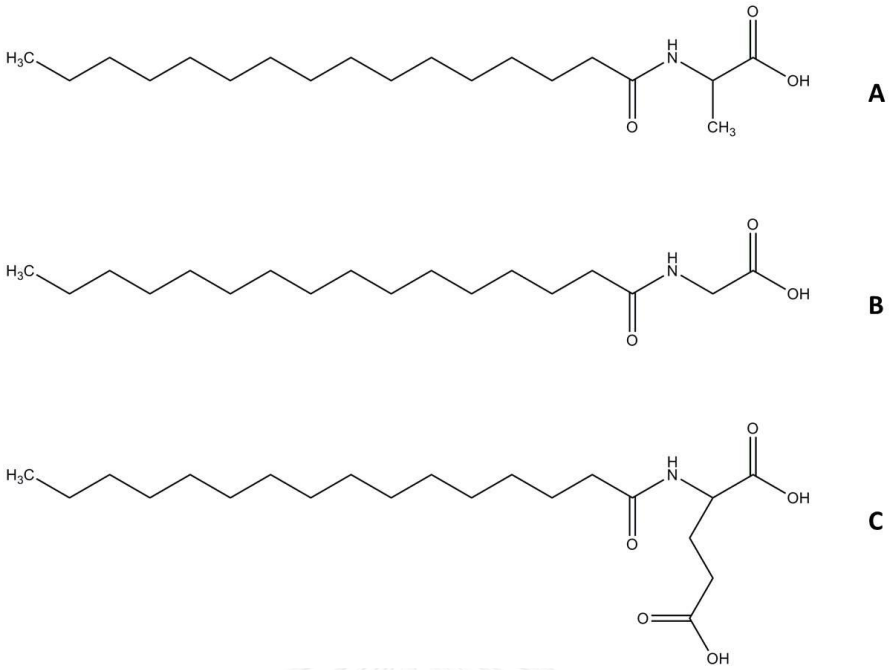


Figure 4.16 Chemical structures of the synthesized N-acyl amino acid
(A) N-palmitoyl alanine, (B) N-palmitoyl glycine, and (C) N-palmitoyl glutamic acid

After the synthesis, the obtained products appeared in the form of white solid. To identify and confirm the synthesized products, several analytical techniques (LC-MS/MS, LC-ELSD, MPLC, and NMR) were performed. In brief, LC-MS/MS was firstly used after the synthesis to check the presence of the synthesized compound by their mass. Then, LC-ELSD was used to monitor the chemical composition of the synthesized product before the purification process with MPLC. After that, the purity of product was confirmed with LC-ELSD, LC-MS/MS, and NMR.

At first, the synthesized products were qualitatively identified by LC-MS/MS in the negative mode on the basis of their molecular mass. The total ion chromatograms of N-palmitoyl alanine, N-palmitoyl glycine, and N-palmitoyl glutamic acid from LC-MS/MS were shown in Figure 4.17- 4.19.

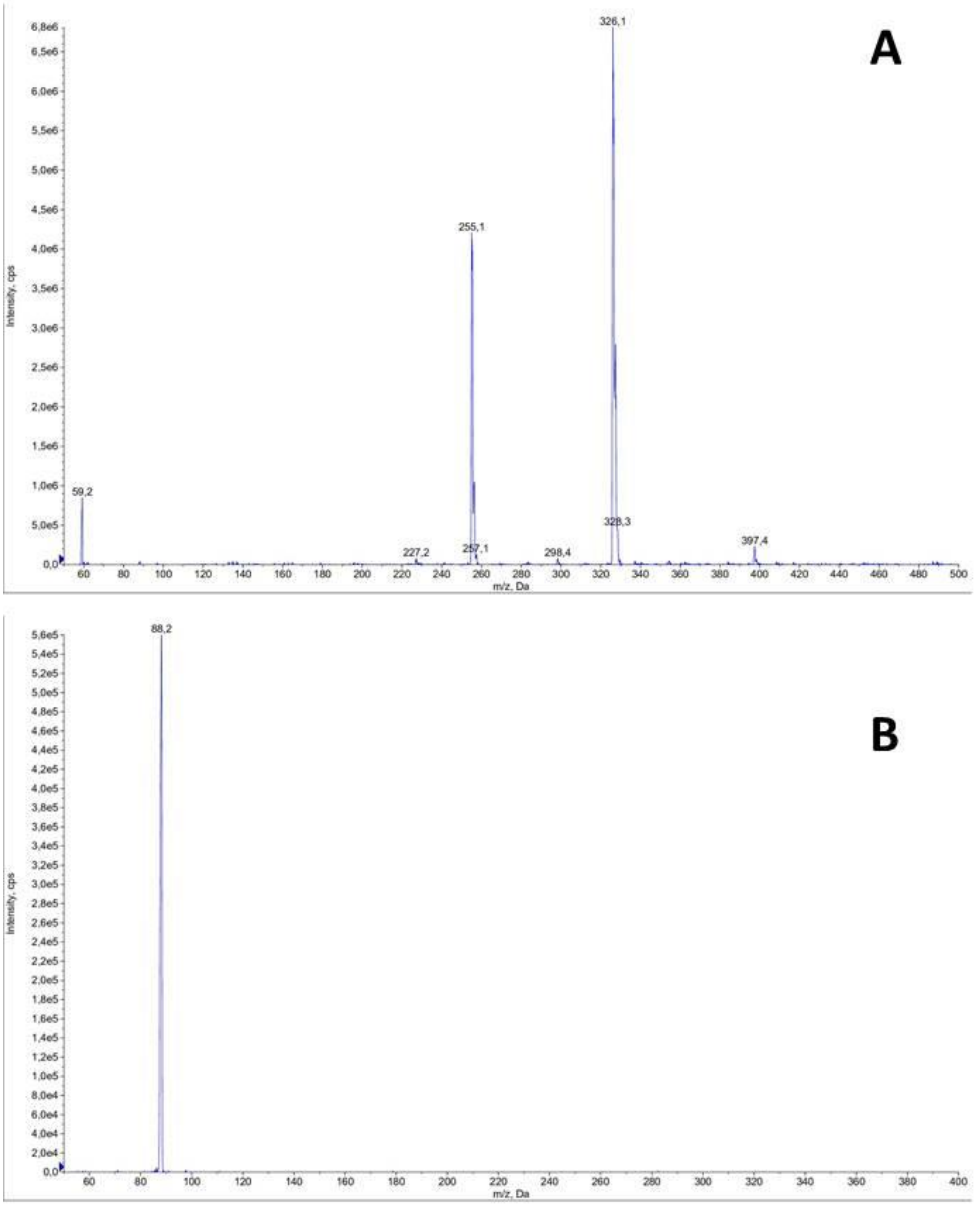


Figure 4.17 LC-MS/MS total ion chromatograms of synthesized N-palmitoyl alanine (MW = 327 g mol^{-1}) (A) from MS1 (B) from MS2

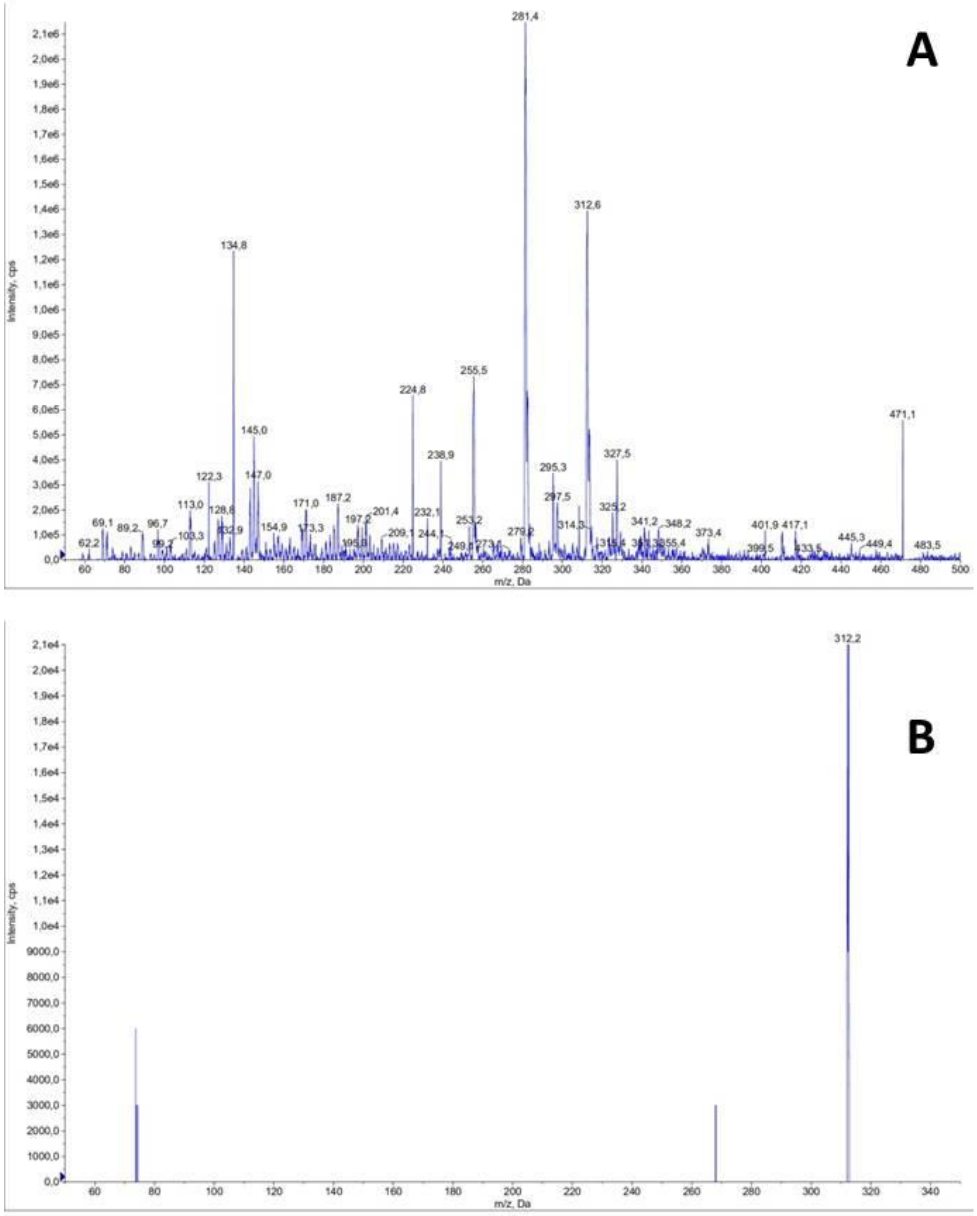


Figure 4.18 LC-MS/MS total ion chromatograms of synthesized N-palmitoyl glycine (MW = 313 g mol^{-1}) (A) from MS1 (B) from MS2

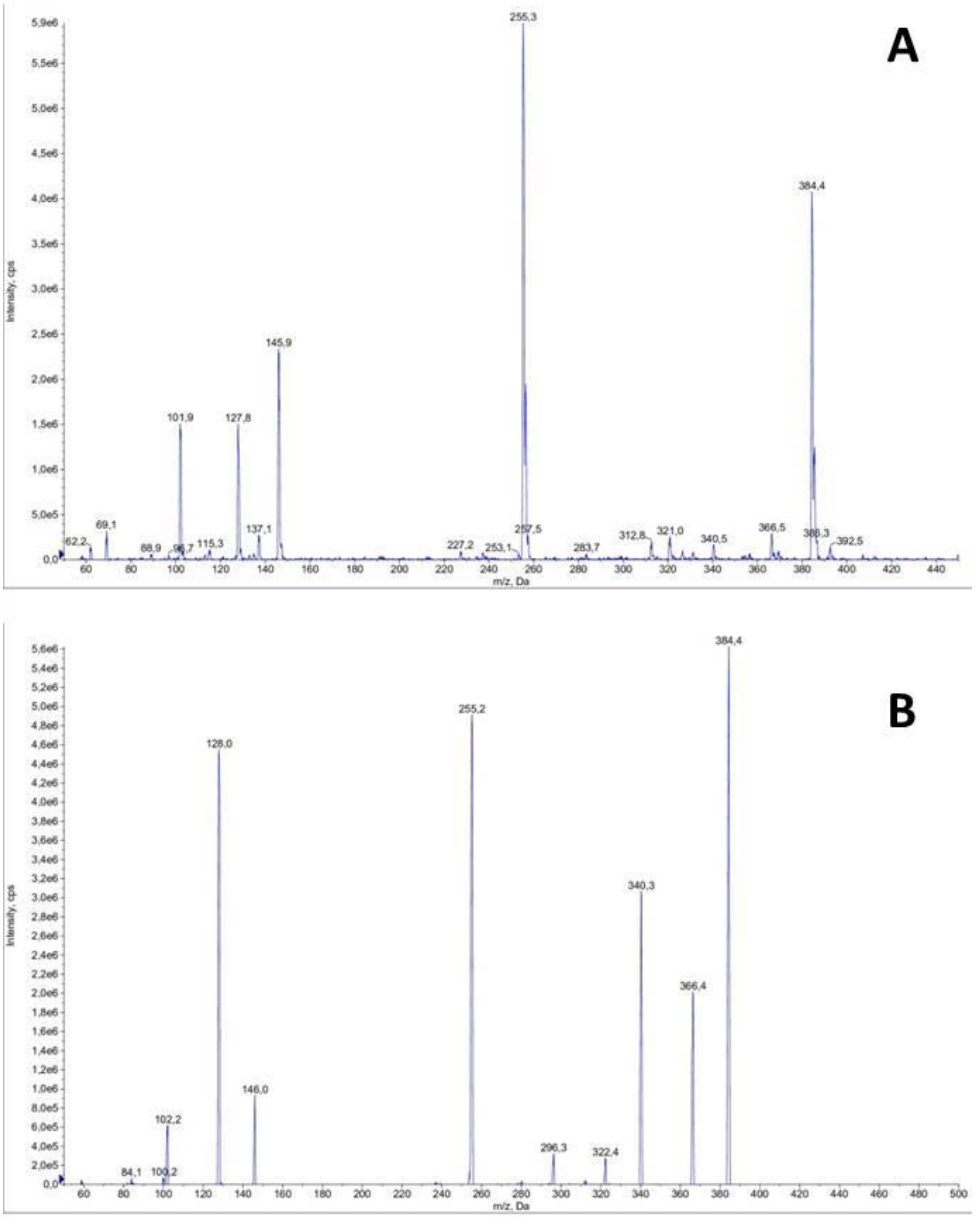


Figure 4.19 LC-MS/MS total ion chromatograms of synthesized N-palmitoyl glutamic acid
(MW = 385 g mol^{-1}) (A) from MS1 (B) from MS2

Figure 4.17-4.19 shows that the molecular mass from MS1 and their fragmented ions from MS2 confirm the existence of the synthesized products. The

success of the synthesis was the presence of the $[M-H]^-$ peaks at m/z 326 in Figure 4.17A, m/z 312 in Figure 4.18A, and m/z 384 in Figure 4.19A, which refer to N-palmitoyl alanine, N-palmitoyl glycine, and N-palmitoyl glutamic acid, respectively. In addition, the confirmation of each product was achieved by the fragmented ion at m/z 88 in Figure 4.17B, m/z 74 in Figure 4.18B, and m/z 146 in Figure 4.19B, which refer to the fragments of alanine, glycine, and glutamic acid, respectively.

The synthesized products were then monitored for the composition by LC-ELSD. The chromatograms were displayed in Figure 4.20A1, B1, and C1 with the presence of the two main peaks. Therefore, every synthesized product had some residue in their composition. For the three synthesized products, a big peak on the right side observed at t_R = 15 min was identified as fatty acid (palmitic acid) by comparing the retention times with commercial standards. The presence of fatty acid may be resulted from the use of excess reactant. Therefore, the peak at t_R = 14 min was assigned as a peak of the synthesized product.

To purify the products, MPLC were used for N-palmitoyl alanine, N-palmitoyl glycine, and N-palmitoyl glutamic acid by collecting the fraction of the products. The MPLC results were presented in Figure 4.21. The presence purified products were qualitatively confirmed by the information from LC-ELSD, LC-MS/MS, and NMR. The confirmation results of the purified products with LC-ELSD were presented as a single peak in Figure 4.20A2, B2, and C2 for N-palmitoyl alanine, N-palmitoyl glycine, and N-palmitoyl glutamic acid, respectively.

