EFFECT OF *PHYLLANTHUS ACIDUS* L. LEAF AND *CARISSA CARANDAS* L. FRUIT EXTRACTS ON QUALITY, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES IN GROUND PORK

NGUYEN THI KIM TUYEN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENT FOR THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE

FACULTY OF AGRICULTURAL TECHNOLOGY

KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG

2017

KMITL-2017-AG-M-065-234

Thesis of Certification

Faculty of Agricultural Technology

King Mongkut's Institute of Technology Ladkrabang

Thesis Title	Effect of *Phyllanthus acidus* L. Leaf and *Carissa carandas* L. Fruit Extracts on Quality, Antioxidant and Antimicrobial Properties in Ground Pork
Student	MISS NGUYEN THI KIM TUYEN
Student ID.	58604006
Degree	Master of Science
Program	Agriculture
Thesis Advisor	Assoc. Prof. Dr. Chamroon Laosinwattana
Thesis Co-advisor	Asst. Prof. Dr. Komkhae Pilasombut

EXAMINERS	SIGNATURES
Asst. Prof. Dr. Teerawat Sarutayophat	Teerawat Sarutayophat
Asst. Prof. Dr. Sasithorn Nakthong	Sasithorn N.
Asst. Prof. Dr. Komkhae Pilasombut	
Assoc. Prof. Dr. Tanimnun Jaenaksorn	
Assoc. Prof. Dr. Chamroon Laosinwattana	

Date 4th May 2017 Time 09.00-12.00 A.M.

Place Meeting room 2, 1st floor, Bunnag Building

Monthon Ganmanee.

(Asst. Prof. Dr. Monthon Ganmanee)

Dean, Faculty of Agricultural Technology

2 June 2017

หัวข้อวิทยานิพนธ์	ผลของสารสกัดจากใบมะยม (*Phyllanthus acidus* L.) และผลมะนาวโห่ (*Carissa carandas* L.) ต่อคุณภาพ ฤทธิ์ในการต้านอนุมูลอิสระและจุลินทรีย์ในเนื้อสุกรบด
นักศึกษา	นางสาว Nguyen Thi Kim Tuyen
รหัสประจำตัว	58604006
ปริญญา	วิทยาศาสตรมหาบัณฑิต
สาขาวิชา	เกษตรศาสตร์
พ.ศ.	2560
อาจารย์ที่ปรึกษาวิทยานิพนธ์	รองศาสตราจารย์ ดร.จำรูญ เล้าสินวัฒนา
อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม	ผู้ช่วยศาสตราจารย์ ดร.คมแข พิลาสมบัติ

บทคัดย่อ

การศึกษาครั้งนี้เพื่อศึกษาหาสัดส่วนของสารสกัด (เอทานอล:น้ำ) ที่เหมาะสมในการสกัดสารออกฤทธิ์ในการต้านอนุมูลอิสระและฤทธิ์ในการต้านจุลินทรีย์ของสารสกัดจากใบมะยมและสารสกัดจากผลมะนาวโห่ และศึกษาผลของการใช้สารสกัดหยาบต่อคุณสมบัติทางกายภาพ เคมี และชีวภาพ ของเนื้อสุกรบดที่ผสมสารสกัดและเก็บรักษาไว้ที่อุณหภูมิ 4 องศาเซลเซียส เป็นระยะเวลา 8 วัน และ ที่อุณหภูมิ -20 องศาเซลเซียส เป็นระยะเวลา 12 สัปดาห์ การศึกษาประสิทธิภาพของการใช้สารสกัดเอทานอลต่อน้ำ ในอัตราส่วนผสมที่แตกต่างกัน (0%, 25%, 50%, 75% และ 100%) ที่มีผลต่อปริมาณสารสกัดหยาบที่สกัดได้ ปริมาณสารฟีโนลิคทั้งหมดและฟลาโวนอยด์ทั้งหมด ผลจากการทดลองพบว่า น้ำบริสุทธิ์สามารถสกัดสารสกัดหยาบจากใบมะยม และผลมะนาวโห่ ได้ปริมาณสารสกัดหยาบสูงสุด โดยปริมาณสารสกัดหยาบที่สกัดได้จะลดลงตามสัดส่วนของเอทานอลที่เพิ่มขึ้น ปริมาณสารสกัดหยาบจากใบมะยมที่สกัดด้วยน้ำบริสุทธิ์ได้ 2.80 กรัมต่อ 100 กรัมใบแห้งในขณะที่สารสกัดหยาบที่สกัดด้วยเอทานอลบริสุทธิ์ได้ 0.53 กรัมต่อ 100 กรัมใบแห้ง ในส่วนของสารสกัดจากผลมะนาวโห่ให้ผลการทดลองไปในทิศทางเดียวกันโดยสารสกัดน้ำมีประสิทธิภาพสูงที่สุดและประสิทธิภาพในการสกัดจะลดลงตามสัดส่วนของเอทานอลที่เพิ่มขึ้น ปริมาณสารสกัดหยาบจากผลมะนาวโห่ที่สกัดด้วยน้ำบริสุทธิ์ได้ 5.28 กรัมต่อ 100 กรัมผลแห้งในขณะที่สารสกัดหยาบที่สกัดด้วยเอทานอลบริสุทธิ์ได้ 3.34 กรัมต่อ 100 กรัมผลแห้ง ปริมาณสารฟีโนลิคทั้งหมดจากการสกัดใบมะยมและผลมะนาวโห่ ค่าอยู่ในช่วง 48.04 ถึง 49.87 มิลลิกรัมสมมูลย์ของกรดแกลลิคต่อสารสกัดหยาบ 1 กรัม และ 15.87 ถึง 20.44 มิลลิกรัมสมมูลย์ของกรดแกลลิคต่อสารสกัดหยาบ 1 กรัม ตามลำดับ ส่วนปริมาณสารฟลาโวนอยด์ทั้งหมดจากการสกัดใบมะยมและผลมะนาวโห่ อยู่ในช่วง 0.51 ถึง 0.70 มิลลิกรัมสมมูลย์เคอร์ซิตินต่อสารสกัดหยาบ 1

กรัม และ 0.11 ถึง 0.17 มิลลิกรัมสมมูลย์เคอร์ซิตินต่อสารสกัดหยาบ 1 กรัม ตามลำดับ ยังพบว่าการต้านอนุมูลอิสระมีค่าสูงสุดจากการสกัดด้วยเอทานอล นอกจากนี้พบว่ามีเพียงสารสกัดจากผลมะนาวโห่เท่านั้นที่มีคุณสมบัติการต้านจุลินทรีย์ โดยไม่พบการต้านจุลินทรีย์ในสารสกัดที่ได้จากใบมะยม จากคุณสมบัติเบื้องต้น ดังนั้นจึงเลือกสารสกัดน้ำจากใบมะยมและผลมะนาวโห่มาใช้ในการศึกษาต่อไป ซึ่งสารสกัดดังกล่าวมีคุณสมบัติต้านอนุมูลอิสระสูงและคุณสมบัติการต้านจุลินทรีย์ รวมถึงพิจารณาความชอบของผู้บริโภค ผลผลิตของสารสกัด ความคุ้มค่า และความปลอดภัยของผู้บริโภค การใช้สารสกัดน้ำจากใบมะยม (PWCE) ผสมในเนื้อสุกรบด แบ่งการทดลองเป็น 4 กลุ่ม ได้แก่ กลุ่มควบคุม (เนื้อสุกรบดไม่เติมสารสกัด) เนื้อสุกรบดที่เติมสาร BHT (0.2 g/kg meat) เนื้อสุกรที่เติม PWCE (2.5 g/kg meat) และเนื้อสุกรที่เติม PWCE (5 g/kg meat) ในทำนองเดียวกัน การศึกษาการใช้สารสกัดจากผลมะนาวโห่ (CWCE) แบ่งการทดลองเป็น 4 กลุ่มเช่นกัน ได้แก่ กลุ่มควบคุม (เนื้อสุกรบดไม่เติมสารสกัด) เนื้อสุกรบดที่เติมสาร BHT (0.2 g/kg meat) เนื้อสุกรที่เติม CWCE (2.5 g/kg meat) และเนื้อสุกรที่เติม CWCE (5g/kg meat) เก็บข้อมูลค่า DPPH scavenging radical activity, ABTS radical cation decolorization, reducing power, TBARS, pH, ค่าสี (CIE L*, a*, b*), จำนวนจุลินทรีย์ทั้งหมด, ยีสต์/รา, แบคทีเรียที่เจริญได้ในอุณหภูมิต่ำ โคลิฟอร์ม ในเนื้อสุกรบด ที่เก็บอุณหภูมิ 4 องศาเซลเซียส นาน 0, 2, 4, 6 และ 8 วัน ผลการทดลองพบว่าค่า DPPH, ABTS และreducing power ในเนื้อสุกรบดที่เติมสารสกัดทั้งสองชนิด (PWCE และ CWCE) ในเนื้อทั้งที่ไม่สุกและเนื้อที่สุก มีค่าสูงกว่ากลุ่มควบคุมและกลุ่มที่เติม BHT อย่างมีนัยสำคัญทางสถิติ ค่าการออกซิเดชั่นของไขมันในไม่แตกต่างกันในกลุ่มควบคุมและกลุ่มที่เติมสารสกัด อย่างไรก็ตาม พบว่าค่าการออกซิเดชั่นของไขมันในเนื้อสุกที่เติมสารสกัดทั้งสองชนิดต่ำกว่ากลุ่มควบคุมและกลุ่มที่เติม BHT อย่างมีนัยสำคัญทางสถิติ นอกจากนี้ยังพบว่าเนื้อสุกรบดกลุ่มที่เติมสารสกัดทั้งสองชนิดสามารถควบคุมค่า pH และค่าสี โดยค่าสีแดงมีค่าสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ การศึกษาผลของการใช้สารสกัดน้ำจากใบมะยมและผลมะนาวโห่ต่อคุณภาพด้านกายภาพ เคมีและชีวภาพในเนื้อสุกรบดระหว่างการเก็บรักษาที่อุณหภูมิ – 20 องศาเซลเซียส เป็นเวลา 0, 4, 8 และ 12 สัปดาห์ ผลการทดลองพบว่าค่า DPPH, ABTS และ reducing power

Thesis Title	Effect of *Phyllanthus acidus* L. leaf and *Carissa carandas* L. fruit extracts on quality, antioxidant and antimicrobial properties in ground pork
Student	Miss. Nguyen Thi Kim Tuyen
Student ID.	58604006
Degree	Master of Science
Program	Agriculture
Year	2017
Thesis Advisor	Assoc. Prof. Dr. Chamroon Laosinwattana
Thesis Co-Advisor	Asst. Prof. Dr. Komkhae Pilasombut

ABSTRACT

The aim of this study was to investigate the antioxidant and antimicrobial activities of *Phyllanthus acidus* L. leaf and *Carissa carandas* L. fruit extracts using various solvent ratios of ethanol in water and their effect on physical, chemical and biological qualities in ground pork during period of storage time for 8 days at 4°C and 12 weeks at -20°C in refrigerator. The effect of various solvent ratios of ethanol (0%, 25%, 50%, 75% and 100%) in water on crude yield, total phenolic content and total flavonoid content was determined. The results showed that the highest crude yield was obtained from water solvent for both *P. acidus* leaf and *C. carandas* fruit extracts. It could be seen that the extraction yield of water was the highest (2.80 g/100 g DW) and the extraction yield of 100% ethanol was the lowest (0.53 g/100 g DW). Whereas, the crude yields of various solvents from *C. carandas* fruits showed that the extraction yield of water was the highest (5.28 g/100 g DW) and the extraction yield of 100% ethanol was the lowest (3.34 g/100 g DW). Moreover, the highest recovery yield of total phenolic content from *P. acidus* leaf and *C. carandas* fruit extracts were ranged from 48.04 to 49.87 mg GAE/g crude and 15.87 to 20.44 mg GAE/g crude, while total flavonoid content from *P. acidus* leaf and *C. carandas* fruit extracts were ranged from 0.51 to 0.70 mg QE/g crude and 0.11 to 0.17 mg QE/g crude, respectively. The *in vitro* antioxidant and antibacterial activities of *P. acidus* leaf and *C. carandas* fruits of various crude extracts were determined. The results indicated that water extract from *P. acidus* leaves had the highest antioxidant activities, but for *C. carandas* fruits, 50% and 75% ethanol extracts had the higher antioxidant activities compared to other extracts. In particular,

only extracts from *C. carandas* fruits showed the strong inhibition on the growth of all the tested bacteria. Thus, the water crude extract from *P. acidus* leaves (PWCE) and *C. carandas* fruits (CWCE) was selected as optimal extract for further studies based on the higher antioxidant activities and preliminary sensory evaluation.

The ground pork samples were subjected to four treatments such as control (non-treated), 0.2 g BHT/kg meat, 2.5 g PWCE/kg meat and 5 g PWCE/kg meat. Also, another group of ground pork samples consisted of control, 0.2 g BHT/kg meat, 2.5 g CWCE/kg meat and 5 g CWCE/kg meat. The effect of PWCE and CWCE on DPPH scavenging radical activity, ABTS radical cation decolonization, reducing power, TBARS, pH, instrumental color (CIE L*, a*, b*), total plate count, yeasts/molds, psychrophilic bacteria and coliforms in ground pork during period of storage time for 0, 2, 4, 6 and 8 days at 4°C and 0, 4, 8 and 12 weeks at -20°C were studied. According to the DPPH, ABTS and reducing power assays, the ground pork samples containing PWCE and CWCE showed significantly higher activity in both raw and cooked samples compared to the control and BHT samples. Moreover, the lower level of lipid peroxidation compared to control and BHT treatments was found in the samples containing PWCE and CWCE. The lightness (L*) and yellowness (b*) values of meat samples contained PWCE and CWCE were lower significantly while the redness (a*) values higher than the control samples at the end of the storage time. The sensory properties of the cooked ground pork mixed with 2.5 and 5 g CE/kg meat were also determined. The results indicated that the addition of PWCE and CWCE had no significantly affected the sensory scores of ground pork samples on overall appearance such as color, odor, texture, flavor and overall quality compared to the control.

These results demonstrated that the extracts from *P. acidus* leaves and *C. carandas* fruits are high potential to be used as natural ingredients to maintain the quality, antioxidant and antimicrobial activities and prolong the shelflife of ground pork as well as develop new functional food safety to satisfy consumers.

ACKNOWLEDGEMENTS

This research was supported by grants from Faculty of Agricultural Technology and King Mongkut's Institute of Technology Ladkrabang Research Fund [KREF125802], King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

I own a deep sense of gratitude to the following organization where I have done the research: Laboratory of Meat Microbiology, Department of Animal Production Technology and Fisheries; Laboratory of Plant Production Technology, Department of Plant Production Technology, King Mongkut's Institute Technology Ladkrabang, Bangkok, Thailand in which providing me necessary technical suggestions.

