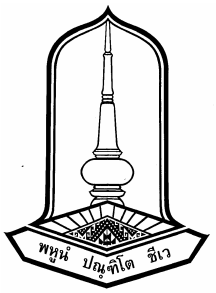

ANTIOXIDATIVE COMPOUNDS FROM SELECTED THAI HERBS AND SPICES AND EFFECTS OF THERMAL PROCESS ON THEIR STABILITIES

PORNPIMOL RAKSAKANTONG

A dissertation submitted in partial fulfillment of the requirements for
The degree of Doctor of Philosophy in Food Technology
Mahasarakham University
April 2012

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The examining committee has unanimously approved this dissertation, submitted by Miss Pornpimol Raksakantong, as a partial fulfillment of the requirements for the Doctor of Philosophy degree in Food Technology, Mahasarakham University

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Pornpimol Raksakantong

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CANDIDATE Miss Pornpimol Raksakantong
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ADVISORS Assoc. Prof. Dr.Sirithon Siriamornpun
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ABSTRACT

The study aimed at determination of antioxidant activities, bioactive compounds and volatile compounds of fourteen Thai herbs and spices. Antioxidant activities were used by two *in vitro* assays, namely DPPH radical scavenging activity and ferric reducing antioxidant power assay (FRAP). Total phenolic content was measured by Folin-Ciocalteu reagent and total flavonoid content by colorimetric method. Identification of phenol compounds were performed using HPLC with the UV-diode array detection. Volatile compounds were performed using GC-MS. The % inhibition (DPPH radical scavenging activity) was in a wide range of 69% in leave *Cassia siamea* Britt. to 87% in *Ocimum sanctum* L. cv. Khao. In leave samples, FRAP value ranged from 245 μmol $FeSO_4$/g in *Piper aurantuacum* to 682 μmol $FeSO_4$/g in *Ocimum sanctum* L. cv. Khao. The highest of total phenolic content (TPC) was found in *Cassia siamea* Britt.(leaf) (502 (GAE)/g DW). The main phenolic acids in all analyzed herbs and spices were ferulic acid, sinapic acid and *p*-coumaric acid. Ferulic acid was the major hydrocinnamic acid derivative, ranging from 2 to 359 mg/g, followed by sinapic acid (16 to 225 mg/g) and *p*-coumaric acid (1 to 130 mg/g). Eucalyptol was the most prominent volatile compound in *Alpinia galanga* (rhizome) (68%). Based on the antioxidant properties, *Ocimum sanctum* L. cv. Khao. (holy basil or kaprow khao) was selected for further studies. Cytotoxic activity of 500 μg/ml kaprow khao crude was 48% in Human hepatocarcinoma cancer cell line (HepG2) and colon cancer cell line (HCT) 46%.

Efficient processes have been proposed to lengthen the shelf life, and to add value to the product as kaprow powder. The quality of the dried product is strongly

dependent on the drying process and processing conditions. Hence, kaprow leaves were dried using three different methods namely, hot-air drying (HA), low relative humidity air drying (LRH) and combined far-infrared radiation with hot-air convection drying (FIR-HA). The most appropriate drying method for kaprow khao was FIR-HA. FIR-HA dried kaprow had higher content of bioactive compounds and antioxidant activities, compared with fresh sample. After drying, eugenol, methyl eugenol and β-caryophyllene were found to be significantly increased ($p<0.05$). Moreover, FIR drying decreased the drying time without having any major effects on color, bioactive compounds, volatile compounds and antioxidant activity of the extracts. Subsequently, FIR-HA dried leaves were ground to powder for further use. Kaprow khao powder of 1%, along with other synthetic antioxidants was added in fish emulsion. Evaluation of antioxidant properties and lipid oxidation of fish emulsion was performed. The results showed that the combination of 1% kaprow powder, vitamin E (100 ppm) and BHT (100 ppm) showed the highest antioxidant activities and lowest lipid oxidation, compared to control.

In conclusions, this study has demonstrated that some of Thai herbs and spices could be considered as valuable sources of bioactive components with high antioxidant properties. Kaprow khao has demonstrated a potential herb and spice for functional food. This could offer good opportunities for food industry in terms of food preservation and product development.

Keywords: Herbs and spices; antioxidant activity; cytotoxic activity; drying process; fish emulsion

ชื่อเรื่อง สารต้านอนุมูลอิสระจากสมุนไพรและเครื่องเทศไทยและผลกระทบของกระบวนการให้ความร้อนต่อคงตัวของสารต้านอนุมูลอิสระ
ผู้วิจัย นางสาวพรพิมล รักษาก้านตง
ปริญญา ปรัชญาดุษฎีบัณฑิต **สาขาวิชา** เทคโนโลยีการอาหาร
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บทคัดย่อ

งานวิจัยครั้งนี้มีวัตถุประสงค์ เพื่อศึกษากิจกรรมการต้านอนุมูลอิสระ ปริมาณสารออกฤทธิ์ทางชีวภาพ และองค์ประกอบของสารระเหยของสมุนไพรและเครื่องเทศไทย 14 ชนิด สารประกอบการทดสอบกิจกรรมการต้านอนุมูลอิสระในหลอดทดลองด้วยวิธีต่างกัน ได้แก่ DPPH radical scavenging activity และferric reducing antioxidant power assay (FRAP) การวิเคราะห์สารประกอบฟีนอลิคทั้งหมดโดยใช้สารละลาย Folin-Ciocalteu reagent และการวิเคราะห์ปริมาณฟลาโวนอยด์ทั้งหมด โดยใช้หลักการ colorimetric method การแยกสารประกอบและวิเคราะห์ปริมาณกรดฟีนอลิค โดยใช้เทคนิค HPLC-DAD และ ปริมาณองค์ประกอบของสารระเหย โดยเทคนิค GC-MS จากผลการทดลองพบว่า ขี้เหล็กมีกิจกรรมการต้านอนุมูลอิสระการยับยั้ง 69% และกะเพราขาว 87% ส่วนค่า FRAP นั้นพบว่าในชะพลู 245 และกะเพราขาว 682 µmol $FeSO_4$/g กะเพราขาวมีกิจกรรมการต้านอนุมูลอิสระสูงกว่าสมุนไพรและเครื่องเทศชนิดอื่นด้วยวิธี DPPH (การยับยั้ง 87%) และวิธี FRAP (682 µmol $FeSO_4$/g) ใบขี้เหล็กมีปริมาณสารประกอบฟีนอลิครวม (502 มิลลิกรัมสมมูลกรดแกลิคต่อกรัม) ส่วนกรดฟีนอลิคหลักที่พบในสมุนไพรและเครื่องเทศได้แก่ กรดferulic, sinapic และ *p*-coumaric โดยกรด ferulic มีปริมาณ 2-359 มิลลิกรัมต่อกรัม ลำดับต่อมาเป็นกรด sinapic มีปริมาณ 16-225 มิลลิกรัมต่อกรัม และกรด *p*-coumaric มีปริมาณ 1-130 มิลลิกรัมต่อกรัม องค์ประกอบของสารระเหยพบว่า eucalyptol นั้นมีปริมาณมากในข่า จากผลการทดลองพบว่ากะเพราขาวมีกิจกรรมการต้านอนุมูลอิสระมากที่สุดจึงเลือกกะเพราขาวเพื่อทำการศึกษาต่อไป สำหรับกิจการทดสอบความเป็นพิษพิษต่อเซลล์มะเร็งของกะเพราขาวพบว่าความสามารถในการยับยั้งเซลล์มะเร็งตับ (HepG2) คือ 44 เปอร์เซ็นต์ และมะเร็งลำไส้ (HCT) เท่ากับ 46 เปอร์เซ็นต์ ที่ความเข้มข้น 500 ไมโครกรัมต่อมิลลิลิตร

ประสิทธิภาพของกระบวนอบแห้งนั้นเป็นการยืดอายุการเก็บรักษาและเพื่อนำไปใช้ในอาหารในรูปแบบกะเพราผง และคุณภาพของผลิตภัณฑ์อบแห้งนั้นก็ขึ้นอยู่กับกระบวนการและสภาวะของการอบแห้ง ดังนั้นดังนั้นจึงนำกะเพรามาอบแห้งด้วยวิธีที่แตกต่างกัน 3 วิธี คือ HA , LRH และ FIR-HA จากการทดลองพบว่าวิธีการอบที่เหมาะสมที่สุดในการอบกะเพราขาวนั้นคือวิธี FIR-HA เพราะเป็นวิธีที่ทำให้ค่าสารประกอบชีวภาพและกิจกรรมการต้านอนุมูลอิสระสูงเมื่อเปรียบกับใบกะเพราขาวสด หลังจากอบแห้งแล้วพบว่า eugenol, methyl eugenol และ β-caryophyllene มีปริมาณเพิ่มขึ้น และยิ่งไปกว่านั้นยังพบอีกว่าการอบด้วยวิธี FIR-HA นั้นลดระยะเวลาในการอบแห้ง แล้วยังไม่มีผลกระทบต่อค่าสี สารประกอบทางชีวภาพ องค์ประกอบของสารระเหย และกิจกรรมการต้านอนุมูลอิสระของสารสกัดกะเพราขาว ดังนั้นจึงเลือกวิธีการอบแห้งด้วยเทคนิค FIR-HA เพื่อเตรียมกะเพราขาวให้อยู่ในรูปผงเพื่อที่จะนำไปใช้เป็นส่วนประกอบในการผลิตปลายอต่อไป และทำการศึกษาเพิ่ม ผงกะเพราขาว 1 เปอร์เซ็นต์ และสารต้านอนุมูลอิสระอื่นในปลายอ ทำการวิเคราะห์ปริมาณสารต้านอนุมูลอิสระและการเกิดลิปิดออกซิเดชันของปลายอ โดยพบว่าการใช้ร่วมกันระหว่างผงกะเพราขาว 1 เปอร์เซ็นต์ วิตามินอี 100 ส่วนในล้านส่วน และ BHT 100 ส่วนในล้านส่วน นั้นมีกิจกรรมต้านอนุมูลอิสระมากที่สุดและเกิดลิปิดออกซิเดชันน้อยที่สุด การศึกษาครั้งนี้ได้แสดงให้เห็นสมุนไพรและเครื่องเทศไทยบางชนิดเป็นแหล่งสำคัญของสารออกฤทธิ์ทางชีวภาพที่มีคุณสมบัติเป็นสารต้านอนุมูลอิสระที่มีศักยภาพ กะเพราขาวเป็นสมุนไพรและเครื่องเทศที่มีศักยภาพสำหรับการนำมาทำเป็นอาหารเพื่อสุขภาพ ดังนั้นจึงเป็นทางเลือกที่ดีในการนำไปใช้ในทางอุตสาหกรรมเพื่อยืดอายุการเก็บรักษาและพัฒนาผลิตภัณฑ์อาหาร

คำสำคัญ: สมุนไพรและเครื่องเทศ; กิจกรรมการต้านอนุมูลอิสระ; กิจกรรมทดสอบความเป็นพิษต่อเซลล์; กระบวนการทำแห้ง; ปลายอ

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List of abbreviations

DPPH	=	1,1-Diphenyl-2-picrylhydrazyl
FRAP	=	Ferric reducing ability power
DMSO	=	Dimethyl sulfoxide
BHA	=	Butylated hydroxyanisol
BHT	=	Butylated hydroxytoluene
FIR	=	Far-infrared radiation
LRH	=	Low relative humidity air drying
HA	=	Hot air
HPLC	=	High Performance Liquid Chromatography
DAD	=	diode array detector
GC	=	Gas chromatography
TFC	=	Total flavonoid content
TPC	=	Total phenolic content
THF	=	Tetrahydrofuran
TEA	=	Triethylamine
TPTZ	=	2,4,6-Tripiridyl-s-triazine
GA	=	Gallic acid
PCCA	=	Protocatechuic acid
p-HO	=	*p*-hydroxybenzoic acid
ChA	=	Chorogenic acid
VA	=	Vanillic acid
CFA	=	Caffic acid
SyA	=	Syringic acid
p-CA	=	*p*-Coumaric acid
FA	=	Ferulic acid
SNA	=	Sinapic acid
HepG2	=	Human hepatocarcinoma cancer cell line
Vero	=	African green monkey kidney (normal cell line)
HCT	=	Colon cancer cell line

CHAPTER 1

INTRODUCTION

1.1 Background

Herbs and spices have always been valued for their ability to add flavor, color and aroma to dishes, but before the advent of refrigeration they were also an important means of food preservation and in some cultures their medicinal and antiseptic characterizes are revered. The regional cuisine of India, Thailand, Indonesia and Malaysia rely a great deal on herbs and spices. Herbs and spices, like vegetables, fruits, and medicinal herbs are known to possess a variety of antioxidant effect and properties, which could be obtained from leave, roots, rhizome, flowers, fruits, seeds or bark. Herbs and spices have also been recognized as having certain health benefit such as digestive stimulant action, carminative action, antimicrobial, anti-inflammatory, anti-mutagenic, anti-carcinogenic potential, etc. (Ceylan and Fung, 2004). With small portion in our dish spices could be an important source of phytochemicals in our daily diet (Carlsen, *et al*., 2010). Flavor is usually the result of the presence of many volatile and nonvolatile components possessing diverse chemical and physicochemical properties (Gramatina, *et al*., 2010). Whereas the nonvolatile compounds contribute mainly to the taste, the volatile ones influence both taste and aroma. A vast array of compounds may be responsible for the aroma of the food products, such as alcohols, aldehydes, esters, dicarbonyls, short to medium chain free fatty acids, methyl ketones, lactones, phenolic compounds and sulphur compounds (Urbach, 1997). Phenolic compounds have strong *in vitro* and *in vivo* antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions and chelate metals (Shahidi and Naczk, 2004). Increased consumption of phenolic compounds has been correlated with a reduced risk of cardiovascular disease and certain cancers (Shahidi, 2000; Barreira *et al*., 2008).

Recently, the demand for ready- to- eat foods, which include herbs and spices as ingredients, has been continually increase (Douglas et al., 2005). For Thai cuisine, selected herbs and spices are among popular ingredients used in various dishes such as Tom yum, Thai stirred fried, Chicken fried with ginger, Curry and Thai spicy soups.

The leaves can be used fresh or dried as herbs and spices. However, the fresh leaves have very short post-harvest life of 3–4 days causing a depreciation in market price (Jiang, 1997; Yueming *et al*., 2002). A number of efficient processes have been proposed to lengthen the shelf life and to add value to the product as herbs and spices powder. In the case of Thailand, to preserve most herbs and spices, drying traditional sun under drying is commonly used. Due to rewetting of the products during drying by rain and also because of too slow drying rate in the rainy season, toxic substances produced by moulds is often found in the dry products. This is one of the main problems, which restricts the growth of exports of herbs and spices to international markets, hence a suitable and efficient drying method is needed. Hot- air drying (HA) is the most commonly employed commercial technique for drying of vegetables and fruits and biological products (Praveen- Kumar et al., 2005). The processing temperature mainly influences the quality changes during drying. The major limitation of hot air drying is that it takes longer for drying, resulting in product quality degradation. Drying is carried out in a mechanical device where food materials may be either static or moving. HA is blown in from different directions, depending on the nature of the products being dried (Mitcham, 2007). Far- infrared radiation (FIR) has been reported to be successfully applied in drying of foods (Sandu, 1986) and agricultural products since the main components of the agricultural products (i.e. proteins, starches and water) have the principal bands of far-infrared radiation absorption (Meeso, 2008). FIR creates internal heating via molecular vibration of the material, i.e., molecules absorb the radiation of certain wavelengths and energy, causing excited vibration. Thus, the mechanism of far-infrared drying is different from that of hot air drying (Sandu, 1986), so that the electromagnetic wave energy is absorbed directly by the dried food with reduced energy loss. FIR is thought to liberate and activate low molecular weight natural antioxidant compounds, because it heats material without degrading the constitutive molecules on the sample surface and it transfers heat evenly to the center of the material (Niwa et al.,1988). Our previous study on mulberry leaves drying has reported that total phenolic content (TPC) increases following combined far- infrared radiation with hot-air convection drying (FIR-HA) of mulberry leaves (75%) with comparison to fresh leaves (Wanyo et al. 2010). Low relative humidity air drying (LRH) is the desiccant wheel, which represents one of the most

promising air humidification of airconditioning systems (Subramanyam et al., 2004; Mazzei et al., 2002; Zhang *et al*., 2003; Neti and Wolfe, 2000; Zhang and Niu, 2002) may be considered an alternative way of drying food products, by incorporation into a conventional hot-air drying system. The mechanism of removing water vapor from the air by desiccant material is analogous to water vapor condensing on an evaporator surface, and the latent heat corresponding to the extracted water liberated into the surrounding air is analogous to energy recovery through the condenser of a heat-pump dryer (Madhiyanon et al., 2007). It is well known that natural antioxidants extracted from herbs and spices (rosemary, oregano, thyme, etc.) have high antioxidant activity and are used in many food applications (Hirasa and Takemasa, 1998). A number of studies deal with the antioxidant activity of extracts from herbs and spices (Cuvelie et al., 1994; Kikuzaki and Nakatani, 1993; Lu and Yeap-Foo, 2001).

Although, some researchers have studied on herbs and spices phenolic compounds, volatile compounds and antioxidant activities of herbs and spices, there has been little information about herbs and spices varieties in Thailand on phenolic compounds, antioxidant activities, antitoxic activity and product development from herb and spice. Therefore, the aims of this study mainly to investigate the antioxidant activity of herbs and spices in Thailand and identified their phenolic compounds. Antiproliferative and phenolic compounds and antioxidant activities of selected herb and spice were also investigated. The effects of different drying methods on the amounts of antioxidant activities, phenolic compounds and volatile compounds of dried herbs and spices were examined. Furthermore, Effect of process and stored time of added kaprow khao powder in fish emulsion on antioxidant properties and lipid oxidation at different storage time were examined.

1.2 Objectives of the research

The objectives of the study are:

1.2.1 To determine antioxidant activity, phenolic compounds and volatile compounds in herbs and spices.

1.2.2 To determine cytotoxic activity, phenolic compounds and antioxidant activity of selected herb and spice.

1.2.3 To study effect of different drying methods on antioxidant activity, phenolic compounds and volatile compounds of potent herbs and spices.

1.2.4 To evaluate the changes in antioxidant properties and lipid oxidation in fish emulsion product (pla-yor) at different storage time.

1.3 Outcomes

1.3.1 Obtained the knowledge of phenolic compounds, including phenolic acids, volatile compounds and their antioxidant activity of herbs and spices.

1.3.2 Obtained the knowledge of cytotoxic activity and phenolic compounds and antioxidant activity of selected herb and spice.

1.3.3 Obtained the knowledge of effect different drying methods on antioxidant activity, phenolic compounds and volatile compounds of potent herbs and spices.

1.3.4 Obtained the knowledge of stability on antioxidant properties and lipid oxidation in fish emulsion product (pla-yor) at different storage time.

1.4 Hypothesis of the research

1.4.1 Extracts of different herbs and spices varieties have different antioxidant activity, volatile compounds and phenolic compounds.

1.4.2 Extracts of selected herb and spice on cytotoxic activity, phenolic compounds and antioxidant activity.

1.4.3 Extracts of potent herbs and spices from different processing have different antioxidant activity, phenolic compounds and volatile compounds.

1.4.4 The levels of antioxidant properties and lipid oxidation in different storage time in fish emulsion product (pla-yor).

1.5 Scope of the research

1.5.1 Analysis of total phenolic content, antioxidant activities and volatile compounds of 14 herbs and spices, namely *Citrus hystrix* DC., *Morinda citrifolia* Linn., *Limnophila aromatica* Merr., *Piper aurantuacum*), *Gynura divaricata* DC.),

Morus alba Linn., *Cassia siamea* Britt., *Centella asiatica* Linn.Urban), *Ocimum sanctum* L. cv. Khao. and *Ocimum sanctum* L. cv. Daeng, rhizome: *Zingiber officinale* Roscoe.), *Alpinia alangal* (Linn.) Swartz., *Curcuma longa* Linn. and *Boesenbergia pandurata* (Roxb.) Schltro.

1.5.2 Analysis of cytotoxic activity, phenolic compounds and antioxidant activity of potent herb and spice.

1.5.3 Study effect of different drying methods on antioxidant activities, phenolic compounds and volatile compounds of potent herbs and spices.

1.5.4 Product development of potent herb and spice and evaluates the changes in, antioxidant properties and lipid oxidation in fish emulsion product (pla-yor) at different storage time.

1.6 Defined words

1.6.1 Herbs and spices: as herbs and spices from Mahasarakham province and other. Examples in this study *Citrus hystrix* DC., *Morinda citrifolia* Linn., *Limnophila aromatica* Merr., *Piper aurantuacum*, *Gynura divaricata* DC., *Morus alba* Linn., *Cassia siamea* Britt., *Centella asiatica* Linn.Urban), *Ocimum sanctum* L. cv. Khao. and *Ocimum sanctum* L. cv. Daeng, rhizome: *Zingiber officinale* Roscoe.), *Alpinia alangal* (Linn.) Swartz., *Curcuma longa* Linn. and *Boesenbergia pandurata* (Roxb.) Schltro.

1.6.2 Phenolic compounds: as compound possess an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of simple phenolic molecules to that of a complex high molecular weight polymer. Examples in this study are phenolic acids and flavonoids

1.6.3 Phenolic acids: are derivatives of benzoic acid and cinnamic acid with hydroxyl groups and methoxy groups substituted at various points on the aromatic ring. Examples in this study are gallic acid, vanillic acid, caffeic acid, *p*-coumaric acid and ferulic acids.

1.6.4 Flavonoids: are polyphenolic compounds that are abundant in fruits, vegetables, red wine, tea and flowers.

1.6.5 Antioxidant activities: can act as scavenge free radicals, bind metal ions are reducing the inhibition rate of lipid peroxidation.