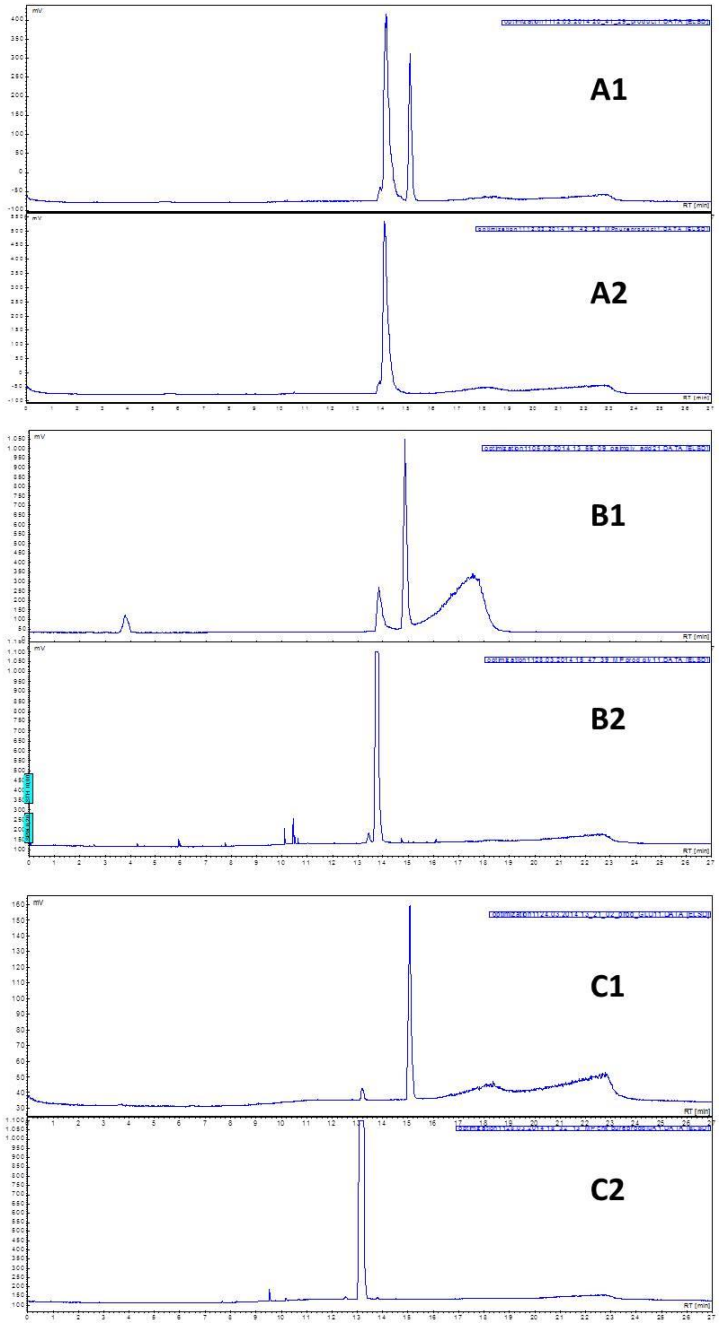


Figure 4.20 LC-ELSD chromatogram of three synthesized products
(A) palmitoylalanine, (B) palmitoylglycine, and (C) palmitoylglutamic acid
(1 = after synthesis (before purified), 2 = after purified)

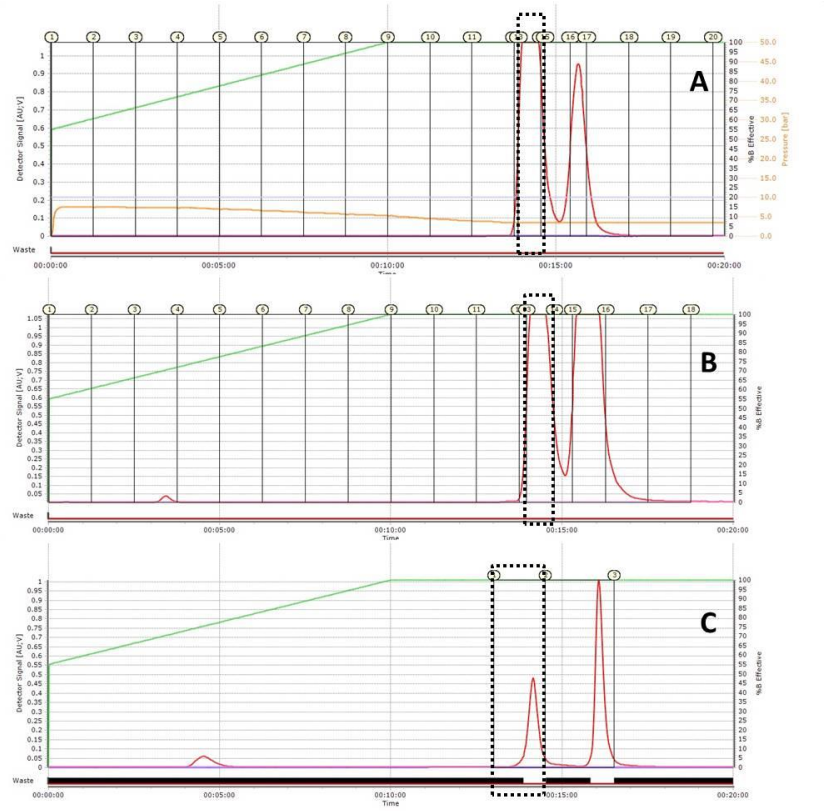


Figure 4.21 Representative MPLC chromatogram of three synthesized products (A) palmitoylalanine, (B) palmitoylglycine, and (C) palmitoylglutamicacid. Dashed square box refers to the collected fraction.

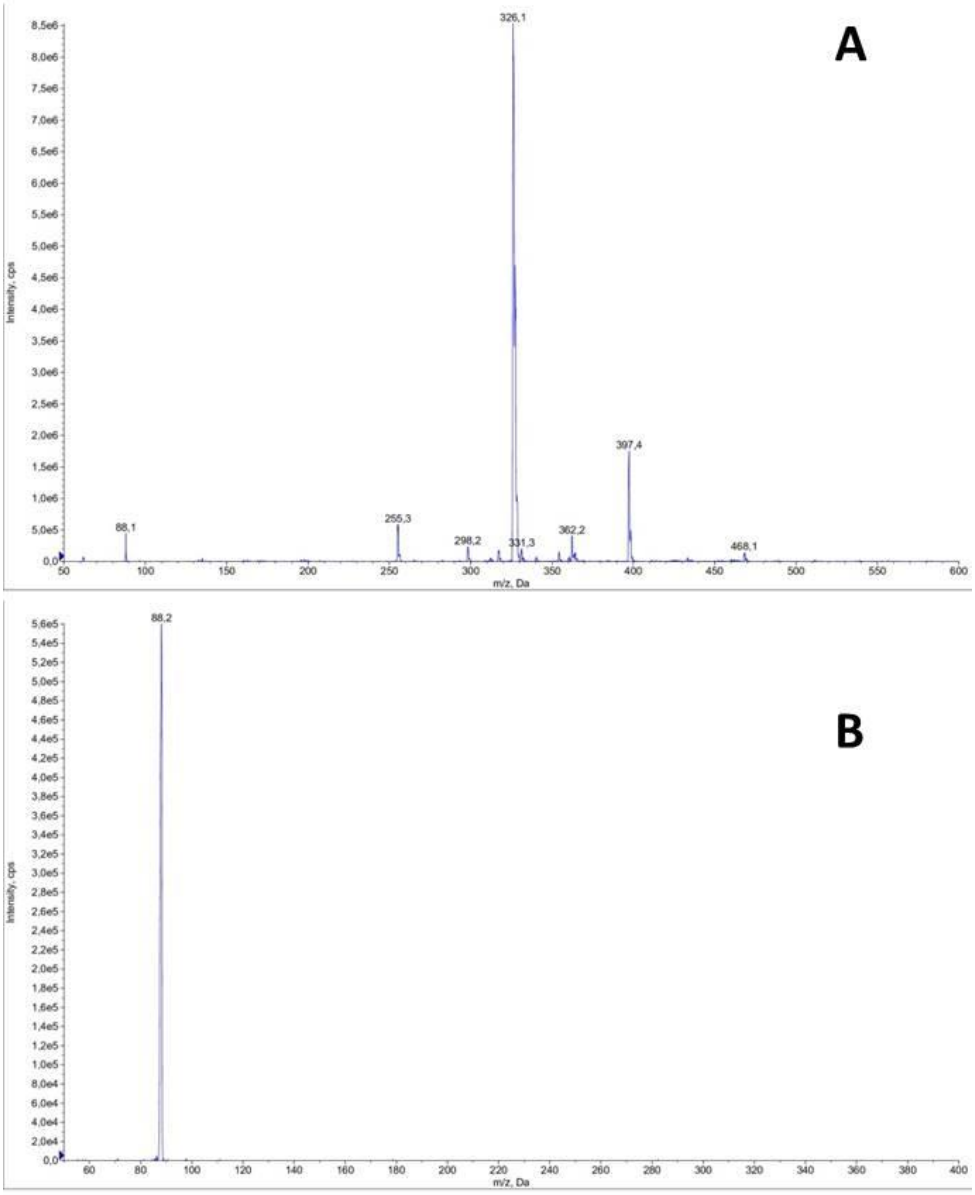


Figure 4.22 LC-MS/MS total ion chromatograms of synthesized N-palmitoyl alanine (MW = 327 g mol^{-1}) after MPLC purification (A) from MS1 (B) from MS2

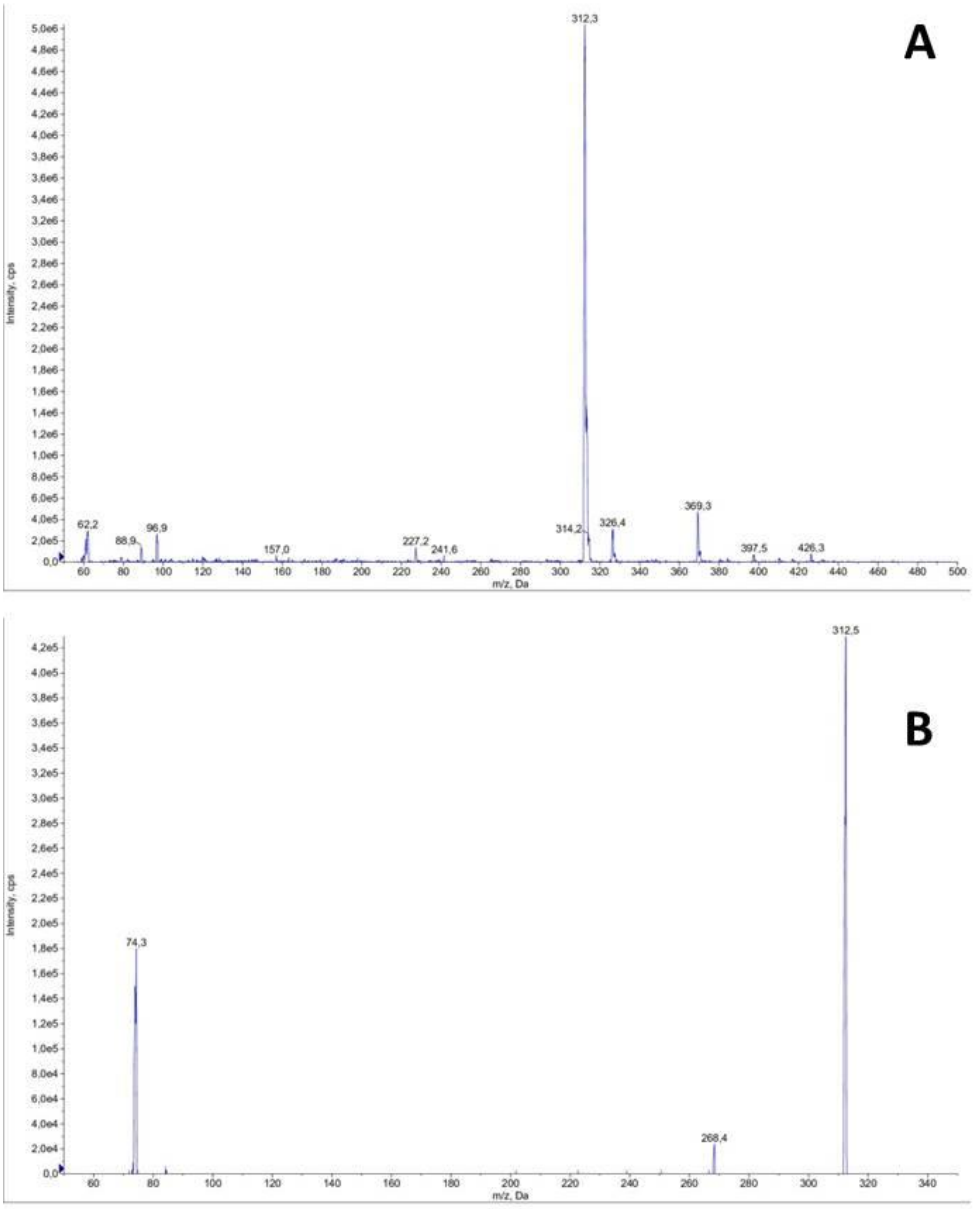


Figure 4.23 LC-MS/MS total ion chromatograms of synthesized N-palmitoyl glycine (MW = 313 g mol^{-1}) after MPLC purification (A) from MS1 (B) from MS2

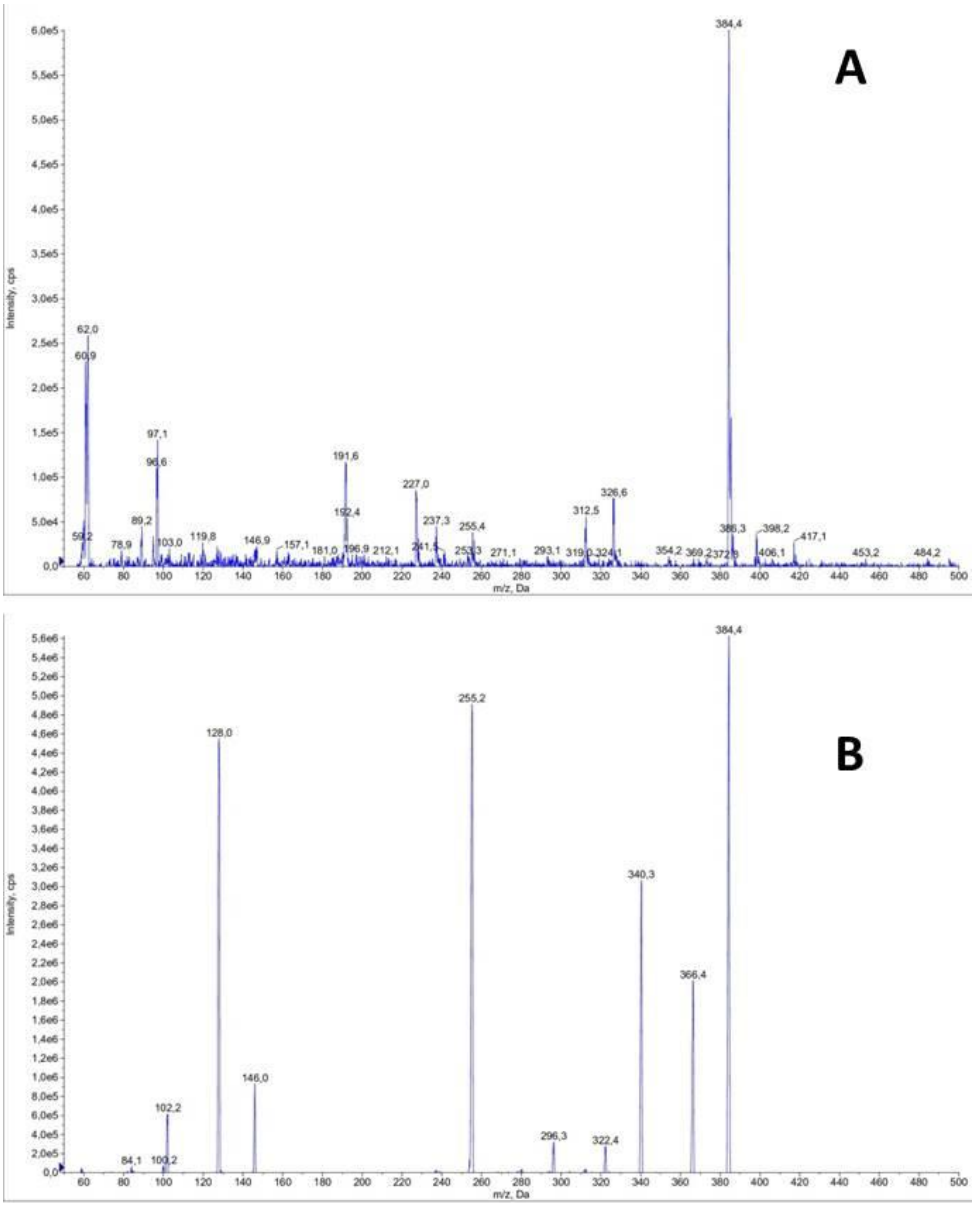
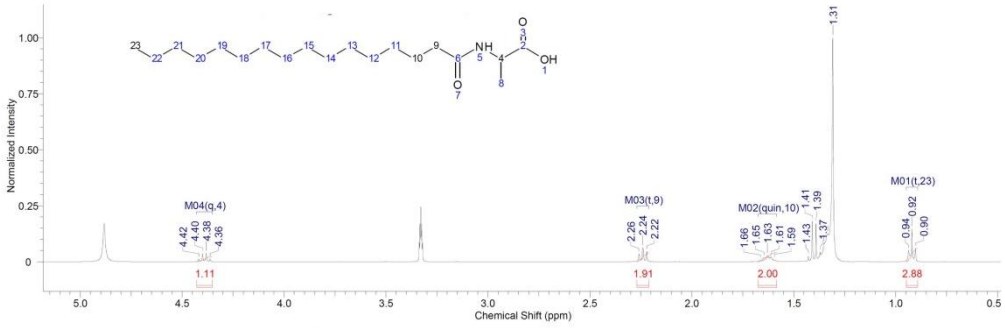


Figure 4.24 LC-MS/MS total ion chromatograms of synthesized N-palmitoyl glutamic acid
(MW = 385 g mol^{-1}) after MPLC purification (A) from MS1 (B) from MS2

From Figure 4.22-4.24, the molecular mass from MS1 and their fragmented ions from MS2 were used to confirm the presence of the purified products. The purified product gives rise to the peaks $[M-H]^-$ at m/z 326 in Figure 4.22A, m/z 312 in Figure 4.23A, and m/z 384 in Figure 4.24A, which refer to N-palmitoyl alanine, N-

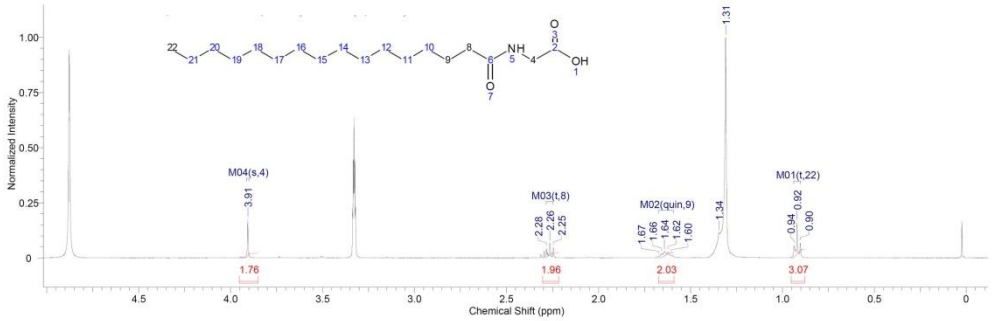
palmitoyl glycine, and N-palmitoyl glutamic acid, respectively. In addition, the presence of each purified product was confirmed by the fragmented ion at m/z 88 in Figure 4.22B, m/z 74 in Figure 4.23B, and m/z 146 in Figure 4.24B, which refer to the fragments of alanine, glycine, and glutamic acid, respectively.

