Doctoral Thesis

Evaluation of Functional Chemical Components and Radical Scavenging Activity in Thailand Foods

Academic year: 2022

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Abstract

Thailand's fermented foods and ingredients are well-liked both domestically and abroad due to their distinct flavor, pleasant aroma, and several health benefits for consumers. Even the most well-known foods, their chemical components and radical scavenging activity have not yet been reported or updated. Moreover, these health benefits of Thailand's food are due to the antioxidant properties of herbs and spices, which could have a considerable inhibitory effect on fish lipid oxidation. However, these data have not yet been clearly revealed, and there is no conventional way to simultaneously analyze the numerous antioxidants present in plants, which is necessary to evaluate the chemical components data of the substances. From the above background, the authors would like to study Thailand's foods, especially fermented fish products and herbs and spices. The results aimed to understand well about Thailand food products' characteristics and benefits related to human health.

Chapter 2 focused on each chemical components and radical scavenging activity in Thailand's fermented food products, including 11 locally fermented fish or aquatic animals as a source. All samples were analyzed contents of lipophilic substances including fatty acids and tocopherol, as well as water-soluble substances including amino acids, purine and pyrimidine monophosphates, and organic acids. Furthermore, the radical scavenging activity of lipophilic and water-soluble substances were evaluated, and the correlation between samples and chemical components was calculated using principal component analysis (PCA). The results of these analyses were very different in each dataset but overall, Nam Pla (fish sauce), Tai Pla (fermented fish organs), and Kapi (shrimp paste) contained higher essential chemical components especially free amino

acids and purine and pyrimidine monophosphates together with higher radical scavenging activity than the other samples. In conclusion, these three samples were essential for human health. The PCA results also showed a strong correlation between the chemical components and samples.

Chapter 3 focused on the development of high-performance liquid chromatography (HPLC) systems for the analysis of antioxidant substances in edible plants from Asia. The newly developed method could separate 14 different antioxidant compounds including gallic acid, epigallocatechin (EGC), caffeine, epigallocatechin gallate (EGCG), coumaric acid, vanillic acid, epicatechin gallate (ECG), rosmarinic acid, gingerol, thymoquinone, carvacrol, thymol, carnosol, and carnosic acid in 70 min, together with a good result of method validation including limit of detection (LOD), limit of quantification (LOQ), intra-, and inter-day precision and recovery. Next, this method was used to analyze 12 edible plants from Asia. The proposed method for analyzing these 14 antioxidant compounds was used to obtain consistent results with other reports and could show more varieties of antioxidant compounds present in each plant, with a visible identification of each antioxidant from each compound.

Chapter 4 focused on the chemical components, total polyphenol content, radical scavenging activity, and effects on lipid oxidation in fish during storage in 11 herbs and spices from Thailand. All samples were analyzed for the contents of lipophilic substances including fatty acids, tocopherols, and sterols, as well as water-soluble substances including amino acids, purine and pyrimidine monophosphates, and organic acids. Furthermore, total polyphenol content and radical scavenging activity of lipophilic, water, and methanol-soluble substances were evaluated. Next, the effects of herbs and spices on lipid oxidation in fish were evaluated. After all experiments, statistical analysis was

conducted including PCA and the correlation between antioxidant compounds and antioxidant capacity. Turmeric, fingerroot, and ginger showed outstanding in terms of total polyphenol content and radical scavenging activity. The addition of herbs and spices to fish also showed good results for prevention of lipid oxidation and aldehyde generation in fish. Moreover, a positive correlation between antioxidant compounds and antioxidant capacity and a strong correlation in PCA between chemical components and samples were also observed.

From the above results, Thailand fermented fish products and herbs and spices have many health benefits, particularly due to their high antioxidant contents. These data are important for further research into food-related topics such as other various chemicals, other locally fermented fish or herbs and spices, comparisons between various production from different areas, and the effects of herbs and spices on different types of meat besides fish in various environments.

Contents

Abbreviation

[Chemicals]

ABTS	2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid
Ace	acetic acid
Ala	alanine
AMP	adenosine 5′-monophosphate
Ans	anserine
Arg	arginine
Asp	aspartic acid
ATP	adenosine 5′-triphosphate
BF_3	boron trifluoride
But	butyric acid
Cit	citric acid
CMP	cytidine 5′-monophosphate
DPPH	2,2-diphenyl-1-picrylhydrazyl
ECG	epicatechin gallate
EGC	epigallocatechin
EGCG	epigallocatechin gallate
FAME	fatty acid methyl esters
Fum	fumaric acid
Glu	glutamic acid
Gly	glycine
GMP	guanosine 5′-monophosphate

HHE	4-hydroxy-trans-2-hexenal
His	histidine
Ile	isoleucine
Isob	isobutyric acid
IMP	inosine 5′-monophosphate
Lac	lactic acid
Leu	leucine
Lys	lysine
Mal	maleic acid
Met	methionine
MUFA	monounsaturated fatty acid
Oxa	oxalic acid
Phe	phenylalanine
Pro	proline
Prop	propionic acid
PUFA	polyunsaturated fatty acid
Pyro	pyroglutamic acid
SFA	saturated fatty acid
Ser	serine
Suc	succinic acid
Tar	tartaric acid
Tau	taurine
Thr	threonine
Tyr	tyrosine

Val	valine
Vale	valeric acid

[Methods]

α	separation factor
Em	emission
Ex	excitation
GC	gas chromatography
HPLC	high-performance liquid chromatography
LOD	limit of detection
LOQ	limit of quantification
UV-VIS	ultraviolet-visible
Rs	resolution factor
RSD	relative standard deviation
SD	standard deviation
tR	retention time

Chapter 1. General Introduction

Thailand has a wide range of plant and animal species owing to its abundant natural resources. These resources are highly beneficial, well-liked, and exported to several countries as fresh, dried, frozen, and processed goods. Together with other countries' cultures, Thai people created many unique foods that are popular around the world because of their distinctive flavor, pleasant aroma, and several health benefits. Because of the popularity, many researchers have studied Thailand's food and other related ingredients for their nutrition, health benefits, and other consequences. For example, numerous studies have discussed various fermented foods available in Thailand, such as Nam Pla (fish sauce), Kapi (shrimp paste), and herbs and spices including coriander, ginger, and turmeric. Most research concludes in the same way that Thailand's fermented products and herbs and spices are very beneficial to human. Others, particularly those that were produced or eaten primarily in smaller areas, were popular only there. As a result, researchers have rarely studied or updated their most recent reports. Additionally, many researchers have reported that many health benefits in Thailand's food are from herbs and spices that are added to them, especially antioxidant contents. Nonetheless, it is difficult to confirm which antioxidant is contained in each herbs and spices due to the large amount of polyphenol, as well as many chemical compounds that can act as antioxidants.

In this thesis, the author evaluates and presents the unique characteristics of local products, particularly fermented fish products and herbs and spices from Thailand, in order to reveal and review information about these products including chemical components and radical scavenging activities. In addition, the author aimed to develop a new simple method for the analysis various antioxidants in plant samples and evaluate

their effects on fish during storage.

In Chapter 2, the author mainly focus on fermented fish products in Thailand because Thailand has an agricultural-based economy and various unique types of fermented foods, including fermented marine fish, freshwater fish, shellfish, crustaceans, meat, vegetables, and fruits, are produced in Thailand (Suphannachart and Warr 2011; Tanasupawat and Komagata 1995). These ingredients are naturally fermented, mainly with salt and some extra ingredients such as fruits, vegetables, rice bran, and so on. The foods are then kept in appropriate condition to allow microorganisms to grow and to ferment them (Tanasupawat and Komagata 1995). For example, Nam Pla (fish sauce) is made from fermented anchovy and salt until a clear brown liquid is produced (Namwong et al. 2005); Pla Ra (fermented fish) is made by fermented mixed fish with salt and roasted rice powder (Sangjindavong et al. 2008); and Pla Som (fermented fish with cooked rice) is made by fermented whole fish with cooked rice, salt, and spices (Kopermsub and Yunchalard 2010). Thailand's traditional foods are well known but to date, there is a lack of data on the functional chemical components and radical scavenging activities of these food types. Although Phithakpol (1993) previously reported the chemical components of fermented food from Thailand, the data were not comprehensive, and the study is now dated. In previous studies, the chemical composition and antioxidant activity of Kapi, a traditional fermented shrimp paste from Southeast Asia, have been reported (Faithong et al. 2010; Peralta et al. 2005; Pongsetkul et al. 2016). Nevertheless, the chemical compositions and antioxidant activities of other fermented foods in Thailand have yet to be reported.

In Chapter 3, to continue to evaluate the chemical components and radical scavenging activity of Thailand's ingredients, the author mainly focus on development of HPLC for analysis of 14 antioxidants in various plants, especially herbs and spices. Herbs and spices

have been widely used since ancient times (Tapsell et al. 2006) particularly in Asia, a region with abundant natural resources. Therefore, people cultivate these herbs and spices and use them in food, cosmetics, and medicines (Chomchalow 2002).

For instance, lemon balm (*Melissa officinalis L.*) is traditionally used for medicinal purposes as an antispasmodic for curing cold sores, and in aromatherapy (Moradkhani et al. 2010). Lemon balm contains many antioxidant compounds, including thymol, carvacrol, rosmarinic acid, and coumaric acid (Moradkhani et al. 2010; Dastmalchi et al. 2008). Similarly, clove (*Syzygium aromaticum*) is widely used as a food preservative and for medicinal purposes. It also exhibits antimicrobial and antiviral properties (Cortés-Rojas et al. 2014). Nassar et al. (2007) also reported that cloves contained thymol. Turmeric (*Curcuma longa*) is another spice used primarily as a food coloring agent and secondarily as a medicine, dietary supplement, and so on (Li et al. 2011). Turmeric has been called the "golden spice" because it is rich in nutrients, and has health benefits, such as its anti-inflammatory, anti-cancer, anti-depression effects (Sahoo et al. 2021). Kumar et al. (2006) also reported that turmeric contained coumaric acid. Concerning antioxidants, most researchers have focused on the total antioxidant activity of a sample but have not paid attention to identifying the antioxidant compounds present in the sample. However, some researchers have used the high-performance liquid chromatography (HPLC) to identify one antioxidant compound at a time. For example, Wang et al. (2004) identified rosmarinic acid and caffeic acid in aromatic herbs, Schwertner and Rios (2007) discovered gingerol and shogaol in ginger, while Türkan et al. (2020) determined catechin, caffeic acid, coumaric acid, ferulic acid, gallic acid, and quercetin in *Achillea schischkinii Sosn*, using HPLC. As mentioned above, each analysis can locate only a specific antioxidant compound and detect only a small number of these compounds. Therefore,

no conventional method has been developed for the simultaneous analysis of many functional components.

In Chapter 4, the leading health benefits of Thailand's foods come from the herbs and spices. Embuscado (2015) described herbs and spices as plants with unique fragrances and tastes composed of various flavonoid, phenolic, and sulfur-related compounds. In addition, the differentiation between herbs and spices is based on the part used. Only the leaves of herbs are used, whereas parts of the plant other than the leaves are used in spices (Embuscado 2015). Many researchers have reported the health benefits of herbs and spices, including reduced the risk of cancer, cardiovascular disease, cholesterol, and inflammation (Jiang 2019; Opara and Chohan 2014; Tapsel et al. 2006). These benefits are attributed to the antioxidants present in herbs and spices that prevent lipid oxidation and the growth of microorganisms. These effects have inspired several researchers to pursue antioxidant-related projects, such as packaging to prevent lipid oxidation and preserve food for a longer time or adding natural extracts containing high contents of antioxidants to food (Gómez-Estaca et al. 2014). All these studies aimed to prevent oxidation, maintain food freshness, and improve food quality. Herbs and spices have antioxidant and antibacterial properties and are mainly composed of polyphenols. Other fat- and water-soluble components are also present but have not been extensively researched. It is possible that these components also have antioxidant properties, similar to those of polyphenols. Although a large number of Thailand's herbs and spices are widely known, some of them remain relatively unknown, resulting in a lack of data on their effects and benefits.

Chapter 2. Evaluation of Functional Chemical Components and Radical Scavenging Activity in 11 Fermented Fish Products from Thailand

2-1. Introduction

Fermented foods are the result of a preservation process aimed at reducing fresh food waste, maintaining food over longer periods, adding value to foods, and providing a more varied diet (Rahman 2007). Different countries have different types of fermented food, depending on their leftover products, fermentation techniques, and the surrounding environment that creates the unique characteristics of each food; examples include Natto (fermented soybean), Kimchi (spicy fermented vegetable), Lassi (yogurt drink), and Nata de Coco (fermented coconut water). Even when the base of the fermented product is the same, different temperatures, fermentation periods, local bacteria colonies, and added ingredients can produce differences between fermentation products. For example, Southeast Asia has many types of local fish sauce (Ly et al. 2018); these may be known as “fish sauce” in English, but other countries use names such as Nam Pla (Thailand), Teuk Trey (Cambodia), Nga Nganpyaya (Myanmar), and Nuoc Mam (Vietnam).

In this study, 11 fermented food products from Thailand were evaluated for functional chemical components and radical scavenging. Such an investigation will not only help to determine which foods are more important for human health but also help to raise the commercial value of the products, increase international exportation, and provide a basis for further relevant research.

2-2. Materials and Methods

2-2-1. Sample Preparation

The 11 fermented food samples were collected from a local market in Thailand. The sample names (with local and English names), source location, and raw materials (with scientific names) of these samples are shown in Table 2-1. The edible part of the samples was pulverized using a blender (TK 435 TESCOM, Tokyo, Japan) and stored at -30°C until further analysis.

2-2-2. Chemicals

Potassium persulfate, L-malic acid, L-lactic acid, and acetonitrile were obtained from Sigma–Aldrich Ltd. (Tokyo, Japan); α-, β-, γ-, and δ-tocopherol were obtained from Eisai Co. Ltd. (Tokyo, Japan); triethylamine and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); AccQ-FBB and AccQ-Fluor reagents were obtained from Waters Corporation (Milford, MA, USA); oxalic acid was obtained from Nacalai Tesque (Kyoto, Japan); pyroglutamic acid was obtained from MP Biomedicals (Illkirch-Graffenstaden, France); and distilled water was obtained from Takasugi Pharmaceutical Co., Ltd. (Fukuoka, Japan). All other chemicals and solvents used in the experiment were obtained from FUJIFILM Wako Pure Chemical Industries (Osaka, Japan).

2-2-3. Lipid Extraction

Lipids were extracted from fermented samples according to the method of Ito et al. (2018). Briefly, 3 g samples were weighed into separate 50-mL glass centrifuge tubes and mixed with 30 mL chloroform:methanol (2:1, v/v). The mixture was homogenized using a homogenizer (PT2100: KINEMATICA Co., Switzerland). An aliquot of 4 mL distilled water was added to each sample. Then, the samples were mixed and centrifuged at 2500 × g for 10 min (Model 4000; KUBOTA Co., Japan). The upper layer was removed using an aspirator, whereas the lower layer was filtered using a cotton plug. The solution was evaporated using a rotary evaporator (N-1300, Tokyo Rikakikai Co. Ltd., Japan), and the residue was resuspended in chloroform:methanol solution (2:1, v/v) to a final volume of 10 mL. The final lipid solutions were used to analyze chemical components and radical scavenging activities, while the remaining solutions were stored at -30°C until further analysis.

2-2-4. Analysis of Fatty Acid Composition

The fatty acid composition of fermented samples was analyzed according to the method of Ito et al. (2018). The extracted lipids were trans-methylated by saponification and then subjected to BF3-catalyzed methylation. Briefly, the extracted lipid solution (3 mg lipid/tube) was added to a 15-mL glass test tube and evaporated using a centrifugal concentrator. The lipid sample was then hydrolyzed in a screw-capped glass tube with 750 μL of 0.5 mol/L potassium hydroxide and 100 μL of 10 mg/mL heptadecanoic acid (internal standard) in methanol at 100°C for 9 min. The reaction mixture was added to 1

mL of 14% BF3 in methanol at 100°C for 7 min. Subsequently, 3 mL hexane and 2.5 mL saturated sodium chloride solution were added to the mixture, which was then centrifuged at 2500 × g for 10 min. The upper layer containing fatty acid methyl esters (FAMEs) was transferred to a Sep-Pak silica column (630 mg; Waters) pre-washed with *n*-hexane, and FAMEs were eluted using 8 mL *n*-hexane:diethyl ether (96:4, v/v). The eluate was evaporated using a centrifugal concentrator (TAITEC, Japan), and FAMEs were reconstituted in 100 μL acetone for gas-liquid chromatography (GLC) analysis. The GLC system was comprised of a gas chromatograph (GC14A; Shimadzu Corporation, Japan) equipped with a flame ionization detector and capillary column (TC-70, 60 m × 0.25 mm i.d.; GL Science, Japan). The column temperature was programmed for a linear increase from 180°C to 230°C at a rate of 1°C/min. The injection and detector port temperatures were maintained at 250°C. The carrier gas used was nitrogen, and the flow pressure was set at 5 mL/min. The FAMEs were identified on the chromatogram by conventional methods using the retention time of standards.

2-2-5. Analysis of Tocopherol Contents

Tocopherol was analyzed according to the method of Ito et al. (2018). Briefly, 50 μL of 1% sodium chloride, 1 mL of 3% pyrogallol-ethanol solution, and 100 μL of 60% potassium hydroxide were added to 3 mg extracted lipid samples in screw-capped tubes. Using an aluminum-block heater, the mixture was gently shaken at 70°C in the dark for 30 min. Subsequently, 2.25 mL of sodium chloride solution and 1.5 mL of ethyl acetate:*n*-hexane (85:15, v/v) were added. The mixture was stirred and centrifuged at 2,500 × g for 5 min. The upper layer was transferred to a clean tube and evaporated using a centrifugal

concentrator. The unsaponifiable substances were dissolved in 500 μL of ethyl acetate:*n*-hexane (85:15, v/v) for high performance liquid chromatography (HPLC) analysis. Tocopherol content was analyzed using HPLC with fluorescence detection (HPLC-FL). This system included a PU-2080 plus pump, an 860-CO column oven (40°C) (JASCO Corporation, Tokyo, Japan), an L-7480 fluorescence detector (Hitachi High-Technologies Corporation, Tokyo, Japan), and a 7725i Rheodyne sample injector connected to a 10 μL sample loop (Rheodyne, Rohnert Park, CA, USA). This system was equipped with an Inertsil NH2 column (250 mm × 4.6 mm × 5 μm particle size, GL Science, Tokyo, Japan) connected to a Guard-Pak guard column containing μBondapak NH2 (Waters, Milford, MA, USA). The mobile phase was *n*-hexane:ethyl acetate (85:15, v/v), and the flow rate was 1 mL/min. The detector was programmed for Ex at 298 nm and Em at 325 nm. This result was identified on chromatograms by conventional methods, i.e., the retention time of standards was compared with the calibration curve of the standard solution.

2-2-6. Water-soluble Extraction

Water-soluble substances were extracted from the fermented samples according to the method of Tanaka et al. (2018). Briefly, the sample (ca. 3 g) was homogenized with 20 mL of 1 mol/L perchloric acid in a 50-mL plastic centrifuge tube. The pH of the resultant solution was then adjusted to 6.7 by mixing it with 2 mol/L potassium hydroxide. The solution was cooled for 30 min at 0°C in a commercial refrigerator, following which the glass tube was centrifuged at 2,000 × g for 15 min. The upper layer was then transferred to a 50-mL volumetric flask and made up to 50 mL with distilled water. A portion of this solution was passed through a 0.8-μm filter (Dismic-25cs; Toyo Roshi Kaisha, Ltd.,

Tokyo, Japan) to obtain the extract solution. The water-soluble solutions were subsequently used for analysis of chemical components and radical scavenging activities, while the remaining solutions were stored at -30°C until further analysis.