1.6.6 Anti-proliferative activity: can inhibits of human cancer cell lines (African green monkey kidney (normal cell line) (Vero), Hepatoma cell line (HepG2) and Colon cancer cell line (HCT).

1.6.7 Potent herbs and spices: as spices possess phenolic compounds and high antioxidant activities.

CHAPTER 2

LITERATURE REVIEW

2.1 Herbs and spices

Herbs and spices have always been valued for their ability to add flavor, color and aroma to dishes, but before the advent of refrigeration they were also an important means of food preservation, and in some cultures their medicinal and antiseptic characterizes are revered. The regional cuisine of India, Thailand, Indonesia and Malaysia rely a great deal on spices. Herbs and spices, like vegetables, fruits, and medicinal herbs are known to possess a variety of antioxidant effect and properties, which could be obtained from leave, roots, rhizome, flowers, fruits, seeds or bark. Herbs and spices have also been recognized as having certain health benefit such as digestive stimulant action, carminative action, antimicrobial, anti-inflammatory, anti-mutagenic, anti-carcinogenic potential, etc. (Ceylan and Fung, 2004).

2.2 Phenolic compounds

Phenolic compounds are a large and diverse group of molecules, which includes many different families of aromatic secondary metabolites in plants (Harborne and Williams, 2000). The presence of phenolic compounds in epidermal tissue, cuticles and accessories is customary. Phenolic compounds are defined as substances possessing a benzene ring bearing one or more hydroxyl substituents, including their functional derivatives (Waterman and Mole, 1994). Phenolis have many favorable effects on human health. They reduce the risk of heart diseases by inhibiting the oxidation of low-density lipoprotein (LDL) (Bonilla et al., 1999). A large range of low and high molecular weight phenols exhibiting antioxidant properties have been studied and proposed to be used as antioxidants against lipid oxidation (Moure et al., 2001). This is particularly true for those phenolics with multiple hydroxyl groups that are generally the most efficient for preventing lipid oxidation. Phenolic compounds are also known to posses antibacterial, antiviral, antimutagenic and anticarcinogenic properties

(Moure et al., 2001). Phenolic compounds, ubiquitous in plants are an essential part of the human diet, and are of considerable interest due to their antioxidant properties. These compounds posses an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer. Phenolic compounds in plants may generally and conveniently be divided in to three major classes based on their sizes. These are phenolic acids, flavonoids and tannins. Structures of some phenolic compounds are shown in figure 2.1

2.2.1 Phenolic acids

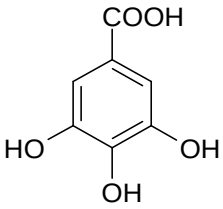
Phenolic acids are derivatives of benzoic acid and cinnamic acid with hydroxyl groups and methoxy groups substituted at various points on the aromatic ring (Marinova and Yanishlieva, 2003). Furulic acid, *p*-coumaric acid, caffeic acid, vanillic acid and syringic acid are all examples of phenolic acids (Pratt and Hudson, 1990).

2.2.2 Flavonoids

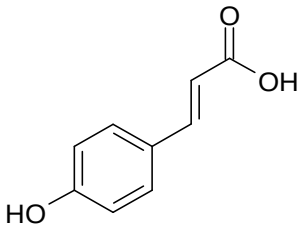
Flavonoids (Figure 2.2) are polyphenolic compounds that are abundant in fruits, vegetables, flowers, red wine, tea and chocolate (Halliwell, 2001; Henning *et al*., 2004). They display a multitude of biological effects *in vitro* and *in vivo* after consumption of flavonoid-containing foods. Epidemiologic studies showed that increased consumption of flavonoids reduces the risk of cardiovascular disease and certain types of cancer (Arts and Hollman, 2005; Koga and Meydani, 2001). Flavonoids may exhibit antioxidant, antimutagenic, and free-radical scavenging activities (Moyers and Kumar, 2004)**.** Moderate consumption of red wine, which contains a high content of polyphenols, is associated with a low risk of coronary heart disease (Donovan *et al*., 2002 ; Bravo, 1998). Consumption of soy and soy products are related to biological effects, including anticarcinogenic, antiatherosclerotic, and antihemolytic effects (Moyers and Kumar, 2004; Bravo, 1998). The daily verage consumption of flavonoids as 1 gram showed higher antioxidant activity than ascorbic acid and α-tocopherol (Bravo, 1998)**.**

Flavonoids are a subset of polyphenol antioxidants, which bear the C6-C3-C6 structure (Madhavi et al., 1996). The C6-C3 is from cinnamic acid and the other C6 fragment is from 3 molecules of malonyl-coenzyme A (Hahn et al., 1984). The general

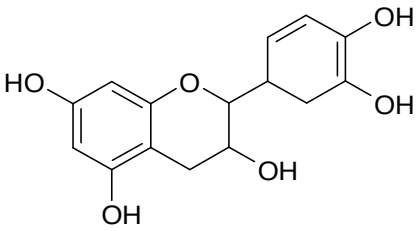
flavonoid structure may be described as consisting of a benzopyran nucleus with an aromatic substituent at carbon number 2 of the C ring.



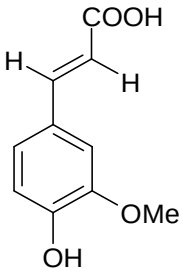
Gallic acid



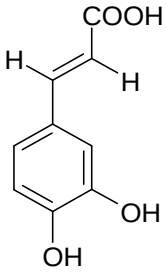
p-Coumaric acid



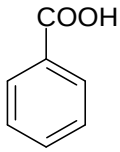
(+)-Catechin acid



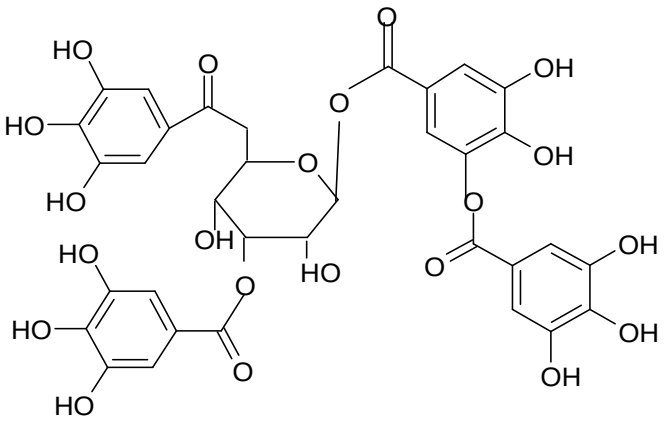
Ferulic acid



Caffeic acid

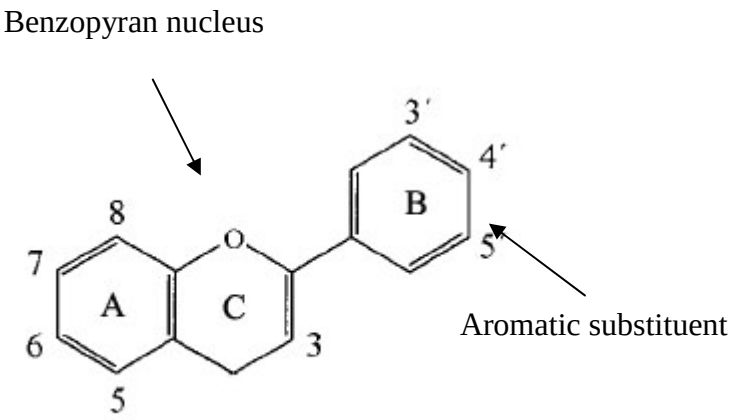


Benzoic acid



Tanic acid

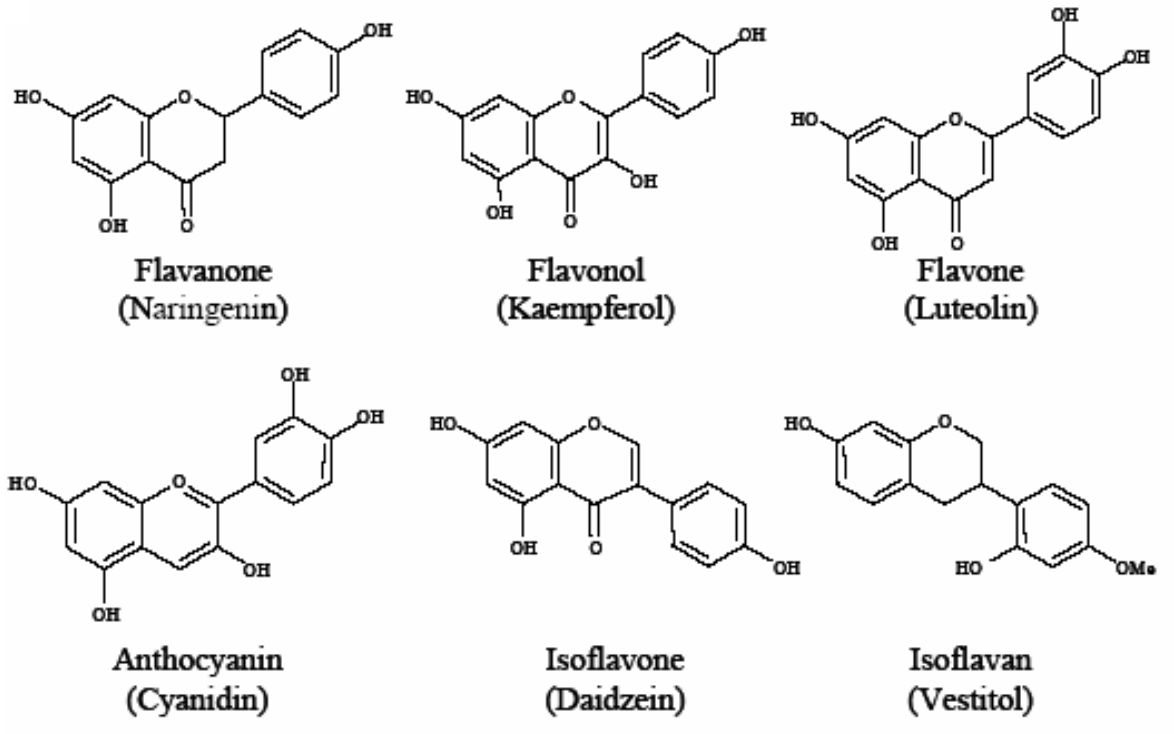
Figure 2.1 Structure of phenolic compounds



Source : Sugihara et al. (1999)

Figure 2.2 Basic structures of flavonoids

The subgroups are classified based on the sustitutional pattern of the C ring and the position of the B ring. The major subgroups include flavonols, flavanones, flavanols (or flavans) and flavones (Figure 2.3). Flavonols, such as quercetin and kaemferol, have a carbonyl at C-4, double bond between C-2 and C-3, and hydroxyl at C-3; flavanones (e.g. taxifolin) have a carbonyl at C-4, no double bond between C-2 and C-3 no hydroxyl at C-3; flavanols (e.g. catechin) have no carbonyl at C-4, no double bond between C-2 and C-3 and a hydroxyl at C-3 and flavones, such as apigenin and luteolin, have a carbonyl at C-4, a double bond between C-2 and C-3 and no hydroxyl at C-3 (Sugihara *et al.*, 1999). Flavonones give rise to other family members such as anthocyanins by undergo a series of transformation that affects the heterocyclic ring (Cao et al.,1996), which are responsible for the color of fruits, legumes and vegetables (Mazza, 1998; Pascual-Teresa and Sanchez-Ballesta, 2008).



Source : Aoki et al. (2000 : 475-488)

Figure 2.3 Flavonoid structures

2.2.3 Tannins

Tannins are substances of vegetable origin capable of transforming fresh hide into leather (Hahn et al., 1984). Tannins are rich in phenolic hydroxyl groups. They are divided into two classes, namely hydrolysable tannins and condensed tannins (Waterman and Mole, 1994). Hydrolysable tannins are phenolic carboxylic acids esterified to sugars such as glucose. They are called hydrolysable tannic since they break down into sugars and a phenolic acid (gallic or ellagic acid) upon hydrolysis with acid, alkali or hydrolytic enzymes (tannase) (Hahn *et al*., 1984). Condensed tannins are polymers of flavan-3-ol units and are also known as proanthocyanins (or proanthocyanidins) because they yield anthocyanins upon heating in acidic media (Santos-Buelga and Scalbert, 2000).

2.3 Phenolic compounds as antioxidants and anticancer

Phenolic compounds have strong *in vitro* and *in vivo* antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions and chelate metals (Shahidi and Naczk, 2004). Increased consumption of phenolic compounds has been correlated with a reduced risk of cardiovascular disease and certain cancers (Shahidi, 2000; Barreira *et al*., 2008). Recent epidemiological studies have suggested that increased consumption of whole grains, herbs, spices, fruits, vegetable and flowers are associated with reduced risks of chronic diseases (Hu, 2002; Nielsen et al., 2002) such as cancer, cardiovascular disease, stroke, Alzheimer's disease and age-related functional decline (Hu, 2003). Phenolic acids are known to be scavengers of oxygen species. The position of the hydroxyl groups in the aromatic ring is important in the efficiency of phenolic acids as antioxidants. For phenolic acids for instance, the presence of hydroxyl group in the para position is important for high antioxidant activity (Pannala et al., 1998 ; Pannala et al., 2001). Phenolic acids have been used as strong inhibitors of carcinogenesis at the initiation and promotion stages induced by different compounds (Kaul and Khanduja, 1998).

The antioxidant activity of phenolic acids depends on the degree of hydroxylation. The derivatives of cinnamic acids are generally more effective than the derivatives of benzoic acid (Marinova and Yanishlieva, 2003). The presence of the CH=CH-COOH group in cinnamic acid derivatives ensures greater efficiency than the COOH group in benzoic acids (Madhavi et al., 1996). The double bond has been reported to participate in stabilizing the phenolxyl radical by resonance (Marinova and Yanishlieva, 2003). Hydroxycinnamic acid ester serve as antioxidant *in vitro*, and it has been suggested that they may serve as natural antioxidants for lipids *in vitro* (Rice-Evans et al., 1997). Although more attention has been paid to flavonoids as potential natural antioxidants (Yamasaki, 1992). Phenolic acid esters, such as chlorogenic acid, also perform well as antioxidant, particularly in protecting lipids from peroxidation (Rice-Evans et al., 1997).

For flavonoids, structure features such as the attachment of the 3-OH group to the 2,3 double bond and adjacent to the 4-carbonyl in the C ring (Rice-Evans et al., 1997), 3,4 dihydroxy arrangement in the B ring and the meta 5,7 dihydroxy

arrangements in the A ring (Rice-Evans et al., 1996) are important for high antioxidant activity. Epidemiological studies suggest an inverse relation between flavonoid intake and incidence of chronic diseases such as coronary heart disease (Rimm *et al*., 1996). Current research has shown that flavonols and flavones are of particularr importance in the human diet as there is evidence that they act as antioxidants, and epidemiological studies have indicated that their consumption is associated with a reduced risk of cancer and cardiovascular disease. Consumption of fruits and vegetables has been highly associated with the reduced risk of cancer. Flavonoids, as one of the most diverse and wide spread group of natural compounds, are probably the most important natural phenolics (Prasad et al., 2009). Besides their antioxidant activity, flavonoids have been demonstrated a wide range of biochemical and pharmacological effects including anti-inflammatory, anti-viral, anti-allergenic, anti-carcinogenic, anti-aging activity (Hülya Orak, 2007), anti-oxidant and anti-allergic effects (Miean and Mohamed, 2001). Phenolic compounds represent a particularly rich family of phytochemicals, consisting of more than 10,000 compounds. Many of these compounds play an important role as antioxidants which effectively reduce the oxidative stress and act as chemo-preventive agents. Polyphenol-rich diets arising from the consumption of fruits and vegetables have been associated with the decrease of cancer incidences (Bonolia et al., 2004). Natural antioxidants occur in all parts of the plant (wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen, and seeds) (Pratt and Hudson, 1990). It is well known that natural antioxidants extracted from herbs and spices (rosemary, oregano, thyme, etc.) have high antioxidant activity and are used in many food applications (Hirasa and Takemasa, 1998). A number of studies deal with the antioxidant activity of extracts from herbs and spices (Cuvelier et al., 1994; Kikuzaki and Nakatani, 1993; Lu and Yeap-Foo, 2001).

Tannins inhibit lipid oxidation by scavenging the initial free radicals or the lipid peroxide radicals. They are also excellent chelators of metals ions such as copper and iron (Soleas et al., 1997).

2.4 Antioxidant capacity assays

Antioxidants have been reported to prevent oxidative damage caused by free radical and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers (Buyukokuroglu et al., 2001). Most natural antioxidants are multifunctional in complex heterogeneous foods; their activity cannot be assessed by any one method (Frankel and Meyer, 2000). No single assay will accurately reflect all of the radical foundations or all antioxidants in a mixed or complex system, and it must be appreciated at the outset that there are no simple universal methods by which antioxidant capacity can be measured accurately and quantitatively (Prior et al., 2005), also too many analytical methods result in inconsistent result, inappropriate application and interpretation of assays, and improper specifications of antioxidant capacities. There are two reaction mechanisms in which antioxidants can deactivate radicals. The first of these methods is the single electron transfer assay (SET) which detects the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls and radicals (Wright et al., 2001). According to Prior et al. (2005), SET reactions are usually slow and require a lengthy time to reach completion, so the antioxidant capacity calculations are based on percent decrease in product rather than kinetics. The second method is the hydrogen atom transfer (HAT), which measures the antioxidant's ability to quench free radicals by hydrogen donation. HAT reaction is solvent and pH dependent and is usually quite rapid. The presence of reducing agents, including metals, is a complication in HAT assays and can lead to erroneously high apparent reactivity (Prior et al., 2005).

2.4.1 The 2, 2- diphenyl-1-picryhydrazyl (DPPH) method a rapid, simple and inexpensive method that has been developed to determine the antioxidant activity of foods utilizes the stable 2, 2-diphenyl-1-picryhydrazyl (DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to

form the reduced DPPH-H. The resulting ecolorization is stochiometric with respect to number of electron captured.

2.4.2 Ferric reducing ability of plasma (FRAP) assay in FRAP assay, ferric ions (Fe^{3+}) are reduced to ferrous (Fe^{2+}) ions by antioxidants (Benzie and Strain. 1996). In this context, antioxidants are considered as reductant and the oxidant is modified by Fe^{3+}. The oxidizing species react with the antioxidant instead of the possible substrate and is reduced to a harmless species. Different mixtures and solutions of antioxidants can be investigated. In the reduction, an intensely colored ferrous- tripyridyltriazine complex is formed and the absorbance of the solution is measured. The values of absorbance of the test mixture are compared to the values of solutions of known concentration of Fe^{2+} in control samples.

2.5 Volatile compounds

Herbs and spices have been used throughout the world mainly for their flavoring and aroma properties. Most studies have reported changes in colour and volatile compounds of the aromatic herbs after drying (Diaz-Maroto et al., 2002). Various physic-chemical changes of aromatic volatiles (oxidation, evaporation) may take during the drying process, influencing aroma intensity and the quality of the dried product (Barbieri et al., 2004). The effect of drying on the composition of volatile flavor constituents of various aromatic plants and vegetables has been the subject of numerous studies, which show that the changes in the concentrations of the volatile compounds during drying depend on several factors, such as the drying method and parameters that are characteristic of the product subjected to drying (Venskutonis, 1997). In certain cases increases in the quantities of some of the components characteristic of a given spice have been observed (Baritaux et al., 1999; Bartley and Jacobs, 2000) or the formation of new compounds after drying has been recorded, probably as a consequence of oxidation reactions, hydrolysis of glycosylated forms, or the release of compounds by the rupture of cell walls (Huopalahti et al., 1985). Certain compounds have been observed to increase in different herbs after drying: for example, eugenol in bay leaf (Diaz-Maroto et al., 2002), thymol in thyme (Venskutonis, 1997), and some sesquiterpenes in different herbs (Baritaux et al., 1999; Yousif et al., 2000). Eugenol is

the main component of kaprow grown in Bangladesh (Mondello et al., 2002), Germany (Laakso et al., 1990), Cuba (Pino et al., 1998), Northeastern Brazil (Machado *et al*., 1999), methyl eugenol from India (Kothari *et al*., 2004). Most studies have reported changes in colour and volatile compounds of the aromatic herbs after drying (Di Cesare et al., 2004; Diaz-Maroto et al., 2002). Citronellal is the main volatile compound found in kaffir lime leaves (Lawrence et al., 1971; Tinjan and Jirapakkul, 2007). The other volatile compounds found in kaffir lime leaves were α-pinene, camphene, β-pinene, limonene, copaene, linalool, β-cubebene, isopulegol, caryophyllene, citronellyl acetate and citronellol (Lawrence et al., 1971; Tinjan and Jirapakkul, 2007).