Additionally, NMR experiments were conducted in one (^{1}H and ^{13}C) and two dimensions (COSY and HSQC) to confirm the presence of the three purified products by determining the molecular structure of various molecules. The ^{1}H NMR spectrum and the interpretation were presented as an example of NMR confirmation result for N-palmitoyl alanine, N-palmitoyl glycine, and N-palmitoyl glutamic acid in Figure 4.25, 4.26 and 4.27 respectively. The results of NMR spectrum from ^{13}C, COSY, and HSQC were shown in Appendix.



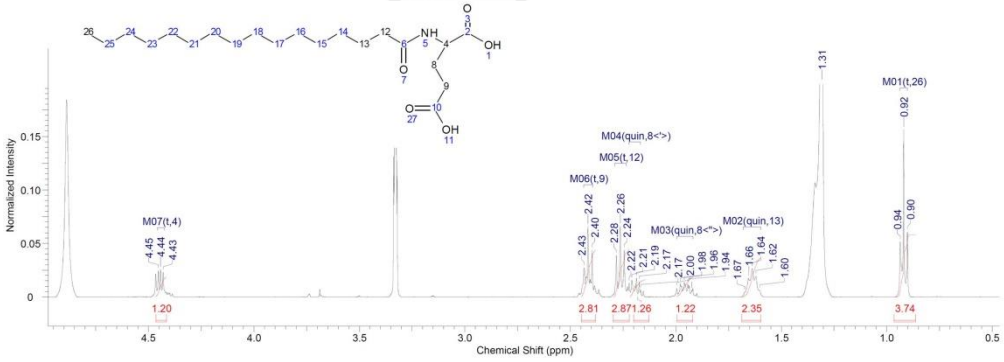
No.	Shift1 (ppm	H's	Type	J (Hz)	Atom1	Multiplet1	(ppm)
1	4.39	1	q	7.32	4	M04	[4.35 .. 4.43]
2	1.63	2	quin	7.00	10	M02	[1.58 .. 1.68]
3	2.24	2	t	1.00	9	M03	[2.21 .. 2.27]
4	0.92	3	t	1.00	23	M01	[0.89 .. 0.95]

Figure 4.25 ^{1}H-NMR spectrum of purified N-palmitoyl alanine
[^{1}H NMR (400 MHz, methanol-d4) δ ppm 0.92 (t, J=1.00 Hz, 3 H) 1.63 (quin, J=7.00 Hz, 2 H) 2.24 (t, J=1.00 Hz, 2 H) 4.39 (q, J=7.32 Hz, 1 H)]



No.	Shift1 (ppm	H's	Type	J (Hz)	Atom1	Multiplet1	(ppm)
1	3.91	2	s	-	4	M04	[3.90 .. 3.91]
2	1.64	2	quin	1.00	9	M02	[1.59 .. 1.67]
3	2.27	2	t	1.00	8	M03	[2.25 .. 2.28]
4	0.92	3	t	1.00	22	M01	[0.90 .. 0.94]

Figure 4.26 ^{1}H-NMR spectrum of purified N-palmitoyl glycine
[^{1}H NMR (400 MHz, methanol-d4) δ ppm 0.92 (t, J=1.00 Hz, 3 H) 1.64 (quin, J=1.00 Hz, 2 H) 2.27 (t, J=1.00 Hz, 2 H) 3.91 (s, 2 H)]



No.	Shift1 (ppm	H's	Type	J (Hz)	Atom1	Multiplet1	(ppm)
1	4.44	1	t	1.00	4	M07	[4.42 .. 4.46]
2	2.20	1	quin	14.00	8<'>	M04	[2.17 .. 2.22]
3	1.96	1	quin	14.30	8<">	M03	[1.92 .. 2.00]
4	1.64	2	quin	7.07	13	M02	[1.60 .. 1.68]
5	2.26	2	t	7.47	12	M05	[2.24 .. 2.29]
6	2.42	2	t	1.00	9	M06	[2.39 .. 2.44]
7	0.93	3	t	1.00	26	M01	[0.90 .. 0.94]

Figure 4.27 ^{1}H-NMR spectrum of purified N-palmitoyl glutamic acid
[^{1}H NMR (400 MHz, methanol-d4) δ ppm 0.93 (t, J=1.00 Hz, 3 H) 1.64 (quin, J=7.07 Hz, 2 H) 1.96 (quin, J=14.30 Hz, 1 H) 2.20 (quin, J=14.00 Hz, 1 H) 2.26 (t, J=7.47 Hz, 2 H) 2.42 (t, J=1.00 Hz, 2 H) 4.44 (t, J=1.00 Hz, 1 H)]

Besides all of the confirming results from LC-ELSD and LC-MS/MS, NMR spectra from one (^{1}H and ^{13}C) and two dimension (COSY and HSQC) experiments was used to confirm the molecular structure and the molecular interaction of the three purified products. Thus, the products was employed as a standard to determine whether these compounds are present in the LUMA beef. The three compounds were identified and determined with the use of LC-MS/MS. The results were shown in Table 4.13.

Table 4.13 Quantitative data (mmol/kg) of three synthesized products in beef cuts with normal and LUMA treatment

Compounds	Rib eye		Short loin		Tenderloin		Sirloin	
	Normal	LUMA	Normal	LUMA	Normal	LUMA	Normal	LUMA
N-palmitoyl alanine	0.0071	0.0039	0.0112	0.0076	0.0066	0.0251	0.0051	0.0087
N-palmitoyl glycine	0.0559	0.0415	0.0528	0.0499	0.0531	0.0493	0.0546	0.0585
N-palmitoyl glutamic acid	0.0046	0.0017	0.0008	0.0073	0.0041	0.0044	0.0140	0.0071

The three synthesized products were qualitatively and quantitatively found in normal and LUMA beef with the variation of concentration in different cuts. When compared to the other components in beef, these three synthesized compounds were present at very low level and the preliminary sensory evaluation indicated that the concentration of these compounds at concentration lower than 0.3 mmol kg^{-1} (0.1 ppm) was not detected by the panelists. Hence, the fractionation experiments of LUMA beef were subsequently conducted with a sensory test to find the possible taste modulator.

4.2.2.3 Fractionation by GPC

In the experiment of fractionation, GPC was firstly employed to separate the chemical components of LUMA beef on the basis of molecular size. The separation is needed for monitoring the specific group of molecules that contribute to the taste of

LUMA beef. The obtained GPC chromatogram was displayed in Figure 4.28. Six GPC fractions were collected from the extract of LUMA short loin.

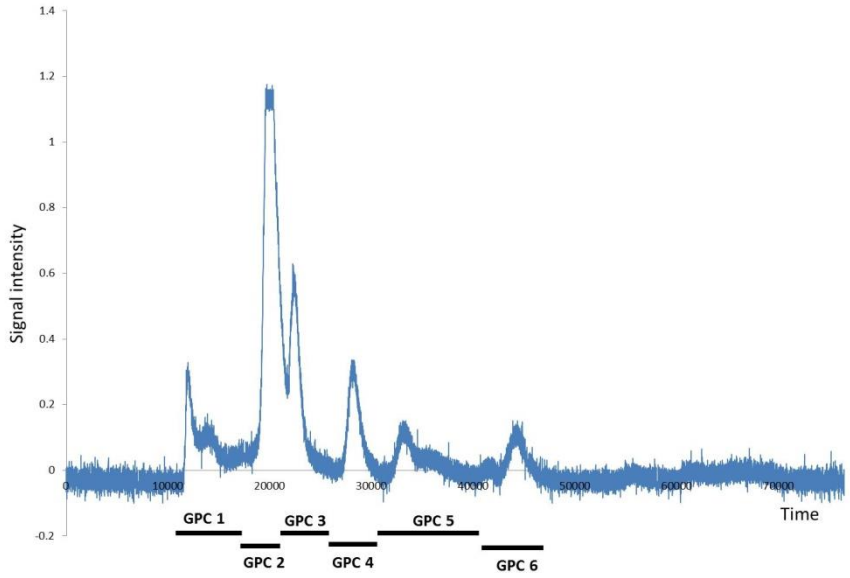


Figure 4.28 GPC chromatogram of short loin beef with LUMA treatment

The six GPC fractions were then analyzed by LC-MS/MS and IC to find the concentration of amino acids and cations which were used to observe the relationship between the molecular information and the sensory results. The quantitative data were shown in Table 4.14.

Table 4.14 Quantitative data (mmol/kg) of amino acids and cations in GPC fractions

Compounds	GPC 1	GPC 2	GPC 3	GPC 4	GPC 5	GPC 6
Alanine	119.98	23.94	117.23	11.04	123.47	4.70
Arginine	18.92	0.16	0.04	0.42	36.53	0.65
Asparagine	0.01	0.27	1.24	0.01	0.09	0.09
Aspartic acid	5.85	1.36	0.52	0.27	3.49	1.97
Glutamine	1.64	4.54	4.40	0.04	0.48	1.07
Glutamic acid	1.25	5.02	0.16	0.02	0.76	0.24
Glycine	4.66	7.48	6.89	0.21	2.35	0.31
Histidine	0.92	25.26	1.48	0.57	18.89	0.77
Isoleucine	1.02	3.78	3.23	0.15	0.86	0.17
Leucine	1.28	2.13	5.05	0.19	0.09	0.02
Lysine	4.56	25.75	0.78	0.30	11.63	2.03
Methionine	0.00	0.05	1.47	0.01	0.00	0.01
Phenylalanine	0.52	0.04	0.07	5.41	2.98	0.00
Proline	42.96	9.76	0.64	0.00	0.64	0.02
Threonine	0.55	9.11	0.93	0.06	0.45	0.09
Tryptophan	0.08	0.00	0.00	0.00	0.03	0.00
Tyrosine	0.97	0.01	0.00	0.03	62.71	0.02
Valine	1.72	11.84	1.64	0.14	1.13	0.05
Carnosine	0.31	11.93	0.28	0.03	7.41	0.11
Anserine	0.05	10.90	0.07	0.01	4.04	0.21
Sodium	811.92	2880.06	373.61	196.31	10678.93	1693.51
Ammonium	158.64	412.78	48.21	43.99	1413.81	569.90
potassium	22.37	50.46	5.39	18.52	290.81	527.38
Magnesium	12.17	75.52	5.19	6.67	251.83	40.02
Calcium	18.90	37.53	8.21	3.41	167.70	28.96

4.2.2.4 Sensory evaluation

To check whether the identified compounds in targeted analysis does produce the typical taste of the LUMA beef, the comparative taste profile analyses were performed. Each GPC fraction was tested for the taste quality and the taste contributor of LUMA beef. The taste recombinant solution (Rec) was prepared with the main molecules (amino acids, cations and chloride) responsible for the taste qualities of LUMA beef.

Rec was used as a matrix solution for the localization of the taste modulators in the GPC fraction and elimination of the matrix effect from a beef extract. The

tested samples were prepared by adding LUMA extract (short loin) and six GPC fractions in Rec. The intensities of the taste descriptors bitter, sweet, sour, salty, umami (savory), and kokumi (mouthfulness, thickness, and a long-lasting taste sensation) were rated on the scale of 0 (not detectable) to 5 (intensely perceived) in comparison to an aqueous Rec (reference) by 10-12 trained panelists. The average values of intensities for individual taste qualities from eight sample solutions were presented in Table 4.15, and their taste profile analysis was displayed in Figure 4.29.

Table 4.15 Average intensities for individual taste qualities of LUMA beef (short loin) and GPC fractions (by 10-12 panelists)

Sample	Solution (1:1, v/v)	Average intensities (≥10 panelists/sample)					
		Sweet	Sour	Salt	Bitter	Umami	Kokumi
1	Rec	0.6	2.5	0.8	0.9	1.5	1.5
2	LUMA extract + Rec	0.5	2.1	0.8	1.0	2.0	1.6
3	GPC1 + Rec	0.8	2.1	1.6	1.6	1.2	0.8
4	GPC2 + Rec	0.6	2.1	1.5	1.4	1.1	0.8
5	GPC3 + Rec	0.7	1.9	1.5	1.4	1.3	0.9
6	GPC4 + Rec	0.6	1.9	1.4	1.2	1.2	0.8
7	GPC5 + Rec	0.5	1.9	1.3	1.1	0.9	0.7
8	GPC6 + Rec	0.6	1.7	1.3	1.3	1.2	0.8

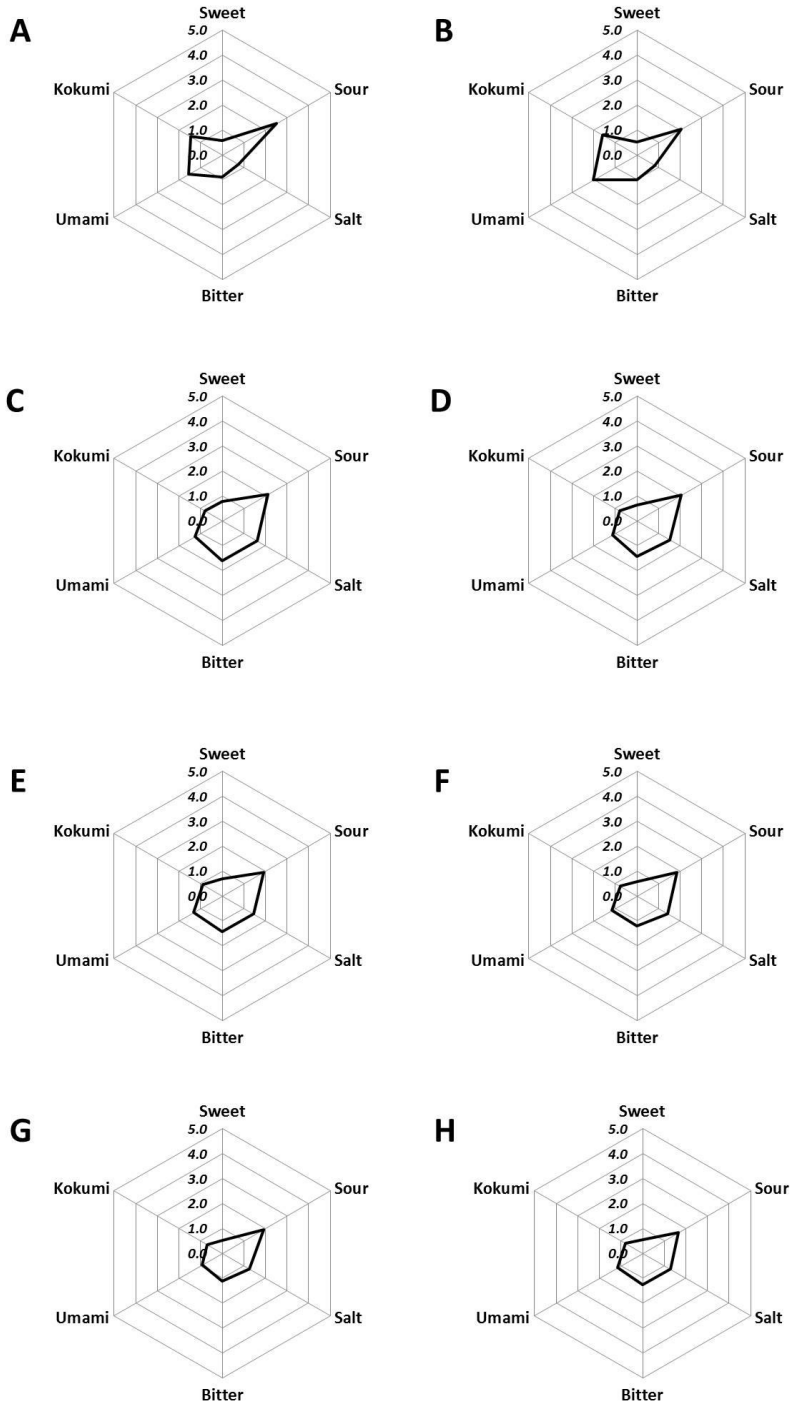


Figure 4.29 Taste profile analysis of LUMA beef and GPC fractions
(A) Rec (B) LUMA extract + Rec (C) GPC1 + Rec (D) GPC2 + Rec (E) GPC3 + Rec (F) GPC4 + Rec (G) GPC5 + Rec (H) GPC6 + Rec

From the charts of sample 1 (Figure 4.29A) and sample 2 (Figure 4.29B), the comparative taste profile analysis revealed that the intensities of sweetness, bitterness, and saltiness for the prepared Rec were close to those of the LUMA extract. The lower intensity of umami in Rec plot shows the lack of a certain compound modulating this taste. However, the similarity between taste profiles of Rec and LUMA indicated that the compounds in the prepared Rec are mainly responsible for the taste quality of LUMA beef. In addition, the taste profile showed that the apparatent tastes of LUMA beef were sour, umami, and kokumi according to the higher value of intensity than the other tastes.