2-2-7. Analysis of Free Amino Acid Content

Free amino acid content was analyzed according to the method of Tanaka et al. (2018). Briefly, free amino acids were converted to AccQ-fluorescence free amino acids and then analyzed with fluorescence HPLC. The HPLC system (Hitachi Co.) included an L-7100 pump, an L-7480 fluorescence detector (excitation wavelength 250 nm, emission wavelength 395 nm), an L-7300 column oven (40° C), an L-7200 autosampler, and a D-7500 integrator. A 4 μm Nova-Pak C18 column (3.9 mm × 300 mm; Waters) was used, with mobile-phase solutions of 0.133 mol/L sodium acetate buffer solution (pH 6.43) (A) and acetonitrile/0.133 mol/L sodium acetate buffer solution (pH 6.32) (60/40) (B) over the following gradient: 0% B to 6% B for 35 min, isocratic at 6% B for 60 min, 6% B to 15% B for 85 min, 15% B to 20% B for 100 min and 20% B to 30% B for 120 min. The flow rate was 1.0 mL/min. This result was identified on chromatograms by conventional methods, i.e., the retention time of standards was compared with the calibration curve of the standard solution.

2-2-8. Analysis of Purine and Pyrimidine Contents

Purine and pyrimidine contents were analyzed according to the method of Ishimaru et al. (2016). Water-soluble extract was injected into the HPLC system. The HPLC system

included a PU-1580 pump, a DG-980-50 degasser, a CO-965 column oven (40°C), a UV-970 UV-VIS detector (wavelength, 270 nm) (JASCO Corporation), a DMC675 Mixer (GL Science), and a 7725i Rheodyne sampling injector connected to a 20 μL sample loop (Rheodyne). This system was equipped with a CAPCELL PAK MG column (250 nm × 4.6 mm, 5 μm particle size, Shiseido, Tokyo, Japan) connected to a Guard-Pak guard column containing Nova Pak-C18 (Waters). The mobile phase consisted of solutions A [water: triethylamine: phosphoric acid, 950:10:5 (v/v); pH 3.8] and B [water: acetonitrile, 90:10 (v/v)]. The gradient employed was 0 min-20 min, isocratic at 0% B; 20 min-35 min, 0% B to 35% B; 35 min-45 min, isocratic at 35% B. The flow rate was 1.0 mL/min. This result was identified on chromatograms by conventional methods, i.e., the retention time of standards was compared with the calibration curve of the standard solution.

2-2-9. Analysis of Organic Acid Content

Organic acid content was analyzed according to the method of Sekizawa et al. (2013). Water-soluble extract was injected into the HPLC system. The HPLC system included an LC-10ADVP pump, DGU4 14A degasser, CTO-10A column oven (60°C), UV-970 SPD-10A UV-VIS detector (wavelength 210 nm) (SHIMADZU Corporation, Kyoto, Japan), and a 7725i Rheodyne sampling injector connected to a 20 μL sample loop (Rheodyne). This system was equipped with a Gelpack GL-C610H-S column (300 mm × 7.8 mm, 6 μm particle size, Hitachi Chemical Co., Ltd.). The mobile phase consisted of 3 mM perchloric acid, and the flow rate was 1.0 mL/min. This result was identified on chromatograms by conventional methods, i.e., the retention time of standards was compared with the calibration curve of the standard solution.

2-2-10. Analysis of Radical Scavenging Activity

The DPPH and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging activities of extracts were analyzed according to the method of Lim et al. (2019). These activities were determined by the difference between a blank and a sample in the absorbance decrease of DPPH and ABTS radicals detected at 520 nm and 740 nm, respectively. The results were calculated against a calibration curve of α-tocopherol, and data were expressed as μg of α-tocopherol equivalent per grams of sample because α-tocopherol is widely used as an index of antioxidant activity.

2-2-11. Principal Component Analysis (PCA)

Principal component analysis was conducted using Bell Curve for Microsoft Excel software (Social Survey Research Information Co., Ltd., Tokyo, Japan). PCA was performed on normalized data to reduce the number of actual variables (42 chemical compounds and radical scavenging activities) and produce fewer derived variables [principal components] that adequately summarized total variance, i.e., to correlate the chemical compositions of fermented fish products with their respective characteristics.

2-3. Result and Discussion

2-3-1. Fatty Acid Composition

Fatty acid composition for each fermented food is shown in Table 2-2ab. Hoy Seab Dong contained the highest amount of saturated fatty acid (SFA). In contrast, the SFA content for the same genus, *Donax trunculus*, has been reported as around 30% (Boussoufa et al. 2011; Gopalsamy et al. 2014). Therefore, the possibility of a high SFA content might come from Nam Pla, which fermented together with Hoy Seab Dong. Tai Pla contained the highest amount of monounsaturated fatty acid (MUFA). Normally, fish organs, rarely eaten because of bitter taste and easy spoilage, will be treated as waste, or used to produce biodiesel (Harsono et al. 2016). Therefore, lipid content and fatty acid composition of internal organs is rarely reported. Polyunsaturated fatty acid (PUFA) can be divided into two groups, including n-3 and n-6 fatty acid. The n-3 fatty acid group, which is mainly contained in fish products, is the focus of this study. The results showed that all samples contained both 22:6 n-3 [docosahexaenoic acid (DHA)] and 20:5 n-3 [eicosapentaenoic acid (EPA)]. Hoy Malang Poo Dong contained the highest amount of DHA (and also the highest n-3 fatty acid composition overall), while Kapi contained the highest amount of EPA. Other types of n-3 fatty acids were also present, but not in large amounts. Hikihara et al. (2020) reported that bivalves tend to be rich in n-3 fatty acids compared to fish, especially the Asian green mussel (*Perna viridis*), which is rich in EPA (26.8%) and DHA (17.25%). This is attributable to bivalves consuming microalgae, which are rich in effective functional components such as EPA, DHA, and other polyunsaturated fatty acids. The raw material of Kapi, namely shrimp, also consumes microalgae rich in EPA and DHA, which explains the high composition of EPA in Kapi observed here (Benemann 1992). For n-6 fatty acid, rice of Pla Som contained the highest content because rice of Pla Som is the only sample to contain a large amount of rice; this rice contains high linoleic acid content, which is a fatty acid mainly found in plants (especially rice) (Wang

et al. 2020). Similarly, many researchers have also reported that both freshwater and marine fish contained only a small amount of n-6 fatty acid (Huynh and Kitts 2009; Özogul et al. 2007; Strobel et al. 2012).

The n-6/n-3 fatty acid ratio is related to depression and inflammatory disease in humans; if this ratio is high, the risk of occurring disease will also increase (Husted and Bouzinova 2016; KiecoltGlaser et al. 2007). According to Table 2-2b, the highest n-6/n-3 fatty acid ratio was found in rice of Pla Som and the lowest ratio in Hoy Malang Poo Dong. Similarly, Li et al. (2007) reported that Asian green mussel (*Perna viridis*) contained a small amount of n-6/n-3 fatty acid ratio.

2-3-2. Tocopherol Content

As shown in Table 2-3, all samples contained α-tocopherol in different amounts, whereas only some samples contained β-tocopherol. Kapi contained the highest amount of α-tocopherol, while Pla Ra contained the highest amount of β-tocopherol. In contrast, none of the samples contained γ- or δ-tocopherol.

According to a previous study of Ozogul et al. 2011, fish contain little β-, γ-, and δ-tocopherol. In our study, we expected that some samples would contain γ-tocopherol because field crabs consume rice and rice was used during fermentation for fish with rice and fermented fish. However, of the four samples, none contained γ-tocopherol. Thus, γ-tocopherol might have been lost during the fermentation process.

2-3-3. Free Amino Acid Content

The free amino acid content of each fermented food is shown in Table 2-4. All samples contained each free amino acid in different amounts. The free amino acids, including histidine (His), taurine (Tau), glutamic acid (Glu), as well as total free amino acid content, are the focus of this study. All samples contained Glu and Tau, whereas some did not contain His. Nam Pla contained the highest amount of His and Tau (and highest free amino acid content overall), while rice of Pla Som contained the highest amount of Glu. Samples that did not contain His were Hoy Seab Dong, Pla Ra, and rice of Pla Som.

Free amino acids are associated with radical scavenging activity. Indeed, Sarmadi and Ismail (2010) reported that free amino acids are related to antioxidant activity by bioactive peptides that bond with free amino acids. Thus, if total free amino acid content is high, antioxidant activities will also be high. In our study, some samples contained a small amount of His, which prevents oxidation, and Tau, which is involved in central nervous system function (Wade and Tucker 1998; Ripps and Shen 2012). Previous studies, however, reported that raw fish meat normally contains high His and Tau content (Antoine et al. 2001; Pyz-Lukasik et al. 2016). Therefore, our study confirms that the free amino acid content is reduced during the fermentation process. In contrast, Glu is mainly found naturally in food, especially in ripening or fermented food, and produces the umami taste (Yamaguchi and Ninomiya 2000). Our study is in agreement with these findings, as the Glu content was high in each fermented sample.

2-3-4. Purine and Pyrimidine Monophosphate Content

As shown in Table 2-5, Nam Pla, Kapi, and Tai Pla contained higher amounts of purine and pyrimidine monophosphates than other samples. All fermented samples contained

cytidine 5′-monophosphate (CMP), whereas some samples did not contain guanosine 5′-monophosphate (GMP), inosine 5′-monophosphate (IMP), and adenosine 5′-monophosphate (AMP). Tai Pla contained the highest levels of CMP and GMP, while Nam Pla contained the highest levels of IMP and AMP. Overall, Tai Pla had a greater variety of purine and pyrimidine than other fish products.

Purine and pyrimidine monophosphates are associated with the umami taste; in particular, IMP and GMP are important nucleic acids because they directly contribute to the umami taste (Ishimaru et al. 2016). In contrast, CMP and AMP do not produce the umami taste alone, but in combination with disodium, they enhance the umami taste, similar to IMP and GMP (Ishimaru et al. 2016). Purine and pyrimidine monophosphates can be found in most foods, especially those that are nearly ripe (Yamaguchi and Ninomiya 2000). In addition, the fermentation process can enhance the umami taste of food (Kijima and Suzuki 2007; Uchiyama et al. 2011). Thus, studies suggest that fermented food is high in purine and pyrimidine monophosphates, and that these compounds can be formed during fermentation.

2-3-5. Organic Acid Content

The organic acid content of each fermented food is shown in Table 2-6. Nam Pla and Kapi contained higher amounts of organic acid than the other samples. For sour tasting acids, Nam Pla contained the highest amount of citric acid, Kapi contained the most succinic acid, and Khem Bak Nad contained the highest lactic acid content (Neta et al. 2007). For foul-smelling organic acids, Kapi contained the highest amounts of pyroglutamic and valeric acid, while Nam Pla contained the highest levels of propionic, isobutyric, and

butyric acid, and none of the samples contained isovaleric acid.

Organic acids are related to the flavor properties of fermented foods and act as natural preservatives (Guzel-Seydim et al. 2000). The present study focused on eight types of organic acid with either a sour taste or foul smell. Some of the present results were consistent with those of previous studies. For example, Khem Bak Nad likely contained the highest amount of sour acids because pineapple has a high volume of such acids (especially lactic acid produced by lactic bacteria) (Cámara et al. 1994). Idris and Suzana (2006) and Yang et al. (2016a) have also reported that fermented pineapple produces lactic acid. In another study, Jo and Park (1985) reported that clam and shellfish contain high levels of succinic acid. Chuon et al. (2014) also reported that Cambodian Kapi (shrimp paste) contains succinic acid at higher levels than other Cambodian fermented fish products such as Prahok (fish paste) and Toeuk Trey (fish sauce). Similarly, we found that Kapi contains succinic acid at higher levels than Hoy Seab Dong and Hoy Malang Poo Dong. Although Nam Pla, Tai Pla, and Kapi contained higher amounts of foul-smelling organic acids than the other samples, the perception of their smell is dependent on a person's sensory capabilities.

2-3-6. Radical Scavenging Activity

The antioxidant activities of lipophilic and water-soluble extracts from the 11 fermented fish products are shown in Table 2-7. Radical scavenging activities are represented by four types of analysis: DPPH and ABTS of lipophilic and water-soluble extracts. The DPPH radical scavenging assay can be used to indicate the hydrogen-donating ability of lipophilic and water-soluble antioxidants (Chandrasekar et al. 2006; Yamaguchi et al.

1998). In fermented products, DPPH radical scavenging should increase during the fermentation period, while IMP (related to umami taste) should decrease. Pongsetkul et al. (2017) reported that shrimp fermented over longer periods had higher DPPH than raw, salted, and dry-salted shrimp. However, in the present study, Tai Pla, Nam Pla, and Kapi contained higher free amino acids, which are associated with radical scavenging. Previously, Zhang et al. (2019) reported that among natural antioxidants, α-tocopherol had the highest DPPH radical scavenging results. Therefore, if α-tocopherol content is high, DPPH radical scavenging is also thought to be high. In terms of α-tocopherol content, our study is in agreement with these findings.

The ABTS radical scavenging assay can also be used to determine both lipophilic and water-soluble antioxidants (Re et al. 1999). In the present study, the ABTS radical scavenging activity of lipophilic extracts from all samples showed a similar trend to DPPH radical scavenging activity; however, that of water-soluble extracts differed. While many studies have indicated that the DPPH and ABTS radical scavenging activities of lipophilic and water-extracts in foods are highly correlated, others have reported a lack of correlation (Faithong et al. 2010; Yu et al. 2002). In the present study, all ABTS activities were higher than DPPH activities, consistent with previous findings for scavenging activity (Arnao 2000).

ABTS are soluble in aqueous and organic media, in which antioxidant activity can be measured because of hydrophilic and lipophilic samples. In contrast, DPPH can only be dissolved in organic media (especially in alcohol-based media), which is an important limitation to consider when interpreting the role of hydrophilic antioxidants. In our study, water-soluble extracts of Hoy Seab Dong contained higher ABTS scavenging capacity than other samples; however, the DPPH scavenging capacity was not equivalently high.

Thus, Hoy Seab Dong likely has a highly water-soluble antioxidant substance that could not be detected by the DPPH method.

Previous antioxidant activity data for fermented fish products from Thailand only exist for Kapi. Prapasuwannakul and Suwannahong (2015) reported that shrimp paste was a good source of natural antioxidants, consistent with our findings. In addition, we found that Nam Pla and Tai Pla had similar radical scavenging activities to Kapi.

2-3-7. Assortment by PCA Analysis

PCA was conducted to evaluate the relationship between 62 variable chemical substances and 11 fermented fish products from Thailand (Figure 2-1). Approximately 51.64% of the variability could be explained by the first two dimensions: PC1 and PC2 accounted for 37.48% and 14.16% of the variance, respectively. Two groups (G1 and G2) were distinguished among the samples (Figure 2-1a). G1, containing Kapi, Tai Pla, Khem Bak Nad, and Nam Pla, was located to the right of the y-axis, whereas G2, containing the other samples, was located to the left of the y-axis. As shown in Figure 2-1b, G1 contained higher levels of chemical components and radical scavenging activities, except for ABTS radical scavenging activity from water-soluble substances, than G2. Kapi was located to the right of the y-axis and above the x-axis (Figure 2-1a). As shown in Figure 2-1b, the same area had lipophilic components such as eicosapentaenoic acid (C20:5), docosahexaenoic acid (C22:5), total n-3 fatty acids, α-tocopherol, and lipophilic radical scavenging activity (DPPH-L and ABTS-L). Hence, samples with higher α-tocopherol content were closely related to radical scavenging activity. Indeed, Kapi contained higher α-tocopherol levels (487.73 ± 13.26 ng/mg lipid) than all other samples. Furthermore,

functional water-soluble substances, such as total free amino acids, purine and pyrimidine monophosphates, and water-soluble radical scavenging activity (DPPH-W), were located to the right of the y-axis and below the x-axis (Figure 2-1b). As shown in Figure 2-1a, the same area had Tai Pla, Khem Bak Nad, and Nam Pla. In fact, these samples contained higher total free amino acids (3603.60-3299.99 μg/g sample) than other samples. They also contained higher IMP, a purine monophosphate associated with umami taste, than other samples. Therefore, samples with higher free amino acid content and total free amino acids are closely related to water-soluble radical scavenging activity (DPPH-W).

2-4. Conclusion

In the 11 fermented fish products, all samples contained similar components and radical scavenging activities but at different levels with a strong correlation in PCA analysis. Additionally, the fermentation process surely influences the lactic acid bacteria to produce various chemical components, particularly the amino acids and organic acids which results in higher content in fermented fish products when compared with fresh fish or soy sauce which made from soybean (Park et al. 2000; Funatsu et al. 2000). Nam Pla contained high levels of free amino acids and purine and pyrimidine monophosphates; Tai Pla contained high levels of free amino acids, purine and pyrimidine monophosphates, and organic acids; and Kapi contained high levels of n-3 fatty acids, α-tocopherol, free amino acids, purine and pyrimidine monophosphates, and organic acid content. Overall, these three samples contained higher chemical components and radical scavenging activities than other samples, suggesting that these three fermented foods are most useful to consume for maintaining human health.

Table 2-1 Fermented fish products from Thailand used in this study

No	Local names (with English name)	Obtained place	Raw material (scientific name)
1	Hoy Seab Dong (Wedge shell in Fish sauce)	Chanthaburi	Wedge shell (*Donax vittatus*)
2	Hoy Malang Poo Dong (Mussel with herbs)	Chanthaburi	Asian green mussel (*Perna viridis*)
3	Nam Pla (Fish sauce)	Chanthaburi	Anchovy (*Engraulidae Gill*)
4	Poo Khem (Salted crab)	Nakhon Ratchasrima	Rice field crabs (*Somanniathelphusa sp.*)
5	Tai Pla (Fermented fish organs)	Nakhon Ratchasrima	Mixed internal fish organs
6	Pla Som (Fermented fish with rice)	Nakhon Ratchasrima	Tilapia (*Oreochromis niloticus*)
7	Pla Too Khem (Salted mackerel)	Chanthaburi	Short Mackerel (*Rastelliger brachysoma*)
8	Kapi (Shrimp paste)	Chanthaburi	Krill (*Euphausiacea sp.*)
9	Khem Bak Nad (Fermented fish with pineapple)	Ubon Ratchathani	Black-ear catfish (*Pangasius larnaudii*)
10	Pla Ra (Fermented fish)	Nakhon Ratchasrima	Mixed small fish
11	Rice of Pla Som	Nakhon Ratchasrima	Cooked rice fermented together with Pla Som

Table 2-2a Fatty acid composition (% of total fatty acids) of fermented fish products from Thailand

Sample	Hoy Seab Dong	Hoy Malang Poo Dong	Nam Pla	Poo Khem	Tai Pla	Pla Som	Pla Too Khem	Kapi	Khem Bak Nad	Pla Ra	Rice of Pla Som
14:0	14.02±1.21	6.97±0.55	23.5±3.30	3.12±0.05	2.83±0.20	2.86±0.15	2.81±0.40	3.76±0.46	3.57±1.93	3.64±0.21	2.86±0.10
15:0	1.77±0.10	0.91±0.10	0.85±0.06	1.99±0.06	1.91±0.10	1.65±0.06	2.25±0.39	0.99±0.12	0.40±0.08	2.42±0.10	0.54±0.01
16:0	41.67±0.56	31.28±2.33	17.94±1.02	33.60±0.63	24.95±0.14	34.60±0.17	35.00±4.14	27.79±1.54	40.07±6.99	27.50±0.29	30.23±0.17
16:1	10.35±0.22	9.75±0.13	0.52±0.06	9.30±0.29	5.61±0.37	6.94±0.69	2.96±0.06	13.80±1.36	2.72±0.77	10.52±0.96	3.62±0.32
18:0	12.31±0.76	7.83±0.50	7.87±0.75	6.19±0.14	7.40±2.01	8.40±0.24	14.97±1.85	8.81±0.83	7.42±3.91	9.14±0.36	3.35±0.09
18:1n-9	6.37±0.30	1.88±0.11	26.34±2.15	17.34±0.24	28.36±0.72	12.60±0.67	8.98±0.32	3.71±0.04	26.20±5.41	16.37±0.15	15.84±0.07
18:1n-7	3.58±0.18	2.83±0.07	5.89±0.32	1.53±0.16	2.45±0.17	4.76±0.36	3.28±0.34	5.09±0.21	1.93±0.32	4.52±0.08	2.39±0.19
18:2n-6	0.68±0.03	2.00±0.01	13.76±0.74	11.37±0.15	12.16±0.33	6.86±0.09	0.94±0.16	1.18±0.06	10.89±1.71	9.54±0.07	33.05±0.61
18:3n-3	1.30±0.11	3.13±0.12	1.16±0.15	8.92±0.18	2.09±0.09	5.60±0.12	1.08±0.05	1.14±0.07	0.90±0.14	5.26±0.01	3.99±0.08
18:4n-3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
20:2n-6	0.63±0.09	0.29±0.01	nd	0.44±0.01	0.33±0.02	0.46±0.01	0.25±0.03	0.48±0.54	0.46±0.08	0.42±0.02	0.18±0.03
20:4n-6	1.25±0.08	3.87±0.24	nd	2.99±0.05	2.02±0.12	4.17±0.48	5.64±1.13	5.38±0.32	1.58±0.31	3.99±0.07	1.20±0.04
22:1n-9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
20:5n-3	3.48±0.37	12.85±1.37	0.70±0.01	1.15±0.04	1.18±0.06	1.00±0.11	4.44±1.57	15.16±0.89	0.16±0.15	1.14±0.04	0.17±0.15
22:4n-6	0.23±0.09	0.89±0.04	nd	0.07±0.00	0.32±0.03	4.17±0.48	1.13±0.46	nd	0.29±0.05	0.55±0.03	0.20±0.01
22:5n-3	0.28±0.03	1.34±0.10	0.30±0.03	0.29±0.02	0.96±0.08	1.97±0.22	2.12±0.92	0.58±0.05	0.42±0.07	1.02±0.05	0.61±0.03
22:6n-3	1.58±0.15	12.53±1.48	1.08±0.10	0.75±0.04	6.11±0.49	4.74±0.87	11.84±2.17	11.29±1.23	1.46±0.28	2.11±0.10	0.92±0.03

Abbreviations: nd, not detected.