I wish to express my sincere gratitude to my super-advisor Assoc. Prof. Dr. Chamroon Laosinwattana, my co-advisor Asst. Prof. Dr. Komkhae Pilasombut and Asst. Prof. Dr. Montinee Teerarak for their keen interest on me at every stage of my research. They prompt inspirations, timely suggestions with kindness, enthusiasm and dynamism have enabled me to complete my thesis.

I thank profusely all Thai friends for their kind help during my research pursuit.

I would like to thank my family for constant encouragement throughout my research period.

Nguyen Thi Kim Tuyen

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Statement and significance of the problems

Meat is an important source for some micronutrients such as trace elements and vitamins, which are either not present in plant derived food or have a poor bioavailability. In addition, meat as a protein-rich and carbohydrate-low product contributes to a low glycemic index (Angiolillo *et al.* 2015). Meat contains several important or even essential nutrients, which are beneficial for human health and development. As an essential part of a mixed diet, meat ensures adequate delivery of essential micronutrients and amino acids and is involved in regulatory processes of energy metabolism. Meat is a good example for a complex structured food whose role often is controversially discussed in the context with health (Velasco and Williams. 2011). However, rich nutrition condition in meat is also the cause for chemical changes and microorganism growth.

Lipid oxidation is one of the main causes of quality deterioration in meat and meat products because it leads to discoloration, off-flavors, texture deterioration, and loss of nutrients, all of which are the major quality factors affecting consumers acceptance of meat (Rajneesh *et al.* 2008). Lipid oxidation is promoted by diverse factors such as heat, light, metal ions, heme (in meat), oxygen, free radicals, and oxidative enzymes (Min and Ahn. 2005). In addition, protein oxidation affects meat quality, including tenderness, water-holding capacity, and nutritional quality, and has attracted considerable attention in recent years after having been ignored for decades (Popova and Marinova. 2013). Complex mechanisms and reaction processes are involved in lipid and protein oxidation, while it is generally accepted that both types of oxidation occur mainly via a radical chain reaction including initiation, propagation, and germination stages (Maqsood *et al.* 2015). Thus, one effective method for slowing oxidation is to use antioxidants to break the radical chain reaction to maintain the nutritional and sensory qualities of meat. Antioxidants can prevent lipid peroxidation using the following mechanisms: decreasing localized oxygen concentrations; preventing chain initiation by scavenging initiating radicals; binding catalysts, such as metal ions to prevent initiating radical generation; decomposing peroxides, by this way-they can not be reconverted to initiating radicals; chain-breaking to prevent continued hydrogen abstraction by active radicals (Foo *et al.* 2015). In industrial processing, mainly synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG), have been widely used to help meat

preservation (Kiokias and Gordon. 2003). However, compared with synthetic antioxidants, natural antioxidants are of great interest because of their safety and health characteristics. The demand for natural antioxidant, especially of plant origin has increased in the recent years due to the growing concern among consumers about these synthetic antioxidants because of their potential toxicological effects. So, over the past few years, increasing consumer demand for more natural, preservative-free products has led the food industry to consider the incorporation of natural antioxidants in a range of products. The use of natural antioxidants has the advantage of being more acceptable by the consumers as these are considered as non-chemical.

Moreover, the contamination of meat products with microorganisms presents a problem of global concern, since the growth and metabolism of microorganisms can cause serious foodborne intoxications and a rapid spoilage of the meat products. Thus, the acceptance and safety of a meat product for the consumers depends in great part on the presence and nature of microorganisms. Besides molds and yeasts, bacteria are the principle responsible for various types of food spoilage and foodborne intoxications (Patra and Baek. 2016). Foodborne pathogens are one of the major causes of morbidity and mortality all over the world. In the United States, 31 foodborne pathogens caused about 9.4 million illness, 56,000 hospitalizations, and 1,300 deaths each year (Patra and Baek. 2016). *Listeria* spp., enterohemorrhagic *E. coli* O157:H7 and *S. aureus* are on the top of the lists of bacterial pathogens related to meat responsible for foodborne illnesses. Moreover, some species of lactic acid bacteria consist of *L. sakie*, *L. mensenterioides*, *Lactococcus* spp., *Enterococcus* spp. and some other Gram-negative bacteria such as *Salmonella* spp., *Pseudomonas* spp., *A. hydrophia*, which caused spoilage in meat (Vashist and Jindal. 2012).

In particular, in the last decade consumer demands in the field of food production has changed considerably. Consumers more and more believe that foods contribute directly to their health (Lee *et al.* 2011). Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers. In this regard, functional foods play an outstanding role. The increasing demand on such foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for improved quality of their later years (Olmedilla-Alonso *et al.* 2013). The terms of functional food and nutraceutical are used interchangeably and are defined as substances which can be considered a food, or part of a food, which provides medicinal properties or healthy benefits, including the prevention and treatment of disease (Angiolillo *et al.* 2015). This awareness has moved consumers to become

more health-conscious, driving a trend towards healthy and nutritious foods with additional health promoting functions. Therefore, urgent requirements of industrial meat are need to concentrate on improvement in the healthy meat to satisfy the consumer concerns.

So, it is increasingly attractive to find out effective and nontoxic measures to delay spoilage and to extend the shelf life of meat and to prevent growth of pathogenic microorganisms in meat, several preservation techniques, such as heat treatment, salting, acidification, drying and chemical treatment have been used in the food industry (Dai *et al.* 2014). Synthetic preservatives have been used as food additives to extend shelf life of meat, but they are strictly regulated due to toxicological concerns and some health problems. To avoid human health problems and respond to the consumer concerns about safety and toxicity risks of chemical preservatives, there is a strong need to replace synthetic compounds with natural compounds from safe natural sources. Numerous efforts are conducted to find natural alternatives to prevent bacterial in meat in recent years, because of the great consumer awareness and concern regarding synthetic chemical additives, foods (Tajkarimi *et al.* 2010).

Plants are generous sources to supply human with a numerous of valuable bioactive compound (Kähkönen *et al.* 1999; Tagne *et al.* 2014). Bioactive compounds range from very polar to very non-polar compounds from plants have been found to possess potent antioxidant and antimicrobial properties (Dailey and Vuong. 2015) and the extraction solvent plays an important role in extraction efficiency of bioactive compounds from the plant materials (Dent *et al.* 2013). Different plant materials such as oilseeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs are being evaluated due to their high content of polyphenols as natural antioxidants to preserve and improve the overall quality of meat (Foo *et al.* 2015). Plant extracts have been used as natural antioxidant and antimicrobial products in meat and meat products by several authors. Different kimchi ethanolic extracts on the antioxidant properties and lipid oxidation of cooked ground pork during storage at 4°C for 14 days (Lee *et al.* 2011). Oregano and sage essential oil treatments significantly reduced the oxidation in meat storage up to 12 days (Fasseas *et al.* 2008). The effects of 10%, 5%, and 2.5% sorrel concentration against *E. coli* O157:H7 strains (Fullerton *et al.* 2011). Leaf extracts of chamnamul, bok choy, fatsia, butterbur, chamnamul, bok choy, crown daisy, and stonecrop extracts exhibited inhibitory activity against *B. subtilis*, *S. aureous*, especially the addition of vegetable extracts resulted in a reduction in growth rate of total viable count (Kim *et al.* 2013a). Clove and rosemary extracts in inhibiting microbial growth, reducing

lipid oxidation, maintaining or improving sensory characteristics and extending the shelf-life of raw chicken meat during storage at 4°C for 15 days (Zhang *et al.* 2016).

Phyllanthus acidus L. belong to the Phyllanthaceae family and commonly grown in Indonesia, South Vietnam, Loa, and Thailand in home gardens (Chakraborty *et al.* 2012). The *P. acidus* leaves are 2 to 7.5 cm long and thin, they are green and smooth on the upper side and blue-green on the underside. Traditionally, *P. acidus* has been used in the treatment of several ailments including inflammatory and oxidative stress-related disorders such as gastric trouble (Chakraborty *et al.* 2012), rheumatism, bronchitis (Shilali *et al.* 2014), respiratory disorders such as asthma ((Sousa *et al.* 2007), hepatic disease (Jain and Singhai. 2011), diabetes (Biswas *et al.* 2011), and gonorrhoea (Habib *et al.* 2011). This plant has also been reported to improve eyesight and memory; to cure cough, psoriasis, and skin disorders; and to be sudorific (Shilali *et al.* 2014). The leaves and roots of this plant are also used as antidotes to viper venom, to treat fever, and to ameliorate hypertension (Jain and Singhai. 2011). A large number of authors found *P. acidus* leaves contain some important chemical constituents including kaempferol, hypogallic acid, gallic acid, quercetin, alkaloids, tannins, flavonoids, phenolics and terpenes (Sousa *et al.* 2007).

Carissa carandas L. (common name Karaunda), is one of the trees with edible small red berries fruit in the Apocynaceae family, Carissa genus, *C. carandas* species. It is a widely used medicinal plant, cultivated as hedges all over India, Thailand, Sri Lanka, Java, Malaysia, Myanmar and Pakistan (Fartyal and Kumar. 2014). Traditionally, whole plant and its parts were used in the treatment of various ailments. The roots were employed as a bitter stomachic, vermifuge and as an ingredient in the remedy for itches. The roots were reported to contain salicylic acid and cardiac glycosides. It also contains carissone, d-glycoside of β-sitosterol, glucosides of odoroside H, carindone, a terpenoid lupeol, ursolic acid and its methyl ester, also carinol, a phenolic lignan. Some other previous studies also reported that the fruits, leaves, barks, and roots of *C. carandas* have been used for ethnomedicine in the treatment of human diseases, such as diarrhea, stomachic, anorexia, intermittent fever, mouth ulcer and sore throat, syphilitic pain, burning sensation, scabies, and epilepsy (Itankar *et al.* 2011; Mehmood *et al.* 2014). Other properties attributed are strengthening tendons, effective against remittent fever, earache and syphilitic pain. Chemical constituents include steroids, terpenes, tannins, flavonoids, benzenoids, phenylpropanoid, lignans, sesquiterpenes, and coumarins (Fartyal and Kumar. 2014; Singh and Uppal. 2015).

1.2 Objectives of the study

1.2.1 Evaluation the effect of various solvent ratios of ethanol (0%, 25%, 50%, 75% and 100%) in water on the extraction crude yield, TPC and TFC of *P. acidus* leaves and *C. carandas* fruits.

1.2.2 Evaluation the effect of various crude extract from *P. acidus* leaves and *C. carandas* fruits on *in vitro* antioxidant and antimicrobial properties of the crude extracts from *P. acidus* leaves and *C. carandas* fruits.

1.2.3 Evaluation the effect of optimal crude extracts from *P. acidus* leaves and *C. carandas* fruits on chemical, physical and biological quality in ground pork storaged at 4°C over 8 days and at -20°C over 12 weeks.

1.2.4 Evaluation the effect of optimal crude extracts from *P. acidus* leaves and *C. carandas* fruits on sensory evaluation of ground pork products.

1.3 Places

1.3.1 Laboratory of Meat Microbiology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

1.3.2 Laboratory of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

1.3.3 Laboratory of Chemical Analysis, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

1.4 Scope of the study

1.4.1 *In vitro* antioxidant and antimicrobial properties of the crude extracts from *P. acidus* leaves and *C. carandas* fruits.

1.4.2 Application of *P. acidus* leaf and *C. carandas* fruit extracts in ground pork.

1.5 Time

The thesis has been studied for 18 months.

1.6 Hypothesis

New natural products from plant resource for antioxidant and antimicrobial properties could be a useful source to prolong the quality of meat for human consumption.

CHAPTER 2

LITERATURE REVIEW

2.1 *Phyllanthus acidus* L.

2.1.1 Background of *Phyllanthus acidus*

Phyllanthus acidus L. is one of the trees with edible small yellow berries fruit in the Phyllanthaceae family, Phyllantheae tribe, Phyllanthus genus, Flueggeinae subtribe, *P. acidus* species (Eldeen *et al.* 2011). *P. acidus* commonly known as the Otaheite gooseberry, Malay gooseberry, Tahitian gooseberry, country gooseberry, star gooseberry, starberry, West India gooseberry, grosella (in Puerto Rico), jimbilin (in Jamaica), damsel (in Grenada), karamay (in the Northern Philippines), chùm ruôt (South Vietnam), cerme and cermai (Laos, northern Peninsular Malaysia, Indonesia), Goanbili (in Maldives), kantuet (in Cambodia) and mayom (Thailand) or simply gooseberry tree, is one of the trees with edible small yellow berries fruit in the Phyllanthaceae family, originated in Madagascar and commonly grown in Indonesia, South Vietnam, Laos, and Thailand in home gardens (Biswas *et al.* 2011). The plant is an intermediary between shrubs and tree, reaching 2 to 9 m high. The tree's dense and bushy crown is composed of thickish, tough main branches, at the ends of which are clusters of deciduous, greenish, 15 to 30 cm long branchlets. The branchlets bear alternate leaves that are ovate or lanceolate in form, with short petioles and pointed ends. The leaves are 2 to 7.5 cm long and thin, they are green and smooth on the upper side and blue-green on the underside. In general, the Otaheite gooseberry tree very much looks like the bilimbi tree (Manju *et al.* 2012).

2.1.2 Medicinal properties

The leaf is analgesic, antipyretic, antirheumatic and cures jaundice, small pox, itching, and gum infection. Traditionally, it is used as liver tonic and blood purifier (Habib *et al.* 2011). The leaf decoction is used by tribal healers of Chittagong Hill Tracts region of Bangladesh to treat liver disease. An aqueo us extract of leaf is reported to have remarkable antiviral and anti cystic fibrosis properties (Sawhney *et al.* 2011). Studies with methanolic extract of *P. acidus* leaf have shown protection against carbon tetrachloride induced hepatotoxicity in rats (Jain *et al.* 2010). Exhaustive literatures showed that *P. acidus* have been used traditionally in the treatment of several pain, inflammatory and oxidative stress related disorders such as rheumatism, bronchitis, asthma, respiratory disorder, hepatic disease, diabetes and gonorrhoea. The plant is also important to improve eyesight, memory and to cure cough, psoriasis, skin disorders, sudorific (Sawhney *et*

al. 2011). Fruits of the plant used as astringent, root and seed are useful as cathartic, leaf and root use as antidote to viper venom (Chakraborty *et al.* 2012). The leaf of the plant found effective in hypertension; leaf, bark and roots are used to treat fever traditionally; the latex of the plant credited with purgative and emetic activity (Chakraborty *et al.* 2012; Jain and Singhai. 2011).

