การต้านอ๊อกซิเดชั่นและการต้านจุลินทรีย์ของสารสกัดจากสมุนไพรและเครื่องเทศปรุงอาหารไทยและการประยุกต์ใช้ในผลิตภัณฑ์เนื้อสัตว์สับละเอียด

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF THAI CULINARY HERB AND SPICE EXTRACTS AND APPLICATION IN COMMINUTED MEAT PRODUCTS

Kanok-on Nugboon

A Thesis Submitted in Partial Fulfillment of the Requirements for the

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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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กนกอร นักบุญ : การต้านอ๊อกซิเดชั่นและการต้านจุลินทรีย์ของสารสกัดจากสมุนไพรและเครื่องเทศปรุงอาหารไทยและการประยุกต์ใช้ในผลิตภัณฑ์เนื้อสัตว์สับละเอียด (ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF THAI CULINARY HERBS AND SPICES AND APPLICATION IN COMMINUTED MEAT PRODUCTS) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.กนกอร อินทราพิเชฐ, 147 หน้า.

วัตถุประสงค์ของการศึกษานี้เพื่อคัดเลือกสมุนไพรและเครื่องเทศปรุงอาหารไทยที่มีฤทธิ์ต้านอ๊อกซิเดชั่นสูงที่สุด 4 ชนิดสำหรับใช้ในผลิตภัณฑ์เนื้อสัตว์สับละเอียดเพื่อตรวจสอบประสิทธิภาพการต้านอ๊อกซิเดชั่นและการต้านจุลินทรีย์ของสารสกัดจากสมุนไพรและเครื่องเทศไทยที่คัดเลือกได้ในผลิตภัณฑ์ลูกชิ้นหมูและเพื่อตรวจสอบความเสถียรของสารประกอบให้กลิ่นสำคัญของสารสกัดแต่ละชนิดในลูกชิ้นหมูระหว่างการเก็บรักษาที่อุณหภูมิเย็น

ปริมาณของฟีนอลทั้งหมด (total phenolic) ฟลาโวนอยด์ทั้งหมด (total flavonoid) และกิจกรรมการต้านอ๊อกซิเดชั่นวิเคราะด้วยวิธี การต้านอนุมูลอิสระพีพีเอช (DPPH radical scavenging) ความสามารถต้านอ๊อกซิเดชั่นด้วยการลดประจุของเฟอริค (ferric reducing antioxidant power; FRAP) และการทำปฏิกิริยากับกรดไธโอบาร์บิทูริค (thiobarbituric acid reactive substances; TBARS) ของเครื่องเทศและสมุนไพรปรุงอาหารไทยที่รวบรวมได้ 22 ชนิด พบว่าสารสกัดด้วยเอธานอล 4 ชนิดที่มีประสิทธิภาพต้านอ๊อกซิเดชั่นสูงตามลำดับประกอบด้วย สารสกัดจากกะเพรา ผักแพว เมล็ดพริกไทยอ่อน และขมิ้น

การทดสอบประสิทธิภาพการต้านจุลินทรีย์ของสารสกัดจากสมุนไพรและเครื่องเทศที่คัดเลือกได้ทั้ง 4 ชนิดกับแบคทีเรียตัวบ่งชี้จากสถาบันวิจัยวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย (วว.) และเชื้อแบคทีเรียที่มีปริมาณมากที่ได้จากลูกชิ้นหมูทางการค้าจากการสร้างวงใสในวุ้นเลี้ยงเชื้อที่เกิดขึ้น พบว่าสารสกัดจากกะเพรา สารสกัดจากพริกไทยอ่อน และสารสกัดจากผักแพวมีความสามารถยับยั้งการเจริญของแบคทีเรียแกรมบวกที่ใช้ทดสอบได้ 2 ชนิด และแบคทีเรียแกรมลบ 1 ชนิดตามลำดับ

การทดลองใช้สารสกัดจากสมุนไพรและเครื่องเทศที่คัดเลือกได้แต่ละชนิดผสมในมวลสับผสมของลูกชิ้น (0.2% w/w) บรรจุในไส้พลาสติกทำให้สุก แล้วทำให้เย็น ลอกไส้พลาสติกออกและตัดให้มีขนาดความยาว 2.5 ซม. แล้วทำการบรรจุลูกชิ้นหมูในบรรจุภัณฑ์แบบมีอากาศปกติ (aerobic) และแบบสุญญากาศ (vacuum) เก็บไว้ที่อุณหภูมิ 4°C เป็นเวลา 9 วันทำการวิเคราะห์ปริมาณจุลินทรีย์และประสิทธิภาพการต้านอ๊อกซิเดชั่นของสารสกัดจากสมุนไพรและเครื่องเทศในตัวอย่างลูกชิ้นหมูทุก 3 วันพบว่า ค่า TBARS และปริมาณ hexanal ของลูกชิ้นหมูที่เติมด้วยสารสกัด

เครื่องเทศต่ำกว่า ($p<0.05$) ตัวอย่างลูกชิ้นควบคุม และพบว่าสารสกัดจากกะเพรา ผักแพว และเมล็ดพริกไทยอ่อนมีประสิทธิภาพต้านอ๊อกซิเดชั่นได้ดีกว่า ($p<0.05$) สารสกัดจากขมิ้นในลูกชิ้นที่บรรจุทั้งแบบมีอากาศปกติและแบบสุญญากาศ ตามข้อกำหนดขององค์การอาหารและยาของไทย มาตรฐานปริมาณจุลินทรีย์ต้องมีไม่เกิน 5.0 log cfu/g ของตัวอย่างอาหารพบว่าสารสกัดจากขมิ้นและผักแพวที่เติมในลูกชิ้นหมูทำให้สามารถเก็บได้นาน 6 วัน และ 9 วัน สำหรับการบรรจุแบบมีอากาศปกติและแบบสุญญากาศตามลำดับ สามารถเก็บได้นานกว่าตัวอย่างควบคุมทั้งในสองสภาวะการบรรจุ ($p<0.05$) ซึ่งเก็บได้น้อยกว่า 6 วันสำหรับค่า Aw ของตัวอย่างลูกชิ้นหมูทั้งหมดไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) และผลการประเมินทางประสาทสัมผัสพบว่าลูกชิ้นหมูที่เติมสารสกัดจากสมุนไพรและเครื่องเทศที่คัดเลือกได้ทั้ง 4 ชนิดมีคะแนนคุณลักษณะด้านกลิ่นออกซิไดซ์ต่ำ ($p<0.05$)

การลดลงของสารประกอบกลิ่นรสสำคัญของสารสกัดสมุนไพรและเครื่องเทศที่คัดเลือกได้ทั้ง 4 ชนิดในตัวอย่างลูกชิ้นหมู ซึ่งวิเคราะห์ด้วยวิธี headspace-gas chromatography และ gas chromatography-mass spectrometry พบอย่างชัดเจนว่าการลดลงของสารกลิ่นรสสำคัญของสารสกัดเครื่องเทศและสมุนไพรในลูกชิ้นบรรจุแบบสุญญากาศต่ำกว่า ($p<0.05$) เมื่อเปรียบเทียบกับการบรรจุแบบมีอากาศปกติ

สาขาวิชาเทคโนโลยีอาหาร ลายมือชื่อนักศึกษา_____

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KANOK-ON NUGBOON : ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF THAI CULINARY HERBS AND SPICES AND APPLICATION IN COMMINUTED MEAT PRODUCTS.
THESIS ADVISOR : ASSOC. PROF. KANOK-ORN INTARAPICHET, Ph.D., 147 PP.

CULINARY HERBS AND SPICES/ANTIOXIDANTACTIVITIES/ ANTIMICROBIAL ACTIVITIES/AROMA-IMPACT COMPOUND STABILITY

The objectives of this study were to screen Thai culinary herbs and spices with high antioxidant activity and to select four of them with the highest activity to be used in comminuted meat products, to investigate antioxidant and antimicrobial efficacy of selected herb and spice extracts used in pork meatballs and to monitor aroma-impact compound stability of each extract in the meatball products during cold storage. The total phenolic and total flavonoid contents and antioxidant activities were determined using DPPH radical scavenging, ferric reducing antioxidant power and thiobarbituric acid reactive substances (TBARS) methods. Out of twenty-two herbs and spices used, four ethanolic extracts were found to contain high antioxidant, which were in order of holy basil, Vietnamese coriander, green peppercorn and turmeric. The antimicrobial properties of the four herb extracts selected were tested against indicator bacteria obtained from the Thailand Institute of Scientific and Technological Research and dominant flora obtained from commercial pork meatballs. Growth of two Gram-positive and one Gram-negative bacteria were inhibited by holy basil and Vietnamese coriander extract, respectively. Individual selected extract was mixed in pork meatball

batter (0.2%, w/w), the batter was stuffed in plastic casing, cooked, cooled and cut into 2.5 cm length, aerobically and vacuum packed, and stored at 4°C for 9 days. The microbial contents and antioxidant efficacy of the extracts in the meatballs were determined every 3 days. The TBARS values and hexanal contents of the meatballs with added herb and spice extracts were lower ($p<0.05$) than those of control samples. Holy basil, Vietnamese coriander and green peppercorn extracts gave a higher ($p>0.5$) antioxidant efficacy than turmeric extract in both aerobically and vacuum packed meatballs. According to the Thai FDA microbial standard count of $\leq$ 5.0 log cfu/g food, the meatballs with added Vietnamese coriander and turmeric extracts could be kept for at least 6 days and 9 days for aerobically and vacuum packed meatballs, respectively, while the control samples could be kept less than 6 days in both packaging conditions. The Aw values of all meatballs were not significantly different ($p>0.05$). The meatballs with the four selected herb and spice extracts had the lowest sensory scores for oxidized flavor attribute.

A decrease in key aroma-impact compounds of all four selected herb and spice extracts in pork meatballs determined by static headspace GC and GC-MS was clearly lower ($p<0.05$) in vacuum packed condition compared with aerobically packed condition

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LIST OF ABBREVIATIONS

ATP	=	Adenosine triphosphates
°C	=	Degree Celsius
Da	=	Dalton
DPPH	=	2,2-diphenyl-1-picryl-hydrazyl radical
IC_{50}	=	The concentration of extract necessary to decrease antioxidant activity by 50%
FRAP	=	Ferric reducing antioxidant power
Fe	=	Iron
$FeCl_3$	=	Ferric chloride
HCl	=	Hydrochloric acid
NaOH	=	Sodium Hydroxide
hr	=	hour
µg	=	microgram
mg	=	milligram
g	=	gram
kg	=	kilogram
M	=	molar
min	=	minute
mL	=	milliliter
mm	=	millimeter

LIST OF ABBREVIATIONS (Continued)

mM	=	millimolar
MW	=	molecular weight
N	=	normal
ppm	=	part per million
L	=	Liter
i.e.	=	That is
e.g.	=	For example
x g	=	Gravitational acceleration
cfu	=	Colony forming unit
pp	=	Page
v/v	=	Volume : volume
w/v	=	Weight : volume
w/w	=	Weight : weight
%	=	Percent

CHAPTER I

INTRODUCTION

1.1 Introduction

Finely comminuted meat products such as pork, beef and chicken meatballs are traditional meat products with a high consumption volume in Thailand. Normally, the meatball is a meat product made of ground meat, flour, ice and seasoning, formed into a ball and then cooked in hot water at about 70-75°C. Lipid oxidation and bacterial contamination are the main factors that determine quality and shelf-life reduction of the meatball products. Therefore, delaying lipid oxidation and preventing bacterial cross-contamination are highly relevant to food processors. The growth of microorganisms in meat products may cause spoilage or food borne diseases. Oxidative processes in meat lead to the degradation of lipids and proteins, imposing an adverse effect on flavor, color and texture as well as nutritional value (Byrne et al., 2001).

The addition of antioxidants is required to preserve food quality. Although synthetic additives have been widely used in food industry to inhibit both, the process of lipid oxidation and microbial growth, the trend is to decrease their use because of the growing concern among consumers about such chemical additives. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) are widely used as antioxidants in the food industry. Their safety, however, has been questioned. BHA

was shown to be carcinogenic in animal experiments. At high doses, BHT may cause internal and external hemorrhaging, which contributes to death in some strain of mice and guinea pigs. This effect is due to the ability of BHT to reduce vitamin K-depending blood-clotting factor (Ito et al., 1986).

Antimicrobial agents are commonly called chemical preservatives and function by controlling the growth of bacteria, yeast and mold in the various food system by extending product shelf life. Chemical preservatives such as potassium sorbate and sodium benzoate have been used to extend the shelf life of processed meats (Choi and Chin, 2003). Sorbic acid and its salts are added to numerous food products. In the meat industry, sorbates are common inhibitors of mold growth in sausage casings. Nitrites are used in meat products for curing, because of their effects on color as well as their antibotulinic activity (Binstok, Campos, Varela and Gerschenson, 1998). Despite the benefits attributed to food additive, for several years there have also been number of concerns regarding the potential short term and long term risks of consuming these substances. Short term acute effects from additives are unlikely. Cancer and reproductive problems resulting from the long term consumption (Branen, Davidson and Salminen, 1990).

Many natural plant extracts contain primarily phenolic compounds which are potent antioxidants (Wong, Hashimoto and Shibamoto, 1995). Some phenolic compounds such as sage, rosemary, thyme, hops, coriander, tea, cloves and basil are known to process antimicrobial effects against foodborne pathogens (Elgayyar, Draughon, Golden and Mount, 2001). Phenols are one of the most important groups of natural antioxidants. They occur only in material of plant origin and they are known to easily protect oxidizable constituents of food from oxidation (Wang, Cao and Prior, 1996).

Therefore, replacing synthetic antioxidants and antimicrobial by natural ingredients from herbs and spices have been increasing awareness of consumers regarding the safety of food additives. Currently, there are many on-going investigations of using natural antioxidants and antimicrobials as the alternative sources for food protection. Thai herbs and spices have been used in cooking for their flavor and fragrance added to many food dishes and shown to have both antioxidant and microbial activities. However, researches on the use of Thai herbs and spices in meat and meat products are limited and still needed more investigations to cover more types of herbs and spices including their effects on product qualities and characteristics. Therefore, investigation of using Thai herbs and spices in meat and meat products in order to gain some information concerning their abilities to retard oxidation and microbial growth including the effects on some physicochemical and sensory properties of the meat products would be very much of interesting. For this research experiment, pork meatballs would be used as a meat product model for the study.

1.2 Research objectives

The objectives of this research are:

1) To screen some of Thai herbs and spices extracts for their potential antioxidant and antimicrobial activities for the use in meat products.
2) To investigate the antioxidant and antimicrobial activities of selected Thai herbs and spices extracts and their effects on some physicochemical and flavor characteristics of finely comminuted meat products. Low fat pork meatballs will be used as the meat product model for this study.

1.3 Research hypothesis

The ethanolic extracts from herbs and spices have an effectiveness of natural antioxidant and antimicrobial as inhibitors of lipid oxidation and spoilage microorganisms in meat products. In addition, the use of herbs and spices extracts in meat products provide better physicochemical and flavor characteristics to the meat products.

1.4 Expected results

The satisfied results of application of some common Thai herb and spice extracts in meat products in the aspects of shelf life extension, some physicochemical properties and sensory characteristics would be obtained. The results from this investigation would lead to fully utilize herbs and spices as a natural antimicrobial and antioxidant in traditional meat products in particularly those are commonly consumed in daily life of Thai consumers.

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CHAPTER II

LITERATURE REVIEWS

2.1 Herbs and spices

Herbs are most often defined as any part of plant that is used in the diet for its aromatic properties. Spices are dried herbs, and condiments are spices and other flavorings add to food at the table (Davison, 1999). Many herbs and spices are usually used in food to contribute many functions to food products including aroma, taste, flavor such as allspice, cinnamon, basil, dill, nutmeg, fennel, parsley, anise, marjoram, cumin, mint, cardamom, mace and tarragon; color such as turmeric, paprika and saffron; masking such as garlic, clove, rosemary, onion, bay leaves, thyme, sage, coriander, caraway and oregano and pungency such as pepper, red pepper, mustard, horseradish and ginger. They are an excellent source of phenolic compounds which have been reported to show good antioxidant activity (Zheng and Wang, 2001). Therefore, they may serve as natural food preservatives. However, herbs and spices usually contain essential oils which show antioxidant activity but also carry flavor (Teissedre and Waterhouse, 2000). It has been shown that herbs and spices including their extracts are useful for reduction of microbial growth and oxidation activity associated with food and food products. In order to promote the use of herbs and spices as potential sources of antimicrobial and antioxidant compounds, it is pertinent to thoroughly investigate their composition and activity and thus, also validate their use. Some phytochemicals produced by plants have antimicrobial activity allowing

these plants to be studied and used for development of new preservative while some of them have a better activity of antioxidants only and some may show both activities (Kim, Cho and Han, 2013). Some local Thai vegetables, which are considered as herbs and spices, were reported to possess either antimicrobial or antioxidant activity or both activity as shown in Table 2.1 (Nanasombat and Techchuen, 2009). Out of 20 plant species, only 5 of them had antimicrobial activity against some microbial strains, especially *Bacillus cereus* and *Staphylococcus aureus*. However, the composition of extracts from the same plant depends on the method of extraction and the properties of the extraction solvent used. Differences of solvent concentration may produce extracts with different effects. Depending on the chemical characteristics of extraction solvents, only certain molecules can be extracted. The efficacy is also different between purified and unpurified extracts. With certain solvent, the unpurified extracts contain a number of different molecules, while the purified extracts contain only one active component. The purified active molecules extracted from plants can be sometimes substituted by synthetic naturally identical molecules. Mainly, plants contain one or more predominant active molecules, which are responsible for certain biological effects. The amount of these molecules varies depending on the variety of plant, growing conditions, harvest times etc. Essential oils of the extract are very potent molecules and must be used in small quantities (Frankič, Voljč, Salobir and Rezar, 2009).

Table 2.1 Antimicrobial and antioxidant of extracts of Thai local vegetable extract.

Botanical names	**Common names/ Thai names**	**Antioxidant, EC_{50} (μg/mg DPPH)**	**Antimicrobial (mm of clear zone)***
Acacia pennata subsp. *insuavia*	Thorny tree/ *Cha-Om*	3,565.7	8.5 / -
Anethum graveolens Linn.	Dill/ *Phak-Chee-Laao*	2,297.8	-
Cassia siamea Britt.	Cassod Tree/ *Khi-Leck*	349.9	9.3 / -
Centella asiatica (Linn.) Urban	Urban Asiatic pennywort/ *Boa-Bok*	6,080.0	-
Coccinia grandis (l.) Voigt.	Ivy gourd/ *Tum-Lueng*	6,640.2	-
Coriandrum spp.	*Hom-Yae*	1,868.8	-
Diplazium esculentum (Retz.) Sw.	Paco/ *Phak-Good*	3,353.2	-
Eryngium foetidum Linn.	Garden parsley/ *Phak-Chee-Farang*	4,739.7	-
Garcinia cowa Roxb.	*Cha-Muang*	1,597.5	20.2 / 10.2
Lasia spinosa Thw.	*Phak-Nam*	3,105.6	-
Limnophila aromatica Merr.	Rice paddy herb/ *Phak- Ka-Yeang*	550.5	21.0 ± 5.2
Morinda citrifolia Linn.	Noni, Indian Mulberry/ *Yau*	4,230.8	-
Momordica charantia Linn.	Bitter cucumber/ *Ma-Ra-Khi-Nok*	990.5	-
Ocinum americanum Linn.	Lemon basil/ *Meang-Luck*	1,817.3	-
Ocimum gratissimum Linn.	Tree basil, Shrubby basil / *Yee-Ra*	3,321.9	-
Sesbania grandiflora Desv.	Vegetable Humming Bird/ *Kae*	13,425.9	-
Polygonum odoratum Lour.	Vietnamese coriander/ *Phak-Paew*	315.4	15.3 / 11.2
Sesbania javanica Miq.	Sesbania flower/ *Sa-No*	2,265.6	-

Table 2.1 (Continued)

Botanical names	**Common names/ Thai names**	**Antioxidant, EC_{50} (μg/mg DPPH)**	**Antimicrobial (mm of clear zone)***
Spilanthes acmella Murr.	Para cress/ *Phak-Krad*	4,963.7	-
Tiliacora triandra Diels.	Bamboo grass/ *Ya-Nang*	3,903.9	-
α-Tocopherol		322.4	-
Penicillin G			17.7 /14.2
Amphotericin B			-

* against *Bacillus cereus* / *Staphylococcus aureus*

- No inhibition observed.

Compiled from Nanasombat and Teckchuen, 2009.

2.1.1 Antimicrobial property of herbs and spices

Plants, including herbs and spices, contain products of secondary metabolism such as phenolics, phenolic acids, quinines, flavonoids, and tannins. Many of these phytochemicals are rich source of antioxidants and provide defense mechanisms to plants against infectious organisms and insects. Many studies have reported a high correlation between antimicrobial efficacy and the level of phenolic components present in certain herb and spice extracts (Salawu, Ogundare, Ola-Salawu and Akindahunsi, 2011; Nitiema, Savadogo, Simpore, Dianou and Traore, 2012; Alves et al., 2013). The possible modes of action for pheonolics as antimicrobial agents have been reported. The effect of phenolic compounds can be concentration dependent. At low concentration, phenols affect enzyme activity, particularly those associated with energy production, while at high concentration, they cause protein denaturation. The antimicrobial effect of phenolic compounds may be due to their ability to alter microbial cell permeability, permitting the loss of macromolecules from the cell. They could also interfere with membrane function such as electron transport, nutrient

uptake, protein, nucleic acid synthesis and enzyme activity, and interact with membrane proteins, causing deformation in structure and functionality (Cox et al., 2000; Dorman and Deans, 2000; Trombetta et al., 2005; Bajpai, Lesperance, Kim and Terskikh, 2008).

Recently, some herbs and spices are reported to available on the preservative and antimicrobial role of herbs, spices and their oils and role of various components of essential oils in the prevention of spoilage in food. The inhibitory effects of spices are mostly due to the volatile oils present in their composition (Sethi and Meena, 1997). The main factors that determine the antimicrobial activity are the type and composition of the herbs and spices, amount used, type of microorganisms, composition of the food, pH value, temperature of the environment, proteins, lipids, salts and phenolic substances present in the food environment (Sağdıç, Yetim and Yilmaz, 2003). Among groups of bacteria, Gram-negative bacteria are more resistant to the antibacterial activity of essential oils than Gram-positive bacteria (Ouattara, Simard, Holley, Piette and Begin, 1997; Tajkarimi, Ibrahim and Cliver, 2010). Briefly, fundamental differences in ultrastructure of the cell wall are responsible for the reaction (+ or -) of bacteria towards the Gram stain. In both types of cell, the *cytoplasmic membrane* is surrounded and supported by a cell wall, which provides strength, rigidity and shape. Schematic cross sections of these structures are given below (Figure 2.1) (Cabeen and Jacob-Wagner, 2005).

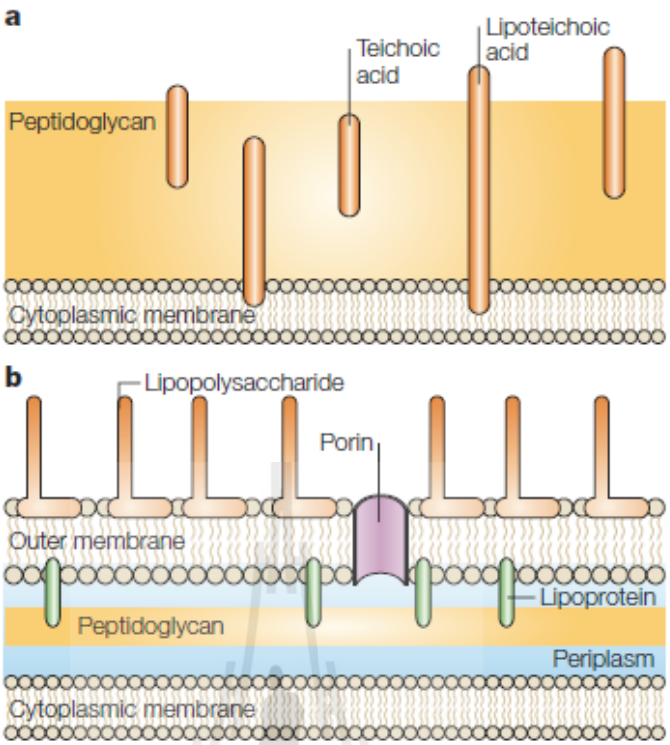


Figure 2.1 Gram-positive (a) and Gram-negative (b) cell wall. (Cabeen and Jacob-Wagner, 2005).

a. The Gram-positive cell wall is composed of a thick, multilayered peptidoglycan sheath outside of the cytoplasmic membrane. Teichoic acids are kinked to and embedded in the peptidoglycan, and lipoteichoics extend into the cytoplasmic membrane.

b. The Gram-negative cell wall is composed of an outer membrane lined by lipoproteins to thin, mainly single-layered peptidoglycan. The peptidoglycan is located within the periplasmic space that is created between the outer and inner membranes. The outer membrane includes porins, which allow the passage of small hydrophic molecules across the membrane, and lippolysaccharide molecules that extend into extracellular space.

- Gram-positive:

• Relatively thick and featureless (electron microscope)

• Major component (~50%) is peptidoglycan

• No lipid and often no protein

• Accessory polymers (*teichoic acid* and/or *teichuronic acid*) covalently linked to peptidoglycan

- Gram-negative:

• The cell envelope consists of a *pair* of membranes (*cytoplasmic* and *outer*) with a thin, intermediate layer of peptidoglycan

• The outer membrane contains lipopolysaccharide (LPS) as well as *lipids* and *proteins*. LPS is located exclusively in the outer leaflet: lipid embedded in the membrane, polysaccharide protruding. This makes the bacteria appear rather fuzzy under an electron microscope.

Essential oils (EOs) (also called volatile or ethereal oils are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, enfleurage or extraction but the method of steam distillation is most commonly used for commercial production of EOs (Burt, 2004). EOs possesses a wide spectrum of different impressive qualities including antiphlogistic, spasmolytic, antinociceptive and antioxidant activity. Moreover, they exert immunomodulant, psychotropic, acaricide and expectorant effects (Lang and Buchbauer, 2011). In addition, EOs shows significant antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria. The use of EOs as biopreservatives is a matter of great interest for the food industry since consumers prefer natural additives instead of synthetic ones.

That is why many studies have been performed on this subject in the last few years. Considering the large number of different groups of chemical compounds present in EOs, it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell. The locations or mechanisms in the bacterial cell thought to be sites of action for EO components are indicated in Figure 2.2, including degradation of the cell wall, damage to cytoplasmic membrane, damage to membrane proteins, leakage of cell contents, coagulation of cytoplasm and depletion of the proton motive force Not all of these mechanisms are separate targets; some are affected as a consequence of another mechanism being targeted (Burt, 2004). An important characteristic of EOs and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable (Burt, 2004; Tajkarimi et al. (2010). Leakage of ions and other cell contents can then occur. Although a certain amount of leakage from bacterial cells may be tolerated without loss of viability, extensive loss of cell contents or the exit of critical molecules and ions will lead to death.

Generally, the EOs possessing the strongest antibacterial properties against food borne pathogens contain a high percentage of phenolic compounds, disturbing the cytoplasmic membrane, disrupting the proton motive force (PMF), electron flow, active transport and coagulation of cell contents. The chemical structure of the individual EO components affects their precise mode of action and antibacterial activity due to the presence of the hydroxyl group in phenolic compounds. EO components may act on the binding sites of proteins. Components of EO also appear to act on cell proteins embedded in the cytoplasmic membrane.