Concerning the GPC fractions in Rec, the fraction 1 was significantly involved in the taste of sweetness and bitterness shown by the increase of intensity from 0.6 to 0.8 and 1.0 to 1.6, respectively. All GPC fractions have a predominant positive impact on the salty taste. On the other hand, there is no increase in taste intensity for sour umami, and kokumi taste by GPC fractions. The GPC fraction 1 mostly pertained to the taste of sweet, salty, and bitter. These sensory results were in agreement with the quantitative results of six GPC fractions in Table 4.14. The compounds responsible for the sweet, salty and bitter taste were determined at the high level in the GPC fraction 1. Therefore, alanine, proline, arginine, and sodium are the tentative compounds highly responsible for the sweetness, saltiness and bitterness of LUMA beef from the taste descriptor information of each compound explained in targeted analysis. Further study is required to identify the specific molecules responsible for the taste of LUMA.

Besides the experimental results, the characteristic of the taste of LUMA beef might be related with the special dry-aged procedure using the novel mold *Thamnidium elegans* treatment [78]. This mold was reported to produce lipids with a variety of saturation structure [127]. The predominant fatty acid generated was oleic acid, followed by palmitic acid which was in agreement with the results in this work (Topic 4.2.1.2). During the aging process, *T. elegans* grew, form the layer on meat surface and transferred the enzymes into the meat. The enzymes from the mold was indicated to impart in meat tenderization, and they led to proteolysis, the breakdown of proteins into smaller amino acids [128]. This shows that the development of the

special LUMA flavor is considerably related with the free amino acids components in LUMA beef.

4.3 Food safety: Matrix effect in pesticide residue analysis

4.3.1 Matrix effect in pesticide residue analysis

In GC analysis, the unexpected high recoveries of pesticides are always occurred from the effect of matrix which is called 'matrix-induced response enhancement effect'. The causes of MEs depend on the active sites from free silanol groups and metal ions on glass surfaces in the liner and column in GC [58]. To observe ME in pesticide residue analysis, 100 pesticides were selected to cover a wide analytical scope, and the final extract of blank chili sample was spiked at 50 ng g-1. Chili sample was chosen for the study of ME because it is well-known as highly pigmented food matrix. From studied 100 compounds, the four pesticides representing the early-, middle-, and late-eluting compounds were chosen and their chromatograms were shown in Figure 4.30 to observe the effect of sample matrix. In addition, the volume of matrix in each sample vial should be similar since the different matrix volumes were added to the solutions in preliminary investigation and the signal deviation appeared at the same pesticide spiking level.

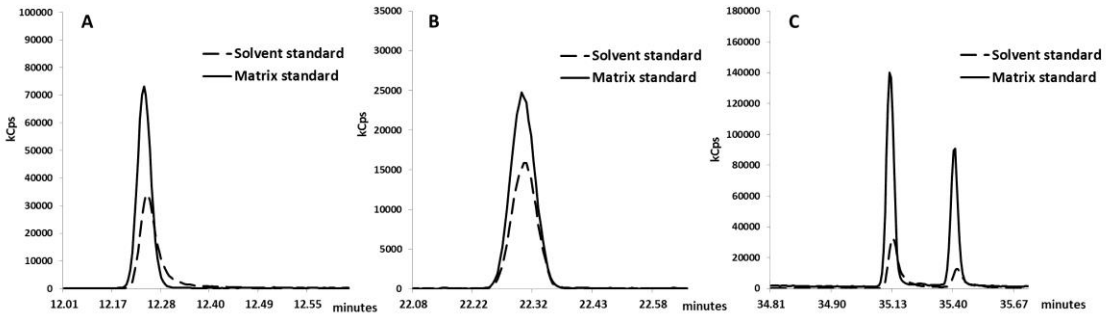


Figure 4.30 Comparison of GC-MS/MS chromatograms from the injections of chili extract at 50 ng g^{-1} with solvent standard (long dashed line) and matrix standard (solid line)
(A) etridiazole (RT = 12.23 min), (B) aldrin (RT = 22.29 min), and (C) fenvalerate I,II (RT = 35.11, 35.40 min)

From Figure 4.30, matrix-induced response enhancement effect was clearly observed for all representative pesticides in the early-, middle-, and late-eluting ranges. The pesticide signals of matrix standard were significantly improved when compared to the signals without matrices (solvent standard). This is because matrix increases the transfer of pesticides from injectors by masking active sites in the injector which are responsible for the adsorption or decomposition of pesticides, while the solvent was unable to afford sufficient protection for the analytes. In common calibration practices, standard in solvents is being used for calibration and recovery calculations [60]. Therefore, overestimation of results from sample analysis may occur. Matrix-matched calibration is recommended by European guidelines to help in protecting analytes from loss in GC system and decrease the method detection limit by increasing sensitivity. However, this approach is a sample-dependent and not practicable in routine multiresidue analysis in which the number of analyzing samples is very large.

4.3.2 AP effect on compensation of ME in pesticide residue analysis

AP is one of the most effective strategies to equalize the response enhancement from matrix. APs play an important role similar to matrix by competing with analytes at those active sites to improve the transfer efficiency of analytes reaching the detector. From the results of studied 100 compounds, the four pesticides representing the early-, middle-, and late-eluting compounds were chosen to present their chromatograms in Figure 4.31 in order to observe the effect of AP on compensation of ME. Mixed APs was added to matrix standard following the classical approach of AP [68].

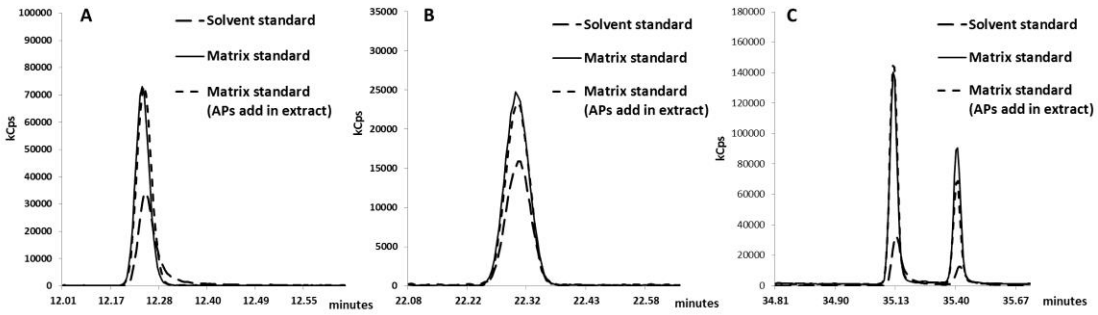


Figure 4.31 Comparison of GC-MS/MS chromatograms from the injections of chili extract at 50 ng g^{-1} with solvent standard (long dashed line), matrix standard (solid line), and matrix standard with mixed APs added in sample (dashed line). (A) etridiazole (RT = 12.23 min), (B) aldrin (RT = 22.29 min), and (C) fenvalerate I,II (RT = 35.11, 35.40 min)

From Figure 4.31, the application of AP was found to compensate matrix-induced response enhancement by providing the almost identical results for the studied pesticides. Considered to the role as masking agent, AP discards the analytical problem from sample-dependent analysis and requires less maintenance of the GC system. Although the efficiency of AP in analyte protection was proved, the approach of AP by adding the solution to each individual sample solution seems to be impractical in routine laboratory because it is time-consuming and requires a large amount of APs. Consequently, the new approach of using AP was firstly investigated in this work. Mixed APs solution was individually prepared in one vial and directly used as a priming agent. Then, the sample can be directly analyzed without adding AP into the solution. To investigate APs in a new type of priming application, mixed APs solution was injected into the GC system as a first vial before the analytical batch of sample extracts. The results of solvent standards, matrix standards and matrix standards with two approaches of AP (mixed APs added in sample and mixed APs made at first injection) were compared by spiking 50 ng g^{-1} studied pesticides in solvent and the final extract of blank chili sample. From studied 100 compounds, the four pesticides representing the early-, middle-, and late-eluting compounds were chosen and their chromatograms were shown in Figure 4.32.

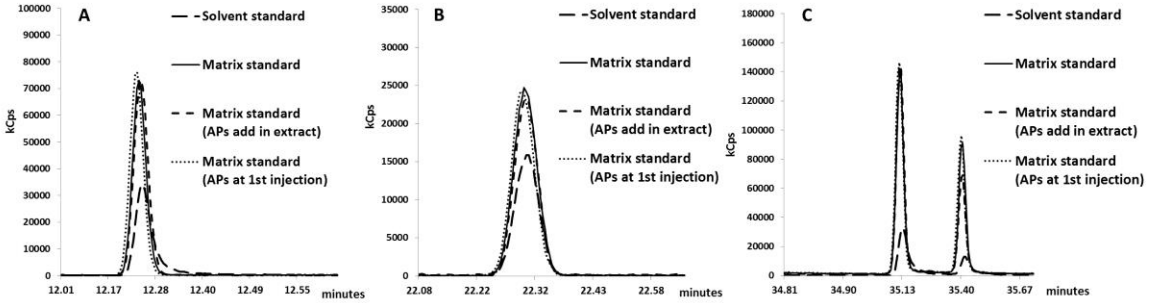


Figure 4.32 Comparison of GC-MS/MS chromatograms from the injections of chili extract at 50 ng g^{-1} with solvent standard, matrix standard, and matrix standard with mixed APs added in sample, and matrix standard with mixed APs in priming. (A) etridiazole (RT = 12.23 min), (B) aldrin (RT = 22.29 min), and (C) fenvalerate I,II (RT = 35.11, 35.40 min)

A similar trend for the chromatograms of matrix standards with two APs used and matrix standards were observed. From the obtained signal, this could be indicated that the use of APs as a priming solution has been provided the same performance as adding APs to each extract.

4.3.2 Long-term performance study

To ensure that the obtained results were from the effect of AP priming and not from the matrix, the effect in a long-term experiment (50 injections over two days) to monitor a matrix and AP priming effect was investigated. A blank chili extract was spiked at 10 ng g^{-1} with 100 pesticides. All 50 replicate injections were performed in two sets of sequence: with and without AP priming. The level of 10 ng g^{-1} was chosen to study because it is a default maximum residue limit for most pesticides in crops and, at this trace level, the stability of quantitative results was obviously monitored. The first sequence with AP priming started with injection of mixed APs vial (3-ethoxy-1,2-propanediol, L-gulonic acid g-lactone, D-sorbitol, and shikimic acid (40:2:1:1)) followed by 50 injections of chili extract. The second sequence without

AP priming has a similar sequence of injection but without priming with the APs at the beginning.

Before starting each sequence, a new, inert liner was used and the system was cleaned with a few injections of solvent (ACN) to renew the GC experimental condition. Additionally, mixed solvent standard solution of 100 pesticides without AP, which is a susceptible case for matrix effect, was injected before starting each sequence analysis in order to observe the effect of active sites inside the column and to check the GC performance during the study. To evaluate the overall obtained results from AP priming effect, the upper and lower limit have been set and the criteria of ±30% vs. the first injection was selected following the SANCO document [129]. A mean value from the first 5 replicates was employed in this work instead of the first injection to provide more consistency of the reference signal from GC analysis. The values of normalized peak area to TPP (I.S.) were calculated and used in this study to compensate for losses during sample preparation or variability during the analytical determination. Figure 4.33 illustrated the comparison between the normalized peak area of 50 injections with and without AP priming and their criteria levels (±30%) of four selected pesticides representing the early-, middle-, and late-eluting compounds.

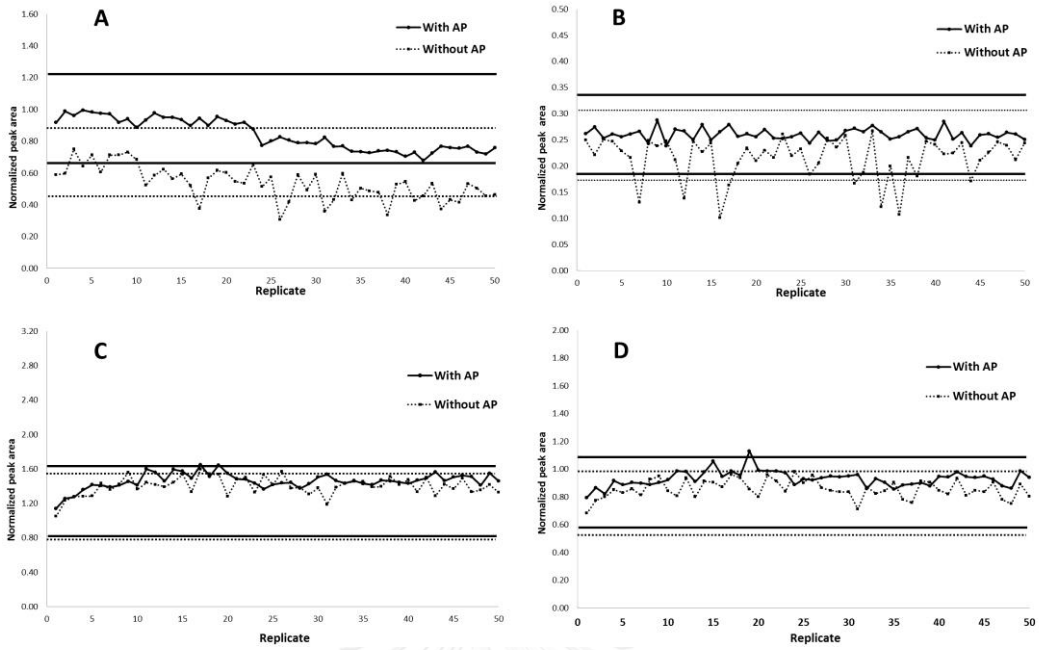


Figure 4.33 Comparison of normalized peak area from the injections of chili extract spiked at 10 ng g^{-1} for long-term study (50 replicates) between with (solid line) and without AP priming (dotted line) and their criteria.
(A) Etridiazole (RT = 12.23 min), (B) aldrin (RT = 22.29 min), (C), fenvalerate I (RT = 35.11 min), and (D) fenvalerate II (RT = 35.40 min)

As seen from Figure 4.33, the sequence of 50 injections without AP priming revealed higher inconsistency of long-term analysis which led to greater inaccuracy of analytical results. The single injection of APs as priming agents before the sequence could significantly improve the results' stability for pesticide residue determination. Owing to the strong hydrogen-bonding capability of the more volatile early-eluting analytes, these ME susceptible compounds tend to have a stronger benefit from AP priming effect, as seen from the result for etridiazole (RT = 12.23 min). Also, the efficiency of AP priming covers the middle-eluting compounds which have moderate hydrogen bonding capability, as observed from aldrin (RT = 22.29 min). For the low volatile late-eluting compounds such as fenvalerate I and II (RT = 35.11 and 35.40 min), AP priming provided only slightly effect because of the non-polar characteristic. This type of compound was not much suffered from matrix effect problem due to a low hydrogen bonding capability with active sites.

To monitor and classify the overall results from AP priming effect, all 100 pesticides were assigned as within criteria and outside criteria and the classification was presented in form of bar graph (Figure 4.34). For 50 injections, if the compound has 3 or more peak areas values fell outside the ±30% criteria, then this compound was classified as not meeting the criteria or outside criteria. Conversely, if at least 47 injections led to peak areas within the ±30% window from the average of the first 5 injections, then the analyte was categorized as within the criteria.

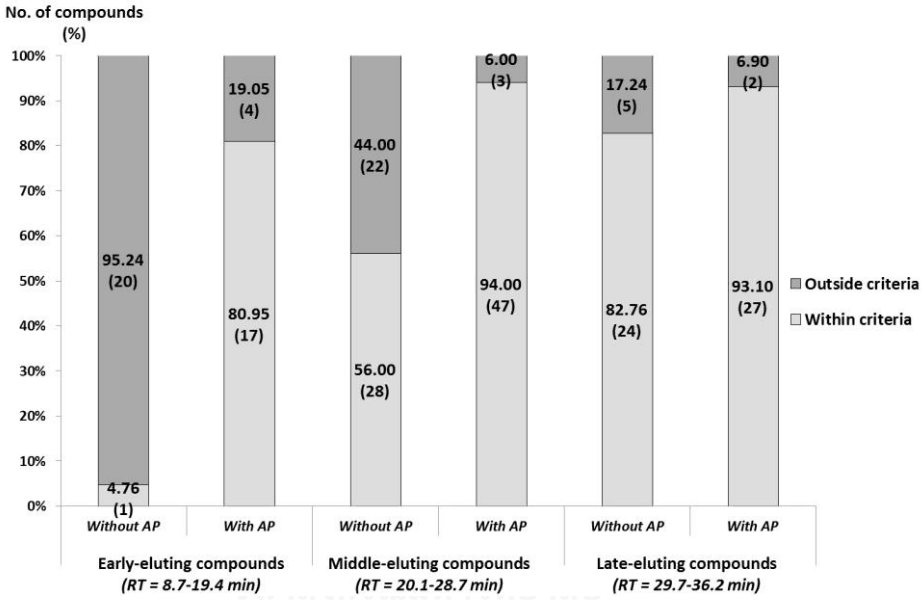


Figure 4.34 The number of pesticides (% of 100) classified as within and outside criteria (±30%) by their peak areas and comparing with and without AP priming use in early-, middle-, and late-eluting time periods.
The peak areas of 100 compounds were obtained from the 50 injections of chili extract spiked at 10 ng g^{-1}. The actual counted number of analytes from early-, middle-, and late-eluting times appear in parentheses

For early-eluting compounds, the number of compounds that met the criteria when using AP priming significantly increased from 5% to 81%, the same as 56% to 94% for middle-eluting compounds, and 83% to 93% for late-eluting compounds. The greater stability of analytical results achieved in long injection sequences is benefit for routine analyses and the effect lasts for at least two days of analysis, which is long

enough for practical suitability. From the comparison of results, it can be concluded that the single injection of AP mix at the beginning of the sequence provided an overall improvement for 100 pesticides and was enough to cover 50 injections. The results supported the view that APs blocked active sites inside the GC system not due to volatility and co-elution with analytes, but they coat in the system.