2.1.3 Antioxidant properties

Ethanolic extract of fruit and leaves was reported to possess antioxidant activity (Jagessar *et al.* 2008). Some important chemical constituents of the leaf include kaempferol, hypogallic acid, gallic acid, quercetin, and adenosine (Lee *et al.* 2011; Sousa *et al.* 2007). *In vitro* screening of petroleum ether extract of fruits showed cytotoxic, antibacterial and antioxidant activity (Habib *et al.* 2011). Different parts of *P. acidus* have been reported for several biological activities, fruits and leaves of the plant showed promising hepatoprotective activity (Lee *et al.* 2011). Phyllanthosols A and B were isolated from roots, which were proposed as promising antitumor activity (Sawhney *et al.* 2011). Adenosine, kaempferol, and hypogallic acid were found in leaves which showed airway chloride secretion, a potential treatment in cystic fibrosis (Sousa *et al.* 2007). *P. acidus* is reach in different secondary metabolite like alkaloids, tannins, flavonoids, lignans, phenolics and terpenes (Jain and Singhai. 2011). *P. acidus* contains 4-hydroxybenzoic acid, caffeic acid adenosine, kaempferol and hypogallic acid (Sousa *et al.* 2007).

2.1.4 Antimicrobial properties

P. acidus leaves potential against important human pathogenic bacteria was investigated in the study of (Rahman *et al.* 2014).

2.2 *Carissa carandas* L.

2.2.1 Background of *Carissa carandas*

Carissa carandas L. commonly known as Karanda belongs to Apocynaceae family. *C. carandas* is large dichotomously branched evergreen shrub with short stem and strong thorn in pairs. Gentianales order, *Carissa* genus, Flueggeinae subtribe, *C. carandas* species. Other names less widely used include: *karau(n)da*, *karanda*, or *carunda*. It is called *kerenda* in Malaya, *karaunda* in Malaya and India; *Bengal currant* or *Christ's thorn* in South India; *nam phrom*, or *namdaeng* in Thailand; and *caramba*, *caranda*, *caraunda* and *perunkila* in the Philippines. In Assam it is called *Karja tenga*. In Bengali it is called as *Koromcha*. This species is a rank-growing, straggly, woody, climbing shrub, usually growing to 3 – 5 m high, sometimes ascending to the tops of tall trees. The plant is native and common throughout India. Fruit is a

drupe, broadly ovoid/ellipsoid, 1.5 to 2.5 centimeters long, bluntly pointed, and blackish or reddish-purple when ripe, and containing 2 to 4 small, flat seeds. Pulp is reddish-purple and sour. Karonda's ripeness depends on its end use. If intended for use as a vegetable, the fruits should be plucked while still unripe. This is apparent by the fruit's greenish white color. When fully ripe, these fruits are selected for canning, preserving and pickling. Some of the fruits grow dark red when fully ripe; others grow dark purple (Kumar *et al.* 2013).

2.2.2 Medicinal properties

The different parts of this plant have been used for various ratios of medicine. Cardiotonic activity was found in root of this plant. This plant has been mentioned in the old chemical literature as purgative, stomachic, antihelmintics and antidote for snake-bite. The physical characteristics of oil from the fruits of *C. carandas* were determined by using standard methods. In addition to this a study of sugars and amino acids from the fruits of this plant was also undertaken by the present authors (Sumbul and Ahmed. 2012). The unripe fruit is bitter, sour, astringent, thermogenic, constipating, appetizer, antipyretic mucolytic and useful in polydypsia, anorexia, diarrhea, diseases of brain intermittent fevers and haematemesis. The ripe fruit is sweet, cooling, appetizing, antiscorbutic and is useful in bilious complaints, expectorant anorexia, burning sensation, scabies, pruritus and other skin diseases, it is useful for cure of anaemia, acts as antidote for poisons. In traditional medicine the fruit is used to improve female libido and to remove worms from the intestinal tract. The fruits have anti-microbial and antifungal properties and its juice is used to clean old wounds which have become infected. The juice can be applied to the skin to relieve any skin problems. Traditionally Karamarda has been used to treat insanity (Kumar *et al.* 2013). In Ayurveda, the unripe fruits were used as an anthelmintic, astringent, appetizer, antipyretic, antidiabetic, aphrodisiac, in biliary disorders, stomach disorders, rheumatism and diseases of the brains (Singh and Uppal. 2015; Sumbul and Ahmed. 2012). It is useful in treatment of diarrhea, anorexia and intermittent fevers. Fruits have also been studied for its analgesic, anti-inflammatory and lipase 1 activities. However, no scientific data is available to validate the folklore claim and keeping the above information in view, the present study has been designed to evaluate its antidiabetic activity.

2.2.3 Antioxidant properties

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants are widely used in dietary supplements and have been investigated for the

prevention of diseases such as cancer, coronary heart disease and even altitude sickness (Fartyal and Kumar. 2014; Sumbul and Ahmed. 2012). Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials of antioxidant supplements including beta-carotene, vitamin A, and vitamin E singly or in different combinations suggest that supplementation has no effect on mortality or possibly increases it (Singh and Uppal. 2015). Randomized clinical trials of antioxidants including beta carotene, vitamin E, vitamin C and selenium have shown no effect on cancer risk or increased cancer risk associated with supplementation (Israr *et al.* 2012; Sadek *et al.* 2013). Randomized clinical trials of antioxidants including beta carotene, vitamin E, vitamin C and selenium have shown no effect on cancer risk or increased cancer risk associated with supplementation (Fartyal and Kumar. 2014).

2.2.4 Antimicrobial properties

Phytochemical studies revealed the presence of glycosides, terpenoids, flavonoids, tannins, saponins, unsaturated sterols, salicylic acid, proteins, vitamin C, phenolic acids, carissol, carissic acid and β-sitosterol as plant constituents (Mehmood *et al.* 2014). In addition, antioxidant activities of this plant are also reported (Singh and Uppal. 2015). The present investigation was undertaken to find out the antibacterial potential of crude extracts of different parts of *C. carandas* against some Gram-positive and Gram-negative bacteria. Antimicrobial activity against *S. aureus, S. epidermidis, E. coli, A. niger, C. albicans* was seen in aqueous, ethanol, methanol, chloroform and acetone extract of *C. carandas* (Salar and Dhall. 2010). Antimicrobial activities of ethanolic extract of fruits of *C. carandas* have been reported against *S. aureus, S. epidermidis, S. pneumoniae, B. subtilis, E. coli* (Israr *et al.* 2012). Therefore, the objectives of this study was to investigate *in vitro* studies of antioxidant and antibacterial properties of *C. carandas* fruits extracts for new natural ingredients that can be further use as functional and food safety in the further.

2.3 Oxidation

The oxidation is a chemical reaction in which an atom, ion or molecule of the electron emits. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols. Its oxidation number is increased

thereby. Another fabric accepts electrons and becomes reduced. Both reactions are together as part of responses to a redox reaction consideration. Oxidation: $A \longrightarrow A^{+} + e^{-}$. Oxidative deterioration in foods involves oxidation in both the aqueous phase (e.g., proteins) and the lipid phase (e.g., polyunsaturated lipids). Formation of free radicals is an early event that occurs prior to the progression of oxidation and is most often associated with the aqueous phase. Linear free energy relationships are found valuable for classification of such early events as electron transfer and hydrogen atom transfer. Inspiration for protection of processed foods against oxidative deterioration of their vulnerable constituents has been found in the antioxidant mechanisms appearing during evolution of aerobic life forms in an increasingly oxidizing atmosphere. A two-dimensional classification of antioxidants opens up for an understanding of the special role of carotenoids, and optimal protection seems to depend on a proper balance between antioxidants and antireductants.

When a hydrogen atom ($H^{\bullet}$) is abstracted from an unsaturated fatty acid (R:H) forming an alkyl radical ($R^{\bullet}$), lipid oxidation is initiated (see nr 1 below). Generation of this lipid radical is thermodynamically unfavorable and is usually initiated by the presence of other radical compounds ($R^{\bullet}$), singletstate oxygen ($^{1}O_2$), decomposition of hydroperoxides (ROOH), or pigments that act as photoensitizers. In order to stabilize, the alkyl radical ($R^{\bullet}$) usually undergoes a shift in the position of the double bond (cis to trans) and production of a conjugated diene ratio. The $R^{\bullet}$ can react with O_2 to form a high-energy peroxyl radical ($ROO^{\bullet}$; see nr 2 below). The peroxyl radical can then abstract a hydrogen atom ($H^{\bullet}$) from another unsaturated fatty acid (see nr 3 below) forming a hydroperoxide (ROOH) and a new, free alkyl radical ($R^{\bullet}$). This process then propagates to another fatty acid (see nr 4 below). Lipid hydroperoxides (ROOH) are the primary products of lipid oxidation. They are tasteless and odorless; however, in the presence of heat, metal ions, and/or light, they can decompose to compounds that contribute off-odors and offtastes. Alkoxy radicals ($RO^{\bullet}$) can also abstract $H^{\bullet}$ from unsaturated fatty acids continuing the chain reaction (see nr 5 below). Hydroxyl radicals ($^{\bullet}OH$) can react with conjugated systems continuing the oxidation process. This chain reaction terminates when 2 radical species combine to form a nonradical species (see nr 6 below). Antioxidants (A:H) inhibit the chain reaction by donating hydrogen atoms ($H^{\bullet}$) to radicals (see nr 7 below). The antioxidant free radical may then form a stable peroxy-antioxidant compound (see nr 8 below).

(1) $R{:}H + O{::}O + \text{Initiator} \longrightarrow R^{\bullet} + HOO^{\bullet}$

(2) $R^{\bullet} + O{::}O \longrightarrow ROO^{\bullet}$

(3) $ROO^{\bullet} + R{:}H \longrightarrow ROOH + R^{\bullet}$

(4) $RO{:}OH \longrightarrow RO^{\bullet} + HO^{\bullet}$

(5) $R{::}R + OH \longrightarrow R{:}R\text{-}O^{\bullet}$

(6) $R^{\bullet} + R^{\bullet} \longrightarrow R{:}R$

(7) $R^{\bullet} + ROO^{\bullet} \longrightarrow ROOR$

(8) $ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O_2$

(9) $ROO^{\bullet} + AH \longrightarrow ROOH + A^{\bullet}$

(10) $ROO^{\bullet} + A^{\bullet} \longrightarrow ROOA$

Ultimately, oxidation depends on the addition of oxygen to a compound; however, the energy level of the oxygen has a significant impact on the ease of the oxidation reaction. Singlet state oxygen ($^1O_2$) has spin-coupled electrons and is a nonradical, high-energy species. It is electrophilic and can react with other electron-rich, nonradical, singlet state compounds containing double bonds (C=C, C=O). However, oxygen in its lowest energy state, triplet state oxygen ($^3O_2$), has 2 unpaired, parallel spin electrons. It is very reactive (primarily with radical species). Most food components, such as carbohydrates and proteins, are nonradical (singlet state) and are relatively unreactive with triplet state oxygen ($^3O_2$); however, they are reactive with singlet state oxygen ($^1O_2$) that can be generated in response to temperature change, reduction of activation energy (presence of transition metals), exposure to UV light, and physical damage to tissues. Singlet oxygen and free radicals can cause biological damage to macromolecules and membrane constituents. The presence of natural antioxidants may help control these degradative reactions (Brewer. 2011).

2.3.1 Antioxidants

Antioxidants are compounds or systems that delay autoxidation by inhibiting formation of free radicals or by interrupting propagation of the free radical by one (or more) of several mechanisms: (1) scavenging species that initiate peroxidation, (2) chelating metal ions such that they are unable to generate reactive species or decompose lipid peroxides, (3) quenching $^{\bullet}O_2^{-}$ preventing formation of peroxides, (4) breaking the autoxidative chain reaction, and/or (5) reducing localized O_2 concentrations (Brewer. 2011). Chain-breaking antioxidants differ in their antioxidative effectiveness depending on their chemical characteristics and physical location within a food (proximity to membrane phospholipids, emulsion interfaces, or in the aqueous phase). The chemical potency of an antioxidant and solubility in oil influence its accessibility to peroxy radicals especially in membrane, micellar and emulsion systems, and the amphiphilic

character required for effectiveness in these systems. Antioxidant effectiveness is related to activation energy, rate constants, oxidation–reduction potential, ease with which the antioxidant is lost or destroyed (volatility and heat susceptibility), and antioxidant solubility. In addition, inhibitor and chain propagation reactions are both exothermic. As the A:H and R:H bond dissociation energies increase, the activation increases and the antioxidant efficiency decreases. Conversely, as these bond energies decrease, the antioxidant efficiency increases. 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. 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. 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, 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 (Brewer. 2011).

2.3.2 Synthetic antioxidants

Oxidation increases at higher ambient temperatures and is catalysed by the presence of heavy metal ions, especially copper. The degree of unsaturation of the oil also influences shelf life, for example oils with a high level of linolenic fatty acid are more prone than those with a higher saturated fatty acid content. Oxidation can be minimized by the presence of anti-oxidants, which inactivate free radicals. Dosages of antioxidants are generally up to 200 mg/kg, based on total fat content. The largest application of antioxidants is found in the processing of oil seeds into oils and fats where refining removes impurities from vegetable oils. With these impurities natural antioxidants can also be removed from the oils, making the products susceptible to oxidation. A range of synthetic antioxidants are available to restore or even improve the oils natural protection against oxidative degradation and thus increasing their shelf life considerably. Other use of antioxidants are found in the rendering of animal fats, the meat industry, in baked goods and practically all foods with a high oil content such as mayonnaise and margarine.

Butylated hydroxyanisole (BHA): is perhaps the most extensively used antioxidant in the food industry. BHA can be easily applied to foods because of its excellent solubility in fats and oils. It is heat stable and of all antioxidants it has the best carry-through effect into baked foods, providing extended shelf life. Vitablend offers BHA as powder, flake, tablet, small pack and liquid solution (with or without synergists BHT and/or propyl gallate). Applications include: vegetable oil, frying oil, animal feed, cereals, chewing gum, potato flakes and cosmetic products.

Butylated hydroxytoluene (BHT): is a synthetic analogue of vitamin E like BHA and operates by reducing oxygen radicals and interrupting the propagation of oxidation processes. Its volatility at higher temperatures makes it especially suitable for products that are stored at moderate temperatures. Vitablend offers BHT as fine crystal, small pack and liquid oil solution (with or without synergistic BHA). Applications include: animal fats, chewing gum, animal feed, vegetable oils.