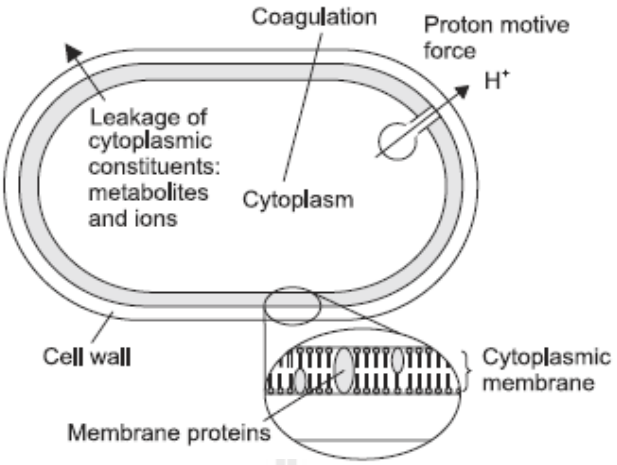


Figure 2.2 Locations and mechanisms in the bacterial cell thought to be sites of action for EO components (Burt, 2004).

Enzymes such as ATPases are known to be located in the cytoplasmic membrane and to be bordered by lipid molecules. Two possible mechanisms have been suggested whereby cyclic hydrocarbons could act on these. Lipophilic hydrocarbon molecules could accumulate in the lipid bilayer and distort the lipid-protein interaction; alternatively, direct interaction of the lipophilic compounds with hydrophobic parts of the protein is possible (Hyldgaard, Mygind, and Meyer, 2012).

Several researches on herbs and spices and their essential oils, the antimicrobial activity of various constituents found in these spices has also been shown to exhibit the strongest antimicrobial activity. Eugenol is the major essential oil component in clove, cinnamon, allspice and basil (Davidson, 1997). Several reports have shown the antimicrobial effect of eugenol against several species of bacteria, such as *Listeria monocytogenes*, *Bacillus cereus*, *Campylobacter jejuni*, *Escherichia coli* 0157:H7, *Salmonella* spp, *Aeromonas hydrophila* and *Enterobacter aerogenes* (Friedman, Henika, and Mandrell, 2002) and fungi such as *Aspergillus* spp. and

Penicillium spp. (Vazquez, Fente, Franco, Vazquez and Cepeda, 2001). Anetol, the major volatile compound of anise seed and thymol which contain in thyme, have been shown to have inhibitory activities against *Aspergillus* spp. and against aflatoxin production (Hirasa and Takemasa, 1998). The inhibitory effects on microbial growth are also reported. The inhibitory effect of cumin on *Micrococcus luteus*, *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* (Çon, Ayar and Gökalp, 1998), cinnamon on *E. coli* and *Klebsiella pneumoniae* (Ouattara, Simard, Holley, Piette and Begin, 1997), cloves on *Candida albicans*, *Enterococcus faecalis*, *E. coli*, *K. pneumoniae*, *M. luteus*, *P. aeruginosa* (Hammer, Carson and Riley, 1999), and *S. aureus* (Nkanga and Uraih, 1981) were reported. Allicin, one of the active principal of garlic was found to inhibit *E. coli*, *C. albicans*, *Entamoeba histolytica* and *Giardia lamblia* (Ankri and Mirelman, 1999). In addition, comparing antimicrobial effects of four garlic-derived organosulfur compounds, i.e., diallyl sulfide (DAS), diallyl disulfide (DADS), *s*-ethyl cysteine (SEC) and *n*-acetyl cysteine (NAC), in ground beef, Yin and Cheng (2003) found that only DAS and DADS showed antimicrobial activity against *Salmonella typhimurium* DT 104, *Escherichia coli* O157:H7, *Listeria moncytogenes*, *Staphyllococcus aureus* and *Campylbacter jejuni*, but not SEC and NAC. The major constituents of garlic and onion are organosulfur-containing compounds. i.e., cysteine sulfoxides, however, it was reported that essential oil extract of garlic showed stronger bacterial inhibition than of onion essential oil extract due to garlic contains nearly three times as much sulfur-containing compounds as onion (Benkeblia, 2004).

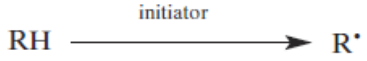
2.1.2 Antioxidant properties

Oxidation in food is one of the major causes of food quality deterioration and

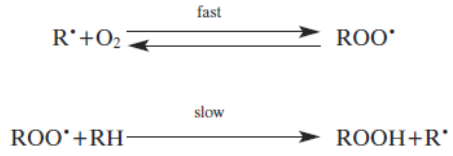
decreases consumer acceptability of foods by producing low-molecular weight off-flavor compounds as well as by destroying essential nutrients. Toxic compounds and dimmers or polymers of lipids and proteins can also be produced, which may be detrimental to the health of consumers (Wasowicz et al., 2004; Choe and Min, 2009). Lipid oxidation is rather complex whereby unsaturated fatty acids reacting with molecular oxygen via a free radical chin mechanism, form fatty acyl hydroperoxides, generally called peroxides or primary products of the oxidation. The primary auto-oxidation is followed by a series of secondary reactions which lead to the degradation of the lipid and the development of oxidative rancidity due to the breakdown products causing rancidity, include complex of aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones (Frankel, 1984; Ladikos and Lougovois, 1990; Min and Ahn, 2005).

There are three different mechanisms, yielding different oxidation products; a free radical mechanism, photo-oxidation and lipoxygenase activity. The first mechanism is an autoxidation due to the reaction of molecular oxygen with lipids, leading to oxidative deterioration. It proceeds by a free radical chain mechanism involving three steps (Wasowicz et al., 2004; Kumar, 2011; Brewer, 2011).

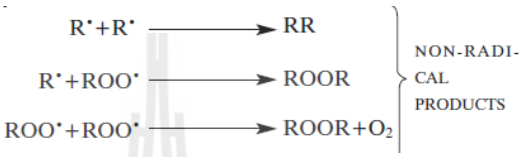
(1) *Initiation step* – homolytic hydrogen atom abstraction from a methylene group that leads to alkyl radical ($R^{\bullet}$) formation;



(2) *Propagation step* – formation of peroxy radicals (ROO•) able to react with unsaturated fatty acids and form hydroperoxides (ROOH);



(3) *Termination step* – formation of non-radical products by interaction of R• and ROO• where: R• – fatty acid radical; ROOH



where: R• – fatty acid radical; ROOH–fatty acid hydroperoxide; ROO• – peroxy radical.

Hydroperoxides, the primary oxidation products, are unstable and easily decompose involving monomolecular or bimolecular reactions. Decomposition products – peroxy and alkoxyl radicals–are highly reactive and may act as initiators of autoxidation. Hydroperoxides formed at the initial stage of autoxidation are non-volatile, odorless and relatively unstable compounds. They decompose to form volatile aromatic compounds, which are perceived as off-flavors and food is no longer edible (Gordon, 2001).

Another mechanism of oxidation occurs in the presence of sensitizer and UV-light. Photo-oxidation pathway is an alternative route leading to the formation of hydroperoxides instead of the free radical mechanism. Excitation of unsaturated fatty acid or oxygen may occur in the presence of light and a sensitizer. There are two types of photo-oxidation (Gordon, 2001): 1) an electron or a hydrogen atom transfers between an excited triplet sensitizer and a sub- strate (PUFA), producing free radicals

or radical ions; and 2) triplet oxygen ($^3O_2$) can be excited by light to singlet oxygen ($^1O_2$), which reacts with the double bond of unsaturated fatty acids, producing an allylic hydroperoxide. This reaction results in a formation of a *trans* configuration. Products of oleate oxidation are 9- nd 10-hydroperoxides, linoleate produces a mixture of 9- 10-(*trans*, *cis*), 12- 3-(*cis*, *trans*) isomers.

The third mechanism of oxidation is based on lipoxygenase activity. Lipoxygenase is an enzyme which is a very important source of hydroperoxides formed during oil extraction. Lipoxygenase produces similar flavour volatiles to those produced during autoxidation. A molecule of lipoxygenase contains an iron atom, which is in high spin state Fe (II) and must be oxidized to Fe (III) by fatty acid hydroperoxides or hydrogen peroxide. The active enzyme abstracts a hydrogen atom from the methylene group of a polyunsaturated fatty acid with the iron being reduced to Fe (II) (Gordon, 2001). A conjugated diene system is formed, followed by reaction with oxygen. Peroxyl radical and finally hydroperoxide are generated. The second type of enzyme reacts with an esterified substrate, before the release of fatty acids by lipase, additionally ketodiene fatty acids are formed.

The fatty acids in the lipids of food tissues may be saturated or unsaturated and may be part of the neutral triglyceride fraction (triacylglycerol) or part of the phospholipid fraction. Free fatty acids are electron-deficient at the oxygen atom of the carbonyl group (C=O); unsaturated fatty acids are also electron-deficient at points of carbon–carbon unsaturation (C=C). These electron deficient regions make fatty acids susceptible to attack by a variety of oxidizing and high-energy agents generating free radicals. Triglycerides contain straight chains of primarily 16- to 18-carbon fatty acids and minimal amounts of unsaturated fatty acids. Phospholipids in tissue membranes

contain up to 15 times the amount of unsaturated fatty acids (C18:4, C20:4, C20:5, C22:5, and C22:6) found in triglycerides. They are much more susceptible to oxidation because of the increase in the number of points of carbon–carbon unsaturation (C=C) (Brewer, 2011).

Food lipids are food components that are very susceptible to oxidation processes leading to deterioration of foods during manufacturing, storage and final preparation. For this reason efforts to reduce oxidation have increased. Most often, the best strategy is the addition of antioxidants to the food products. There are one (or more) of several mechanisms of antioxidant in food system. Antioxidants are compounds or systems that slow down the oxidation rates of foods by a combination of scavenging free radicals, chelating prooxidative metals, quenching singlet oxygen and photosensitizers, and inactivating lipoxygenase (Choe and Min, 2009; Brewer, 2011).

2.1.2.1 Free radical scavenging

Antioxidants scavenge free radicals of foods by donating hydrogen to them, and they produce relatively stable antioxidant radicals with low standard reduction potential, less than 500 mV (Choe and Min 2005). The higher stability of antioxidant radicals than that of food radicals is due to resonance delocalization throughout the phenolic ring structure (Choe and Min 2006). Examples of antioxidants to scavenge free radicals are phenolic compounds (tocopherols, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), propyl gallate (PG), lignans, flavonoids, and phenolic acids), ubiquinone (coenzyme Q), carotenoids, ascorbic acids, and amino acids. The most effective antioxidants are those that interrupt the free radical chain reaction. Usually containing

aromatic or phenolic rings, these antioxidants donate $H^{\bullet}$ to the free radicals formed during oxidation becoming a radical themselves. These radical intermediates are stabilized by the resonance delocalization of the electron within the aromatic ring and formation of quinone structures (Nawar 1996). In addition, many of the phenolics lack positions suitable for molecular oxygen attack. Both synthetic antioxidants (BHA, BHT, and propyl gallate) and natural botanicals contain phenolics (flavonoids) function in this manner. Botanical extracts with antioxidant activity generally quench free radical oxygen with phenolic compounds as well (Choe and Min, 2009; Brewer, 2011). Ascorbic acid and glutathione scavenge free radicals by donating hydrogen to food radicals, producing more stable ascorbic acid and glutathione radicals than food radicals. Ascorbic acid radicals become dehydroascorbic acid by loss of proton. Amino acids containing sulfhydryl or hydroxy groups such as cystein, tyrosine, phenylalanine, and proline also inactivate free radicals (Choe and Min, 2009).

2.1.2.2 Metal chelating

Because bivalent transition metal ions, Fe^{2+} in particular, can catalyze oxidative processes, leading to formation of hydroxyl radicals, and can decompose hydroperoxides via Fenton reactions ($Fe^{2+} \rightarrow Fe^{3+}$) (Caillet et al., 2007), chelating these metals can effectively reduce oxidation. Food materials containing significant amounts of these transition metals (red meat) can be particularly susceptible to metal-catalyzed reactions. Metal chelators decrease oxidation by preventing metal redox cycling, forming insoluble metal complexes, or providing steric hindrance between metals and food components or their oxidation intermediates. EDTA and citric acid are the most common metal chelators in foods (Choe and Min, 2009; Brewer, 2011).

2.1.2.3 Singlet oxygen quenching

Singlet oxygen having high energy of 93.6 kJ above the ground state triplet oxygen reacts with lipids at a higher rate than triplet oxygen. Sensitizers are dyes such as methylene blue, eosin, and curcumin, pigments such as chlorophylls and hematoporphyrin, and aromatic hydrocarbons such as rubrene and anthracene. Riboflavin also acts as a sensitizer. The major pathway for singlet oxygen formation in food is photosensitization. Tocopherols, carotenoids, phenolics, urate, and ascorbate can quench singlet oxygen. Curcumin, a major component of food flavoring turmeric, was also reported to be a good singlet oxygen quencher at physiologically low concentrations (2.75 ~ 3.12 μM). Carotenoids with 9 or more conjugated double bonds are good singlet oxygen quenchers by energy transfer. The singlet oxygen quenching activity of carotenoids depends on the number of conjugated double bonds in the structure and the substituents in the *β*-ionone ring. *β*-Carotene and lycopene which have 11 conjugated double bonds are more effective singlet oxygen quenchers than lutein which has 10 conjugated double bonds. The quencher donates electron to singlet oxygen to form a singlet state charge transfer complex and then changes the complex to the triplet state by intersystem crossing. Finally, the triplet state charge transfer complex is dissociated into triplet oxygen (Min and Boff, 2002; Choe and Min, 2005).

2.1.2.4 Inactivating lipoxygenase

Lipoxygenase is a catalytic enzyme in the oxidation of lipids and is inactivated by tempering, which is heat treatment with moisture. Steaming of ground soybeans at 100ºC for 2 min or 116ºC under 44.5 N for 1 min decreases the

ipoxygenase activity by 80% to 100%, with a decrease in peroxide values, which improves the sensory quality of crude soybean oil (Choe and Min, 2009).

Polyphenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Kähkönen et al., 1999). Crude extracts of herbs and spices, and other plant materials rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. Many spices have been investigated for their antioxidant properties for at least 50 years. The results showed that rosemary, oregano, sage and others belonging to the Labiatae family, exhibited antioxidant properties. Some researchers reported that other species such as clove, cinnamon and coriander also exhibited antioxidant properties (Wu et al., 2004).

Antioxidant components contain in thyme, ginger, red pepper, turmeric and other herbs and spices have been identified to some extent. Eugenol and thymol, which are the major compounds found in the essential oils of clove and thyme, respectively, are shown to retard the increase in peroxide value (POV) of dehydrated pork. Quercetin, the main compound in onion, has been shown to reduce lipid oxidation in cooking and storage of the cooked meat (Tang and Cronin, 2007). The extracts of garlic and shallot bulbs has significant antioxidant potential, as measured by a decrease in free radicals and an ability to inhibit lipid oxidation (Leelarungrayub, Rattanapanone, Chanarat and Janusz, 2006). Shogaol and zingerone, the pungent compounds found in ginger, exhibit strong antioxidant activities (Kikuzaki and Nakatani, 1993). Some curcuminoids, including curcumin, 4-hydroxycinnamoyl (feruloyl) methane and bis (4-hydroxycinnamoyl) methane were found to have

antioxidant activities. Capsaicin and dihydrocapsaicin, the pungent principles of red pepper, were also found to be responsible for its antioxidant activity. Essential oil treatments of oregano and sage showed significantly reduce the oxidation, while the heat treatment and storage time significantly affect the antioxidant activity of the meat (Fasseas, Mountzouris, Tarantilis, Polissiou and Zervas, 2007). The ginger extract showed an antioxidant activity comparable with that of BHT in inhibiting the lipid peroxidation both at 37°C, and at a high temperature of 80°C. Most inhibition was reported to occur at the stage of formation of secondary products of the auto-oxidation of fats (Stoilova, Krastanov, Stoyanova, Denev and Gargova, 2007). Holy basil and galangal extracts was also reported to give strong superoxide anion scavenging activity, Fe^{2+}chelating activity, and reducing power in a concentration-dependent manner (Juntachote and Berghofer, 2005).

2.2 Use of herb and spice for improving meat product quality

Nowadays, in response to recent claims that synthetic antimicrobials and antioxidants have the potential to cause toxicological effects and consumers are demanding for more natural (organic) foods, it is an obliging of the industry to use, in some means, and include natural preservatives in foods. Consumers' increased interest in purchasing natural products, hence, food industry has been searching for sources of natural preservatives to replace the use of synthetic preservatives and to improve food products. Herbs and spices have been used as food additives since ancient times, as flavoring agents but also as natural food preservatives. Some studies have demonstrated that shelf life and food quality can be improved by using herb and spice extracts in some stages of production. The main effects of these compounds are to

retard microbial growth and lipid oxidation during storage. Nevertheless, more research is needed to determine herb and spice efficacies, particularly for their antimicrobial and antioxidant, in food products during processing and storage, and their effects on other product quality parameters. Many alternatives from natural sources have been investigated with demands to reformulate products without synthetic additives. Herbs and spices are the natural alternatives that have been intensively studied for the use in food in particular for their antioxidant and antimicrobial properties. Herbs and spices are the group of minor food adjuncts that have been in used for thousands of years to enhance sensory quality of foods extensively in tropical countries. These ingredients impart characteristic flavor, aroma and color to foods. Some of them can also modify the texture of food. In addition, herbs and spices receive much attention according to the decrease trends of using synthetic food additives due to high cost, storage stability and consumer risk of additive residues in food products. For instance, synthetic preservatives such as sodium benzoate, which has been used safely and successfully for many years, primarily to prevent fungal spoilage in acid foods, research in UK found that a cocktail of synthetic colors together with sodium benzoate could be linked to hyperactivity in some children. In 2009, the British government requested that artificial dyes and benzoic acid and sodium benzoate to be removed from foods and beverages (Kanarek, 2011).

Meat and meat products being the highly perishable commodity require special attention during handling, storage and transportation. Their qualities can be altered starts from farm, slaughtering, cutting, transportation, further processing and during display for sale in the markets. Quality changes of meat can lead to risk of consumer

health and economical loss. Numerous studies have been published on the antimicrobial and antioxidant activities herbs and spices including their extracts to improve animal nutrition and meat quality. Herbs and spices have been used in reducing the use of antibiotic growth promoters and no residues in animal products. Herbs are also cheaper in the long run and may lead to lower feed costs. Due to the wide variety of active compounds, different herbs and spices affect animal digestion processes differently. Some herbs and spices often used in animal production, its active components and functions as compiled by Frankič et al. (2009) are shown in Table 2.2

In Thailand, herbs and spices have also been used as alternative supplements in animal feed in order to replace chemicals and antimicrobial drugs and to improve meat quality. In addition, using Thai herbs can also reduce dangerous residues in animal meat, lower production cost and can be claimed as organic or natural origin (Piboonpunyachote, 2012). Some herbal medicine is a Thai local wisdom that has been long use for prevention and treatment of diseases and found to be safe with less toxic. Some herbs and spices reported to be used in animal feeds such as *Andrographis paniculata* (kariyat), *Citrus hystrix* DC (kaffir lime), *Garcinia mangostana* Linn (mangosteen), *Centella asiatica* (asiatic pennywort), *Alpinia galangal* (galanga), *Psidium guajava* Linn.(guava), *Curcuma longa* L. (turmaric), *Zingiber montanum* (Koenig) Link ex Dietr. (cassumunar ginger), *Stevia rebaudiana* Bertoni (stevia), *Doragag Staph* (lemon grass), *Tinospora crispa* (L.) Miers ex Hook.f. & Thomson (Menispermaceae), *Stephania pierrei* (diels), *Boesenbergia rotunda* (L.) Mansf. (Kaempfer) *Allium sativum* Linn. (garlic) and Anglegrass (Ketpanyapong, 2007; Piboonpunyachote, 2012).

Table 2.2 Often used plants, its active components and functions

Plant	**Used parts**	**Major active component**	**Function**
Aromatic spices			
Nutmeg	Seed	Sabinene	Digestion stimulant, antidiarrhoeic
Cinnamon	Bark	Cinnamaldehyde	Appetite and digestion stimulant, antiseptic
Cloves	Cloves	Eugenol	Appetite and digestion stimulant, antiseptic
Cardamom	Seed	Cineol	Appetite and digestion stimulant
Coriander	Leaves, Seed	Linalool	Digestion stimulant
Cumin	Seed	Cuminaldehyde	Digestive, carminative, galactagogue
Anise	Fruit	Anethol	Digestive, carminative, galactagogue
Celery	Fruit, Leaves	Phtalides	Appetite and digestion stimulant
Parsley	Leaves	Apiol	Appetite and digestion stimulant, antiseptic
Fenugreek	Seed	Trigonelline	Appetite stimulant
Pungent spices			
Capsicun	Fruit	Capsaicin	Digestion stimulant
Pepper	Fruit	Piperine	Digestion stimulant
Horsradish	Root	Allyl isothiocyanate	Appetite stimulant
Mustard	Seed	Allyl isothiocyanate	Digestion stimulant
Ginger	Rhizome	Zingerone	Gastric stimulant
Garlic	bulb	Allicin	Digestion stimulant, antiseptic
Herbs			
Rosemary	Leaves	Cineol	Digestion stimulant, antiseptic, antioxidant
Thyme	Whole plant	Thymol	Digestion stimulant, antiseptic, antioxidant

Table 2.2 (Continued)

Plant Aromatic spices	Used parts	Major active component	Function
Sage	Leaves	Cineol	Digestion stimulant, antiseptic, carminative
Laurel	Leaves	Cineol	Appetite and digestion stimulant, antiseptic
Mint	Leaves	menthol	Appetite and digestion stimulant, antiseptic

Compiled by Frankič et al., 2009.

There are reports of studies on the use of herbs and spices and their extracts in meat and meat products as compiled by Tajkarimi et al. (2010) and shown in Table 2.3 According to polyphenolics, flavanoids, lignans, and terpenoids, spices have been shown to have antioxidant properties and demonstrated their antioxidant effects in raw and cooked pork (El-Alim, Lugasi, Hóvári and Dworschák, 1999; Jayathilakan, Sharma, Radhakrishna and Bawa, 2007) raw chicken (El-Alim et al., 1999), raw and cooked beef (Du and Li, 2008; Dwivedi, Vasavada, and Cornforth, 2006; Jayathilakan et al., 2007; Vasavada, Dwivedi, and Cornforth, 2006) and cooked mutton (Jayathilakan et al., 2007).

El-Alim et al. (1999) investigated the use of ground spices and spice extracts as antioxidants in raw ground chicken and ground pork, aerobically packed and stored at 4°C for 7 d, or -18°C for 6 months. Ground chicken was treated with 10 g/kg of the following dried spices: marjoram, wild marjoram, caraway, clove, peppermint, nutmeg, curry, cinnamon, basil, sage, thyme, and ginger. The formation of TBARS was inhibited in refrigerated and frozen samples that were treated with spices. During refrigerated storage, cloves showed the largest reduction in TBARS values compared

with the control. Peppermint and caraway were not significantly different from the control.

Table 2.3 Application of essential oil (EOs) or their components in meat and poultry*

Food group	EO or component	Bacterial species	Inhibitory effect
Meat	Clove oil , eugenol and coriander, oregano, thyme oils, encapsulated rosemary EO, clove and tea tree	*Listeria monocytogenes, Aeromonas hydrophila, Escherichia coli* O157:H7	Yes (+_++)
Fried meat	Oregano and thyme, oregano with marjoram and thyme with sage	*B. cereus* and *P. aeruginosa, Escherichia coli* O157:H7 and *Listeria monocytogenes*	Yes (+)
Ground beef	Chinese cinnamon and winter savory Eos	Pathogenic micro-organisms	Yes (increased radiosensitivity)
Meat	EOs and nisin	*Listeria monocytogenes*	Yes
Meat surfaces	Oregano, pimento, or oregano:pimento	*Escherichia coli* O157:H7 or *Pseudomonas* spp.	Yes
Fresh sausages	Marjoram (*Origanum majorana* L.) EO	Several species of bacteria	Yes
Chicken	Eugenol	*Listeria monocytogenes, Aeromonas hydrophila*	Yes (++_+++)
Chicken	Sage oil	*Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium*	No
Chicken	Oregano	Increase shelf life	Yes (with modified atmosphere packaging)

Table 2.3 (Continued)

Food group	EO or component	Bacterial species	Inhibitory effect
Pâté	Mint oil	*Listeria monocytogenes, Salmonella enteritidis*	No
Minced pork	Thyme oil	*Listeria monocytogenes*	No
Vacuum-packed ham	Cilantro oil	*Listeria monocytogenes*	No
Vacuum-packed minced pork	Oregano oil	*Clostridium botulinum* spores	No
Liver pork sausage	Rosemary	*Listeria monocytogenes*	Yes (encapsulated has more effect than standard)
Minced pork	Winter savory (*Satureja montana*) EO	Food-borne bacteria and improve quality	Yes (in combination with other techniques)

*Compiled by Tajkarimi et al. (2010).

Jayathilakan et al. (2007) showed that cinnamon and cloves were effective at inhibiting TBARS formation in cooked ground meat of beef, pork, and mutton. In this study, ground meat samples of various species were treated with synthetic antioxidants, e.g., tertiary butyl hydroxyl quinone (TBHQ), butylated hydroxy anisole (BHA) and propyl gallate (PG), at 0.02% level each and with 250 mg/100 g meat of either ground cinnamon or ground cloves, packed in polypropylene pouches, and cooked in a boiling water bath under atmospheric pressure for 35 min. Samples were stored at 5°C for 6 d. TBARS values were measured and antioxidant potential was reported. No difference was observed ($p>0.05$) between samples treated with cinnamon and samples treated with butylated hydroxyanisole (BHA), or propyl gallate (PG) at 0.02% in ground beef and pork. Cloves exhibited higher antioxidant activity than BHA and PG. However, tertiary butyl hydroxyquinone (TBHQ) demonstrated the

highest antioxidant activity of all tested antioxidants in all three species of meat. Data from this study revealed that cloves had antioxidant potential similar to synthetic antioxidant BHA and could be used as a substitute to enhance shelf life of meat products.

Dwivedi, Vasavada and Cornforth (2006) evaluated the antioxidant effects of Chinese 5-spice blend and the components of this blend (obtained from a local supermarket) on cooked ground beef. Ground beef (15% fat) was treated with a retail 5-spice blend and its individual components: cinnamon, clove, fennel, pepper, and star anise at 0.1%, 0.5% and 1%, cooked to an internal temperature of 82 to 85°C and stored in plastic bags for 15 days at 2°C. At lowest spice concentration, TBARS values of cooked meat blended with spices were lower than that of the control. They found that the optimum levels were 0.1% clove, 0.5% fennel, 0.5% cinnamon, 0.5% pepper, 0.5% star anise, and 0.5% retail 5-spice blend.

Thailand is in the tropical region; there are wide varieties of herbs and spices available. Culinary herbs and spices are a group of edible plants that have been used as vegetable and cooking ingredients. There are very few experiments reported the use of local Thai herbs and spices in meat and meat products published in terms of antimicrobial and antioxidant activity. Therefore, it is very interesting to search for those culinary herbs and spices which have good antioxidant and antimicrobial efficacy to use for improving the quality of meat and meat products. The outcome results of those herbs and spices and their extracts may be effectively used to replace the synthetic food preservatives.