2.6 Infrared radiation

Infrared refers to that part of the electromagnetic spectrum between the visible and microwave regions. Electromagnetic spectrum refers to the seemingly diverse collection of radiant energy, from cosmic rays to X-rays to visible light to microwaves, each of which can be considered as a wave or particle traveling at the speed of light. These waves differ from each other in the length and frequency, as illustrated in figure 2.4

Frequency, ν (nu), is the number of wave cycles that pass through a point in one second. It is measured in Hz, where 1 Hz = 1 cycle/sec. Wavelength, λ (lambda), is the length of one complete wave cycle. It is often measured in cm (centimeters). Wavelength and frequency are inversely related:

$$\nu = \frac{c}{\lambda} \text{ and } \lambda = \frac{c}{\nu}$$

where c is the speed of light, 3×10^{10} cm/ sec

Energy is related to wavelength and frequency by the following formulas:

$$E = h\nu = \frac{hc}{\lambda}$$

where h = Planck's constant, 6.6 x 10^{-34} joules-sec

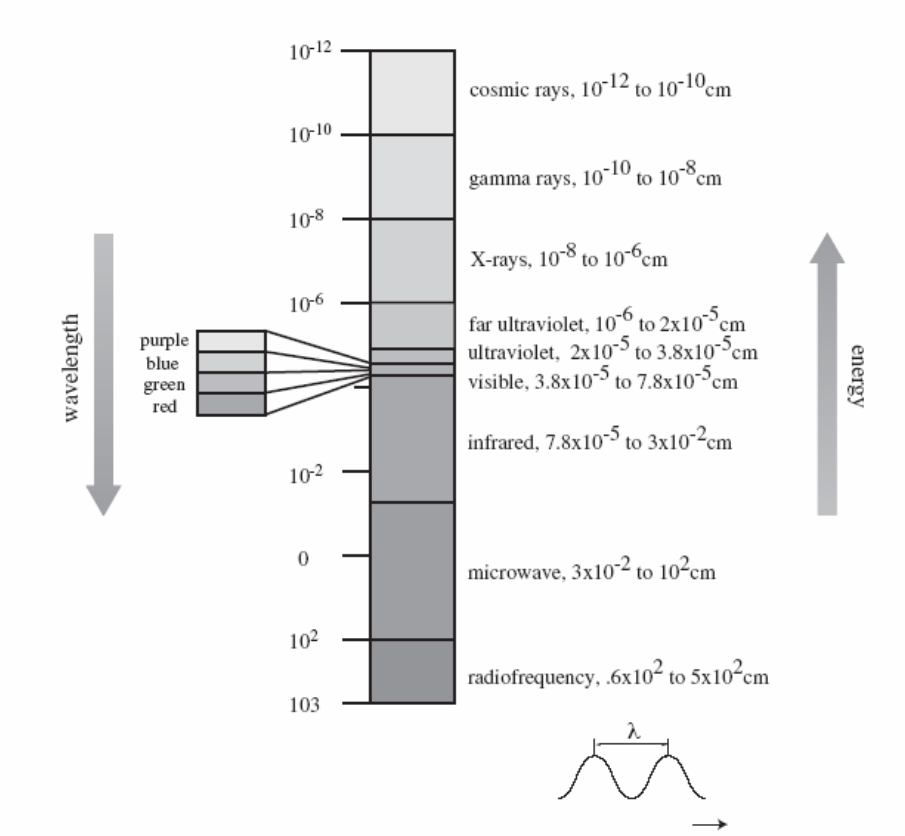


Figure 2.4 The electromagnetic spectrum

The IR region is divided into three regions: the near, mid, and far IR (Figure 2.5). The mid IR region is of greatest practical use to the organic chemistry. This is the region of wavelengths between 3 x 10^{-4} and 3 x 10^{-3} cm. Chemists prefer to work with numbers which are easy to write; therefore IR spectra are sometimes reported in μm, although another unit, $\bar{\nu}$ (nu bar or *wavenumber*), is currently preferred.

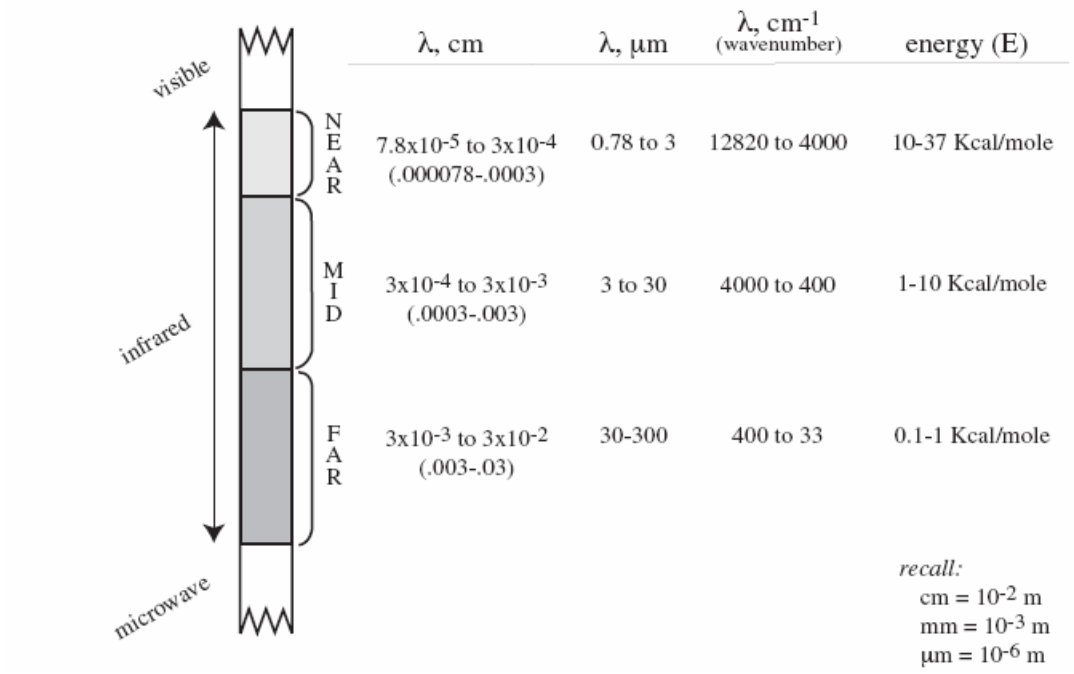


Figure 2.5 The IR regions of the electromagnetic spectrum.

2.7 Drying by using Far- infrared radiation

Far-infrared radiation (FIR) has been reported to be successfully applied in drying of foods (Sandu, 1986) and agricultural products since the main components of the agricultural products (i.e. proteins, starches and water) have the principal bands of far-infrared radiation absorption (Meeso, 2008). FIR creates internal heating via molecular vibration of the material, i.e., molecules absorb the radiation of certain wavelengths and energy, causing excited vibration. Thus, the mechanism of far-infrared drying is different from that of hot air drying (Sandu, 1986), so that the electromagnetic wave energy is absorbed directly by the dried food with reduced energy loss. FIR is thought to liberate and activate low molecular weight natural antioxidant compounds, because it heats material without degrading the constitutive molecules on the sample surface and it transfers heat evenly to the center of the material (Niwa et al., 1988). It is widely used in agricultural, chemical, pharmaceutical, textile and many other industries. Far-infrared radiation creates internal heating with molecular vibration of material, i.e., molecules absorb the radiation of certain wavelengths and energy, and cause vibration

excitedly. Moreover, the mechanism of far-infrared drying is different from hot air drying (Mongpraneet et al., 2002), and the electromagnetic wave energy is absorbed directly by the dried food with less energy loss. At present, various driers have been developed by using far-infrared radiators. The utilization of far-infrared radiation is a novel process that could increase the drying efficiency, save working space, and result in a clean working environment, etc. (Ratti and Mujumdar, 1995). Yagi and Kunii (1951) attempted to apply far-infrared radiation to the drying of agricultural materials and improved results were reported. Combination of far-infrared radiation with air convection or vacuum drying had also been tested (Abe and Afzal, 1997; Hasatani et al., 1983; Mongpraneet et al., 2002). Far-infrared radiation drying of potato had attained high drying rates by using infrared heaters of high emissive power (Masamura *et al*., 1988). Although significant product value increases would occur if vacuum or freeze drying methods were combined with far-infrared radiation treatment, only studies combining far-infrared radiation and vacuum operation has been studied (Itoh and Chung, 1995; Mongpraneet et al., 2002). Lin Tsen and King (2005) studied effects of far-infrared radiation on the freeze drying of sweet potato. Previous studies found that antioxidant activities and total phenolic contents increased after exposure of rice hulls to FIR radiation (Lee et al., 2003) and peanut hull (Lee et al., 2006) and mulberry tea and mulberry leaves drying has reported that total phenolic content (TPC) increases following combined FIR-HA treatment of mulberry leaves (75%) with comparison to fresh leaves (Wanyo et al., 2011). Many antioxidant phenolic compounds in plants are most frequently present in a covalently bound form with insoluble polymers / (Peleg et al., 1991). If this bonding is not strong FIR treatment could liberate and activate low-molecular-weighted natural antioxidants in plants (Jeong et al., 2004; Lee et al., 2003).

FIR rays are biologically active (Inoue and Kabaya, 1989) and transfer heat to the center of materials evenly without degrading the constituent molecules of surface (Niwa et al., 1988). FIR may have capability to cleave covalent bonds and liberate antioxidants such as flavonoids, carotene, tannin, ascorbate, flavoprotein or polyphenols from repeating polymers (Niwa et al., 1988). In previous study (Lee et al., 2003) showed that simple heat treatments could not cleave covalently bound phenolic compounds from rice hulls but FIR treatments could.

2.8 Hot- air drying (HA)

Hot- air drying (HA) is the most commonly employed commercial technique for drying of vegetables and fruits and biological products (Praveen- Kumar et al., 2005). The processing temperature mainly influences the quality changes during drying. The major limitation of hot air drying is that it takes longer for drying, resulting in product quality degradation. However, HA drying has long been used for agricultural products, there are some limitations such as degrade of change of color. Drying is carried out in a mechanical device where food materials may be either static or moving (Syahral et al., 2003). Erbay and Icier (2009) that TPC and total antioxidant activity of olive leaves were decreased by 9 and 44%, respectively after dried by using heat pumps drying at 53°C, process time of 288 min. In addition it has been reported that outer leaves of white cabbage (*Brassica oleracea* L. var. capitata) dried under hot air drying resulted in a decrease of total phenolic content (TPC) more than 60% (Tanongkankit et al., 2010). Longer drying time, damage to sensory characteristics and nutritional properties of foods, oxidation of pigments and destruction of vitamins by HA, and solute migration from the interior of the food to the surface are described as the main disadvantages of HA drying (Afzal et al., 1999; Reyes et al., 2010). Food processing can improve the properties of naturally occurring antioxidants or induce the formation of new compounds with antioxidant properties, so that the overall antioxidant activity increases or remains unchanged (Tomaino et al., 2005). Therefore degradation of polyphenol compounds by thermal process may result in releasing antioxidant compounds which have different chemical and biological properties (Tsai et al., 2002). Effects could vary from little or no change to significant losses, or even enhancement in antioxidant properties (Nicoli et al., 1999).

2.9 Low relative humidity air drying (LRH)

Low relative humidity air drying (LRH) is the desiccant wheel, which represents one of the most promising air humidification of airconditioning systems (Subramanyam et al., 2004; Mazzei et al., 2002; Zhang et al., 2003; Neti and Wolfe, 2000; Zhang and Niu, 2002) may be considered an alternative way of drying food

products, by incorporation into a conventional hot-air drying system. The mechanism of removing water vapor from the air by desiccant material is analogous to water vapor condensing on an evaporator surface, and the latent heat corresponding to the extracted water liberated into the surrounding air is analogous to energy recovery through the condenser of a heat-pump dryer. (Madhiyanon et al., 2007).

2.10 Herbs and spices use in food product

Flavoring agents, herbs and spices are most often added to improve not only the flavor, but also the shelf life, as many of them are potent antioxidants. They are usually added in small amounts, about 1%, so that the addition of the antioxidant fraction is much lower. Nevertheless, their effect should not be ignored, as they act in combination with various native antioxidants or synergists. Sometimes, these additives require stabilization, for instance, essential oils from citrus fruits. Lemon oil was stabilized with α-tocopherol and citric acid (Sato and Sudo, 1969), Essential oil from *Citrus hystrix* from Indonesia and bergamot oil were stabilised with rosemary extract (Pudil et al., 1998). The activity of antioxidants is weaker but still persists in mixtures with proteins or polysaccharides (Volfoá et al., 1998), simulating real flavored foods. A combination of BHT or tocopherols with citric acid has been proposed for the stabilization of citrus essential oil (Kieronczyk and Kamiňski, 1999). The addition of spices, such as extracts from lemongrass, clove leaves, black peper leaves and turmeric increased the shelf life of cakes and also contributed to their characteristic flavor (Lim and Suhaila, 1999). The keeping quality of crackers and cookies is of great economic importance since these products are often stored for extended periods before before they are consumed (sometimes after opening the packaging) and they are not protected from oxidation. A soda cracker biscuit was processed using a fine powder of marjoram, spearmint, peppermint and basil, and their purified diethyl ether.

CHAPTER 3

METHODOLOGY

This research was experimental research to determine and analyse phenolic compounds, including phenolic acids, flavonoids and volatile compounds in herbs and spice as well as evaluate their antioxidant activities. Antitoxic activity of selected herb and spice was examined. In addition, determination of phenolic compounds, volatile compounds and antioxidant activity of the extracts from potent herbs and spices of different drying methods were investigated. Finally, changes in antioxidant properties and lipid oxidation in fish emulsion product (pla-yor) at different storage time were examined. Therefore, experimental design was followed by:

3.1 Experimental plan

3.2 Instruments and equipments

3.3 Materials

3.4 Chemicals

3.5 Methods

3.6 Statistical analysis

3.1 Experimental plan

This research was divided into four experiments including (1) determination of phenolic compounds such as total phenolic content, phenolic compounds, volatile compounds and evaluation their antioxidant activities of herbs and spices, (2) determination of antitoxic activity, antioxidant activities and phenolic compounds of potent herb and spice, (3) analysis of antioxidant activity, phenolic compounds and volatile compounds in potent herbs and spices with different drying methods and (4) determination of antioxidant properties and lipid oxidation in fish emulsion product (pla-yor) at different storage time. All experiments were done in triplicate.

Experiment 1 was to determine phenolic compounds including total phenolic content, phenolic acids and volatile compounds of fourteen herbs and spices, namely herbs and spices were analyzed, divided into two types of leave and rhizome are

fourteen namely, leave: kaffir lime (*Citrus hystrix* DC.), indian mulberry (*Morinda citrifolia* Linn.), finger grass (*Limnophila aromatica* Merr.), variegatum (*Piper aurantuacum*), purple velvet plant (*Gynura divaricata* DC.), mulberry (*Morus alba* Linn.), thai copper pod (*Cassia siamea* Britt.), asiatic pennywort (*Centella asiatica* Linn.) Urban), holy basil /*Ocimum sanctum* L. cv. Khao and holy basil */Ocimum sanctum* L. cv. Daeng, rhizome: ginger (*Zingiber officinale* Roscoe.), galangal (*Alpinia alangal* (Linn.) Swartz.), turmeric (*Curcuma longa* Linn.) and boesenbergia (*Boesenbergia pandurata* (Roxb.) Schltro). and evaluation their antioxidant activity.

Experiment 2 was to determine antitoxic activity, antioxidant activity and phenolic compounds of potent herb and spice (*Ocimum sanctum* L. cv. Khao).

Experiment 3 was to analyze antioxidant activity, phenolic compounds and volatile compounds in potent herbs and spices (holy basil or kaprow leaves) different drying methods, namely low relative humidity air drying (LRH), hot air drying (HA) and combined far- infrared radiation with hot-air convection drying (FIR-HA).

Experiment 4 was to evaluate antioxidant properties and lipid oxidation in fish emulsion product (pla-yor) at different storage time.

For experimental plan used in this research was completely randomized design (CRD). Analysis of variance was used to test any difference in resulting from these methods. Duncan method was used to determine significant differences at $p < 0.05$.

3.2 Instruments and equipments

3.2.1 High performance liquid chromatography system with diode array detector (LC 20A, Shimadzu)

3.2.2 Gas chromatography system with flame ionization detector (GC-2014, Shimadzu) and GC/MS-QP2010 (Shimadzu, Japan)

3.2.3 Ultraviolet-Visible spectrophotometer (Lambda 12, Perkin Elmer, USA)

3.2.4 UV spectrometer (Anthos 2010; Biochrom, UK)

3.2.5 Centrifuge (Rotina 48 R)

3.2.6 Rotary evaporator (Buchi)

3.2.7 Column Inetsil ODS-3, C18 (4.6 mm x 250 mm, 5 µm)

3.2.8 Column DB-Wax (0.25 mm x 30 m)

3.2.9 Hot air oven (Memmert)

3.2.10 Incubator shaker

3.2.11 A coffee blender (model CG-2, 230-240).

3.2.12 Beaker

3.2.13 Erlenmeyer flask

3.2.14 Volumetric flask

3.2.15 Pipette

3.2.16 Vial

3.3 Materials

Fourteen herbs and spices available in northeastern Thailand (Mahasarakham province and other) were studied (Table 3.1).

3.4 Chemicals

3.4.1 2,2-Diphenyl-1-picrylhydrazyl , DPPH (Fluka)

3.4.2 6-Hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid, Trolox (Fluka)

3.4.3 2,4,6-Tripiridyl-s-triazine, TBTZ (Fluka)

3.4.4 Folin-Ciocalteu's reagent (Fluka)

3.4.5 Ferrous sulphate (Carlo)

3.4.6 Acetic acid (Fisher Scientific)

3.4.7 Sodium sulphate (Merck)

3.4.8 Absolute ethanol (BDH)

3.4.9 Acetone (BDH)

3.4.10 Phosphoric acid (BDH)

3.4.11 Methanol (Merck)

3.4.12 Hydrochloric acid (HCL)

3.4.13 Dimethyl sulfoxide (DMSO)

3.4.1.4 Acetonitrile (Merck)

3.41.15 Standard phenolic acids (gallic acid, ferulic acid, *p*-hydroxybenzoic acid, protocatechuic acid, *p*-coumaric acid, caffeic acid, syringic acid, sinapic acid, chlorogenic acid and vanillic acid (Sigma)

3.4.16 Standard volatile compounds: eugenol (Fluka), methyl eugenol (Sigma) and β-caryophyllene (Sigma)

3.4.17 Fluorescence dye 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich (USA)

3.4.18 Sodium bicarbonate (NaHCO3)

3.4.19 Neutral red, 4-(4'-nitrobenzyl) pyridine (NBP)

3.5 Methods

The experimental methods in this research study will be divided into four experiments following: phenolic content, phenolic compounds including total phenolic content, phenolic acids, antitoxic activity and volatile compounds and evaluation of antioxidant properties and lipid oxidation.

3.5.1 Experiment one: Determination of antioxidant activity, phenolic compounds and determination of volatile compounds in herbs and spices.

3.5.1.1 Fourteen herbs and spices were collected from northeastern region of Thailand (Table 3.1). The extracts prepared from dried herbs and spices (1 g) were extracted for 2 hr with 10 ml of 80% ethanol at room temperature on an orbital shaker set at 180 rpm. The mixture was centrifuged at 1400 x g for 20 min and the supernatant was decanted into a 30 ml vial. The pellet was re-extracted under identical conditions. Supernatant was combined and used for antioxidant activity and used for analyzing antioxidant activity *in vitro*. All analyses were performed in triplicates.

3.5.1.2 Determination of phenolic compounds

1) Total phenolic content

Total phenolic content was determined using Folin–Ciocalteu reagent as followed by Abu Bakar et al. (2009) as adapted from Velioglu et al. (1998). Briefly, 0.3 ml of extract was mixed with 2.25 ml of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min; 2.25 ml of sodium carbonate (60 g/ l) solution were added to the mixture. After 90 min at

room temperature, absorbance was read at 725 nm using spectrophotometer. Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/ g dry weight).

3.5.1.3 Determination of antioxidant activity

1) DPPH$^{\bullet}$ radical-scavenging activity

The hydrogen atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-coloured methanol solution of DPPH (Gulluce *et al*., 2007). The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca et al. (2001). Aqueous extract (0.1 ml) was added to 2.9 ml of a 0.004% DPPH in methanol. Absorbance at 517 nm was determined after 30 min, and the percent inhibition of activity was calculated as $[(Ao-Ae)/Ao]\times100$
(Ao = absorbance without extract; Ae = absorbance with extract).

2) Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is a method of measuring the ability of reductants (antioxidants) to reduce Fe^{3+}– Fe^{2+}. The formation of bluecoloured Fe^{2+}- TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. The method of Butsat and Siriamornpun (2009) was used with some modifications. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was warmed at 37 ºC before using. Flower extracts (100 µl) were allowed to react with 1.9 ml of the FRAP solution. The absorbance at 593 nm of the mixture was measured after 60 min of reaction (Butsat and Siriamornpun, 2009). The results were calculated by standard curves prepared with known concentrations of $FeSO_4$, and were expressed as µmol $FeSO_4$/ 100 g DW.

3.5.1.4 Identification and quantification of phenolic compounds

1) Phenolic compounds extraction

The phenolic compounds in the samples were extracted using a modification of the procedure described by Bengoechea *et al*. (1997) as adapted from Uzelac *et al*. (2005). Each sample (5 g) was mixed with 50 mL of methanol/HCl (100:1, v/v) which contained 2% tertbutylhydroquinone,in inert atmosphere (N_2) during 12 h at 35˚C in the dark. The extract was then centrifuged at 4000 rpm/min, and the supernatant

was evaporated to dryness under reduced pressure (35–40°C). The residue was redissolved in 25 mL of water/ethanol (80:20, v/v) and extracted four times with 25 mL of ethyl acetate. The organic fractions were combined, dried for 30–40 min with anhydrous sodium sulfate, filtered through the Whatman-40 filter, and evaporated to dryness under vacuum (35–40˚C). The residue was redissolved in 5 mL of methanol/ water (50:50, v/v) and filtered through a 0.45 µm filter before injection (20 µl) into the HPLC aperture. Samples were analyzed in triplicate.

2) High-performance liquid chromatography – diode array detector (HPLC–DAD) system for analysis of phenolic compounds

RP-HPLC system for analysis of phenolic compounds HPLC analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A with diode array detector and chromatographic separations were performed on a LUNA C-18 column (4.6×250 mm i.d., 5 µm). The composition of solvents and the gradient elution conditions used were described previously by Bengoechea et al. (1997); Schieber, Keller and Carle (2001) and Butsat et al. (2009) with some modifications. The mobile phase consisted of purified water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5 to 9% solvent B; from 5 to 15 min, 9% solvent B; from 15 to 22 min, linear gradient from 9 to 11% solvent B; from 22 to 38 min, linear gradient from 11 to 18% solvent B; from 38 to 43 min, from 18 to 23% solvent B; from 43 to 44 min, from 23 to 90% solvent B; from 44 to 45 min, linear gradient from 90 to 80% solvent B; from 45 to 55 min, isocratic at 80% solvent B; from 55 to 60 min, linear gradient from 80 to 5% solvent B and a re-equilibration period of 5 min with 5% solvent B used between individual runs. Operating conditions were as follows: column temperature, 38˚C, injection volume, 20 µL, and UV-diode array detection at 280 nm (hydroxybenzoic acids), 320 nm (hydroxycinnamic acids) and 370 nm (flavonols) at a flow-rate of 0.8 ml / min. Spectra were recorded from 200 to 600 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds and were detected using an external standard method. Standards namely: gallic, ferulic, phydroxybenzoic, protocatechuic, *p*-coumaric, caffeic, syringic, sinapic, chlorogenic and vanillic acids were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO).