Tert-butylhydroquinone (TBHQ): is a general-purpose antioxidant used in many applications. Its strength increases with a higher degree of unsaturation, making it widely used in vegetable oils. Other applications are margarine, fish oil, fried foods, essential oils, nuts, edible animal fats, butterfat and packaged fried foods. Vitablend offers TBHQ as powder and as liquid solutions with or without added synergists to improve antioxidant capability.

Propyl gallate (PG): is made from natural gallic acid and shows excellent antioxidant activity in foods and vegetable oils, especially in combination with ascorbyl palmitate. It is also synergistic with BHA. Propyl gallate shows lower solubility in oils compared to BHA and BHT. Vitablend offers propyl gallate as powder and as a liquid solution (with or without other synergistic antioxidants and citric acid).

2.3.3 Natural antioxidants

Food tissues, because they are (or were) living, are under constant oxidative stress from free radicals, reactive oxygen species, and prooxidants generated both exogenously (heat and light) and endogenously (H_2O_2 and transition metals). For this reason, many of these tissues have developed antioxidant systems to control free radicals, lipid oxidation catalysts, oxidation intermediates, and secondary breakdown products (Chang *et al.* 2007; Mariutti *et al.* 2011). These antioxidant compounds include flavonoids, phenolic acids, carotenoids, and tocopherols that can inhibit Fe^{3+}/AA-induced oxidation, scavenge free radicals, and act as reductants (Chang *et al.* 2007). Spices and herbs, used in foods for their flavor and in medicinal mixtures for their

physiological effects, often contain high concentrations of phenolic compounds that have strong H-donating activity (Brewer. 2011).

Some plantderived compounds (carnosol, rosmanol, rosmariquinone, and rosmaridiphenol) are better antioxidants than BHA (Richheimer *et al.* 1996). The major antioxidative plant phenolics can be divided into 4 general groups: phenolic acids (gallic, protochatechuic, caffeic, and rosmarinic acids; Figure 2.1), phenolic diterpenes (carnosol and carnosic acid; Figure 2.2), flavonoids (quercetin and catechin; Figure 2.3), and volatile oils (eugenol, carvacrol, thymol, and menthol). Phenolic acids generally act as antioxidants by trapping free radicals; flavonoids can scavenge free radicals and chelate metals as well (Ho, 1992). The common characteristic of the flavonoids (flavones, flavonols, flavanols, and flavanones) is the basic 15-carbon flavan structure ($C_6C_3C_6$).

These carbon atoms are arranged in 3 rings (A, B, and C). Classes of flavonoids differ in the level of saturation of the C ring. Individual compounds within a class differ in the substitution pattern of the A and B rings that influence the phenoxyl radical stability and the antioxidant properties of the substances (Khoddami *et al.* 2013). The free radical-scavenging potential of natural polyphenolic compounds appears to depend on the pattern (both number and location) of free ˙OH groups on the flavonoid skeleton (Kumar and Jain. 2015). The B-ring substitution pattern is especially important to free radical-scavenging ability of flavonols. Studying the ability of 4 flavonols substituted at different points on the B-ring (galangin, kaempferol, quercetin, and myricetin) to quench the intrinsic fluorescence of bovine serum albumen.

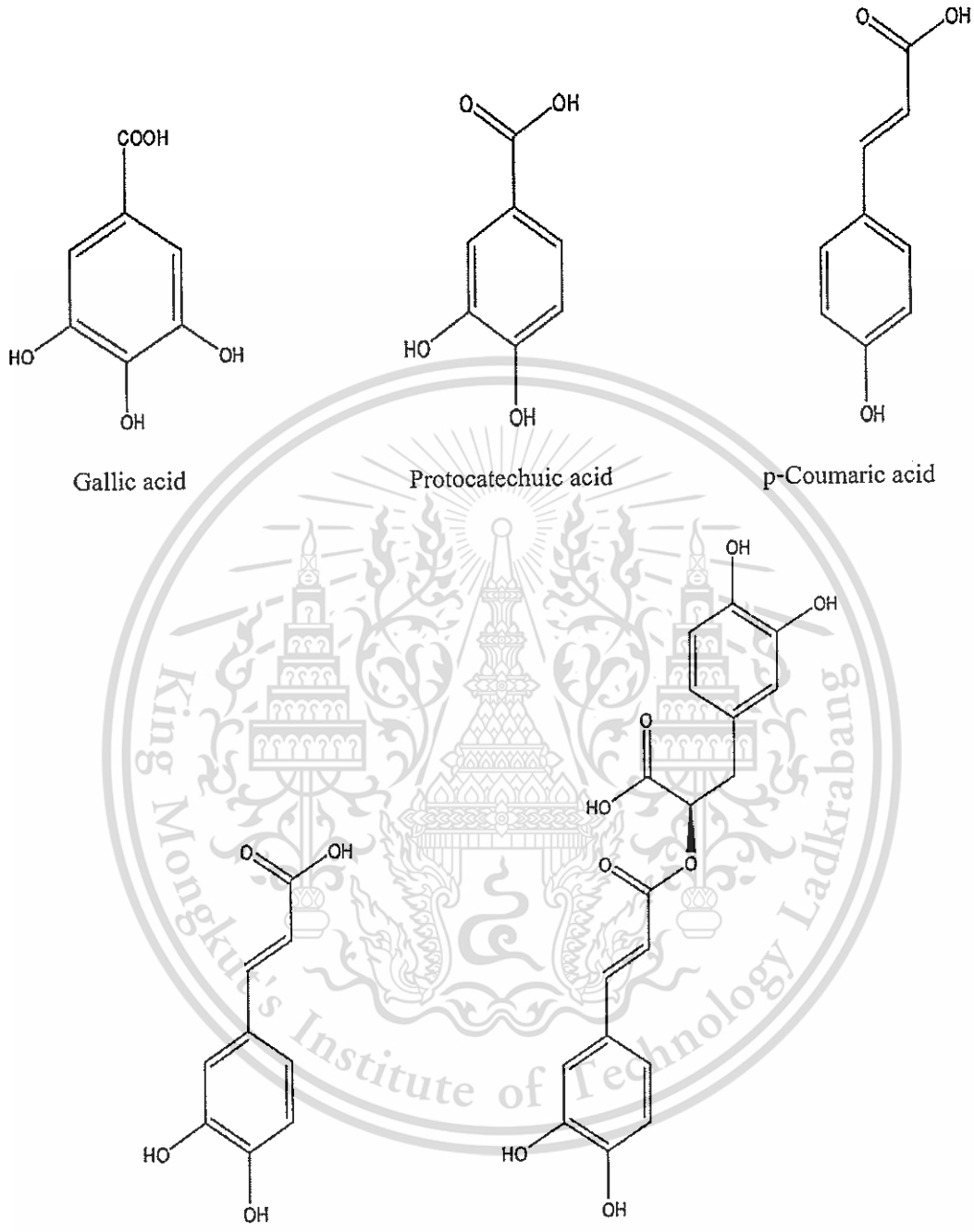
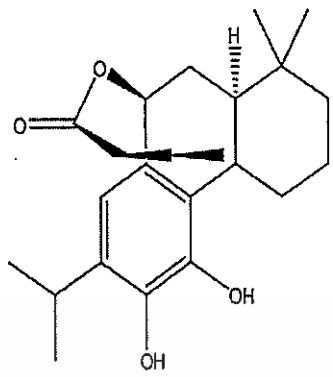
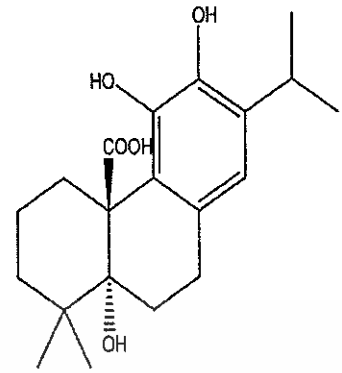


Figure 2.1 Antioxidative phenolic acids (gallic, protochatechuic, p-coumaric, rosmarinic and caffeic) found in plant extracts. All structures generated using ChemBioOffice (2008)

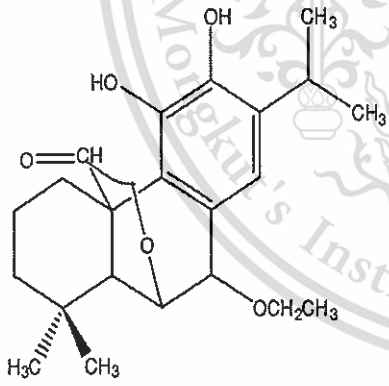
Source: Gheldof and Engeseth. (2002)



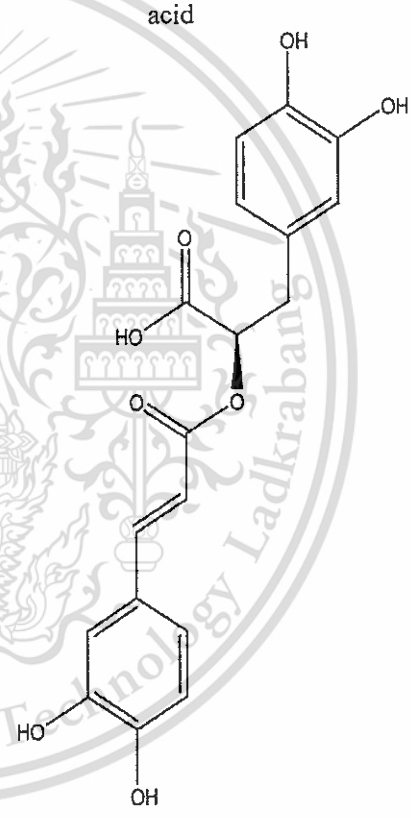
Carnosol



Carnosic acid



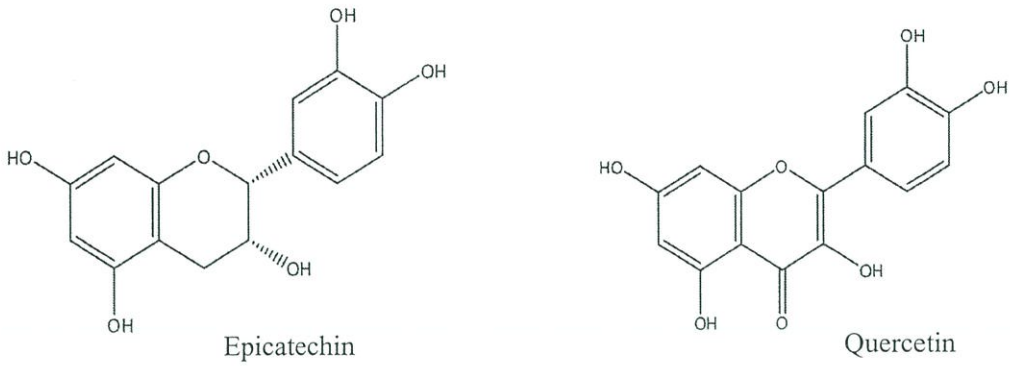
Rosmanol



Rosmarinic acid

Figure 2.2 Antioxidative phenolic diterpenes (carnosol, carnosic acid, rosmanol, and rosmarinic acid) found in plants

Source: Gheldof and Engeseth. (2002)



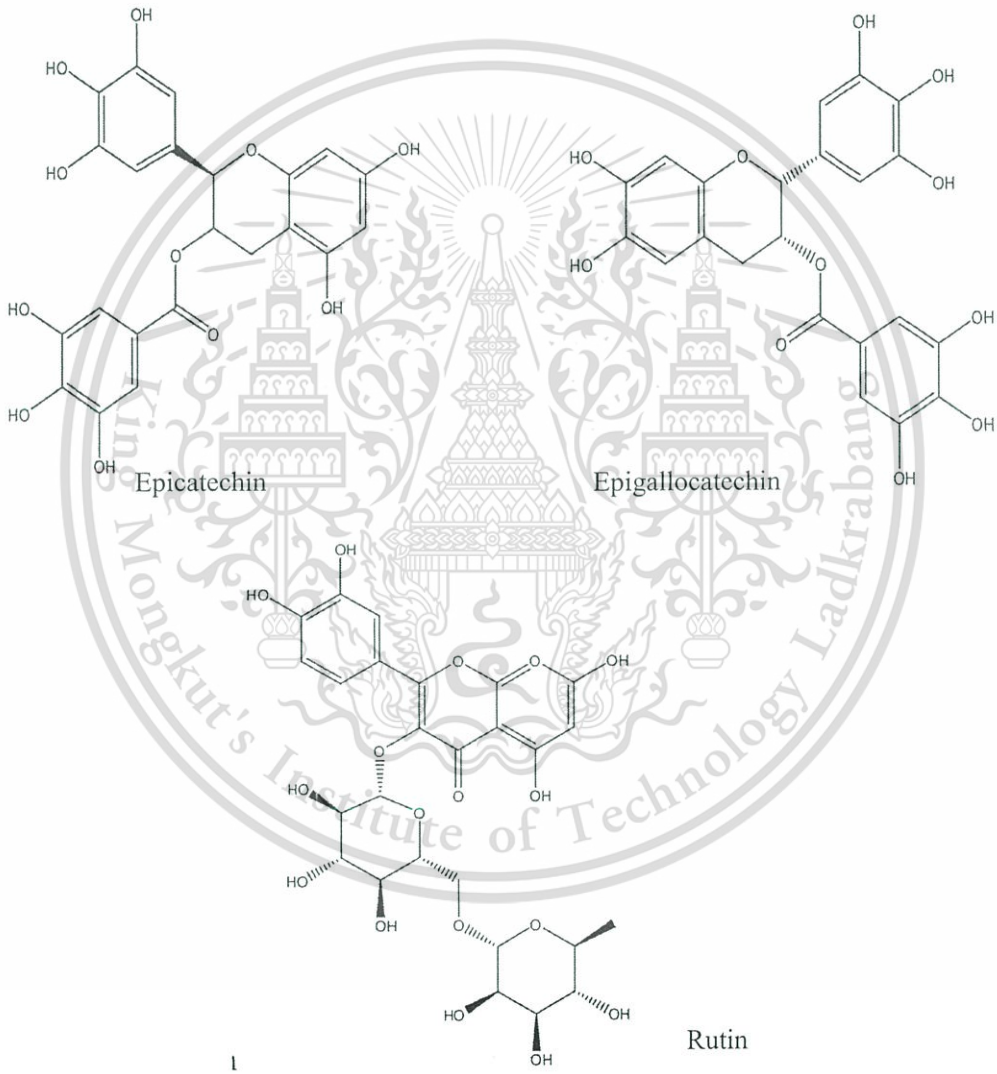


Figure 2.3 Antioxidative flavonoids (epicatechin, quercetin, epicatechin gallate, epigallocatechin gallate, and rutin) found in plant extracts

Source: Gheldof and Engeseth. (2002)