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CHAPTER III

SCREENING OF THAI CULINARY HERBS AND SPICES FOR THEIR ANTIOXIDANT ACTIVITIES

3.1 Abstract

Almost all of the herbs and spices contribute their properties in many health aspects including antioxidant activities. Many herbs and spices are used as the main seasonings and flavoring ingredients in Thai foods. In order to obtain the antioxidant efficacy of Thai culinary herbs and spices for further studies in other aspects in meat products. Preliminary screening of 22 Thai culinary herbs and spices commonly used for cooking were performed. Extraction was done using 95% ethanol. Analyses were performed for total phenolic compounds and total flavonoid contents. Antioxidant activities were evaluated using three methods: DPPH radical scavenging activity (DPPH assay), ferric reducing antioxidant power (FRAP assay) and thiobarbituric acid reactive substances (TBARS assay) for aldehyde production. It was found that turmeric contained highest amount of total phenolic compounds (579.83 µg gallic acid/g extract) and total flavonoid contents (129.62 µg catechin/g extract). Vietnamese coriander showed the highest antioxidant activity determined by DPPH assay with an IC_{50} of 380.40 µg/ml, which lower than that of trolox and FRAP assay of 3,395 µg Trolox/g extract. From TBARS assay, turmeric was found to have the highest antioxidant activity with an IC_{50} of 347.57 µg/ml.

Keywords: antioxidants, culinary herbs and spices, total phenolic content, total flavonoid content, DPPH, FRAP, TBARS

3.2 Introduction

Oxidation reaction is one of the mechanisms causing food spoilage reducing quality and shelf life of food products. An addition of antioxidants is generally required to preserve food quality. Although synthetic additives have been widely used in food industry to inhibit lipid oxidation but the trend of using is decreasing according to the growing concerns among consumers for their health and toxicity of them. Most effective synthetic antioxidants normally used in food systems for preservation purposes are butylated hydroxyanisloe (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ) and proply gallate (PG). These antioxidants are widely used due to their low molecular weights and non-polar properties. But nowadays, their uses are being restricted in very low concentration because of their toxic and carcinogenic effects (MacFarlane et al., 1997; Iverson, 1999; Shahidi, 2000; Jeong et al., 2005). Thus, interest in finding natural antioxidants has increased tremendously. Herbs and spices for seasoning and cooking are one of the plant groups that gain a great interest for their benefits of containing active antioxidant properties, particularly phenolic compounds. A number of studies deal with an assessment of antioxidant ability of phenolic compounds using several assay methods including DPPH (2,2-diphenyl-1-picrylhydrazyl), ABST (2,2′-azinobis (3-ethlybenzothiazoline 6-sulfonate)), FRAP (ferric reducing antioxidant power) and ORAC (oxygen radical absorption capacity), and total phenolic contents of herbs and spices extracts using different solvent systems. Many studies revealed that antioxidant

activities of most herbs and spices showed strong correlation (r^2>0.800) with their total phenolic contents (Shan, Cai, Sun and Corke, 2005; Dudonné, Vitrac, Coutière, Woillez and Mérillon, 2009; Mustafa, Hamid, Mohamed and Bakar, 2010; Lu, Yuan, Zeng and Chen, 2011). It appeared that relationship between antioxidant activities and phenolic compounds also depend on diversion of studied plant species, solvents and extraction procedures and assay methods that could give low correlation (Hinneburg, Dorman andHiltunen 2006; Silva, Souza, Rogez, Rees and Larondelle, 2007; Daduang, Vichiphan, Daduang, Hongsprabhas and Boonsiri, 2011). It has been known since an ancient time that herbs and spices process antioxidant capacities, improving shelf life and taste of food products. Some of the temperate herbs and spices extracts have been evaluated for their antioxidant activities, and some extract products are already available commercially and used in meat products such as rosemary, sage, grape seed, green tea and pine bark extracts (Wong, Hashimoto and Shibamoto, 1995; Wanasundra, and Shahidi, 1998; Zin, Abdul-Hamid and Osman, 2002; Mielnik, Aaby and Skrede, 2003; Ahn**,** Grün and Mustapha, 2007).

Many tropical herbs and spices have been traditionally used in Thai cooking to flavor dishes differently. Numerous studies reported that not only the benefit of making food tasty, they also contain excellent source of antioxidants (Juntachote and Berghofer, 2005; Maisuthisakul, Pasuk, and Ritthiruangdej,2008; Nanasombat and Teckchuen, 2009; Puangsombat, Gadgil, Houser, Hunt, and Smith, 2011). There are a few reports published results of study on antioxidant activities of some Thai food containing indigenous vegetables (Tangkanakul, Trakoontivakorn, Auttaviboonkul, Niyomvit, and Wongkrajang, 2006; Maisuthisakul et al., 2008; Nanasombat et al., 2009). In Thailand, normally herbs and spices are used as ingredients in home cooking

such as curry soup and chili paste. Only few of them are investigated for using in modern trade meat products such as meatballs and sausages (Juntachote, Berghofer, Siebenhandl, and Bauer, 2006; Juntachote et al., 2007a; Juntachote et al., 2007b). Moreover, the knowledge about the antioxidant capacities of herbs and spices particularly those are used in Thai cooking is very limited. As a part of present ongoing investigations on the use of culinary herbs and spices in modern trade meat products, preliminary assessment of antioxidant capacities and relationships among total phenolic and flavonoid contents of herbs and spices commonly used in Thai cooking were performed for further uses. Therefore, the objective of this study was to evaluate antioxidant capacities of culinary herbs and spices commonly used in Thai cuisine cooking in order to screen and select those having relatively high antioxidant activities for further use as natural antioxidant in meat products

3.3 Materials and Methods

3.3.1 Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and soybean phosphatidylcholine were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA), TPTZ (2,4,6-tripyridyl-S-tri-azine) was purchased from Fluka (Buchs, Switzerland). Gallic acid was purchased from Fluka (Madrid, Spain). Other chemicals and solvents used in this experiment were analytical grade, purchased from Sigma-Aldrich Co., Ltd. (Steinheim, Germany).

3.3.2 Preparation for herb and spice extracts

Twenty-two indigenous herbs and spices commonly used in Thai cooking cuisine were selected for this study as shown in Table 3.1. They were purchased from

local markets in Nakhon Rachsima and Sakon Nakhon Province. The herbs and spices were cleaned, cut into small pieces, freeze-dried (LYOVAC GT2, GEA Lyophil GmbH, Hürth, Germany), finely ground (Retsch ZM 1000, Retsch GmbH, Haan, Germany) and stored at -20^{o}C for further application.

Ethanolic extraction of dried herbs and spices were performed by mixing 50 g of freeze-dried power of each herb and spice in 750 ml of 95% ethanol at room temperatures for 24 h. After extraction, the extract was filtered through a Whatman No.1 filter paper, the residue was re-extracted twice with 750 ml and 500 ml of 95% ethanol. The collected supernatant was evaporated to dryness using a rotary evaporator at 40^{o}C (Rotavapor-R114, BÜCHI, Flawil, Switzerland). The extract was stored at -20^{o}C until use.

Table 3.1 Indigenous Thai culinary herbs and spices collected and used for evaluation of their antioxidant activities.

Common name/Thai name	**Scientific name**	**Part of plant**
1. Green shallot/Ton hom	*Alliumcepa* var. aggregatum	Leaves
2. Coriander/ Pak chee	*Coriandrum sativum* Linn	Leaves and branches
3. Dill/ Phak chi lao	*Anethum graveolens* Linn.	Leaves and branches
4. Garden parsley/ Yira	*Eryngium foetidum* Linn.	Leaves
5. Kaffir lime / Ma grood	*Citrus hystrix* DC	Leaves
6. Celery/ Khuen chai	*Apium graveolens* Linn.	Leaves and branches
7. Holy basil/ Kra phrao	*Ocimum sanctum* Linn	Leaves
8. Sweet basil/ Ho ra pa	*Ocimum basilicum* Linn.	Leaves.
9. Vietnamese coriander/Pak paeow	*Polygonum odoratum* Lour.	Leaves
10. Lemon balm/ Sa ra nae	*Melissa officinalis* Linn.	Leaves
11. Lemon basil/Maenglak	*Ocimum basilicum* L.f. var. citratum Back.	Leaves.
12. Ginger/Khing	*Zingiber officinale* Roscoe	Tubers

Table 3.1 (Continued)

Common name/Thai name	Scientific name	Parts of plant
13. Galangal/ khaa	*Alpiniagalanga* Linn.	Tubers
14. Fingerroot/ Kra Chai	*Boesenbergia pundurata* (Roxb) Schitr	Tubers
15. Garlic/ Kra thiam	*Allium sativum* Linn.	Bulbs
16. Shallot/ Hom daeng	*Allium ascalonicum* Linn.	Bulbs
17. White curcuma / Ka min	*Curcuma mangga* Val.and.Zijp.	Tubers
18. Turmeric/ Ka min khao	*Curcuma longa* Linn	Tubers
19. Green pepper/ Prik thai	*Piper nigrum* Linn.	Young fruits
20. Kaffir lime /Ma grood	*Citrus hystrix* DC	Fruit peels
21. Long red chili/ Phrik chi fa daeng	*Capsicum annuum* L.var.grossum	Tubers
22. Lemon grass/ Ta krai	*Cymbopogon citratus* Stapf.	Stems

3.3.3 Determination of total phenolic compounds

Total phenolic compounds (TPC) were estimated according to the Folin-Ciocalteu method (Matthaus, 2002). Briefly, the extract at concentration of 1000 µg/ml in ethanol was prepared. To 100 µl sample, 2 ml of 2% (w/v) aqueous Na_2CO_3 was added. After 2 min, 100 µl of 0.1 N Folin-Ciocalteu reagent was added, mixed well and incubated for 30 min at room temperature and then, the absorbance was measured at 760 nm using UV-visible spectrophotometer (Smartspec^{tm} Plus, Bio-RAD, Hercules, CA, USA). The TPC was calculated and expressed as an equivalent of gallic acid (µg GAE/g extract) using a standard calibration curve based on concentration of gallic acid solutions. All determinations were performed in triplicate which two replications.

3.3.4 Determination of total flavonoid contents

Total flavonoid contents (TFC) were determined using the modified of Zhishen, Mengcheng and Jianming(1999) with some modification. The extract of 2000 µg /ml in ethanol was prepared. An aliquot of 0.5 ml of diluted extract or standard solution of catechin was added to 2 ml of deionized distilled water and 0.15 ml of 5% $NaNO_2$, mixed well and kept for 6 min and then 0.15 ml of 10% $AlCl_3$ was added. After 6 min, 1 ml of 1 M NaOH solution was added and the total volume was made up to 5 ml with water and mixed well and then, the absorbance was measured against prepared reagent blank at 510 nm. The TFC was calculated from the standard calibration curve based on concentration of catechin solutions and expressed as µg catechin equivalents (QE)/g extract. All determinations were performed in triplicate which two replications.

3.3.5 Antioxidant capacity assays

3.3.5.1 Free radical scavenging activity by DPPH method

Free radical scavenging activity of the extract was determined using 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) according to the method described by Sanchez-Moreno, Larrauri and Saura-Calixto (1999) with some modification. Stock solution of each herb and spice extract was prepared and diluted to obtain the final concentrations of 10, 50, 100, 500 and 1000 µg/ml in ethanol. Trolox was used as a positive control. Each diluted extract of 75 ml was added to 2.925 ml of a 0.025 g/l DPPH solution in ethanol. The reaction mixture was incubated in the dark for 30 min and the absorbance at 515 nm was measured using UV-visible spectrophotometer. The remaining DPPH concentration in the reaction mixture was calculated from the DPPH

standard curve and the remaining DPPH (%) was calculated using the following equation:

Scavenging activity (%) = (1- absorbance $_{sample}$/absorbance $_{control}$) x 100

The DPPH radical scavenging activity (%) was plotted against the extract concentration (µg /ml) to determine the concentration of extract necessary to decrease DPPH radical scavenging activity by 50% (IC_{50}). All determinations were performed in triplicate which two replications.

3.3.5.2 Ferric reducing antioxidant power (FRAP Assay)

The FRAP assay was determined according to the method described by Katalinic, Milos, Modum, Music and Bodan (2004) and Woraratphoka, Intarapichet, and Indrapichate (2007) with some modification. The working FRAP reagent was prepared by mixing 10 volumes of 1.0 mol/l acetate buffer, pH 3.6 with 1 volume of 10 µmol/l TPTZ (2,4,6-tripyridyl-S-tri-azine) in 40 µmol/l hydrochloric acid and with 1 volume of 20 µmol/l ferric chloride. A 100 µl of the sample extract (1000 µg /ml in ethanol) was mixed with 3 ml of FRAP reagent and an absorbance was measured at 593 nm after 8 min. The antioxidant efficiency of the sample solution was calculated with reference to the standard curve of known concentrations (100-1000 µg /ml) of Trolox. The FRAP of the sample was expressed as Trolox equivalent (µg/ g extract). All determinations were performed in triplicate which two replications.

3.3.5.3 Thiobarbituric acid reactive substances (TBARS) assay

TBARS assay was monitored for lipid oxidation by the method modified by Maikhunthod and Intarapichet (2005). Stock solution of extract was prepared and diluted to obtain the final concentrations of 10, 50, 100, 500 and 1000 µg /ml in 95%

ethanol. Trolox was used as a positive control. Briefly, the extract was dissolved in soybean phosphatidylcholine liposome suspension to give various concentrations. After 10 min, sodium ascorbate and $FeCl_3$ solutions were added, incubated in a 37^{o}C water bath (Julabo SW22, JULABO GmbH, Seelbach, Germany) for 30 min, and then 2 ml of thiobarbituric acid (TBA) reagent (0.02 M in water) was added. The reaction tube was heated in boiling water bath for 15 min, cooled and centrifuged at 4000 x g (Thermo Sciencetific, Waltham, MA, USA) for 15 min. The absorbance of supernatant was measured at 532 nm using UV-visible spectrophotometer. Concentration of malondialdehyde (MDA) in oxidation system was calculated from the MDA standard curve. The percentage of inhibition was calculated as follow:

$$\% \text{ inhibition} = \frac{(\text{MDA in absence of ext}) \quad (\text{MDA in presence of ext})}{\text{MDA in absence of ext}} \text{ x } 100$$

The antioxidant activity of extract was expressed as amount of extract used in the system to obtain 50% inhibition of oxidation (IC_{50}).

3.3.6 Statistical Analysis

The statistical analyses were performed using statistical package SPSS (version 16, SPSS Inc., USA). Analyses of variance were performed by ANOVA procedure with one-way analysis. Differences among the means were compared based on Duncan's Multiple Range Test with a level of $p<0.05$ was used as the criterion for statistical significance. The values obtained were means of three replicate determinations ± standard deviations. The 50% inhibitory concentration (IC_{50}) was calculated according to concentration-effect regression line. Correlation analysis was carried out to determine the relationship between the antioxidant capacity and total phenolic compounds or Total flavonoid contents.

3.4 Results and Discussion

3.4.1 Total phenolic compounds and total flavoniod contents

Total phenolic compounds are very important due to they exhibit antioxidant activity. The amount of total phenolic compounds (TPC), measured by the Folin-Ciocalteu method is rapid and widely-used assay (Kähkönen et al., 1999). Therefore, in this work, the TPC of 22 herbs and spices extracts tested varied from 31.17-579.83 µg gallic acid/g extract ($p<0.05$) as shown inFigure3.1 and Table 3.2. Turmeric extract contained highest level of TPC (579.83µg gallic acid/g extract) while the other extracts considering contained the lowest amounts were shallot, garlic, long red chili, green shallot and ginger (42.33, 39.83, 34.50, 33.67 and 31.17 µg gallic acid/g extract, respectively). The extract of Vietnamese coriander contained very high level of TPC of 389.00 µg gallic acid/g extract. Other spices that had good levels of TPC were green pepper, finger root, holy basil, galangal, lemon balm, kaffir lime (leaves), sweet basil and kaffir lime (skin) (187.67, 167.33, 148.33, 140.33, 139.83, 116.00, 114.00 and 110.17 µg gallic acid/g extract, respectively). The extracts having TPC in the range of 55.17-92.50 µg gallic acid/g extract were garden parsley, lemon basil, dill, coriander, lemon grass, white curcuma and celery.

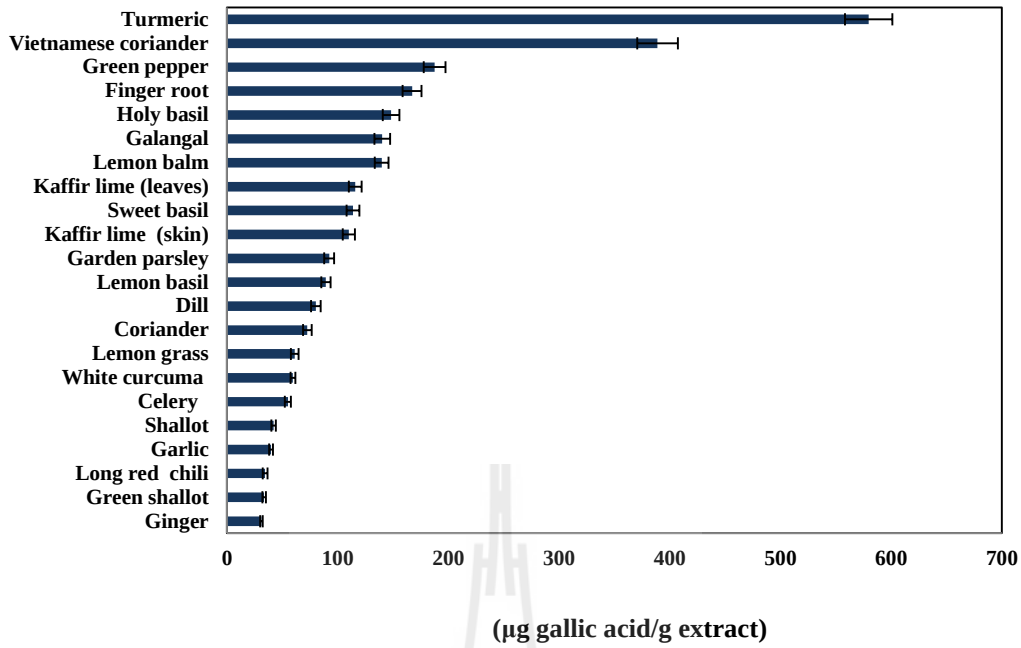

Figure 3.1 Total phenolic compounds of 22 Thai culinary herb and spice ethanolic extracts, arranged from the highest to the lowest amounts.

Flavonoids are the largest class of phenolic compounds, and they are ubiquitous in the plants. The basic flavonoid structure is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings (C6-C3-C6) with different oxidation and antioxidant activity and influences phenoxyl radical stability (Wojdylo, Oszmiański and Zemerys, 2007). Total flavonoid contents (TFC) exhibits antioxidant activity and their mechanisms of action are through free radical scavenging or chelating process (Kessler, Ubeaud and Jung, 2003). In this study, the flavonoid contents ranged of 1.21-129.62 µg catechin/gextract ($p<0.05$) as shown in Figure 3.2 and Table 3.2. Turmeric extract contained highest amount of TFC (129.62 µg catechin/g extract). Herb and spice extracts that contained TFC in the second highest range of 49.58-62.24 µg catechin/g extract were Vietnamese coriander (62.24 µg

catechin/g extract), green pepper, holy basil, white curcuma, sweet basil, lemon balm, lemon basil, coriander, lemon grass, garden parsley and green shallot. The extracts having TFC in the range of higher than 10 µg catechin/g extract but less than 30 µg catechin/g extract were dill (25.30 µg catechin/g extract), finger root, celery, galangal and long red chili. The group of herb and spice extracts having lowest TFC, less than 10 µg catechin/g extract, were kaffir lime (skin) (8.25 µg catechin/g extract), ginger (4.20 µg catechin/g extract) and shallotand garlic (1.21 µg catec hin/g extract).

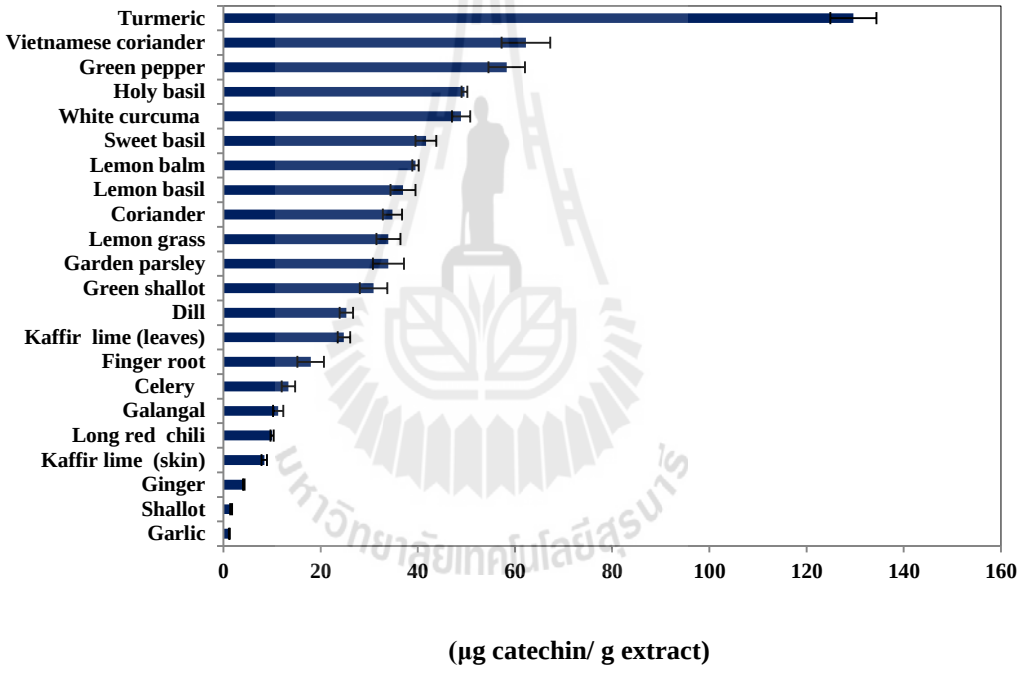

Figure 3.2 Total flavonoid contents of 22 Thai culinary herb and spice ethanolic extracts, arranged from the highest to the lowest amounts.

Considering the top five culinary herbs and spices found in this study having highest total phenolic compounds were turmeric, Vietnamese coriander, green pepper, finger root and holy basil while those had highest total flavonoid contents were

turmeric, Vietnamese coriander, green pepper, holy basil and white curcuma. Due to flavonoids are the subset of phenolic compounds, hence, total flavonoid contents significantly increases with the presence of high concentration of total phenolic compounds. Maisuthisakul et al., (2008) suggested that the total phenolic compounds correlated well with total flavonoid contents. Similarly Mustafa, Hamid, Mohamed and Bakar (2010) reported the similar results.

3.4.2 Antioxidant capacity of culinary herbs and spices

In this study, the antioxidant activities of the extract of culinary herbs and spices were focused on phenolic and flavonoid compounds and three common assays used to evaluate their antioxidant capacities were based on different radicals and mechanisms of reaction.

DPPH assay is a free radical compound that has been widely used to determine the free radical-scavenging ability of various samples (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, and Weil, 2004). This assay is based on the principle that a hydrogen donor is an antioxidant. The antioxidant effect is proportional to the disappearance of DPPH radicals in test samples. $DPPH^{\bullet}$ shows color and strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant (Moon and Shibamoto, 2009). The DPPH free radical-scavenging activity of 22 Thai local culinary herbs and spices were presented in Figure 3.3 and Table 3.2, expressed as IC_{50} values ranging from 294.97 to 5,769.64 µg/ml ($p<0.05$). Trolox tested as standard reference, showed the most active with the IC_{50} values of 294.97 µg/ml. Significant radical scavenging activities among all 22 culinary herbs and spices were found ($p<0.05$). The Vietnamese coriander extract showed strongest inhibition activity

of IC_{50} value (380.40 µg/ml) ($p<0.05$), followed by green pepper (527.94 µg/ml) and turmeric (628.71 µg/ml). The extracts shown to have medium inhibition activity having an IC_{50} higher in the range from 1,000 to 2,000 µg/ml were holy basil (1,258.96 ug/ml), lemon balm (1,634.67 µg/ml), finger root (1,636.52 µg/ml), garden parsley (1,887.30 µg/ml), lemon grass (1,969.38 µg/ml) and sweet basil (2,065.47 µg/ml). The extracts of herbs and spices having IC_{50} higher than 2,000 µg/ml found in garlic (2,272.32 µg/ml), white curcuma (2,311.60 µg/ml), dill (2,433.08 µg /ml), galangal (2,456.14 µg/ml), lemon basil (2,553.57 µg/ml), kaffir lime (leaves) (3,657.08 µg/ml), green shallot (5,302.23 µg/ml), and celery extract (5,769.64 µg /ml).

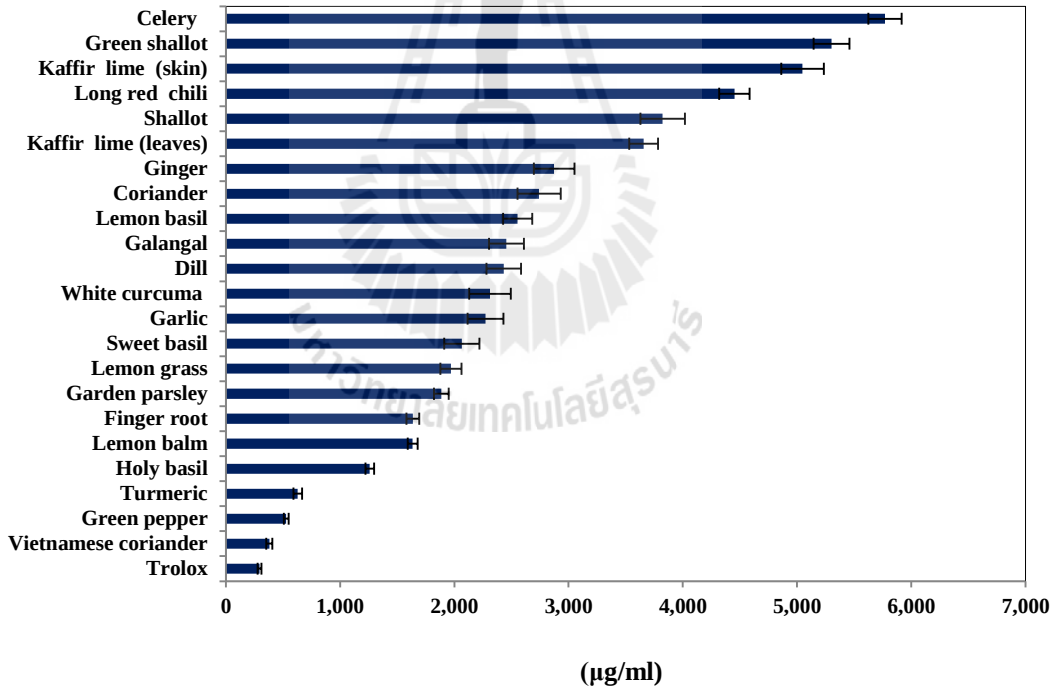

Figure 3.3 Antioxidant capacity based on IC_{50} free radical scavenging on $DPPH^{\bullet}$, (µg/ml) of 22 Thai culinary herb and spice ethanolic extracts, arranged from the lowest to the highest capacity.