Also, RSDs of 50 injections were also calculated to estimate the repeatability of all peak areas in the sequence. The results are presented in Table 4.16. The criterion of RSDs at 10 ng g^{-1} is ±20% in accordance with the SANCO document [129]. The RSDs of 100 compounds were classified into two categories on the basis of their results into in criteria and over criteria.

Table 4.16 The number of pesticides (%) classified as in and over criteria (±20%) by their RSDs and comparing between with and without AP priming use in three elution time ranges.
The number of pesticides (%) classified as in and over criteria (±20%) by their RSDs and comparing between with and without AP priming use in three elution time ranges.

Elution time range[a]		No. of compound (%[b])	
		In criteria	Over criteria
1	Without AP	0 (0.0)	21 (100.0)
	With AP	17 (81.0)	4 (19.1)
2	Without AP	15 (30.0)	35 (70.0)
	With AP	43 (86.0)	7 (14.0)
3	Without AP	27 (93.1)	2 (6.9)
	With AP	27 (93.1)	2 (6.9)

[a] Elution time range (1-3) represented early-, middle-, and late-eluting compounds

[b] The percentage counted number of compounds from early-, middle-, and late-eluting time were in bracket.

The 100% of early-eluting compounds have the RSDs outside the criteria, but the number of compounds with RSDs meeting the criteria extremely rose up when using AP priming. The increasing number of compounds with RSDs passing the

criteria also found in middle-eluting compounds (from 30% to 86%). For late-eluting pesticides, AP priming had little effect because they did not suffer as much from ME. The tendency of these overall RSDs of 100 pesticides are in correspondence with the results of peak area classification of all compounds and could be explained by the polarity and volatility properties.

4.3.3 Investigation of AP priming practical use for routine analysis

The duration time of AP priming effects was studied to determine the time in which AP remained in the system and to select the use of AP as priming agent in routine analysis. The injections of 50 replicates of chili extract spiked with 100 pesticides at 10 ng g^{-1} were performed among 2 days of analysis. One AP injection at the beginning of batch analysis were compared with two AP injections every 25 injections (at the beginning of each day). The results were presented in Figure 4.35

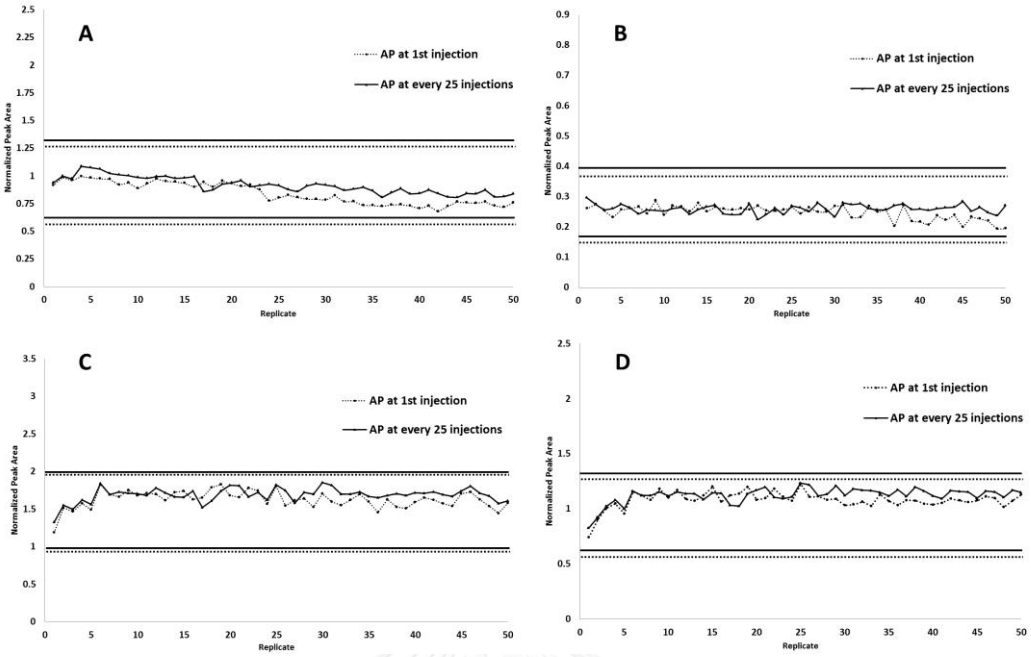


Figure 4.35 Comparison of normalized peak area from the injections of chili extract spiked at 10 ng g^{-1} for long-term study (50 replicates) between two AP injections at every 25 injections and one AP injection at the beginning of batch and their criteria. (A) Etridiazole (RT = 12.23 min), (B) aldrin (RT = 22.29 min), (C), fenvalerate I (RT = 35.11 min), and (D) fenvalerate II (RT = 35.40 min)

The results showed no difference between two approaches of AP priming use. Therefore, only one injection of AP before sequence was enough for priming the long-term batch analysis of sample. Furthermore, the stability of AP solution was investigated. The same AP vial was used in priming for two long sequence analyses (50 injections each) in two different weeks with changing the inlet liner. The obtained signals of pesticides from the second week analysis showed the high inconsistency of signals which were similar to the experiments without AP priming. Thus, the effect of AP as priming agent existed only one week and it is recommended to prepare AP solution freshly.

4.3.4 Comparison of mixed APs and single AP

From the observation of AP priming characteristics, the mechanism of AP for its protecting performance inside GC seems to differ from the explanation of traditional approach. Interestingly, AP was not co-eluted with analytes but still coat and block active sites in the system. Therefore, the use of mixed AP compounds to cover analytes with various volatilities might not necessary in the analysis. To support the hypothesis and to confirm the priming effect, mixed AP (3-ethoxy-1,2-propanediol, L-gulonic acid g-lactone, D-sorbitol and shikimic acid) and individual AP (L-gulonic acid g-lactone and D-sorbitol) were employed at the same concentration and monitored for their priming effect on the signal and RSD values of 50 injections at 10 ng g^{-1} pesticides. The classification of analytical results based on the same criteria as in Topic 4.3.2 was shown in Figure 4.36 and Table 4.17.

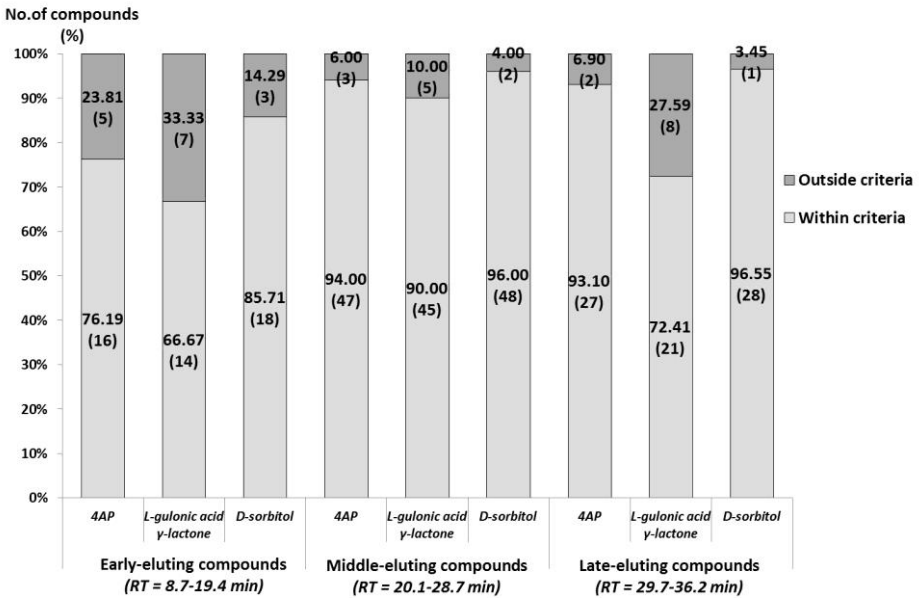


Figure 4.36 The number of pesticides (% of 100) classified as within and outside criteria (±30%) by their peak areas in early-, middle-, and late-eluting times from the use of 4 mixed APs, and L-gulonic acid g-lactone and sorbitol in priming. The peak areas of 100 compounds were obtained from the 50 injections of chili extract spiked at 10 ng g^{-1}. The actual counted number of analytes from early-, middle-, and late-eluting time appear in parentheses

Table 4.17 The number of pesticides (%) classified as in and over criteria (±20%) by their RSDs and comparing between the use of 4 mixed APs, and L-gulonic acid g-lactone and sorbitol in priming within three elution time ranges.

Elution time range[a]		No. of compound (%[b])	
		In criteria	Over criteria
1	4AP	18 (85.7)	3 (14.3)
	L-gulonic acid g-lactone	18 (85.7)	3 (14.3)
	Sorbitol	19 (90.5)	2 (9.5)
2	4AP	47 (94.0)	3 (6.0)
	L-gulonic acid g-lactone	49 (98.0)	1 (2.0)
	Sorbitol	49 (98.0)	1 (2.0)
3	4AP	27 (93.1)	2 (6.9)
	L-gulonic acid g-lactone	27 (93.1)	2 (6.9)
	Sorbitol	28 (96.5)	1 (3.5)

[a] Elution time range (1-3) represented early-, middle-, and late-eluting compounds

[b] The percentage counted number of compounds from early-, middle-, and late-eluting time were in bracket.

From Figure 4.36 and Table 4.17, there is no significant difference of the results from using mixed APs and individual AP for all pesticide residue analyses in three elution time ranges. These data supported the AP priming hypothesis because the mixture of various volatilities of AP to co-elute with the analyte was no longer a key parameter when AP primed the GC system for the long term without co-eluting. Considering the results of all elution times, sorbitol was selected as a AP of choice in this work because the greater numbers of compounds that passed the criteria (within criteria) and the similar RSDs results were achieved when compared to the other APs. Moreover, sorbitol contains multiple hydroxy groups which able to efficiently bind with active sites in GC. Sorbitol also gives the benefit of a lower price and wider commercial availability.

CHAPTER V
SUMMARY

In this work, the food quality and safety were investigated under the topic of the authentication of ginger origin, the molecular taste profile in beef, and the matrix effect in pesticide residue analysis. Chromatography techniques play a major role in both qualitative and quantitative analysis. GC and LC combined with various types of detector were successfully applied to analyze food profile and solve the analytical problem. Moreover, several statistical methods were combined for data treatment in order to develop the better interpretation.

Under the topic of the food authentication, HPLC-based fingerprinting method for origin labelling of ginger was effectively established. Gingers from five different cities in Thailand (Chiangmai, Chiangrai, Leoy, Nakonpatom and Petchaboon) and five countries (China, India, Malaysia, Thailand, and Vietnam) were analyzed and identified with LC-DAD and LC-MS/MS. The application of unsupervised and supervised pattern recognition of chemometrics techniques improved the understanding about the obtained data. Nine chromatographic peaks were measured with LC-DAD, identified with LC-MS/MS, and used as ginger profiles for origin discrimination based on the quantitative data. The optimization of the sample preparation and chromatographic conditions were performed for the analysis of 152 ginger samples. Due to the complex interpretation of 152 x 9 data matrix, the methods of similarity analysis, HCA, PCA and LDA were used for data exploratory and classification purposes. The results were discussed in the term of the city and the country of ginger production. The unsupervised pattern recognition methods (similarity analysis, HCA, PCA) were performed to describe the relationship among ginger profiles and to monitor data in case of mean, representative and, whole data, respectively. The three selected statistical methods provided a similar trend of results. Gingers from the different cities but in the same country are difficult to discriminate due to the closeness of production area while gingers from the different countries were successfully distinguished on the basis of their chromatographic profiles. After that, the prediction models were constructed by the LDA method. The lower value of

recognition ability (84%) and predictive ability (81%) were found in the ginger profiles from different cities, whereas the recognition ability and predictive ability of gingers from different countries were found to be higher (97% and 91%). This indicated that gingers from China, India, Malaysia, Thailand, and Vietnam tend to have the correct classification from the created model. Due to the high percentage of recognition ability and predictive ability, this method is suitable for tracing unclassified ginger samples and for quality control of ginger. In addition, the study of marker discovery showed that methyl [6]-gingerol, [8]-gingerol, methyl diacetoxy-[8]-gingerdiol, and acetoxy-[8]-gingerol can be used as marker for gingers from Nakonpatom. Also, Methyl [6]-gingerol and diacetoxy-[6]-gingerdiol are marker for Chiangrai gingers, while diacetoxy-[6]-gingerdiol can be used as marker for Leoy gingers. For discrimination of gingers from the different countries, [8]-gingerol and methyl [6]-gingerol are significant markers for specifying ginger from India and Thailand, respectively while methyl diacetoxy-[8]-gingerdiol, [10]-gingerol, and diacetoxy-[8]-gingerdiol can be individually employed as a marker for Chinese ginger. The developed food authentication method was proven to be an effective tool to label ginger origin. The procedure can be modified for the authentication study of other food samples.

For another food quality research, this work studied the molecular taste profiles of a specially treated beef with a sensomic approach. The dry-aged beef treated with a noble mold from LUMA company in Switzerland was selected due to because of its unique taste. Both targeted and non-targeted analyses were performed with several statistical data processing methods and a sensory evaluation. The compositions of the normally treated and dry-aged beefs were compared with four different cuts (rib eye, short loin, tenderloin and sirloin). In the targeted analysis, the contents of the basic taste compounds (amino acids, fatty acid, organic acids, cations and anions) which are related to the ordinary beef taste were examined. The LC-MS/MS technique was chosen to define the amount of amino acids, fatty acids, and organic acids in the studied beefs. In addition, various cations and anions were determined by IC. Amino acids which are well-known as taste contributors for bitter, sweet and umami taste were found as the major component in both normal and LUMA beefs. The established sensomic heatmap for data processing of amino acids

revealed the clear differentiation between normal and LUMA beef. On the other hand, non-targeted analysis required the combination of various analytical methods (LC-TOF-MS, synthesis of tentative compounds, GPC, and sensory test) to obtain the modulating compounds for the characteristic taste of LUMA. The non-targeted screening with LC-TOF-MS presented the existing chemical components in two types of beef. The calculation with OPLS-DA method yields the score plot and the loading S-plot based on the LC-TOF-MS profiles of normal and LUMA beef. The clear separation between two groups was shown in thebscore plot while the S-plot shows the possible potent marker of each group. Additionally, the three possible taste contributors were synthesized. Three N-acyl amino acids (N-palmitoyl alanine, N-palmitoyl glycine and N-palmitoyl glutamic acid) were formed by the reaction between a specific pair of fatty acid and amino acids. These synthesized products were confirmed and purified with several analytical techniques (LC-MS/MS, LC-ELSD, MPLC and NMR). LC-MS/MS was used to determine the products in normal and LUMA beef. However, the sensory experiment for the study of taste contributor could not have been performed owing to the trace amount of the synthesized products. Additionally, the fractionation experiment by GPC was conducted, and the six fractions were obtained from LUMA extract. Each fraction was evaluated with a sensory test to find the taste modulator by comparing the intensity of the basic tastes with the prepared recombinant solution of amino acids and cations. The increase in sweetness, saltiness, and bitterness were significant shown in the GPC fraction 1. The determination of amino acids and cations in the GPC fraction 1 revealed the relationship between the concentration and the taste intensity of LUMA beef. Therefore, the tentative compounds for sweet, salt, and bitter taste contributors in LUMA beef were predicted from this information. Further studies to define the specific compounds responsible for the taste of LUMA beef should be performed.