Authors interpret these findings as indicating that hydrogen bond force plays an important role. Flavonoids with multiple hydroxyl groups are more effective antioxidants than those with only one. The presence of the ortho-3,4-dihydroxy structure increases the antioxidative activity (Gheldof and Engeseth. 2002). Flavonoids can dampen transition metal enhancement of oxidation by donating a $H^{\bullet}$ to them, rendering them less proxidative. In addition, flavones and some flavanones (naringenin) can preferentially bind metals at the 5-hydroxyl and 4-oxo groups (Birt *et al.* 2001) evaluated the antioxidative activity of structurally related polyphenols, anthocyanidins, and phenolic acids at physiologically relevant concentrations (100 to 1000 nM) using a Cu^{2+}-mediated low-density lipoprotein oxidation model. Polyphenols with an ortho-dihydroxy substituted arrangement (cyanidin-3-glucoside, cyanidin, and protocatechuic acid) were the most effective, while trihydroxy-substituted compounds (gallic acid) had only intermediate efficacy. This was explained, in part, by their ability to chelate Cu^{2+} ions. It seems likely that the steric relationship of these OH groups and their arrangement on the ring(s) both play a role in the ability of the substance to chelate metal ions. However, differences in lipid/hydrophilic phase partitioning and in H-donating abilities were also hypothesized to have contributed to the structure-activity relationships. (Tinoi *et al.* 2006) reported that the order of free radicalscavenging activity of a group of polar compounds was ferulic acid > coumaric acid > propyl gallate > gallic acid > AA; the free radical-scavenging activity of a group of nonpolar compounds was rosmarinic acid > BHT, tert-butylhydroquinone (TBHQ) > tocopherol. Only propyl gallate, TBHQ, gallic acid, and rosmarinic acid inhibited lipid oxidation in an oil-in-water emulsion that may reflect the ability of these compounds to orient at the interface of the oil droplet in the emulsion. Evaluating the antioxidative activity of hydroxycinnamic acids with similar structures (caffeic, chlorogenic, o-coumaric, and ferulic acids) in a fish muscle system, (Medina *et al.* 2007) found that the capacity of these compounds to donate electrons (bond dissociation energies) appeared to play the most significant role in delaying rancidity, while the ability to chelate metals and the distribution between oily and aqueous phases were not correlated with inhibitory activities. The latter finding may reflect the type of matrix, fish muscle, in which the oxidative activity was studied. Caffeic acid was the most effective of this antioxidant group (similar to propyl gallate). (Kumar and Jain. 2015) reported that, of a variety of flavonoids (rutin, dihydroquercetin, quercetin, epigallocatechin gallate, and epicatechin gallate), the catechins were most effective in inhibiting microsomal lipid peroxidation. All were able to chelate Fe^{2+}, Fe^{3+}, and Cu^{2+} and were effective O^{2-} scavengers to varying degrees. Authors speculate that the relative

ability to scavenge O^{2-} may be responsible for the relative antioxidative difference among these compounds. Many of the antioxidative flavonoid compounds are naturally occurring pigments. It appears that chloroplast-located flavonoids perform a photoprotective role against O^{2-} in plants. Anthocyanins are the glycosides of polyhydroxy or polymethoxy derivatives of the flavylium cation. Hydrolysis of the sugar moiety yields an aglycone, anthocyanindin. Anthocyanins and anthocyanindins exhibit visual color because of the extreme mobility of the electrons within the molecular structure (double bonds) in response to light in the visible spectrum (approximately 400 to 700 nm). The pigments are quite watersoluble and 4 ˉOH groups are bound to the aromatic rings. pH has a significant effect on anthocyanin pigments. These ˉOH groups can give up H^+ (in a basic solution) or H to an oxidizing lipid (ROO•). Proanthocyanidins also contain multiple ˉOH groups that can donate hydrogen, quench O^{2-}, and chelate metals. Free radicalscavenging ability increases as the number of phenolic ˉOH groups increases. Some phenols can polymerize into polyphenols that can bind minerals. Proanthocyanidins often occur as oligomers or polymers of monomeric flavonoids, polyhydroxy flavan-3-ols such as 228 [+]-catechin and [-]-epicatechin.

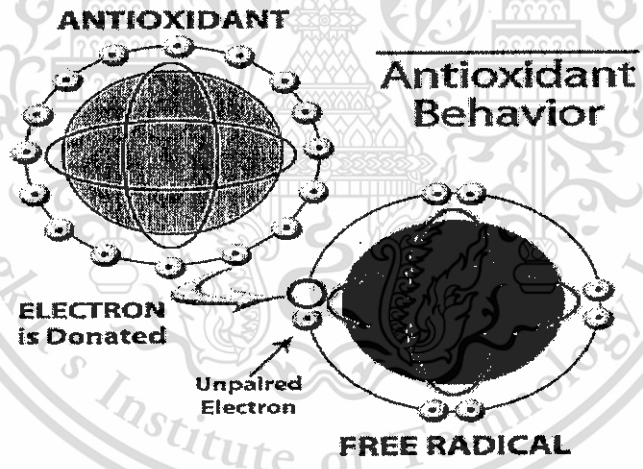


Figure 2.4 Antioxidant process

(Souce: www.amazing-glutathione.com/what-are-antioxidants.html)

The polymeric procyanidins are better antioxidants than the corresponding monomers, catechin, and epicatechin. Catechin and epicatechin can combine to form esters, such as catechin/epicatechin gallate, or bond with sugars and proteins to yield glycosides and polyphenolic proteins. Glycosylation of flavonoids at the 3 ˉOH group usually decreases the antioxidative activity due to the reduction of the number of phenolic groups (quercetin/rutin). Proanthocyanidins with demonstrated antioxidant activity and potential biologically therapeutic effects occur in fruits (apples and cherries), some berries (rosehips, raspberries, blackberries, and

strawberries), as well as in the leaves (tea), seeds (grape, sorghum, soy, and cocoa bean), and bark of many plants.

2.4 Microbes in meat

2.4.1 Role of microbes in meat

Producers and manufacturers have been challenged by the increasing demand for safe and high quality meat and meat products over the past few decades. Meat and meat products are highly prone to microbial contamination since they are rich in essential nutrients and perishable in nature. This is further accelerated by some intrinsic factors including pH and water activity of fresh meat. In general, most fresh meat has a water activity value higher than 0.85 and its pH value falls within the favorable pH range for spoilage bacteria of meat (Fullerton *et al.* 2011). Hence, deterioration in quality and potential public health issues is common if these products are not properly handled and preserved (Tajkarimi *et al.* 2010).

A significant level of spoilage of meat and meat products takes place every year at different levels of the production chain including the preparation, storage, and distribution. Besides lipid oxidation and autolytic enzymatic spoilage, microbial spoilage plays a significant role in this deterioration process leading to a substantial economic and environmental impact (Dave and Ghaly. 2011). Microorganisms associated with the spoilage of meat and meat products including bacteria such as *Pseudomonas, Acinetobacter, Brochothrix thermosphacta, Lactobacillus* spp., *Enterobacter* etc., and yeast and mold cause quality defects such as off-flavor, off-odor etc.

Additionally, foodborne diseases have emerged as important and growing public health and economic problems in many countries over the last few decades. Foodborne diseases are not limited to a particular age group or country (Bozoglu *et al.* 2004). Regarding the meat and meat products, several pathogenic microorganisms including *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Clostridium* spp. and *Aeromonas hydrophila* can result in foodborne illnesses to consumers if the products are not preserved and handled properly. Vegetative cells of the microbes are destroyed with the thermal processing, but not the spores of food pathogens such as *Bacillus* spp. and *Clostridium* spp. They can survive and need much harsher conditions to be inactivated. With the absence of competitive microflora, these spores can germinate and grow under favorable conditions caused by mishandling of the heat

treated products. Hence, it is vital to apply other hurdles such as low temperature storage to avoid this problem (Mor-Mur and Yuste. 2010).

2.4.2 Microbial deterioration of meat and meat products

Depending on the preservation method used, the growth and metabolism of spoilage and pathogenic microorganisms can cause a rapid spoilage of meat and meat products and serious foodborne intoxications (Ngoci *et al.* 2014; Parsaeimehr *et al.* 2010; Radha Krishnan *et al.* 2014). Bacteria are generally considered as the principle agents responsible for these deteriorations and health issues in addition to molds and yeasts. Table 2.1 presents the major genera of bacteria, yeasts and molds found in meat and meat products. Main spoilage bacteria including *Pseudomonas*, *Acinetobacter*, *B. thermosphacta*, *Moraxella*, *Enterobacter*, *Lactobacillus* spp., *Leuconostoc* spp., *Proteus* spp. etc, yeast and mold decompose meat and meat products and develop unpleasant quality characteristics (Böhme *et al.* 2012; Rawat. 2015) when they grow in large number in these perishable products. This can be attributed to the degradation of proteins and lipids present in meat and meat products (Velasco and Williams. 2011) resulting in off-odors, off-flavors, discoloration, texture defects, slime and gas productions, and changes in pH (Filgueras *et al.* 2010). Although spoilage microorganisms normally do not cause illness, they can result in gastrointestinal disturbances when consumed in high concentrations. However, the rate of meat spoilage is affected by several factors including hygiene, storage temperature, the acidity of the meat and meat products (Addis. 2015).

In addition, Russell *et al.* (1996) reported that the growth of spoilage bacteria is favored at a pH range of 5.5 to 7.0. Mor-Mur and Yuste. (2010) reported that *C. jejuni*, *S.* Typhimurium, *E. coli* O157:H7, other enterohemorrhagic *E. coli* (EHEC), *L. monocytogenes*, *Arcobacter butzleri*, *Mycobacterium avium* subspp. *paratuberculosis* and *A. hydrophila* are the most prevalent and serious emerging pathogens in meat and meat-derived products. The color, odor, taste or texture of meat and meat products are not often changed by these pathogenic bacteria. However, they are mainly responsible for food poisoning and food intoxications. Hence, microbial deterioration of meat and meat products can be considered as one of the main limitations in the meat industry. Proper protective practices should be, therefore, applied for meat and meat products (Jalosinska and Wilczak. 2009; Min *et al.* 2008) to produce the highest quality products possible.

Table 2.1 Common spoilage and pathogenic microorganisms associated with fresh meat and meat products

Microorganism	Genera/Species
Spoilage microorganism	
Bacteria	*Pseudomonas*
	Acinetobacter
	Brochothrix thermosphacta
	Moraxella
	Enterobacter
	Lactobacillus spp.
	Leuconostoc spp.
	Proteus spp.
	Flavobacterium
	Alcaligenes
Yeasts	*Candida*
	Torulopsis
Molds	*Zhizopus*
	Sporotrichum
	Fusarium
	Monilia
Pathogenic microorganism	*Salmonellas* spp.
	Staphylococcus aureus
	Listeria monocytogenes
	Clostridium perfringens
	Escherichia coli O157:H7
	Enterohemorrhagic *E. coli* (EHEC)
	Campylobacter spp.
	Bacillus cereus
	Yersinia enterocolitica

Source: Jayasena and Jo. (2013)

2.4.2.1 Pathogenic bacteria in meat

Pathogens are organisms, frequently microorganisms, or components of these organisms that cause disease. Microbial pathogens include various species of bacteria, viruses, and protozoa. Many diseases caused by pathogenic microorganisms, and the frequency of these diseases, are a national security issue. Foodborne diseases have emerged as important and growing public health and economic problems in many countries over the last few decades. Foodborne diseases are not limited to a particular age group or country (No. 2012). It is estimated that 76 million cases of foodborne diseases occur each year in the United States result in illnesses, with 325,000 hospitalizations and 5000 deaths, leading to high medical costs and productivity losses in the range of US$ 6.6 billione 37.1 billion (Böhme *et al.* 2012). The pathogens of greatest concern in fresh and frozen meat and meat products are *Salmonella* spp., *E. coli* O157:H7 and other enterohemorrhagic *E. coli* (EHEC), *L. monocytogenes*, *S. aureus*, and the potential for *C. botulinum* in cured hams and sausages. Vegetative cells of the microbes are destroyed with the thermal processing, but not the spores of food pathogens such as *Bacillus* spp. and *Clostridium* spp. They can survive and need much harsher conditions to be inactivated. With the absence of competitive microflora, these spores can germinate and grow under favorable conditions caused by mishandling of the heat treated products. Hence, it is vital to apply other hurdles such as low temperature storage to avoid this problem.

(1) *Salmonella* spp.

Salmonella spp. is an enteric pathogen associated with animal and slaughter hygiene. In the EU, eggs and egg products are the most frequently implicated sources of human salmonellosis. Meat is also an important source, with poultry and pork implicated more often than beef and lamb. The two most common *Salmonella* serotypes are Typhimurium and Enteritidis. In human salmonellosis, *S.* Typhimurium is the most frequent serotype. *Salmonella* Enteritidis is associated primarily with poultry and eggs. It has been observed that *Salmonella* spp. usually persist during chilling. Human salmonellosis infections can lead to uncomplicated enterocolitis and enteric (typhoid) fever, the latter being a serious disease that may involve diarrhea, fever, abdominal pain, and headache. *Salmonella* spp. can also cause systemic infections, resulting in chronic reactive arthritis (Mor-Mur and Yuste. 2010). Developed countries have used for a long time systems of surveillance of food safety problems. However, many outbreaks of food poisoning are never recognized because known pathogens are not accurately diagnosed or reported, and other causative foodborne agents are unknown and therefore unreported. This

causes underestimation of foodborne disease incidences. Furthermore, industries check their products but usually do not report positive findings. Most foodborne disease outbreaks and deaths with an undetermined cause are likely caused by known pathogens that are not detected, particularly viruses, often members of the Norovirus genus. This situation can be corrected through initiation of new and improvement of existing epidemiological monitoring programs. Furthermore, as most foodborne diseases are due to mishandling of foods in ways we know we should avoid (e. g., improper cooling, inadequate heating/reheating, and poor personal hygiene), education of food handlers and consumers about the importance of food hygienemay improve safety and so prevent many illness (Mor-Mur and Yuste. 2010).

(2) *Escherichia coli* O157:H7

Enterohemorrhagic *E. coli* (EHEC), i.e., *E. coli* O157:H7 and other serotypes of Shiga toxin-producing *E. coli*, are foodborne pathogens of primary concern. They are etiological agents of hemorrhagic colitis. In some cases, complications may occur, e.g., hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. EHEC other than *E. coli* O157:H7 have been increasingly associated with such complications. The severity of the illness and the low infective dose (<100 organisms) make *E. coli* O157:H7 among the most serious foodborne pathogens. *Escherichia coli* O157:H7 is an enteric organism associated with animal and slaughter hygiene. It may be present in the feces and intestines of healthy meat (Fullerton *et al.* 2011). Swine and poultry are also possible reservoirs of *E. coli* O157:H7 because the organism can colonize the ceca. The pathogen has also been isolated from other domestic and wildlife animals sheep, goats, deer, dogs, horses, and cats (Mith *et al.* 2014). Therefore, meat can be contaminated during slaughter operation and processing (Zhang *et al.* 2010). Most people infected with *E. coli* O157:H7 pick up the organism from cattle, which are a major reservoir, either through direct contact with feces or by consuming meat. *E. coli* strains from food sources (e.g., animal carcasses and derived meat products) can harbor potentially significant virulence determinants, such as cytotoxic necrotizing factors in uropathogenic strains, and cytolethal distending toxins in strains which are not certain causes of human infection (Fullerton *et al.* 2011).