The FRAP assay is a measure of antioxidant activity according to their reducing ability/antioxidant power of the herb and spice extracts. When a Fe^{3+}-TPTZ complex is reduced to the Fe^{2+} formed by an antioxidant under acidic conditions, an intense blue color with absorption maximum develops at 593 nm (Moon et al., 2009). In this study, the FRAP values of extracts of culinary herbs and spices ranged from 134.17 to 3,395.00 µg Trolox/g extract ($p<0.05$) are shown in Figure 3.4 and Table 3.2. It could be divided in five groups as followings: (1) very high FRAP values which were Vietnamese coriander (3,395.00 µg Trolox/g extract) green pepper (2,254.67 µg Trolox/g extract) and turmeric (1,357.80 µg Trolox/ g extract), (2) high FRAP values which were holy basil (671.83 µg Trolox/g extract), lemon balm (610.33 µg Trolox

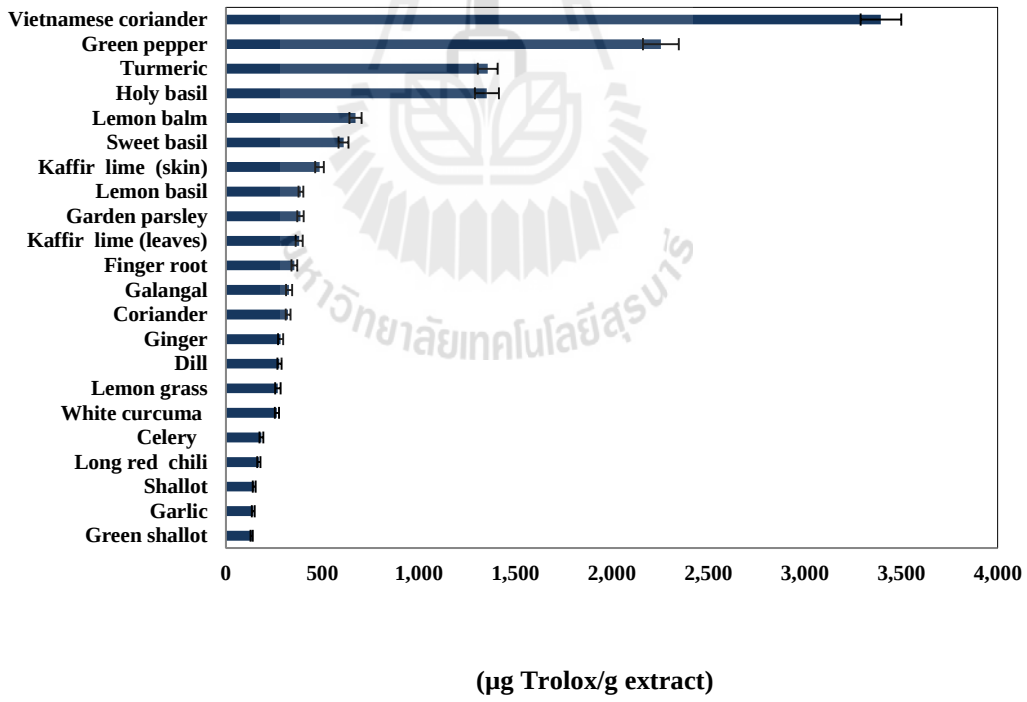

Figure 3.4 Ferric reducing antioxidant power (FRAP, µg Trolox/g extract) of ethanolic extracts of 22 Thai culinary herbs and spices, arranged from the highest to the lowest capacity.

/g extract), (3) moderate FRAP values which were sweet basil (485.67 µg Trolox/g extract), kaffir lime skin (388.67 µg Trolox/g extract), lemon basil (387.23 µg Trolox/g extract), garden parsley (380.50 µg Trolox/g extract) and kaffir lime (leaves) (355.67 µg Trolox/g extract), (4) low FRAP values which were finger root (327.67 µg Trolox/g extract), galangal (322.50 µg Trolox/g extract), coriander (283.83 µg Trolox/g extract), ginger (278.11 µg Trolox/g extract), dill (269.50 µg Trolox/g extract), lemon grass (264.83 µg Trolox/g extract), white curcuma (238.13 µg Trolox/g extract), and (5) very low FRAP values which were celery (184.17 µg Trolox/g extract), long red chili (171.17 µg Trolox/g extract), shallot (147.33 µg Trolox/g extract), garlic (142.53 µg Trolox/g extract) and green shallot (134.17 µg Trolox/g extract).

TBARS assay has been commonly used to measure lipid oxidation. This method measures the malonaldehyde (MDA) formed after lipid hydroperoxide decomposition. Synthesized MDA was also used as a standard for determination. The sample reaction was characterized as the color complex formed due to the condensation adducted between thiobarbituric acid (TBA) and MDA. The result was expressed as percentage of inhibition and concentration of 50% of inhibition activity was determined as IC_{50} values, µg MDA/ml extract. The TBARS values as IC_{50} values of 22 culinary herbs and spices tested were presented in Figure 3.5 and Table 3.2, ranged from 347.57 to 4,424.44 µg/ml ($p<0.05$). Turmeric was found to have the strongest antioxidant activity in term of TBARS value (347.57 µg/ml) and found to be better than Trolox (427.59 µg/ml) ($p<0.05$). Vietnamese coriander (808.54 µg/ml), holy basil (887.95 µg/ml) and green pepper (909.64 µg/ml) were the next strongest to turmeric extract. The next high inhibition activity was identified for lemon grass

(997.59 µg/ml), finger root (1,068.40 µg/ml) white curcuma (1,234.44 µg/ml), celery (1,314.88 µg/ml), galangal (1315.82 µg/ml), coriander (1,425.19 µg/ml) and lemon balm (1,563.48 µg/ml). The herb and spice extracts considered as medium inhibitors were green shallot (1961.34µg/ml), sweet basil (1,975.81µg/ml), ginger (1,998.70 µg/ml), dill (2,042.83 µg/ml) and kaffir lime (leaves) (2,150.92 µg/ml). The extracts considered having low inhibition activity were kaffir lime (skin) (2,252.26µg/ml), garden parsley (2,423.86 µg/ml), shallot (2,744.67 µg/ml), garlic (2,899.26 µg/ml) and lemon basil (2,937.56 µg/ml). Lastly, the extract having lowest inhibition activity was the extract from long red chili (4,424.44µg/ml).

The results of antioxidant activity of herbs and spices are mainly contributed by the active compounds present in them mainly as phenolic compounds and flavonoids. Phenolic constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts. Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolic (Agrawal, 1989). Due to the fact that antioxidant activities are influenced by many factors, it cannot be fully described by only a single method (Maisuthisakul et al., 2008) for evaluation an antioxidant mechanisms for the plant extracts.

In this study, the antioxidant activities of all culinary herb and spice extracts were determined using FRAP, DPPH and TBARS assay. Among 22 herbs and spices, it could be concluded that Vietnamese coriander and turmeric possessed the highest antioxidant activities and had high TPC and TFC (Table 3.2). Overall, spices that contained a high amount of TPC tended to have high scavenging activity. However, finger root, which had slightly higher TPC than holy basil, had lower scavenging

activity than holy basil did. Prior, Wu, and Schaich (2005) suggested that some of inorganic substances may also interact with Folin-Ciocalteu reagent, giving an inaccurate result of the TPC of the samples.

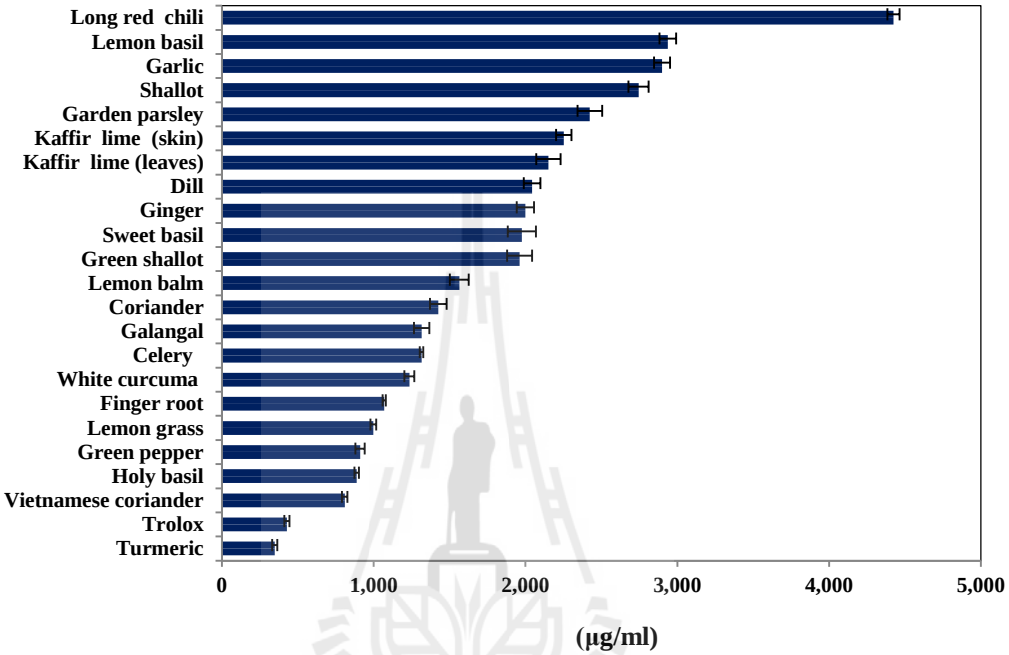

Figure 3.5 Antioxidant capacity based on IC_{50} of TBARS values (MDA µg/ml) of ethanolic extracts of 22 Thai culinary herbs and spices, arranged from the lowest to the highest.

In general, antioxidant activity of phenolic and flavonoid depends on the structure and substitution pattern of hydroxyl groups. Vietnamese coriander possessed strong antioxidant activity and anti-breast cancer activity. Significant amounts of flavonoids exist in its leaves such as rutin was the most abundant constituent (3.77% w/w dry extract), followed by catechin (0.34%), quercetin (0.079%), kaempferol (0.009%) and isorhamnetin (0.007%). Rutin in combination with other flavonoids may

cause strong antioxidant activity (Nanasombat et al., 2009). Sun, Zhang, Lu, Zhang, and Zhang (2011) reported that rutin was the significant compound in scavenging of $DPPH^{\bullet}$ free radical. A group of phenolic compounds including curcumin with the highest level (17.61 µg/g) was found to be the active principal in turmeric along with demethoxycurcumin (3.91 µg/g) and bis-methoxycurcumin (3.88 µg/g) (Puangsombat et al., 2011). Curcumin is well known for its strong antioxidant activitygroup of phenolic compounds (Miquel, Bernd, Sempere, Diaz-Alperi and Ramiraz, 2000). In green pepper, the significant components having antioxidant activity were reported to be 3,4-dihydroxyphenyl ethanol glucoside (0.076 µg/ml), 3,4-dihydroxy-6-(*N*-ethylamino) benzamide (0.27 µg/ml) and phenolic acid glycosides (0.12 µg/ml), suggesting a high radical scavenging activity of these phenolics (Orav, Stulova, Kailas and Müürisepp, 2004; Chatterjee et al., 2007). Phytochemical investigations on holy basil leaf extract have been shown to possess potent antioxidant. Phenolic compounds in holy basil were reported to be eugenol, cirsilineol, isothymucin, isothymonin, apigenin and vosamarinic acid and flavonoids compounds were orientin and vicenin (Yanpallewar, Rai, Kumar and Acharya, 2004). Pharmacologically active compounds isolated from the rhizomes of finger root are reported to be flavonoid compounds (pinostrobin), flavanones (pinostrobin, pinocembrin and alpinetin) and chalcones (cardamonin and boesenbergin A) (Mahmood, Abdalbasit, Siddig, Salmah and Fouad, 2010).

Table 3.2 Total phenolic contents, total flavonoid contents, FRAP values, DPPH values and TBARS values

Herbs and spices	Phenolic (ug gallic acid/g extract)	Flavonoid (ug catechin/g extract)	FRAP value (ug Trolox/ g extract)	DPPH (IC_{50})(µg/ml)	TBARS (IC_{50})(µg/ml)
1. Green shallot	33.67±1.51 o	30.88±0.81 k	134.17±6.19 n	5,302.23±156.49 b	1,961.34±82.81 h
2. Coriander	72.67±3.86 k	34.80±0.75 i	283.83±13.38 j	2,742.04±188.61 h	1,425.19±54.46 j
3. Dill	80.33±4.37 j	25.30±0.66 l	269.50±14.12 j	2,433.08±152.29 j	2,042.83±54.40 g
4. Garden parsley	92.50±4.47 i	33.95±0.54 j	380.50±17.87 gh	1,887.30±164.66 n	2,423.86±80.38 d
5. Kaffir lime (leaves)	116.00±5.90 g	24.79±0.39 l	355.67±14.93 h	3,657.08±125.66 f	2,150.92±80.92 f
6. Celery	55.17±2.74 m	13.38±0.32 n	184.17±9.86 l	5,769.64±145.33 a	1,314.88±12.05 k
7. Holy basil	148.33±7.50 e	49.58±0.29 d	671.83±31.17 d	1,258.96±38.08 p	887.95±14.31 o
8. Sweet basil	114.00±5.79 g	41.68±0.43 f	485.67±22.63 f	2,065.47±155.23 l	1,975.81±93.76 h
9. Vietnamese coriander	389.00±18.37 b	62.24±0.82 b	3,395.00±104.85 a	380.40±25.95 s	808.54±17.19 p
10. Lemon balm	139.83±6.14 f	39.50±0.60 g	610.33±25.75 e	1,634.67±144.01 o	1,563.48±61.76 i
11. Lemon basil	89.50±4.23 i	36.98±0.63 h	387.23±16.78 g	2,553.57±127.51 i	2,937.56±55.11 b
12. Ginger	31.17±1.17 o	4.20±0.07 r	278.11±10.40 j	2,873.75±178.38 g	1,998.70±57.05 gh
13. Galangal	140.33±7.03 f	11.27±0.12 o	322.50±12.68 i	2,456.14±153.63 j	1,315.82±50.85 k
14. Finger root	167.33±8.55 d	17.96±0.17 m	327.67±16.19 i	1,636.52±55.66 o	1,068.40±10.29 m
15. Garlic	39.83±1.75 n	1.21±0.01 s	142.56±7.75 n	2,272.32±156.24 k	2,899.26±52.73 b
16. Shallot	42.33±2.03 n	1.59±0.01 s	147.33±7.47 mn	3,824.09±195.91 e	2,744.67±66.56 c

Table 3.2 (Continued)

Herbs and spices	Phenolic (ug gallic acid/g extract)	Flavonoid (ug catechin/g extract)	FRAP value (ug Trolox/ g extract)	DPPH (IC_{50})(µg/ml)	TBARS (IC_{50})(µg/ml)
17. White curcuma	59.67±2.21 l	48.89±0.12 e	238.17±11.94 k	2,311.60±182.97 k	1,234.44±33.65 l
18. Turmeric	579.83±21.47 a	129.62±0.47 a	1,357.80±51.47 c	628.71±37.93 q	347.57±17.60 r
19. Green pepper	187.67±9.75 c	58.30±0.05 c	2,254.67±93.27 b	527.94±20.82 r	909.64±29.90 o
20. Kaffir lime (skin)	110.17±5.49 h	8.25±0.05 q	388.67±12.34 g	5,048.36±187.19 c	2,252.26±51.17 e
21. Long red chili	34.50±2.17 o	10.01±0.06 p	171.17±8.47 kl	4,452.44±132.89 d	4,424.44±39.52 a
22. Lemon grass	61.33±3.37 l	33.95±0.45 j	264.83±11.47 jk	1,969.38±92.58 m	997.59±19.18 n
23. Trolox	-	-	-	294.97±17.62 t	427.59±16.01 q

Different letters in the same day of storage are significantly different ($p<0.05$), each value in the table was expressed as mean ± standard deviation (n = 6).

3.5 Conclusions

The study showed antioxidant activities of some indigenous and local culinary herbs and spices commonly consumed in Thailand. Some of herbs and spices could be considered as good sources of natural antioxidants since their extracts were found to possess high antioxidant activities. Among 22 herbs and spices studied, those posse good antioxidant activities, considered in terms of DPPH, FRAP and TBARS, were in the order of Vietnamese coriander, turmeric, green pepper and holy basil, although their total phenolic compounds and total flavonoid contents were not in the same order as their antioxidant activities.

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CHAPTER IV

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF SELECTED THAI CULINARY HERB AND SPICE EXTRACTS: APPLICATION IN PORK MEATBALLS

4.1 Abstract

The ethanolic extracts from four selected Thai culinary herbs and spices, i.e., holy basil, Vietnamese coriander, turmeric and green pepper were used as natural bioactive ingredients in pork meatballs for shelf life extension. The batter of pork meatball was prepared with 0.2 % (w/w) ethanolic extract, and then, the pork meatball model was formed in plastic casing and cooked in hot water till internal temperature reached 70ºC. The pork meatball model was cut in defined length, aerobically and vacuum packaged and stored at 4ºC for 9 days. Thiobarbituric acid reactive substances (TBARS) and microbial enumeration were performed every 3 days. It was found that holy basil, Vietnamese coriander and green pepper showed stronger antioxidant effects in the pork meatballs than did turmeric throughout 9 days of storage period for both aerobically and vacuum packaged ($p<0.05$). For antimicrobial activity, the meatballs made with the extracts from holy basil and green pepper and packaged in both aerobic and vacuum conditions had the highest shelf life of 9 days while those made with Vietnamese coriander and turmeric extracts had the shelf life of about 6-9 days and

less than 6 days for control meatballs. In addition, water activity values of all meatballs were not significantly different ($p>0.05$) which were in the range of 0.969-0.980. Antimicrobial inhibition of selected culinary herb and spice extracts were tested against indicator bacteria by clear zone forming. Holy basil extract could inhibit two Gram-positive bacteria, i.e., *Enterococcus faecalis* (AP-31) and *Micrococcus luteus* TISTR 745 with clear zone diameter ranging from 7.4 to 7.6 mm and green pepper extract could inhibit *Micrococcus luteus* TISTR 745 with clear zone of 7.6 mm, while Vietnamese coriander extract was highly potent in inhibition of *Acinetobacter calcoaceticus* TISTR 1264 with clear zone of 7.6 mm. By sensory evaluation, the pork meatballs with herb and spice extracts showed higher score of the spicy and off-flavor attributes than control sample but was not significant ($p>0.05$) at the end of storage times. The pork meatballs with holy basil and green pepper extracts showed the lowest oxidized intensity and bacterial counts which indicated that these two plants could be the good sources of antioxidant and antimicrobial for use in comminuted pork products.

Keywords: Culinary herb/spice extracts, antioxidant, antimicrobial, TBARS, pork meatballs, aerobically and vacuum packaged.

4.2 Introduction

Finely comminuted meat products such as pork, beef and chicken meatballs are traditional meat products with a high consumption volume in Thailand. Normally, the meatball is a meat product made of ground meat, flour, ice and seasoning, formed into a ball and then cooked in hot water at about 70-75°C. Lipid oxidation and bacterial contamination are the main factors that determine quality and shelf-life reduction of

the meatball products. Therefore, delaying lipid oxidation and preventing bacterial cross-contamination are highly relevant to food processors. The growth of microorganisms in meat products may cause spoilage or food borne diseases. Oxidative processes in meat also lead to the degradation of lipids and proteins, imposing an adverse effect on flavor, color and texture as well as nutritional value (Byrne et al., 2001).

In addition, antioxidants are required to preserve food quality. Although synthetic additives have been widely used in food industry to inhibit both, the process of lipid oxidation and microbial growth the trend is to decrease their use because of the growing concern among consumers about such chemical additives. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) are widely used in food industry. Their safety, however, has been questioned. BHA was reported to be carcinogenic in animal experiments. At high doses, BHT may cause internal and external hemorrhage, which contributes to death in some strains of mice and guinea pigs. This effect is due to the ability of BHT to reduce vitamin K-depending blood-clotting factor (Ito et al., 1986).

Antimicrobial agents are commonly called chemical preservatives and their functions are to control the growth of bacteria, yeast and mold in the various food systems leading to extending product shelf life. Chemical preservatives such as potassium sorbate and sodium benzoate have been used to extend the shelf life of processed meats (Choi and Chin, 2003). Sorbic acid and its salts are added to numerous food products. In meat industry, sorbates are the common inhibitors of mold growth in sausage casings. Nitrites are used in meat products for curing, because of

their effects on color as well as their antibotulinic activity (Binstok, Campos, Varela and Gerschenson, 1998). Despite the benefits attributed to food additives, for several years there have also been a number of concerns regarding the potentially short term and long term risks of consuming these substances. Short term acute effects from additives are unlikely, cancer and reproductive problems resulting from the long term consumption of them (Branen, Davidson and Salminen, 1990).

Many natural plant extracts contain primarily phenolic compounds which are potent antioxidants (Wong, Hashimoto and Shibamoto, 1995). The phenolic compounds from some plants such as sage, rosemary, thyme, hops, coriander, tea, cloves and basil are known to process antimicrobial effects against foodborne pathogens (Elgayyar, Draughon, Golden and Mount, 2001). Phenols are one of the most important groups of natural antioxidants. They occur only in material of plant origin and they are known to easily protect oxidizable constituents of food from oxidation (Wang, Cao and Prior, 1996).

Therefore, replacing synthetic antioxidants and antimicrobs by natural ingredients from herbs and spices have been increasing awareness of consumers regarding to the safety of food additives. Currently, there are many on-going investigations of using natural antioxidants and antimicrobs as the alternative sources for food protection. Thai herbs and spices have been used in cooking for their flavor and fragrance added to many food dishes and shown to have both antioxidant and microbial activities. However, researches on the use of Thai herbs and spices in meat and meat products are limited and still needed more investigations to cover more types of herbs and spices including their effects on product qualities and characteristics. Therefore, investigation of using Thai herbs and spices in meat and meat products in order to gain

some information concerning their abilities to retard oxidation and microbial growth including the effects on some physicochemical and sensory properties of the meat products are very much of interesting. For this experiment, pork meatballs were used as the meat product model for the study.

The objective of this study was to investigate the use of some Thai culinary herb and spice extracts for their antioxidant and antimicrobial activities to extend the shelf life of pork meatballs aerobically and vacuum packaged during storage at 4ºC.

4.3 Materials and methods

4.3.1 Preparation for herb and spice extracts

Four selected herbs and spices namely; holy basil (*Ocimum sanctum* Linn), Vietnamese coriander (*Polygonumodoratum* Lour.), turmeric (*Curcuma longa* Linn) and green pepper (*Piper nigrum* Linn) were purchased from market places in Nakhon Rachsima and Sakon Nakhon Province. The herbs and spices were cut into small pieces, freeze dried (LYOVAC GT2, GEA Lyophil GmbH, Hürth, Germany) and finely ground (Retsch ZM 1000, Retsch GmbH, Haan, Germany) and stored at -20°C for further experiment.

Ethanolic extraction of dried herbs and spices are performed by mixing 50 g of ground plant in 750 ml of 95% ethanol at room temperatures for 24 h. After extraction, the extract was filtered through a Whatman No.1 paper, the residue was re-extracted twice with 750 ml and 500 ml of 95% ethanol, respectively. The extract was evaporated to dryness using a rotary evaporator at 40°C (Rotavapor-R114, BÜCHI, Flawil, Switzerland). The extracts were stored at -20°C until use. Extraction was done in duplicate.

4.3.2 Preparation for bacteria from pork meatballs

To obtain dominant bacterial flora contaminated in pork meatballs, microorganisms were collected from one-day old homemade pork meatballs and local markets. Briefly 10 g sample was homogenized in 90 ml (0.85% NaCl) using a stomacher (Stomacher 400, Seward laboratory blender, Worthing, West Sussex, England), and aliquots was plated out directly at 1:10 dilution in 0.1% peptone water. After serially diluting each sample in sterile peptone solution, 0.1 ml portions was separately plated onto plates. The total bacterial count was determined on plate count agar (PCA, Hi-media, Mumbai, MH, India) and incubated at 37°C for 24 h or deMan, Rogosa and Sharpe agar (MRS, Merck, Darmstadt, Germany) for lactic acid bacteria and incubated at 35°C for 24 h with incubator (Binder BD400, Bohemia, NY, USA). Microbial colonies were counted and expressed as $\log_{10}$ cfu/g sample (Sriwira and Intarapichet, 2007; Sriwira, 2008).

Dominant bacterial colonies obtained from the above was isolated, purified and identified according to bacterial morphology and biochemical reactions with analytical profile index system (API system). These identified bacterial strains were subjected to the tests of herb and spice extracts in addition to those obtained from Thailand Institute of Scientific and Technological Research (TISTR) culture collection (Sriwira, 2008).

4.3.3 Determination of antimicrobial activity of selected herb and spice extracts

Selected foodborne bacteria obtained from Thailand Institute of Scientific and Technological Research (TISTR) were used as indicator bacteria for testing antibacterial activity of herb and spice extracts. The Gram-positive indicator bacteria

were *Lactobacillus plantarum* TISTR 850, *Leuconostoc mesenterroides* TISTR 942, *Staphylococcus aureus* TISTR 029, *Enterococcus faecalis* TISTR 379, *Micrococcus luteus* TISTR 745 and *Listeria monocytogenes* TISTR 17303 while the Gram-negative indicator bacteria were *Pseudomonas aeruginosa* TISTR 1467, *Klebsiella oxytoca* TISTR 556, *Pseudomonas fluorescens* TISTR 358, *Escherichia coli* TISTR 887, *Acinetobacter calcoaceticus* TISTR 1264 and *Enterobacter aerogenes* TISTR1 540. In addition, spoilage bacteria from pork meatballs were collected from pork meatballs available in local markets, isolated, identified and used as indicator strains for testing of herb and spice extracts.

Top four herb and spice extracts having high antioxidant capacity obtained from screened plants as in Chapter III which were selected for preparation of the pork meatball models. Stock solution of each herb and spice extract was prepared by diluted in 10% dimethyl sulfoxide (DMSO, Riedel-de Haën, Seelze, Germany) solution to obtain a final concentration of 5,000 ppm. Antimicrobial activity of the extracts was determined by agar diffusion method (Sriwira, 2008). The inhibitory effect was assessed by measuring the diameter of inhibition clear zone around the extract. Wells of approximately 6 mm were made in agar plates using a sterilized stainless steel borer and each well was filled with 40 μl of individual extract solution. The agar plates were incubated at appropriate temperatures for each type of indicator strain used. Microorganisms showing a clear zone were considered as being inhibited (Arora and Kaur, 1999; Nanasombat and Teckchuen, 2009).

4.3.4 Preparation for pork meatballs

Pork meatball batter model was prepared using 5 kg lean ground pork, 2% salt, 0.25% sodium phosphate, 15% ice, 2% tapioca starch and 0.2% herb and spice

extracts. After chopping, the meatball batter was stuffed in a 20 mm diameter plastic casing, cooked in hot water at 70°C for 30 min, cooled in chilled water and aseptically cut into pieces with the length of about 2.5 cm. The meatballs were aerobically and vacuum packaged in plastic bags, stored at 4°C. The meatball samples were randomly taken every 3 days during 9 days of storage for microbial enumeration and antioxidant activity determination.

4.3.5 Microbial enumeration

Microbial enumeration of the meatball samples were performed every 3 days during storage. Total plate counts (TPC) and lactic acid bacterial (LAB) contents were performed using Petrifilm^{TM} (3M, St. Paul, MN, USA.) and incubated at 37°C for 24 h for TPC or incubated at 35°C for 24 h for LAB. The number of colony count was expressed as log cfu/g sample.

4.3.6 Determination of antioxidant activity

4.3.6.1 Thiobarbituric acid reactive substances (TBARS) assay

Lipid oxidation of pork meatball sample was determined for TBARS values by Buege and Aust (1978) with some modification. A 5 g of sample was homogenized (IKA$^{®}$T25, T25D, Staufen, Germany) in 15 ml of deionized distilled water. Meatballs homogenate (1ml) was transferred into a test tube and 50 µl of 7.2 % butylated hydroxyanisole (Sigma, St. Louis, MO, USA) and 2 ml of 20 mM 2-thiobarbituric acid (TBA, Fluka, Buchs, St. Gallen Rheintal, Switzerland) in 15% trichloroacetic acid (TCA, Qrec, Auckland, New Zealand) solution were added, mixed, incubated in a boiling water bath (Julabo SW22, JULABO GmbH, Seelbach, Germany) for 15 min to develop color and then, centrifuged for 15 min at 2000×g using Sorvall® RC SC-plus centrifuge (Thermo Sciencetific, Waltham, MA, USA).

The absorbance was measured at 532 nm (Smartspec^{tm} Plus, Bio-RAD, Hercules, CA, USA) and TBARS values were calculated as mg malondialdehyde (MDA) /kg sample. Known concentrations of MDA (Sigma, St. Louis, MO, USA) were used for calibration.