3.5.1.5 Determination of volatile compounds

The samples were ground and 0.2 g was put in vials. The vials were sealed with an aluminium–rubber septum (Supelco, Bellefonte, PA, USA) and analysed by the headspace sampling technique (Barcarolo and Casson, 1997). GC-MS analysis was carried out using a GC-2010 chromatograph coupled to a GC/MS-QP2010 (Shimadzu, Japan). Samples were analyzed on a fused-silica capillary column Rtx-5Ms (5% diphenyl 95% dimethyl polysiloxane, 30 m length, 0.25 mm internal diameter, 0.25 μm film thickness; Restek, U.S.) and Rtx-5 (5% diphenyl 95% dimethyl polysiloxane, 30 m length, 0.25 mm internal diameter, 0.25 μm film thickness; Restek, U.S.). Carrier gas, helium; constant pressure, 134.2 kPa; injector temperature, 250 °C; split ratio, 1:5; temperature program, 80 to 250 °C at 10 °C /min then held isothermal (2 min) at 250 °C; ion source temperature, 200 °C; transfer line temperature, 250 °C; ionization energy, 70 eV; electron ionization mass spectra were acquired over the mass range 35-550 u. Standard namely: eugenol, methyl eugenol and β-caryophyllene and others volatile compounds compare in library.

3.5.2 Experiment two: Determination of cytotoxic activity, antioxidant activity and phenolic compounds of potent herb and spice

3.5.2.1 Plant Material

Ocimum sanctum L. cv. Khao was obtained from a market in the Mahasarakham province of Thailand. Dried plants were cut and macerated with 50% ethanol and water (1 g:6 ml) for seven days with occasional shaking. The solvent was filtered, distilled in vacuo with a rotary evaporator below 40°C, and freeze-dried to obtain the crude extracts.

3.5.2.2 Determination of antioxidant activity

1) $DPPH^{\bullet}$ radical-scavenging activity

The hydrogen atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-coloured methanol solution of DPPH (Gulluce *et al*., 2007). The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al*. (2001). Aqueous extract (0.1 ml) was added to 2.9 ml of a 0.004% DPPH in

methanol. Absorbance at 517 nm was determined after 30 min, and the percent inhibition of activity was calculated as [(Ao-Ae)/Ao]×100
(Ao = absorbance without extract; Ae = absorbance with extract).

IC50, the amount of sample extracted into 1 ml solution necessary to decrease by 50% the initial DPPH concentration was derived from the % disappearance vs. concentration plot. (Concentration here means mg of holy basil extracted into 1 ml solution.)

2) Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is a method of measuring the ability of reductants (antioxidants) to reduce Fe^{3+}– Fe^{2+}. The formation of bluecoloured Fe^{2+}- TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. The method of Butsat and Siriamornpun (2009) was used with some modifications. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was warmed at 37 ºC before using. Flower extracts (100 µl) were allowed to react with 1.9 ml of the FRAP solution. The absorbance at 593 nm of the mixture was measured after 60 min of reaction (Butsat and Siriamornpun, 2010). The results were calculated by standard curves prepared with known concentrations of $FeSO_4$, and were expressed as µmol $FeSO_4$/ 100 g DW.

3.5.2.3 Assessment of phenolic compounds

1) Total phenolic content

Total phenolic content was determined using Folin–Ciocalteu reagent as followed by Abu Bakar et al. (2009) as adapted from Velioglu et al. (1998). Briefly, 0.3ml of extract was mixed with 2.25 ml of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min; 2.25 ml of sodium carbonate (60 g/ l) solution were added to the mixture. After 90 min at room temperature, absorbance was read at 725 nm using spectrophotometer. Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/ g dry weight).

2) Total flavonoid content

Total flavonoid content was determined using the colorimetric method described by Abu Bakar et al. (2009) as adapted from Dewanto et al. (2002). Briefly, 0.5 ml of the extract was mixed with 2.25 ml of distilled water in a test tube followed by

addition of 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.3 ml of a 10% $AlC_{l3}·6H_2O$ solution was added and allowed to stand for another 5 min before 1.0 ml of 1 M NaOH was added. The mixture was mixed well by vortex. The absorbance was measured immediately at 510 nm using a spectrophotometer. Results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/ g dry weight).

3.5.2.4 Identification and quantification of phenolic compounds

1) Phenolic compounds extraction

The phenolic compounds in the samples were extracted using a modification of the procedure described by Bengoechea et al. (1997) as adapted from Uzelac et al. (2005). Each sample (5 g) was mixed with 50 mL of methanol/HCl (100:1, v/v) which contained 2% tertbutylhydroquinone,in inert atmosphere (N_2) during 12 h at 35˚C in the dark. The extract was then centrifuged at 4000 rpm/min, and the supernatant was evaporated to dryness under reduced pressure (35–40°C). The residue was redissolved in 25 mL of water/ethanol (80:20, v/v) and extracted four times with 25 mL of ethyl acetate. The organic fractions were combined, dried for 30–40 min with anhydrous sodium sulfate, filtered through the Whatman-40 filter, and evaporated to dryness under vacuum (35–40˚C). The residue was redissolved in 5 mL of methanol/ water (50:50, v/v) and filtered through a 0.45 µm filter before injection (20 µl) into the HPLC aperture. Samples were analyzed in triplicate.

2) High-performance liquid chromatography – diode array detector (HPLC–DAD) system for analysis of phenolic compounds

RP-HPLC system for analysis of phenolic compounds HPLC analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A with diode array detector and chromatographic separations were performed on a LUNA C-18 column (4.6×250 mm i.d., 5 µm). The composition of solvents and used gradient elution conditions were described previously by Uzelac et al. (2005) and Butsat et al. (2009) with some modifications. The mobile phase consisted of purified water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 ml/ min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5 to 9% solvent B; from 5 to 15 min, 9% solvent B; from 15 to 22 min, linear gradient from 9 to 11% solvent B; from 22 to 38 min, linear gradient from 11 to 18%s olvent B; from 38 to 43 min, from 18 to 23% solvent B; from 43 to 44 min, from 23 to 90% solvent B; from 44

to 45 min, linear gradient from 90 to 80% solvent B; from 45 to 55 min, isocratic at 80% solvent B; from 55 to 60 min, linear gradient from 80 to 5% solvent B and a re-equilibration period of 5 min with 5% solvent B used between individual runs. Operating conditions were as follows: column temperature, 38 ˚C, injection volume, 20 µl, UV-diode array detection at 280 nm (for hydroxybenzoic acids), 320 nm (for hydroxycinnamic acids) and 370 nm (for flavonols). Phenolic acids in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds and were detected using an external standard method.

3.5.2.5 Cytotoxic activity

The crude extracts were dissolved in dimethyl sulfoxide (DMSO) at 20 mg/ml as stock solutions which were then diluted with DMEM to desired concentrations ranging from 10 to 500 µg/ml. The final concentration of DMSO in each sample did not exceed 1% v/v, to keep the cytotoxicity of DMSO at less than 10%. The Hepatoma cell line (HepG2), Colon cancer cell line (HCT) and normal Vero cell line were used as cell models. Cytotoxicity testing was performed with a neutral red (NR) method (Fotakis and Timbrell, 2006). Briefly, the cells were seeded in 96-well plates (100 µl/well at a density of 3×10^5cells/ml) and treated with various concentrations of the samples for 24 hours. Then, cells were washed twice with 1× PBS and the supernatant was discarded. A total of 100 µl NR solution (50 µg/ml) was added to each well and incubated at 37 °C for another hour. NR was then dissolved by 100 µl of 0.33% HCl. Absorbance of NR dye was detected by a dual-wavelength UV spectrometer (Anthos, 2010; Biochrom, UK) at 520 nm with a 650 nm reference wavelength. The percentage of cytotoxicity compared to the untreated cells was determined with the equation given below. A plot of % cytotoxicity versus sample concentrations was used to calculate the concentration which showed % cytotoxicity. Cytotoxicity (%) = [100×(Absorbance of untreated group−Absorbance of treated group]/Absorbance of untreated group

The selectivity, which indicates the cytotoxic selectivity (safety) of the crude extract against cancer cells versus normal cells (Prayong et al., 2008), also determined.

3.5.3 Experiment three: Determination of antioxidant activity, phenolic compounds and determination of volatile compounds in potent herbs and spices different drying methods.

3.5.3.1 Plant Materials

Holy basil (*Ocimum sanctum* L. cv. Khao; kaprow khao) and (*Ocimum sanctum* L. cv. Daeng; kaprow daeng and kaffir lime leaves (*Citrus hystrix* D.C.) were bought from the market from Mahasarakham Province, Thailand. Herbs and spices were cleaned and the petals were separated. At each market, 2–3 kg samples were sampled from three representative outlets. Single composite samples for each representative market, were prepared by combining about 500 g of homogenised single sample of the same kaffir lime variety from three representative outlets and then homogenising again to obtain a uniform single composite sample. Leaves were washed and drained. Afterwards, the leaves were dried under hot-air (HA), low relative humidity air drying (LRH) (21% relative humidity) and combined far- infrared radiation with hot-air convection drying (FIR-HA).

3.5.3.2 Drying treatment

Samples were subject to three different drying methods, i.e., HA, LRH and FIR-HA. For each drying method, 100 g of fresh leave was used. The protocols used in this present study were selected from the optimal conditions of each method, which were preliminary studied in our lab (Raksakantong et al., 2011). A laboratory scale dryer using combination of far-infrared Radiation, hot-air convection and low relative humidity air drying used in this study was developed in the Research Unit of Drying Technology for Agricultural Product, Faculty of Engineering, Mahasarakham University, Thailand, as shown in Figure 3.1. In FIR-HA drying, the kaffir lime was FIR-HA dried in the FIR dryer at FIR intensity of 5 kW/m^2 (FIR energy irradiated per FIR heater surface area).

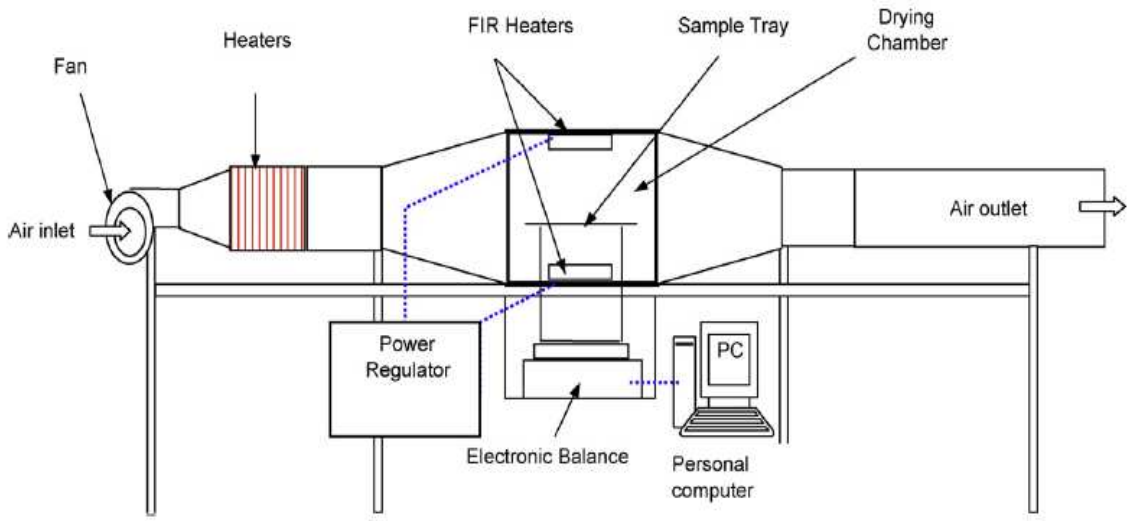


Figure 3.1 Combined far- infrared radiation with hot-air convection drying, hot-air convection and low humidity air drying system

3.5.3.3 Drying instruments

1) Hot-air (HA)

A laboratory scale dryer using in this study was developed in the Research Unit of Drying Technology for Agricultural Product, Faculty of Engineering, Mahasarakham University, Thailand. The sample tray (25.4 x 37 cm^2), the sample tray was placed midway between, and parallel to, the top and bottom heaters, and the distance between each set of heaters and a tray was fixed at 15 cm. The sample tray was supported on a balance which enabled continuous recording of the mass the product throughout the test (Wanyo et al., 2011). Drying temperature was set at 40º C and air velocity at 1.5 m/sec.

2) Low relative humidity air drying (LRH)

A hot air dryer integrating a desiccant wheel operates with two air circuits: an air circuit carrying air for drying the product, which can operate in closed-system or partially open-system modes, and an air circuit providing hot air for silica gel regeneration after adsorption. The desiccant wheel is divided into adsorption and regeneration sections that work simultaneously by means of continuous rotation between the process humid air and a heated regeneration air stream. The process humid air needing to be dehumidified flows through the adsorption section; meanwhile, the

heated air delivers energy to the desiccant in the regeneration section, so that the vapor pressure is greater than the surrounding air, causing water trapped in the saturated desiccant to be driven off (Madhiyanon et al., 2007). Drying temperature was set at 40º C, air velocity at 1.5 m/sec and controlled relative humidity at 21%.

3) Combined far- infrared radiation with hot-air convection drying (FIR-HA)

A laboratory scale dryer using far-infrared radiation in this study was developed in the Research Unit of Drying Technology for Agricultural Product, Faculty of Engineering, Mahasarakham University, Thailand. The dryer consists of a stainless steel drying chamber with inner dimensions of 30 x 51 x 50 cm^3. A far-infrared heater was 12.2x6.0 cm^2 in area, and maximum power of 250 W (Sang Chai Meter Co., Ltd., Bangkok, Thailand) Two sets of three-FIR heaters were installed, one at the top and nother one at the bottom of the drying chamber. The FIR intensity was varied by power regulator. The sample tray (25.4 x 37 cm^2), the sample tray was placed midway between, and parallel to, the top and bottom heaters, and the distance between each set of heaters and a tray was fixed at 15 cm. The sample tray was supported on a balance which enabled continuous recording of the mass the product throughout the test. The temperature of inlet air passing through a hot-air heater was controller by a PID controller (accuracy of ± 1^oC). The air was passed through the drying chamber by a centrifugal fan. The hot-air temperature was measured by K-type thermocouples connected to a data logger (accuracy of ± 1^oC). Besides, air velocity was measured by a hot-wire anemometer (accuracy of ± 0.1 m/s) (Wanyo et al., 2011). After drying, these samples were allowed to cool to ambient temperature before extraction. Drying temperature was set at 40º C, air velocity at 1.5 m/sec. and FIR intensity of 5 kW/m^2 (FIR energy irradiated per FIR heater surface area).

3.5.3.4 Colorimetric parameters

Color changes in sample were determined by a Minolta CR-300 Chroma Meter (Minolta, Japan) in *L**, *a**, *b** color scale. Parameters *L**, *a** and *b** determine a three-dimensional color space, in which *L** represents brightness (on a lightness–darkness scale) whereas positive and negative a values determine the redness and greenness, and positive and negative b values determine yellowness and blueness, respectively. The instrument was calibrated against a white-standard. Measurements

were individually taken for ten samples per treatment and the average of ten readings was calculated. The color difference Δ*E* was calculated from the *L*, a*, b** parameters, using the Hunter-Scotfield equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

3.5.3.5 Determination of volatile compounds

The samples were ground and 0.2 g was put in vials. The vials were sealed with an aluminium–rubber septum (Supelco, Bellefonte, PA, USA) and analysed by the headspace sampling technique (Barcarolo and Casson, 1997). GC-MS analysis was carried out using a GC-2010 chromatograph coupled to a GC/MS-QP2010 (Shimadzu, Japan). Samples were analyzed on a fused-silica capillary column Rtx-5Ms (5% diphenyl 95% dimethyl polysiloxane, 30 m length, 0.25 mm internal diameter, 0.25 μm film thickness; Restek, U.S.) and Rtx-5 (5% diphenyl 95% dimethyl polysiloxane, 30 m length, 0.25 mm internal diameter, 0.25 μm film thickness; Restek, U.S.). Carrier gas, helium; constant pressure, 134.2 kPa; injector temperature, 250 °C; split ratio, 1:5; temperature program, 80 to 250 °C at 10 °C /min then held isothermal (2 min) at 250 °C; ion source temperature, 200 °C; transfer line temperature, 250 °C; ionization energy, 70 eV; electron ionization mass spectra were acquired over the mass range 35-550 u. Standard namely: eugenol, methyl eugenol and β-caryophyllene and others volatile compounds compare in library.

3.5.3.6 Assessment of antioxidant activity

1) Sample extraction

The extracts prepared from dried herb and spice (1g) were extracted for 2 hr with 10 ml of 80% ethanol at room temperature on an orbital shaker set at 180 rpm. The mixture was centrifuged at 1400 x g for 20 min and the supernatant was decanted into a 30 ml vial. The pellet was re-extracted under identical conditions. Supernatant was combined and used for antioxidant activity and used for analyzing antioxidant activity *in vitro*. All analyses were performed in triplicates.

2) Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is a method of measuring the ability of reductants (antioxidants) to reduce Fe^{3+}–Fe^{2+}. The formation of bluecoloured Fe^{2+}-TPTZ complex

(Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. The method of Butsat and Siriamornpun (2009) was used with some modifications. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was warmed at 37 ºC before using. Flower extracts (100 µl) were allowed to react with 1.9 ml of the FRAP solution. The absorbance at 593 nm of the mixture was measured after 60 min of reaction (Butsat and Siriamornpun, 2009). The results were calculated by standard curves prepared with known concentrations of $FeSO_4$, and were expressed as µmol $FeSO_4$/ 100 g DW.

3.5.3.7 Assessment of phenolic compounds

1) Total phenolic content

Total phenolic content was determined using Folin–Ciocalteu reagent as followed by Abu Bakar et al. (2009) as adapted from Velioglu et al. (1998). Briefly, 0.3ml of extract was mixed with 2.25 ml of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min; 2.25 ml of sodium carbonate (60 g/ l) solution were added to the mixture. After 90 min at room temperature, absorbance was read at 725 nm using spectrophotometer. Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/ g dry weight).

2) Total flavonoid content

Total flavonoid content was determined using the colorimetric method described by Abu Bakar *et al*. (2009) as adapted from Dewanto *et al.* (2002). Briefly, 0.5 ml of the extract was mixed with 2.25 ml of distilled water in a test tube followed by addition of 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.3 ml of a 10% $AlC_{l3} \cdot 6H_2O$ solution was added and allowed to stand for another 5 min before 1.0 ml of 1 M NaOH was added. The mixture was mixed well by vortex. The absorbance was measured immediately at 510 nm using a spectrophotometer. Results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/ g dry weight).

3.5.4 Experiment four: Evaluation of antioxidant properties and lipid oxidation of potent herb and spice at different storage time.

3.5.4.1 Plant Materials

Holy basil (*Ocimum sanctum* L. cv. Khao; kaprow khao) was bought from the market at Mahasarakham Province, Thailand. kaprow khao were cleaned and the petals were separated. The raw holy basil leaves were washed and kept at room temperature to drain. The samples were dried by combined far- infrared radiation with hot-air convection drying (FIR-HA). The samples were used for fish emulsion product (pla-yor) in experiment 4.

3.5.4.2 Fish emulsion processing

Fish were cleaned by rinsing in tap water. Fillets were separated and coarsely ground in a food processor. Three samples of fish emulsion were produced using a recipe. All ingredients were mixed in a bowl chopper while ice was gradually added into the mix. The mixture was blended for approximately 15 min until a homogenous mass was obtained. We added kaprow khao powder (1%) and 200 ppm of vitamin E and BHT and combination of kaprow khao powder (1%), BHT(100 ppm) and E (100 ppm). The products were cooked in a steamer at 100ºC for 45 min. Color values of the cooked products were measured as mentioned above.

3.5.4.3 Colorimetric parameters

Color changes in sample were determined by a Minolta CR-300 Chroma Meter (Minolta, Japan) in L^*, a^*, b^* color scale. Parameters L^*, a^* and b^* determine a three-dimensional color space, in which L^* represents brightness (on a lightness–darkness scale) whereas positive and negative a values determine the redness and greenness, and positive and negative b values determine yellowness and blueness, respectively. The instrument was calibrated against a white-standard. Measurements were individually taken for ten samples per treatment and the average of ten readings was calculated. The color difference ΔE was calculated from the L^*, a^*, b^* parameters, using the Hunter-Scotfield equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

3.5.4.4 Determination of antioxidant activity

1) DPPH radical-scavenging activity

The hydrogen atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-coloured methanol solution of DPPH (Gulluce et al., 2007). The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca et al. (2001). Aqueous extract (0.1 ml) was added to 2.9 ml of a 0.004% DPPH in methanol. Absorbance at 517 nm was determined after 30 min, and the percent inhibition of activity was calculated as [(Ao-Ae)/Ao]×100 (Ao = absorbance without extract; Ae = absorbance with extract).

2) Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is a method of measuring the ability of reductants (antioxidants) to reduce Fe^{3+} to Fe^{2+}. The formation of the blue-coloured Fe^{2+}-TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. The method of Butsat and Siriamornpun (2009) was used with some modifications. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was warmed to 37 ºC before use. Flower extracts (100 µl) were allowed to react with 1.9 ml of the FRAP solution. The absorbance at 593 nm of the mixture was measured after 60 min of reaction (Butsat and Siriamornpun, 2009). The results were calculated by standard curves prepared with known concentrations of $FeSO_4$, and were expressed as µmol $FeSO_4$/ 100 g DW.