Each value represents the mean ± SD of three technical replicates.

Table 2-2b Fatty acid composition (% of total fatty acids) of fermented fish products from Thailand

Sample	Hoy Seab Dong	Hoy Malang Poo Dong	Nam Pla	Poo Khem	Tai Pla	Pla Som	Pla Too Khem	Kapi	Khem Bak Nad	Pla Ra	Rice of Pla Som
SFA	69.77±2.62	47.00±3.48	50.16±5.12	44.90±0.88	37.09±2.45	47.51±0.63	55.03±6.79	41.35±2.95	51.46±12.91	42.70±0.96	36.98±0.38
MUFA	20.30±0.71	14.46±0.31	32.76±2.53	28.17±0.69	36.42±1.26	24.29±1.73	15.22±0.72	22.60±1.61	30.85±16.5	31.41±1.20	21.85±0.58
PUFA	9.42±0.96	36.90±3.38	17.01±1.03	25.98±0.49	25.18±1.23	25.51±2.00	27.44±6.48	35.21±3.16	16.16±2.79	24.02±0.48	40.32±0.98
n-6	2.78±0.30	7.04±0.31	13.76±0.74	14.87±0.21	14.84±0.50	12.20±0.67	7.95±1.77	7.05±0.92	13.21±2.15	14.49±0.19	34.62±0.69
n-3	6.63±0.67	29.86±3.07	3.24±0.28	11.11±0.28	10.35±0.73	13.30±1.33	19.48±4.70	28.16±2.24	2.95±0.64	9.53±0.29	5.69±0.29
n-6/n-3	0.42±0.02	0.24±0.01	4.28±0.59	1.34±0.01	1.44±0.05	0.92±0.04	0.41±0.01	0.25±0.03	4.48±0.08	1.52±0.02	6.08±0.12

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; n-6, total of n-6 fatty acids; n-3, total of n-3 fatty acids; n-6/n-3, ratio of total n-6 and n-3 fatty acids.

Each value represents the mean ± SD of three technical replicates.

Table 2-3 Tocopherol contents (ng/mg lipid) of fermented fish products from Thailand

Sample	Hoy Seab Dong	Hoy Malang Poo Dong	Nam Pla	Poo Khem	Tai Pla	Pla Som	Pla Too Khem	Kapi	Khem Bak Nad	Pla Ra	Rice of Pla Som
α-Toc	26.86±2.62	162.66±5.23	0.03±0.05	193.74±6.50	37.12±2.46	13.42±0.32	3.76±0.66	487.73±13.26	12.40±4.22	20.35±1.15	6.37±1.58
β-Toc	nd	nd	nd	nd	2.67±2.70	6.04±0.86	1.86±3.23	nd	0.98±1.70	59.5±6.12	4.18±3.96
γ-Toc	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
δ-Toc	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Abbreviations: α-Toc, α-Tocopherol; β-Toc, β-Tocopherol; γ-Toc, γ-Tocopherol; δ-Toc, δ-Tocopherol; nd, not detected.

Each value represents the mean ± SD of three technical replicates.

Table 2-4 Free amino acid contents (μg/g sample) of fermented fish products from Thailand

Sample	Hoy Seab Dong	Hoy Malang Poo Dong	Nam Pla	Poo Khem	Tai Pla	Pla Som	Pla Too Khem	Kapi	Khem Bak Nad	Pla Ra	Rice of Pla Som
Asparagine	52.2±1.2	95.6±0.8	132.9±2.2	50.6±0.7	183.4±1.3	7.7±0.3	69.6±2.8	161.0.7±0.7	101.0±3.0	61.9±2.4	9.2±0.2
Glutamic acid	111.9±1.9	426.2±0.9	288.5±4.9	119.1±1.3	294.1±2.4	467.8±1.6	123.7±1.3	682.2±10.1	245.1±1.6	110.1±2.5	1011.7±11.0
Serine	15.6±1.1	70.1±1.1	23.9±4.1	28.5±0.7	120.2±3.3	7.9±0.5	58.7±1.0	89.3±2.2	87.3±1.0	8.0±0.3	5.1±0.5
Glycine	50.3±0.4	82.9±0.6	687.0±2.1	98.3±1.7	91.2±0.6	71.3±0.7	33.7±0.6	170.6±1.3	50.6±0.1	15.2±0.6	27.6±0.2
Histidine	nd	28.3±0.7	382.4±507.4	15.5±3.2	77.1±4.6	14.7±0.3	161.0±6.6	55.9±2.0	33.2±2.3	nd	nd
Taurine	597.4±5.3	84.4±4.4	829.0±30.7	198.5±6.5	525.8±1.0	151.0±6.0	175.7±3.6	546.8±21.0	121.8±5.3	382.1±6.5	109.9±0.8
Threonine	55.1±0.5	95.7±8.7	177.6±2.4	48.2±0.5	198.3±15.1	8.6±6.4	79.4±8.5	180.0±2.6	125.6±0.8	26.8±3.9	5.3±0.7
Alanine	76.6±4.3	82.5±0.8	190.0±5.6	65.8±4.2	183.5±4.5	31.0±0.1	81.5±1.5	301.2±5.4	112.9±3.2	52.3±3.0	20.5±3.0
Arginine	25.9±0.8	4.6±0.2	nd	nd	549.8±13.5	68.5±2.1	464.2±19.2	nd	691.4±32.5	24.0±1.4	nd
Anserine	nd	nd	nd	22.4±7.7	nd	nd	nd	nd	nd	nd	nd
Proline	35.6±0.6	30.5±3.2	121.4±1.8	33.8±0.6	101.8±1.0	12.8±0.6	29.4±0.3	145.5±2.3	29.2±0.2	32.2±2.0	3.7±0.2
Tyrosine	7.8±0.6	86.1±1.7	10.0±7.1	22.5±0.5	94.6±1.9	5.0±0.1	35.7±1.6	107.4±5.7	127.7±2.0	6.8±0.7	5.4±0.1
Valine	58.8±1.5	74.3±2.8	151.4±2.5	34.5±0.3	169.5±1.2	10.8±0.4	63.4±0.6	173.5±1.4	97.7±1.2	39.2±0.3	6.7±0.1
Methionine	12.2±0.7	28.7±3.1	66.7±2.1	5.7±0.5	66.4±2.7	5.5±0.7	34.9±1.0	87.7±4.4	87.7±3.2	5.0±0.5	2.7±0.3
Isoleucine	65.5±0.4	77.4±0.9	88.0±0.5	27.8±0.3	151.2±2.1	7.5±0.1	55.7±0.4	156.0±1.8	94.6±1.0	37.6±0.4	4.7±0.3
Leucine	110.6±1.1	111.7±2.7	158.0±4.6	63.4±0.5	262.1±6.81	24.2±0.4	119.1±2.4	340.3±3.8	180.6±1.4	91.6±0.5	23.7±0.6
Lysine	61.2±3.3	127.7±6.0	296.1±4.4	63.3±1.0	230.2±10.	18.9±0.8	110.1±2.8	294.9±7.6	207.8±2.5	58.0±0.5	14.4±0.6
Phenylalanine	34.7±0.9	65.2±1.5	84.8±2.3	27.7±0.4	117.3±1.7	9.2±0.1	55.8±0.7	137.1±6.2	92.3±0.8	23.1±0.7	6.5±0.2
Total	1337.4±12.7	1988.3±50.9	3603.6±480.4	898.5±14.2	3299.9±46.7	913.7±11.8	1696.4±31.4	3493.7±11.7	2394.9±35.8	951.5±8.2	1251.2±9.0

Abbreviations: nd, not detected. Each value represents the mean ± SD of three technical replicates.

Table 2-5 Purine and pyrimidine monophosphate contents (μg/g sample) of fermented fish products from Thailand

Sample	Hoy Seab Dong	Hoy Malang Poo Dong	Nam Pla	Poo Khem	Tai Pla	Pla Som	Pla Too Khem	Kapi	Khem Bak Nad	Pla Ra	Rice of Pla Som
CMP	173.9±10.5	290.4±3.4	476.9±26.8	75.2±1.2	1080.1±46.7	73.5±5.8	100.2±86.7	850.9±7.9	10.97±1.1	16.44±0.5	76.43±0.5
GMP	26.9±2.0	124.9±2.3	554.4±53.7	82.8±1.1	915.0±22.3	nd	206.1±1.8	216.7±23.7	3.9±0.1	nd	nd
IMP	58.4±1.4	70.9±0.6	458.9±34.6	36.4±1.1	183.3±3.8	nd	66.9±0.8	238.2±8.0	166.9±6.6	nd	nd
AMP	14.8±1.7	53.1±5.2	1487.5±120.2	211.1±9.5	396.0±17.5	nd	65.6±0.5	416.1±5.6	nd	nd	nd

Abbreviations: CMP, cytidine monophosphate; GMP, guanosine monophosphate; IMP, inosine monophosphate; AMP, adenosine monophosphate; nd, not detected.

Each value represents the mean ± SD of three technical replicates.

Table 2-6 Organic acid contents (mg/g sample) of fermented fish products from Thailand

Sample	Hoy Seab Dong	Hoy Malang Poo Dong	Nam Pla	Poo Khem	Tai Pla	Pla Som	Pla Too Khem	Kapi	Khem Bak Nad	Pla Ra	Rice of Pla Som
Oxa	1.17±0.09	0.88±0.17	0.72±0.04	1.81±0.01	2.26±0.01	0.41±0.01	0.55±0.01	2.42±0.02	0.38±0.02	0.84±0.03	1.01±0.17
Mal	nd	nd	nd	0.01±0.01	0.01±0.01	nd	nd	0.02±0.01	nd	nd	nd
Cit	0.62±0.05	nd	2.80±0.08	nd	2.40±0.08	0.26±0.01	0.81±0.03	1.42±0.09	0.73±0.08	1.94±0.02	0.10±0.08
Tar	0.87±0.07	nd	1.68±0.04	0.37±0.01	0.58±0.04	0.01±0.01	0.09±0.01	3.05±0.15	0.82±0.06	0.18±0.01	0.06±0.02
Suc	2.73±0.11	11.86±1.12	10.26±0.21	8.22±0.01	11.23±0.59	0.33±0.17	8.57±0.01	30.70±0.50	3.96±0.12	0.51±0.01	0.15±0.60
Lac	1.69±0.05	0.57±0.51	4.21±0.13	1.79±0.23	3.24±0.39	2.03±0.01	3.93±0.01	6.72±0.26	16.34±0.26	7.21±0.04	1.89±0.03
Fum	0.01±0.01	nd	nd	nd	nd	0.07±0.01	nd	0.05±0.01	nd	nd	nd
Ace	5.85±0.63	nd	7.64±0.17	nd	nd	0.54±0.08	nd	8.39±0.19	1.52±0.05	1.55±0.07	0.27±0.01
Pyro	0.77±0.02	0.25±0.01	2.75±0.01	0.21±0.01	1.36±0.11	nd	0.14±0.01	2.92±0.03	2.25±0.03	0.14±0.01	0.12±0.01
Prop	3.39±0.08	nd	7.35±0.07	nd	2.05±0.26	nd	nd	nd	nd	4.83±0.02	nd
Isob	nd	nd	3.36±0.17	nd	nd	nd	nd	nd	nd	0.83±0.01	nd
But	0.18±0.01	nd	3.74±0.21	nd	nd	nd	nd	2.68±0.11	1.74±0.03	0.66±0.02	nd
Vale	nd	nd	0.58±0.06	nd	nd	nd	nd	5.63±0.08	nd	nd	nd

Abbreviations: Oxa, oxalic acid; Mal, maleic acid; Cit, citric acid; Tar, Tartaric acid; Suc, Succinic acid; Lac, Lactic acid; Fum, fumaric acid; Ace, acetic acid; Pyro, pyroglutamic acid; Prop, propionic acid; Isob, isobutyric acid; But, butyric acid; Vale, valeric acid; nd, not detected.

Each value represents the mean ± SD of three technical replicates.

Table 2-7 Radical scavenging activities of fermented fish products from Thailand *

Sample	Hoy Seab Dong	Hoy Malang Poo Dong	Nam Pla	Poo Khem	Tai Pla	Pla Som	Pla Too Khem	Kapi	Khem Bak Nad	Pla Ra	Rice of Pla Som
DPPH											
Lipophilic	26.6±0.9	22.7±0.9	29.6±2.0	38.1±3.1	129.1±1.9	18.2±1.9	58.4±5.2	125.4±1.2	34.8±4.7	48.3±0.8	2.4±0.9
Water	160.2±7.0	297.2±2.0	380.0±2.5	35.6±5.7	273.3±5.7	76.4±2.7	372.0±5.1	327.0±15.4	325.0±18.1	115.7±3.8	128.7±10.7
ABTS											
Lipophilic	121.0±22.4	47.7±3.4	151.7±18.9	480.7±10.0	438.7±2.3	15.3±5.4	112.3±6.3	457.4±2.5	92.4±7.3	102.0±6.6	4.4±2.1
Water	10147.9±393.7	453.3±18.9	432.3±72.8	857.2±249.1	1153.1±44.4	1329.4±43.2	552.2±22.9	5068.9±102.0	1433.9±15.5	1050.5±459.7	4792.6±183.8

*Radical scavenging activity are expressed as μg of α-tocopherol equivalent per g of each samples.

Each value represents the mean ± SD of three technical replicates.

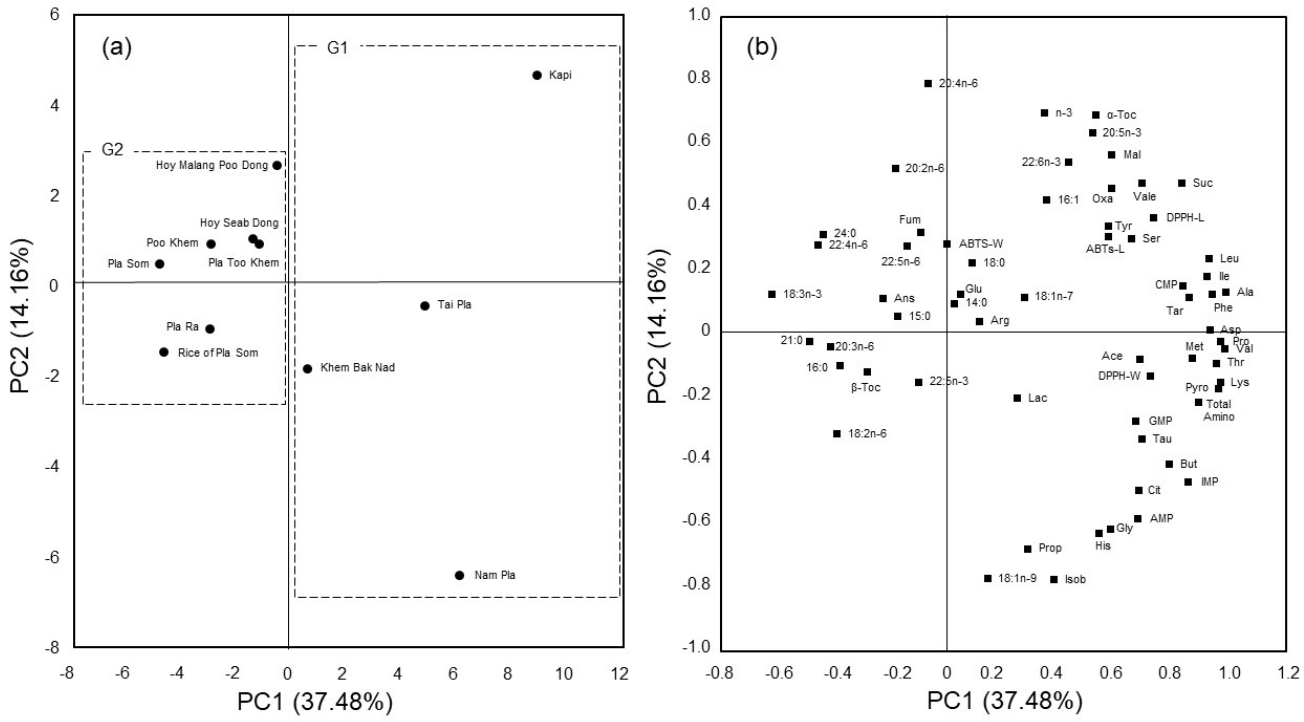

Figure 2-1 Score and loading plots of principal component analysis for 11 fermented fish products from Thailand. (a) Score plot of the 11 fermented fish products from Thailand. Detailed information on each sample is shown in Table 2-1. (b) Loading plot showing the distribution of the 62 functional chemical compounds analyzed in this study: 14:0 (myristic acid), 15:0 (pentadeaylic acid), 16:0 (palmitic acid), 16:1 (palmitoleic acid), 18:0 (stearic acid), 18:1 n-9 (oleic acid), 18:1 n-7 (vaccenic acid), 18:2 n-6 (linoleic acid), 18:3 n-3 (α-linolenic acid), 21:0 (heneicosanoic acid), 20:2 n-6 (eicosadienoic acid), 20:3n-6 (dihomo-γ-linolenic acid), 20:4 n-6 (arachidonic acid), 20:5 n-3 (eicosapentaenoic acid), 24:0 (lignoceric acid), 22:4 n-6 (docosatetraenoic acid), 22:5 n-6 (osbond acid), 22:5 n-3 (clupanodonic acid), 22:6 n-3 (docosahexaenoic acid), n-3 (total n-3 fatty acids), α-Toc (α-tocopherol), β-Toc (β-tocopherol), Asp (asparagine), Glu (glutamic acid), Ser (serine), Gly (glycine), His (histidine), Tau (taurine), Thr (threonine), Ala (alanine), Arg (arginine), Ans (anserine), Pro (proline), Tyr (tyrosine), Val (valine), Met (methionine), Ile (isoleucine), Leu (leucine), Lys (lysine), Phe (phenylalanine), CMP (cytidine monophosphate), GMP (guanosine monophosphate), IMP (inosine monophosphate), AMP (adenosine monophosphate), Oxa (oxalic acid), Mal (maleic acid), Cit (citric acid), Tar (Tartaric acid), Suc (Succinic acid), Lac (Lactic acid), Fum (fumaric acid), Ace (acetic acid), Pyro (pyroglutamic acid), Prop (propionic acid), Isob (isobutyric acid), But (butyric acid), Vale (valeric acid), DPPH-L (DPPH scavenging activity of the lipophilic extracts), DPPH-W (DPPH scavenging activity of the water-soluble extracts), ABTS-L (ABTS scavenging activity of the lipophilic extracts), and ABTS-W (ABTS scavenging activity of the water-soluble extracts).