4.3.6.2 Determination of hexanal content.

Hexanal content was determined by the method of Ahn, Grün and Mustapha (2007) with some modification. One gram of sample was weighed into a 22 mL headspace vial and 3 mL of deionized distilled water was added. The vial was crimped with aluminum caps with Teflon septa after purging with nitrogen. The sample was equilibrated in the headspace autosampler (Tekmar HT3, Teledyne Tekmar, Mason, OH, USA) at a platen temperature of 75°C (the sample temperature was 75°C when the equilibrium was reached). After thermal equilibration, samples were mixed for 2 min during the mix mode preprogrammed in the Teckmar HT3 autosampler. The vial was shaken during this mode, which may reduce the mean diffusion path length of solutes as they migrate to the gas/sample interface within the vial. Samples were then stabilized for 2 min, pressurized for 0.3 min, and equilibrated for 0.05 min in the autosampler. After the loop was filled and equilibrated for 0.3 min, the carrier gas (helium) back flushed the loop and carried the compound through the heated transfer line (150°C) into the GC. The released volatiles were automatically injected and separated on the Gas Chromatography (CP-3800 GC, Varian Inc., Walnut Creek, CA, USA) capillary column (CP8924, 30 m×0.32 mm×0.25 μm). A split injection ratio of 1:10 at 220°C flow rate of the carrier gas was 2.0 mL/min. The oven temperature was programmed from 35°C for 5 min, increased 45°C at 8°C /min and increased to 200°C at 40°C/min for 6 min and FID was set at 250°C. Hexanal

concentration was quantitated using pure hexanal (Sigma-Aldrich, St. Louis, MO, USA) for standard curve and calculated as mg of hexanal/g sample.

4.3.7 Water activity determination

The water activity (Aw) of the meatball samples were performed every 3 days during storage, the sample was measured at room temp. An adequate amount of 5 g ground sample was placed into a sample cup to thoroughly cover the bottom and using the water activity meter (AQUA LAB CX3TE, Decagon Devices, Inc., Pullman, WA, USA).

4.3.8 Color determination

Color determination of pork meatball samples was performed immediately in triplicates per sample by hand color meter (Minolta CR-300, Minolta Camera Co., Ltd., Osaka, Japan). Color values were presented in terms of CIE, L*, a*, b*. Color was described as coordinates; lightness (L*), redness (a*; + red, - green) and yellowness (b*; + yellow, - blue), following the guidelines for color measurements from American Meat Science Association (Hunt et al., 1991). Two replications of the experiment were performed every 3 days with ten measurements per replication taken for each sample.

4.3.9 Texture analysis

Texture analysis was performed using texture analyzer (TA.XT. plus, Stable Micro Systems, Ltd., Hamilton, MA, USA), compression force (g) was evaluated with a spherical probes (P/0.5s, 1.2 cm diameter ball probe) speed was 2 mm/s, their depth range from 10 mm, Six representative cores (22 mm in diameter and height 2 cm). Hardness values were reported in gram (g) (Huang, Shiau, Liu, Chu and Hwang,

2005). Two replications of the experiment were performed every 3 days with ten measurements per replication taken for each sample.

4.3.10 Sensory evaluation

Sensory evaluation of pork meatballs was performed using the quantitative descriptive analysis (QDA) method with unstructured line score of 10 cm, from less intensity to high intensity (Stone and Sidel, 1985). The trained sensory panel consisted of 9 members of scientists and post graduate students of School of Food technology, Suranaree University of Technology, Thailand. The panelists were trained and well acquainted with different sensory attributes. The pork meatball samples held at refrigeration temperature (4°C) were evaluated every 3 days during 9 days of storage. The stored samples were reheated in boiling water for 5 min and served to the panelists. The panelists evaluated the samples for oxidized, spicy, off-flavor and texture including hardness and springiness.

4.3.11 Statistical Analysis

Statistical analysis was evaluated in completely Randomized design (CRD) using SPSS for Windows and means comparison by Duncan's Multiple Range Tests (DMRT) were analyzed (Montgomery, 1991). Two replications of the experiment were performed with triplicate analyses per replication. Statistical difference was determined at $p \leq 0.05$.

4.4 Results and discussion

4.4.1 Isolation and selection of bacteria from meatball

The in-house meatballs were aerobically and vacuum packaged and stored at 4°C for 14 days in order to let them spoiled for microbial collection. Enumerations of

contaminated bacteria were done on plate count agar (PCA) for aerobic bacteria and MRS agar for lactic acid bacteria counts. The result showed total bacterial counts ranged 7.57-8.59 log cfu/g (Table 4.1).

Table 4.1 Microorganism counts of spoiled pork meatballs after 14 days storage at 4°C

Sample	Total viable counts (cfu/g)	
	PCA	MRS
Aerobic package	7.57	8.59
Vacuum package	8.35	8.43

Different 18 isolates from the spoilage meatballs; 3 isolates of short rod, 10 isolates of cocci, and 5 isolates of ovoid cocci were identified. Morphological characteristics were performed and found that all isolates were Gram-positive bacteria. The different morphological characteristics of 18 isolates are shown in Table 4.2. The highest bacteria population for aerobically and vacuum packaged meatballs on PCA and MRS for AP-31, AM-31, VP-51 and VM-21 were 15.91%, 19.05%, 83.19% and 72.52%, respectively. These 4 isolates were used as representative bacteria for isolation and identification for dominant spoilage bacteria present in the pork meatballs used for this study.

Table 4.2 Morphological characteristics and basic biochemical tests of isolates collected from spoiled pork meatballs

Bacterial isolate code	Cell shape	Gram stain	Oxidase	Catalase	Oxidation/Fermentation test
AP-11	Short Rods	+	-	-	+/+
AP-21	Cocci	+	-	-	+/+
AP-31	Ovoid, Cocci	+	-	-	+/+
AM-11	Cocci	+	-	-	-/-
AM-21	Ovoid, Cocci	+	-	-	-/-
AM-31	Ovoid, Cocci	+	-	-	-/-
AM-41	Cocci	+	-	-	-/+
VP-11	Rods	+	+	-	-/-
VP-21	Cocci	+	-	+	-/-
VP-31	Cocci	+	-	-	-/-
VP-41	Cocci	+	-	-	+/+
VP-51	Cocci	+	-	-	+/+
VP-61	Cocci	+	-	+	-/-
VM-11	Cocci	+	-	-	-/-
VM-21	Rods	+	-	-	-/-
VM-31	Cocci	+	-	-	-/-
VM-41	Cocci	+	-	-	-/-
VM-51	Oviod	+	-	-	-/-

Note: AP=Bacteria population for aerobically packaged meatballs on PCA, AM= Bacteria population for aerobically packaged meatballs on MRS, VP= Bacteria population for vacuum packaged meatballs on PCA, VM= Bacteria population for vacuum packaged meatballs on MRS.

4.4.1.1 Isolation of bacteria species

Isolation and selection of dominant bacterial species from pork meatballs were performed according to their morphological characteristic and basic biochemical reactions as results shown in Table 4.3. From biochemistry aspects, colony of AP-31,

AM-31, VP-51 and VM-21 showed similar circular, smooth, and small size (0.5-1 mm) but different in color. The AM-31 isolate showed white-yellow colony but other isolates showed white colonies. The AP-31 and AM-31 isolates were ovoid and cocci, the VP-51 isolate was circular while, the VM-21 isolate was rod. The cell arrangement of all four isolates was single and pair. From biochemical test of all four isolates, they were not able to produce catalase oxidase. Morphological characteristics and biochemical test of bacteria isolated from pork meatballs showed differences in details as shown in Table 4.3.

Identification of selected dominant bacteria from pork meatballs were performed according to their morphological characteristics and biochemical reactions and results shown in Table 4.3. By using test kit in API system, the AP-31 was similar to *Enterococcus faecalis* with 98% ID, AM-31 isolate was similar to *Leuconostoc citreum* with 94% ID and VP-51 was similar to *Lactococcus garvieae* with 99% ID, while VM-21 isolate was not identified because of obscure results in the group of lactic acid bacteria.

These four dominant floras obtained from pork meatballs; *Enterococcus faecalis* (AP-31), *Leuconostoc citreum* (AM-31), *Lactococcus garvieae* (VP-51) and unidentified (VM-21) were also used as indicator bacteria along with those obtained from TISTR for testing against all selected Thai culinary herbs and spice extracts in the study.

Table 4.3 Morphological characteristic and some biochemistry of dominant bacteria collected from spoiled pork meatballs

Test	**AP-31**	**AM-31**	**VP-51**	**VM-21**
Colony:				
Color	White	White-yellow	White	White
From	Circular	Circular	Circular	Circular
Size	1 mm	0.5 mm	0.5-1 mm	0.5-1 mm
Margin	Entire	Entire	Entire	Entire
Surface	Smooth	Smooth	Smooth	Smooth
Cell:				
Shape	Ovoid,Cocci	Ovoid, Cocci	Cocci	Rods
Gram stain	+	+	+	+
Arrangement	Pair/ short chain	Pair/ short chain	Pair/ short chain	Single/ Pair
Motility	+	-	-	+
Oxidase	-	-	-	-
Catalase	-	-	-	-
Growth in 6.5% NaCl	+	+	-	ND
Phosphatase	-	-	-	ND
Urease	-	-	-	ND
Arginine dihydrolase	+	-	+	ND
Ala-Phe-Pho arylamidase	-	-	+	ND
β-galactosidase	-	-	-	ND
α –galactosidase	-	-	-	ND
β-galactopyranosidase	-	-	-	ND
α-glucosidase	+	+	-	ND
β-glucuronidase	-	-	-	ND
α-mannosidase	-	-	-	ND
Leucinearyamindase	-	-	+	ND
L-prolinearylamidase	-	-	-	ND

Table 4.3 (Continued)

Test	AP-31	AM-31	VP-51	VM-21
L-aspartate arylamidase	+	-	(-)	ND
Alanine arylamidase	-	-	+	ND
Tyrosine arylamidase	+	-	(+)	ND
Phosphatidylinositol phospholipase C	-	-	-	ND
O/129 resistance	+	+	+	ND
Novobiocin resistance	+	+	+	ND
Fermentation production of acid form:				
D-amygdalin	+	+	+	ND
D-sorbitol	+	-	-	ND
D-galactose	+	-	+	ND
D-ribose	+	-	+	ND
Lactose	-	-	-	ND
D-maltose	+	-	+	ND
D-mannitol	+	-	+	ND
D-raffinose	-	-	-	ND
Pullulan	-	-	-	ND
D-mannose	+	+	+	ND
Salicin	+	+	+	ND
Sucrose	+	+	+	ND
D-trehalose	+	+	+	ND
Methyl-β-D-glucopyranoside	+	+	+	ND
N-Acetyl-D-glucosamine	+	+	+	ND
Bacitracin resistance	+	+	+	ND
Polymixin_B resistance	+	+	+	ND
Cyclodextrin	+	-	+	ND

Note: + = Positive test, - = Negative test, ND = Not detect, (+) = Week-positive reaction, (-) = Week-negative reaction.

4.4.1.2 Inhibition of selected herb and spice extracts against indicator bacteria

For testing the capability of antimicrobial of selected herb and spice extracts against indicator bacteria and dominant bacteria isolated from pork meatballs; *Enterococcus faecalis* (AP-31), *Leuconostoc citreum* (AM-31), *Lactococcus garvieae* (VP-51) and VM-21 (Figure 4.1) were used comparing with streptomycin 10 µg/ml and nisin 10 µg/ml. Results from clear zones of bacterial inhibition showed that holy basil extract could inhibit two Gram-positive bacteria; *Enterococcus faecalis* (AP-31) and *Micrococcus luteus* TISTR 745 with the clear zone diameter ranging from 7.4 to 7.6 mm.

While Vietnamese coriander extract was able to inhibit *Acinetobacter calcoaceticus* TISTR 1264 with a clear zone of 7.6 mm (Table 4.4). Holy basil extract could inhibit two Gram-positive bacteria, i.e., *Enterococcus faecalis* (AP-31) and *Micrococcus luteus* TISTR 745 with clear zone diameter ranging 7.4-7.6 mm and green pepper extract could inhibit *Micrococcus luteus* TISTR 745 with a clear zone of 7.6 mm while Vietnamese coriander extract was able to inhibit *Acinetobacter calcoaceticus* TISTR 1264 with a clear zone of 7.6 mm.

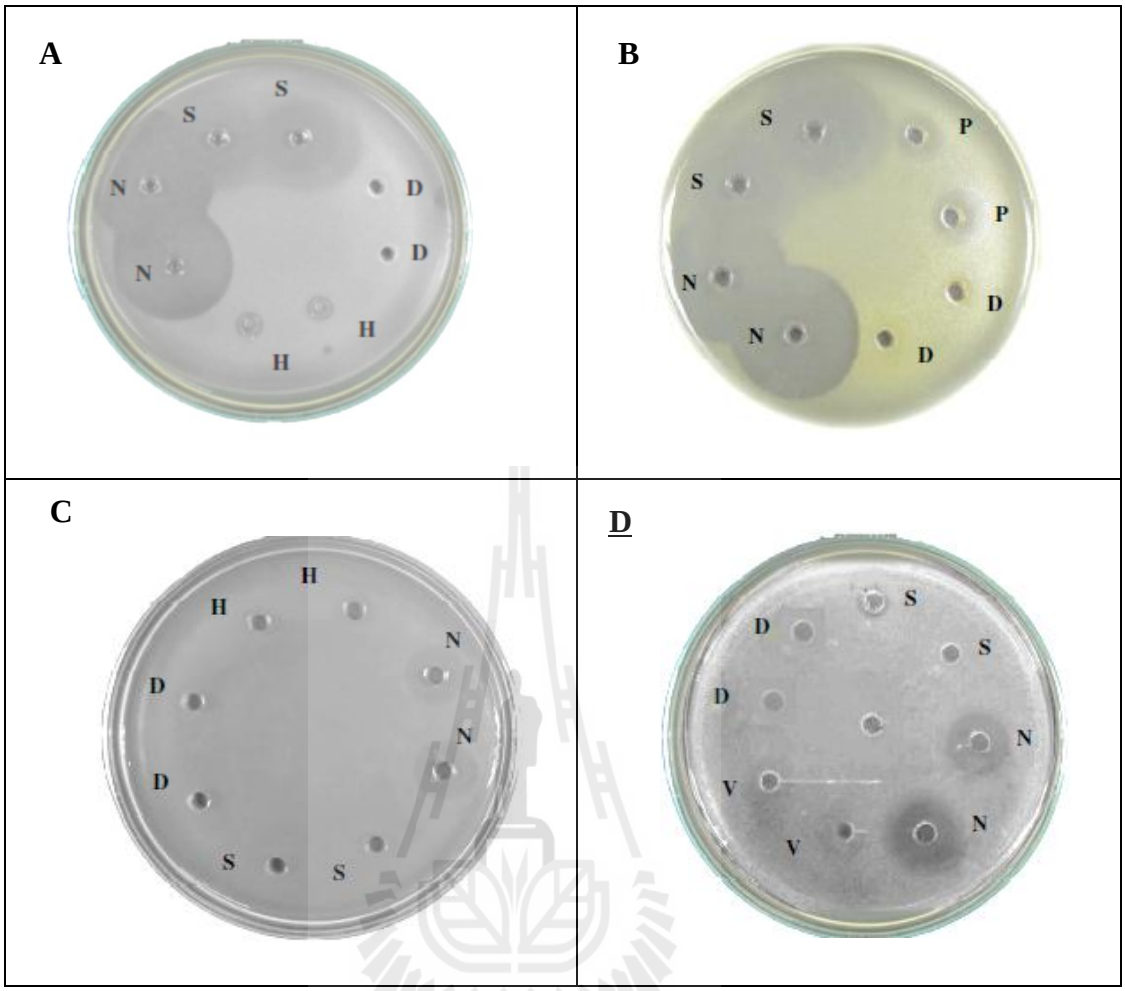

Figure 4.1 The inhibition activity of *Micrococcus luteus* TISTR 745; N = nisin, 10 µg/ml; S = streptomysin, 10 µg/ml; D = 10 % DMSO; H = holy basil extract, 5000 ppm (A). The inhibition activity of *Micrococcus luteus* TISTR 745; N = nisin, 10 µg/ml; S = streptomysin, 10 µg/ml; D = 10 % DMSO; P = green pepper extract, 5000 ppm (B). The inhibition activity of *Enterococcus faecalis* (AP-31), N = nisin 10 µg/ml, S = streptomysin 10 µg/ml, D = 10% DMSO, H = holy basil extract, 5000 ppm (C). The inhibition activity of *Acinetobacter calcoaceticus* TISTR1264, N = nisin 10 µg/ml, S = streptomysin 10 µg/ml, D = 10% DMSO, V = Veitnamese coriander extract 5,000 ppm (D).

4.4.2 Antimicrobial activity of herb and spice extracts used in pork meatballs

Microbial changes of pork meatball samples packaged under different conditions (aerobically and vacuum) during refrigerator storage at 4°C are shown in Figure 4.2 and 4.3.

Initial Total plate counts (TPC) of all pork meatballs sampled at day 0 were less than 4 log cfu/g sample. Figure 4.2, A and B shows the microbial profiles of meatball samples during storage in both aerobic and vacuum packages, respectively. Similar trend of microbial growth was found in both packaging conditions. The meatballs without any herb and spice extracts had higher counts than those herb and spice extracts added at all time of sampling. Among four selected culinary herbs and spices used in this study, holy basil and green pepper extracts gave similar and lowest TPC. Hence, it could be mentioned that they had the highest efficacy in extending the meatball shelf-life packaged in both aerobic and vacuum conditions. According to the Food and Drug Administration Thailand (FDA) standard for acceptable marginal microbial counts of ≤ 5.0 log cfu/g food samples, the control meatballs packaged in both conditions could be kept less than 5 days while the meatballs treated with turmeric, Vietnamese coriander, green pepper and holy basil extracts could be kept for 6, 7, 8 and 9 days, respectively, when packaged in aerobic condition.

Table 4.4 Inhibition of antimicrobial extracts from Vietnamese coriander, holy basil, green peppercorn, and turmeric against selected bacteria by measuring clear zone diameter in millimeter (mm)

Indicator bacteria	Average diameter of inhibition zone (mm.) of indicator bacteria					
	Streptomycin	**Nisin**	**Holy basil**	**Green paper**	**Turmeric**	**Vietnamese coriander**
Pseudomonas aeruginosa TISTR1467	9	-	-	-	-	-
Lactobacillus plantarum TISTR850	-	9.8	-	-	-	-
Leuconostoc mesenterroides TISTR942	8.4	8.8	-	-	-	-
Klebsiella oxytoca TISTR556	9.4	-	-	-	-	-
Pseudomonas fluorescens TISTR358	8.4	-	-	-	-	-
Staphylococcus aureus TISTR029	9.4	-	-	-	-	-
Escherichia coli TISTR887	9.0	-	-	-	-	-
Acinetobacter calcoaceticus TISTR1264	9.0	-	-	-	-	7.6
Enterobacter aerogenes TISTR1540	8.8	-	-	-	-	-
Enterococcus faecalis TISTR379	9.4	-	-	-	-	-
Micrococcus luteus TISTR745	10.0	12.0	7.6	7.6	-	-
Listeria monocytogenes TISTR17303	7.6	7.6	-	-	-	-
Leuconostoc citreum NV-MC	9.6	9.2	-	-	-	-
Lactococcus garvieae PV-F	-	8.0	-	-	-	-
Enterococcus faecalis NV-PC	9.8	8.6	7.4	-	-	-
MV-B	-	8.0	-	-	-	-

- = Negative test.

However, it was obvious that under vacuum condition the meatballs treated with holy basil, Vietnamese coriander and green pepper had similar shelf life of about 9 days while turmeric extract could extend the shelf life only up to 6 days of storage.

Jałosińska and Wilczak (2009) studied the possibility of applying different plant extracts as additives to meat products of the meatball type in order to extend their microbiological shelf life. It was found that the production of meatball model with the addition of 0.2% plant extracts, the highest total content of polyphenols among the extracts used was found in the preparation with rosemary (14.5 mg/g extract), exhibited stronger antibacterial properties than those with the addition of the cranberry extract (5.1 mg of polyphenols per gram of extract) and lovage extract (2.7 mg/g extract). The phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects (Hara-Kudo, Kobayashi, Sugita-Konishi and Kondo, 2004).

This inhibitory effect is due probably to the action of the major compound of extracts, which is believed to work by inhibiting oxidative respiration, inducing membrane deformation (dilatation) with consequent changes in membrane permeability (Cox et al., 2000). Although some reports regarding the efficiency of natural plant antimicrobial agents in foods can be found in the reports such as rosemary and marjoram essential oil. However, the action of Thai culinary herb and spice extracts in food systems has not been previously documented.

Lactic acid bacteria (LAB) as facultative anaerobic bacteria can grow under high concentrations of CO_2 and is the important competitors of other spoilage related microbial groups under vacuum or modified atmosphere packaging conditions (Tsigarida, Skandamis and Nychas, 2000).

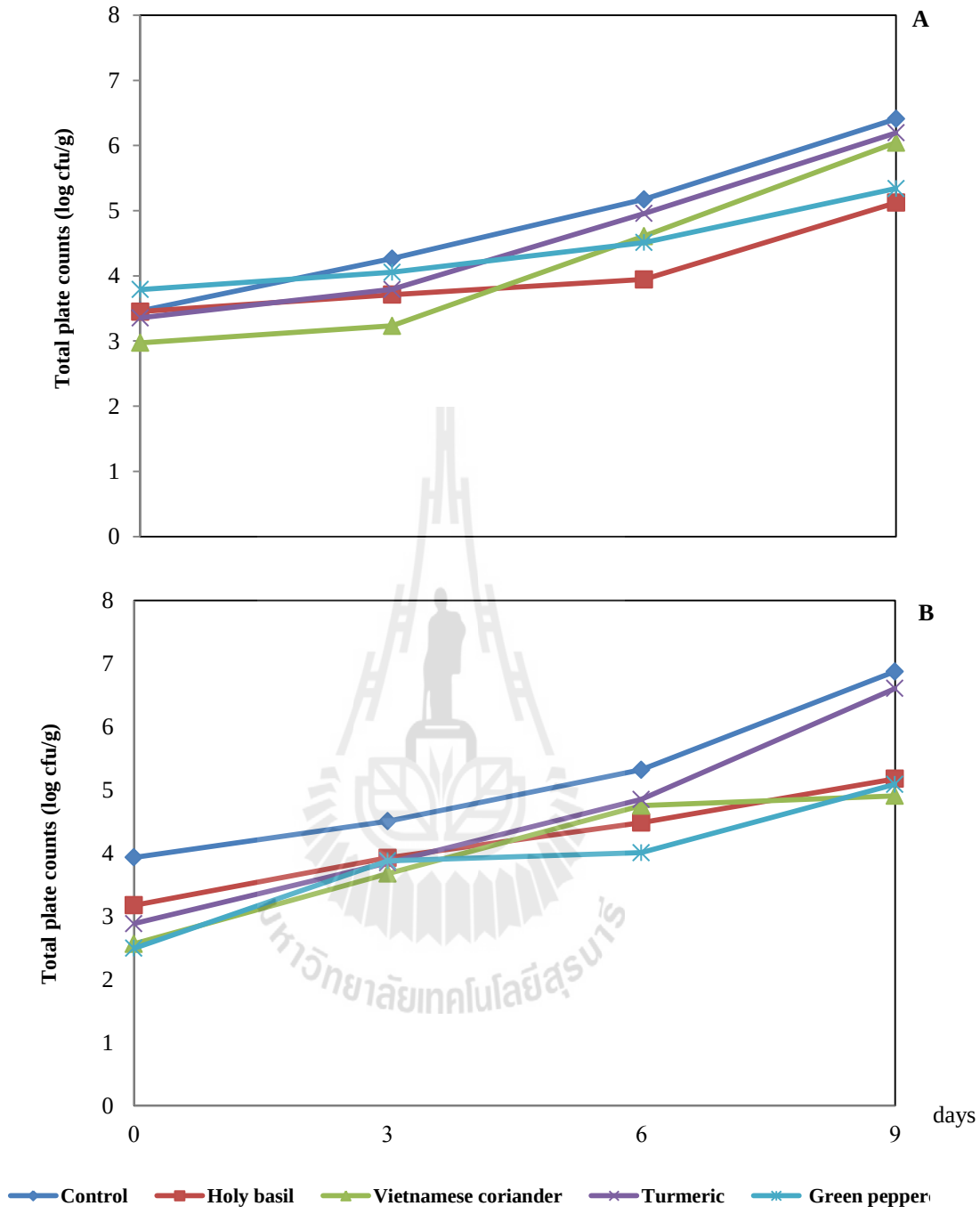

Figure 4.2 Total plate counts of pork meatballs added herb and spice extracts and stored at 4°C for 9 days; A= aerobically packaged and B=vacuum packaged.

They are associated to the spoilage of refrigerated raw meat and they can also dominant throughout storage in reduced O_2 availability (Labadie, 2000; Lambert, Smith and Dodds, 1991). Similarly, Lactobacillus is the major component of the microbiota in chilled pork for vacuum packaged (Blixt and Borch, 2002). More species of lactobacilli can be found during the storage under vacuum at 4°C (Pavelková et al, 2013). Many spices and herbs are considered as alternative means of delaying the onset of spoilage or preventing the growth of foodborne pathogens since their essential oils possess antimicrobial activity (Nychas and Tassou, 2000).