(3) *Listeria monocytogenes*

L. monocytogenes is an environmentally transmitted pathogen. It is a psychrotroph and ubiquitous, and grows well in poor substrates, which enables contamination during any phases of food chain. *L. monocytogenes* is able to survive and multiply on plants and in soil and water. The incidence of listeriosis is relatively low, but it is of major public health concern because of the

severity and nonenteric nature of the disease, which reveals as meningitis or meningoencephalitis, septicemia, and abortion, mainly in populations such as young children, the elderly, pregnant women, and other immunocompromised persons. It is also a major public health concern because of the ability of the pathogen to grow at refrigeration temperature. Pediatric lymphocytic meningoencephalitis due to *L. monocytogenes* is a serious form of brain infection, even in immunocompetent childhood, especially when an important inflammatory syndrome appears. Most persons frequently ingest listeriae, but they are apparently resistant to infection. The infective dose depends on the immunological status of the human host and characteristics of the organism such as its virulence factors. The dose is usually high, but in some cases it may be as low as several hundred or even less organisms (Tajkarimi *et al.* 2010). Cooked, ready-to-eat meat and poultry products have been the source of sporadic and outbreak-associated cases of listeriosis in North America and Europe. Contaminated frankfurters and turkey deli meat caused multistate outbreaks of listeriosis in the USA in 1998, 2000, and 2002 (Kim *et al.* 2013a). Thus, ready-to-eat meals, unreheated frankfurters, and undercooked chicken can be vehicles for the pathogen. It has been found that 16% of salamis are contaminated with the pathogen. The organism tends to concentrate in organs. Therefore, eating undercooked organ meat may be more hazardous than eating undercooked muscle tissue (Kim *et al.* 2013a). *L. monocytogenes* is not significantly affected by vacuum packaging and certain modified atmospheres because it is a facultative anaerobe. There is very little or no *L. monocytogenes* multiplication at pH 5.0 (Mor-Mur and Yuste. 2010). The organism grows well in some refrigerated ready-to-eat foods if stored for a long period, and thus consumer practices may determine the level of *L. monocytogenes*. Ready-to-eat meat and poultry products that have received heat treatment followed by cooling in brine before packaging may provide a particularly favorable environment for *L. monocytogenes* because of the reduction of competitive microbiota and the high salt tolerance of the organism. Therefore, ready-to-eat foods are of great risk and it is not practical to expect them to *be L. monocytogenes* free. If industries do not report positive findings, the incidence of the pathogen is underestimated, and therefore the Administration keeps the zero-tolerance policy, which has been questioned by industries of many countries as unattainable. Food regulatory agencies in many countries have accepted the argument that it is impossible to produce *L. monocytogenes* free foods and have given tolerance levels for the pathogen. For this reason, levels of risk for different meat and poultry products have been established.

(4) *Staphylococcus aureus*

Food-poisoning staphylococci are widely distributed; meat contamination being generally associated with highly manual-handled foods. The most common etiological agent is *S. aureus* and its related heat stable enterotoxins (Castellano *et al.* 2008). Studies in slaughtered pigs showed that *S. aureus* was the pathogenic bacteria most frequently detected in slaughterhouses and processing rooms. Although the microbiological quality of pork is highly dependent on the hygiene of slaughterhouses, dressing operations and processing lines, where workers' hands and the environment of locations associated with the evisceration process are the principal sources. In addition, the presence of staphylococci in bioaerosols from red-meat abattoirs as well as pork or beef mastitis may also constitute a risk of foodborne pathogen contamination (Tajkarimi *et al.* 2010). Pig carcasses are often important sources of contamination with *S. aureus* mainly due to the sequential steps of the slaughter, which involve scalding, dehairing, polishing, trimming, washing, chilling and cold chain systems for transportation and merchandising. Time and temperature abuse of a food product contaminated with enterotoxigenic staphylococci can result in enterotoxin formation. The occurrence of enterotoxigenic *S. aureus* in foods of animal origin was reported to be extremely variable, ranging from 6% to 90% (Tajkarimi *et al.* 2010).

(5) Other pathogenic bacteria in meat

Species within the genus *Campylobacter* and *Yersinia* have also emerged as pathogens of human public health concern (Castellano *et al.* 2008). *Campylobacter* is the most common cause of human foodborne illness in the United States, this being related to its high degree of virulence and its widespread prevalence in foods of animal origin. *Campylobacter* has been isolated from beef at retail sale, indicating that beef can be a potential vehicle for the transmission of this pathogen to humans. *C. jejuni* can be transferred from hides to meat during slaughter and dressing of beef carcasses. Even after high initial carcass prevalence, chilling showed to be an efficient critical control point to eliminate *Camplylobacter* from carcass surfaces. A high prevalence of antimicrobial resistance was frequently observed in *Campylobacter* strains, *C. coli* being generally more resistant than *C. jejuni*. On the other hand, *Y. enterocolitica* is frequently associated with pigs and pork products and can be transmitted to humans through the consumption of raw, undercooked or recontaminated processed meats. The prevalence of *Y. enterocolitica* in pig herds has been reported to range between 40% and 65%, tonsils and oral cavity being important reservoirs (Castellano *et al.* 2008).

Other pathogens of human health concern that may be present but remain undetected in slaughtered animals involve streptococci, clostridia and corinebacteria. Among these, *Clostridium perfringes* is the leading cause of bacterial foodborne illness in countries where meat and poultry consumption is high. Meat animals are subjected to a number of clostridial diseases and these bacteria may be present in their carcasses. Enterotoxigenic type A strains carrying a chromosomal *cpe* gene, necessary for food poisoning, have been strongly associated with food poisoning outbreaks (Omojate *et al.* 2014). In a study carried out in England and Wales, the consumption of red meats was implicated in infectious intestinal outbreaks in which *C. perfringes* was the most frequently reported organism. A decrease in the number of cases linked to foods containing red meat is in agreement with the steady decline in red meat consumption due to the BSE crisis (Zhang *et al.* 2016a). After slaughter and dressing of carcasses, bacterial growth will depend on storage conditions. During storage, environmental factors such as temperature, gaseous atmosphere and meat pH will select certain bacteria for growth. Cold storage of meat will decrease bacterial growth, only 10% of the bacteria initially present being able to grow at refrigeration temperatures. Oxygen restriction using vacuum or modified atmospheres will drastically reduce the presence of *Pseudomonas* while bacterial flora will be gradually selected towards CO_2 tolerant organisms. Under these conditions, the dominating microorganisms are Brochothrix thermosphacta, lactic acid bacteria (LAB), mainly *Lactobacillus, Leuconostoc* and *Carnobacterium*. The presence of *Enterococcus* in beef, poultry and pork carcasses or fresh meat, indicating fecal contamination during slaughter, has also been reported. Both beneficial and detrimental roles in foods have been ascribed to *Enterococcus*. They play an important role in the development of flavor in traditional cheeses and sausages and, as probiotics, can provide important health benefits. As detrimental organisms, they have been implicated in outbreaks of foodborne illness through their antibiotic resistance and as carriers of virulence factors. The prevalence of antibiotic resistance in streptococci and enterococci isolated from the production chain of swine commodities has been extensively reported (Castellano *et al.* 2008).

2.4.2.2 Spoilage of meat

A significant level of spoilage of meat and meat products takes place every year at different levels of the production chain including the preparation, storage, and distribution. Besides lipid oxidation and autolytic enzymatic spoilage, microbial spoilage plays a significant role in this deterioration process leading to a substantial economic and environmental impact (Böhme *et al.* 2012). Main spoilage bacteria including *Pseudomonas*, *Acinetobacter*, *B.*

thermosphacta, Moraxella, Enterobacter, *Lactobacillus* spp., *Leuconostoc* spp., *Proteus* spp. etc, yeast and mold decompose meat and meat products and develop unpleasant quality characteristics when they grow in large number in these perishable products. This can be attributed to the degradation of proteins and lipids present in meat and meat products resulting in off-odors, off-flavors, discoloration, texture defects, slime and gas productions, and changes in pH (Castellini *et al.* 2006). Although spoilage microorganisms normally do not cause illness, they can result in gastrointestinal disturbances when consumed in high concentrations. However, the rate of meat spoilage is affected by several factors including hygiene, storage temperature, the acidity of the meat and meat products (Jayasena and Jo. 2013).

2.4.3 Common preservation methods used against microbial deterioration in meat

Due to the increasing demand for precooked, refrigerated, ready-to-eat meat, and meat products convenient for modern and busy lifestyles, meat manufacturers must overcome many challenges, including the control of microbial deterioration of these products (Falowo *et al.* 2014). Several factors such as microbial growth, color, and lipid oxidation affect the shelf life and consumer acceptance of fresh meat and meat products (Kim *et al.* 2013a; Mhalla *et al.* 2017; Zhang *et al.* 2016b). Therefore, to produce the safest and highest quality products possible, these factors must be controlled. Several thermal and nonthermal food preservation techniques have been used, alone or in combination, to prevent or minimize the growth of spoilage and pathogenic microorganisms in meat and meat products (Böhme *et al.* 2012; Mith *et al.* 2014). Table 2.2 shows the common physical and chemical preservation techniques used to control bacterial activity (Mor-Mur and Yuste. 2010). In brief, low temperature preservation methods such as chilling and freezing which offer temperatures below the optimal range for microbial growth (Rawat. 2015), inhibit their growth and proliferation and thereby limit the spoilage rate. In addition, water activity in meat is controlled by several preservation methods including drying, adding chemicals or a combination of these methods leading to inhibition of cell growth. Sodium chloride is usually used for this purpose (Patra and Baek. 2016). High pressure processing effectively inactivates spoilage and pathogenic microorganisms by modification of morphology and of several components such as cell membranes, ribosomes and enzymes (Jayasena and Jo. 2013). Meanwhile, microbial genetic materials such as DNA are damaged by different irradiation methods leading microbial cells to lose normal cellular functions and die. Different packaging methods are involved in meat and meat products manufacturing. They often control the microenvironment of the microorganisms and thereby limit their growth and proliferation. The

preservation effect of heat treatments such as conventional heating, microwave heating etc. is generally achieved through reducing the growth of, or inactivating, spoilage and pathogenic microflora. Chitosan has also been used as antimicrobial films and coatings against gram-positive and gram-negative bacteria, yeasts and molds due to its positive charges which interfere with microbial cell surface resulting in the membrane leakage. Additionally, a broad range of synthetic food additives with antimicrobial properties is widely techniques used in the meat industry to prevent the growth of foodborne pathogens and spoilage microorganisms and to extend the refrigerated storage time of fresh meat and meat products (Sebranek *et al.* 2005). Common synthetic food additives used as antimicrobial compounds consist of chlorides, nitrites, sulfites, lactic acid, ascorbic acid and sorbic acid (Abdulmumeen *et al.* 2012). However, some of the traditional preservation techniques such as brining and fermentation are being challenged due to an increasing consumer demand for products with lower salt content, which ultimately increases the need for effective food preservatives. Additionally, the use of some synthetic food additives such as nitrate salt in meat products has been questioned due to possible toxic and carcinogenic effects on human health. Several countries have restricted or banned the use of chemical additives that are harmful to human health (Ebrahimabadi *et al.* 2010). Hence, consumers now prefer natural food additives over traditional synthetic preservatives (Qi *et al.* 2015; Sebranek *et al.* 2005) thereby driving research for identifying natural food additives with broadspectrum antimicrobial activities. These additives may also improve the quality and shelf life of meat products (Jalosinska and Wilczak. 2009). Many studies have examined the use of aromatic phytochemical preparations with dual functionality against microbial spoilage and lipid oxidation in meat and meat products (Radha Krishnan *et al.* 2014), particularly plant extracts (Kim *et al.* 2013a; Nascimento *et al.* 2000). Numerous experimental applications of plant extracts have shown their suitability as preservatives in meat and meat products (Qi *et al.* 2015; Reddy *et al.* 2013), particularly as effective natural antimicrobial agents against foodborne pathogenic and spoilage bacteria (Jalosinska and Wilczak. 2009; Patra and Baek. 2016).

Table 2.2 Common preservation methods used against microbial deterioration in meat

Physical treatments	Chemical treatments
Conventional heating	(Spray) washing with water, steam or solution (e.g., organic acids, such as lactic acid, trisodium phosphate)
Chilling/freezing/drying	Other agents in solution, such as fatty acid esters, parahydroxybenzoic acid esters, lysozyme, phenolic compounds, isothiocyanates, ascorbic acid
Canning/microwave heating	Nitrites
Ohmic heating	Sulfites
Ultrasound	Spices, condiments, plant essential oils and plant extracts
Ultraviolet radiation	Chitosan
Ionizing radiation	Bacteriocins
High pressure processing	Bacteriophages
Pulsed electric fields	
Oscillatory magetic fields	
Packageing: Vacuum or modified atmosphere, and/or active packaging	

Source: Mor-Mur and Yuste. (2010); Addis. (2015)

2.5 Application of plant extract in meat

Strong consumer demand for safe and high-quality meat can be attributed in part to the widespread availability and accessibility of quality health data and information. There are also new concerns about food safety due to increasing occurrence of new foodborne disease outbreaks caused by pathogenic microorganisms. This raises considerable challenges, particularly since there is increasing unease regarding the use of chemical preservatives and artificial antimicrobials to inactivate or inhibit growth of spoilage and pathogenic microorganisms (Munuswamy *et al.* 2013; Obeidat *et al.* 2012; Sokmen *et al.* 2004). As a consequence, natural antimicrobials are receiving a good deal of attention for a number of microorganism control issues. Reducing the need for antibiotics, controlling microbial contamination in food, improving shelf-life extension

technologies to eliminate undesirable pathogens and/or delay microbial spoilage, decreasing the development of antibiotic resistance by pathogenic microorganisms or strengthening immune cells in humans are some of the benefits (Böhme *et al.* 2012; Mor-Mur and Yuste. 2010).