3.5.4.5 Proximate composition

Proximate composition analysis of the fish emulsion was performed according to AOAC (AOAC, 1999). Moisture, protein, fat, fiber and ash parameters were determined in triplicate from product of fish emulsion.

3.5.4.6 Measurement of peroxide value

Peroxide value (PV) was determined according to the AOAC International (2000). The sample (5 g) was weighed in a 250-ml glass stoppered Erlenmeyer flask and heated in a water bath at 60 ˚C for 3 min to melt the fat, then thoroughly agitated for 3 min with 30 ml acetic acid–chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered under vacuum through Whatman filter paper.

Saturated potassium iodide solution (0.5 ml) was added to the filtrate, which was transferred into the burette. The titration was allowed to run against standard solution of sodium thiosulfate (25 g/l). PV was calculated and expressed as milliequivalent peroxide per kg of sample: POV (meq/kg) = $(S \times N) / W \times 1000$, where S is the volume of titration (ml), N the normality of sodium thiosulfate solution (N = 0:01), and W the sample weight (kg).

3.5.4.7 Measurement of TBA value

The 2-thiobarbituric acid (TBA) assay was carried out according to the procedure of Schmedes and Holmer (1989). Fish emulsion sample (0.15 g) was mixed with 25 ml. of trichloroacetic acid solution (0.2 g/l of TCA in 100 ml/l n-Butanol solution) and homogenized in a blender for 30 s. After filtration, 5 ml of the filtrate were added to 5 ml TBA solution in a test tube. The test tubes were incubated at water bath 95 °C in the dark for 2 h; then the absorbance was measured at 530 nm by using UV–VIS spectrophotometer (model UV-1200, Shimadzu, Japan). TBA value was expressed as mg malonaldehyde per kg of fish emulsion. Bank was no sample.

3.5.4.8 Textural profile analysis (TPA)

TPA was performed using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, England) with cylindrical aluminum probe (50 mm diameter).
The samples were cut into cylinders (30 mm height 20 mm diameter) and placed on the instrument's base. The tests were performed with two compression cycles. TPA textural parameters were measured at room temperature with the following testing conditions: crosshead speed 5.0 mm/s, 50% strain, surface sensing force 99.0 g, threshold 30.0 g, and time interval between the first and the second compressions was 1 s. The Texture Expert version 1.0 software (Stable Micro Systems, Surrey, England) was used to collect and process the data. Hardness, springiness, cohesiveness, gumminess and chewiness were calculated from the force–time curves generated for each sample (Bourne, 1978).

3.5.4.9 Sensory analysis

The sensory evaluation was performed by 30 untrained panelists, who were the students and lecturer in Food Science and Technology programme with the age of 20–48 years and were familiar with fish emulsion consumption. Panelists were asked to evaluate for color, taste, texture and overall likeness of fish emulsion samples using a

9-point hedonic scale (Mailgaad et al., 1999): 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely.

3.6 Statistical analysis

3.6.1 Resulting data was expressed as means and standard deviation of three replicates.

3.6.2 Analysis resulting was presented in tables, bar and line graphs.

3.6.3 The F-test was used to test hypothesis of data results of dependence variables.

3.6.4 Analysis of variance (ANOVA) and least significant difference tests were conducted to identify differences among means using one-way analysis of variance and comparison of paired samples mean was conducted according to Duncan's multiple range test method. The p values of < 0.05 were regarded as significant.

Table 3.1 Description of Thai erbs and spices and their uses

Common name /scientific name	Thai name	Food
Leave		
Thai copper pod /*Cassia siamea* Britt.	Ke-lheg	Light curry (Kaeng khe lhek) , Curry with coconut milk
Asiatic pennywort /*Centella asiatica* Linn.) Urban	Bua-bog	Side dishes, Salad, Drink
Kaffir lime/*Citrus hystrix* DC.	Ma-grood	Spicy chicken or beef salad, Fried fish cakes with cucumber sauce, Tom yum, Hot Thai curry with chicken/beef/pork, Red Curry ,Savory curry with fish/chicken/beef, Curried fish cake, Seamed fish
Purple velvet plant /*Gynura divaricata* DC.	Patumpuang	Side dishes
Finger grass /*Limnophila aromatica* Merr.	Kayang	Soup Nor Mai (Bamboo Shoot Salad Northeastern Style), Side dishes
Indian mulberry /*Morinda citrifolia* Linn.	Yor	Fish curry in banana leaves
Mulberry /*Morus alba* Linn.	Mon	Steamed curried fish, Kaeng Kai Ban
Variegatum /*Piper aurantuacum*	Chaploo	Kaeng Oom Kreueng Nai Moo (Northern Thai Spicy Soup with Pork Giblets)

Table 3.1 (continue)

Common name /scientific name	Thai name	Food
Holy basil /*Ocimum sanctum* L. cv. Khao	Kaprow khao	Thai stirred fries, Stirred fried rice with kaprow, Thai spicy soups chicken curry
Holy basil */Ocimum sanctum* L. cv. Daeng;	Kaprow daeng	Thai stirred fries, Stirred fried rice with kaprow, Thai spicy soups
Rhizome		
Galangal / *Alpinia alangal* (Linn.) Swartz.	Kha	Soup with chicken, galangal root and coconut,Tom yum, Side dishes
Boesenbergia /*Boesenbergia pandurata* (Roxb.) Schltro	Grachay	Noodles with fish curry
Turmeric /*Curcuma longa* Linn.	Kamin	Yellow curry
Ginger /*Zingiber officinale* Roscoe.	Khing	Chicken fried with ginger, Gutinious Dumpling in Hot Ginger Syrup , Soft Bean Curd in Hot Ginger Syrup , Nam khing

CHAPTER 4

RESULTS AND DISCUSSIONS

The results of data analysis and describing were sequent expressed followed by

4.1 Symbols used for data resulting expression

4.2 Phenolic compounds, antioxidant activity and volatile compounds of Thai herbs and spices

4.3 Results and discussions

4.1 Symbols used for resulting data expression

In this study, expression of data analysis results was conducted as various symbols.

SD = Standard deviation

$\overline{X}$ = Means

df = Degrees of freedom

F = F-distribution

p = Probability

4.2 Phenolic compounds, antioxidant activity and volatile compounds of Thai herbs and spices

The present study was to generate information about phenolic compounds antoxidant activity and volatile compounds of 14 Thai herbs and spices from northeastern Thailand. We expected to shed light on their potential health benefits that could be useful for consumers and public health workers.

4.3 Results and discussions

4.3.1 Results and discussion of experiment 1

4.3.1.1 Total phenolic content

Recently, phenolic compounds have been received considerable attention, due to their potential antioxidant activities and free-radical scavenging abilities, which potentially have beneficial implications in human health (Imeh and Khokhar, 2002; Kaisoon et al., 2011). Ethanolic extracts obtained from the samples plants were evaluated for the presence of phenolic compounds. The samples were evaluated using the Folin-Ciocalteu assay, which was suggested as a fast and reliable method to quantify phenolics in foods (Konczak et al., 2010). TPC was determined in comparison with standard gallic acid and the results expressed in terms of mg gallic acid equivalent (GAE)/g DW. The levels of total phenolic compounds (TPC) in the evaluated spices varied significantly from 3 mg GAE/gDW in boesenbergia (*Boesenbergia pandurata* (Roxb.) Schltro) to 19 mg GAE/gDW in *Limnophila aromatic* (Lam.)Merr.(Figure 4.1). The highest value of TPC was found that *Limnophila aromatica* Merrill. (leave), followed by *Morinda citrifolia* L. (leave) and *Curcuma longa* Linn. (rhizome), while boesenbergia (*Boesenbergia pandurata* (Roxb.) Schltro) (rhizome) contained the lowest TPC compared to other herbs and spices studied.

4.3.1.2 Identification of phenolic acids

RP-HPLC analysis was used to identify the phenolic compounds of spices extracts, by comparison with standard compounds. Phenolic acids are hydroxylated derivatives of hydrobenzoic acid and hydrocinnamic acid, which often occur in plants as esters, glycosides and bound complexes (Germano *et al*., 2006). In the 14 spices varieties analyzed, it was possible to identify 10 phenolic acid: gallic acid, protocatechuic acid, *p*-hydroxy benzoic acid, chorogenic acid, vanilic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid and sinapic acid. The distribution of phenolic acids in all spices is presented in Table 4.1. The highest value of total phenolic acids was found in *Cassia siamea* Britt. (leave) (502 mg/g DW), followed by *Piper sarmentosum* Roxb.(leave) (435 mg/g DW), *Morinda citrifolia* Linn. (leave) (258 mg/g DW) and *Centela asiatica* Linn. (leave)(123 mg/g DW). The smallest amounts of

phenolic acids were found in *Alpinia galangal* (Linn.) Swartz. (rhizome), *Curcuma Longa* Linn. (rhizome) and *Gynura divaricata* Subsp.(leave) (Table 4.1). The main phenolic acids (hydrocinnamic acids) in herbs and spices were ferulic acid, sinapic acid and p-coumaric acid. Ferulic acid was the major hydrocinnamic acid derivative, ranging from 2 to 359 mg/g, followed by sinapic acid (16 to 225 mg/g) and p-coumaric acid (1 to 130 mg/g). The highest content of ferulic acid was found in *Piper sarmentosum* Roxb. (leave) (Table 2). High levels of ferulic acid are found in spices, herbs, vegetables, fruits, cereals, and coffee. (Zhao and Moghadasian, 2008). Ferulic acid is an abundant dietary antioxidant which may offer beneficial effects against cancer, cardiovascular disease, diabetes and alzheimer's disease (Zhao and Moghadasian, 2008). *Morinda citrifolia* Linn. (leave) had the most dominant contents of sinapic acid (Table 4.1). The hydroxybenzoic acid, gallic acid, vanilic acid and *p*-hydroxy benzoic acid occurred in small quantities, but not in all spices investigated (Table 4.1).
The highest content of protocatechuic acid and chlorogenic acid were found in *Cassia siamea* Britt. Natural antioxidants are important ingredients that facilitate the control of the oxidative deterioration of foods. Herbs and spices extracts, which exhibited strong antioxidant activities, were found to contain high amounts of total and individual phenolics that may contribute to this activity. Phenolic compounds are commonly found in plants and they have been reported to have a strong antioxidant activity (Elzaawely *et* al., 2005; Mansouri et al., 2005). The antioxidant potential of phenolic compounds is dependent on the number and arrangement of the hydroxyl groups as well as the presence of electron donating substituent in the ring structure (Elzaawely et al., 2005).

4.3.1.3 DPPH radical scavenging activity

DPPH radical is a free radical compound which has been widely used to test free-radical scavenging ability (Sakanaka et al., 2005). Anti-oxidants, on interaction with the DPPH radical, transfer either an electron or hydrogen atom to DPPH, thus neutralizing its free-radical character (Naik et al., 2003). The reagent colour changes from purple to yellow and its absorbance at wavelength 517 nm decreases. The DPPH radical-scavenging activity, percentage inhibition of twelve Thai herbs and spices are given in Table 4.2. The % inhibition (DPPH radical scavenging activity) was in a wide

range of 69% in leave *Cassia siamea* Britt. to 87% in *Ocimum sanctum* L. cv. Khao, while *Curcuma longa* Linn. (rhizome) had the highest % inhibition (DPPH radical scavenging activity) of 86% (Table 4.2).

4.3.1.4 Ferric reducing anti-oxidant potential (FRAP)

The FRAP assay directly measures reductants or anti-oxidants (Abu Bakar et al., 2009) by determining the reducing potential of anti-oxidant reacting with a ferric tripyridyltriazine (Fe3+–TPTZ) complex and producing a coloured ferrous tripyridyltriazine (Fe2+–TPTZ) (Benzie and Strain, 1996). Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free-radical chain via donating a hydrogen atom (Duh, Du and Yen, 1999). According to Benzie and Strain (1996), the reduction of Fe3+–TPTZ complex to blue-coloured Fe2+–TPTZ occurs at low pH. FRAP values of the fruit extracts are shown in Table 3. In leave samples, FRAP value ranged from 245 μmol FeSO4/100 g in *Piper aurantuacum* to 682 μmol FeSO4/ 100 g in *Ocimum sanctum* L. cv. Khao (Table 4.2).

4.3.1.5 Volatile compounds

The extraction of the volatile compounds from herbs and spices were carried out following the headspace sampling and analysis by means of coupled GC–MS method. Forty volatile compounds were identified in the leave (Table 4.3), while fifteen volatile compounds were identified in the rhizome (Table 4.3). There were significant differences among different varieties tested. Eucalyptol was the most prominent volatile compound in *Alpinia alangal* (Linn.) Swartz. (rhizome) (68%). *Piper aurantuacum* (leave) had the highest content of 7-Hydroxy-5,6,7,8-tetrahydroind (67%) (Table 4.3). Valeric aldehyde was the most dominant volatile compound in *Morinda citrifolia* Linn.(leave) (65%). Only one volatile compound found both leave and rhizome was isoserine. Isoserine was found to be a volatile in *Curcuma longa* Linn.(rhizome) (34%), *Cassia siamea* Britt.(leave) (16%) and *Alpinia alangal* (Linn.) Swartz. (rhizome) (10%) (Table 4.3). Many compounds are responsible for plant flavor and some of the herbs and spices are used by humans to season food yield (Quintavalla and Vicini, 2002). Herbs and spices have been used for providing humans with tastes in foods. In addition to taste, their beneficial health effects have also been widely attracted by food scientists.

4.3.1.6 Summary of experiment 1

Thai herbs and spices are traditional foods in most cultures which play important roles in human nutrition. This study has demonstrated the informative results of phenolic compounds, antioxidant activity and volatile compounds. The main phenolic acids in these spices were ferulic acid, sinapic acid and *p*-coumaric acid. There were significant differences among different varieties tested. This study generated useful information for consumers and many encourage researchers to utilize herbs and spices as sources of bioactive compounds.

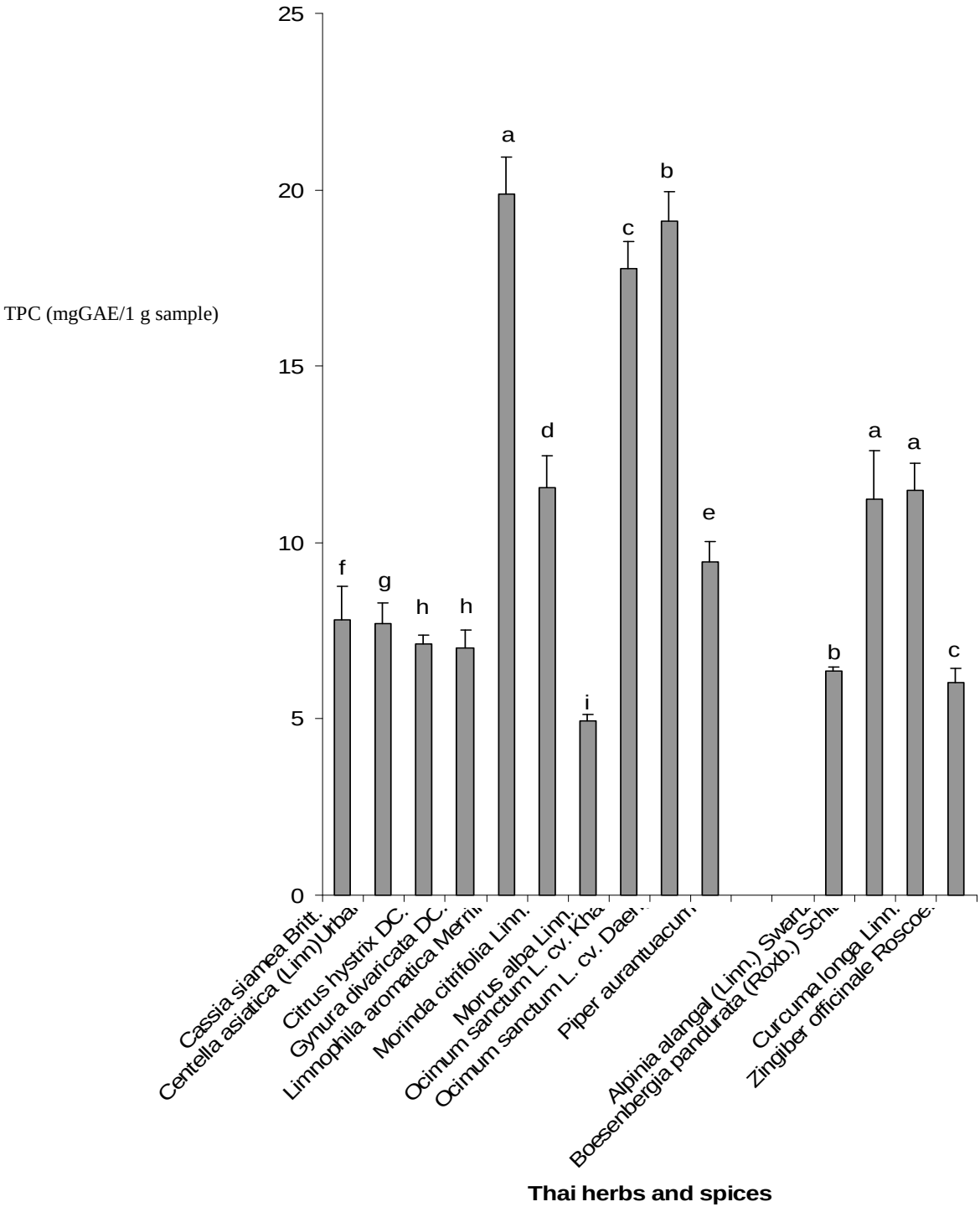


Figure 4.1 Total phenolic content of Thai herbs and spices

Table 4.1 Phenolic acids of Thai herbs and spices

Herbs and spices	Hydrobenzoic acids (mg/g DW)					Hydrocinnamic acids (mg/g DW)					Total phenolic acids
	GA	PCCA	p-HO	ChA	VA	CFA	SyA	p-CA	FA	SNA	
Leave											
Cassia siamea Britt.	1.91±0.15^{f}	273.83±27.12^{a}	26.93±1.26^{c}	83.48±2.45^{a}	nd	45.85±3.18^{a}	41.40±8.53^{a}	7.14±0.59^{d}	34.07±1.71^{c}	23.81±1.46^{h}	502.69±27.35^{a}
Centela asiatica Linn.	1.64±0.09^{i}	1.52±0.10^{g}	2.88±0.11^{e}	21.92±0.65^{b}	nd	2.38±0.09^{c}	10.87±0.96^{d}	2.53±0.06^{f}	60.76±9.45^{b}	19.01±1.12^{i}	123.51±7.48^{g}
Citrus hystrix DC.	2.39±0.45^{e}	2.92±0.41^{e}	nd	nd	8.96±0.68^{a}	3.33±0.48^{b}	24.08±0.34^{b}	8.29±0.32^{c}	18.85±0.79^{e}	31.73±1.36^{g}	100.55±5.60^{j}
Gynura divaricata subsp.	6.02±0.16^{c}	nd	nd	11.71±0.94^{c}	0.54±0.05^{e}	nd	8.75±0.08^{g}	1.44±0.06^{g}	5.39±0.02^{g}	18.96±0.09^{i}	52.81±2.38^{k}
Limnophila aromatica (Lam.) Merr.	1.83±0.20^{g}	nd	1.54±0.39^{f}	nd	nd	nd	2.11±0.07^{h}	nd	25.43±3.54^{d}	72.53±9.47^{e}	103.44±9.42^{i}
Morinda citrifolia Linn.	Nd	2.72±0.28^{f}	3.15±0.59^{d}	nd	6.07±0.51^{b}	1.77±0.54^{d}	17.04±0.19^{f}	2.47±0.32^{f}	nd	225.44±10.60^{a}	258.66±11.46^{f}
Morus alba Linn.	1.76±0.06^{h}	1.44±0.06^{h}	nd	nd	5.15±0.11^{c}	nd	21.62±3.46^{e}	2.33±0.21^{a}	4.25±0.09^{h}	75.72±12.17^{d}	112.27±8.72^{h}
Ocimum sanctum L. cv. Khao	7.78±1.24^{b}	14.59±1.07^{b}	54.1±2.13^{b}	8.97±1.18^{d}	4.67±1.03^{d}	nd	29.89±1.86^{c}	130.56±5.42^{b}	6.41±1.02^{f}	85.92±0.84^{b}	342.89±17.28^{c}
Ocimum sanctum L. cv. Daeng	9.26±1.72^{a}	12.73±1.84^{c}	61.5±2.84^{a}	7.49±0.86^{e}	nd	nd	31.36±2.04^{b}	127.84±5.63^{e}	5.69±0.74^{g}	78.74±1.45^{c}	334.61±15.43^{d}

Table 4.1 (continue)

Herbs and spices	Hydrobenzoic acids (mg/g DW)					Hydrocinnamic acids (mg/g DW)					Total phenolic acids
	GA	PCCA	p-HO	ChA	VA	CFA	SyA	p-CA	FA	SNA	
Leave											
Piper sarmentosum Roxb.	2.85±0.10[d]	3.71±0.15[d]	nd	nd	nd	2.53±0.06[c]	2.27±0.08[h]	4.61±0.07[c]	359.29±22.50[a]	60.25±9.08[f]	435.51±24.63[b]
Rhizome											
Alpinia alangal (Linn.) Swartz.	nd	1.72±0.29[b]	nd	nd	nd	1.52±0.39[b]	1.47±0.44[c]	1.48±0.05[c]	2.46±0.22[b]	16.34±1.45[c]	24.99±1.26[c]
Boesenbergia pandurata (Roxb.) Schltro.	nd	3.96±0.56[a]	nd	nd	3.35±0.34[a]	1.70±0.27[a]	8.34±0.51[b]	6.36±0.34[a]	52.28±3.46[a]	24.38±2.66[a]	100.37±5.73[a]
Curcuma Longa Linn.	nd	1.65±0.05[c]	nd	nd	nd	1.64±0.39[a]	1.67±0.22[c]	1.74±0.28[b]	2.29±0.14[b]	17.41±1.52[c]	26.40±1.18[c]
Zingiber officinale Roscoe.	nd	nd	1.79±0.19[a]	nd	nd	Nd	17.58±1.37[a]	nd	nd	21.86±2.66[b]	41.23±2.83[b]

Values are expressed as mean ± SD of triplicate measurement. nd= not detected; GA= Gallic acid; PCCA=Protocatechuic acid; p-HO=p-hydroxy benzoic acid; ChA= Chorogenic acid; VA= Vanilic acid; CFA= Caffic acid; SyA=Syringic acid; p-CA= *p*-Coumaric acid; FA=Ferulic acid; SNA=Sinapic acid.