Chapter 3. The Simultaneous Analysis of 14 Antioxidant Compounds Using HPLC with UV Detection and Their Application to Edible Plants from Asia

3-1. Introduction

An antioxidant compound is a substance that detects, prevents, and reduces oxidative damage to a cell's structure (Santos Sánchez et al. 2019). These compounds are essential and have many health benefits, such as their ability to reduce the incidence of diseases, such as cancer, neurological decline, and type 2 diabetes (Wootton-Beard and Ryan 2011). Typically, antioxidants can be found easily in most foods, particularly in herbs, spices, and traditional medicinal plants, which have a high content of natural antioxidant compounds (Carlsen et al. 2010).

In this study, we developed a method to simultaneously analyze 14 antioxidative compounds including gallic acid, epicatechin gallate (ECG), caffeine, vanillic acid, epigallocatechin gallate (EGCG), coumaric acid, epigallocatechin (EGC), rosmarinic acid, gingerol, thymoquinone, carvacrol, thymol, carnosol, and carnosic acid, using a simple HPLC system. We subsequently evaluated the antioxidant compounds in popular edible herbs and spices from Asia using this method.

3-2. Materials and Methods

3-2-1. Samples

Sundried plant samples including kaffir lime *Citrus hystrix* (leaves), galangal *Alpinia galanga* (rhizome), turmeric *Curcuma longa* (rhizome), fingerroot *Boesenbergia rotunda* (rhizome), ginger *Zingiber officinale* (rhizome), lemongrass *Cymbopogon nardus* (stem and leaves), pandan *Pandanus amaryllifolius* (leaves), white pepper *Piper nigrum* (seeds), sweet basil *Ocimum basilicum* (leaves), Climbing wattle *Senegalia pennata* (stem and leaves mixture), and coriander *Coriandrum sativum* (stem and leaves mixture) were purchased from local supermarkets in Thailand. Japanese tea (dried leaves) were purchased from local supermarkets in Japan. These purchased dried samples were pulverized using blender (Hi-Power Blender MX1200XTM, Osaka Chemical Co., Ltd., Japan) and kept in -30°C until start the analysis.

3-2-2. Reagents

Methanol, vanillic acid, EGCG, and acetonitrile were purchased from Sigma-Aldrich Ltd. (Tokyo, Japan); acetic acid, gallic acid, caffeine, and ECG were purchased from Fujifilm Wako Pure Chemical Industries (Osaka, Japan); rosmarinic acid was purchased from Chromadex Standards (Colorado, USA); and distilled water was purchased from Takasugi Pharmaceutical Co., Ltd. (Fukuoka, Japan). All other chemicals and solvents used in this experiment were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

3-2-3. Preparation of Standard Solutions

For specificity, linearity, range, the limit of detection (LOD), the limit of quantitation (LOQ), and intra-, including inter-day precision analysis, 14 antioxidant compound

solutions (gallic acid, EGC, caffeine, vanillic acid, EGCG, coumaric acid, ECG, rosmarinic acid, gingerol, thymoquinone, carvacrol, thymol, carnosol, and carnosic acid was prepared by dissolving 10 mg of each compound in 10 mL methanol (each 1 mg/mL). Then, the mixed standard solution was prepared by mixing 1 mL EGC solution, 500 μL thymoquinone solution, 50 μL coumaric acid solution, and 100 μL of all other standard solutions. Subsequently, methanol was added to make the final volume up to 5 mL. For the recovery test, the mixed standard solution was prepared by mixing 5 mg of EGC with 1 mL thymoquinone solution (5 mg/mL), 100 μL coumaric acid solution, and 200 μL of all other standard solutions. Methanol was then added again to make the final volume up to 5 mL.

3-2-4. Extraction of Antioxidants from Samples

Methanol is used to extract antioxidants from plants (Li et al. 2019; Mamati et al. 2006; Troncoso et al. 2005). Therefore, we adapted the extraction of antioxidants from plant samples using methanol in this study. All pulverized plant samples (1 g) were weighed into glass centrifuge tubes containing 40-mL methanol. The mixtures were subsequently homogenized using a homogenizer, then vortexed. Next, the sample was incubated in a 37°C water bath for 1 h with shaking. After incubation, the mixture was centrifuged at 2500 ×g for 10 min. The upper layer was filtered using a cotton plug and transferred to pear-shaped flasks. Then, methanol was evaporated using a rotary evaporator. The resulting substance was resuspended in methanol to obtain a final volume of 10 mL. The solution was kept in a brown glass bottle and stored at -30°C until HPLC analysis. All measurements were performed in triplicate for each sample, and results were expressed

as means ± standard deviations.

3-2-5. The HPLC System

The antioxidant and caffeine standard solution was analyzed using an HPLC system. This system was equipped with an LC-10ADVP pump, a DGU-14A degasser, a CTO-10A column oven (30°C), an SPD-10A UV-Vis detector (wavelength 284 nm) (Shimadzu Corporation, Kyoto, Japan), a 7725i Rheodyne sampling-injector with 20-μL sample loop (Rheodyne, Rohnert Park, CA, USA), and a Capcell Pak C18 UG120 column (250 mm × 4.6 mm, 5 μm particle size; Osaka Soda Co., Ltd. Osaka, Japan). The mobile phase contained solutions A (1% acetic acid in water) and B (acetonitrile), with a flow rate of 1.0 mL/min. The gradient program was 5% B to 25% B from 0 to 20 min, and 25% B to 100% B from 20 to 60 min, which was immediately decreased to 5% B from 60 to 70 min. The data were processed using the chromatography software; CDS-Lite v.5.0. To identify the 14 antioxidants compounds in samples, the HPLC system was connected to a diode array detector (5430 DAD, Hitachi High-Tech Science Corporation, Tokyo, Japan). Identification of the 14 antioxidant compounds was subsequently accomplished following the retention times of each peak and comparing between the UV-vis absorption spectrum (scan range: 200-600 nm) of the standard compounds and the detected peaks in these samples.

3-2-6. Method Validation

To validate the proposed HPLC method, precision, specificity, linearity, range, LOD,

LOQ, intra- and inter-day precision, including recovery were evaluated. To test precision and specificity, 20 μL of the standard solution was analyzed using HPLC five times. The retention time (tR), peak area, the capacity factor (K′), resolution factor (Rs), separation factor (α), and tailing factor (TF) was then estimated based on the results of the five replicate analyses. The definition of each parameter was presented in our previous report (Sasaki et al. 2020). Subsequently, the intra-day precision test was conducted five times daily, while the inter-day precision test was conducted once for 3 days. The precision of each test was evaluated by calculating their relative standard deviations (RSDs). To test linearity, the range, LOD, and LOQ, analytical solutions were prepared by diluting with methanol in an appropriate concentration. A 20 μL aliquot of each solution was then analyzed using HPLC. LOD was defined as the concentration at which each peak was visibly separable from the baseline noise (greater than 3 times the noise baseline). Alternatively, LOQ was defined as the concentration at which the analytes were greater than 10 times the baseline noise. Therefore, to evaluate recovery, fortified samples were prepared by adding 0.1, 0.25, or 0.5 mL of mixed standard solutions to Japanese tea and coriander, respectively. Next, the inherent 14 antioxidant compounds from these fortified samples were analyzed following the methods described in the "Extraction of antioxidants from Sample" and "The HPLC System" sections. Recovery was estimated on the basis of the results from this analysis.

3-3. Results and Discussion

3-3-1. Method Validation

In this study, 10 antioxidant compounds were selected based on the result of other researchers' reports on HPLC analysis for antioxidant from edible plants from Asia, including rosmarinic acid, carnosic acid, carnosol, EGC, EGCG, caffeine, thymoquinone, thymol, vanillic acid, and carvacrol (Hadad et al. 2013; Choi et al. 2019). Later, 4 antioxidant compounds including gallic acid from pandan leaf, coumaric acid from pandan leaf, and gingerol from ginger, and ECG from Japanese tea were also added. Therefore, the proposed method required the separation of antioxidants from caffeine to determine the accuracy of HPLC in separating the 14 antioxidants compounds. First, the method of Hadad et al. (2013) was applied to analyze the standard mixture (containing the 14 antioxidants compounds). This method can separate each catechin compound, thymoquinone, thymol, and carvacrol, using a conventional octadecyl-silica column and gradient program. However, this method was difficult to separate the 14 standard mixtures to produce similar solubility and polarity. Therefore, the column, the elution solvent, and gradient program was changed and adjusted following the method of Choi et al. (2019). This method used a Capcell Pak C18 UG120 separation column and adapted a gradient program using solutions A (1% acetic acid in water) and B (methanol) as the mobile phase. This method can detect not only antioxidant compounds, such as rosmarinic acid, carnosol, and carnosic acid, but also catechins, such as EGC, EGCG, and ECG. However, this method also did not separate the 14 standard mixture, and the baseline of the HPLC chromatogram was unstable. Therefore, the solution B was also changed from methanol to acetonitrile to improve the resolution of the standard mixture, which resulted in a more superior resolution of the 14 standard mixture than that obtained using methanol as the mobile phase B. However, the resolution was still unsuitable for determining the 14 standard mixture in this study. Thus, to improve the chromatogram resolution, the

gradient program was adjusted by changing the concentration of mobile phase B at each different time. The best gradient program was described in “The HPLC System” section. Furthermore, we tried to separate other catechins such as epicatechin (EC) and catechin (C) using the HPLC system. However, the EC and C could not be separated by the method due to the diastereomeric substances each other. It is necessary to apply a chiral column to separate EC and C (Machonis et al. 2019). Therefore, we excluded EC and C analysis using the method. As a result, the chromatogram of the 14 antioxidant compounds and those of each spectra are shown in Figure. 3-1 and Figure. 3-2, respectively. As a result, the 14 standard mixture was separated with reasonable specificity in a 70 min cycle. According to the based methods used first, both Hadad et al. (2013) and Choi et al. (2019) could analyze antioxidant substances for a shorter time than the developed method. However, the developed method could analyze simultaneously 14 antioxidant compounds at an analysis that save more time and can identify many varieties of antioxidant compounds that are rarely analyzed together too.

To verify the precision and specificity of this newly developed HPLC method, the tR, k, RS, α, and TF were investigated (Table 3-1). All RS values of each compound were greater than 1.5, and all TF values were around 0.99, which meant that this method perfectly and reliably separated each compound in the sample. The linearity, range, LOD, and LOQ were also investigated (Table 3-2). The correlation factors of all compounds were around 0.9993. Also, the LOD range was 0.02-1.75 μg/mL, whereas, the LOQ range was 0.07-5.85 μg/mL. The LOD and LOQ are important as one of the indexes for evaluating the performance of HPLC. Compared with other researchers’ reports, the LOD and the LOQ of gallic acid, catechin groups, rosmarinic acid, and caffeine were superior to these reports that applied UV or DAD-HPLC method (Wang et al. 2003; Wang et al.

2004; Aucamp et al. 2000); whereas the LOD and the LOQ of gingerol, vanillic acid, and coumaric acid were same levels or not superior to other researchers' HPLC method (Seo and Shin 2021; Sinha et al. 2007). However, Seo and Shin (2021) applied LC-MS/MS, which was higher sensitivity than UV or DAD, to analysis 6-gingerol in medicinal herbs. Overall, the developed method was satisfied to detect and quantify antioxidant compounds in popular edible herbs and spices from Asia. In proving the intra- and inter-day precision, the tR, and peak areas from the two types of standard solutions were investigated (Table 3-3). For the ×0.5 standard solution, the RSD range of the intra-day precision was 0.040%-0.064%, whereas, that for inter-day precision was 0.003%-0.016%. However, for the ×0.1 standard solution, the RSD range of the intra-day precision was 0.002%-0.028%, while it was 0.004%-0.055% for the inter-day precision. On the basis of these results, we concluded that the developed method showed high reproducibility. Recovery tests using Japanese tea and coriander were conducted as well to confirm the efficiency of the proposed method (Table 3-4). The overall recovery rate of all antioxidant compounds for Japanese tea was 90.68%-115.91%, whereas that for coriander was 80.92%-95.01%, which meant that this method was reliable and quantitative.

3-3-2. Contents of the 14 Antioxidant Compounds in Plant Samples

Contents of antioxidant compounds in 12 plant samples were also evaluated using the developed method of this study (Table 3-5). Typical chromatograms of samples analyzed, such as Japanese tea, sweet basil, and ginger are shown in Figure. 3-3. To identify each antioxidant compound in these samples, we compared the retention times and spectra of each standard compound. However, some plant samples were diluted with methanol 5-

100 times before analysis to prevent combined peaks and for easy identification. From our results, ginger and sweet basil contained the most varieties of antioxidant compounds, which were noticeable. Ginger contained gingerol (12.27±0.24 mg/g), thymoquinone (57.57±1.52 mg/g), carvacrol (0.75±0.02 mg/g), thymol (0.78 ± 0.06 mg/g), carnosol (19.60±0.20 mg/g), and carnosic acid (6.62±0.10 mg/g). Other compounds as discussed above were also detected. Sweet basil contained vanillic acid (0.10±0.01 mg/g), EGCG (0.10 ± 0.01 mg/g), coumaric acid (0.02±0.01 mg/g), ECG (0.19±0.01 mg/g), rosmarinic acid (0.08±0.01 mg/g), carvacrol (1.44±0.02 mg/g), carnosol (8.74±0.06 mg/g), and carnosic acid (0.17±0.02 mg/g). For the catechin group, Japanese tea was the only sample that contained a high content of catechin and caffeine: EGC (38.93±0.09 mg/g), caffeine (25.08±0.07 mg/g), EGCG (49.52±0.21 mg/g), and ECG (9.61±0.03 mg/g). Several plants were already analyzed for antioxidant substances but as mentioned before, many researchers will mainly focus on antioxidant activity, not contents of each antioxidant were contained. As a result, interesting several results were found. Semwal et al. (2015), Tanweer et al. (2020), and Ghafoor et al. (2020) has identified gingerol as the main antioxidant compound of ginger, and also reported that small amounts of gallic acid and coumaric acid were contained. When compared with our analysis, we found that ginger contained not only gingerol and coumaric acid but also thymoquinone, thymol, carvacrol, carnosic acid, and carnosol which were not mentioned in previous researchers' reports. Mahae and Chaiseri (2009) reported that galangal contained catechin and a small amount of coumaric acid. In our analysis, galangal contained also EGCG, one of the catechin group compounds, and carnosic acid. Elansary et al. (2020) reported that sweet basil contained rosmarinic acid and vanillic acid. Złotek et al. (2015) also highlighted in their report that sweet basil contained small content of rosmarinic acid and coumaric acid. Bae

and Lee (2010), including Suzuki et al. (2005) also reported that Japanese tea contained many catechins (including EGC, EGCG, and ECG) and caffeine. On the basis of the data from other researchers, and compared with other analysis methods, the proposed method for analyzing these 14 antioxidant compounds was used to obtain consistent results and can show more varieties of antioxidant compounds present in each plant, with a visible identification of each antioxidant from each compound.

3-4. Conclusion

In this study, a simple HPLC method was successfully developed for simultaneously analyzing 14 antioxidant compounds. One cycle was completed within 70 min, with sensitive, quantitative, and reliable results. With this method, the 14 antioxidant compounds including catechin group (EGC, EGCG, ECG), caffeine and others in Asian herbs and spices were identified and quantified simultaneously, Antioxidant compounds have attracted wide attention because of their ability of antioxidant. Furthermore, antioxidant compounds have been commercialized in the form of supplements. Therefore, we propose that since our method evaluated the 14 antioxidant compounds in the samples studied, other samples, such as fruits, meat, dairy products, fish, or unknown biological tissues including unexplored resources also can evaluated in future analysis.

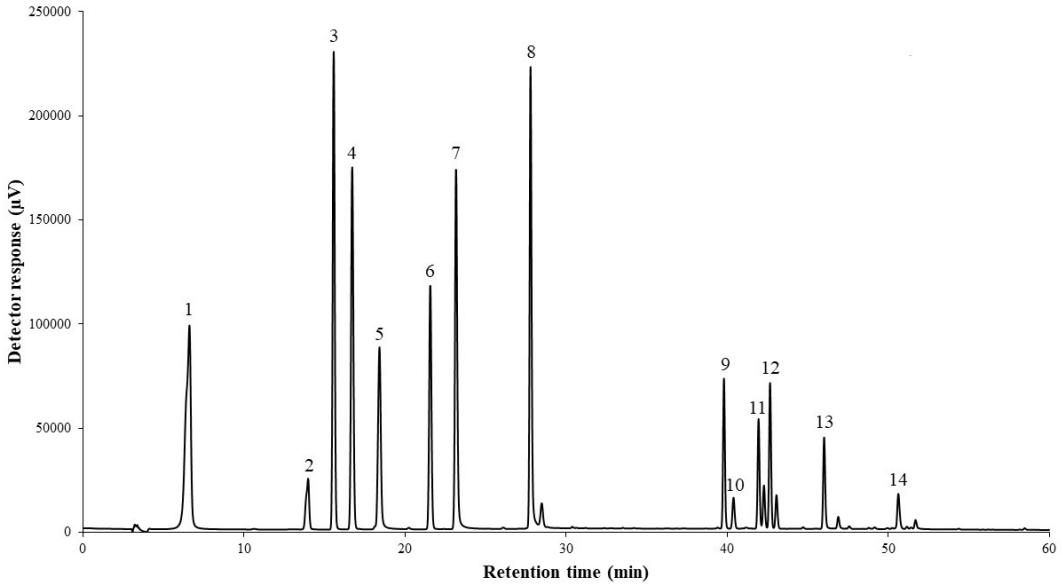

Figure 3-1 HPLC chromatogram of 14 antioxidant compounds monitored at 284 nm. 1, gallic acid; 2, EGC; 3, caffeine; 4, vanillic acid; 5, EGCG; 6, coumaric acid; 7, ECG; 8, rosmarinic acid; 9, gingerol; 10, thymoquinone; 11, carvacrol; 12, thymol; 13, carnosol; and 14, carnosic acid.

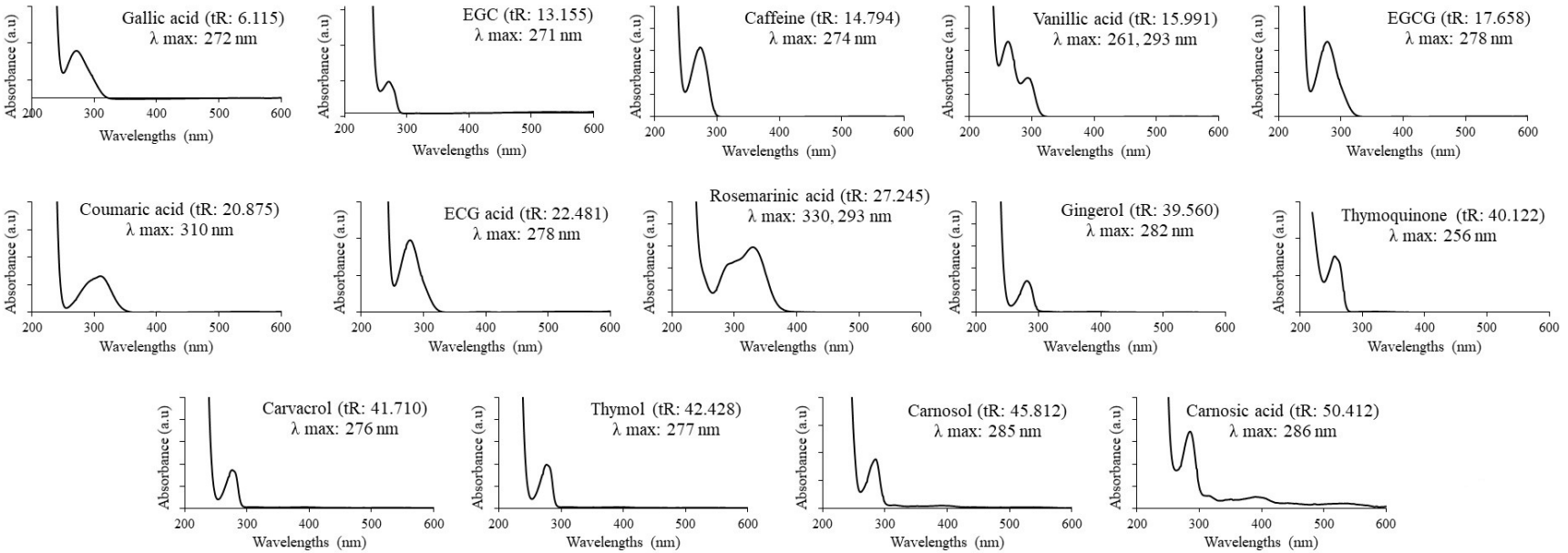

Figure 3-2 Retention time (tR), UV-Vis absorbance spectrum, and the maximum absorption wavelength of 14 antioxidant compound.