At present, meat industry uses chemical additives in several meat processes to prevent the growth of food-borne pathogens and extend the shelf life of refrigerated storage. Since concern over the safety of chemical additives has arisen in recent years, consumers increasingly demand the use of natural products as alternative preservatives in foods (Negi. 2012). This massive wastage which has become a major concern to consumers, governments and food industries is however associated with the outbreak of foodborne diseases.

The use of natural compounds such as plant extracts has been identified for decontamination of meat and meat products against *Salmonella* spp., *E. coli, S. aureus* (Boskovic *et al.* 2015; Vashist and Jindal. 2012). The antimicrobial activities of plant crude extracts in Table 2.3 have been reported in several studies. The effectiveness of these medicinal plants for example: *Artemisia absinthium, Hypericum perforatum, Oleoresin rosemary, Origanum vulgare, Satureja horvatii, Syzygium aromaticum, Fatsia* spp., and olive among others, against microbial growth in meat and meat products has been reported in several studies in Table 2.3. Some interesting results were however found by combining different plants together to test their efficacy against food borne organisms that are prevalent in meat and meat products (Vashist and Jindal. 2012). Krishnan *et al.* (2014) found a stronger antimicrobial effect of the combination of *S. aromaticum*, *O. vulgare* extracts in pork meat than individual spices, and they attributed this to synergistic actions of each specific compounds present in the mixed spices.

The presence and level of concentration of different phytochemical compounds such as phenolic, flavonoid, alkaloids, saponins, tannins, carvacrol, terpenes, and thymol among others, have been recognised as the potential source of antimicrobial activities in plant materials (Falowo *et al.* 2014). Further study should be concentrated on the combination and application of different natural antioxidants to reduce meat spoilage and to extend the storage time, as these will greatly help to reduce financial loss, labour costs, ensure safety and ultimately improve the functional properties of the meat.

Table 2.3 The antimicrobial activities of plant crude extracts in meat and meat products

Plant materials	Meat	Effect on foodborne pathogenic organisms	References
Fresh leaves of butterbur (*Petasites japonicus*)	Ground beef patties	*E. coli* O157:H7	(Kim *et al.* 2013a)
		Bacillus anthracis	
Clove, Cinnamon, Oregano and Mustard	Raw chicken meat	*L. monocytogenes*	(Radha Krishnan *et al.* 2014)
		L. lactis	
		L.mesenteroides,	
		P. fluorescens	
		S.putrifaciens	
		E. coli	
		S. typhimurium	
Rumex tingitanus extracts	Minced beef meat	*Bacillus* spp.	(Mhalla *et al.* 2017)
		S. aureus	
		L. monocytogenes,	
		E. coli	
		P. aeruginosa	
Rosemary, cranberry and lovage extracts	Pork meat	*Y. enterocolitica*	(Jalosinska and Wilczak. 2009)
		S. aureus	

Source: Vashist and Jindal. (2012)

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant and ground pork samples

Fresh *P. acidus* leaves were collected from the local area in Ladkrabang, Bangkok, Thailand. Mature fresh *C. carandas* fruits were collected from the local area at Samut Songkhram, Thailand. Ground pork meat and fat were purchased at the Makro supermarket in Bangkok, Thailand.

3.1.2 Media and growth conditions of bacteria

Table 3.1 List of indicator strains and their growth conditions

Indicator strains	Media	Temperature (°C)	Condition
Pathogenic bacteria			
Salmonella Typhimurium TISTR 292	TSB-YE	37	Aerobic
Staphylococcus aureus TISTR 118	TSB-YE	37	Aerobic
Escherichia coli TISTR 780	TSB-YE	37	Aerobic
Aeromonas hydrophila TISTR 1321	TSB-YE	37	Aerobic
Spoilage bacteria			
Pseudomonas fluorescens TISTR 358	TSB-YE	37	Aerobic
Lactobacillus plantarum ATCC 14947^T	MRS	30	Anaerobic
Lactobacillus sakei TISTR 890	MRS	30	Anaerobic
Leuconostoc mesenteroides subsp. *mesenteroides* TISTR 942	MRS	30	Anaerobic
Streptococcus sp. TISTR 1030	MRS	30	Anaerobic
Lactococcus cremoris TISTR 1344	MRS	30	Anaerobic
Bacillus coagulans TISTR 1447	MRS	30	Anaerobic

MRS = de Man, Rogosa and Sharpe media

TSB-YE = Trypticase soy broth with 0.6% yeast extract media

TISTR = Thailand Institute of Scientific and Technological Research, Thailand

ATCC = American Type Culture Collection, Rockville, Md

3.1.3 Equipments and chemicals

(1) Autoclave (Hirayama model HVE 50, Japan)

(2) Bag vacuum-type (K-Nylon/LLDPE)

(3) Centrifuged machine (Avanti J-E, Beckman coulter, California)

(4) Color measurement spectrophotometers (HunterLab MiniScan 4500S, The Stothard Group Ltd., United Kingdom)

(5) Electronic balance (Tanita model 1144, Tanita Corporation, Japan)

(6) Rotary evaporator (Buchi Rotavapor R215, Canada)

(7) Homogenizer (Ultra tarrax, Germany)

(8) Hot-air oven (Memmert model CM500, Germany)

(9) Chopper machine (Guangdong, China)

(10) HunterLab MiniScan 4500S Spectrophotometer (EZ, USA)

(11) Incubator (WTB Binder model BD, Germany)

(12) pH meter (Mettler Toledo, Greifensee, Switzerland)

(13) pH meter (NANA-107121, Germany)

(14) Spectrophotometer (Shimadzu model UV-1601, Japan)

(15) Stomacher Bag Mixer (400 model VW, France)

(16) Vacuum packing machines (Ramon, Germany)

(17) Vortex Mixer (KMC-1300V, Korea)

(18) Water Bath (Memmert, Germany)

3.1.4 Chemicals and media

(1) (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Sigma, Russian)

(2) 1,1,3,3 – Tetraethoxypropane (Sigma, Germany)

(3) 2 – Thiobarbituric acid (Sigma, Germany)

(4) 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Sigma, Canada)

(5) 2,2-Diphenyl-1-picrylhydrazyl (Sigma, Germany)

(6) 2,6-Di-tert-butyl-4-methylphenol (Acros, Belgium)

(7) Agar (Criterion, USA)

(8) Analytical balance (Basic, Germany)

(9) Chromocult (Merck, Germany)

(10) Ethanol 99% (Merck, Germany)

(11) Malt extract (Merck, Germany)

(12) MRS agar (Merck, Germany)

(13) Plate count agar (Merck, Germany)

(14) Potassium persulphate (Hazardous, New Zealand)

(15) Simmons citrate agar (Merck, Germany)

(16) Trichloroacetic acid (Merck, Germany)

(17) Tryptic Soy Broth (Merck, Germany)

(18) Yeast extract granulated (Merck, Germany)

3.2 Methods

This study was divided into 4 experiments:

Objectives	Experiments
Experiment 1 Evaluation the effect of various solvent ratios of ethanol (0%, 25%, 50%, 75% and 100%) in water on the extraction crude yield, TPC and TFC of *P. acidus* leaves and *C. carandas* fruits	1.1 Preparation of ethanol extracts at the concentrations of 0, 25, 50, 75, 100% (v/v) from *P. acidus* leaves and *C. carandas* fruits 1.2 Determination of crude extract content of *P. acidus* and *C. carandas* extracts using the solvent ratio containing 0, 25, 50, 75, 100% (v/v) of ethanol aqueous solution — Total phenolic content (TPC) — Total flavonoids content (TFC)
Experiment 2 Evaluation the effect of various solvent ratios of ethanol (0%, 25%, 50%, 75% and 100%) in water on *in vitro* antioxidant and antimicrobial properties of the crude extracts from *P. acidus* leaves and *C. carandas* fruits	2.1 *In vitro* antioxidant activity of the extracts from *P. acidus* leaves and *C. carandas* fruits: — Radical scavenging activity (DPPH) — Lipid peroxidation assay — Reducing power ability — Metal chelating activity 2.2 *In vitro* antimicrobial activity of the extracts from *P. acidus* leaves and *C. carandas* fruits:

	– Microorganism test – Agar well diffusion
Experiment 3 Evaluation the effect of optimal crude extracts from *P. acidus* leaves and *C. carandas* fruits on chemical, physical and biological quality in ground pork storaged at 4°C up to 8 days and at -20°C up to 12 weeks	3.1 Preparation of meat samples such as: – Group 1: + Control (Non-treated) + BHT 0.2 g/kg meat + 2.5 g *P. acidus* extract/kg meat + 5 g *P. acidus* extract/kg meat – Group 2: + Control (Non-treated) + BHT 0.2 g/kg meat + 2.5 g *C. carandas* extract/kg meat + 5 g *C. carandas* extract/kg meat 3.2 Quality of ground pork samples The samples were storaged at 4°C during period time of 0, 2, 4, 6, 8 days and at -20°C during period time of 0, 4, 8, 12 weeks. – Chemical analysis: + Free scavenging radical activity (DPPH) + Radical cation decolorization assay (ABTS) + Reducing power ability + Thiobarbituric acid reactive substances (TBARS) – Physical analysis: + pH values + Color parameters – Biological analysis: + Total plate count

	+ Yeasts/Molds + Psychrophilic bacteria + Coliforms/*E. coli*
Experiment 4 Evaluation the effect of optimal crude extracts from *P. acidus* leaves and *C. carandas* fruits on sensory evaluation of ground pork products	Sensory evaluation

3.2.1 Evaluation the effect of various solvent ratios of ethanol (water, 25%, 50%, 75% and 100%) in water on the extraction crude yield, TPC and TFC of *P. acidus* leaves and *C. carandas* fruits

3.2.1.1 Preparation of ethanol extracts at the concentrations of 0, 25, 50, 75, 100% (v/v) from *P. acidus* leaves and *C. carandas* fruits

After washing under running tap water, Fresh *P. acidus* leaves and *C. carandas* fruits were dried in a hot-air oven at 45°C for 3 days, and then ground to small pieces. The dried leaves and fruits were extracted with different solvent ratios (water, 25%, 50%, 75% and 100% (v/v) ethanol in water) using 1 part of powder to 9 parts of each solvent (w/v) for 72 h at 4°C. Extraction was repeated three times and the extracts were then combined after being filtered through three layers of cheesecloth to remove large debris and re-filtered through Whatman No.1 filter paper. The filtrates were evaporated in a rotary evaporator (BUCHI Rotavapor R255, Lausanne, Switzerland) at 45°C, to leave a sticky residue and stored at 4°C until use. The sticky crude extracts (CE) were dissolved in their extraction solvent before use.

3.2.1.2 Determination of crude extract content of *P. acidus* and *C. carandas* extracts using the solvent ratio containing 0, 25, 50, 75, 100% (v/v) of ethanol aqueous solution

(1) Total phenolic content (TPC)

TPC was determined by the Folin-Ciocalteu method (Chumyam *et al.* 2013). The calibration curve was established using gallic acid (0 - 200 mg/l). One ml of each extract solution (1000 mg/l) or gallic acid was individually combined with 0.5 ml of 2N Folin-Ciocalteu reagent and 4 ml of 7.5% (w/v) sodium carbonate solution. The mixtures were allowed to stand for an hour at room temperature in the dark, and then measured at 765 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). TPC was calculated on the basis of the calibration curve of

gallic acid standard. The results were expressed as mg gallic acid equivalent per 1 gram of crude extract (mg GAE/g crude).

(2) Total flavonoids content (TFC)

TFC was measured with the aluminum chloride colorimetric assay (Jain and Singhai. 2011). The calibration curve was established using quercetin (0 - 200 mg/l). In brief, 0.5 ml of each stock extract solution (1000 mg/l) or quercetin was individually mixed with 0.1 ml of 10% (w/v) aluminum nitrate, 0.1 ml of 1M potassium acetate; the total volume was then added to more ethanol to reach a total of 10 ml. The combination was thoroughly mixed and allowed to stand for 40 mins at room temperature. The maximum absorbance of the mixture was measured at 415 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The results are expressed as mg quercetin equivalent per 1 gram crude extract (mg QE/g crude extract).

3.2.2 Evaluation of the effect of various solvent ratios of ethanol (0%, 25%, 50%, 75% and 100%) in water on *in vitro* antioxidant and antimicrobial properties of the crude extracts from *P. acidus* leaves and *C. carandas* fruits

3.2.2.1 *In vitro* antioxidant activity of the extracts from *P. acidus* leaves and *C. carandas* fruits:

There are abundant antioxidant methods for determination of antioxidant activity. Of these, inhibition of lipid peroxidation, reducing power, DPPH radical scavenging activity and metal chelating were used to determine the antioxidant activity of *P. acidus* and *C. carandas* crude water extracts. The sticky crude of each extract was dissolved in their extraction solvent to contain the stock extract solution at concentrations of 100, 250, 500, 750 and 1000 mg/l (w/v) for the following antioxidant determination:

(1) Lipid peroxidation assay

Lipid peroxidation inhibition of the crude extracts in the egg yolk was determined using a modified (Rajneesh *et al.* 2008). In brief, one ml of each concentration (100, 250, 500, 750 and 1000 mg/l (w/v)) of each crude extract was individually added to 50 ml of egg yolk prepared in phosphate buffered saline (PBS) at a ratio of 1:4 (w/v), then 0.5 ml of 24 mM ferrous sulfate and 0.5 ml of PBS were added. The mixture was shaken vigorously and incubated at 37°C for 15 mins. Next, 0.5 ml of 20% (w/v) trichloroacetic acid and 1 ml of 0.8% (w/v) thiobarbituric acid was added to the mixture. After boiling at 95°C and cooling for 30 mins, the mixture was centrifuged at 2200 *g* for 20 mins at 25°C. The absorbance was measured at 532 nm by

spectrophotometer (Shimadzu model UV-1601, Japan). The results were expressed as IC_{50} values, which required 50% inhibition of lipid peroxidation.

(2) Reducing power ability

The reducing power of *P. acidus* and *C. carandas* extracts was measured by the method described by Vijayalakshmi and Ruckmani. (2016). In brief, one ml of each concentration (100, 250, 500, 750 and 1000 mg/l (w/v)) of each crude extract was individually mixed with 2.5 ml of 0.2M phosphate buffer pH 6.6 and 2.5 ml of 1% (w/v) potassium ferricyanide, then incubated at 50°C for 20 mins. Next, 2.5 ml of 10% (w/v) trichloroacetic acid was added and centrifuged at 2200 *g* for 10 mins at 25°C. Finally, 2.5 ml of upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride. The absorbance was measured at 700 nm by a spectrophotometer against a blank. The results are expressed in IC_{50} values, which required 50% inhibition of reducing power activity.