Table 4.2 Antioxidant activity of Thai herbs and spices at concentration 0.2 g/ml

Thai herb and spice	Antioxidant activity	
	DPPH (% inhibition)	FRAP (μmol $FeSO_4$/100g sample)
Leave		
Cassia siamea Britt.	69.18±0.32[h]	397.26±1.15[f]
Centella asiatica Linn.) Urban	76.45±0.57[f]	407.34±1.58[e]
Citrus hystrix DC.	82.56±0.17[d]	525.38±3.23[c]
Gynura divaricata DC.	75.30±0.67[f]	348.81±2.19[h]
Limnophila aromatica Merr.	83.45±0.23[c]	498.62±3.21[d]
Morinda citrifolia Linn.	72.13±0.64[g]	373.28±1.75[g]
Morus alba Linn.	78.26±0.21[e]	325.38±2.43[i]
Piper aurantuacum	79.09±0.38[e]	245.89±1.23[j]
Ocimum sanctum L. cv. Khao	87.16±0.87[a]	682.43±4.16[a]
Ocimum sanctum L. cv. Daeng	84.04±0.52[b]	644.35±4.09[b]
Rhizome		
Alpinia alangal (Linn.) Swartz.	85.29±0.18[b]	606.39±2.25[a]
Boesenbergia pandurata (Roxb.)Schltro	78.26±0.74[d]	421.24±1.78[d]
Curcuma longa Linn.	86.42±0.32[a]	563.09±2.46[b]
Zingiber officinale Roscoe.	82.57±0.94[c]	472.06±1.46[c]

Table 4.3 volatile compounds (% area) of Thai herbs and spices

Thai herbs and spices	Volatile compounds
Leave	
Cassia siamea Britt.	Cadinene (16.62%) Isoserine (16.07%) Carotol (15.56%) Furfural (10.01%) Oxymetholone (8.52%) Selina-6-en-4-ol (8.24%) Spathulenol (6.84%) Farnesene (5.71%) 1-Heptadecene (5.67%) Spinacen (4.03%)
Centella asiatica Linn.) Urban	3-Chloromethylfuran (53.39%) Tetradecanal (19.42%) Citronellal (11.82%) Octadecanal (7.27%)
Citrus hystrix DC.	Citronellal (53.76%) Propylenediamine (13.68%) Phytol (13.55%) Linalool (10.58%) Squalene (4.99%) Farnesene (3.44%)
Gynura divaricata DC.	Butanal (41.84%) Alpha-pinene (39.37%) Cadinene (11.02%) Stellasterol (7.78%)
Limnophila aromatica Merr.	Stereoisomer (54.28%) Cycloheptene (19.95%) Alpha-pinene (16.47%) Neodihydrocarveol (9.30%)
Morinda citrifolia Linn.	Valeric aldehyde (65.31%) Benzaldehyde (10.32%) Benzenacetaldehyde (10.25%) Palmitaldehyde (7.90%) Cyclohexanol (6.15%)
Morus alba Linn.	n-Pentanal (42.73%) 2,3,3-Trimethyloctane (17.86%) 2,2,4-Trimethyl (13.62%) Columbin (10.16%) 1,3,7-Octatriene (9.70%) Farnesol (5.93%)

Table 4.3 (continue)

Thai herbs and spices	Volatile compounds
Piper aurantuacum	7-Hydroxy-5,6,7,8-tetrahydroind (67.35%) Cysteine (26.70%) 1,2,3-Butanetriol (3.69%) Xexanol (1.16%) 2-propylheptanol (0.58%)
Ocimum sanctum L. cv. Khao	Eugenol (20.54%) Beta-elemene (7.08%) Methyl eugenol (20.5%) Benzene (4.94%) Caryophyllene (23.3%) Alpha-humulene (4.62%) Beta-elemene (4.77%) Neophytadiene (5.58%) Phytol (4.26%) Hexasiloxane (1.48%)
Ocimum sanctum L. cv. Daeng	4-Cyclopropyl-2-methoxyphenol (1.25%) Beta-elemene (8.48%) Methyl eugenol (35.35%) Caryophyllene (31.91%) Alpha-humulene (5.77%) Helminthogermacrene (6.88%) Caryophyllene oxide (4.43%) Neophytadiene (5.94%)
Rhizome *Alpinia alangal* (Linn.) Swartz.	Eucalyptol (68.80%) Isoserine (10.33%) Caryophyllene (8.02%) 4-Carvomenthenol (6.88%) Alpha-humulene (5.97%)
Boesenbergia pandurata (Roxb.)Schltro	Alpha-tumerone (41.85%) Alpha-phellandrene (26.98%) Zingiberene (16.06%) Camphene (15.11%)
Curcuma longa Linn.	Isoserine (34.38%) 1-heptadecene (22.91%) Carotol (12.65%) Alpha-phellandrene(10.85%)
Zingiber officinale Roscoe.	Zingiberene (26.84%) Eucalyptol (26.07%) Camphene (18.19%) Beta-sesqulphellandrene (12.09%) Citral (8.55%) Hexaldehyde (8.27%)

4.3.2 Results and discussion of experiment 2

4.3.2.1 Antioxidant activities, phenolic compounds and cytotoxic activity of crude kaprow khao

We investigated the compositions of antioxidant activities, phenolic compounds and cytotoxic activity of crude kaprow khao.

1) Antioxidant activity

The DPPH radical-scavenging and FRAP assays were used to evaluate the antioxidant capacities of kaprow khao. DPPH is a stable free-radical compound widely used to test the free-radical-scavenging ability of various samples (Sakanaka et al., 2005). The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe^{2+}–TPTZ) (Benzie and Strain, 1996). Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free-radical chain by donating a hydrogen atom (Gordon, 1990). The reduction of Fe^{3+}–TPTZ complex to blue-colored Fe^{2+}–TPTZ occurs at low pH (Benzie and Strain, 1996). DPPH scavenging activities of kaprow khao are shown in Table 4.4. The % inhibition (DPPH radical scavenging activity) of kaprow khao was 87%.

The FRAP assay directly measures reductants or anti-oxidants (Abu Bakar *et al*., 2009) by determining the reducing potential of anti-oxidant reacting with a ferric tripyridyltriazine (Fe3+–TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe2+–TPTZ) (Benzie and Strain, 1996). Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free-radical chain via donating a hydrogen atom (Duh et al., 1999). According to Benzie and Strain (1996), the reduction of Fe3+–TPTZ complex to blue-coloured Fe2+–TPTZ occurs at low pH. FRAP values of kaprow khao are shown in Table 4.4. FRAP value of the crude holy basil was 725 μmol FeSO4/100 g. It is well known that natural antioxidants extracted from herbs and spices (rosemary, oregano, thyme, etc.) have high antioxidant activity and are used in many food applications (Hirasa and Takemasa, 1998). A number of studies deal with the antioxidant activity of extracts from herbs and spices (Cuvelier et al., 1994; Kikuzaki and Nakatani, 1993; Lu and Yeap-Foo, 2001). Previous study has shown that ethanolic extracts of kaprow

exhibited strong antioxidant activity against a β-carotene linoleic system, superoxide anion scavenging activity, Fe^{2+} -chelating activity and reducing power, and also acted as radical scavenger and lipoxygenase inhibitors (Juntachote and Berghofer, 2005).

2) Determination of total flavonoid content (TFC)

Large amounts of natural phenolic compounds are found in teas, fruits, and vegetables. Flavonoids are the most common and widely distributed group of plant phenolic compounds; they are characterized by a benzo pyrene structure, which is ubiquitous in fruits and vegetables (Yao et al., 2004). Total flavonoid content (TFC) can be determined in the sample extracts by reaction with sodium nitrite, followed by the development of colored flavonoid-aluminum complex formation using aluminum chloride which can be monitored spectrophotometrically at 510 nm. The amounts of TFC in kaprow khao are shown in Table 4.4. The total flavonoid content of kaprow khao was 252 mg GAE/g DW.

3) Determination of total phenolic content (TPC)

Phenolic compounds have been received considerable attention, due to their potential antioxidant activities and free-radical scavenging abilities, which potentially have beneficial implications in human health (Imeh and Khokhar, 2002; Kaisoon *et al*., 2011). Ethanolic extracts obtained from the samples plants were evaluated for the presence of phenolic compounds. The samples were evaluated using the Folin-Ciocalteu assay, which was suggested as a fast and reliable method to quantify phenolics in foods (Konczak et al., 2010). TPC was determined in comparison with standard gallic acid and the results expressed in terms of mg gallic acid equivalent (GAE)/g DW. The total phenolic content of kaprow khao was 100 mg GAE/ g DW (Table 4.4).

Table 4.4 Phenolic compounds and antioxidant activities of kaprow khao

Phenolic compounds		Antioxidant activity		
TPC	TFC	FRAP (μmol $FeSO_4$/100g sample)	DPPH (% inhibition)	IC_{50}
100.38±3.03	252.21±5.16	725.88±1.24	87.16±0.87	1.05±0.02

4) Determination of phenolic acids

Natural antioxidants are important ingredients that facilitate the control of the oxidative deterioration of foods. Holy basil extracts, which exhibited strong antioxidant activities, were found to contain high amounts of total and individual phenolics that may contribute to this activity. RP-HPLC analysis was used to identify the phenolic compounds of crude kaprow khao, by comparison with standard compounds. Phenolic acids are hydroxylated derivatives of hydrobenzoic acid and hydrocinnamic acid, which often occur in plants as esters, glycosides and bound complexes (Germano et al., 2006). In the crude holy basil analyzed, it was possible to identify 9 phenolic acid: gallic acid, protocatechuic acid, *p*-hydroxy benzoic acid, chorogenic acid, vanilic acid, syringic acid, *p*-coumaric acid, ferulic acid and sinapic acid. The distribution of phenolic acids in crude holy basil was presented in Figure 4.2. The concentration of total phenolic acids was 215.86 mg/g. The main phenolic acids (hydrocinnamic acids) in crude holy basil were p-coumaric acid (140 mg/g) and syringic acid (11 mg/g) (Figure 8). The hydroxybenzoic acid, *p*-hydroxy benzoic (16 mg/g) acid and Chorogenic acid (10 mg/g) investigated (Figure 4.2). *p*-Coumaric acid (CA) widely exists in fruits, such as apples and pears, and in vegetables and plant products, such as beans, potatoes, tomatoes, and tea. It is an intermediate product of the phenylpropanoid pathway in plants. CA has been suggested to exhibit antioxidant

properties (Castelluccio et al., 1996). It was reported that CA *in vitro* can provide antioxidant protection to LDL as a result of the chain-breaking activity of CA (Castelluccio et al., 1996). Diet supplementation with a crude extract of CA isolated from pulses resulted in the reduction of ester cholesterol, providing a protective mechanism against the development of atherosclerosis (Sharma, 1979). The ability of CA to prevent excessive lipid peroxidation on the basis of its chain-breaking activity of alpha-tocopherol oxidation has also been demonstrated (Laranjinha, 1996). More recently, Castelluccio et al. (1996) reported that CA was effective in enhancing the resistance of LDL to oxidation. If CA is an efficient antioxidant for LDL, it may play a key role in the purported effect of oxidized lipoprotein on platelet activity to inhibit atherogenesis. In addition, the dehydrogenation polymer of CA was reported to have anti-human immunodeficiency virus activity (Shimizu, 1993). Phenolic compounds are commonly found in plants and they have been reported to have a strong antioxidant activity (Elzaawely et al., 2005). The antioxidant potential of phenolic compounds is dependent on the number and arrangement of the hydroxyl groups as well as the presence of electron donating substituent in the ring structure (Elzaawely et al., 2005).

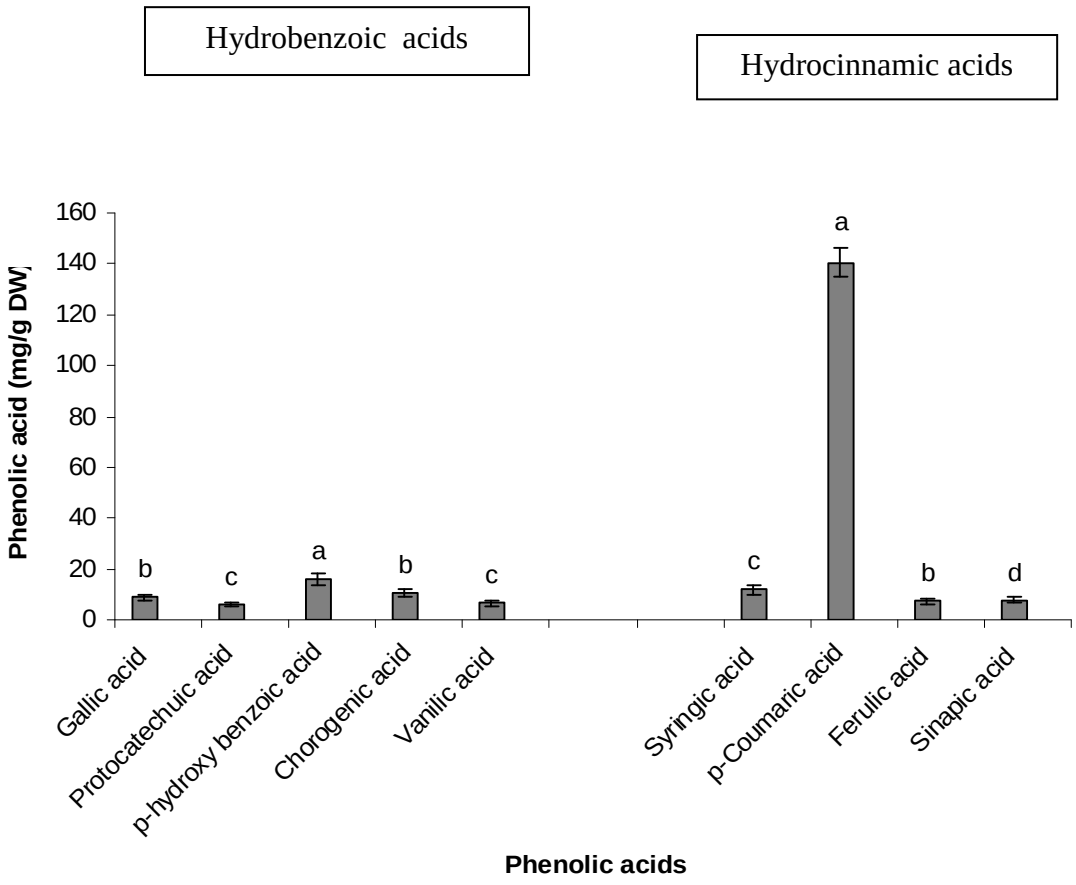


Figure 4.2 Phenolic acids of kaprow khao

5) Cytotoxic activity of kaprow khao

In vitro cytotoxicity test is mainly performed to screen potentially toxic compounds that affect basic cellular functions. This cytotoxicity is measured with cellular damage using neutral red (NR) which is a weak cationic dye that penetrates and accumulates in the lysosomes of living cells (Fotakis and Timbrell, 2006). Therefore, NR assay was used to determine the cell viability or, in other words, the toxicity of the test compounds. Several studies of cytotoxicity of herbal plants have been reported. One example showed that the extract *Pinus kesiya* had the highest selectivity and potent cytotoxicity in the Hepatoma cell line (HepG2), with an IC_{50} value of 52.0 ± 5.8 μg/ml (mean ± standard deviation). Machana *et al.* (2011) reported that *Catimbium speciosum* exerted cytotoxicity with an IC_{50} value of 55.7 ± 8.1 μg/ml. Crude extracts from

Glochidion daltonii, *Cladogynos orientalis*, *Acorus tatarinowii* and *Amomum villosum* exhibited cytotoxicity with IC_{50} values ranging 100-500 µg/ml. The phytochemicals present in several herbal products and plants were have shown to potential as preventive or therapeutic agents against various human cancer. Study was also shown that Kaprow khao was cytotoxic against Hepatoma cell line (HepG2) and Colon cancer cell line (HCT) at 500 µg/ml with less cytotoxic against normal Vero cells. The result indicated the selective cytotoxicity of kaprow khao in cancer cells tested. The % cytotoxicity of 500 µg/ml kaprow khao was 48% in HepG2 cells and 46% in HCT cells (Figure 4.3).

4.3.2.2 Summary of experiment 2

Crude kaprow khao extracts, which exhibited strong antioxidant activities, were found to contain high amounts of total and individual phenolics that may contribute to this activity. Study was also shown that Kaprow khao was cytotoxic against Hepatoma cell line (HepG2) and Colon cancer cell line (HCT) at 500 µg/ml with less cytotoxic against normal Vero cells. Kaprow khao has demonstrated a potential herb and spice for functional food.

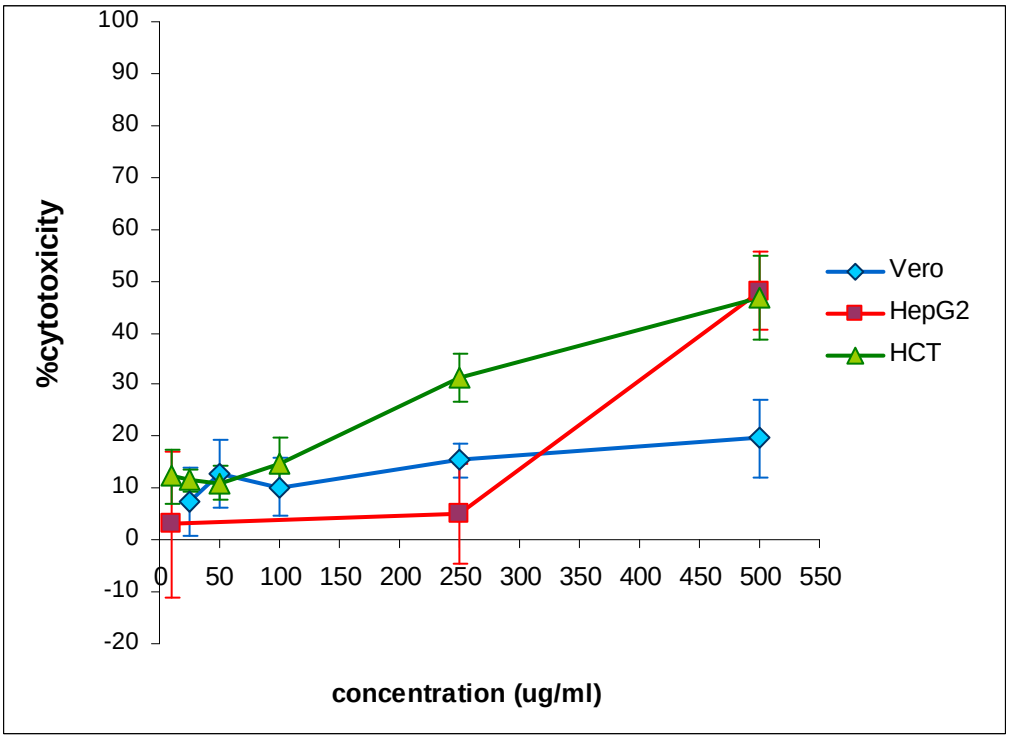


Figure 4.3 Cytotoxicity (%) of kaprow khao

4.3.3 Results and discussion of experiment 3

4.3.3.1 Optimized drying of kaprow leaves for industrial production of holy basil spice powder.

Three drying methods used were hot-air drying (HA), low relative humidity air drying (LRH) and combined far- infrared radiation with hot-air convection drying (FIR-HA) drying. Quality assessment of kaprow made from the different drying methods included antioxidant property, total flavonoid content (TFC), total phenolic content (TPC) and volatile compounds.

1) Drying efficiency

Moisture content was fixed at 7% dry basis, according to the industrial standards for herbs and spices dried product (Jayaraman and Das-Gupta,1995). For HA drying, the drying process is conducted by using a hot air dryer at 50 °C for 15-16 hr and the dry kaprow had moisture content of kaprow daeng and kaprow khao (6.2 and 6.6%). For LRH drying, the drying process is a desiccant wheel dehumidification system to a conventional hot-air system had moisture content of kaprow daeng and kaprow khao (6.8 and 6.9%). FIR-HA drying process took only 27-30 min to achieve dry kaprow containing 6.9 and 6.1% (daeng and khao) moisture content (Table 4.5). Low relative humidity air drying (LRH); desiccant cooling and air dehumidification is a good alternative to the conventional vapour compression system for air conditioning. Therefore, the reduction in drying time decreased within the regeneration temperature (Madhiyanon et al., 2007) and far- infrared radiation (FIR) drying, the heat is generated deep inside the kernel and tends to be selectively highest in the regions having the highest moisture content. Thus the vapour pressure would be largest in these regions, and diffusion will be toward the areas of lower vapour pressure such as the kernel surface therefore water is removed away rapidly. While, hot air drying is a slow process relying on heat conduction from the outer surface towards the interior. The use of FIR and air temperature resulted in much faster drying and considerable reduction in energy than at comparable convective temperatures
(Afzal et al., 1999)

2) Color parameters

Color is a psychological property of food products that affects to the enjoyment of eating. Temperature during drying is one of the causes of color degradation in dehydrated products (Lozano and Ibarz, 1997). Color parameters of kaprow dried by different methods compared to fresh leaves are shown in Table 4.5. Overall, when compared with holy basil fresh leaves, a smaller decrease in L^* values of the three methods dried kaprow khao than those of kaprow daeng of FIR-HA dried was observed. The total color difference ΔE^*, which is a combination of parameters L^*, a^* and b^* values, is a colorimetric parameter extensively used to characterize the variation of colors in food during processing. The results presented in this work suggest that the change in ΔE^* of LRH and FIR-HA dried kaprow daeng were smaller as compared to HA drying. As the color of LRH and FIR-HA, dried samples of two cultivars, appeared to be more like fresh leaf, these may imply that these drying methods could better preserve bioactive compounds and activities. The color changes in kaprow caused by the thermal may be due not only the non-enzymatic browning reaction, but also to the destruction of pigments present in the leaf (Wanyo et al., 2010).