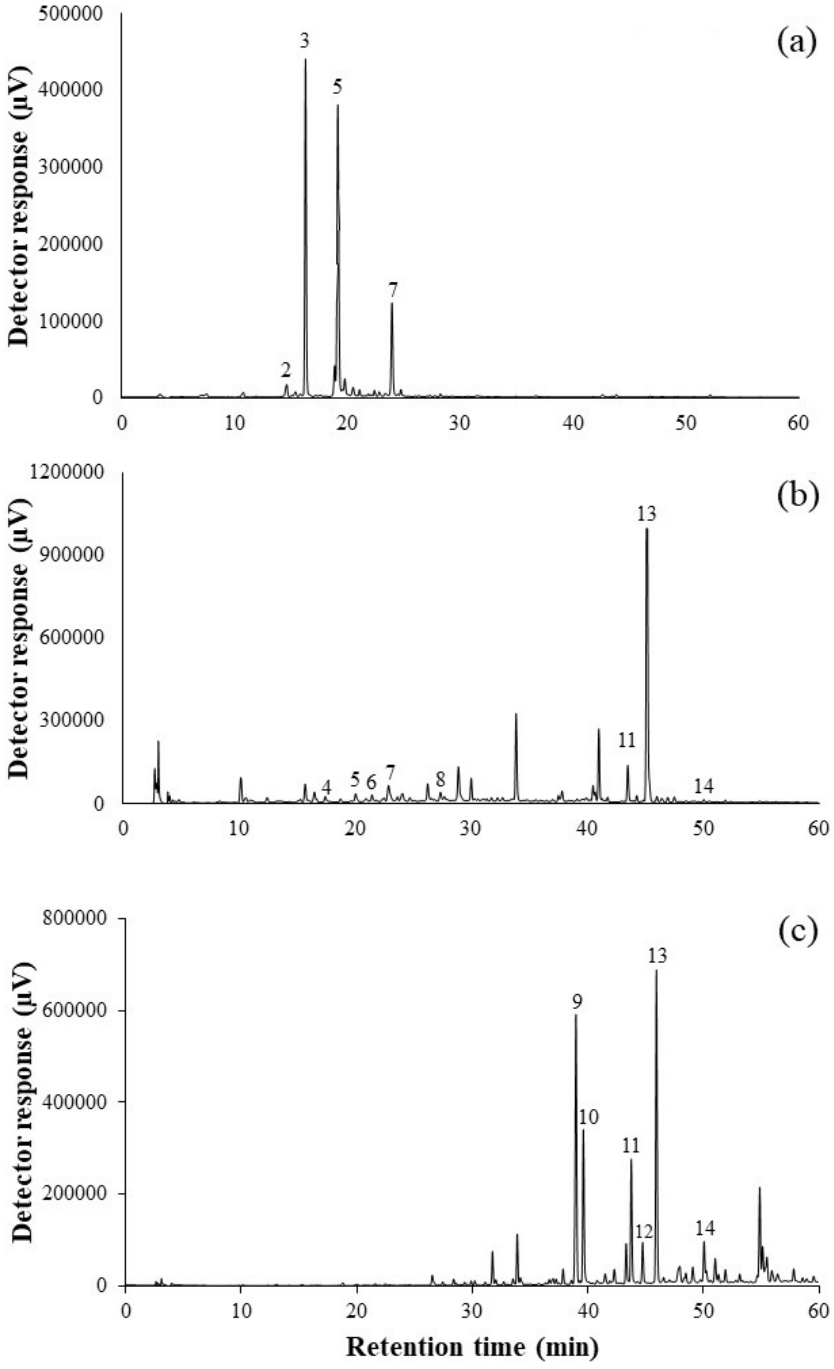

Figure 3-3 HPLC chromatograms of antioxidant compounds in edible plant samples monitored at 284 nm. (a) Japanese tea, (b) sweet basil, and (c) ginger. Japanese tea contains the following: 2, EGC; 3, caffeine; 5, EGCG; and 7, ECG. Sweet basil contains the following:4, vanillic acid; 5, EGCG; 6, coumaric acid; 7, ECG; 8, rosmarinic acid; 11, carvacrol; 13, carnosol; and 14, carnosic acid. Ginger contains the following: 9, gingerol; 10, thymoquinone; 11, carvacrol; 12, thymol; 13, carnosol; and 14, carnosic acid.

Table 3-1 Results from the evaluation of the specificity of the LC method [a)]

Compound	tR (min)	*k*	RS	α	TF
Gallic acid	6.188	0.934	6.089	-	0.744
EGC	13.280	3.150	14.877	3.379	0.782
Caffeine	14.917	3.661	5.018	1.162	0.943
Vanillic acid	16.099	4.031	4.544	1.101	1.000
EGCG	17.778	4.556	5.460	1.130	0.985
Coumaric acid	20.983	5.557	10.712	1.220	1.003
ECG	22.598	6.062	6.181	1.091	1.067
Rosmarinic acid	27.381	7.556	19.220	1.247	1.053
Gingerol	39.634	11.386	54.373	1.507	1.030
Thymoquinone	40.192	11.560	2.375	1.015	0.999
Carvacrol	41.790	12.059	6.657	1.043	0.980
Thymol	42.511	12.285	1.721	1.010	1.010
Carnosol	45.910	13.344	12.821	1.086	1.056
Carnosic acid	50.556	14.799	17.376	1.109	1.011

[a)] Chromatographic parameters of 14 antioxidant compounds with UV-Vis HPLC (detection, wavelength 284 nm), mixed 14 antioxidant compounds (concentration ×0.1) per 20 µL injection (n=5).
tR; retention time, k; retention factor, Rs; resolution factor, α; separation factor, TF; tailing factor

Table 3-2 Results from the evaluation of the linearity and range of the LC method

Compound	Regression equation	Correlation factor	Linear range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
Gallic acid	y [a)] $= 3.84\times10^{3}x$ [b)] $+ 1.25\times10$	0.9988	0.43-50	0.13	0.43
EGC	$y = 2.33\times10^{2}x + 1.68\times10^{2}$	0.9989	7.09-200	2.13	7.09
Caffeine	$y = 9.25\times10^{3}x - 6.17\times10^{2}$	0.9996	0.18-50	0.05	0.18
Vanillic acid	$y = 7.14\times10^{3}x - 1.15\times10^{3}$	0.9999	0.23-50	0.07	0.23
EGCG	$y = 3.72\times10^{3}x - 1.41\times10^{2}$	0.9976	0.07-50	0.02	0.07
Coumaric acid	$y = 2.38\times10^{4}x + 7.74\times10^{2}$	0.9996	0.44-10	0.13	0.44
ECG	$y = 7.19\times10^{3}x + 7.96\times10^{2}$	0.9996	0.23-50	0.07	0.23
Rosmarinic acid	$y = 9.58\times10^{3}x - 1.58\times10^{3}$	0.9984	0.17-50	0.05	0.17
Gingerol	$y = 2.92\times10^{3}x - 3.73\times10^{2}$	1.0000	0.56-50	0.17	0.56
Thymoquinone	$y = 2.82\times10^{2}x + 5.84\times10^{2}$	0.9990	5.85-100	1.75	5.85
Carvacrol	$y = 2.10\times10^{3}x - 1.60\times10^{2}$	1.0000	0.78-50	0.24	0.78
Thymol	$y = 2.89\times10^{3}x - 4.35\times10^{2}$	0.9997	0.57-50	0.17	0.57
Carnosol	$y = 1.85\times10^{3}x - 1.31\times10^{3}$	0.9995	0.89-50	0.27	0.89
Carnosic acid	$y = 8.58\times10^{2}x + 2.33\times10$	0.9998	1.92-50	0.58	1.92

[a)] y = mass concentration (µg/mL)

[b)] x = HPLC peak area

Table 3-3 Results from the evaluation of the intra- and inter-day precision of the LC method

Compound	×0.5 standard solution				×0.1 standard solution			
	tR-RSD (%)		AREA-RSD (%)		tR-RSD (%)		AREA-RSD (%)	
	Intra-day [a)]	Inter-day [b)]	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
Gallic acid	0.064	0.016	0.021	0.024	0.028	0.014	0.012	0.007
EGC	0.049	0.012	0.024	0.019	0.019	0.010	0.010	0.010
Caffeine	0.045	0.010	0.021	0.020	0.017	0.010	0.008	0.004
Vanillic acid	0.040	0.010	0.020	0.023	0.015	0.009	0.007	0.008
EGCG	0.038	0.009	0.021	0.024	0.014	0.008	0.007	0.017
Coumaric acid	0.032	0.008	0.020	0.024	0.012	0.007	0.008	0.006
ECG	0.030	0.007	0.018	0.023	0.011	0.006	0.012	0.013
Rosmarinic acid	0.019	0.006	0.025	0.028	0.007	0.001	0.007	0.019
Gingerol	0.007	0.003	0.018	0.019	0.002	0.002	0.010	0.018
Thymoquinone	0.008	0.003	0.009	0.010	0.003	0.003	0.009	0.027
Carvacrol	0.007	0.003	0.019	0.017	0.002	0.002	0.012	0.004
Thymol	0.006	0.021	0.021	0.017	0.002	0.002	0.012	0.006
Carnosol	0.005	0.019	0.019	0.026	0.002	0.002	0.013	0.024
Carnosic acid	0.004	0.027	0.025	0.025	0.002	0.001	0.051	0.055

[a)] Intra-day at five times in 1 day (n=5)

[b)] Inter-day on 3 different days (n=3)

tR; retention time, AREA; peak area

Table 3-4 Results from the evaluation of the recovery in plant samples of the LC method

Sample	Coriander					Japanese tea				
Spiked standard	Orig. [a)]	1.0 [b)]	0.5 [b)]	0.2 [b)]	Ave. [c)]	Orig.	1.0	0.5	0.2	Ave.
Gallic acid	nd	94.82	78.23	88.88	87.31	nd	107.92	101.57	138.22	115.91
EGC	nd	87.61	74.50	80.65	80.92	38.93	93.10	86.61	105.99	95.23
Caffeine	0.04	98.98	80.76	93.56	91.10	0.50	100.82	84.74	106.53	97.36
Vanillic acid	nd	96.18	82.64	97.66	92.16	nd	105.41	104.71	124.08	111.40
EGCG	nd	87.57	76.99	106.50	90.35	0.99	101.85	93.06	128.79	107.90
Coumaric acid	nd	97.56	84.66	102.73	94.98	nd	101.33	80.17	90.55	90.68
ECG	0.10	34.31	71.15	119.57	95.01	0.19	101.12	82.32	97.65	93.70
Rosmarinic acid	nd	95.06	81.66	95.98	90.90	nd	102.58	93.31	109.89	101.92
Gingerol	0.12	94.53	78.93	92.24	88.57	nd	99.77	91.69	104.22	98.65
Thymoquinone	nd	92.35	78.52	89.33	86.73	nd	97.99	99.56	101.41	99.65
Carvacrol	0.19	80.51	98.08	95.33	91.31	nd	106.14	88.56	105.57	100.09
Thymol	nd	101.26	80.83	87.85	89.98	nd	106.26	93.95	100.66	100.29
Carnosol	0.12	76.40	92.86	94.55	87.94	nd	114.77	93.41	121.77	109.98
Carnosic acid	0.39	80.93	90.97	80.27	84.06	nd	104.96	81.68	95.03	93.89

a) The average levels of original analytes in each sample (mg/g), nd; not detected.

b) Spiked standard 1.0; this solution contained 0.5 mL of the original standard solution, 5 mg plant sample, and methanol to fill up 8 mL, 0.5; this solution contained 0.25 mL of the original standard solution, 5 mg plant sample, and methanol to fill up 8 mL, 0.2; this solution contained 0.1 mL of the original standard solution, 5 mg plant sample, and methanol to fill up 8 mL. Each data; Recoveries of each antioxidant compounds at three concentrations (%).

c) The average recoveries for the analyses of three concentrations of spiked standard solutions (%).

Table 3-5 Analytical results of the antioxidants content in plant samples (mg/g sample) [a)]

	Sample name											
Compound	Kaffir lime leaf	Galangal	Turmeric	Fingerroots	Ginger	Lemongrass	Pandan leaf	White pepper	Sweet basil	Climbing wattle	Coriander	Japanese Tea
Gallic acid	nd	nd	nd	nd	nd	nd	0.08±0.02	nd	nd	nd	nd	nd
EGC	nd	nd	nd	2.41±0.01	nd	nd	nd	nd	nd	2.35±0.36	nd	38.93±0.09
Caffeine	nd	nd	nd	nd	nd	nd	0.57±0.01	nd	nd	nd	0.04±0.01	25.08±0.07
Vanillic acid	nd	nd	nd	nd	nd	nd	nd	nd	0.10±0.01	nd	nd	nd
EGCG	nd	0.04±0.01	nd	nd	nd	nd	nd	nd	0.10±0.01	nd	nd	49.52±0.21
Coumaric acid	0.11±0.01	nd	nd	nd	nd	nd	0.22±0.01	nd	0.02±0.01	nd	nd	nd
EGC	nd	nd	nd	nd	nd	nd	nd	nd	0.19±0.01	0.20±0.01	0.10±0.01	9.61±0.03
Rosmarinic acid	0.21±0.06	nd	nd	0.75±0.01	nd	0.13±0.01	nd	nd	0.08±0.01	nd	nd	nd
Gingerol	1.31±0.04	0.51±0.01	nd	13.84±0.11	12.27±0.24	nd	nd	nd	nd	nd	0.12±0.01	nd
Thymoquinone	nd	nd	nd	239.15±0.75	57.57±1.52	nd	nd	nd	nd	nd	nd	nd
Carvacrol	nd	nd	48.66±2.38	nd	0.75±0.02	0.64±0.01	nd	2.31±0.03	1.44±0.02	nd	0.19±0.01	nd
Thymol	nd	nd	78.45±2.64	nd	0.78±0.06	0.52±0.01	nd	nd	nd	nd	nd	nd
Carnosol	nd	nd	nd	nd	19.60±0.20	nd	nd	7.17±0.05	8.74±0.06	nd	0.12±0.01	nd
Carnosic acid	nd	0.07±0.01	nd	1.96±0.08	6.62±0.10	nd	nd	7.07±0.25	0.17±0.02	nd	0.39±0.01	nd

[a)] Mean ± standard deviations (n=3), nd; not detected

Chapter 4. Evaluation of Chemical Components of Herbs and Spices from Thailand and Effect on Lipid Oxidation of Fish During Storage

4-1. Introduction

Thailand's food is inspired by various cultures and cuisines to produce unique flavors in delectable combinations (Tan 2005). Sriwattana et al. (2002) summarized the popularity of Thailand's food and found that the most popular were Tom Yum Kung (hot and sour shrimp soup), Pad Thai (Thai style fried noodles), and Kaeng Kew Wan (green curry). These foods contain various herbs and spices, including kaffir lime leaves, galangals, chilis, lemongrass, chives, garlic, and coriander, which make Thailand's food healthy. According to Khanthapok and Sukrong (2019), Thailand's food contains several antioxidants and biological compounds that have benefits such as anti-aging effects. Danyuthasilpe et al. (2009) studied elderly individuals in northern Thailand. The survey showed that Thai cooking commonly contains a variety of organic products, such as vegetables, herbs, and spices. In addition, Siwarungson and Lertpringkop (2017) investigated Thai curry soups without coconut milk. They determined that all the soups contained several antioxidant, anti-aging, and anti-hypertensive compounds, which provide a variety of health benefits.

In this study, 11 herbs and spices from Thailand were evaluated for other fat- and water-soluble components and total polyphenolic content to investigate their radical scavenging activities. The effects of herb and spice powders on lipid oxidation in fish were evaluated. This study aimed to collect general data on the chemical components in herbs and spices and investigate their ability to scavenge free radicals and to prevent lipid

oxidation for improved food preservation.

4-2. Materials and Methods

4-2-1. Sample Preparation

Eleven fresh herb and spice samples were obtained from a local market in Nakhon Ratchasrima, Thailand. The sample names (with scientific names), analytical parts, and obtained locations are listed in Table 4-1. All samples were cut and sun-dried until all water was completely removed. They were then pulverized using a blender (MX1200XTSLJ; Waring Commercial, McConnellsburg, Pennsylvania, USA) and stored at -30 °C until further analysis. The resulting herb and spice powders are shown in Figure 1. Raw fish of the species, *Seriola dumerili* (amberjack), were purchased from a local market and homogenized using a food processor (TK 435; TESCOM, Tokyo, Japan). Homogenized fish were stored in a plastic bag at -30 °C until further analysis.

4-2-2. Chemicals

Potassium persulfate, L-malic acid, L-lactic acid, Folin-Ciocalteu reagent, campesterol, β-sitosterol, desmosterol, stigmasterol, ergosterol, triphenyl phosphine, and acetonitrile were purchased from Sigma-Aldrich Ltd. (Tokyo, Japan). α-, β-, γ-, and δ-Tocopherol were purchased from Eisai Co. Ltd. (Tokyo, Japan). Triethylamine and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). AccQ-FBB and AccQ-Flour reagents were purchased from Waters Corporation

(Milford, Massachusetts, USA). 1,3-Cyclohexanedione and oxalic acid were purchased from Nacalai Tesque (Kyoto, Japan). Pyroglutamic acid was purchased from MP Biomedicals (Illkirch-Graffenstaden, France). 4-Hydroxy-trans-2-hexenal (HHE) were purchased from Cayman Chemical Company (Ann Arbor, Michigan, USA). Distilled water was purchased from Takasugi Pharmaceutical Co. Ltd. (Fukuoka, Japan). Other chemicals and solvents used in the experiments were purchased from FUJIFILM Wako Pure Chemical Industries (Osaka, Japan). All reagents used in this experiment were of reagent grade.

4-2-3. Analysis of Lipophilic Components

Lipophilic components were extracted from the herbs and spices by following the method described by Ito et al. (2018). Each herb and spice powder (1 g) was mixed with a chloroform:methanol solution (30 mL, 2:1 v/v) and homogenized. The upper layer was centrifuged and removed using an aspirator. The lower layer was filtered and evaporated until approximately 5 mL remained. The chloroform:methanol solution (up to final volume of 10 mL, 2:1 v/v) was added to the final solution and stored at -30 °C until further analysis. To determine the fatty acid composition, 3 g of lipid was measured, trans-methylated through saponification, and subjected to BF3-catalyzed methylation according to Ito et al. (2018) method. These fatty acid methyl esters (FAMEs) were resuspended in acetone and analyzed using gas-liquid chromatography (GLC). To determine the tocopherol content, 3 mg of extracted lipid was saponified and the unsaponifiable substance was extracted according to Ito et al. (2018). The unsaponifiable substance was dissolved in an ethyl acetate:hexane solution (200 μL, 85:15 v/v) for

analysis by high-performance liquid chromatography (HPLC). Sterols were analyzed following the method described by Yoshida et al. (2006). The unsaponifiable substance, which was extracted following a similar method as for tocopherol analysis, was dissolved in acetone (100 μL) and analyzed using GLC.

4-2-4. Analysis of Water-soluble Components

Water-soluble components were extracted from herbs and spices following the method described by Tanaka et al. (2018). The herb and spice powder samples (1 g) were mixed with 1% perchloric acid (20 mL) and homogenized. Then, the pH was adjusted to 6.8 using a potassium hydroxide solution before being centrifuged. Distilled water (up to final volume of 50 mL) was added to the solution, and it was filtered through a 0.8 μm filter. The final solution was stored at -30 °C until further analysis. The free amino acid content was analyzed following the method described by Tanaka et al. (2018). The processed solution was analyzed using HPLC. Purine and pyrimidine were extracted as described by Ishimaru et al. (2016). The water-soluble solution was injected into the HPLC system and analyzed at a wavelength of 270 nm. Organic acids were analyzed following the method described by Sekizawa et al. (2013). The water-soluble solution was directly injected and analyzed using HPLC at a wavelength of 210 nm.