Regarding food safety, the method was developed to solve the problem of food matrix on the quantitative analysis of pesticide residue, which is always present at a low trace level. For routine analysis, the problem of matrix-induced response enhancement effect predominantly occurs in GC due to the presence of the active sites in the system. These active sites could bind and lead to the loss of analytes via hydrogen bonding. However, the sample matrix can modify those active sites and

helps to protect the analytes. Therefore, the detected analyte concentration in a pure solvent (matrix-free solution) negatively differed from those of in the sample matrix, causing an overestimation of the quantitative results. Analyte protectant (AP) is a chemical of choice developed to solve the problem of matrix-induced response enhancement effect by binding with the active sites in the GC system instead of the matrix. Traditionally, the AP solution is added into every sample extracts before the instrumental analysis which is an inconvenient practice for a routine laboratory. This work developed a new approach of using AP which is called AP priming. The AP solution was injected into the GC system as a first vial before the sequence analysis of the sample extracts. The performance of using the new AP process was compared with the classical approach in the determination of the spiked 100 pesticides in red chili. Additionally, the analysis of these 100 compounds in the solvent was also performed to observe the matrix effect and the effect of using two AP approaches to compensate the problem. The studied 100 pesticides were classified into early-, middle-, and late-eluting compounds for monitoring. The effect of the sample matrix was found and two AP applications provided a similar performance for problem solving. For the AP priming evaluation, long-term experiments (50 injections) were conducted with and without the use of AP priming for 100 pesticide residues in chili matrix at the trace level. Due to the capability to bind hydrogen bond with the active sites, the early- and middle-eluting pesticides tend to suffer more from matrix effect than late-eluting compounds. Therefore, AP priming significantly affect the matrix compensation for 50 injections especially for the early- and middle-eluting pesticides because of their polarity. The intensities and reproducibility of the results at the trace level was improved by the AP priming approach in the long-term injection which is practical suitability. Moreover, the uses of mixed and single AP to determine the 100 studied pesticides were compared and the results showed that D-sorbitol is considered as a suitable AP for priming in the case of its priming performance, price and commercial availability. This procedure is fit-for-purpose in a routine practice in which large amounts of samples were analyzed because it is simpler and cheaper to use; it offers high accuracy and precision, it gives applicability to a wide variety of sample batches; and it induces less system maintenance needs. For Thai food samples, AP priming might be an effective approach to determine the chemical residue because

several types of food have complex matrices which could lead to inaccuracy of results. This work investigated the new approach of using AP. Extending the experiment of AP priming evaluation is recommended to find the other possibilities in food application.

The study of food research in case of food quality and food safety issues in this work proved the effectiveness and the versatility of chromatographic and analytical techniques as a potential tool for obtaining new information or solving the analytical food problem. These studies could be used as a model for extended applications of analytical chemistry to other fields of research, for product inspection, or development in food industry.

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APPENDIX

Appendix

4.1.2 Optimization of extraction methods

Peak are data for MeOH volume optimization = 10,20,30,40,50,60,70,80 ml

MeOH volume (mL)	6-gingerol	8-gingerol	10-gingerol
10	223.11	50.94	59.41
	208.18	48.48	58.51
	213.56	48.20	56.04
average	214.95	49.21	57.99
%RSD	3.52	3.07	3.01
20	279.52	59.66	68.72
	282.78	63.74	70.39
	287.45	59.22	71.09
average	283.25	60.88	70.06
%RSD	1.41	4.09	1.74
30	349.71	73.16	81.75
	356.71	76.63	82.57
	364.48	75.39	88.05
average	356.96	75.06	84.12
%RSD	2.07	2.34	4.07
40	378.68	79.08	79.98
	409.61	82.77	88.17
	453.13	95.02	105.23
average	413.81	85.62	91.13
%RSD	9.04	9.75	14.13
50	431.01	94.31	92.30
	420.86	99.24	101.02
	393.81	93.82	87.55
average	415.23	95.79	93.62
%RSD	4.63	3.13	7.30
60	399.39	94.34	91.75
	431.19	99.90	93.14
	424.99	101.77	92.95
average	418.52	98.67	92.61
%RSD	4.03	3.92	0.82
70	416.51	114.79	102.18
	403.59	115.06	103.30
	382.84	116.76	109.24
average	400.98	115.54	104.90
%RSD	4.24	0.92	3.62
80	386.71	123.73	109.96
	406.93	110.58	99.43
	403.51	119.27	111.01
average	399.05	117.86	106.80
%RSD	2.71	5.67	6.00

4.1.3 Method validation

Peak area data for intra-day and inter-day precision

	Replicate	**peak1**	**peak2**	**peak3**	**peak4**	**peak5**	**peak6**	**peak7**	**peak8**	**peak9**
DAY1	1	254.52	217.14	368.92	19.05	18.03	42.06	103.57	357.60	209.40
	2	248.60	214.55	360.00	19.09	17.41	42.38	101.66	351.51	206.09
	3	251.05	215.88	367.40	19.65	17.11	43.12	103.54	358.92	210.30
	4	251.34	215.52	367.33	20.19	18.08	41.96	101.74	357.35	209.33
	5	249.87	215.82	365.45	19.35	17.84	42.44	102.44	354.93	207.72
	6	248.08	215.01	365.69	19.29	17.39	43.47	103.32	357.65	209.68
	Average	250.58	215.65	365.80	19.44	17.64	42.57	102.71	356.32	208.75
	SD	2.32	0.89	3.11	0.43	0.40	0.60	0.89	2.69	1.56
	%RSD	0.93	0.41	0.85	2.19	2.25	1.41	0.86	0.76	0.75
DAY2	1	263.01	222.38	369.22	20.77	17.16	40.13	105.57	359.15	210.49
	2	260.60	215.47	359.15	20.34	18.61	41.14	103.37	353.16	212.47
	3	249.30	218.36	362.30	20.79	17.00	39.16	100.22	354.56	215.38
	4	252.34	217.47	365.70	19.31	17.28	38.19	102.48	350.57	211.25
	5	259.16	216.23	367.63	20.27	18.33	40.88	104.25	356.84	206.58
	6	247.38	215.35	360.22	20.28	17.69	41.36	101.85	355.74	208.26
	Average	255.30	217.54	364.04	20.29	17.68	40.14	102.96	355.00	210.74
	SD	6.48	2.64	4.10	0.54	0.66	1.25	1.87	2.98	3.11
	%RSD	2.54	1.21	1.13	2.65	3.75	3.12	1.82	0.84	1.48
DAY3	1	243.34	212.14	362.20	20.27	17.96	41.35	99.35	349.34	203.64
	2	245.73	217.36	355.16	19.26	17.27	40.34	104.25	352.25	207.37
	3	252.59	214.26	361.63	19.37	17.46	39.24	100.25	345.88	205.74
	4	249.36	210.27	359.27	20.57	18.15	40.35	103.35	355.75	203.68
	5	251.57	218.37	366.35	19.27	18.26	39.32	99.35	352.36	200.37
	6	248.27	212.57	350.27	20.25	17.14	41.53	101.86	353.37	204.48
	Average	248.48	214.16	359.14	19.83	17.71	40.35	101.40	351.49	204.21
	SD	3.50	3.16	5.69	0.60	0.48	0.97	2.09	3.44	2.35
	%RSD	1.41	1.47	1.58	3.00	2.70	2.40	2.06	0.98	1.15
Overall	Average	251.45	215.78	362.99	19.85	17.68	40.80	102.36	354.27	207.90
	SD	5.11	2.69	5.07	0.59	0.49	1.11	1.75	3.55	3.62
	%RSD	2.03	1.25	1.40	2.98	2.79	2.73	1.70	1.00	1.74

4.1.5 Sample analysis

The peak area of characteristic peaks of ginger samples from different origins

Country of origin	Sample no.	Relative peak area								
		Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9
China	1	70.487	22.089	60.683	14.593	16.767	20.333	48.743	55.583	123.485
	2	130.031	50.55	87.603	24.654	44.669	39.231	180.304	107.265	248.881
	3	115.928	59.722	133.668	26.17	39.921	39.95	133.188	137.01	212.695
	4	140.092	39.024	94.029	15.124	17.57	31.47	42.093	79.769	163.944
	5	130.458	31.275	68.152	18.229	25.869	28.607	58.25	87.191	171.189
	6	112.222	18.439	46.431	14.035	17.885	16.481	33.684	61.426	116.785
	7	130.542	48.734	84.866	21.569	33.727	36.465	108.224	99.777	189.026
	8	179.821	71.605	136.096	30.008	40.16	46.92	117.136	134.252	247.331
	9	144.655	27.164	47.231	17.849	28.021	30.608	47.401	67.39	144.976
	10	123.227	31.496	51.296	19.088	33.302	31.199	95.719	78.967	180.995
	11	91.076	18.46	28.638	15.91	11.327	20.007	18.266	44.007	84.849
	12	92.289	21.071	34.667	11.166	16.089	21.794	28.678	53.081	88.684
	13	100.573	37.331	65.045	27.012	12.126	23.353	37.124	54.184	106.557
	14	107.35	45.944	77.055	17.076	27.595	29.486	111.634	77.334	178.055
	15	79.943	44.95	70.232	22.322	12.867	25.873	34.364	57.181	96.319
	16	191.862	104.952	157.346	28.213	43.109	57.405	189.046	152.792	314.368
	17	147.272	111.726	173.412	25.36	42.307	43.958	175.364	143.11	242.466
	18	107.647	47.79	77.399	13.642	22.059	27.242	60.586	66.267	147.124
India	19	240.649	170.205	656.417	14.916	40.988	53.916	172.361	402.221	212.361
	20	212.71	113.187	456.818	19.481	43.143	35.78	128.688	313.389	162.268
	21	219.417	165.85	498.642	15.834	46.599	38.446	155.429	383.399	194.951
	22	154.597	91.582	291.132	12.567	32.649	27.331	94.487	247.765	121.598
	23	173.232	142.881	402.101	13.615	31.757	36.11	128.143	269.12	144.226
	24	353.305	304.986	587.916	17.374	75.351	78.741	275.572	513.894	303.984
	25	313.554	158.261	542.817	9.966	57.796	62.196	137.477	332.147	202.665
	26	286.388	187.706	578.746	14.871	64.667	66.239	148.708	443.37	217.344
	27	121.523	57.454	214.465	14.228	24.388	45.026	52.667	148.651	103.244
	28	179.764	118.443	363.162	23.231	33.253	72.587	125.914	249.149	168.918
	29	146.485	88.44	215.474	18.07	23.444	46.571	77.605	216.608	121.447
	30	282.762	223.249	618.24	46.572	58.452	94.576	225.573	545.419	263.434
	31	346.346	283.911	614.447	44.444	52.399	110.875	222.46	473.963	289.349
	32	196.687	201.434	554.978	41.051	51.447	86.677	164.959	368.596	181.102
	33	258.536	231.419	483.235	36.527	38.594	88.45	128.403	395.327	218.332
	34	205.509	106.94	469.648	23.846	33.195	76.595	118.498	317.277	207.747
	35	234.994	277.708	489.094	42.457	53.552	92.093	228.405	374.17	199.958
	36	129.661	133.18	261.621	19.824	24.44	49.894	100.268	250.417	133.333
Malaysia	37	216.81	147.838	272.72	10.884	27.378	45.391	91.164	324.455	155.068
	38	162.377	127.273	244.641	12.13	20.642	37.183	75.906	274.397	130.908

	39	196.689	129.02	364.323	25.469	35.61	72.307	119.713	375.873	231.604
	40	195.869	116.268	200.89	18.62	26.579	62.212	100.646	321.274	213.62
	41	181.246	135.711	236.549	27.564	27.406	57.641	96.284	390.151	164.133
	42	149.197	115.729	208.074	15.065	18.287	51.602	90.482	281.599	166.827
	43	173.397	85.933	359.65	26.156	25.474	65.284	116.403	351.301	170.794
	44	230.154	156.926	333.985	35.644	36.285	73.547	157.606	486.259	205.019
Vietnam	45	322.332	186.147	331.41	61.496	87.754	98.669	348.902	390.44	395.191
	46	270.203	160.446	279.422	43.053	72.001	93.742	392.582	392.03	368.063
	47	183.917	93.81	165.89	17.211	20.411	56.178	97.472	186.705	213.636
	48	473.89	212.807	364.338	82.381	97.31	124.126	350.885	615.966	643.172
	49	245.304	156.515	328.680	30.786	26.250	85.417	153.437	241.150	313.581
	50	325.526	232.199	389.185	50.167	60.383	110.346	276.749	475.577	464.962
	51	280.264	152.384	260.563	37.79	51.179	82.616	220.363	314.651	388.392
	52	180.988	115.145	206.927	21.628	20.513	62.108	107.198	183.405	225.910
	53	264.319	95.641	147.233	23.026	29.309	51.817	121.365	247.565	336.415
	54	110.529	43.567	127.356	17.531	14.092	35.847	40.597	133.947	93.300
	55	98.387	47.373	85.148	15.437	14.810	36.112	30.363	103.853	105.008
	56	114.292	42.295	79.545	18.676	21.919	36.655	35.075	127.929	111.589
	57	129.801	47.009	84.892	14.618	16.893	29.817	70.226	133.324	173.823
	58	171.220	76.806	133.735	34.010	48.679	37.039	109.361	178.905	256.529
	59	82.779	49.072	80.708	17.864	14.673	32.363	33.612	119.618	90.634
	60	155.175	59.235	99.941	20.573	22.648	40.006	38.677	147.903	129.073
	61	158.184	42.398	108.095	22.823	42.041	52.993	65.336	133.449	210.786
	62	258.500	122.880	184.526	27.933	33.226	62.981	127.777	290.667	327.537
Chiangmai	63	308.507	185.140	401.773	26.627	49.071	44.321	127.923	335.571	224.459
	64	281.543	76.007	292.716	30.687	30.687	31.370	72.504	198.875	227.752
	65	890.890	506.192	1615.948	25.821	219.216	133.495	656.794	1095.220	760.340
	66	315.591	120.657	549.402	21.076	36.098	41.805	91.363	280.099	217.953
	67	780.152	541.756	1147.468	69.765	91.144	95.661	336.557	728.899	631.301
	68	912.152	380.034	1312.808	41.967	126.312	85.327	298.923	662.031	587.053
	69	359.806	169.595	477.858	21.886	45.045	40.717	95.842	238.081	206.794
	70	531.881	205.624	1102.141	28.368	82.628	83.240	264.764	611.487	433.355
	71	938.006	348.607	1314.730	45.115	152.339	112.490	300.714	641.302	686.569
	72	635.553	349.928	785.945	22.885	132.745	86.500	326.157	550.665	562.320
	73	624.789	397.163	812.851	27.197	126.638	74.098	270.142	483.927	478.854
	74	850.058	507.850	1280.790	75.105	109.904	117.930	365.698	721.091	640.142
	75	488.108	297.053	696.403	56.931	67.431	64.673	237.553	502.195	280.172
	76	642.802	341.002	741.920	19.215	165.432	79.495	306.667	497.877	495.220
	77	604.484	352.250	823.270	23.454	123.775	92.670	450.136	658.010	594.120
	78	490.901	287.486	702.533	55.429	72.033	69.712	201.213	523.261	276.050
	79	629.733	378.015	894.949	30.679	134.611	96.993	387.336	668.513	629.503
	80	825.478	423.461	1709.706	80.428	143.938	116.339	431.230	839.365	634.730
Chiangrai	81	241.152	236.431	397.600	7.301	25.061	44.178	106.839	341.887	206.424
	82	156.574	165.756	273.933	6.499	9.315	31.608	76.503	163.281	136.749

	83	340.211	354.543	596.251	10.896	30.650	66.352	154.122	422.461	272.834
	84	161.185	100.658	177.839	5.054	13.115	21.844	39.206	128.320	109.831
	85	236.390	189.457	328.763	6.059	20.197	37.212	89.358	305.163	190.929
	86	416.759	398.732	681.910	12.758	36.079	87.468	198.899	427.913	375.038
	87	310.424	324.609	547.591	15.240	22.814	62.604	135.615	317.912	246.070
	88	191.822	154.834	265.123	5.533	11.764	31.175	80.007	190.905	127.673
	89	161.393	133.036	221.835	8.246	7.481	28.222	62.611	145.439	119.042
	90	328.606	330.503	561.645	9.260	21.631	72.085	152.132	366.819	295.291
	91	230.080	186.481	311.142	6.433	16.859	30.419	86.779	216.892	189.295
	92	264.976	250.025	434.567	5.196	22.872	46.723	97.059	333.664	201.039
	93	242.117	240.312	412.185	6.267	18.479	44.891	94.927	310.981	199.936
	94	271.205	232.149	394.343	9.984	15.810	43.561	90.442	295.166	201.518
	95	348.318	251.326	427.886	8.746	22.685	49.196	99.132	340.813	237.008
	96	309.354	269.274	465.981	11.877	28.983	54.642	146.728	416.746	257.224
	97	315.725	294.067	511.709	10.803	20.963	59.303	119.697	425.049	244.671
	98	427.685	315.757	548.399	16.471	23.269	69.960	126.386	446.985	293.226
Leoy	99	180.374	65.024	236.224	12.373	28.483	11.675	59.259	144.406	140.821
	100	230.923	125.587	216.864	14.142	24.592	13.914	64.948	176.848	133.343
	101	191.014	83.974	159.104	12.436	28.469	12.525	59.365	171.855	102.196
	102	110.798	42.805	97.9272	10.19	16.44352	16.165	37.52804	95.084	80.76
	103	182.667	89.353	183.259	10.265	22.699	19.968	69.416	173.264	124.317
	104	342.527	201.275	367.049	33.568	38.232	22.561	111.414	275.598	267.084
	105	338.573	170.776	319.004	20.983	60.889	18.823	90.235	211.229	110.617
	106	174.167	75.045	156.144	11.503	25.935	10.099	46.209	129.228	106.474
	107	267.174	158.149	286.135	20.386	39.149	16.359	75.221	219.653	163.007
	108	256.056	145.616	259.959	12.111	40.598	17.765	82.507	200.156	144.985
	109	240.337	141.554	272.482	15.504	40.339	31.212	77.018	196.902	151.019
	110	163.589	78.134	175.964	16.633	27.931	29.066	61.306	152.678	126.342
	111	170.325	78.529	124.415	9.596	31.903	40.979	75.235	185.487	116.627
	112	175.098	87.414	134.621	21.143	36.331	38.367	87.906	177.282	123.091
	113	186.967	99.04	141.828	25.771	42.387	40.486	87.072	212.376	122.75
	114	179.872	151.022	224.817	20.586	32.681	47.979	82.038	238.392	157.521
	115	207.945	128.098	184.353	19.711	33.561	46.044	81.189	211.961	165.29
	116	165.144	95.056	147.352	24.996	28.707	36.749	62.338	168.758	111.882
Nakonpatom	117	112.584	102.649	200.391	10.230	21.877	50.714	79.761	210.854	99.939
	118	120.504	98.539	172.602	14.908	13.578	120.259	113.123	245.059	142.703
	119	173.109	206.814	392.692	26.600	29.491	90.233	153.171	435.840	184.526
	120	194.770	132.125	282.650	30.402	38.156	60.268	140.236	343.579	201.188
	121	87.376	61.325	110.258	13.322	17.907	32.000	62.909	191.450	99.116
	122	108.245	124.514	205.618	16.949	23.422	50.635	121.908	250.964	128.819
	123	129.754	140.482	216.023	17.306	24.427	50.758	112.232	247.410	117.200
	124	101.026	70.469	104.304	10.900	14.721	35.609	72.029	163.647	99.975
	125	173.385	129.665	291.469	29.668	32.801	67.532	123.184	285.530	176.846
	126	113.879	107.442	185.335	13.803	23.119	43.067	66.094	240.158	113.572