3) Effect of total phenolic content (TPC) and total flavonoid content (TFC)

Phenolic compounds are widely distributed in fruits and vegetables (Li *et al*., 2006), which have received considerable attention, due to their potential antioxidant activities and free-radical scavenging abilities, which potentially have beneficial implications in human health *(Govindarajan et al., 2007; Li et al., 2006; Lopez-Velez et al., 2003). Ethanolic extracts obtained from the samples plants were evaluated for the presence of phenoli*c compounds. The samples were evaluated using the Folin-Ciocalteu assay, which was suggested as a fast and reliable method to quantify phenolics in foods (Konczak et al., 2010; Prior et al., 2005). TPC was determined in comparison with standard gallic acid and the results expressed in terms of mg GAE/g DW. The total phenolic content of the fresh kaprow daeng was 19.12 mg GAE/g DW. With different drying methods, the total phenolics contents in the HA, LRH and FIR-HA were 14.23, 20.62 and 22.36 mg GAE/g DW, respectively, while the total phenolic content of the fresh kaprow khao was 17.78 mg GAE/g DW. With different

drying methods, the total phenolics contents in the HA, LRH and FIR-HA were 12.47, 22.36 and 18.64 mg GAE/g DW, respectively (Table 4.6).Thermal processing may release more bound phenolic acids from the breakdown of cellular constituents. Although disruption of cell walls also releases the oxidative and hydrolytic enzymes that can destroy the antioxidants in fruits and vegetables (Chism and Haard, 1996). Therefore degradation of polyphenol compounds by thermal process may result in releasing antioxidant compounds which have different chemical and biological properties (Tsai et al., 2002). However, there were increases in TPC following combined FIR–HA treatment of mulberry leaves (Wanyo, et al., 2010). Sliced carrots were dried by convective drying (CD), microwave-convective drying (MCD), infrared-convective drying (IRCD), and freeze drying (FD). Dried carrots of cv. Deep Purple were characterized by an approximately 2.5 times higher antioxidant capacity and higher contents of anthocyanins and polyphenols than dried carrots of cv. Purple Haze (Witrowa-Rajchert et al., 2005).Food processing can improve the properties of naturally occurring antioxidants or induce the formation of new compounds with antioxidant properties, so that the overall antioxidant activity increases or remains unchanged (Tomaino et al., 2005). Flavonoids are the most common and widely distributed group of plant phenolic compounds, that are characterized by a benzo pyrene structure, which is ubiquitous in fruits and vegetables. Total flavonoid can be determined in the sample extracts by reaction with sodium nitrite, followed by the development of coloured flavonoid-aluminum complex formation using aluminum chloride which can be monitored spectrophotometrically at 510 nm. The HA dried of two kaprow cultivars contained the lowest amount of TFC as compared to the fresh and two processed. Amounts of TFC in kaprow daeng FIR-HA dried contained had the highest flavonoid content (14.15 mg RE/g DW), followed by the kaprow khao FIR-HA dried (12.76 mg RE/g DW), and LRH dried kaprow daeng (12.37 mg RE/g DW). Flavonoids, as one of the most diverse and wide spread group of natural compounds, are probably the most important natural phenolics (Prasad et al., 2009). Besides their antioxidant activity, flavonoids have been demonstrated a wide range of biochemical and pharmacological effects including anti-inflammatory, anti-viral, anti-allergenic, anti-carcinogenic, anti-aging activity (Hülya Orak, 2007), anti-oxidant and anti-allergic effects (Miean and

Mohamed, 2001). Importantly, in our study, the values of TPC and TFC were higher after FIR-HA and LRH drying, compared to the results for HA dried or even for fresh kaprow.

4) Effect of Ferric reducing antioxidant power (FRAP)

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe3+–TPTZ) complex and producing a coloured ferrous tripyridyltriazine (Fe2+–TPTZ) (Benzie and Strain, 1996). Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free-radical chain by donating a hydrogen atom (Gordon, 1990). The reduction of Fe3+–TPTZ complex to blue-coloured Fe2+–TPTZ occurs at low pH (Benzie and Strain, 1996). FRAP values of the extracts of kaprow different drying methods are shown in Table 4.6. The FRAP value of different drying methods indicated that the FIR dried had the greatest reducing power both kaprow daeng and kaprow khao (872.59 and 916.87 μmol $FeSO_4$/100 g DW), followed by LRH dried (787.90 and 812.85 μmol $FeSO_4$/100 g WD), fresh (644.35 and 682.43 μmol $FeSO_4$/100 g), and then HA dried (513.57 and 543.25 μmol $FeSO_4$/100 g). A significant decrease in FRAP value was found in HA dried compared to fresh leaves. Similar results were found in *Alpinia zerumbet* and *Etlingera elatior* leaves which significant decreases of FRAP values (50 and 53%) were observed in HA dried samples (Chan et al., 2009). In addition, HA drying resulted in drastic declines of 80% for leaves of *Curcuma longa* and 71% for leaves of *Kaempferia gala* and 70-75 % for vegetables (Roy *et al*., 2007) and 27% for mulberry leaves (Wanyo et al., 2010). This behaviour could be related to drying process at low temperatures, which implies long drying times that may promote a decrease of antioxidant capacity (Garau et al., 2007). Losses in antioxidant properties of heat-treated samples have been attributed to thermal degradation of phenolic compounds (Larrauri et al., 1997). Declines in antioxidant activity are often accompanied by loss of other bioactive properties (Roy et al., 2007). The different drying methods of kaprow showed significant ($p<0.05$) difference in FRAP value, indicating that the drying methods significantly influenced the ferric reducing power evaluation.

5) Percentage losses or gains for antioxidant activity, total flavonoid and total phenolic content

Percentage losses or gains for TPC, TFC and FRAP of kaprow dried by different methods compared to fresh leaves are shown Figure 4.4-4.5. There were increases in TPC, TFC and FRAP value following LRH and FIR-HA dried of kaprow daeng (LRH: 7.85, 11.54 and 22.27 %, FIR-HA: 1.77, 27.59 and 35.42%) and kaprow khao (LRH: 25.75, 12.54 and 19.11%, FIR-HA: 4.83, 41.62 and 34.35%), respectively, while TPC, TFC and FRAP values decreased (kaprow daeng (25.57, 27.77 and 28.12%) and kaprow khao (28.12, 16.42 and 20.39%) of HA drying. Our findings are similar to the data from previous studies by Erbay and Icier (2009) that TPC and total antioxidant activity of olive leaves were decreased 9.77 and 44.25%, respectively after dried using by heat pumps drying at 53°C, process time of 288 min. In addition, outer leaves of white cabbage (*Brassica oleracea* L. var. capitata) by hot air drying found that TPC less than 40% retention after dried (Tanongkankit et al., 2010).

Table 4.5 Total phenolic content (TPC), Total flavonoid content (TFC) and FRAP value of kaprow.

Drying method	TPC (mg GAE/g)		TFC (mg RE/g)		FRAP (μmolFeSO$_4$/100 g)	
	Kaprow daeng	Kaprow khao	Kaprow daeng	Kaprow khao	Kaprow daeng	Kaprow khao
Fresh	19.12±0.84^{b}	17.78±0.74^{c}	11.09±0.42^{c}	9.01±1.82^{c}	644.35±18.02^{c}	682.43±14.32^{c}
HA	14.23±0.69^{c}	12.47±0.39^{d}	8.01±0.27^{d}	7.53±0.45^{d}	531.57±16.21^{d}	543.25±12.61^{d}
LRH	20.62±1.05^{a}	22.36±1.24^{a}	12.37±0.31^{b}	10.14±0.82^{b}	787.90±13.14^{b}	812.85±18.83^{b}
FIR	19.46±0.98^{b}	18.64±0.11^{b}	14.15±0.90^{a}	12.76±0.90^{a}	872.59±23.17^{a}	916.87±24.61^{a}

Values are expressed as means ± standard deviation. Means with different letters in the same column were significantly different at the level $p < 0.05$.

Food processing can improve the properties of naturally occurring antioxidants or induce the formation of new compounds with antioxidant properties, so that the overall antioxidant activity increases or remains unchanged (Tomaino et al., 2005). Therefore degradation of polyphenol compounds by thermal process may result in releasing antioxidant compounds which have different chemical and biological properties (Tsai et al., 2002). Effects could vary from little or no change to significant losses, or even enhancement in antioxidant properties (Nicoli et al., 1999). Importantly, in our study, the values of TPC, TFC and FRAP were higher after LRH and FIR-HA drying, compared to the results for HA drying or even for fresh leaves. FIR-HA creates internal heating with molecular vibration of material, i.e., molecules absorb the radiation of certain wavelengths and energy, and cause vibration excitedly. Moreover, the mechanism of far-infrared drying is different from hot air drying (Sandu, 1986), and the electromagnetic wave energy is absorbed directly by the dried food with less energy loss. FIR was thought to liberate and activate low molecular weight natural antioxidant compounds, because it heats materials without degrading the constitutive molecules of the surface and contributes to an even transfer of heat to the center of the materials (Niwa et al., 1988). Previous studies found that antioxidant activities and total phenolic contents increased after exposure of rice hulls to FIR radiation (Lee et al., 2003) and peanut hull (Lee et al., 2006) and mulberry tea (Wanyo et al., 2010). Many antioxidant

phenolic compounds in plants are most frequently present in a covalently bound form with insoluble polymers (Niwa and Miyachi, 1986; Peleg et al., 1991). If this bonding is not strong FIR treatment could liberate and activate low-molecular-weighted natural antioxidants in plants (Jeong et al., 2004; Lee et al., 2003).

6) Effect of volatile compounds

In this work, the extraction of the volatile compounds from kaprow was carried out following the headspace sampling and analysis by means of coupled GC–MS method. Twenty one volatile compounds were identified in the kaprow (Table 4.7). Methyl eugenol, and β-caryophyllene were typically found two varieties. The main compounds were β-caryophyllene followed by methyl eugenol and eugenol. Drying brought about an increase in the concentrations of many of the volatile components such as β-caryophyllene with HA, LRH and FIR-HA drying. Only kaprow khao contained eugenol as a major compound (20.54 %) while kaprow daeng had a high percentage of methyl eugenol all drying methods (42.51, 47.62 and 49.05 %, respectively), compared to the results for fresh leaves. Certain compounds have been observed to increase in different herbs after drying: for example, eugenol in bay leaf (Diaz-Maroto et al., 2002), thymol in thyme (Venskutonis, 1997), and some sesquiterpenes in different herbs (Baritaux et al., 1999; Yousif et al., 2000). Most studies have reported changes in colour and volatile compounds of the aromatic herbs after drying (Di Cesare et al., 2004; Diaz-Maroto et al., 2002). We found that eugenol was a major volatile compound ranging from 18% in HA dried to 23% in FIR-HA dried samples in kaprow khao while methyl eugenol was the maian volatile compound in kaprow daeng ranging from 35% in fresh to 49% in FIR-HA dried samples.Eugenol is the main component of kaprow grown in Bangladesh (Mondello et al., 2002), Germany (Laakso et al., 1990), Cuba (Pino et al., 1998), Northeastern Brazil (Machado *et al*., 1999), methyl eugenol from India (Kothari et al., 2004). Kaprow khao is an economic culinary herb in Thailand, containing unique aromatic flavor. The predominant volatile compound of kaprow was found to be methyl eugenol (Raseetha Vani et al., 2009). Methyl eugenol is a chemical compound used extensively in various application including perfumes, food seasoning and flavoring, aromatheraphy and medicinal application (Harborne and Baxter, 1993; Hopp and Mori, 1993; Laskar and Majumdar,

1998; Harris, 2002). Methyl eugenol also shows DNA binding activities, spasmolytic and gives muscle relaxant effects (Harborne and Baxter, 1993). Various physic-chemical changes of aromatic volatiles (oxidation, evaporation) may take during the drying process, influencing aroma intensity and the quality of the dried product (Barbieri *et al*., 2004). The effect of drying on the composition of volatile flavor constituents of various aromatic plants and vegetables has been the subject of numerous studies, which show that the changes in the concentrations of the volatile compounds during drying depend on several factors, such as the drying method and parameters that are characteristic of the product subjected to drying (Venskutonis, 1997).

4.3.3.2 Summary of experiment 3

The present study has demonstrated that different drying methods resulted in individual bioactive compound differently. The kaprow khao samples with LRH dried and FIR-HA dried had higher content of bioactive compounds and antioxidant activities, compared with fresh sample. FIR-HA gave highest content of eugenol, methyl eugenol and β-caryophyllene. Surprisingly, eugenol was only found in kaprow khao as a major compound (20.54 %) while kaprow daeng had a high percentage of methyl eugenol all drying methods (42.51, 47.62 and 49.05 %, respectively), compared to the results for fresh leaves. After drying, eugenol, methyl eugenol and β-caryophyllene were found to be significantly increased. Moreover, FIR-HA drying decreased the drying time without having any major effects on color, bioactive compounds, volatile compounds and antioxidant activity of the extracts. According to the results from our present study, LRH and FIR-HA should be considered as a suitable drying method for kaprow with respect to preserving its color, antioxidant properties and volatile compounds. The present study has provided useful information for industrial use of dried kaprow or kaprow powder production. Further application is to use kaprow powder as soup stock, curry paste and food additive. This could offer good opportunities for food industry in terms of food preservation and product development.

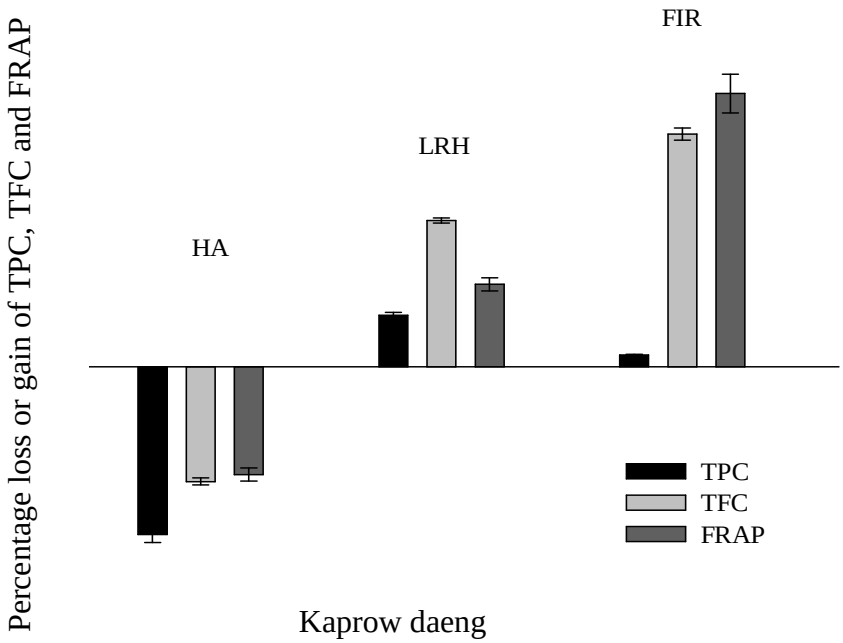


Figure 4.4 Percentage loss or gain of total phenolic content (TPC), total flavonoid content (TFC) and ferric reducing/antioxidant power (FRAP) for kaprow daeng (*Ocimum sanctum* L. cv. Daeng) samples after drying by hot-air (HA), low relative humidity air drying (LRH) and combined far- infrared radiation with hot-air convection drying (FIR-HA) compared to fresh leaves.

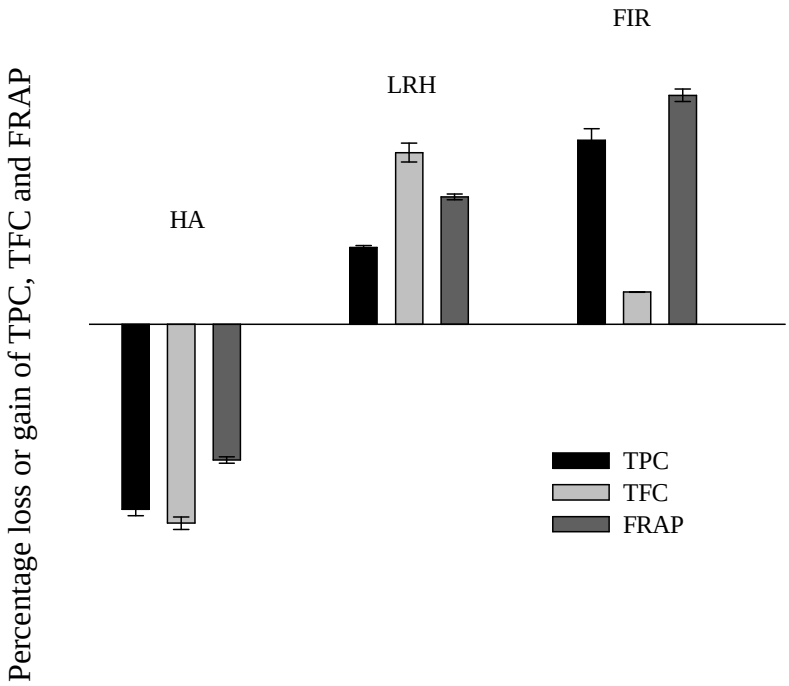


Figure 4.5 Percentage loss or gain of total phenolic content (TPC), total flavonoid content (TFC) and ferric reducing/antioxidant power (FRAP) for kaprow khao (*Ocimum sanctum* L. cv. Khao) samples after drying by hot-air (HA), low relative humidity air drying (LRH) and combined far- infrared radiation with hot-air convection drying (FIR-HA) compared to fresh leaves.

Table 4.6 Color parameters of kaprow of different drying method

Drying methods	%MC (dry basis)	Drying time	Color value				%MC (dry basis)	Drying time	Color value			
			Kaprow daeng						Kaprow khao			
			*L**	*a**	*b**	*ΔE**			*L**	*a**	*b**	*ΔE**
Fresh	80.37±0.31[a]	-	26.33±2.66[a]	3.54±0.03[a]	9.63±0.15[a]	-	85.26±0.42[a]	-	33.37±2.58[a]	7.04±0.06[a]	12.48±0.24[a]	-
HA	6.19±0.15[c]	15 hr	17.13±1.07[d]	0.68±0.04[c]	4.85±0.13[d]	10.75±0.29[a]	6.58±0.24[c]	16 hr	29.34±1.07[d]	2.02±0.01[c]	6.02±0.18[d]	9.11±0.21[a]
LRH	6.79±0.23[b]	9 hr	24.36±2.03[b]	1.28±0.06[b]	6.27±0.87[c]	4.50±0.12[b]	6.93±0.26[b]	9.5 hr	30.69±2.16[c]	3.00±0.05[b]	8.16±0.37[c]	6.49±0.16[b]
FIR-HA	6.86±0.18[d]	27 min	23.22±1.05[c]	0.19±0.02[d]	7.85±0.73[b]	4.90±0.16[b]	6.07±0.13[d]	30 min	31.36±1.22[b]	3.73±0.04[b]	9.74±0.76[b]	4.74±0.13[c]

Values are expressed as means ± standard deviation. Means with different letters in the same column were significantly different at the level $p < 0.05$.

4.3.4 Results and discussions of experiment 4

4.3.4.1 Effect of process and stored time of added kaprow khao powder in fish emulsion on antioxidant properties and lipid oxidation

In this study, we investigated kaprow khao powder of 1%, along with other synthetic antioxidants was added in fish emulsion. Evaluation of antioxidant properties and lipid oxidation of fish emulsion was performed.

Table 4.7 Sensory property of fish emulsion with added kaprow khao powder (0.5%, 1%, 1.5% and 2.0%)

Samples	Likeness score			
	Color	Taste	Texture	Overall acceptability
0.5%	7.04±0.67^{b}	6.34±0.48^{b}	6.85±0.42^{b}	6.74±0.56^{b}
1.0%	7.37±0.71^{a}	7.34±0.62^{a}	7.26±0.51^{a}	7.31±0.64^{a}
1.5%	7.42±0.68^{a}	6.53±0.55^{b}	6.42±0.46^{c}	6.52±0.57^{b}
2.0%	6.75±0.64^{b}	6.42±0.52^{b}	6.27±0.49^{c}	6.41±0.59^{b}

Values are expressed as means ± standard deviation. Means with different letters in the same column were significantly different at the level $p < 0.05$.

Table shows Sensory property of fish emulsion with added kaprow khao powder (0.5%, 1%, 1.5% and 2.0%). Based on the highest overall acceptability, kaprow khao powder (1%) was selected for further studies. Therefore, we selected added kaprow khao powder (1%) in fish emulsion (pla-yor) for study effect of process and stored time on antioxidant properties and lipid oxidation.

1) Effect of kaprow khao powder, BHT and vitamin E on color of fish emulsion.

Color expressed as *L*,a*, b* and ΔE** of fish emulsion added with holy basil (1.0%), vitamin E and BHT at day 0 of refrigerated storage is shown in Table 4.9. After addition of holy basil, BHT and vitamin E, there was change in *L** (lightness) values of the fish emulsion, compared to the control ($p < 0.05$). After addition of kaprow khao powder (1%), BHT and vitamin E resulted in the decrease in the *L** (lightness) and an increase in the *b**(yellowness). *ΔE** values of the kaprow khao

powder added samples were higher than those all samples, except for sample BHT (100 ppm).