Total lactic acid bacteria (LAB) counts of all meatballs, aerobically and vacuum packaged, are shown in Figure 4.3. The initial LAB counts for both packaging conditions were less than 4 log cfu/g sample. However, throughout the storage period LAB reduction by all selected culinary herb and spice extracts was better in vacuum packaging than in aerobic packaging condition. At the end of storage time, the meatballs added with holy basil, Vietnamese coriander and green pepper extracts had lower LAB counts than control meatballs of about 2 log and 1.5 log cycles in aerobic and vacuum packaging condition, respectively while those with turmeric extract had similar counts to control meatballs of about 6.61-6.88 log cfu/g and 5.28-6.04 log cfu/g, respectively. This was similar to the experiment of Viuda-Martos, Ruiz-Navajas, Fernández-López and Pérez-Álvarez (2011) which reported the effect of treatments, packaging conditions and time on the growth of aerobic and lactic bacteria on the microbiological quality of mortadellas, a bologna-type sausage. On day 0, the 5.00% citrus fiber washing water (CFWW) + 0.02 % thyme essential oil (TEO) and 5.00% citrus fiber washing water (CFWW) + 0.02% rosemary essential oil (REO) samples showed lower lactic acid bacteria and aerobic bacteria growth values ($p<0.05$)

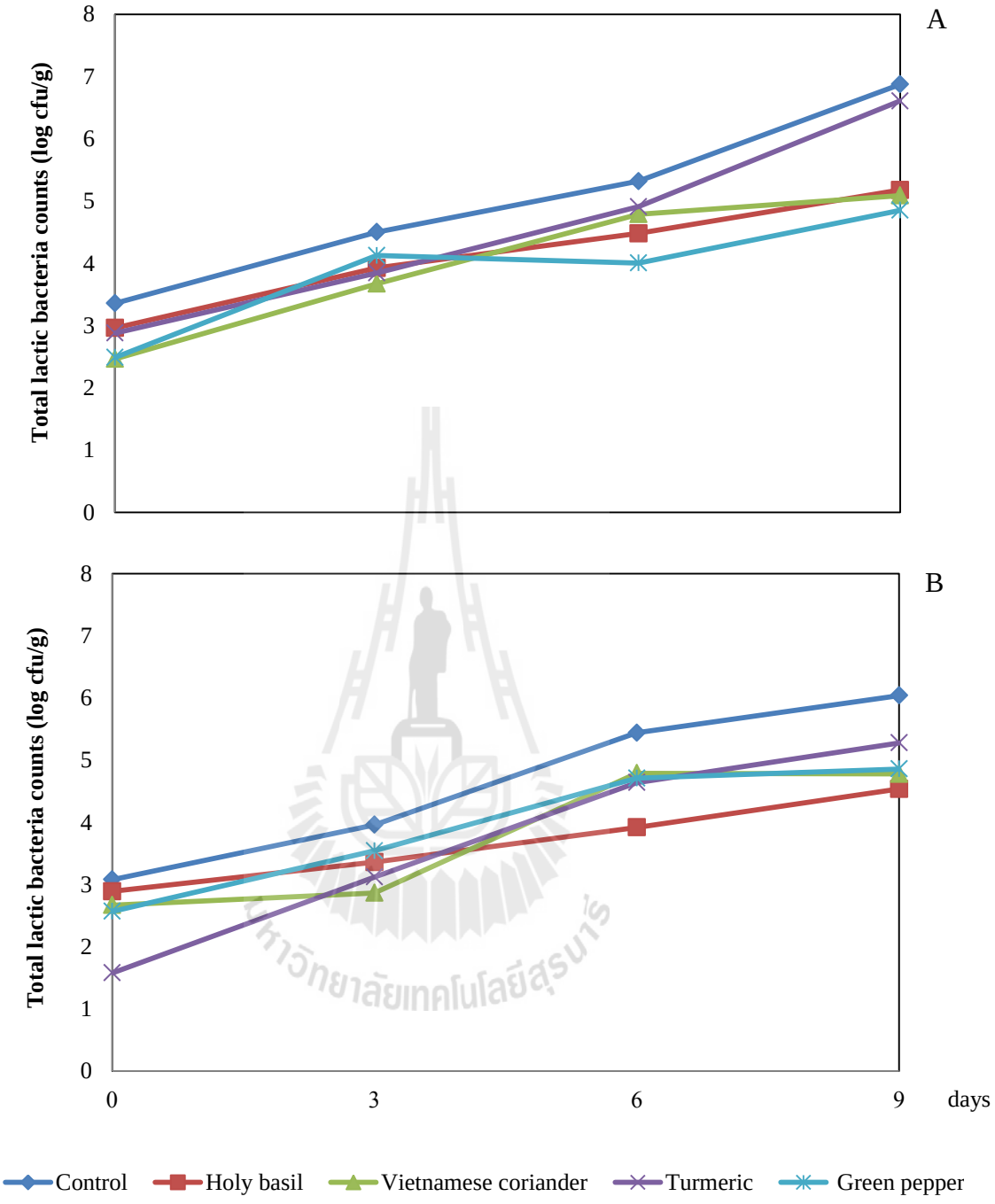

Figure 4.3 Total lactic acid bacteria contents of pork meatballs added herb and spice extracts and stored at 4°C for 9 days; A=aerobically packaged and B= vacuum packaged.

than control samples in all types (air, vacuum and modified atmosphere) of packaging with no statistically significant differences ($p>0.05$) between the CFWW + TEO and CFWW + REO samples. On day 12, the vacuum packaged control sample, CFWW + TEO and CFWW + REO vacuum packaged samples showed the lowest ($p<0.05$) aerobic bacteria counts (3.79, 3.62 and 3.51 log cfu/g, respectively) and the lowest ($p<0.05$) lactic acid bacteria counts (2.97, 2.63 and 2.54 log cfu/g, respectively). The air packaged control sample showed the highest ($p<0.05$) aerobic bacteria and lactic acid bacteria counts in all packaging types.

Pavelková et al (2013) hypothesized antibacterial mechanisms of thymol and carvacrol components that they had hydroxyl group on the phenolic ring. This could be similar mechanisms of the selected Thai culinary herb and spice extracts added in pork meatballs. For antibacterial mechanisms, first, the components could increase the permeability of the cytoplasmic membrane, and probably enable some components to be more easily transported into the cell. Second, the components could increase the number, size or duration of existence of the pores created by the components binding to proteins in the cell membrane, so that a synergistic effect is achieved when the two are used together.

4.4.3 Antioxidation capacity of herb and spice extracts used in pork meatballs

All four selected Thai culinary herb and spice extracts could inhibit oxidation of the pork meatballs longer than control meatballs in both aerobically and vacuum packages during storage at 4°C. Such natural extracts contain high level of bioactive phenolic compounds that can help to control inhibition of lipid oxidation.

Green pepper, holy basil and Vietnamese coriander extracts provided superior efficacy in oxidation and microbial growth inhibition to the extracts from turmeric

(Table 4.3). Antioxidant assay with lipid peroxidation involves the monitoring of hydroperoxides or specific oxidative secondary products. Normally, lipid peroxidation product used for antioxidant assay, malonaldehyde (MDA) has been most widely used to evaluate the antioxidant activity in lipid peroxidation systems on the thiobarbituric acid reactive substances (TBARS) values (Pryor, Stanley and Blair, 1976).

The effects of adding selected herb and spice extracts, packaging conditions and storage times on the lipid oxidation on the TBARS values of pork meatballs are shown in Table 4.5. At all storage times, the pork meatballs with the selected herb and spice extracts showed lower TBARS values than control ones in both packaging conditions with statistically significant differences ($p<0.05$) but among pork meatballs with added selected herb and spice extracts, the pork meatballs with turmeric extract showed lower antioxidative activity than other herb and spice extracts ($p<0.05$) in aerobic condition, followed by pork meatballs with added Vietnamese coriander, holy basil and green pepper, consecutively. Storage period had significant influence on the development of lipid oxidation in the pork meatballs resulting in intensive increase in TBARS values during the storage times of refrigeration.

The samples stored under vacuum packaging conditions and storage times showed that the TBARS values of the pork meatballs with added holy basil, Vietnamese coriander, turmeric and green pepper extracts were lower than the control samples ($p<0.05$). In all sample added selected herb and spice extracts, the pork meatballs with turmeric extract showed higher TBARS value than other herb and spice extracts ($p<0.05$) followed by the sample with Vietnamese coriander extract but the sample added holy basil and green pepper extracts showed similar TBARS values ($p>0.05$).

No statistically significant difference ($p<0.05$) was observed between control and pork meatballs added turmeric extract packaged in both conditions. Oxidative rancidity measured as TBARS for all samples increased during storage, but the highest increases in TBARS over time were found in control samples. It was in agreement with Lee and Ahn (2003) who reported that TBARS values of turkey breast meat patties were affected by external antioxidant, packaging atmosphere, and storage period. They found that antioxidants reduced lipid oxidation effectively both in aerobically and vacuum packaged while aerobically packaged control meat had considerably higher TBARS values than the vacuum-packaged control meat stored for 5 days at 4°C. Vacuum packaged was more resistant to lipid oxidation than aerobically packaged. The TBARS value of aerobically packaged increased during the storage of pork meatballs. The presence of oxygen was the most critical factor in influencing lipid oxidation of aerobically packaged (Nam and Ahn, 2003). Due to the comminuted nature of the raw materials and thermal processing that such products undergo spoilage by lipid oxidation. The oxidative deterioration of lipid and proteins is a major concern for food technologists due to the loss of quality associated with these processes (Estévez and Cava, 2006). Chopping and heating may catalyze lipid oxidation because they disrupt cellular protective compounds contained in cell membranes such as vitamin E, electron, and hydrogen donors (Keokamnerd, Acton, Han and Dawson, 2008). The agents responsible for antioxidant activity in essential oil are the bioactive compounds they contain, and mainly, polyphenols and terpenes (Viuda-Martos, Ruiz-Navajas, Fernández-López and Pérez-Álvarez, 2011). Ruberto and Baratta (2000) reported that monoterpene hydrocarbons had a significant antioxidant protective effect, with several variants due to the different functional

groups.

Lipid oxidation is one of the main causes of deterioration in the quality of meat products during storage and processing (Este´vez, Morcuende, Ventanas and Cava, 2003). Certain lipid-derived volatiles have been demonstrated to be potent odorants and contribute to the overall aroma of cooked meats (Morrissey, Sheehy, Galvin, Kerry, and Buckley, 1998). The use of herbs and spices has been widespread in recent years to inhibit the development of oxidative reactions in food systems as the natural antioxidants. In general, the hexanal content, which is a major breakdown product of linoleic acid oxidation (Frankel, 1996), has been used to determine of lipid oxidation and off-flavor development in cooked foods (Dupuy, Bailey, St Angelo, Legendre and Vercelotti, 1987). Byrnea, Brediea, Mottramb and Martensa (2002) found hexanal, heptanal, octanal and nonanal volatile compounds while studying the effect of oven cooking on warmed over flavors in chicken meat, but Marques et al (2013) clearly demonstrated that hexanal was the best indicator for lipid oxidation. The hexanal contents of pork meatballs packaged in aerobic and vacuum conditions during storage at 4°C are shown in Table 4.6.

Table 4.5 TBARS values (mg MDA/kg sample) of pork meatballs added herb and spice extracts and stored at 4°C for 9 days (mean±SD)

Storage time (days)	CON	HOB	VNC	TMR	GPP
Aerobically packaged					
0	1.36±0.05 Ad	0.20±0.01 Dd	0.26±0.03 Cd	0.61±0.04 Bd	0.18±0.04 Dc
3	1.53±0.06 Ac	0.31±0.03 Dc	0.41±0.05 Cc	0.90±0.06 Bc	0.23±0.02 Ebc
6	1.73±0.08 Ab	0.35±0.02 Db	0.44±0.03 Cb	1.00±0.99 Bb	0.25±0.03 Eb
9	2.09±0.17 Aa	0.43±0.03 Da	0.52±0.03 Ca	1.25±0.95 Ba	0.34±0.06 Ea
Vacuum packaged					
0	1.30±0.11 Ad	0.13±0.01 CDd	0.17±0.02 Cd	0.51±0.02 Bd	0.11±0.02 Dd
3	1.54±0.08 Ac	0.15±0.01 Dc	0.25±0.03 Cc	0.61±0.03 Bc	0.14±0.01 Dc
6	1.64±0.09 Ab	0.18±0.03 Db	0.31±0.04 Cb	0.76±0.03 Bb	0.17±0.01 Db
9	1.82±0.12 Aa	0.25±0.03 Da	0.36±0.04 Ca	0.90±0.04 Ba	0.23±0.03 Da

Within packaging condition; uppercase letters indicate significantly different in the row ($p<0.05$), lowercase letters indicate significantly different in the significantly different in the column ($p<0.05$), n = 6. CON = control, HOB = holy basil, VNC = Vietnamese coriander, TMR = turmeric, and GPP = green pepper.

Under the aerobic condition, storage period had significant influence on the development of lipid oxidation in the pork meatballs resulting in intensive increase in hexanal contents during the first day of refrigeration. At day 0, the pork meatballs with added selected herb and spice extracts had significantly ($p<0.05$) lower hexanal contents than control sample throughout storage times (day 9). However, among pork meatballs with selected herb and spice extracts, the pork meatballs with Vietnamese coriander extract had higher hexanal contents than did the meatballs with other herb and spice extracts ($p<0.05$) at day 0 of storage in aerobic conditions followed by pork meatballs added with holy basil, green pepper and turmeric extracts, which their hexanal contents were not significantly different ($p>0.05$). At day 3, the pork meatballs added with selected herb and spice extracts showed no significant difference ($p>0.05$) but lower than the control sample ($p<0.05$). The pork meatballs added with Vietnamese coriander and turmeric extracts had higher hexanal contents than the pork meatballs with holy basil and green pepper extracts ($p<0.05$) at day 6. At the end of storage, pork meatballs with Vietnamese coriander extract had higher hexanal content than the pork meatballs with holy basil and green pepper extracts ($p<0.05$) but no significant difference was found for the sample with turmeric extract ($p>0.05$).

The stability of cooked, refrigerated pork meatballs is also influenced by packaging systems, such as the vacuum packaging. The product is placed in a plastic bag with low gas permeability, the air is removed and the bag is sealed hermetically. Air removal enables to extend the shelf life of foodstuffs (Škrinjar and Nemet, 2009). Restricted access to air has previously been reported to limit oxidation in poultry meat (Ahn, Ajuyah, Wolfe and Sim, 1993).

In vacuum condition, hexanal contents of pork meatballs with selected herb and spice extracts had significantly ($p<0.05$) lower than control sample throughout storage times. Nevertheless, at day 0 and 3 of storage, hexanal contents of pork meatballs with selected herb and spice extracts were not significantly different ($p>0.05$) but at day 6 and 9 the meatballs with Vietnamese coriander and turmeric extracts had higher hexanal contents than those with holy basil and green pepper extracts ($p<0.05$). However, hexanal contents of all pork meatballs made with all four selected culinary herb and spice extracts and stored under vacuum packaging also increased throughout the storage ($p<0.05$). Mielnik, Olsen, Vogt, Adeline, and Skrede (2006) studied the interaction between packaging and antioxidant concentrations and reported that supplementation of low concentrations of grape seed extract needed to support packaging technique to delay the oxidation process in the cooked meat. The samples stored under vacuum maintained the initial levels of lipid oxidation nearly unchanged, while samples packaged in air contained three times higher levels of lipid oxidation at the end of the storage period. In addition, they illustrated that relationships between volatile compounds and TBARS values were positively correlated and highest correlation was found for hexanal and TBARS values. Therefore, they could serve as markers for the oxidation process in the cooked turkey breast meat. The result of correlation, TBARS value in the cooked turkey meat was highly correlated with concentration of hexanal which was $r^2 = 0.96$. Similarly, Juntachote, Berghofer, Siebenhandl and Bauer (2007) reported that changes in hexanal content were similar to changes in TBARS value. TBARS values and hexanal contents correlated well over the storage period, with a correlation coefficient of 0.96 ($p<0.05$).

Table 4.6 Hexanal contents (mg/kg sample) of pork meatballs added herb and spice extracts, aerobically and vacuum packaged, stored at 4°C for 9 days (mean ± SD)

Storage time (days)	CON	HOB	VNC	TMR	GPP
Aerobically packaged					
0	2.04±0.14 Ad	0.12±0.01 Cd	0.24±0.04 Bd	0.13±0.01 Cd	0.07±0.01 Cd
3	3.47±0.39 Ac	0.19±0.01 Bc	0.38±0.03 Bc	0.39±0.03 Bc	0.14±0.02 Bc
6	5.09±0.44 Ab	0.25±0.02 Cb	0.85±0.06 Bb	0.65±0.03 Bb	0.25±0.01 Cb
9	6.79±0.89 Ac	0.37±0.06 Ca	1.38±0.12 Ba	0.90±0.07 BCa	0.50±0.03 Ca
Vacuum packaged					
0	0.96±0.24 Ad	0.07±0.01 Bd	0.09±0.01 Bd	0.10±0.01 Bd	0.05±0.01 Bc
3	2.44±0.40 Ac	0.13±0.03 Bc	0.30±0.04 Bc	0.28±0.05 Bc	0.06±0.01 Bc
6	3.99±0.34 Ab	0.21±0.02 Cb	0.50±0.08 Bb	0.47±0.07 Bb	0.19±0.04 Cb
9	5.35±0.45 Aa	0.30±0.01 Da	0.66±0.07 BCa	0.71±0.05 Ba	0.34±0.03 CDa

Within packaging condition; uppercase letters indicate significantly different in the row ($p<0.05$), lowercase letters indicate significantly different in the significantly different in the column ($p<0.05$), n = 4. CON = control, HOB = holy basil, VNC = Vietnamese coriander, TMR = turmeric, and GPP = green pepper.

4.4.4 Water activity

Measurement of the water activity (Aw) has been identified as the primary factor in meat products and becomes increasingly more important because of Aw value influences different chemical reactions in the product as well as the surviving and the resistance of microorganisms. Most spoilage and pathogenic microorganisms cannot grow below Aw values of 0.91 (Naveena, Muthukumar, Muthulakshmi, Anjaneyulu and Kondaiah, 2012). Addition of salt and sugar during the processing of emulsion products in our study might have resulted in increase of osmotic pressure leading to reduction in Aw (Troller and Christian 1978). Lower Aw values in the present experiment might be due to the use of herb/spice extracts. Karpinska-Tymoszczyk (2007) also found that mixture of sage extract and sodium-isoascorbate added to turkey meatballs reduced their water activity compared with control samples. Thus, while raw meat samples had Aw of greater than 0.99, cooked samples had Aw of 0.98 (McDonald, Sun and Kenny, 2000). However, most investigators did not directly consider Aw as an important parameter of emulsion meat products (Leistner, Rodel, and Krispien, 1981; Sureshkumar, Venkataramanujam, Dushyyanthan and Kalaikannan, 2006). Labuza, Mcnally, Gallangher, Hawkes, and Hurtado (1972) reported a decrease in Aw might inhibit the growth of microorganisms and slow down the rate of chemical reactions. Aw in the turkey meatballs was 0.990-0.992 and decreased considerably at day 0 of storage times for 15 days to 0.983-0.985.

A gradual reduction in Aw in vacuum packaged meat products over storage was observed by Fernández-Fernández, Romero-Rodríguez and Vázquez-Odériz (2001). In this study, the Aw remained high throughout storage time. Table 4.7 shows the Aw values of pork meatballs with and without addition of herb and spice extracts.

The Aw values of meatballs in all treatments slightly decreased as time of storage went by but differences were not found ($p>0.05$). The values were in the range of 0.969-0.981. These results partly corresponded to those obtained by Nussu, Goncalves, Pereire Da Silva and Beserra (2003), who found that rosemary extract added 0.025% (w/w) and 0.050% (w/w) to fermented goat meat sausages, reduced Aw of the products to 0.886 and 0.881, respectively, as compared with that of control sample of 0.912.

4.4.5 Color of pork meatballs

The effects of selected herb and spice extracts on color changes of pork meatballs during storage at 4°C under aerobic packaging condition are shown in Table 4.8. The effects on color parameters of the pork meatballs after addition selected herb and spice extracts were compared with control samples. At the initial day (day 0), the addition holy basil, green pepper and Vietnamese coriander extracts significantly decreased the lightness (L*) values ($p<0.05$) but no significant difference ($p>0.05$) was not found when turmeric extract was added as compared with control sample. As storage time progressed, the pork meatballs addition of selected herb and spice extracts in all samples caused significant decrease in lightness (L*) values ($p<0.05$). The lightness (L*) values of all samples were in the range of 58.09 to 74.76. Satterlee, Brown and Lycometros (1972) suggest that this phenomenon results from the oxidation of myoglobin to metmyoglobin. It is also known that aerobic storage of meat oxidizes oxymyoglobin to metmyoglobin, causing meat to lose its bright red color and turn dullish gray. It was in agreement with Chouliara, Karatapanis, Savvaidis and Kontominas (2007) who reported that color values of all chicken meat treatments at selected sampling days. The L* value which refers to the lightness, decreased

progressively up to day 25 of storage due to the fact that the color of the product became more dull.

The redness (a*) values of all samples decreased as the storage time progressed ($p<0.05$). The redness color (a*) values of control samples were higher ($p<0.05$) than those of samples added with herb and spice extracts because of the color of extracts, holy basil was dark green-yellow color, green pepper was dark green color and Vietnamese coriander was dark black-green color but turmeric was bright yellow-orange color which creased redness of meat pigments and increased greenness. The highest decreasing in redness was found with the addition of holy basil extract followed by the extracts of green pepper, Vietnamese coriander and turmeric. Decreasing in redness values throughout the storage period was observed for all treatments of the meatballs. In addition, oxidation of myoglobin to metmyoglobin could be another factor due to denaturation of protein globin and accelerates the oxidation process of myoglobin to metmyoglobin in the sample after the heating process (Rosario Ramirez, Morcuende, Eztevez, and Cava, 2004). The a* values of all samples were in the range of -8.16 to 4.46.

The yellowness (b*) values of all samples of pork meatballs with storage time and the addition of turmeric extract significantly highest and increased the b* values ($p<0.05$) followed by holy basil extract. However the samples with added Vietnamese coriander and green pepper extract not significant ($p>0.05$) when compared with the control sample. In all samples of pork meatballs significantly increased the b* values ($p<0.05$), which were maintained throughout the storage period. The b* values of all samples was ranging from 12.19 to 25.53.

Table 4.7 The water activity (Aw) of pork meatballs added herb and spice extracts, aerobically and vacuum packaged, stored at 4°C for 9 days (mean ± SD)

Storage time (days)	CON	HOB	VNC	TMR	GPP
Aerobically packaged					
0	0.975±0.03 Ab	0.975±0.13 Aa	0.980±0.11 Aa	0.975±0.05 Aa	0.980±0.03 Aa
3	0.972±0.08 Aab	0.972±0.11 Aab	0.980±0.08 Aa	0.975±0.08 Aa	0.977±0.08 Aab
6	0.971±0.11 Ab	0.970±0.06 Ac	0.975±0.08 Aa	0.974±0.05 Aa	0.975±0.10 Ab
9	0.969±0.12 Ab	0.970±0.11 Ac	0.975±0.08 Aa	0.971±0.12 Ab	0.973±0.01 Ab
Vacuum packaged					
0	0.975±0.04 Aa	0.975±0.17 Aa	0.980±0.13 Aa	0.975±0.09 Aa	0.980±0.13 Aa
3	0.975±0.10 Aa	0.972±0.10 Aa	0.975±0.08 Aa	0.970±0.08 Ab	0.975±0.07 Ab
6	0.970±0.08 Ab	0.970±0.06 Aa	0.975±0.07 Aa	0.970±0.08 Ab	0.973±0.06 Ab
9	0.970±0.11 Ab	0.970±0.12 Aa	0.973±0.12 Aa	0.970±0.01 Ab	0.973±0.01 Ab

Within packaging condition; uppercase letters indicate significantly different in the row ($p<0.05$), lowercase letters indicate significantly different in the significantly different in the column ($p<0.05$), n = 6. CON = control, HOB = holy basil, VNC = Vietnamese coriander, TMR = turmeric, and GPP = green pepper.

The color values of all pork meatballs with added selected herb and spice extracts during storage at 4°C under vacuum packaging condition are shown in Table 4.9. The patterns of color changes under vacuum packaging condition were similar to the samples under aerobic packaging condition. Ahn and Lee (2004) reported no changes in color values for both aerobic and vacuum packaging condition turkey breast meat during 15 days of storage. These results were in agreement with finding by Chouliara et al. (2007) who reported that The combined effect of oregano essential oil (0.1% and 1% w/w) and modified atmosphere packaging (MAP) (30% CO2/70% N2 and 70% CO2/30% N2) on shelf-life extension of fresh chicken meat stored at 4°C had a no significant ($p>0.05$) effect on color changes with the exception of day 25 of storage when compared to the aerobic packaging condition. The L* value which refers to the lightness, decreased progressively up to day 9 of storage, the addition holy basil, green pepper and Vietnamese coriander extracts significantly decreased the lightness (L*) values ($p<0.05$) but addition turmeric extract was no significant difference ($p>0.05$) when compared to the control. As storage time progressed, the pork meatball samples addition selected herb and spice extracts in all samples, significantly decreased the lightness (L*) values ($p<0.05$). The lightness (L*) values of all samples were in the range of 58.22 to 74.74.34.

The redness (a*) values of all samples decreased as the storage time progressed ($p<0.05$). The redness color (a*) values of control samples were higher ($p<0.05$) than the samples with herb and spice extracts due to the color of extracts, the addition of holy basil extract had the lower a* values ($p<0.05$) than control sample at the day 0 followed by the pork meatballs with the extracts of green pepper, Vietnamese coriander and turmeric, respectively. The redness values of all samples decreased

throughout the storage period, the a* values of all samples were in the range of -8.09 to 5.43. The same pattern holds for parameter b* the values of which varied between 12.22 and 25.98. The addition of turmeric extract significantly gave the highest and increased the b* values ($p<0.05$) of the meatballs followed by holy basil extract. In contrast, the samples added with Vietnamese coriander and green pepper extracts were not significantly different ($p>0.05$) compared with control samples. However, b* values of all samples significantly increased ($p<0.05$) and maintained throughout storage period. The b* values of all samples were ranging from 12.19 to 25.98.

4.4.6 Texture analysis

The average force required to compress aerobically packaged and vacuum packaged meatballs are shown in Table 4.10. In general, texture of all pork meatballs of all treatments gradually increased with increasing storage time ($p<0.05$) for both packaging conditions. This could be due to all the herb and spice extracts were prepared in dry powder form which could absorb free water within the product (Fernández-López, Pérez-Alvarez and Aranda-Catalá, 2000), subsequently increasing in compressive force. However, adding all selected herb and spice extracts to the meatballs and packaged in aerobic condition caused an increase of compressive forces when compared with control meatballs ($p<0.05$). Karpinska-Tymoszczyk (2007) reported that after thermal processing, the conciseness of turkey meatballs was desirable, but deteriorated during storage, probably because of juice drip. The juiciness of heat-treated samples was also desirable; they became less juicy during storage. The texture of the turkey meatballs with sage extract at day 0 was soft and easy to bite, but during storage the meatballs became tougher or less springiness during storage.

Table 4.8 The color values of pork meatballs added herb and spice extracts, aerobically packaged, stored at 4°C for 9 days (mean ± SD)

Storage time (days)	Aerobically packaged				
	CON	HOB	VNC	TMR	GPP
The lightness (L*) values					
0	74.76 ± 3.55 Aa	60.40±1.61 Ca	71.22±2.10 Ba	74.16±1.71 Aa	71.64±1.05 Ba
3	73.82±1.13 Ab	59.93±1.21 Ea	69.62±1.14 Db	72.63±1.69 Bb	71.58±1.27 Ca
6	72.91±1.66 Ab	58.41±1.10 Eb	68.30±0.82 Dc	71.74±1.13 Bbc	71.07±1.74 Cab
9	72.90±1.08 Ab	58.09±1.67 Db	68.21±1.53 Cc	71.31±1.27 Bc	70.53±1.80 Bb
The redness (a*) values					
0	4.46±1.00 Aa	-6.17±1.57 Da	1.40±0.20 Ca	1.80±0.52 Ba	1.12±0.30 Ea
3	3.50±1.33 Ab	-6.47±0.68 Ea	1.38±0.19 Bb	1.48±0.30 Bb	-0.31±0.49 Cb
6	3.43±1.13 Abc	-6.46±0.40 Ea	1.33±0.24 Bbc	1.43±0.28 Bbc	-0.92±0.43 Cbc
9	2.75±0.99 Ac	-8.16±1.25 Db	1.34±0.21 Cc	1.38±0.42 Bc	-1.08±0.35 Cc
The yellowness (b*) values					
0	12.22±1.86 Cb	21.59±2.88 Bb	12.75±1.91 Cb	24.30±2.08 Ab	12.56±1.96 Cb
3	14.75±0.64 Ca	23.66±0.79 Ba	14.99±1.01 Ca	24.94±2.14 Aa	14.52±0.79 Ca
6	14.66±0.76 Ca	24.01±0.81Ba	14.87±0.95 Ca	25.40±0.61 Aa	14.45±0.81 Ca
9	15.02±0.55 Ca	24.06±0.35 Ba	15.06±0.66 Ca	25.53±0.78 Aa	14.76±0.35 Ca

Within packaging condition; uppercase letters indicate significantly different in the row ($p<0.05$), lowercase letters indicate significantly different in the significantly different in the column ($p<0.05$), n = 20. CON = control, HOB = holy basil, VNC = Vietnamese coriander, TMR = turmeric, and GPP = green pepper.