	127	226.220	182.126	327.636	36.107	47.056	92.332	255.368	463.890	256.124
	128	126.924	118.443	180.606	17.727	23.766	49.153	106.546	258.525	130.425
	129	106.019	103.128	152.520	15.291	21.587	45.500	79.491	271.636	96.967
	130	139.560	118.418	177.247	15.433	22.458	49.571	115.295	215.983	142.955
	131	154.137	129.052	192.016	17.611	27.059	48.822	121.075	233.971	133.428
	132	125.782	100.686	148.280	16.109	20.406	45.375	67.936	195.858	124.650
	133	163.237	169.616	255.131	30.710	32.729	66.385	135.555	360.446	137.324
	134	153.558	155.759	234.404	26.786	28.280	60.690	153.292	328.217	131.251
Petchaboon	135	97.396	64.656	118.714	11.269	19.934	36.184	96.883	198.300	115.385
	136	105.317	80.941	128.452	12.347	20.291	51.603	102.424	207.700	124.604
	137	102.198	52.098	83.514	11.923	17.976	30.198	70.644	194.221	91.415
	138	160.157	109.522	251.879	23.580	30.570	57.549	116.323	298.796	136.460
	139	136.529	106.200	160.206	15.876	23.619	44.306	91.071	243.892	109.182
	140	153.305	100.492	163.038	15.140	21.293	41.550	102.552	213.341	110.712
	141	159.827	116.415	180.178	18.635	27.975	49.835	126.360	256.949	153.391
	142	101.021	81.574	130.179	12.712	19.983	38.915	85.041	176.708	103.109
	143	153.243	141.556	213.987	23.589	33.776	66.182	182.210	289.904	128.755
	144	136.438	113.050	175.528	14.501	21.950	47.342	107.121	262.225	137.993
	145	108.476	62.551	99.737	13.020	15.278	34.386	72.721	152.407	101.722
	146	117.556	56.654	97.023	9.981	16.556	32.623	53.713	142.296	101.264
	147	201.008	152.057	260.312	12.842	49.199	43.240	154.351	382.649	189.164
	148	213.238	182.285	300.134	27.600	40.240	65.510	161.274	395.378	193.416
	149	146.255	95.734	146.976	17.924	26.727	44.367	112.483	223.118	143.639
	150	109.315	80.020	122.180	11.965	20.423	25.772	59.012	163.130	88.639
	151	122.602	97.244	145.110	11.713	25.185	35.103	84.252	205.561	115.638
	152	137.534	105.963	166.280	30.474	36.048	40.349	128.697	297.818	153.901

4.1.6 Data analysis

4.1.6.5 Marker discovery

1) The calculated T-value of ginger profiles from five cities with T-test (T critical~2)

	peak1	peak2	peak3	peak4	peak5	peak6	peak7	peak9
Chiangmai vs all	5.655	0.179	8.163	0.940	5.124	-3.910	3.469	5.013
Chiangrai vs all	1.430	9.433	4.888	-12.220	-10.747	-1.441	-4.265	1.570
Leoy vs all	5.917	0.709	-0.100	4.717	6.236	-2.329	-2.444	2.246
Nakonpatom vs all	-10.862	-5.308	-7.440	1.018	-5.071	3.345	-0.129	-6.724
Petchaboon vs all	5.413	0.179	8.163	0.940	5.124	-3.910	3.469	5.013

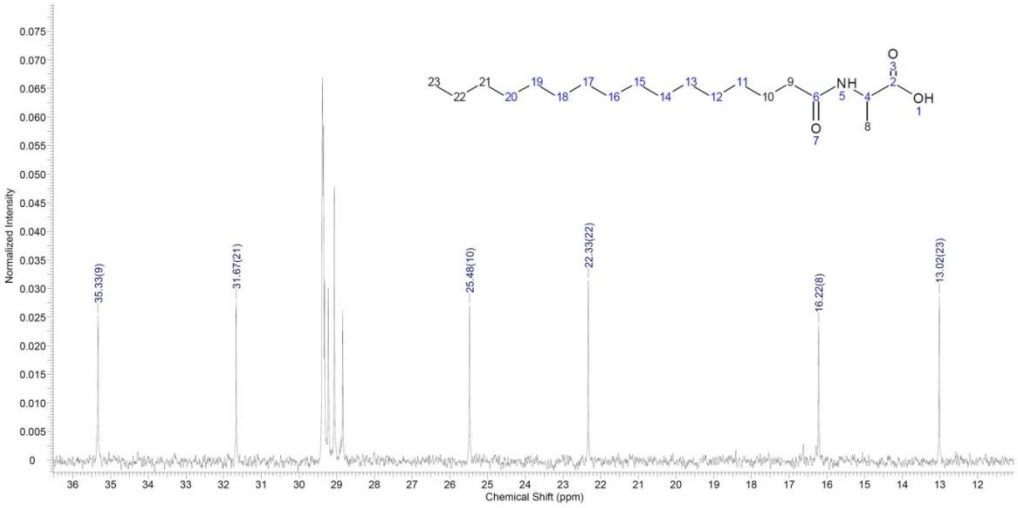
2) The calculated T-value of ginger profiles from five countries with T-test (T critical~2)

	peak1	peak2	peak3	peak4	peak5	peak6	peak7	peak9
China vs all	8.516	1.056	-1.549	7.871	10.247	11.796	5.489	19.636
India vs all	-8.144	-0.568	7.356	-5.865	-5.472	-2.974	-3.246	-8.136
Malaysia vs all	-9.939	-4.602	-3.751	-6.813	-11.050	-5.188	-7.284	-7.612
Thailand vs all	2.236	3.783	1.400	-3.504	-0.132	-6.556	-4.533	-4.603
Vietnam vs all	-1.981	-2.685	-4.834	0.137	-1.742	2.164	-0.168	0.828

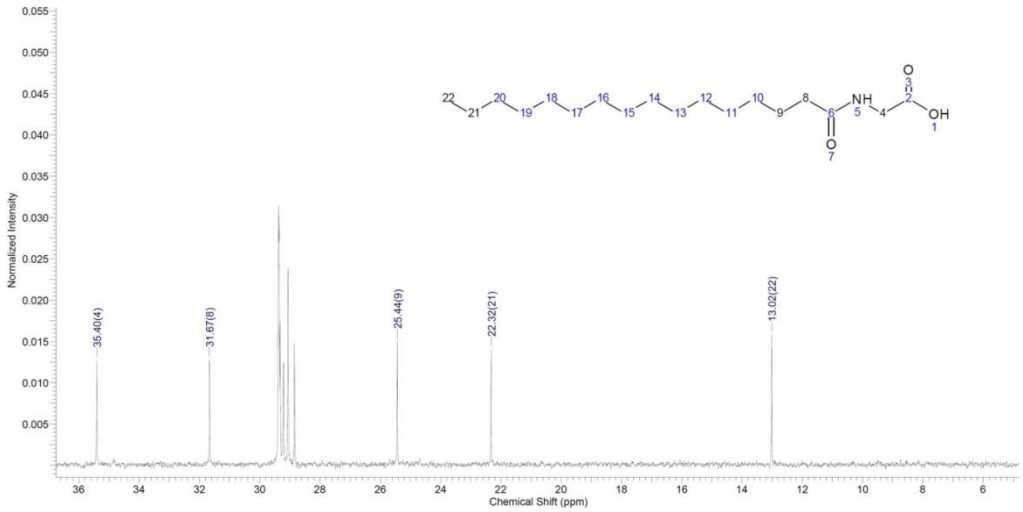
4.2.2.2 Synthesis of possible taste modulators

13C NMR spectrum for N-palmitoyl alanine, N-palmitoyl glycine, and N-palmitoyl glutamic acid

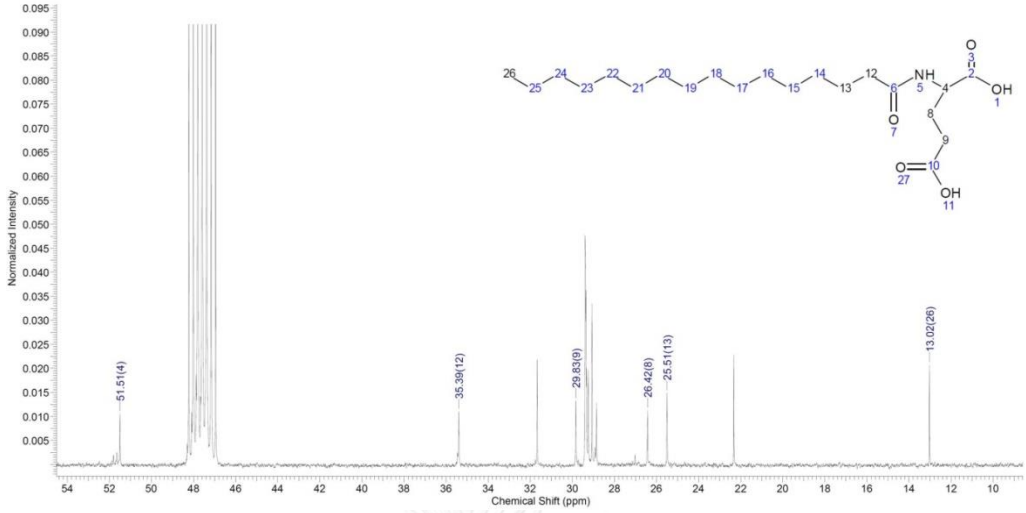
N-palmitoyl alanine



N-palmitoyl glycine

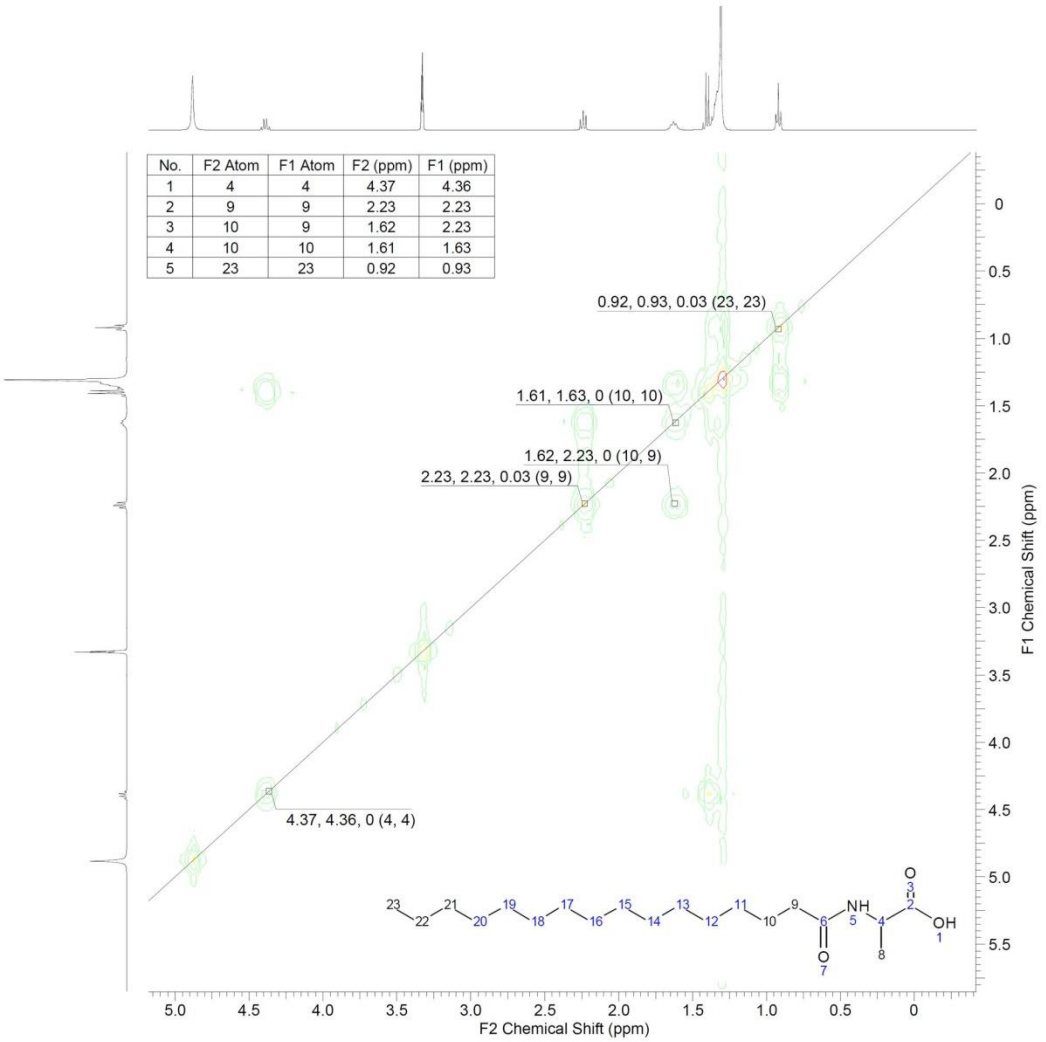


N-palmitoyl glutamic acid

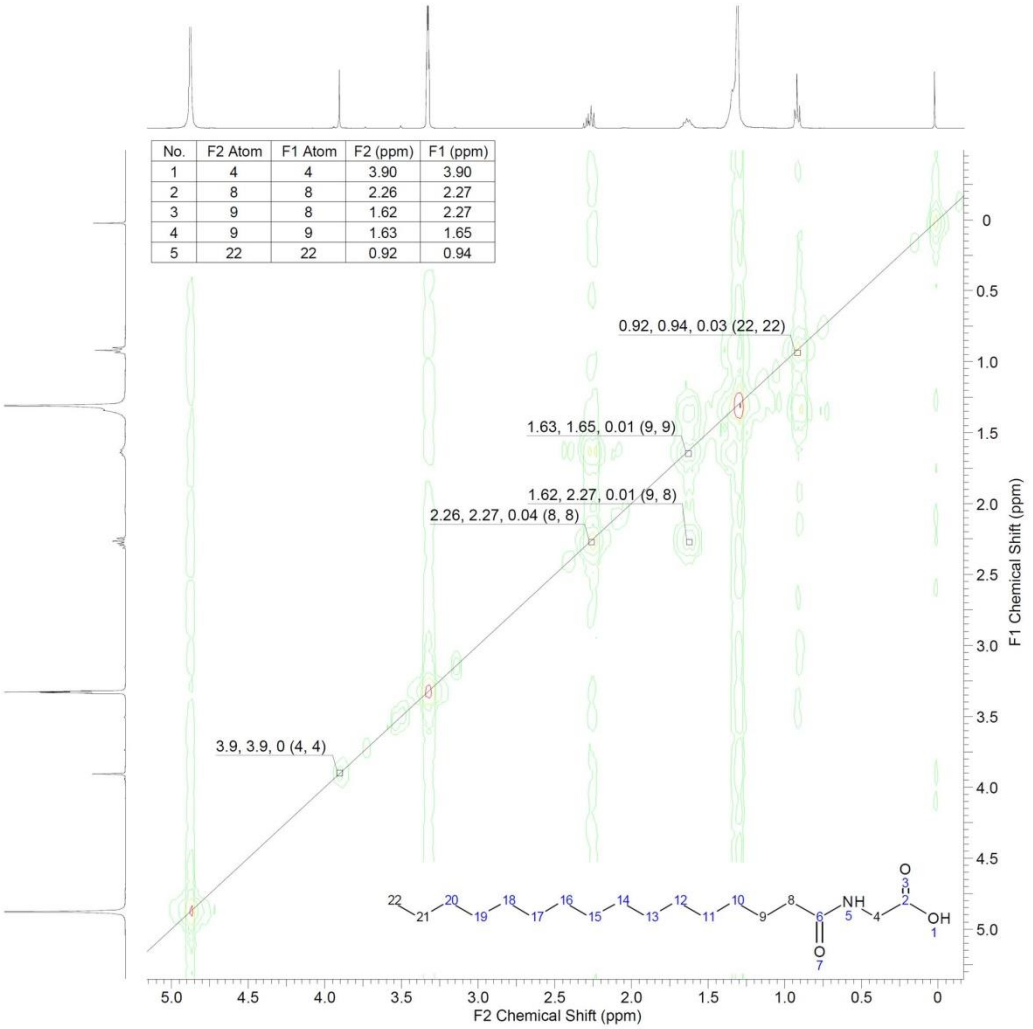


COSY NMR spectrum for N-palmitoyl alanine, N-palmitoyl glycine, and N-palmitoyl glutamic acid