4-2-5. Analysis of Total Polyphenol Content

The total polyphenol content was compared with the lipophilic, water-soluble, and methanol-soluble components. The extraction of lipophilic and water-soluble components

was previously described in the extraction of lipophilic and water-soluble component sections, respectively. Methanol-soluble substances were extracted from herbs and spices following the method described by Jongsawatsataporn and Tanaka (2022).

The total polyphenol content was analyzed following a modified version of the method described by Lim et al. (2019) with modifications. First, methanol (1.9 mL) was added to the extract solutions (100 μL of lipophilic, water-soluble, and methanol-soluble component solutions), followed by the addition of the Folin-Ciocalteu reagent (500 μL). The resulting solution was incubated for 5 min at 30 °C in a water bath. Thereafter, a 20% sodium carbonate solution (5 mL) was added to the solution, after which it was incubated again for 40 min at 30 °C. Finally, the solution was centrifuged at 3000 × g for 10 min. The total polyphenol content was determined by measuring the absorbance increase at 730 nm between the blank and sample solutions. The results were calculated against a calibration curve of pyrogallol, and the data was expressed in micrograms of pyrogallol equivalent per gram of sample.

4-2-6. Analysis of Radical Scavenging Activity

The radical scavenging activities of the DPPH and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acids (ABTS) in the extracts were analyzed following the method described by Lim et al. (2019). For DPPH analysis, a DPPH solution (2.5 mL) was added to a mixture of the extract solutions (100 μL) and ethanol (1.9 mL). The samples were incubated for 30 min at 30 °C in a water bath. The activities were determined by measuring the decrease in absorbance of DPPH radicals at 520 nm based on the difference between the blank and sample solutions. The results were calculated against a calibration

curve of α-tocopherol and the data was expressed in micrograms of α-tocopherol equivalent per gram of sample because α-tocopherol is widely used as an index of antioxidant activity.

For ABTS analysis, an ABTS solution (4.75 mL) was added to the sample extracts (0.25 mL). The samples were then incubated for 6 min at 30 °C in a water bath. The activities were determined by measuring the decrease in absorbance of ABTS radicals detected at 740 nm based on the difference between the blank and sample solutions. The results were calculated against a calibration curve of α-tocopherol and the data was expressed in micrograms of α-tocopherol equivalent per gram of sample.

4-2-7. Effect of Herbs and Spices Powders Addition to Fish on Lipid Oxidation

In this study, we referred to Wang et al. (2019) to investigate the effect of herb and spice powders on the suppression of lipid oxidation in fish meat. To accomplish this goal, three types of fish meat samples were prepared. Fresh and frozen minced fish meat were prevented from undergoing oxidation. For the control, frozen minced fish meat (3 g) was weighed into a 50 mL glass centrifuge tube and capped. The samples were subjected to oxidation in a water bath at 30 °C for 15 h. For the preparation of the fish meat with herb and spice powder samples, minced frozen fish meat (10 g) and a powder of herb and spice (0.5 g) were weighed into a mortar and mixed using a pestle. The sample was transported into a 50 mL glass centrifuge tube, capped, and subjected to oxidation in a water bath at 30 °C for 15 h. After preparation, the samples were analyzed for lipid peroxide (LPO) and aldehydes. LPO was analyzed according to the method described by Nakamura et al. (1998). Cyclohexane and triphenylphosphine reagent (100 μL each) were added to an

aliquot (3 mg) of the lipids in a screw-capped tube. The mixture was shaken gently at 30 °C in the dark for 30 min. The resulting stoichiometrically generated triphenylphosphine oxide was determined using an HPLC system at a wavelength of 260 nm. The aldehyde content was analyzed according to the method described by Tanaka et al. (2013a). The samples that contained aldehydes were reacted with a 1,3-cyclohexanedione (CHD) reagent. CHD-aldehydes were analyzed using an HPLC system with a fluorescence detector at an excitation and emission wavelength of 385 and 450 nm, respectively. The results were calculated by comparing the level of lipid oxidation of the fresh fish meat, control, and fish meat with herb and spice powder samples.

4-2-8. Statistical Analysis

In this study, the data was presented as the mean ± standard deviation (SD). Principal component analysis (PCA) was conducted using a Bell Curve in Microsoft Excel software (Social Survey Research Information Co., Ltd.). PCA was conducted on normalized data to reduce the number of actual variables (44 chemical compounds, total polyphenol content, radical scavenging activities, and LPO and aldehyde contents in fish) and derived variables (principal components) that summarize the total variance, that is, to correlate the chemical compositions of herbs and spices with their respective characteristics.

4-3. Results and Discussion

4-3-1. Lipophilic Components

The fatty acid composition of the herbs and spices is shown in Table 4-2. All samples contained various fatty acids except palmitoleic acid (16:1), which is commonly found in plants, especially oil crops and nuts like macadamias (Aquino-Bolaños et al. 2017; Reszczyńska and Hanaka 2020). Similarly, other researchers have rarely reported or did not mention the palmitoleic acid content in herbs and spices (Nengroo and Rauf 2019; Savych et al. 2021). Neffati and Marzouk (2009) reported that a small amount of palmitoleic acid content was observed in coriander. The primary focus of this experiment was linolenic acid (18:3 n-3), which is a greatly beneficial fatty acid that acts as and is converted to eicosapentaenoic acid and is predominantly found in plants. Sweet basil had the highest percentage of linolenic acid (52.96±0.92% fatty acid), followed by coriander (36.41±0.52% fatty acid) and pandan leaf (28.28±0.94% fatty acid) (Rajaram 2014). According to a report by Daga et al. (2022), sweet basil contains a higher concentration of linolenic acid than coriander, but that a prominent amount was still present in both herbs. In addition, Saini et al. (2021a) reported linoleic acid (18:2 n-6) in galangal, turmeric, ginger, lemongrass, white pepper, and sweet basil. Most of the results from the aforementioned reports contained similar information and conclusions, including similar percentages of fatty acids in galangal, ginger, and white pepper. Moreover, there are many reports on the essential oil components of pandan leaves. However, there are only a few reports on the fatty acid composition of raw pandan leaves. Our data may be the first to show that pandan leaves contain a higher linolenic acid content than the more commonly studied herbs such as sweet basil. In case of n-6/n-3 ratio, all samples except for white pepper contained less than 6% of the n-6/n-3 ratio. A higher n-6/n-3 ratio increases the risk of obesity, inflammation, and cancer (Simopoulos 2016; Yang et al. 2016b; Xia et al. 2005). This means that most of the herbs and spices in this experiment were safe for adult

consumption (Wijendran and Hayes 2004).

The tocopherol content of the herbs and spices is shown in Table 4-3. Plants are described as living organisms that can accumulate and store lipids in their cells. However, when compared with oil crops, including sunflower, palm, and canola oils, a lower lipid content was observed, which was scarcely reported (Singer and Weselake 2016). Herbs and spices mainly contain α- and γ-tocopherols, which are the major forms of vitamin E. These tocopherols can prevent inflammation in cells and act against prostate cancer (Jiang et al. 2001; Reiter et al. 2007). In this study, kaffir lime leaves contained the highest α-tocopherol content (517.15±8.89 ng/mg lipid), whereas ginger contained the highest γ-tocopherol content (86.07±1.13 ng/mg lipid) among the samples. However, none of the samples contained β- or δ-tocopherols. Dertyasasa and Tunjung (2017) also reported that kaffir lime leaves contain both α- and γ-tocopherols. In addition, Jelled et al. (2015) confirmed that ginger contains high levels of α- and γ-tocopherols. Other herbs and spices are also consistent with other researchers' reports, including Gómez-Coronado et al. (2004), who reported the contents of α- and γ-tocopherols in basil and coriander.

The sterol content of the herbs and spices are shown in Table 4-4. All samples contained stigmasterol and β-sitosterol, which are important compounds commonly found in plants (Babu and Jayaraman 2020; Chaudhary et al. 2011). Stigmasterol is related to hormone synthesis in humans, whereas β-sitosterol plays an important role in anti-diabetic and anticancer activities, and can act as an antioxidant (Babu and Jayaraman 2020; Chaudhary et al. 2011). However, campesterol, ergosterol, and desmosterol were present in small amounts or were absent from some samples, such as coriander, which contained stigmasterol, β-sitosterol, and campesterol. Saini et al. (2021ab) similarly reported the sterol content of coriander. The turmeric sample contained the highest

stigmasterol (8.30±1.20 μg/g lipid) and β-sitosterol (14.69±2.00 μg/g lipid) content with the highest among the samples. This result is consistent with the reports of Gupta et al. (2013) and Li et al. (2011). Many researchers focus on the sterol content of oil crops, including palm, sunflower, and soybean oils (Derek et al. 2017). Therefore, the data indicates that non-oil crops contain relatively small amounts of fatty acids and sterol. This is also consistent with the result that relatively small amounts of sterol content exist in the herb and spice samples that are not oil crops.

4-3-2. Water-soluble Components

The free amino acid content of the herbs and spices is shown in Table 4-5. The results showed that climbing wattle contained the highest total free amino acid content among the samples, followed by sweet basil and kaffir lime leaves. Other researchers have reported that some amino acids, including arginine (Arg), glycine (Gly), lysine (Lys), alanine (Ala), tyrosine (Tyr), serine (Ser), and histidine (His), could function as antioxidants (Martínez-Tomé et al. 2001). This implies that most of the herbs and spices used in the experiments contained the abovementioned amino acids, especially climbing wattle, which contained the highest Arg content (13,061.48±87.9 μg/g sample). Some researchers have reported the amino acid content of other plant samples. For example, Ajayi et al. (2013) and Ifeanyi et al. (2014) reported the amino acid content in ginger and Almuhayawi et al. (2021) reported the amino acid content in lemongrass. Our results are consistent with these reports. When we focused on human essential amino acids, including phenylalanine (Phe), valine (Val), tryptophan (Trp), threonine (Thr), isoleucine (Ile), methionine (Met), leucine (Leu), His, and Lys but excluding Met and Trp, which

were not present in any sample, and Thr, which was not present in fingerroots, ginger, lemongrass, and white pepper, the samples contained high levels of these essential amino acids (Lopez and Mohiuddin 2021).

The purine and pyrimidine monophosphate contents of the herbs and spices are shown in Table 4-6. None of the herbs and spices in this experiment contained guanosine 5′-monophosphate (GMP) or inosine 5′-monophosphate (IMP), whereas small amounts of cytidine 5′-monophosphate (CMP) and adenosine 5′-monophosphate (AMP) were present in some samples. Ginger did not contain any purine or pyrimidine monophosphate. Overall, sweet basil, climbing wattle, and coriander contained significantly higher levels of purine and pyrimidine monophosphate than the other samples. Coriander had the highest CMP content (198.14±4.6 μg/g sample) and climbing wattle contained the highest AMP content (368.75±18.2 μg/g sample). Purine and pyrimidine contents have been used to determine the level of freshness of edible animals and fish. However, CMP, GMP, IMP, and AMP can also determine the umami taste in various foods (Ishimaru et al. 2016). Duan et al. (2020a) reported the amounts of purine and pyrimidine monophosphate in sweet basil and coriander. These results might be slightly different from those of the present study. However, they also concluded that both sweet basil and coriander contained lesser amounts of purine and pyrimidine monophosphate than the other samples. In another study, Duan et al. (2020b) reported the purine and pyrimidine content of turmeric. However, only CMP was present in this experiment, in contrast to Duan et al.'s reports, which reported very small amounts of all types of purine and pyrimidine monophosphate (Duan et al. 2020a; Duan et al. 2020b).

The organic acid content of the herbs and spices is shown in Table 4-7. All samples contained fumaric acid, especially coriander, which had the highest content among the

samples (54.98±1.12 mg/g sample). Fumaric acid is present in several plants because it has an important function in the fixed carbon procedure. Older plants tend to have a higher content of fumaric acid compared to younger plants (Chia et al. 2000). Other organic acids were also present in the samples, including oxalic, citric, and succinic acids. However, relatively small amounts of unpleasant smelling organic acid groups, including propionic, butyric, isovaleric, and valeric acids, were present in the herb and spice samples that were analyzed (Amoore 1977; Hey et al. 2009; Turner-Walker 2012). The results indicated that the galangal sample was the only one that contained butyric acid. It was also determined that sweet basil contained a relatively small amount of isobutyric acid. As previously mentioned, both Duan et al. (2020ab) reports conclude that the purine, pyrimidine monophosphate, and organic acid contents in turmeric, sweet basil, and coriander. The results remained consistent with this analysis despite the fact that only a few of the organic acids were analyzed. In particular, a high oxalic acid content was present in turmeric (20.64±0.35 mg/g sample). Oxalic acid is present in various plants and can act as an antioxidant (Kayashima and Katayama 2002). A higher radical scavenging activity is typically associated with a higher oxalic acid content.

4-3-3. Total Polyphenol Content

The total polyphenol content of herbs and spices are shown in Table 4-8. This experiment used three types of herb and spice extracts, including lipophilic, water-, and methanol-soluble solutions. Each type of extract had different total polyphenol content. In particular, the lipophilic extract of turmeric, and the methanol-soluble extracts of fingerroot and ginger expressed higher polyphenol content than the other extracts. The result clearly

showed that turmeric contains the highest total polyphenol content, followed by fingerroot and ginger. Herbs and spices are sources of polyphenols. When consuming herbs and spices, the level of polyphenol intake also increases (Tantipopipat et al. 2010). Thailand's herbs and spices are widely used in Thai cuisine and in supplementary and commercial snacks (Wangcharoen et al. 2002). Many studies have reported the antioxidative ability and polyphenol content of turmeric and ginger. Alafiatayo et al. (2014) reported the polyphenol content of ten selected species of *Zingiberaceae* rhizomes. The report also showed that turmeric and ginger contained higher polyphenol content than other samples. Jongsawatsataporn and Tanaka (2022) reported higher antioxidant contents in turmeric, fingerroot, and ginger. Słowianek and Leszczynska (2016) also reported antioxidant properties in methanol-extracts of various herbs and spices, including ginger, sweet basil, and turmeric. Among all samples of herbs and spices, the polyphenol content of turmeric remained the highest compared to the others in this study.

4-3-4. Radical Scavenging Activity

The radical scavenging activities of herbs and spices are shown in Table 4-8. In this study, DPPH and ABTS radical scavenging activity in lipophilic, water- and methanol-soluble extracts were observed. Among the results, the DPPH and ABTS in water-soluble extracts exhibited the highest radical scavenging activity. Regarding DPPH, the lipophilic extract of turmeric (221.39±2.65 μg α-tocopherol equivalent), water-soluble extract of kaffir lime leaves (325.63±17.24 μg α-tocopherol equivalent, and methanol-soluble extract of ginger (296.65±25.69 μg α-tocopherol equivalent) exhibited the highest equivalent contents. The lipophilic and methanol-soluble extracts of turmeric and the water-soluble extracts of

climbing wattle exhibited the highest equivalent contents of ABTS. The lipophilic extract of turmeric contained 746.23±6.61 μg α-tocopherol equivalent, the water-soluble extract of climbing wattle contained 3946.6±293.20 μg α-tocopherol equivalent, and the methanol-soluble extract of turmeric contained 757.76±39.98 μg α-tocopherol equivalent. Similar to polyphenols, many researchers have reported DPPH and ABTS radical scavenging activities of herbs and spices. According to the previous topic, Słowianek and Leszczynska (2016) also reported the percentage inhibition of DPPH in methanol-extracts of ginger, sweet basil, and turmeric. Compared with our results, the DPPH and ABT radical scavenging activities of ginger, sweet basil, and turmeric were excellent and showed a high inhibition rate. Nikolic et al. (2019) reported on the DPPH and ABT content of sweet basil. The results showed a relatively small amount of equivalent content. Assefa et al. (2018) also reported on various herbs and spices, including galangal, coriander, turmeric, sweet basil, white pepper, and ginger. Lu et al. (2011) reported the DPPH activity of galangal, ginger, and white pepper. In conclusion, turmeric was mostly reported because of its high efficiency in both DPPH and ABTS, equivalent to all types of extracts, and the results were consistent with findings from other researchers.

4-3-5. Effect of Herbs and Spices Powders Addition to Fish on Lipid Oxidation

The LPO levels in fish mixed with herbs and spices are shown in Figure 4-2a. This bar graph represents the LPO levels in fresh fish meat, the control, and fish meat with herb and spice powder. The LPO content of the control increased when compared with fresh fish meat. The LPO content of fish meat with kaffir lime leaves, pandan leaves, sweet basil, climbing wattle, and coriander increased. In other samples which included galangal,

finger root, ginger, and lemongrass, the LPO levels decreased, whereas only turmeric and white pepper samples were not different from the control. In agreement with the total polyphenol content and radical scavenging activity sections, galangal, fingerroot, and ginger had a higher total polyphenol content and radical scavenging activity than the other samples. Normally, lipid oxidation can easily occur in food, especially food that contains considerable amounts of oil emulsion, such as salad dressing, mayonnaise, and milk, which could worsen food quality, including taste, smell, texture, and nutritional quality (Waraho et al. 2011). Notably, lipid oxidation in fish can occur anytime between feeding in the aquaculture process and the cooking of the fish, meaning that without careful handling, the fish will get spoiled (Secci et al. 2016). Because of this condition, many researchers have studied lipid oxidants in fish and examined them by adding several components, such as tea catechins for minced meat or grape extract in fish (Tang et al. 2001; Sánchez-Alonso et al. 2007). Herbs and spices are widely used to prevent lipid oxidation in food. For example, Nikousaleh and Prakash (2016) reported the effect of cloves and cinnamon on meat sausage, and Juntachote et al. (2007) used dried holy basil to cook ground pork. The studies all reported that the addition of a high antioxidant-containing substance could better prevent lipid oxidation. In this experiment, 5% of fish weight herbs and spices powder was added to fish according to Wang et al. (2019) report which confirm that adding 5% weight of mushrooms to Cantonese sausages did not significantly change the lipid content or nutrition composition of the sausages while slightly affecting the color, texture, and sensory profile, together with good results of LPO prevention. In conclusion, the results of preventing lipid oxidation by galangal, fingerroot, and ginger in this study were consistent with other researchers' reports.

The aldehyde levels in fish mixed with herbs and spices are shown in Figures 4-2b

and 4-2c. As mentioned in the lipid peroxide level in the fish mixed with herbs and spices section, Figures 2b and 2c represent the amounts of HHE and propanal in fresh fish meat, the control, and fish meat with herb and spice powder. In this study, we focused on HHE and propanal as oxidation indicators in fish. These aldehydes are produced by the oxidation of n-3 fatty acids, which are abundant in fish products, and their levels increase owing to quality deterioration. In a previous study, we evaluated the quality of frozen saury, frozen tuna, and seaweed by using HHE and propanal levels as oxidative indicators (Tanaka et al. 2013b; Tanaka et al. 2016a; Tanaka et al. 2016b). In the present study, the HHE and propanal levels of the samples with herbs and spices were decreased. Moreover, aldehyde levels in most of the samples decreased. Lemongrass contained the highest aldehyde content among the herbs and spices that were examined, but still showed a significant difference between the fresh fish meat and control. Pushpakumari et al. (1987) and Mathew and Joseph (2008) reported that lemongrass had a high aldehyde content. These results can explain the higher HHE and propanal levels in lemongrass-mixed samples compared to the others. Aldehyde group components were produced as the main product during lipid oxidation, indicating that higher lipid oxidation occurred and higher contents of the aldehyde group were present (Miyasaki et al. 2011; Zhang et al. 2020). As mentioned in the section on lipid peroxide levels in fish mixed with herbs and spices, if lipid oxidation prevention by herbs and spices was successful, the aldehyde content in fish with herbs and spices with high polyphenol content and radical scavenging activities should be lower than that of the control and fresh fish samples. In particular, the results of this study are presented in a similar manner and a superior result for lipid oxidation could be observed. This suggests that herb and spice powders could potentially prevent aldehyde creation during lipid oxidation in fish or reduce the lipid oxidation activity.