Table 4.9 The color values of pork meatballs added herb and spice extracts, vacuum packaged, stored at 4°C for 9 days (mean ± SD)

Storage time (days)	Vacuum packaged				
	CON	HOB	VNC	TMR	GPP
The lightness (L*) values					
0	74.34±3.23 Aa	60.22±2.89 Da	70.83±1.93 Ca	74.22±1.78 Aa	73.43±3.32 Ba
3	72.41±1.13 Ab	59.83±1.08 Dab	70.13±1.24 Ca	72.92±1.34 Ab	71.63±1.17 Bb
6	71.80±1.56 Ab	58.03±1.01 Dbc	68.37±1.36 Cb	71.32±0.86 Ac	70.44±1.86 Bb
9	71.48±1.67 Ab	58.22±3.20 Dc	68.04±1.83 Cb	71.11±1.51 Ac	70.29±1.90 Bb
The redness (a*) values					
0	5.43±1.08 Aa	-6.16±0.49 Da	1.57±0.40 Ca	2.74±1.48 Ba	1.39±0.28 Ca
3	3.55±1.18 Ab	-6.47±0.68 Ea	1.46±0.70 Ca	2.08±0.21 Bab	0.11±0.46 Db
6	3.40±1.41 Ab	-6.46±0.40 Ea	1.43±0.38 Ca	2.48±0.32 Bab	-0.21±0.49 Dc
9	2.79±0.89 Ab	-8.09±1.23 Db	1.41±0.56 Ba	1.63±0.28 Bc	-0.29±0.46 Cc
The yellowness (b*) values					
0	12.22±1.86 Cb	21.57±4.45 Bb	12.53±3.44 Db	23.96±3.10 Ab	13.05±3.56 Cb
3	14.75±0.64 Ca	23.58±1.51 Ba	14.58±1.06 Ca	24.91±2.48 Ab	15.18±0.95 Ca
6	14.66±0.76 Ca	23.91±0.96 Ba	14.66±0.71 Da	25.03±1.28 Ab	15.16±1.02 Ca
9	15.02±0.55 Ca	24.46±2.75 Ba	15.36±0.38 Ca	25.98±2.66 Aa	15.41±0.35 Ca

Within packaging condition; uppercase letters indicate significantly different in the row ($p<0.05$), lowercase letters indicate significantly different in the significantly different in the column ($p<0.05$), n = 20. CON = control, HOB = holy basil, VNC = Vietnamese coriander, TMR = turmeric, and GPP = green pepper.

The compressive force of all samples was ranging from 399.87 to 470.02 g. The compressive forces (g) were also the same trend for both packaging conditions which increased with increasing storage time.

4.4.7 Sensory evaluation

Sensory attributes consisted of oxidized, spicy, off-flavor and texture; hardness and springiness. The results of a sensory evaluation of pork meatballs with selected herb and spice extracts during storage at 4°C under aerobic packaging condition are shown in Table 4.11. Control sample showed higher ($p<0.05$) score for oxidized flavor than the samples added with selected herb and spice extracts, indicating that the oxidation reaction began during product processing. The addition of selected herb and spice extracts in pork meatballs could delay such reactions as the control samples were already oxidized since the beginning of meatballs processing. Differences of oxidized flavor ($p<0.05$) between storage times were obviously detected by panelists. When oxidation reactions began to occur in the samples with selected herb and spice extracts, detection of the panelists mainly in the treatments added green pepper extract which received the lowest oxidized flavor scores followed by the pork meatballs with holy basil, Vietnamese coriander and turmeric extracts, which were not significantly different ($p>0.05$). Within the treatments, the oxidized flavor scores of the meatballs with selected herb and spice extracts were not significantly different ($p>0.05$). These results were not in agreement with TBARS values (Table 4.5) hexanal contents (Table 4.6).

Table 4.10 Compression force (g) of pork meatballs added herb and spice extracts, aerobically and vacuum packaged, stored at 4°C for 9 days (mean ± SD)

Storage time (days)	CON	HOB	VNC	TMR	GPP
Aerobically packaged					
0	378.03±10.18 Cb	395.09±11.32 BCb	427.64±12.16Ab	421.12±10.50 ABc	392.01±10.54 Cb
3	389.52±10.32 Bb	412.76±11.75 ABb	437.87±12.68 Ab	439.25±11.97 Abc	412.47±11.28 ABb
6	451.49±11.49 Aa	456.23±11.88 Aa	471.46±12.79 Aa	466.89±12.52 Aab	455.43±12.41 Aa
9	456.23±12.44 Ba	469.14±12.20 ABa	487.54±12.86 Aa	472.69±13.15 ABa	461.96±12.85 ABa
Vacuum packaged					
0	388.63±9.19 Bb	425.76±10.57 Ab	414.25±11.04 ABc	408.05±9.97 ABb	410.78±11.00 ABc
3	400.78±10.47 Bb	443.60±10.74 Aab	447.48±11.98 Ab	428.92±11.00 Aab	422.92±11.93 ABc
6	403.68±11.66 Cb	449.39±10.82 ABab	470.69±12.15 Aab	435.50±11.85 Ba	450.37±12.14 ABb
9	442.18±13.06 Aa	458.36±12.38 ABb	485.89±13.50 Aa	445.74±12.71 Aa	480.23±12.34 Aa

Within packaging condition; uppercase letters indicate significantly different in the row ($p<0.05$), lowercase letters indicate significantly different in the significantly different in the column ($p<0.05$), n = 20. CON = control, HOB = holy basil, VNC = Vietnamese coriander, TMR = turmeric, and GPP = green pepper.

It could be that panelists may not be sensitive enough to detect minor differences at high oxidized flavor levels (Ahn, Grün and Fernando, 2002). Nussu, Goncalves, Pereire Da Silva and Beserra (2003) reported that only oxidized flavor attribute showed a significant correlation ($p<0.01$) with TBARS values, indicating that the trained sensory panel evaluated samples containing different antioxidant levels during storage time of the control treatment. Ahn, Grün and Fernando (2002) reported significant correlation coefficients were between TBARS values and hexanal contents ($r=0.96$; $p<0.01$), between warmed-over flavor (WOF) scores or oxidized flavor scores and TBARS values ($r=0.90$; $p<0.01$), and between WOF scores and hexanal content ($r=0.87$; $p<0.01$), indicating that TBARS values and hexanal contents could be used as reliable indicators of WOF in cooked ground beef. Moreover, pork meatballs with herb and spice extracts showed the lowest oxidized intensity scores which not significantly different ($p>0.05$) indicating that good sources of antioxidant adding in the meatballs could reduce oxidized flavor.

The spicy scores of control sample was significantly ($p<0.05$) lower than those of pork meatballs with holy basil, green pepper and turmeric extracts, except for Vietnamese coriander extract during day 0-6 of storage but at day 9, the pork meatballs with holy basil and Vietnamese coriander extract were not significant different ($p>0.05$) when compared with control sample. Among all treatments, spicy flavor of the meatballs were not significantly different ($p>0.05$) throughout the storage times. The spicy scores showed a similar decreasing pattern when storage times increased.

The off-flavor of control sample and pork meatballs added with turmeric extract were lower ($p<0.05$) than those added with holy basil, Vietnamese coriander

and green pepper throughout storage time. The samples from among all treatments were not significant different ($p>0.05$) throughout the storage time. Moreover, the panelists were detected slight bitter taste in the samples with herb and spice extracts. Ferna´ndez-Lo´pez, Zhi, Aleson-Carbonell , Pe´rez-Alvarez and Kuri (2005) reported that off odor was observed between treatments ($p<0.05$) but not between storage days which suggested that off odor perception could be attributed to the peculiar composition of each extract but was not mainly due to type of extract. Therefore, optimized application of herb and spice extracts in foods was important to sensory acceptability. Gutierrez, Rodriguez, Barry-Ryan and Bourke (2008), reported that lettuce samples treated with thyme and lemon balm at concentrations of 500 and 1000 ppm, respectively, were rejected by panelists as they perceived strong chemical odors from these samples. Therefore, to apply herb and spice extracts in food products as natural antioxidant and antimicrobial agents, organoleptic impact should be considered as the use of naturally derived preservatives could alter the taste of food or exceed acceptable flavor thresholds. The problem may occur if high concentrations required achieving useful essential oil antimicrobial activity, resulting in unacceptable levels of flavors and odors (Gutierrez, Barry-Ryan and Bourke, 2009; Škrinjar and Nemet, 2009).

Hardness and springiness characteristics of the pork meatballs in aerobic packaging condition by panelists were not different ($p>0.05$) among treatments and throughout storage time within each treatment. In general, the hardness scores showed a similar increasing pattern when storage times increased. Karpińska-Tymoszczyk (2007) reported that after thermal processing, the conciseness of turkey meatballs was desirable, but deteriorated during storage, probably because of juice drip. The

juiciness of heat-treated samples was also desirable; they became less juicy during storage. Similarly, texture of these experimental products was at first soft and easy to bite, but during storage the meatballs became tough. As a result, the hardness in all samples increased with increasing storage time. Mittal and Barbut (1994) reported that low fat meat products were considered to be tenderer than the high fat products. This is probably because the low fat products had more moisture, which requires less force for biting. The tenderness results showed the same trend as the objective springiness results.

Table 4.11 The sensory evaluation of pork meatballs added herb and spice extracts, aerobically packaged, stored at 4°C for 9 days (mean ± SD)

Storage time (days)	Aerobically packaged				
	CON	**HOB**	**VNC**	**TMR**	**GPP**
Oxidized scores					
0	2.45±2.66 Ab	0.79±1.94 Ba	1.13±1.49 Ba	0.59±0.80 Ba	0.92±2.11 Ba
3	3.28±3.03 Aa	0.83±0.88 Ba	1.17±1.32 Ba	0.71±0.81 Ba	0.97±1.27 Ba
6	3.57±3.30 Aa	0.98±1.35 Ba	1.47±1.49 Ba	0.77±0.94 Ba	1.11±1.46 Ba
9	3.65±3.11 Aa	1.01±1.33 Ba	1.69±1.98 Ba	1.01±1.41 Ba	1.29±1.13 Ba
Spicy scores					
0	0.47±0.78 Ba	1.69±1.94 Aa	1.43±1.49 ABa	2.01±1.41 Aa	2.31±2.11 Ba
3	0.38±0.92 Ba	1.63±0.88 Aa	1.41±1.32 ABa	1.70±0.94 Aa	2.29±1.27 Aa
6	035±0.74 Ba	1.59±1.34 Ba	1.17±1.49 ABa	1.67±0.81 Ba	1.61±1.46Aa
9	0.35±0.76 Ba	1.10±1.33 ABa	0.95±1.98 ABa	1.58±0.80 Aa	1.57±1.13 Aa
Off-flavor scores					
0	2.08±2.39 Ba	4.50±3.33 Aa	4.27±2.24 Aa	2.29±2.77 Ba	4.42±3.44 Aa
3	2.41±2.63 Ba	5.16±3.77 Aa	4.42±3.11 Aa	2.54±2.24 Ba	4.68±3.27 Aa
6	2.56±2.74 Ba	5.33±3.65 Aa	4.76±3.21 Aa	2.61±2.66 Ba	4.83±3.59 Aa
9	3.04±2.79 Ba	5.79±3.46 Aa	5.2±2.91 Aa	2.66±2.59 Ba	5.26±3.20 Aa

Table 4.11(Continued)

Storage time (days)	Aerobically packaged				
	CON	**HOB**	**VNC**	**TMR**	**GPP**
Hardness scores					
0	2.94±1.59 Ab	2.85±1.81 Ab	2.92±1.90 Ab	3.09±1.63 Aa	3.06±1.89 Aa
3	3.58±2.25 Aab	3.43±1.97 Aab	3.38±2.38 Aab	3.51±1.80 Aa	3.36±1.99 Aa
6	3.96±2.20 Aa	3.63±2.00 Aab	3.39±1.72 Aab	3.53±2.11 Aa	3.42±2.25 Aa
9	4.32±2.11 Aa	3.95±1.84 Aa	4.05±1.86 Aa	3.58±1.92 Aa	3.70±1.82 Aa
Springiness score					
0	4.37±2.32	4.38±1.88	4.56±2.03	4.53±1.46	4.16±1.87
3	4.07±2.11	4.31±2.05	4.34±1.80	4.38±1.67	4.07±2.15
6	3.82±2.13	4.12±2.46	4.22±2.24	4.37±2.23	3.80±2.25
9	3.88±2.40	4.08±2.02	4.08±2.52	4.28±1.98	3.56±1.82

Uppercase letters indicate significantly different in the row ($p < 0.05$), lowercase letters indicate significantly different in the significantly different in the column ($p < 0.05$), n = 18. CON = control, HOB = holy basil, VNC = Vietnamese coriander, TMR = turmeric, and GPP = green pepper.

Texture of pork meatballs kept under vacuum packaging condition as evaluated by panelists are shown in Table 4.12. The attributes were evaluated similarly to the samples kept under aerobic packaging condition. The score of oxidized flavor of control sample was higher ($p<0.05$) than those samples with herb and spice extracts. At day 0, the pork meatball added Vietnamese coriander extract had similar oxidized flavor scores to control sample. However, samples added holy basil, turmeric and green pepper extracts had lower ($p<0.05$) oxidized flavor scores, and similarly between day 3 to day 9 of storage although slight increase was observed as storage time progressed.

The spicy scores of control sample was significantly ($p<0.05$) lower than those of pork meatballs added holy basil, green pepper and turmeric extracts, except for

Vietnamese coriander extract ($p>0.05$) when compared with control sample. Within treatments, control sample and meatballs added holy basil and turmeric extracts were not significant ($p>0.05$) throughout the storage times. However, the pork meatballs added Vietnamese coriander and green pepper extracts decreased significantly ($p<0.05$) the spicy scores at day 0 and day 9.

The off-flavor scores of control sample and pork meatballs with added turmeric extract were significantly ($p<0.05$) lower than the pork meatballs added holy basil and green pepper extracts but did not differ compared with the meatballs added Vietnamese coriander ($p>0.05$) at day 0. At day 3 and 6, the control sample and pork meatballs added turmeric extract were significantly ($p<0.05$) lower than those with holy basil, Vietnamese coriander and green pepper extracts. However, off-flavor of all meatball samples at day 9 was not differ ($p>0.05$). In addition, off-flavor of all meatballs within treatments was not significant different ($p>0.05$) throughout the storage time. However, off-flavor of all samples increased with increasing storage time.

Hardness and springiness characteristics of vacuum packaged pork meatballs were not differed ($p>0.05$) among and within treatments throughout storage time. However, increasing in hardness and less springiness along the storage time was observed.

Table 4.12 The sensory evaluation of pork meatballs added herb and spice extracts, vacuum packaged, stored at 4°C for 9 days (mean ± SD)

Storage time (days)	Vacuum packaged				
	CON	**HOB**	**VNC**	**TMR**	**GPP**
Oxidized scores					
0	2.24±2.69 Aa	0.77±2.09 Ba	1.32±2.13 ABa	0.83±2.08 Ba	1.09±2.48 Ba
3	3.17±3.15 Aa	0.89±1.03 Ba	1.37±1.57 Ba	0.97±1.44 Ba	1.11±1.35 Ba
6	3.34±3.30 Aa	1.06±0.91 Ba	1.48±1.68 Ba	1.01±1.50 Ba	1.31±1.32 Ba
9	3.68±3.09 Aa	1.01±1.24 Ba	1.91±1.93 Ba	1.11±1.34 Ba	1.45±1.66 Ba
Spicy scores					
0	0.62±1.41 Ba	2.04±2.87 Aa	1.55±2.44 ABa	2.28±2.98 Aa	2.73±2.68 Aa
3	0.40±0.71 Ca	1.60±1.83 ABa	1.05±1.11 BCab	1.71±2.49 ABa	2.67±2.22 Aab
6	0.32±0.74 Ca	1.24±1.60 ABa	1.05±3.10 BCab	1.66±2.58 ABa	2.07±1.94 Aab
9	0.36±0.83 Ca	1.19±2.24 ABa	0.81±3.24 BCb	1.60±2.22 ABa	1.66±2.35 ABb
Off-flavor scores					
0	2.16±2.95 Ba	5.10±3.37 Aa	3.77±2.48 ABa	2.08±2.78 Ba	4.49±3.34 Aa
3	2.33±2.90 Ba	5.27±3.71 Aa	4.08±3.21 Aa	2.09±2.46 Ba	4.73±3.43 Aa
6	2.21±2.78 Ba	5.48±3.76 Aa	4.43±3.10 Aa	2.72±1.74 Ba	4.81±3.54 Aa
9	2.35±2.67 Ca	5.84±3.45 Aa	4.46±3.25 ABa	2.79±2.92 BCa	4.97±3.38 Aa
Hardness scores					
0	3.12±1.92	3.16±1.85	2.95±1.60	2.69±1.57	2.78±1.55
3	3.35±2.29	3.76±2.07	3.07±1.91	2.96±2.02	3.13±1.99
6	3.43±2.12	3.69±2.42	3.37±1.82	3.25±1.74	3.13±1.96
9	3.97±2.38	3.56±2.02	3.53±1.77	3.32±1.70	3.21±1.85
Springiness score					
0	4.68±1.91 Aa	6.10±1.62 Aa	4.44±1.96 Aa	5.26±1.70 Aa	4.52±2.02 Aa
3	4.26±2.67 Aa	6.06±8.79 Aa	4.49±2.16 Aa	4.97±2.16 Aa	4.47±2.38 Aa
6	4.09±2.43 Aa	4.68±8.84 Aa	4.41±2.09 Aa	4.62±2.09 Aab	4.32±2.16Aa
9	3.25±2.58 Aa	4.06±2.21 Aa	4.22±2.33 Aa	3.99±2.02 Ab	4.07±2.02 Aa

Uppercase letters indicate significantly different in the row ($p < 0.05$), lowercase letters indicate significantly different in the significantly different in the column ($p < 0.05$), n = 18. CON = control, HOB = holy basil, VNC = Vietnamese coriander, TMR = turmeric, and GPP = green pepper.

4.5 Conclusions

The antioxidant and antibacterial activities of the ethanolic extracts from four selected Thai culinary herbs and spices, i.e., holy basil, Vietnamese coriander, turmeric and green pepper were used as natural bioactive ingredients in pork meatballs for improving shelf life. Antimicrobial inhibition of selected culinary herb and spice extracts were tested against indicator bacteria. From clear zone forming, holy basil extract could inhibit two Gram-positive bacteria, i.e., *Enterococcus faecalis* (AP-31) and *Micrococcus luteus* TISTR 745 and green pepper extract could inhibit *Micrococcus luteus* TISTR 745 while Vietnamese coriander extract was able to inhibit *Acinetobacter calcoaceticus* TISTR 1264. For antimicrobial activity of herb and spice extracts added in pork meatballs made with the extracts from holy basil and green pepper and packaged in both aerobic and vacuum conditions had the highest shelf life of 9 days while those made with Vietnamese coriander and turmeric extracts had the shelf life of about 6-9 days and the control meatballs had the shelf life less than 6 days.

From TBARS values, holy basil, Vietnamese coriander and green pepper showed stronger antioxidant effects in the pork meatballs than did turmeric throughout 9 days of storage period for both aerobic and vacuum packages. However, it was clear that all four selected Thai culinary herb and spice extracts were found to influence the hexanal contents significantly ($p<0.05$). Initial hexanal contents for all treatments were significantly lower than those of control ($p<0.05$). Hexanal contents increased with increasing storage time. The treated samples of all treatments had lower hexanal contents than the control sample in both aerobic and vacuum packaging conditions during storage time. The green pepper extract significantly decreased lipid oxidation

and showed stronger in both aerobic and vacuum packaging conditions. In addition, hexanal contents of all four selected extracts were lower in vacuum condition in aerobic packaging condition during storage at 4°C. In addition, the water activity values of all meatballs were not significantly different ($p>0.05$) which were in the range of 0.969-0.980.

Color of pork meatball samples measured in term of L* (lightness) were no significant differences ($p>0.05$) of L* values among all pork meatball samples packaged under both aerobic and vacuum conditions throughout storage period, except the pork meatballs with holy basil extract showed lower lightness values of both packaging conditions. The redness (a*) values of pork meatballs with holy basil and turmeric extracts decreased in both packaging conditions because of color of the extracts, green pepper is dark green color and turmeric is yellow color. The yellowness (b*) values were also showed the same tend as the redness but the pork meatballs with holy basil and turmeric extracts were increased in yellowness values of aerobic and vacuum packaging. Compressive force (g) of pork meatballs in aerobically and vacuum packaged conditions throughout storage period were no significant differences ($p>0.05$) among treatments. By sensory evaluation, the pork meatballs with herb and spice extracts showed the spicy and off-flavor attributes higher score than control sample were not significant ($p>0.05$) at the at the end of storage times. The spicy and off-flavor scores showed a similar decreasing pattern when increasing storage times in both packaged. The pork meatballs with holy basil and green pepper showed the lowest oxidized intensity and bacterial counts which indicated that these two spices might be the good sources of antioxidant for adding in the pork meatballs to antioxidant and antibacterial.

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CHAPTER V

STABILITY OF AROMA-IMPACTCOMPOUNDS OF SELECTED THAI CULINARY HERB AND SPICE EXTRACTS IN COMMINUTED MEAT PRODUCT

5.1 Abstract

The objectives of this study was to investigate the changes of aroma-impact compounds of ethanolic extracts from four selected Thai culinary herbs and spices, i.e., holy basil, Vietnamese coriander, turmeric and green pepper incorporated in pork meatballs, aerobically and vacuum packed and storage at refrigerated temperatures (4ºC). Each of the herb and spice extract was mixed in pork meatball batter in the proportion of 0.2 % (w/w), the batter was stuffed in plastic casing, cooked to an internal temperature of 70ºC then, cooled in ice water. The pork meatball model was cut into pieces of 2.5 cm length, aerobically and vacuum packed and stored at 4ºC for 9 days. Changes of major aroma-impact compounds of each herb and spice extract were identified and quantified by using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) for every 3 days.

The authentic chemical of major aroma-impact compound of each herb and spice was used as chemical standard and for confirmation were △-3-carene for green pepper extract; methyl eugenol for holy basil extract; *ar*-turmarone for turmeric

extract, and (*Z*)-3-hexanal for Vietnamese coriander extract. The results showed that decreasing in concentration of all major aroma-impact compounds were obviously higher ($p<0.05$) in aerobically packed meatballs than those in the vacuum packed ones.

Keywords: - Thai culinary herbs and spices, ethanolic extracts, major aroma-impact compounds, pork meatballs, aerobic and vacuum packaging.

5.2 Introduction

In recent years, there has been an increasing concern about the safety of synthetic food additives, such as butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT), including the possible toxicity of the synthetic chemicals used as antioxidants. Naturally occurring compounds particularly herbs and spices have been chosen as a safe alternative to synthetic antioxidants. Furthermore, many herbs, spices, and their extracts have been added in a variety of foods to improve their sensory characteristics and extend shelf-life by retarding lipid oxidation and microbial growth (Shahidi, Janitha, and Wanasundara, 1992). The antioxidant activity of these plants is attributed to their phenolic compound contents, which includes volatile compounds also known as essential oils (Teissedre and Waterhouse, 2000).

Apart from the above mentioned, many herbs and spices are also used in food products to contribute aroma, taste, and flavor such as allspice, cinnamon, basil, dill, nutmeg, fennel, parsley, anise, marjoram, cumin, mint, cardamom, mace and tarragon (Davison, 1999). Therefore, herbs and spices usually contained essential oils which shownot only antioxidant activity but also carry flavor (Teissedre and Waterhouse, 2000).

Essential oils are very complex natural mixtures which can contain about 20-60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20-70%) compared to others components present in trace amounts. The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular weight (Bakkali, Averbeck, Averbeck and Idomar, 2008).

The major volatile compounds found in the essential oils of clove and thyme is eugenol and thymol, respectively, both of them also retarded the increasing of peroxide value (POV) of dehydrated pork. Quercetin, the main compound in onion, has been shown to reduce lipid oxidation in cooking and storage of cooked meat (Tang and Cronin, 2007). The extracts of garlic and shallot bulbs have significant antioxidant potential, as measured by decreasing in free radicals and an ability to inhibit lipid oxidation (Leelarungrayub, Rattanapanone, Chanarat and Janusz, 2006). Shogaol and zingerone, the pungent volatile compounds found in ginger, have been shown to exhibit strong antioxidant activities (Kikuzaki and Nakatani, 1993). Essential oil of oregano and sage shows significantly reduces the oxidation, while the heat treatment and storage time significantly affect the antioxidant activity of the meat (Fasseas, Mountzouris, Tarantilis, Polissiou and Zervas, 2007).

The major volatile compounds found in essential oil of holy basil are reported to bemethyl eugenol, methyl chavicol, and eugenol (Vani, Cheng and Chuah, 2009). Vietnamese coriander, a strong coriander leaf with lemony and green type of smell, is one of those numerous herbs that give its unique touch due to (*Z*)-3-hexenal, (*Z*)-3-hexenol, and decanal (Starkenmann, Luca, Niclass, Praz, and Roguet, 2006). Delta-3-carene constitutes in high content in green pepper extract followed by beta-pinene and

limonene (Orav, Stulova, Kailas and Müürisepp, 2004). The major volatile component in turmeric extract has been reported to be aromatic-turmerone followed by alpha-turmarone and beta-turmarone (Singh et al., 2010). By nature, aroma compounds in herbs and spices are volatile causing reduction of their flavor intensity from time-to-time during food storage. Because of volatility of the character-impact compounds appeared in herbs and spices, gas chromatography with flame ionizationdetector (GC-FID) or gaschromatography-mass spectrometry (GC-MS) are normally used to monitor the changes of these compounds during storage. Therefore, the objective of this study was to investigate the changes of some principal aroma character-impact compounds of culinary herbs and spices generally used in Thai cooking. Pork meatball models mixed with individual ethanolic extract form four selected Thai culinary herbs and spices were used for monitoring the major aroma-impact compounds during refrigerated storage in aerobic and vacuum packaging.

5.3 Materials and methods

5.3.1 Chemical

Methyl eugenol**,** (z)-3-hexenal, *ar*-turmerone and △-3-carene were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA).

5.3.2 Spiced pork meatball models

Spiced pork meatballs were prepared as described in 4.2.1, Chapter IV. The meatballs were aerobically and vacuum packaged in plastic bags and stored at 4°C for 9 days. The meatball samples were randomly taken for monitoring the stability of aroma-impact compound of each herb and spice extract used every 3 day during storage.

5.3.3 Identification for confirmation of aroma-impact compounds

One gram of pork meatball sample and one gram of sodium chloride were weighed into a 22 mL headspace vial and 3 mL of distilled water was added. The vial was crimped with magnetic caps and Teflon septum. The headspace volatiles in the vial above the meatball sample were analyzed using solid phase microextraction-gas chromatrography-mass spectrometry (SPME-GC-MS). Volatile compounds were extracted from headspace of the sample using a 3-phase SPME fiber (1cm-50/30 μm Stable Flex Divinyl-benzene/Carboxen/PDMS, Supelco, PA, USA) at 50ºC for 30 min in a heating block. Volatile compounds were desorbed for 3 min into the injection port of a gas chromatograph at 250ºC. Separation of the desorbed volatile compounds are achieved using GC-MS (CP-3800 GC, Varian Inc., Walnut Creek, CA, USA connected to a capillary column (DB-Wax, 60 m×0.25 mm×0.25 μm Agilent Technologies, Redwood, CA., U.S.A.). The oven temperature was increased from 25 to 200ºC at 4ºC/min. Volatile compounds were identified using a quadrupole mass detector (Mass spectrometer 1200L quadrupole, Varian Inc., Walnut Creek, CA., USA). Mass spectra of volatile compounds were obtained by electron ionization (EI) at 70 eV.