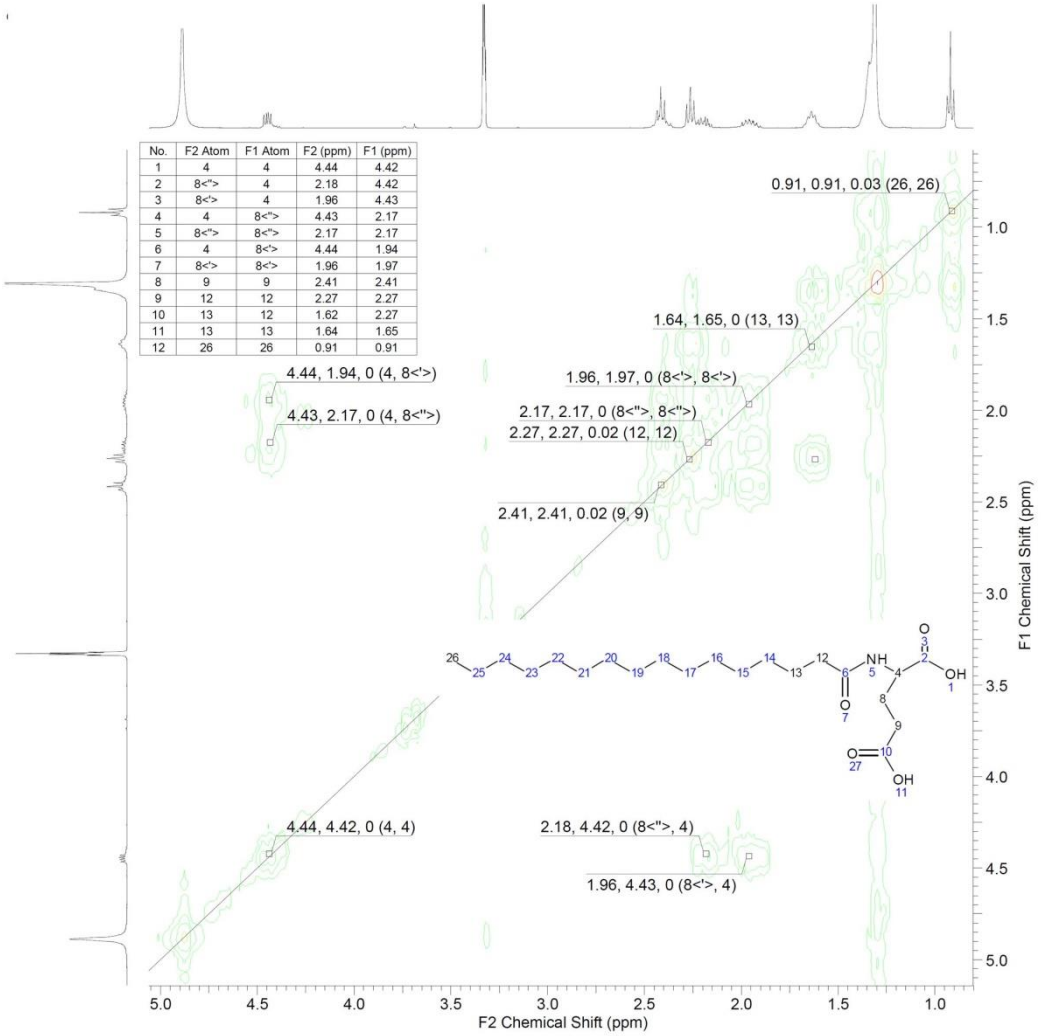
N-palmitoyl alanine



N-palmitoyl glycine



N-palmitoyl glutamic acid



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3	8<'>	4	1.96	4.43
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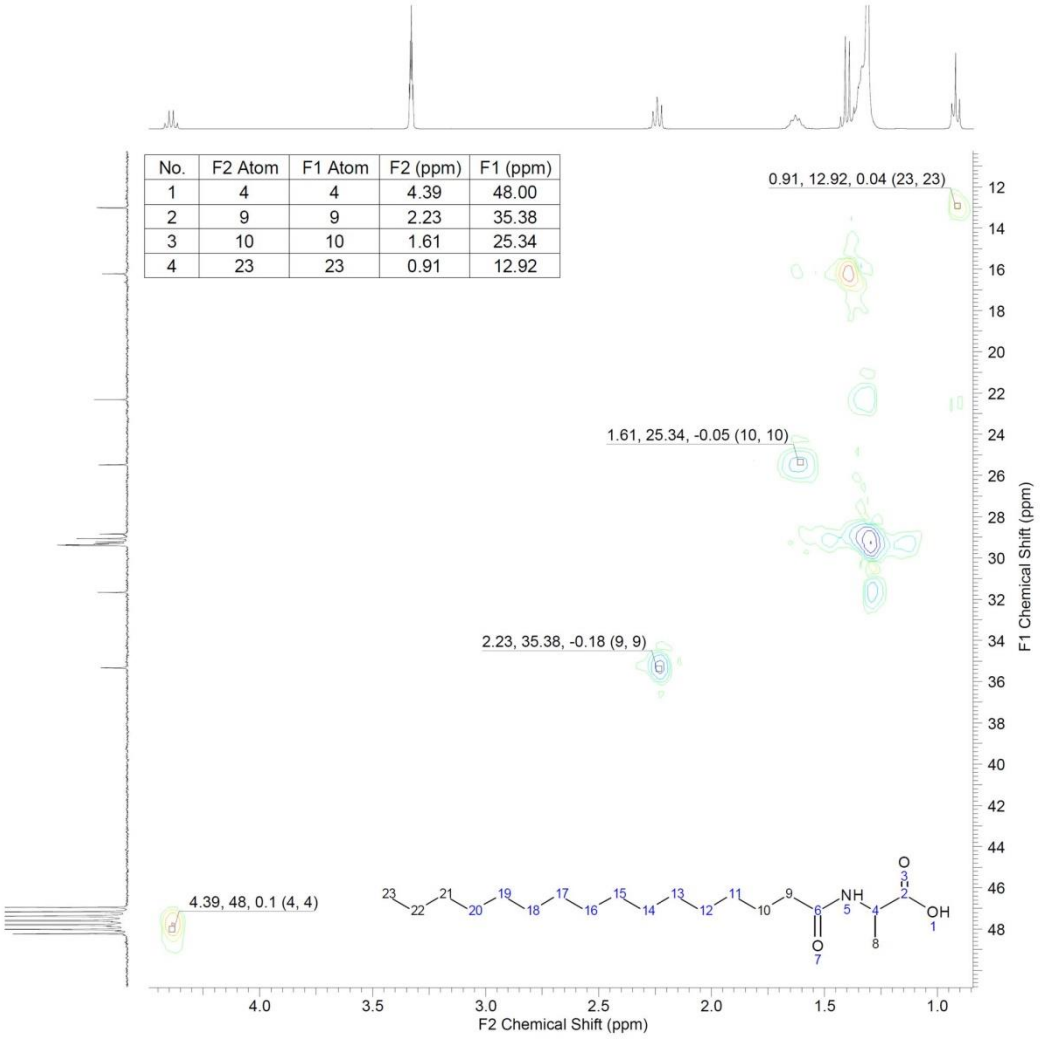
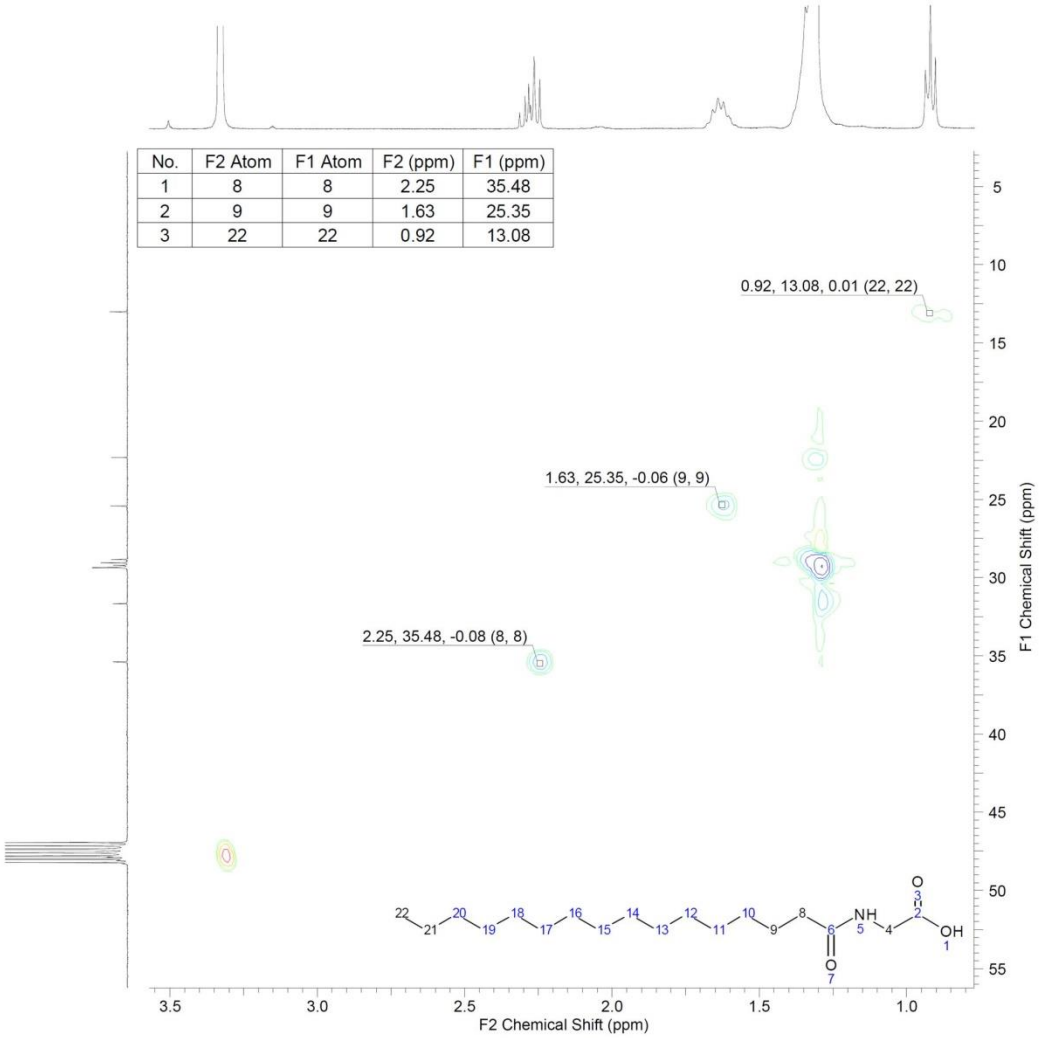
5	8<">	8<">	2.17	2.17
6	4	8<'>	4.44	1.94
7	8<'>	8<'>	1.96	1.97
8	9	9	2.41	2.41
9	12	12	2.27	2.27
10	13	12	1.62	2.27
11	13	13	1.64	1.65
12	26	26	0.91	0.91

HSQC NMR spectrum for N-palmitoyl alanine, N-palmitoyl glycine, and N-palmitoyl glutamic acid

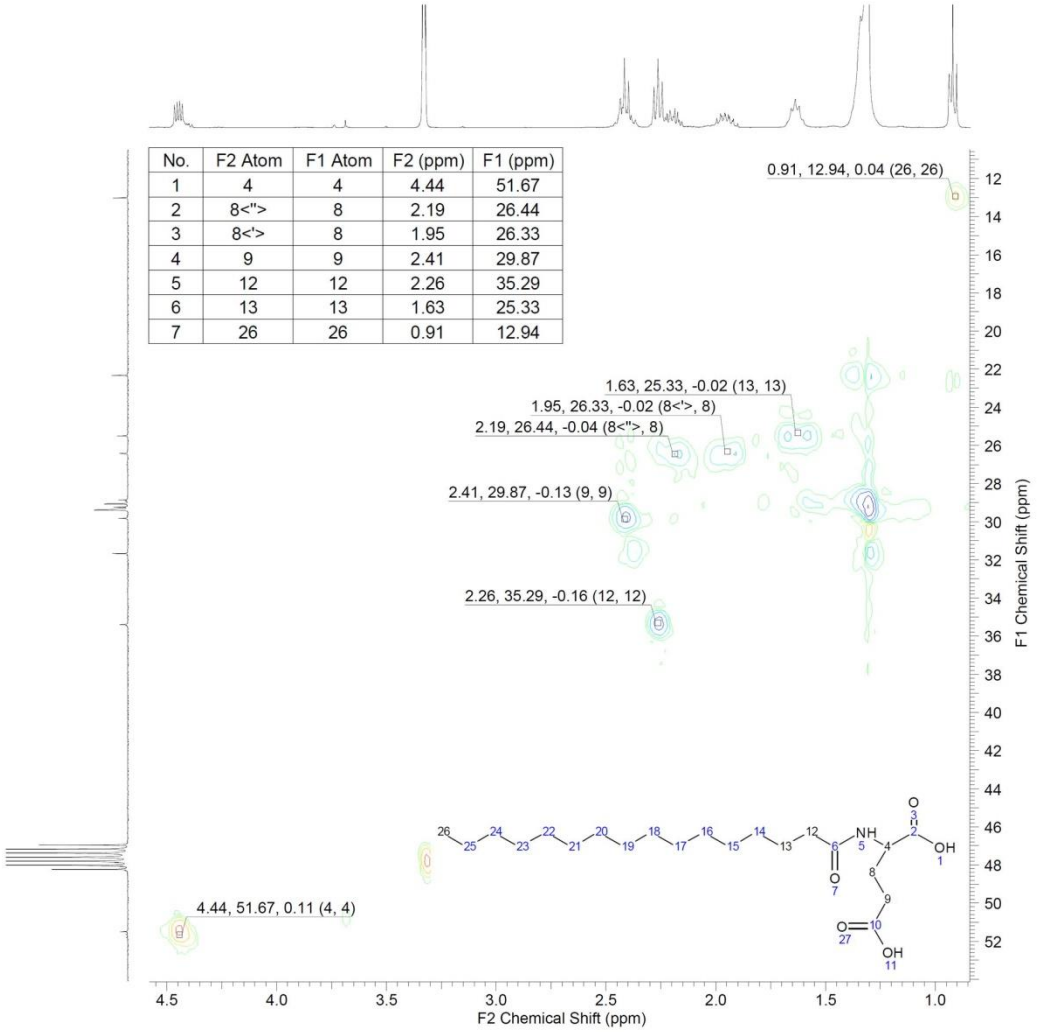
N-palmitoyl alanine



N-palmitoyl glycine



N-palmitoyl glutamic acid



VITA

Miss Soparat Yudthavorasit was born on June 25, 1985, in Bangkok, Thailand. She had graduated a Bachelor's degree of Science in Chemistry from Mahidol University in 2007. Afterwards, she continued her academic education for Master degree at Department of Chemistry, Faculty of Science, Chulalongkorn University. She had completed the program in September 2009 and received her Master's degree of Science in Analytical Chemistry in July 2010. For Ph.D. study, she finished her Ph.D. at Department of Chemistry, Faculty of Science, Chulalongkorn University in June 2015 and received her Doctor of Philosophy in Analytical Chemistry in October 2015.

Poster presentation and proceeding

- S. Yudthavorasit, C. Chiaochan, S. Jayanta, N. Leepipatpiboon Determination of Macrolide Antibiotic Residues in Water Using Hollow-Fiber Liquid-Phase Microextraction. 35th Congress on Science and Technology of Thailand, 15-17 October 2009, The Tide Resort (Bangsean Beach), Chonburi, Thailand

- S. Yudthavorasit, C. Chiaochan, N. Leepipatpiboon Hollow-Fiber Liquid-Phase Microextraction for the determination of Macrolides and Tetracyclines residue in water sample. 4th International Symposium on Recent Advances in Food Analysis (RAFA 2009), 4-6 November 2009 , Diplomat Hotel Conference Centre, Prague, Czech Republic

- S. Yudthavorasit, K. Wongravee, N. Leepipatpiboon Geographical Classification of Volatile Components in Ginger(Zingiber officinale) by Gas Chromatography-Mass Spectrometry and Chemometric Method. Pure and Applied Chemistry International Conference 2013 (PACCON2013), 23-25 January 2013, The Tide Resort (Bangsean Beach), Chonburi, Thailand

- S. Yudthavorasit, W. Meecharoen, N. Leepipatpiboon Practical Approach for Using Analyte Protecant in Routine Analysis. 6th International Symposium on Recent Advances in Food Analysis (RAFA 2009), 5-8 November 2013 , Clarion Congress Hotel, Prague, Czech Republic (Poster: Poster Excellence Award)

Publication

- S. Yudthavorasit, C. Chiaochan, N. Leepipatpiboon Simultaneous determination of multi-class antibiotic residues in water using carrier-mediated hollow-fiber liquid-phase microextraction coupled with ultra-high performance liquid chromatography tandem mass spectrometry. Microchimica Acta (2011) 172:39–49.

- C. Chiaochan, U. Koesukwiwat, S. Yudthavorasit, N. Leepipatpiboon Efficient hydrophilic interaction liquid chromatography–tandem mass spectrometry for the multiclass analysis of veterinary drugs in chicken muscle. Analytica Chimica Acta (2010) 682:117-129.

- S. Yudthavorasit, K. Wongravee, N. Leepipatpiboon Characteristic fingerprint based on gingerol derivative analysis for discrimination of ginger (Zingiber officinale) according to geographical origin using HPLC-DAD combined with chemometrics. Food Chemistry (2014) 158:101-111.

- S. Yudthavorasit, W. Meecharoen, N. Leepipatpiboon New practical approach for using an analyte protectant for priming in routine gas chromatographic analysis. Food Control (2015) 48:25-32.