Table 4.8 Color parameters *L**, *a** and *b** values of fish emulsion at day 0 of refrigerated storage.

Samples	*L**	*a**	*b**	ΔE^*
A	71.59±0.07[a]	1.89±0.03[b]	14.73±0.04[d]	2.24[c]
B	66.75±0.08[c]	-1.08±0.04[c]	19.99±0.08[b]	7.27[a]
C	70.74±0.06[b]	2.05±0.02[a]	15.55±0.07[d]	1.50[d]
D	69.78±0.07[b]	2.50±0.04[a]	18.16±0.08[c]	3.15[b]
E	69.50±0.08[b]	-1.74±0.02[d]	21.35±0.10[a]	7.04[a]

Values are expressed as means ± standard deviation. Means with different letters in the same column were significantly different at the level $p < 0.05$. A: control; B: kaprow khao powder (1%); C: vitamin E (200 ppm); D: BHT(200 ppm); E: combination of 1% kaprow khao powder, vitamin E (100 ppm) and BHT (100 ppm)

2) Proximate compositions of the fish emulsion with kaprow khao powder, BHT and vitamin E

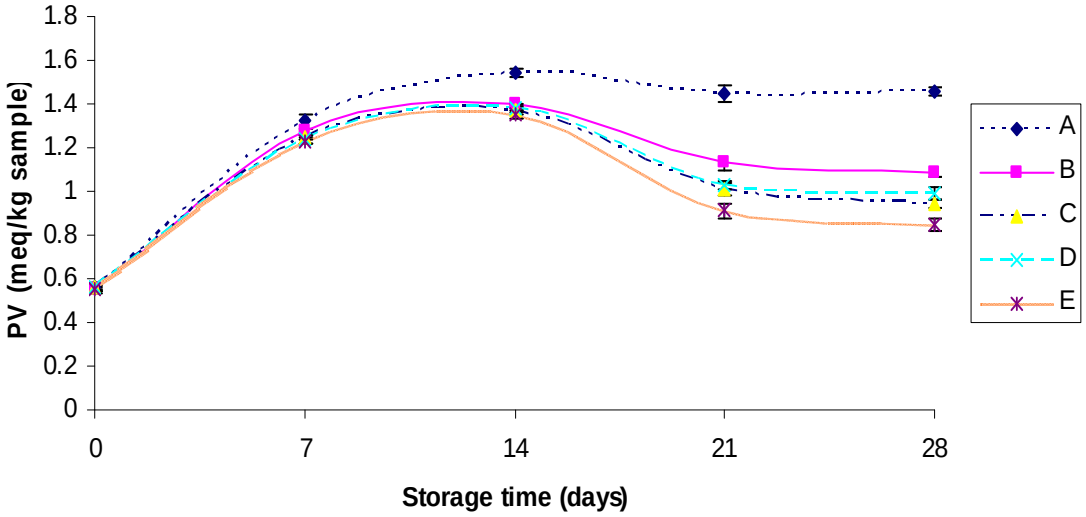
Proximate compositions of the fish emulsion with holy basil, BHT and vitamin E are shown in Table 4.10. The moisture content (71%-72%), protein (19-20 %), lipid (1%), carbohydrate (2-3%), ash (2%) and crude fiber (1%) of all samples.

3) Effect of kaprow khao powder, BHT and vitamin E on lipid oxidation in The oxidative process was evaluated by the peroxide value (PV) and thiobarbituric acid (TBA) in fish emulsion order to determine how it was affected by the different storage time. Impact of kaprow khao powder (1%) (B), vitamin E (200 ppm) (C), BHT (200 ppm) (D), and holy basil (1%), BHT (100 ppm) and vitamin E (100 ppm) (E) on lipid oxidation of fish emulsion during 28 days of refrigerated storage is shown in Figure 4.6. A sharp increase in PV was observed in all samples up to 7 days of refrigerated storage ($p < 0.05$) (Figure 4.6 (a)). However, decrease changes in PV

were found in sample B, C, D and E during 14 and 21 days ($p < 0.05$). Thereafter, the decrease in PV was found in those samples. The decrease in PV was most likely due to the decomposition of hydroperoxide formed into the secondary oxidation products (Boselli et al., 2005). During day 21–28 of storage, the constant PV was found in all sample. This was plausibly due to the similar rate of decomposition and formation of hydroperoxide in this sample.

TBARS values no changes in TBARS were found in all samples up to 14 days storage ($p > 0.05$) (Figure 4.6 (b)). At day 0, TBARS values of all sample ranged from 0.2 to 0.25 mg MAD/kg of sample, indicating that the lipid oxidation occurred during the processing and cooking of the fish emulsion. Control samples (A) showed the higher formation of TBARS throughout the storage of 28 days, compared with other samples ($p < 0.05$), which had the similar value during the 21– 28 days of storage ($p > 0.05$). The results showed that the combination of 1% kaprow powder, vitamin E (100 ppm) and BHT (100 ppm) showed the lowest lipid oxidation, compared to control. Especially at higher was therefore very effective in retarding the lipid oxidation. Apart from acting as a radical scavenger,could chelate iron, which might be released during cooking.

(a)



(b)

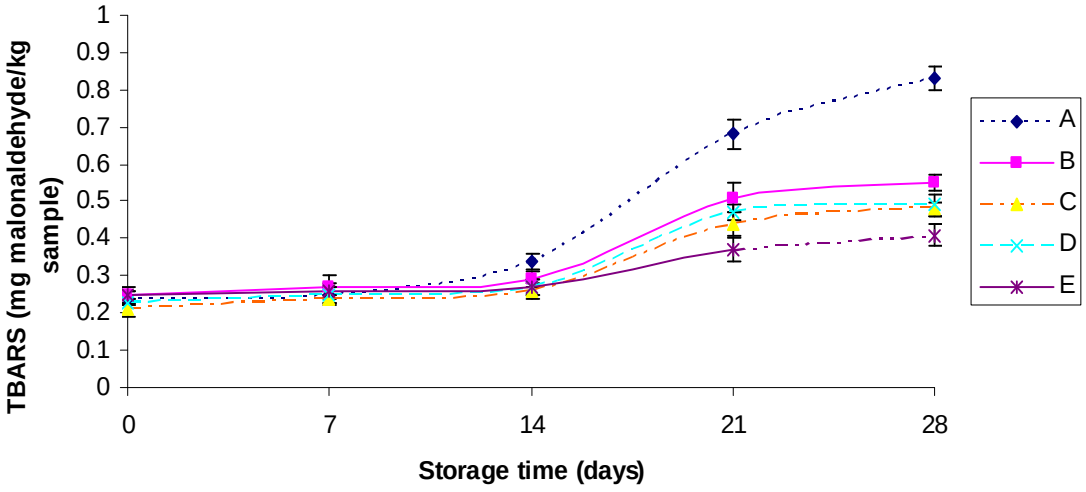


Figure 4.6 Peroxide value (PV) (a) and thiobarbituric acid-reactive substance (TBARS) (b) of fish emulsion during 28 days of refrigerated storage. Bars represents the standard deviation (n = 3). A: control; B: kaprow khao powder (1%); C: vitamin E (200 ppm); D: BHT(200 ppm); E: combination of 1% kaprow khao powder, vitamin E (100 ppm) and BHT (100 ppm)

4) Antioxidant activity

4.1) DPPH radical scavenging activity

The DPPH assay is a preliminary test to investigate the antioxidant potential of extracts. This assay has been widely used to test the free radical scavenging ability of various samples (Shimoji et al., 2002; Sakanaka et al., 2005). DPPH, a free radical compound, is a stable organic radical with a characteristic absorption at 517 nm; it was used to study the radical scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases. Antioxidants, on interaction with DPPH, either transfer an electron or hydrogen atom to DPPH, thus neutralising its free radical character (Naik et al., 2003). The color changes from purple to yellow and its absorbance at wavelength 517 nm decreases. The DPPH radical-scavenging activity (percentage inhibition) of fish emulsion after different storage time is given in Figure 4.7. In the test with DPPH radical, there was a decrease in antioxidant activity during 4 weeks of storage at 4 °C in fish emulsion with added kaprow khao powder (1%), BHT and vitamin E and with a combination of BHT and vitamin E.

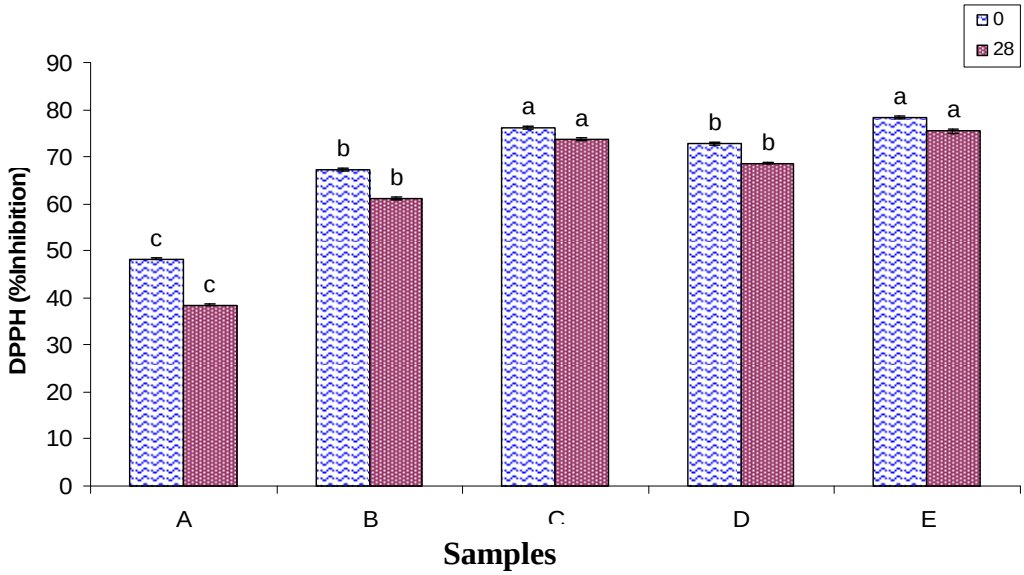


Figure 4.7 DPPH radical-scavenging activity of fish emulsion at day 0 and 28 of refrigerated storage. Different letters on different bars indicate significant differences between treatments ($P<0.05$). A: control; B: kaprow khao powder (1%); C: vitamin E (200 ppm); D: BHT(200 ppm); E: combination of 1% kaprow khao powder, vitamin E (100 ppm) and BHT (100 ppm)

4.2) Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex, producing a coloured ferrous tripyridyltriazine (Fe^{2+}–TPTZ) (Benzie and Strain, 1996). Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom (Gordon, 1990; Duh et al., 1999). According to Benzie and Strain (1996), the reduction of Fe^{3+}–TPTZ complex to blue colored Fe^{2+}–TPTZ occurs at low pH. In the test with FRAP, there was a decrease in antioxidant activity during 4 weeks of storage at 4 °C in fish emulsion with added kaprow khao powder (1%), BHT and vitamin E and with a combination of BHT and vitamin E. The ferric reducing power of fish emulsion after different storage times expressed as FRAP values (µmol $FeSO_4$/ 100 g) is shown in Figure 4.8. Natural antioxidant can be used to replace the synthetic antioxidant in the food industry such as BHT, BHA and vitamin, which may possess mutagenic activity (Skrinjar et al., 2007; Namiki, 1990).

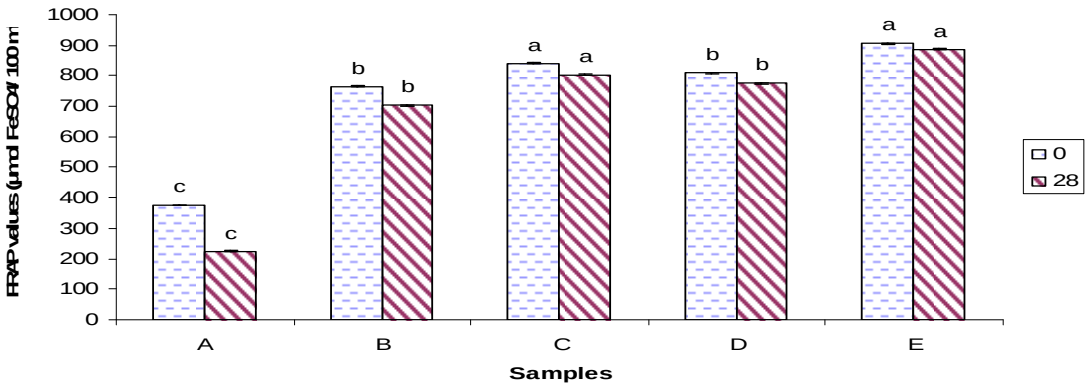


Figure 4.8 FRAP value of fish emulsion at day 0 and 28 of refrigerated storage. Values are expressed as means ± standard deviation. Different letters on / different bars indicate significant differences between treatments ($p<0.05$). A: control; B: fish emulsion with added holy basil (1%); C: fish emulsion with added vitamin E (200 ppm); D: fish emulsion with added BHT (200 ppm); E: fish emulsion with added holy basil (1%), vitamin E (100 ppm) and BHT(100 ppm).

5) Effect of kaprow khao powder, BHT and vitamin E on textural property of fish emulsion

Texture profile analysis of the emulsion added fish emulsion with added kaprow khao powder (1%), vitamin E (200 ppm) and BHT (200 ppm) and with a combination of 1% kaprow khao powder, vitamin E (100 ppm) and BHT (100 ppm) at day 0 of refrigerated storage is shown in Table 4.11. There was no difference in all textural parameters among all samples tested ($p > 0.05$).

6) Effect of kaprow khao powder, BHT and vitamin E on sensory property of fish emulsion

At the beginning of the storage, the similar texture and overall acceptability was found between the control and those treated with holy basil, BHT and vitamin E (Table 4.9). Hayes et al. (2010) reported that overall texture, tenderness and flavour in cooked pork sausage during storage at 4 °C were not significantly affected by the addition of lutein, ellagic acid and sesamol. Similarity tannic acid and ethanolic kiam wood extract (EKWE) can be incorporated into fish emulsion sausages without having any detrimental effect on the organoleptic quality of products (Maqsood et al., 2012).

Table 4.9 Sensory property of fish emulsion at day 0 of refrigerated storage

Samples	Likeness score			
	Color	Taste	Texture$^{(ns)}$	Overall acceptability$^{(ns)}$
Control	7.09±0.74^{b}	7.07±0.62^{b}	7.02±0.64	7.06±0.71
Holy basil (1%)	7.20±0.89^{a}	7.51±0.69^{a}	7.12±0.71	7.16±0.72
Vitamin E (200 ppm)	7.11±0.78^{b}	7.19±0.64^{b}	7.06±0.83	7.08±0.69
BHT(200 ppm)	7.07±0.87^{b}	7.07±0.59^{b}	7.10±0.86	7.12±0.74
Holy basil (1%), vitamin E (100 ppm) and BHT(100 ppm)	7.21±0.84^{a}	7.45±0.63^{a}	7.03±0.84	7.14±0.71

Values are expressed as means ± standard deviation. Means with different letters in the same column were significantly different at the level $p < 0.05$.The panelists evaluated each characteristic of the sample using a 9-point hedonic scale (n=30).

4.3.4.2 Summary of experiment 4

Evaluation of antioxidant properties and lipid oxidation of fish emulsion was performed. The results showed that the combination of 1% kaprow powder, vitamin E (100 ppm) and BHT (100 ppm) showed the highest antioxidant activities and lowest lipid oxidation, compared to control.

Table 4.10 Proximate compositions of fish emulsion at day 0 of refrigerated storage.

Samples	Composition (% by weight)					
	Moisture$^{(ns)}$	Protein$^{(ns)}$	Lipid$^{(ns)}$	Carbohydrate	Ash	Fiber
A	71.65±0.84	20.16±0.41	1.74±0.09	3.64±0.21^{a}	2.16±0.02^{a}	0.65±0.01^{b}
B	71.61±0.76	20.76±0.35	1.66±0.03	2.03±0.07^{b}	1.28±0.05^{b}	2.66±0.03^{b}
C	71.33±0.78	20.74±0.38	1.71±0.08	2.85±0.17^{b}	2.35±0.04^{a}	1.02±0.03^{a}
D	72.40±0.86	19.89±0.33	1.67±0.05	2.58±0.13^{b}	2.42±0.02^{a}	1.04±0.04^{b}
E	72.02±0.79	20.32±0.31	1.62±0.02	2.11±0.06^{b}	1.21±0.06^{b}	2.70±0.05^{a}

Values are expressed as means ± standard deviation. Means with different letters in the same column were significantly different at the level $p < 0.05$. A: control; B: fish emulsion with added holy basil (1%); C: fish emulsion with added vitamin E (200 ppm); D: fish emulsion with added BHT(200 ppm); E: fish emulsion with added holy basil (1%), vitamin E (100 ppm) and BHT(100 ppm).

Table 4.11 Textural properties of fish emulsion at day 0 of refrigerated storage

Samples	Hardness (N)	Springiness (cm)$^{(ns)}$	Cohesiveness (Ratio) $^{(ns)}$	Gumminess (N) $^{(ns)}$	Chewiness (N cm) $^{(ns)}$
A	34.37±0.14^{b}	0.79±0.18	0.49±0.15	19.35±0.36	17.14±0.36
B	35.68±0.27^{a}	0.81±0.17	0.50±0.17	20.17±0.38	18.82±0.38
C	34.85±0.18^{b}	0.80±0.19	0.49±0.14	19.78±0.33	18.21±0.35
D	35.94±0.21^{a}	0.81±0.21	0.52±0.18	20.65±0.37	19.47±0.39
E	36.27±0.25^{a}	0.82±0.22	0.51±0.16	20.42±0.34	19.21±0.31

Values are expressed as means ± standard deviation. Means with different letters in the same column were significantly different at the level $p < 0.05$. A: control; B: fish emulsion with added holy basil (1%); C: fish emulsion with added vitamin E (200 ppm); D: fish emulsion with added BHT(200 ppm); E: fish emulsion with added holy basil (1%), vitamin E (100 ppm) and BHT(100 ppm)

CHAPTER 5

CONCLUSIONS

In this study was to determine bioactive compounds and antioxidant activities of fourteen Thai herbs and spices varieties. In addition, monitoring changes in bioactive compounds, antioxidant activities and antitoxic activity of herbs and spices was also investigated. Study thermal process of three drying methods namely, hot-air drying (HA), low relative humidity air drying (LRH) and combined far- infrared radiation with hot-air convection drying (FIR-HA) drying. Finally, Kaprow khao powder of 1%, along with other synthetic antioxidants was added in fish emulsion. Evaluation of antioxidant properties and lipid oxidation of fish emulsion was performed.

The findings of the present thesis can be concluded as follows:

1. Thai herbs and spices are traditional foods in most cultures which play important roles in human nutrition. This study has demonstrated the informative results of phenolic compounds, antioxidant activity and volatile compounds. Thai herbs and spices are traditional foods in most cultures which play important roles in human nutrition. This study has demonstrated the informative results of phenolic compounds, antioxidant activity and volatile compounds. The main phenolic acids (hydrocinnamic acids) in these spices were ferulic acid, sinapic acid and *p*-coumaric acid. There were significant differences among different varieties tested. This study generated useful information for consumers and many encourage researchers to utilize herbs and spices as sources of bioactive compounds.

2. Crude kaprow khao extracts, which exhibited strong antioxidant activities, were found to contain high amounts of total and individual phenolics that may contribute to this activity. Antiprolificative of kaprow khao at 500 µg/ml was found to be 48% in Hepatoma cell line (HepG2) and 46% colon cancer cell line (HCT). The phytochemicals present in several herbal products and plants were have shown to potential as preventive or therapeutic agents against various human cancer.

3. The present study has demonstrated that different drying methods resulted in individual bioactive compound differently. The kaprow khao samples with LRH dried and FIR-HA dried had higher content of bioactive compounds and antioxidant

activities, compared with fresh sample. FIR-HA gave highest content of eugenol, methyl eugenol and β-caryophyllene. Surprisingly, eugenol was only found in kaprow khao as a major compound (20.54 %) while kaprow daeng had a high percentage of methyl eugenol all drying methods (42.51, 47.62 and 49.05 %, respectively), compared to the results for fresh leaves. After drying, eugenol, methyl eugenol and β-caryophyllene were found to be significantly increased. Moreover, FIR-HA drying decreased the drying time without having any major effects on color, bioactive compounds, volatile compounds and antioxidant activity of the extracts. According to the results from our present study, LRH and FIR-HA should be considered as a suitable drying method for kaprow with respect to preserving its colour, antioxidant properties and volatile compounds. The present study has provided useful information for industrial use of dried kaprow or kaprow powder production. Further application is to use kaprow powder as soup stock, curry paste and food additive. This could offer good opportunities for food industry in terms of food preservation and product development.

4. Kaprow khao powder of 1%, along with other synthetic antioxidants was added in fish emulsion. Evaluation of antioxidant properties and lipid oxidation of fish emulsion was performed. The results showed that the combination of 1% kaprow powder, vitamin E (100 ppm) and BHT (100 ppm) showed the highest antioxidant activities and lowest lipid oxidation, compared to control.

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Biography

Biography

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Research output

I. Raksakantong, P., Siriamornpun, S., Ratseewo, J. & Meeso, N.(2011). Optimized Drying of Kaprow Leaves for Industrial Production of Holy Basil Spice Powder. *Drying Technology*, 29, 974–983.

II. Raksakantong, P., Siriamornpun, S., & Meeso, N.(2011) Effect of drying methods on volatile compounds, fatty acids and antioxidant property of Thai kaffir lime (Citrus hystrix D.C.). International Journal of Food Science and Technology. *Inpress*