4-3-6. Correlation Between Antioxidant Compounds and Antioxidant Capacity

The correlation between antioxidant compounds and antioxidant capacity is shown in Table 4-9. This table presents the correlation coefficient (r) between antioxidant capacity (DPPH or ABTS radical scavenging activity) and antioxidative compounds (total polyphenol content, tocopherol, and amino acids). The correlation between ABTS radical scavenging activity and the polyphenol content in lipophilic extracts had the strongest positive correlation with r=0.968, followed by the correlation between ABTS radical scavenging activity and the polyphenol content in methanol-extracts (r=0.954), ABTS radical scavenging activity of water-soluble Arg (r=0.815), and ABT radical scavenging activity of water-soluble Gly (r=0.750). According to Gogtay and Thatte (2017), these strong positive correlations imply that the factors are positively related to each other. In this case, if the total polyphenol content of the lipophilic or methanol-soluble extract is high, the ABTS radical scavenging activity results of the lipophilic or methanol-soluble extracts are also high. As mentioned in the amino acid analysis results section, this also confirms that Arg and Gly act as antioxidants that can affect the ABTS radical scavenging activity of the water-soluble extracts. Similarly, an r-value higher than 0.5 also confirmed that the relationship between the two factors was still present (Gogtay and Thatte 2017). Thus, the results of the total polyphenol content and DPPH radical scavenging activity of lipophilic extracts (r=0.618) and ABTS radical scavenging activity of water-soluble Lys (r=0.614) showed positive relationships with each other. However, the results of other interactions, including negative relationships, showed a weak relationship with each other due to an r-value near 0, which means that they are not affected by each other.

4-3-7. Assortment by Principal Component Analysis

PCA was used to evaluate the relationship between 56 variable chemical substances and 11 herbs and spices from Thailand (Figure 4-3). An estimated 48.52% of the variability could be attributed to the first two dimensions. PC1 and PC2 accounted for 29.99% and 18.93% of the variance, respectively. The results were divided into three groups: G1, G2, and G3 (Figure 3a). G1, which included pandan leaves, sweet basil, climbing wattle, and coriander, was located to the right of the y-axis. G2, including turmeric, was located at the top of the x-axis and to the left of the y-axis. G3, which is comprised of the remaining samples, was located at the bottom of the x-axis and to the left of the y-axis. Compared with Figure 3b, G1 contained a larger variety of amino acids, organic acids, AMP, linoleic acid (18:3n-3), and ABTS in the water-soluble extracts. However, this area also included LPO, which represents a higher lipid oxidation content in fish. For G2, most of the radical scavenging activities, including that of ABTS in lipophilic and methanol-soluble extracts and the total polyphenol content of lipophilic, water-, and methanol-soluble extracts, were located along with stearic acid (18:0), oxalic acid, and campesterol. G3 contained various sterol groups, DPPH of water- and methanol-soluble extracts, oleic acid (18:1n-9), asclepic acid (18:1n-7), α- tocopherol, acetic acid, butyric acid, and cysteine (Cys-Cys). This group also showed elevated levels of HHE and propanal. As shown in Figure 3b, most components located in G1 were water-soluble. This result is consistent with the prediction that the ABTS radical scavenging activity of the water-soluble extracts in G1 were higher than that of G2 and G3. The G2 extracts, excluding those that were water-soluble, had a strong relationship with the total polyphenol content and ABTS radical

scavenging activity, which agreed with the result that turmeric had the highest radical scavenging activities. In addition, most of the G3 extracts contained a variety of chemical components and the position of the DPPH radical scavenging activity could be determined, excluding lipophilic extracts, acetic acid, butyric acid, Cys-Cys, and Pro, which represented the components in water- and methanol-soluble extracts, and α-tocopherol, 18:1n-7, and ergosterol, which represented the components in lipophilic extracts. Both kaffir lime leaves and lemongrass were used as aroma oils, meaning that they were dominant in lipophilic extracts, whereas galangal, fingerroot, and ginger were more dominant in water-soluble extracts. White pepper was the only sample that showed favorable results for the methanol-soluble extract.

4-4. Conclusion

11 Thai herbs and spices were investigated for their ability to prevent fish lipid oxidation and the production of aldehydes, as well as their total polyphenol content, radical scavenging properties, and chemical components including lipophilic and water-soluble. The results are all excellent and in agreement with those of other researchers, particularly turmeric, ginger, and fingerroot, which have stronger radical-scavenging abilities as well as greater lipid oxidation and aldehyde prevention outcomes. Confirm the health benefits of eating herbs and spices. Along with a strong correlation between samples and chemical components in PCA, the correlation between antioxidant compounds and antioxidant capacity has also shown strongly favorable results, mainly in water-soluble components.

Table 4-1 Herbs and spices analyzed in this study

No	English name (with scientific name)	Analytical part	Obtained place
1	Kaffir lime leaf (*Citrus hystrix*)	Leaves	Nakhon Ratchasrima (Thailand)
2	Galangal (*Alpinia galanga*)	Rhizomes	Nakhon Ratchasrima (Thailand)
3	Turmeric (*Curcuma longa*)	Rhizomes	Nakhon Ratchasrima (Thailand)
4	Fingerroot (*Boesenbergia rotunda*)	Rhizomes	Nakhon Ratchasrima (Thailand)
5	Ginger (*Zingiber officinale*)	Rhizomes	Nakhon Ratchasrima (Thailand)
6	Lemongrass (*Cymbopogon citratus*)	Stems, leaves	Nakhon Ratchasrima (Thailand)
7	Pandan leaf (*Pandanus amaryllifolius*)	Leaves	Nakhon Ratchasrima (Thailand)
8	White pepper (*Piper nigrum L.*)	Seeds	Nakhon Ratchasrima (Thailand)
9	Sweet basil (*Ocimum basilicum*)	Stems, leaves	Nakhon Ratchasrima (Thailand)
10	Climbing wattle (*Senegalia pennata*)	Stems, leaves	Nakhon Ratchasrima (Thailand)
11	Coriander (*Coriandrum sativum*)	Stems, leaves	Nakhon Ratchasrima (Thailand)

Table 4-2 Fatty acid composition (% of total fatty acids) of herbs and spices from Thailand

	Kaffir lime leaf	Galangal	Turmeric	Fingerroot	Ginger	Lemongrass	Pandan leaf	White pepper	Sweet basil	Climbing wattle	Coriander
14:0	4.50±0.23	0.61±0.01	2.85±0.21	1.02±0.28	1.59±0.04	0.77±0.01	0.89±0.11	2.17±0.43	0.45±0.20	0.95±0.05	0.50±0.03
15:0	2.13±0.06	0.93±0.02	3.00±0.03	1.09±0.24	0.56±0.03	0.83±0.01	3.80±0.04	0.31±0.06	4.57±0.01	1.77±0.04	1.53±0.01
16:0	37.29±0.60	44.77±0.40	9.97±0.98	42.59±7.04	32.65±0.86	45.21±0.37	36.39±1.58	22.62±1.42	28.35±1.02	55.45±0.56	21.08±0.72
16:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
18:0	6.27±0.21	6.40±0.11	58.94±1.37	7.60±1.25	5.44±0.14	7.33±0.06	4.85±0.13	3.85±0.34	2.76±0.05	14.17±0.06	10.52±0.16
18:1n-9	13.61±0.20	30.79±0.36	6.78±0.08	18.54±3.51	21.43±0.29	14.10±0.25	4.01±0.09	32.84±0.78	2.12±0.04	4.51±0.04	1.95±0.03
18:1n-7	0.82±0.15	2.08±0.06	2.66±0.01	4.51±0.86	1.64±0.05	1.16±0.23	0.61±0.07	3.70±0.65	0.25±0.01	0.86±0.03	0.28±0.01
18:2n-6	14.01±0.13	10.58±0.05	7.68±0.67	18.51±14.46	31.21±0.39	22.44±0.07	21.17±0.47	31.21±0.48	8.54±0.12	13.35±0.46	27.71±0.37
18:3n-3	21.37±0.23	3.84±0.03	8.11±0.18	6.15±1.38	5.48±0.12	8.16±0.08	28.28±0.94	3.29±0.39	52.96±0.92	8.94±0.14	36.41±0.52
SFA	50.19±1.10	52.71±0.54	74.76±2.59	52.29±8.81	40.24±1.07	54.14±0.46	45.93±1.85	28.96±2.25	36.13±1.28	72.34±0.71	33.64±0.91
MUFA	64.62±1.45	85.58±0.96	84.21±2.69	75.34±13.19	63.32±1.41	69.4±0.93	50.55±2.02	65.49±3.67	38.51±1.33	77.71±0.78	35.87±0.96
PUFA	35.38±0.36	14.42±0.07	15.79±0.84	24.66±15.84	36.68±0.50	30.6±0.14	49.45±1.41	34.51±0.87	61.49±1.04	22.29±0.60	64.13±0.89
n-6/n-3	0.66±0.54	2.75±1.53	0.95±3.78	3.01±10.45	5.70±3.34	2.75±0.88	0.75±0.50	9.48±1.22	0.16±0.13	1.49±3.32	0.76±0.71

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; n-6, total of n-6 fatty acids; n-3, total of n-3 fatty acids; n-6/n-3, ratio of 18:2n-6 and 18:3n-3; nd, not detected.

Each value represents the mean ± SD (n=3).

Table 4-3 Tocopherol contents (ng/mg lipid) of herbs and spices from Thailand

Sample	Kaffir lime leaf	Galangal	Turmeric	Fingerroot	Ginger	Lemongrass	Pandan leaf	White pepper	Sweet basil	Climbing wattle	Coriander
α-Toc	517.15±8.89	18.60±0.93	4.22±0.78	57.30±4.29	27.92±1.38	10.14±0.94	27.98±0.87	190.54±4.89	146.36±3.31	14.16±0.55	91.24±4.71
β-Toc	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
γ-Toc	30.97±3.99	33.61±3.24	6.58±1.08	5.47±1.29	86.07±1.13	nd	32.21±3.12	nd	nd	nd	11.77±4.69
δ-Toc	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Abbreviations: α-Toc, α-Tocopherol; β-Toc, β-Tocopherol; γ-Toc, γ-Tocopherol; δ-Toc, δ-Tocopherol; nd, not detected.

Each value represents the mean ± SD (n=3).

Table 4-4 Sterol contents (μg/g sample) of herbs and spices from Thailand

Sample	Kaffir lime leaf	Galangal	Turmeric	Fingerroot	Ginger	Lemongrass	Pandan leaf	White pepper	Sweet basil	Climbing wattle	Coriander
Desmosterol	0.14±0.05	nd	0.30±0.05	1.00±0.17	0.04±0.01	nd	0.03±0.01	nd	nd	nd	nd
Ergosterol	0.14±0.04	0.12±0.03	nd	0.11±0.02	0.26±0.05	0.11±0.03	nd	nd	nd	nd	nd
Campesterol	0.35±0.06	0.16±0.04	2.99±0.45	0.39±0.07	0.35±0.02	1.44±0.10	1.96±0.18	nd	2.12±0.43	0.19±0.08	0.05±0.02
Stigmasterol	0.52±0.14	0.78±0.16	8.30±1.20	1.29±0.22	0.58±0.03	1.81±0.15	6.07±0.55	0.21±0.01	3.71±0.76	2.21±0.36	3.65±1.47
β-Sitosterol	2.80±0.45	1.67±0.32	14.69±2.00	1.70±0.29	1.63±0.07	3.90±0.14	5.76±0.45	0.18±0.05	5.65±1.11	7.57±1.31	2.10±0.87

Abbreviations: nd, not detected.

Each value represents the mean ± SD (n=3).

Table 4-5 Free amino acid contents (μg/g sample) of herbs and spices from Thailand

Sample	Kaffir lime leaf	Galangal	Turmeric	Fingerroot	Ginger	Lemongrass	Pandan leaf	White pepper	Sweet basil	Climbing wattle	Coriander
Asparagine	61.62±2.3	73.79±1.6	251.85±14.1	183.29±10.6	307.96±6.3	200.57±13.4	534.84±5.8	13.14±1.4	3737.31±121.4	5800.26±76.5	519.91±27.8
Glutamic acid	35.17±4.4	22.97±3.8	221.36±6.2	139.23±8.6	76.90±1.5	90.24±0.5	102.88±6.7	8.48±1.9	945.55±32.6	910.55±3.9	101.22±1.1
Serine	117.97±3.9	70.29±2.0	43.02±4.2	80.03±2.9	45.52±1.6	32.28±1.4	169.10±0.7	10.76±1.0	118.18±3.9	335.64±6.9	949.12±29.3
Glycine	17.68±1.4	20.35±1.2	24.20±1.2	51.06±3.0	23.56±0.9	32.95±0.6	32.10±1.5	5.29±0.8	133.18±1.5	370.16±7.7	113.90±1.3
Histidine	84.06±2.6	57.93±8.4	45.11±2.2	65.13±4.8	38.43±1.0	61.71±6.0	81.73±0.6	42.51±2.0	228.63±3.0	314.2±30.8	235.08±4.0
Taurine	445.87±9.4	411.94±12.5	620.11±13.8	2218.73±40.0	1688.79±21.1	940.96±19.9	1148.67±26.5	228.25±9.7	10916.79±64.5	8378.77±96.9	1070.58±29.5
Threonine	63.03±3.5	63.59±1.7	20.06±5.1	nd	nd	nd	124.32±9.4	nd	753.17±17.9	229.15±8.1	691.93±20.4
Alanine	208.92±2.3	56.21±2.0	232.23±51.3	85.01±2.8	31.22±4.0	148.07±3.8	nd	nd	nd	676.54±8.0	915.70±20.2
Arginine	809.53±66.5	1819.06±128.2	nd	564.50±93.3	nd	1540.83±18.7	637.13±13.4	nd	1659.85±45.0	13061.48±87.9	nd
Anserine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Proline	6739.46±233.6	24.21±10.9	7.30±1.9	19.13±4.1	nd	3003.94±124.9	69.88±1.0	6.50±3.4	228.34±14.6	401.27±6.0	575.53±18.4
Tyrosine	115.27±3.3	11.19±0.5	nd	11.80±3.3	nd	nd	30.21±3.5	6.95±4.6	117.77±5.2	nd	508.29±0.4
Valine	70.02±5.2	15.36±2.3	28.19±2.4	24.82±1.4	5.25±1.1	34.58±2.5	154.27±4.4	8.94±1.9	62.82±5.3	383.7±8.8	987.66±9.0
Cysteine	nd	nd	nd	nd	nd	410.60±15.3	nd	nd	nd	nd	nd
Methionine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Isoleucine	28.60±2.3	9.88±0.6	7.40±0.8	8.95±0.1	1.66±0.7	6.88±0.3	77.38±4.5	5.04±0.4	22.14±2.1	111.70±8.8	654.85±12.3
Leucine	27.20±1.6	11.30±0.8	14.74±1.5	58.60±76.9	3.12±1.0	23.56±1.1	56.23±2.4	12.30±0.7	84.41±2.1	192.52±5.9	604.25±6.2
Lysine	34.15±1.0	11.11±1.1	5.55±1.3	11.62±2.5	1.60±0.7	89.01±3.2	11.74±2.6	5.97±0.8	43.50±2.9	538.83±4.0	288.37±4.9
Phenylalanine	71.17±3.0	13.54±1.6	7.84±1.6	31.52±1.6	2.11±1.9	12.91±0.7	116.23±2.8	10.03±0.6	493.19±3.2	586.94±20.7	542.56±9.6
Total	8858.53±199.5	2679.16±139.2	1521.14±17.6	3521.9±86.8	2224.03±22.1	6616.18±155.1	3230.47±24.0	354.14±17.6	19051.63±205.1	31704.77±177.3	8216.40±141.4

Abbreviations: nd, not detected. Each value represents the mean ± SD (n=3).

Table 4-6 Purine and pyrimidine monophosphate contents (μg/g sample) of herbs and spices from Thailand

Sample	Kaffir lime leaf	Galangal	Turmeric	Fingerroot	Ginger	Lemongrass	Pandan leaf	White pepper	Sweet basil	Climbing wattle	Coriander
CMP	82.90±3.9	36.71±2.8	143.21±3.5	nd	nd	50.26±1.2	4.61±4.1	24.00±0.6	64.18±1.6	nd	198.14±4.6
GMP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
IMP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
AMP	74.16±0.8	8.44±4.4	nd	12.27±0.8	nd	nd	18.52±8.0	37.67±1.1	159.45±4.4	368.75±18.2	324.64±10.7

Abbreviations: CMP, cytidine monophosphate; GMP, guanosine monophosphate; IMP, inosine monophosphate; AMP, adenosine monophosphate; nd, not detected.

Each value represents the mean ± SD (n=3).

Table 4-7 Organic acid contents (mg/g sample) of herbs and spices from Thailand

Sample	Kaffir lime leaf	Galangal	Turmeric	Fingerroot	Ginger	Lemongrass	Pandan leaf	White pepper	Sweet basil	Climbing wattle	Coriander
Oxa	2.73±0.05	6.72±0.12	20.64±0.35	10.23±0.21	7.82±0.20	3.00±0.04	2.92±0.04	2.38±0.09	11.23±0.46	5.87±0.13	nd
Mal	nd	nd	nd	nd	nd	nd	nd	nd	0.06±0.01	0.07±0.01	0.23±0.01
Cit	1.55±0.01	5.17±0.82	1.11±0.58	2.63±0.47	nd	nd	4.24±0.23	1.58±0.19	7.41±1.06	11.32±0.95	32.56±2.66
Tar	0.30±0.01	nd	nd	nd	nd	nd	2.90±0.02	0.61±0.14	6.68±0.77	nd	nd
Suc	4.03±0.01	2.82±0.38	nd	5.96±0.93	0.60±0.04	35.07±3.22	175.73±2.88	2.28±0.99	16.19±2.78	60.05±2.79	59.79±12.71
Lac	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Fum	0.31±0.01	1.48±0.17	1.20±0.15	0.04±0.01	0.08±0.02	0.12±0.01	0.17±0.03	0.10±0.01	0.53±0.14	1.16±0.05	54.98±1.12
Ace	nd	14.34±1.56	1.85±0.35	nd	1.48±0.35	1.13±0.13	0.68±0.07	nd	nd	nd	nd
Pyro	nd	nd	nd	0.33±0.07	0.70±0.08	0.22±0.01	nd	nd	2.23±0.10	0.46±0.03	0.61±0.14
Prop	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Isob	nd	nd	nd	nd	nd	nd	nd	nd	1.42±0.66	nd	nd
But	nd	4.49±0.91	nd	nd	nd	nd	nd	nd	nd	nd	nd
Vale	nd	nd	nd	ndn	nd	nd	nd	nd	nd	nd	nd

Abbreviations: Oxa, oxalic acid; Mal, maleic acid; Cit, citric acid; Tar, Tartaric acid; Suc, Succinic acid; Lac, Lactic acid; Fum, fumaric acid; Ace, acetic acid; Pyro, pyroglutamic acid; Prop, propionic acid; Isob, isobutyric acid; But, butyric acid; Vale, valeric acid; nd, not detected.

Each value represents the mean ± SD (n=3).