Compound identification was based on comparing retention indices, mass spectra in mass spectral library (National Institute of Standards; NIST data) and with those of authentic standards analyzed under identical conditions in the case of positive identification. Tentative identification was based on matching retention indices and against authentic standard. The relative retention index was performed using a homologous series of *n*-alkanes and calculated according to (Harris, 1987) under the same chromatographic conditions as following equation:

$$I = 100\left[n + (N - n)\frac{\log t'_r(\text{unknown}) - \log t'_r(n)}{\log t'_r(N) - \log t'_r(n)}\right]$$

Where n is the number of carbon atoms in the smaller alkane, N the number of carbon atoms in the larger alkane, t'_r (n) the adjust retention time of the smaller alkane, and t'_r (N) is the adjusted retention time of the larger alkane (Harris, 1987).

5.3.4 Quantitation of aroma-impact compounds

One gram of sample and one gram of sodium chloride were weighed into a 22 mL headspace vial and 3 mL of distilled water was added. The vial was crimped with aluminum caps and Teflon septa and equilibrated in the headspace autosampler (Tekmar HT3, Teledyne Tekmar, Mason, OH, USA) at a platen temperature of 75°C (the sample temperature was 75°C when the equilibrium was reached). After thermal equilibration, sample was mixed for 2 min during the mix mode pre-programmed in the Teckmar HT3 autosampler. The vial was shaken during this mode, which may reduce the mean diffusion path length of solutes as they migrate to the gas/sample interface within the vial. Sample was then stabilized for 2 min, pressurized for 0.3 min, and equilibrated for 0.05 min in the autosampler. After the loop was filled and equilibrated for 0.3 min, the carrier gas (helium) back flushed the loop and carried the volatiles through the heated transfer line (150°C) into the GC. The released volatiles were automatically injected and separated in the GC capillary column (DB-Wax, 60 m×0.25 mm×0.25 μm Agilent Technologies, Redwood, CA., USA). Column temperature was 120°C, isothermal; injector temperature was set at 220°C and FID was at 250°C. Flow rate of the helium carrier gas was 28 mL/min with an inlet pressure of 10 psi and a split injection ratio of 1:10. Air, hydrogen flows were adjusted to 300 and 30 mL/min, respectively. The oven temperature was programmed from 35

to 200 at 10°C/min and then 200 to 240 at 20°C/min. The key volatile compounds in pork meatballs were quantified using the external standard curve as following equation:

$$\text{Concentration}\left(\frac{\mu g}{g}\right)=\text{Area ratio of}\left(\frac{\text{sample}}{\text{standard}}\right)\times\left(\frac{\text{amount of standard}}{\text{g of sample}}\right)$$

5.3.5 Statistical analysis

Statistical analysis was evaluated in completely Randomized design (CRD) using SPSS for Windows and means comparison by Duncan's Multiple Range Tests (DMRT) were analyzed. All experiments were performed in duplicate. Each replicate was chemically analyzed in duplicate samples. Statistical difference was determined at $p \leq 0.05$.

5.4 Results and discussion

5.4.1 Identification for confirmation of major aroma-impact compound

Determination of unknowns in gas chromatography (GC) requires two independent forms of identification such as the retention time on two different chromatographic columns, retention time and mass spectral match, or retention time and aromatic match (Harris, 1987). Because retention times vary depending on the temperature programming of the GC, the relative retention index using a series of standards n-alkanes by solid-phase microextraction (SPME) with DB-wax column and quadrupole mass spectrometric detector was performed.

The authentic chemical standard of aroma-impact compound of each herb and spice was used as chemical standard and for confirmation. The compound selected

from the highest concentration of each extract such as such as △-3-carene (Delta-3-carene) for green pepper extract; methyl eugenol for holy basil extract; *ar*-turmarone for turmeric extract, and (*Z*)-3-hexanal for Vietnamese coriander extract.

Volatile compounds were identified by comparison of their mass spectra with library as well as comparing retention index as shown in table 5.1.

Table 5.1 Authentic aromatic compounds used as chemical standards and its retention index obtained for compound identification

Herb/spice	**Aroma compound**	**Retention index**
Holy basil	Methyl eugenol	2007
Vietnamese coriander	(*Z*)-3-hexenal	1135
Green pepper	△-3-carene	1187
Turmeric	*Ar*-turmerone	1640

5.4.1.1 The major aroma-impact compound in holy basil extract

The major aroma-impact compound in holy basil extract identified by GC-MS was methyleugenol and confirmed with authentic chemical standard. Methyleugenol has a molecular ion of 178 and the RI of 2007 (Figure 5.1and Table 5.1).

5.4.1.2 The major aroma-impact compound in Vietnamese coriander extract

(*Z*)-3-hexenal is one of the major aroma-impact compounds of Vietnamese coriander extract. It was also identified by GC-MS and confirmed by using authentic chemical compound as it mass spectrum shown in Figure 5.2. The molecular ion of (*Z*)-3-hexenal is 98 and its RI was 1135 as shown in Table 5.1.

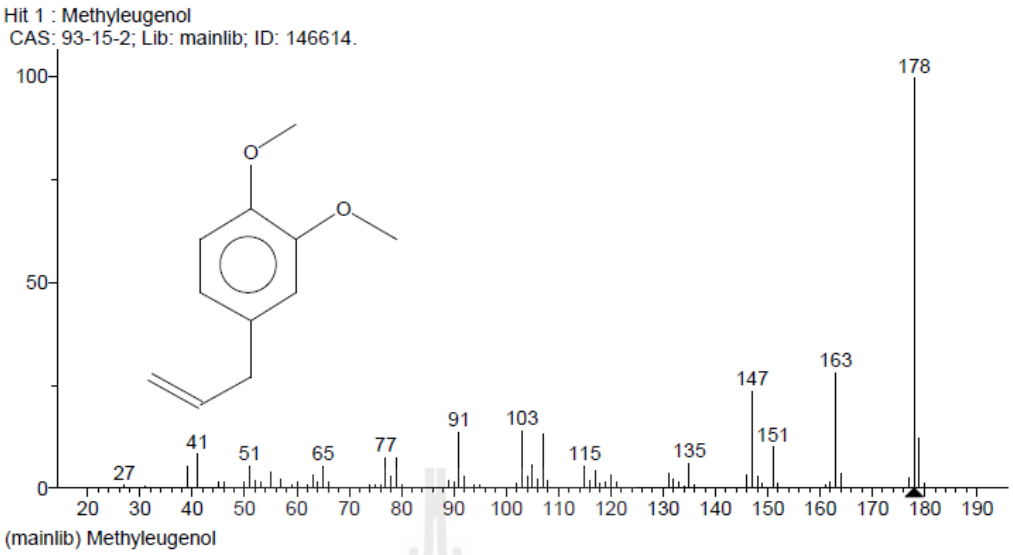

Figure 5.1 Mass spectrum of methyleugenol, the major aroma-impact compound in holy basil extract.

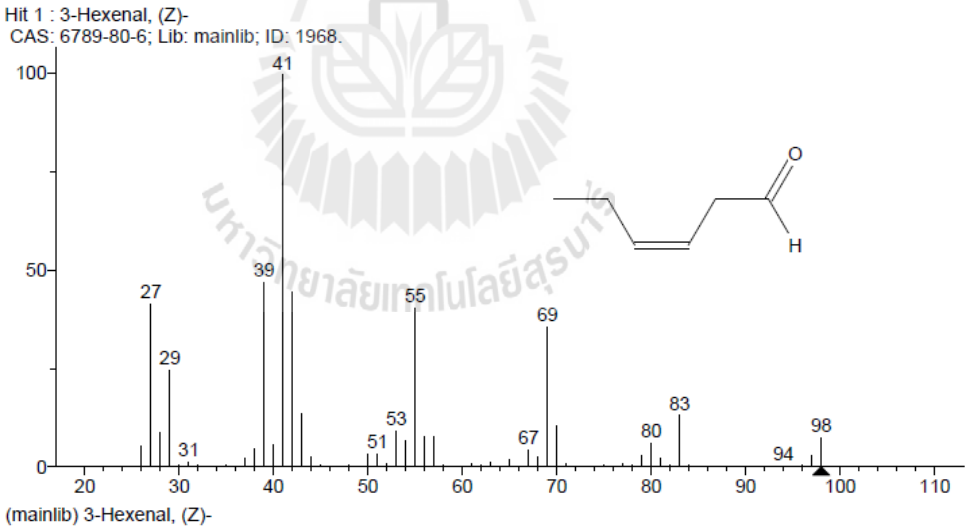

Figure 5.2 Mass spectrum of (*Z*) 3-Hexenal, the major aroma-impact compound in Vietnamese coriander extract.

5.4.1.3 The major aroma-impact compound in green pepper extract

The major aroma-impact compound used for monitoring changes of flavor in pork meatballs added green pepper extract was △-3-Carene. Its molecular ion identified and confirmed by GC-MS was 136 and the RI of 1187 (Figure 5.3 and Table 5.1).

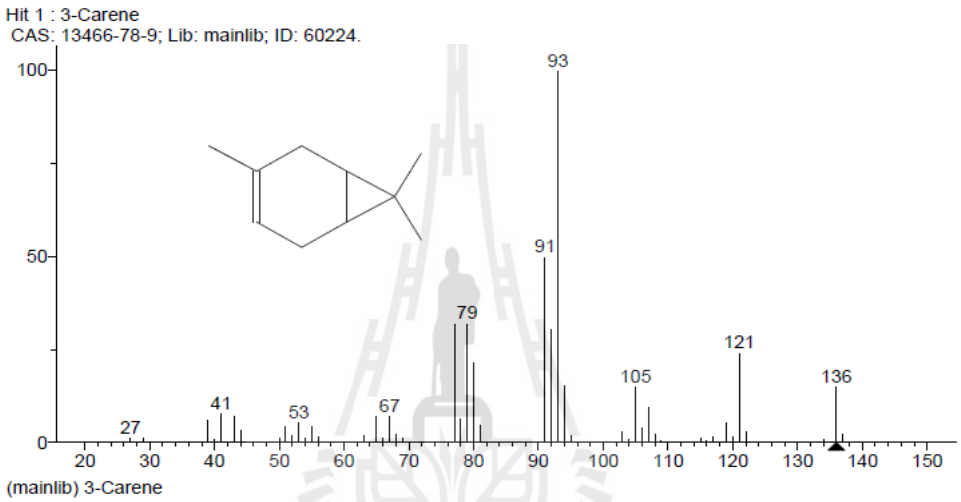

Figure 5.3 Mass spectrum of △-3-carene, major aroma-impact compound in green pepper extract.

5.4.1.4 The major aroma-impact compound in turmeric extract

The major aroma-impact compound used for the study of turmeric extract was *ar*-turmerone. The mass spectrum of *ar*-turmerone was identified by GC-MS and confirmed by using an authentic compound with molecule ion of 216 and RI of 1640 (Figure 5.4, Table 5.1).

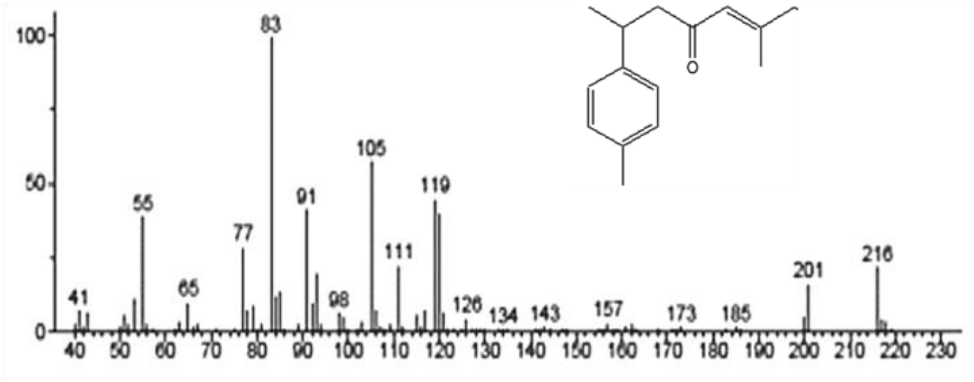

Figure 5.4 Mass spectrum of *ar*-turmarone, the major aroma-impact compound in turmeric extract (modified from Qin, Yang, Wang and Li, 2007; Singh, Rajesh, Sahoo, Subudhi and Nayak, 2011).

5.4.2 Changes of major aroma-impact compounds of selected Thai culinary herb and spice extracts used in pork meatballs

The changes of major aroma-impact compounds of selected Thai culinary herb and spice extracts in pork meatballs were determined by using static headspace gas chromatography and compared with their authentic chemical compounds.

5.4.2.1 Changes methyleugenol in pork meatballs added holy basil extract

The concentration of methyleugenol in pork meatballs added holy basil extract decreased as storage time progressed. Reduction of this aroma compound was found higher ($p<0.05$) in the meatballs aerobically packed than in vacuum packed as shown in Figure 5.5.

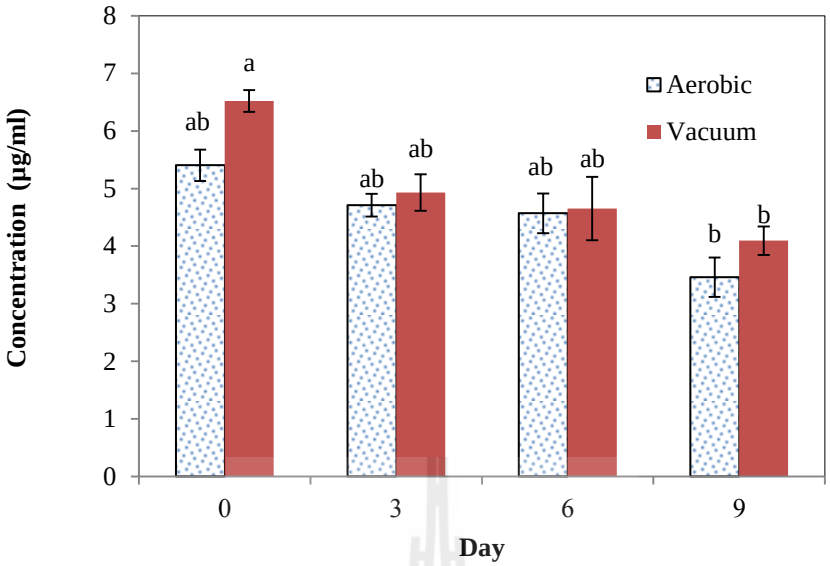

Figure 5.5 Changes of methyleugenol concentrations in aerobically and vacuum packed pork meatballs added holy basil extract during storage at 4ºC.

5.4.2.2 Changes (*Z*) 3-hexenal in pork meatballs added Vietnamese coriander extract

(*Z*) 3-hexenal also showed the similar trend as methyleugenol that was the concentration of (*Z*) 3-Hexenal decreased with increasing storage time. The vacuum packaged pork meatballs added Vietnamese coriander extract also showed better condition than aerobically packaged one ($p<0.05$) (Figure 5.6).

5.4.2.3 Changes of △-3-carere in pork meatball added green pepper extract

△-3-carere concentration in pork meatballs added green pepper extract decreased similarly to those of methyleugenol in holy basil extract and (*Z*) 3-hexenal in Vietnamese coriander extract added in the pork meatballs. Reduction in concentration of this compound was found higher in aerobically packaged meatballs than in vacuum packed ones ($p<0.05$) (Figure 5.7).

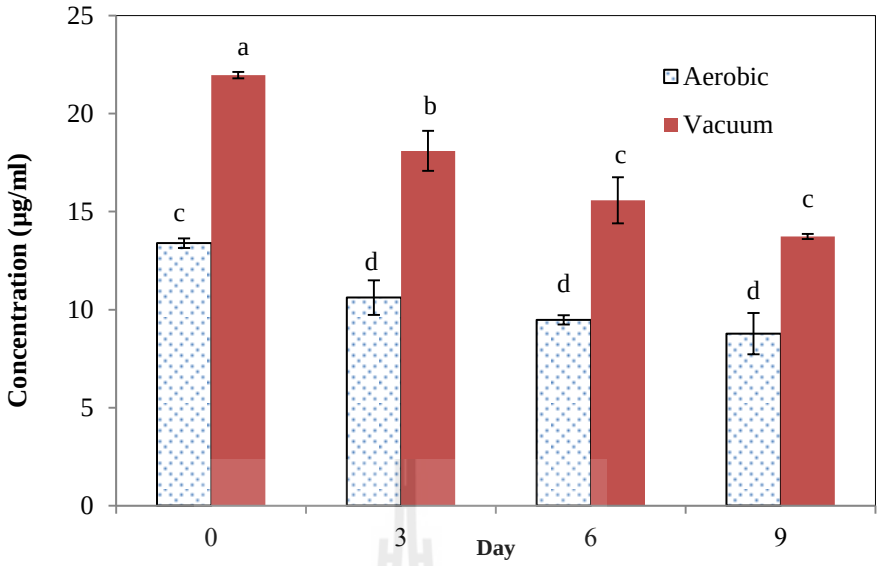

Figure 5.6 Changes of (Z) 3-hexenal concentrations in aerobically and vacuum packed pork meatballs added Vietnamese coriander extract during storage at 4ºC.

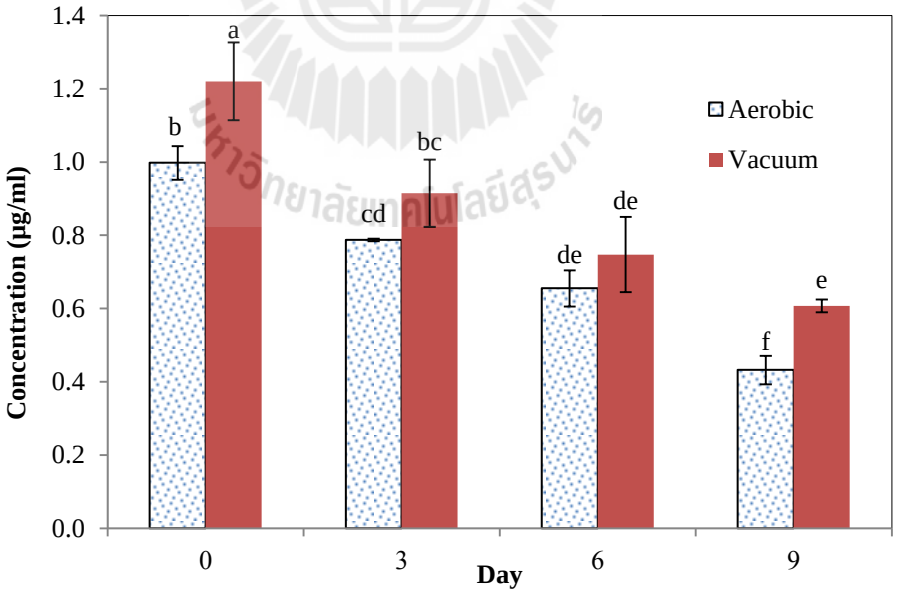

Figure 5.7 Changes of △-3-carene concentrations in aerobically and vacuum packed pork meatballs added green pepper extract during storage at 4ºC.

5.4.2.4 Changes of *ar*-turmarone in pork meatballs added turmeric extract

ar-Turmarone, one of the major aroma-impact compound in turmeric extract was found to decrease as time of storage progressed in both aerobically and vacuum packed. In addition, vacuum packaging condition could retain more *ar*-turmarone than in aerobic condition ($p<0.05$) (Figure 5.8).

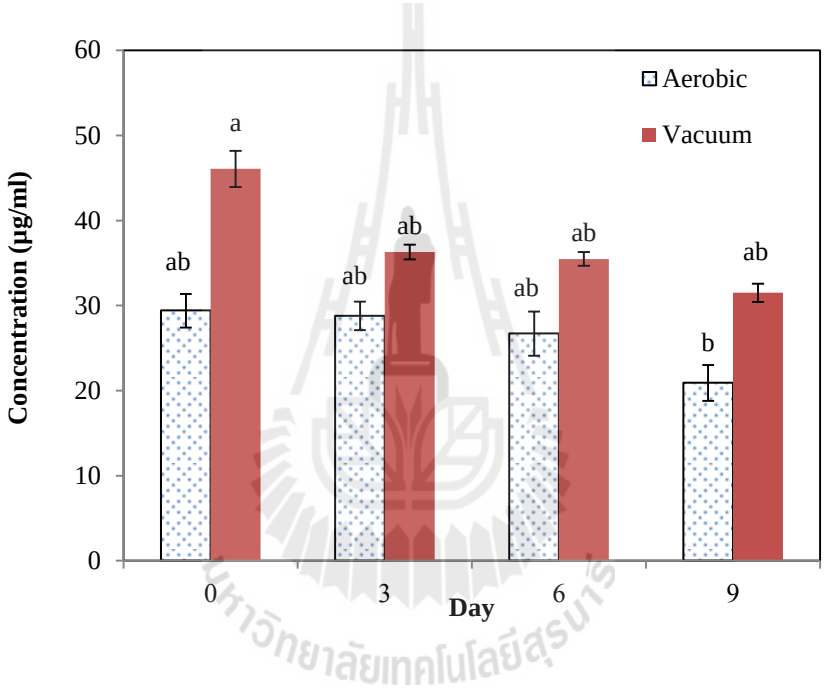

Figure 5.8 Changes of *ar*-Turmarone concentrations in aerobically and vacuum packed pork meatballs added turmeric extract during storage at 4ºC.

The key or major aroma-active compound of each herb and spice extract used to mix in pork meatballs was firmly identified by the use of GC-MS comparing mass spectrum with data available in the library and RI indices. Authentic chemicals also provided a confirmation of identification of all the aroma compounds in the study. The results showed that decreasing in concentration of all major aroma-impact

compounds were obviously higher in aerobically packed meatballs than those in the vacuum packed ones.

The analysis of major aroma-impact compound using solid-phase microextraction and GC-MS for identification and for confirmation however is not always possible using MS data alone (Shellie, Mondello, Marriott and Dugo, 2002). Differences in mass spectra are obtained using a quadrupole MS, as opposed to using an ion trap MS. Often different spectra are reported in a library for a single compound, with different common names, or systematic name, corresponding to an individual component sometimes apparent. Chromatographic retention data can support MS data, providing an independent parameter on which to base compound identity. The reproducibility and reliability of retention indices allows assignment of identity to unknown components with greater confidence, both retention indices and MS data of essential oil (Verzera, Trozzi, Cotroneo, Lorenzo and Dellacassa, 2000). The GC-MS analyses and the GC-flame ionization detection (FID) for quantitative analyses in the present investigation were all performed using two independent temperature programs. The sample complexity demanded two independent analyses, on dissimilar temperature programs. Adequate resolution of many individual components was not possible in a single analysis (Shellie, et al., 2002).

The stability of major aroma-impact compound in pork meatballs added different herb and spice extracts were found better in vacuum package condition than in aerobic package one throughout storage time. Vacuum packed condition was more resistant to lipid oxidation than aerobically packed (Nam and Ahn, 2002) and aromatic compound of plant has been known to possess biological activity as antioxidant (Baratta, Dorman, Deans, Figueiredo, Barroso and Ruberto, 1998). Therefore, less

interaction and releasing of volatile compounds of the herb and spice extract in vacuum packaging condition could be also observed.

Packaging is a critical factor that affects the quality stability of aroma compound, and thus, modification of packaging methods can minimize the quality defect in stability of aroma compound. Pork meatballs added different herb and spice extracts in aerobic conditions and time during storage had off-odor volatiles higher than vacuum packed.

Therefore, vacuum-packaging conditions could be the effective packing method providing good stability of aroma compound during storage of comminuted meat products added herb and spice extracts as such the pork meatballs for commercial purposes.

5.5 Conclusion

The major aroma-impact compound of each herb and spice extracts used in the pork meatballs models were found and confirmed in corresponding with those authentic chemical standards used for study which were △-3-carene for green pepper extract; methyleugenol for holy basil extract; *ar*-turmarone for turmeric extract, and (Z)-3-hexanal for Vietnamese coriander extract. The results showed that decreasing in concentration of all major aroma-impact compounds were obviously higher in aerobically packed pork meatballs than those in the vacuum packed ones.

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CHAPTER VI

SUMMARY

Most of herbs and spices contribute their properties of many health aspects including their antioxidant activities and also used as the main seasonings and flavoring ingredients in Thai foods. Preliminary screening of 22 Thai culinary herbs and spices commonly used for cooking were performed in order to obtain the antioxidant efficacies by using 95% ethanol. Analyses were performed for total phenolic and total flavonoid contents. Antioxidant activities were evaluated using three methods: DPPH radical scavenging activity (DPPH assay), ferric reducing antioxidant power (FRAP assay) and thiobarbituric acid reactive substances (TBARS assay). The result showed that turmeric contained the highest amount of total phenolic (579.83 µg gallic acid/g extract) and total flavonoid (129.62 µg catechin/g extract). While Vietnamese coriander showed the highest antioxidant activity determined by DPPH assay with an IC_{50} of 380.40 µg/ml. However, the DPPH assay of Vietnamese coriander was lower than trolox and FRAP assay of 3,395 µg Trolox/g extract. From TBARS assay, turmeric showed the highest antioxidant activity with an IC_{50} of 347.57 µg/ml.

The ethanolic extracts from four selected Thai culinary herbs and spices – holy basil, Vietnamese coriander, turmeric and green pepper – were used as natural bioactive ingredients in pork meatballs for study on shelf life extension. The batter of pork meatball was prepared with 0.2 % (w/w) ethanolic extract and then the pork

meatball model was formed in plastic casing and cooked in hot water till internal temperature reached 70^{o}C. The pork meatball model was cut in defined length, aerobically and vacuum packaged and stored at 4^{o}C for 9 days. Thiobarbituric acid reactive substances (TBARS), microbial enumeration and changes of major aroma-impact compounds of each herb and spice extract using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) were performed every 3 days.

It was found that holy basil, Vietnamese coriander and green pepper showed stronger antioxidant effects in the pork meatballs than turmeric throughout 9 days of storage period for both aerobic and vacuum packages. For antimicrobial activity, the meatballs adding with holy basil and green pepper and packed in both aerobic and vacuum conditions showed the highest shelf life of 9 days while the shelf life of meatballs adding with Vietnamese coriander and turmeric extracts were about 6-9 days and less than 6 days for the control meatballs. In addition, the water activity values of all meatballs were not significantly different ($p>0.05$) which were in the range of 0.969 – 0.980. The selected culinary herb and spice extracts were tested for antimicrobial inhibition against indicator bacteria. The result of clear zone forming showed that holy basil extract could inhibit two Gram-positive bacteria, i.e., *Enterococcus faecalis* (AP-31) and *Micrococcus luteus* TISTR 745 with clear zone diameter ranging 0.7-0.8 mm and green pepper extract could inhibit *Micrococcus luteus* TISTR 745 with clear zone of 0.8 mm, while Vietnamese coriander extract could inhibit *Acinetobacter calcoaceticus* TISTR 1264 with clear zone of 0.8 mm. By sensory evaluation, the pork meatballs with herb and spice extracts showed the spicy and off-flavor attributes higher score than control sample were not significant ($p>0.05$). The pork meatballs with holy basil and green pepper showed the lowest

oxidized intensity and bacterial counts which indicated that these two spices might be the good sources of antioxidant for adding in the pork meatballs to antioxidant and antibacterial.

The chromatographic data using authentic chemical of major aroma-impact compound of each herb and spice was used as chemical standard and for confirmation △-3-carene for green pepper extract; methyl eugenol for holy basil extract; *ar*-turmarone for turmeric extract; and (*Z*)-3-hexanal for Vietnamese coriander extract. The decreasing in concentration of all major aroma-impact compounds was observed during storage which obviously higher ($p<0.05$) in aerobically packed meatballs than those in the vacuum packed ones.

BIOGRAPHY

Ms. Kanok-on Nugboon was born in April 15th, 1974 at Nakhon Phanom Province. In 1997, she received bachelor's degree in B.Sc. (Food Technology) from Suranaree University of Technology, Thailand. In 2003, she received master's degree in M.Sc. (Food Technology) from Khon Kaen University, Thailand. She received scholarship for Ph.D program from Office of the Higher Education Commission for providing financial support and Rajamangala University of Technology Isan, Sakon Nakhon Campus, Thailand.