Table 4-8 Polyphenol content and radical scavenging activities of herbs and spices from Thailand

Sample	Kaffir lime leaf	Galangal	Turmeric	Fingerroot	Ginger	Lemongrass	Pandan leaf	White pepper	Sweet basil	Climbing wattle	Coriander
Polyphenol [1)]											
Lipophilic	3.44±0.08	4.58±0.14	138.56±6.69	11.85±0.31	5.62±0.29	1.98±0.15	4.00±0.20	2.09±0.09	2.38±0.05	2.79±0.11	2.56±0.03
Water	6.82±0.23	6.00±0.11	38.29±7.65	1.32±0.04	1.31±0.01	2.27±0.14	2.28±0.02	0.74±0.05	1.82±0.08	4.56±0.07	2.76±0.04
Methanol	4.32±0.10	4.53±0.15	128.27±4.55	28.88±1.13	29.89±0.77	3.87±0.09	3.82±0.25	2.27±0.16	2.94±0.08	4.96±0.06	2.98±0.15
DPPH [2)]											
Lipophilic	104.46±14.11	36.39±1.14	221.39±2.65	174.35±4.16	168.77±4.23	61.85±6.21	167.1±16.96	63.77±7.04	60.6±1.67	25.72±0.47	75.12±6.04
Water	325.63±17.24	95.67±28.01	143.31±10.45	300.17±10.09	205.19±11.65	165.71±4.63	249.96±14.01	147.03±11.60	255.91±5.05	207.82±13.94	64.48±22.05
Methanol	143.41±8.84	65.12±5.31	145.92±6.78	236.63±7.30	296.65±25.69	228.76±15.97	132.53±19.16	72.13±11.14	108.28±13.65	47.18±10.18	112.17±11.83
ABTS [2)]											
Lipophilic	149.85±6.35	3.84±1.20	746.23±6.61	181.27±5.39	162.29±4.59	54.71±5.40	122.9±5.61	49.48±2.54	93.58±3.50	17.25±3.60	108.11±4.54
Water	175.41±2.53	794.85±19.21	1844.50±27.19	931.64±12.75	1578.74±16.25	222.17±13.75	1022.37±21.41	18.71±5.40	693.21±81.44	3946.6±293.20	94.48±23.52
Methanol	187.00±9.35	58.99±4.22	757.76±39.98	149.95±2.78	359.58±37.90	164.58±1.02	120.76±4.60	52.84±2.86	140.34±7.57	148.99±4.43	135.39±3.85

1) Polyphenol contents are expressed as μg of pyrogallol equivalent per g of each samples.

2) Radical scavenging activity are expressed as μg of α-tocopherol equivalent per g of each samples.

Each value represents the mean ± SD (n=3).

Table 4-9 Correlation coefficients between antioxidant compounds and antioxidant capacity

		Poly-L	Poly-W	Poly-M	α-Toc	γ-Toc	Arg	Gly	Lys	Ala	Tyr	Ser	His
DPPH	Lipophilic	0.618	-	-	-0.127	0.339	-	-	-	-	-	-	-
	Water	-	-0.202	-	-	-	0.068	-0.023	-0.197	-0.398	-0.352	-0.414	-0.047
	Methanol	-	-	0.219	-	-	-	-	-	-	-	-	-
ABTS	Lipophilic	0.968	-	-	0.119	-0.035	-	-	-	-	-	-	-
	Water	-	0.261	-	-	-	0.815	0.750	0.614	0.291	-0.363	-0.017	0.448
	Methanol	-	-	0.954	-	-	-	-	-	-	-	-	-

Abbreviations: DPPH, 2,2-diphenyl-1picrylhydrazyl; ABTS, 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acids; Poly-L, Polyphenol of a lipophilic extract; Poly-W, Polyphenol of a water-soluble extract; Poly-M, Polyphenol of a methanol-soluble extract; α-Toc, α-Tocopherol; γ-Toc, γ-Tocopherol; Arg, Arginine; Gly, Glycine; Lys, Lysine; Ala, Alanine; Tyr, Tyrosine; Ser, Serine; His, Histidine.
'-' represents correlation coefficients that were not evaluated.

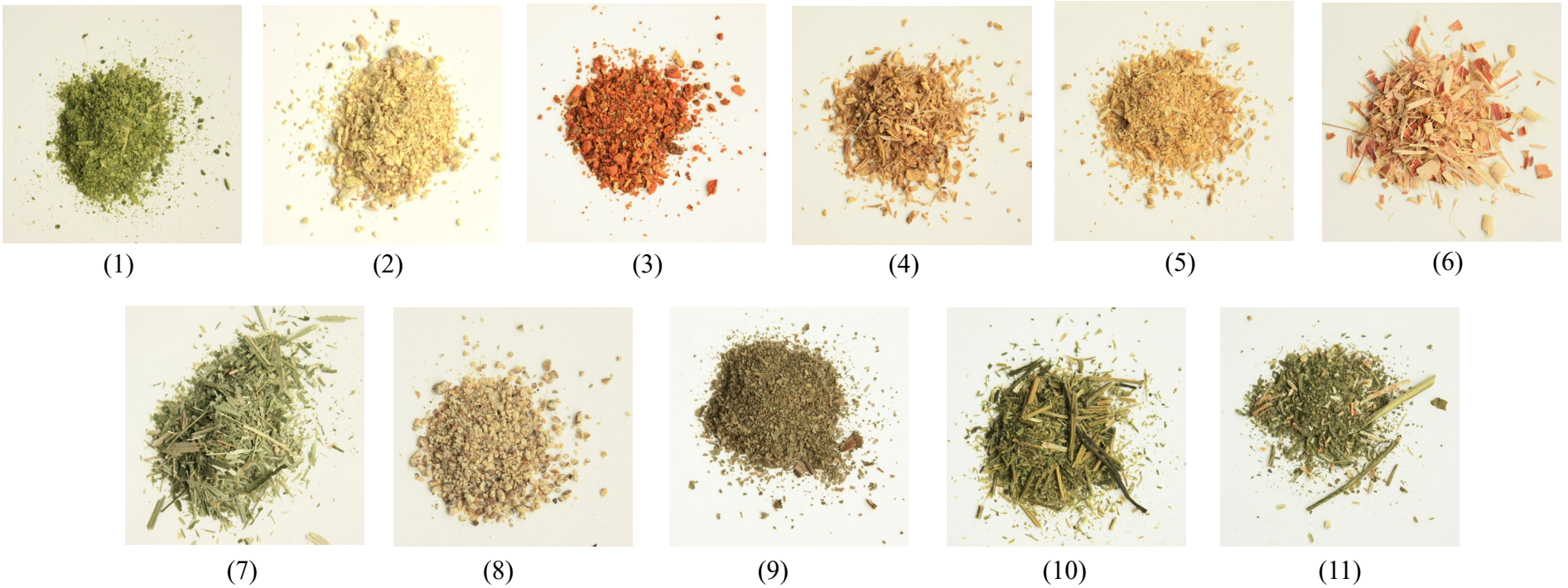

Figure 4-1 Picture of the herbs and spices powders used in this study: (1) Kaffir lime leaf (*Citrus hystrix*), (2) galangal (*Alpinia galanga*), (3) turmeric (*Curcuma longa*), (4) fingerroot (*Boesenbergia rotunda*), (5) ginger (Zingiber officinale), (6) lemongrass (*Cymbopogon citratus*), (7) pandan leaf (*Pandanus amaryllifolius*), (8) white pepper (*Piper nigrum L.*), (9) sweet basil (*Ocimum basilicum*), (10) climbing wattle (*Senegalia pennata*), and (11) coriander (*Coriandrum sativum*). Detailed information on these samples is presented in Table 4-1.

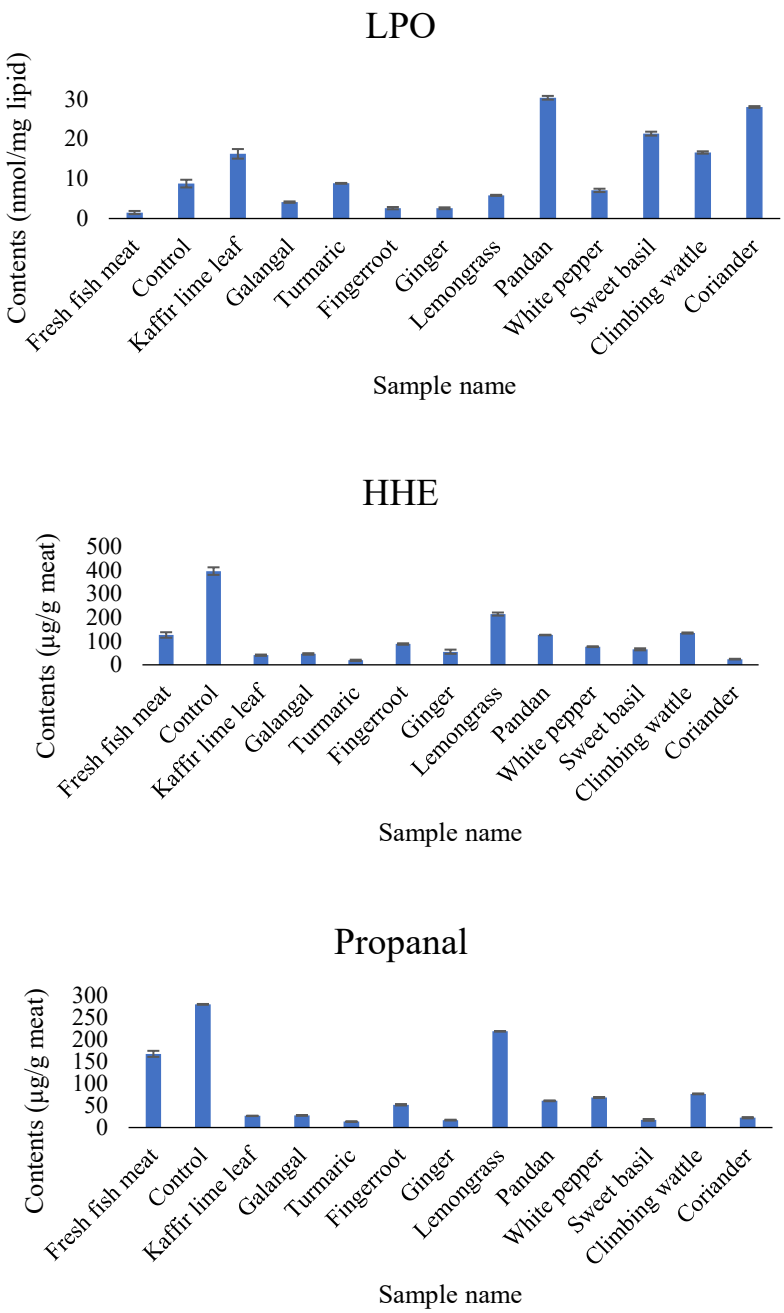

Figure 4-2 Effect of herbs and spices powders on lipid oxidation of fish. (a) LPO (lipid oxidation), (b) HHE (4-hydroxy-trans-2-hexenal), and (c) propanal. Fresh fish meat; frozen minced fish meat was not subjected to oxidation. Control; 10 grams of frozen minced fish meat was subjected to oxidation in a water bath at 30°C for 15 h. Fish meat with herbs and spices powder; 10 grams of frozen minced fish meat and 0.5 g of herbs and spices powder were mixed well, then the mixed was subjected to oxidation in a water bath at 30°C for 15 h. Each data are presented as the mean ± SD (standard deviation).

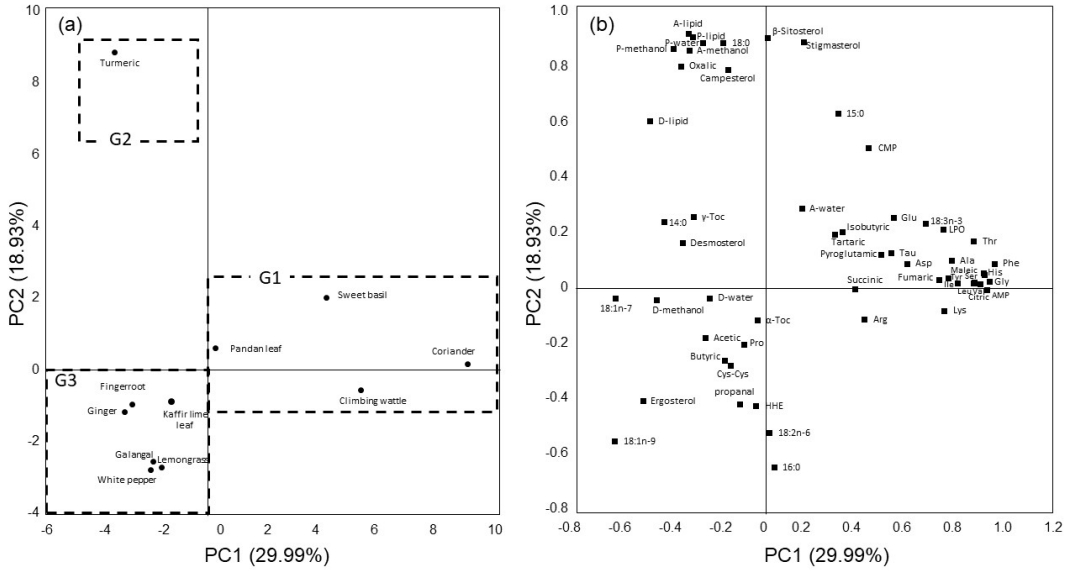

Figure 4-3 Score and loading plots of principal component analysis of 11 herbs and spices in Thailand (a) Score plot showing 11 herbs and spices from Thailand. The detail information each sample were shown in Table 1. (b) Loading plot showing the distribution of the 56 functional chemical compounds analyzed in this study: 14:0 (myristic acid), 15:0 (pentadeaylic acid), 16:0 (palmitic acid), 18:0 (stearic acid), 18:1 n-9 (oleic acid), 18:1 n-7 (vaccenic acid), 18:2 n-6 (linoleic acid), 18:3 n-3 (α-linolenic acid), α-Toc (α-Tocopherol), γ-Toc (γ -Tocopherol), Desmosterol (desmosterol), Ergosterol (ergosterol), Campesterol (campesterol), Stigmasterol (stigmasterol), β-Sitosterol (β-sitosterol), Asp (asparagine), Glu (glutamic acid), Ser (serine), Gly (glycine), His (histidine), Tau (taurine), Thr (threonine), Ala (alanine), Arg (arginine), Pro (proline), Tyr (tyrosine), Cys-Cys (cysteine), Val (valine), Ile (isoleucine), Leu (leucine), Lys (lysine), Phe (phenylalanine), CMP (cytidine monophosphate), AMP (adenosine monophosphate), Oxalic (oxalic acid), Maleic (maleic acid), Citric (citric acid), Tartaric (tartaric acid), Succinic (succinic acid), Fumaric (fumaric acid), Acetic (acetic acid), Pyroglutamic (pyroglutamic acid), Isobutyric (isobutyric acid), Butyric (butyric acid), P-Lipid (total polyphenol of the lipophilic extracts), P-Water (total polyphenol of the water-soluble extracts), P-Methanol (total polyphenol of the methanol-soluble extracts), D-Lipid (DPPHs scavenging activity of the lipophilic extracts), D-Water (DPPH scavenging activity of the water-soluble extracts), D-Methanol (DPPH scavenging activity of the methanol-soluble extracts), A-Lipid (ABTS scavenging activity of the lipophilic extracts), A-Water (ABTS scavenging activity of the water-soluble extracts), A-Methanol (ABTS scavenging activity of the methanol-soluble extracts), LPO (lipid oxidation), HHE (4-hydroxy-trans-2-hexenal), and propanal.

Chapter 5. General Discussion

In Chapter 2, the author focused on each chemical components and radical scavenging activity in Thailand's fermented food products. Many interesting results were presented during the analysis. Including Hoy Seab Dong, fermented *Donax trunculus* in Nam Pla, showed the highest SFA, citric acid, and ABTS radical scavenging of water-soluble solution. Hoy Malang Poo Dong contained the highest DHA content. Nam Pla contained the highest histidine, taurine, lysine, IMP, AMP, and DPPH radical scavenging activity of the lipophilic solution. Poo Khem showed the highest ABTS radical scavenging activity of the lipophilic solution. Tai Pla showed the highest MUFA, CMP, GMP, acetic acid, propionic acid, and DPPH radical scavenging activity of the lipophilic solution. Kapi showed the highest EPA, α-tocopherol, phenylalanine, isobutyric acid, and isovaleric acid. Khem Bak Nad showed the highest content of arginine and lactic acid. Butyric acid was the most abundant in Pla Ra and n-6 fatty acids were the most abundant in rice of Pla Som. Each sample had a different highlight content, but when compared overall, Nam Pla, Tai Pla, and Kapi were the most mentioned among all samples.

In Chapter 3, the author developed a method to simultaneously analyze 14 antioxidative compounds using HPLC system. To verify the precision and specificity of this newly developed HPLC method, the validation test was executed. The limit of quantitation range was between 0.07 and 5.85 μg/mL, the relative standard deviation of intra- and inter-day precision were 0.040%0.064% and 0.003%-0.0016%, recovery tests were between 90.60%-115.91% and 80.92%-95.01%. Next, the antioxidant compounds in popular edible herbs and spices from Asia was evaluated using the method. The samples included kaffir lime leaves, galangal, turmeric, fingerroot, ginger, lemongrass,

pandan leaves, white pepper, sweet basil, climbing wattle, coriander, and Japanese tea. Several interesting results were obtained from the analysis. For example, ginger contained gingerol, which is consistent with other reports, but this analysis also found other antioxidant compounds, such as thymoquinone, thymol, carvacrol, that have not been mentioned in any previous report (Semwal et al. 2015; Tanweer et al. 2020; Ghafoor et al. 2020). In another study, galangal contained a little amount of catechin and coumaric acid; we also found EGCG, a catechin group compound, and coumaric acid. Overall, the results were consistent with the those of other studies (Suzuki et al. 2005; Mahae and Chaiseri 2009; Elansary et al. 2020).

In Chapter 4, the author focused on each chemical components, total polyphenol content, radical scavenging activity in Thailand's herbs and spices, and effect of herbs and spices powders addition to fish on lipid oxidation. Popular edible herbs and spices from Asia are known to contain many types of polyphenols. However, there are few reports on water-soluble components, including amino acids, purines, pyrimidines, and organic acids, as well as fat-soluble components, such as fatty acids, sterols, and tocopherol. These chemical components may possess antioxidant properties and contain polyphenols. In this thesis, a variety of chemical components in herbs and spices which are rarely reported, were identified. Good results were obtained for total polyphenol and radical scavenging activities, leading to the addition of herbs and spices to fish and oxidation. This result involved the polyphenols present in herbs, spices, and other antioxidant substances. The PCA results also showed a strong correlation between the chemical components and samples. Among the samples, turmeric, fingerroot, and ginger showed the best results, especially for antioxidant-related compounds. All the results were consistent with those reported by other researchers (Słowianek and Leszczynska

2016; Lu et al. 2011). Herbs and spices are used in food processing to handle livestock and fish to prevent quality deterioration and suppress odors. In addition, the nutritional components of the herbs and spices may be ingested by eating livestock and fish. In particular, it is possible to ingest plant sterols that are rarely found in livestock and fish. This data was used for the development of food preservation methods and other applications that could improve human health. More data on herbs and spices is required for future experimentation on, for example, the differentiation of each type, including fresh or edible extracts that produce similar results or other herbs and spices that were not mentioned in this report because most of the local herbs and spices in Thailand are not well known.

In conclusion, the chemical components and radical scavenging activities of Thailand's fermented fish products and herbs and spices were revealed. Even though the level of each component was different for each ingredient, they still led to the conclusion that these products were good for consumption because they are essential for human health. The development of HPLC to analyze antioxidants was also successful with good validation, quantitative, and suitability for analysis not only for plant samples but also for other samples like meat, fruits, dairy products, and so on. Moreover, herbs and spices from Thailand showed high total polyphenol content and good results for lipid oxidation prevention in fish especially turmeric, fingerroot, and ginger. These results are important to improve or build on ideas for other studies on products and ingredients in Thailand. Furthermore, these data can lead to further studies that can be conducted in the future such as comparison between each product in different brand including both local and commercial products, comparison between fresh, dried, and cooked herbs and spices, effects of lipid oxidation prevention in other ingredients or food, antioxidant contained in

fermented fish products, and so on. These concepts are not just for study or experimentation; they could also result in future commercial activities like quality confirmation, control for products produced on a large scale, addition of nutrition in fermented products and more.

Acknowledgement

The author would like to express her deepest gratitude to her advisor, Prof. Ryusuke Tanaka of the faculty of agriculture at the University of Miyazaki, who always provides enormous supports and suggests many insightful comments. The author would like to express appreciation to examiners of her doctoral dissertation: Associate Prof. Yousuke Taoka, Prof. Terutoyo Yoshida, and Prof. Katsuhisa Uchida from the faculty of agriculture, and Associate Prof. Jun Hirose of the faculty of engineering at the University of Miyazaki. Also, the author would like to thank the rest of Tanaka laboratory's past and present members. Without them, these researches could not have been accomplished. Finally, the author would like to thank her family and friends. Their encouragement and warm supports always support the author throughout the period of this researches.

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