(3) Radical scavenging activity (DPPH)

DPPH free radical-scavenging activity was determined using the method described by Ebrahimzadeh *et al.* (2008). In brief, 2 ml of each concentration (100, 250, 500, 750 and 1000 mg/l (w/v)) of each crude extract was individually mixed with 2 ml of 100 µM 2,2-diphenyl-1-picrylhydrazyl (DPPH) prepared in ethanol. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 mins. The absorbance of the mixture was measured at 517 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The results were expressed as IC_{50} values, which required 50% scavenging inhibition of DPPH radical.

(4) Metal chelating activity

The chelating ability of the crude extracts for ferrous ions (Fe^{2+}) was quantified according to the modified method of Jamuna *et al.* (2012). Briefly, one ml of each concentration (100, 250, 500, 750 and 1000 mg/l (w/v)) of each crude extract was individually mixed with 50 µl of 2mM ferrous sulfate heptahydrate, and then added 100 µl of 5mM ferrous solution. The mixture was shaken vigorously and incubated at room temperature for 10 mins. The absorbance was then measured at 562 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The results were presented by IC_{50} values, which required 50% inhibition of chelate ferrous ions.

3.3.2.2 *In vitro* antimicrobial activity of the extracts from *P. acidus* leaves and *C. carandas* fruits:

(1) Microbial preparation

Pathogenic strains of *Salmonella* Typhimurium TISTR 292, *Staphylococcus aureus* TISTR 118, *Escherichia coli* TISTR 780, *Aeromonas hydrophila* TISTR 1321 and spoilage strains of *Pseudomonas fluorescens* TISTR 358, *Lactobacillus plantarum* ATCC 14947^T, *Lactobacillus sakei* TISTR 890, *Leuconostoc mesenteroides* subsp. *mesenteroides* TISTR 942, *Streptococcus* sp. TISTR 1030, *Lactococcus cremoris* TISTR 1344, *Bacillus coagulans* TISTR 1447 and were obtained from Thailand Institute of Scientific and Technological Research, Thailand; and American Type Culture Collection, Rockville, Md. The bacteria strains were grown and maintained in glycerol eppendorfs containing MRS broth for lactic acid bacteria and TSB-YE (Trypticase Soy broth with 0.6% Yeast Extract) for pathogenic bacteria. All the stock bacteria strains were stored at -80°C for further use.

(2) Preparation of crude extracts

The sticky crude of each extract was dissolved in their extraction solvent to contain the stock extract solution at concentrations of 6.25, 12.5, 25, 50 and 100 mg/ml (w/v).

(3) Agar well diffusion

Antibacterial property of the *P. acidus* leaf and *C. carandas* fruit crude extracts against 11 strains of food pathogenic and spoilage bacteria was determined using the method of Biswas *et al.* (2013). Bacterial strains were cultured on petridishes of MRS agar for lactic acid bacteria and TSB-YE for pathogenic bacteria for 48 h to obtain single colony. The bacterial suspensions were adjusted with sterile 0.85% sodium chloride solution to contain 10^8 CFU/ml of tested bacteria according to 0.5 McFarland standards. Consequently, 25 µl of these inoculums were transferred to 25 ml of proper media and poured into sterile petridish. Later, agar plates were allowed to become solid, wells were prepared in the plates with the help of a 6 mm sterile cork-borer. A total of 50 µl of each stock extract solution (range from 6.25 to 100 mg/ml) was added into the well. 10% of ethanol was used as negative control. The plates were incubated overnight at proper conditions for each strain. Microbial growth was determined by measuring the diameter of inhibition zone (mm). The experiment was done three times and the mean values are presented.

3.2.3 Evaluation the effect of optimal crude extracts from *P. acidus* leaves and *C. carandas* fruits on chemical, physical and biological quality in ground pork storaged at 4°C up to 8 days and at -20°C up to 12 weeks

3.2.3.1 Preparation of meat samples

Raw pork and fat were obtained from Makro supermarket, Thailand. After being cut to small pieces, the pork was combined to contain 30% fat (w/w), and then the mixture was ground by a chopper machine (Guangdong, China). The experiment consisted of four treatments: none added (control); 0.2 g BHT/kg meat (positive lipid peroxidation); ground pork plus 2.5 and 5.0 g CE/kg meat; and mixed vigorously. Cooked meat samples were prepared by boiling at 95°C for 20 mins. Meat samples were packed in polyethylene bags and tested period in 0, 2, 4, 6, 8 days at 4°C for chilled meat condition and 0, 4, 8, 12 weeks at -20°C for frozen meat condition.

3.2.3.2 Quality of ground pork samples

(1) Chemical analysis:

Free scavenging radical activity (DPPH)

The radical scavenging activities of the meat samples were measured according to the method of Qwele *et al.* (2013). Briefly, 2 ml of the meat extracts was individually added to 2 ml of 0.2 mM DPPH prepared in ethanol. The mixture was vortexed and left to stand in the dark at room temperature for 30 mins. The mixture of ethanol (2 ml) and meat extract (2 ml) serve as a blank. The control solution was prepared by mixing ethanol (2 ml) and DPPH radical solution (2 ml). The absorbances were measured at 517 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The results were expressed as inhibiting percent and calculated as in:

$$\text{DPPH activity (\%)} = \left[1 - \frac{A_{sample} - A_{blank}}{A_{control}}\right] \times 100$$

Radical cation decolorization assay (ABTS)

The total antioxidant activity of the meat samples was measured by the method described by Re *et al.* (1999). The stock [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] $ABTS^{\bullet+}$ solution was produced by mixing 7 mM $ABTS^{+}$ aqueous solution with 2.4 mM potassium persulfate in the dark for 12-16 hours at room temperature. The mixture was incubated in the dark at room temperature 12-16 h before use. Prior to assay, this solution was diluted in 80% ethanol and equilibrated at 30°C to give an absorbance of 0.70 ± 0.02 at 734 nm. Then, 3 ml of diluted $ABTS^{\bullet+}$ solution was added to 300 µl of the meat extract. After 6 mins of incubation at room

temperature, the absorbance were recorded at 734 nm using a spectrophotometer (Shimadzu model UV-1601, Japan) against a blank (3 ml of ethanol plus 300 µl of sample extract), and a control (3 ml of $ABTS^{\bullet+}$ solution plus 300 µl of ethanol). The scavenging activity of meat samples against ABTS radical cation decolorization was expressed as inhibiting percent and calculated as in:

$$\text{ABTS activity (\%)} = \left[1 - \frac{A_{sample} - A_{blank}}{A_{control}}\right] \times 100$$

Reducing power ability

Reducing power ability was measured by the method described by Vijayalakshmi and Ruckmani. (2016). Briefly, one ml of the meat extracts was individually mixed with 2.5 ml of 0.2M phosphate buffer pH 6.6, 2.5 ml of 1% potassium ferricyanide and then incubated at 50°C for 30 mins. Afterwards, the mixture was added to 2.5 ml of 10% trichloroacetic acid and centrifuged at 2200 *g* for 10 mins at 25°C. Finally, 2.5 ml of upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The absorbance of the sample solutions were measured at 700 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The results were expressed in the absorbance values.

Thiobarbituric acid reaction substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) were measured by the method described by Buege and Aust. (1987). Briefly, 2 g of meat samples was individually homogenized with 10 ml of thiobarbituric acid solution reagent (a mixture of 0.375% (w/v) 2-thiobarbituric acid, 0.25N hydrogen chloride, and 15% (w/v) trichloroacetic acid was prepared with a silent crusher for 1 h at high speed), and heated at 95°C for 15 mins in a boiling water bath. Subsequently, the mixtures were cooled in ice-cold water and centrifuged at 1500 × *g* for 15 mins at 4°C. The absorbances were measured at 532nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The results were reported as mg malondialdehyde per kg meat sample (mg MDA/kg meat).

(2) Physical analysis:

pH

pH levels were determined according to AOAC (1995). Specifically, 2 g of samples were homogenized in 20 ml of distilled water. The mixtures were filtered using Whatman No.1 filter

paper. The pH of the filtrate was measured using a pH meter (Mettler Toledo, Greifensee, Switzerland).

Color

The color of the samples was determined using a color measurement spectrophotometers (HunterLab MiniScan 4500S, The Stothard Group Ltd., United Kingdom). Color was described in terms of the L^* (lightness), a^* (redness), and b^* (yellowness) color space values. Measurements were made perpendicular to the sample surfaces at five different locations per sample; mean values (L^*, a^*, and b^*) from the samples were analyzed to obtain an average colorimetric value.

(3) Biological analysis:

Briefly, 25 g of meat samples were mixed into the polypropylene bags containing 225 ml of 0.85% sodium chloride (NaCl) using a stomacher bag mixer (400 model VW, France) in 2 min to get the 10^{-1} dilutions. Then, one ml of these 10^{-1} dilutions was pipetted into a test tube containing 9 ml of 0.85% sodium chloride to get a 10^{-2} dilution. This step was repeated to get 10^{-3}, 10^{-4} and 10^{-5} dilutions. The sample solutions were used for bio-microbial parameters:

Total plate count (TPC)

One ml of the sample solutions of each dilution as described above were pour into sterilized plates which contain 20 ml of plate count agar to determine total viable counts after incubation for 24 - 48 h at 37°C. Microbial colonies were counted and expressed as log 10 CFU/g meat samples (AOAC. 2005).

Yeasts/Molds (Y/M)

One ml of the sample solutions of each dilution as described above were pour into sterilized plates which contain 20 ml of malt agar for determining total yeast and mold count after incubation for 3 - 5 days at 26°C. Microbial colonies were counted and expressed as log 10 CFU/g meat samples (AOAC. 2005).

Psychrophilic bacteria

0.1 ml the sample solutions of each dilution as described above was smeared on plate count agar to determine the aerobic psychrophilic bacteria after incubation for 7 -10 days at 7°C. All plates were examined visually for colony type and morphological characteristics associated with each growth medium. Select the plate with counts between 30 - 300 colonies forming units (CFU). Microbial colonies were counted and expressed as log 10 CFU/g meat samples (AOAC. 2005).

Coliforms/*E. coli* (Chromocult)

0.1 ml the sample solutions of each dilution as described above was smeared on plate count agar to determine the aerobic *E. coli*/coliforms after incubation for 24 – 48 h at 37°C. All plates were examined visually for colony type and morphological characteristics associated with each growth medium. Select the plate with counts between 30 - 300 colonies forming units (CFU). Microbial colonies were counted and expressed as log 10 CFU/g meat samples (AOAC. 2005).

3.2.4 Evaluation of the effect of optimal crude extracts from *P. acidus* leaves and *C. carandas* fruits on sensory evaluation of ground pork products

Sensory evaluation was carried out to evaluate the overall appearance, color, ordor, texture, flavor, and overall quality of each ground pork sample, following a descriptive hedonic scale method of Beinner *et al.* (2010). The sensory panel consisted of 30 consumer panel from Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Thailand. The ground pork samples were boiled in a water bath at 95°C for 20 mins, including time for defrosting and evaluated as soon as the samples were completed. Water was provided for cleaning the palate between samples. Panelists were asked to evaluate the sensory properties of each sample using a 7-point descriptive Hedonic scale ranging from extremely like to extremely dislike: 1 = extremely dislike; 2 = very dislike; 3 = dislike; 4 = neither like nor dislike; 5 = like; 6 = very like, 7 = extremely like was used to score the samples.

3.2.5 Statistical analysis

Analysis of variance was performed using raw data with the mean values and standard deviation of the means (SD) was calculated. Differences among the means were analyzed using the Tukey's Multiple Range Tests with a significance defined at $P<0.05$ level.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of solvent ratio on extraction yield and bioactive compounds from *P. acidus* leaves and *C. carandas* fruits

4.1.1 Extraction yield

Selection of solvent is an important step for obtaining extracts with acceptable yields and strong antioxidant and antimicrobial activity (Dent *et al.* 2013). In this study, the extraction solvents significantly ($P<0.05$) affected the amount of crude yields of the extracts from *P. acidus* leaves and *C. carandas* fruits and the results were expressed as gram per 100 g of dry weight (g/100 g DW) in Table 4.1 and 4.2. The extraction yields of various solvents decreased in the following order: water > 25% ethanol > 50% ethanol > 75% ethanol > 100% ethanol and it could be seen that the extraction yield of 0% ethanol (water) was the highest (2.80 g/100 g DW) and the extraction yield of 100% ethanol was the lowest (0.53 g/100 g DW) for *P. acidus* leaf extracts; water = 25% ethanol > 50% ethanol > 75% ethanol > 100% ethanol and water (5.28 g/100 g DW) and 25% ethanol (4.81 g/100g DW) solvents were higher the crude yield than 50% ethanol (4.58 g/100 g DW), 75% ethanol (3.97 g/100 g DW) and 100% ethanol (3.34 g/100 g DW) for *C. carandas* fruit extracts.

This result was similar to the study of Zielinski and Kozlowska. (2000), who reported that water solvent extracted higher crude yield than organic solvent. Dent *et al.* (2013); Sun *et al.* (2015); Tan *et al.* (2013) who reported that the different extract yield could be explained by the distinct polarities of extraction solvents and solubility of compounds in the extraction solvent. Iloki-Assanga *et al.* (2015) also indicated that the polarity of used solvent and the chemical constituents in tested materials greatly impacted the yield of extract. Moreover, these studies demonstrated that the crude extract yield was enhanced with the increasing of water concentration in solvent, which could be ascribed to the increased solubility of other components. However, higher crude yield does not necessarily imply that it will also be higher bioactive compounds as well as antioxidant activity because the antioxidant activity depends on the active antioxidant compounds present in the extract. These studies investigated the increasing water concentration in the solvent increase extraction yield, which can be explained by the increased solubility of other components such as proteins and carbohydrates (Do *et al.* 2014).

4.1.2 Total phenolic content (TPC) and total flavonoid content (TFC)

4.1.2.1 TPC and TFC of *P. acidus* crude extracts

The recovery amount of TPC and TFC was expressed in Table 4.1. The results showed that the TPC and TFC were increased with increasing water concentration in solvent. The TPC value of the crude extracts range from 48.04 mg GAE/g crude for 100% ethanol extract to 49.87 mg GAE/g crude for water extract). The TFC values of the crude extracts range from 0.51 mg QE/g crude for 100% ethanol extract for to 0.70 mg QE/g crude for water extract. The increase of TPC and TFC in the following order: water > 25% ethanol > 50% ethanol > 75% ethanol > 100% ethanol. These results were in agreement with the study of Jagajothi *et al.* (2013) and Munuswamy *et al.* (2013), who found flavonoids and phenols in *P. acidus* leaf extract. Moreover, the previous studies also demonstrated that extraction solvents significantly influenced the recovery amount of bioactive compounds from plant materials (Dailey and Vuong. 2015; Dent *et al.* 2013). These results could be explained by polyphenols are often extracted in higher amounts in more polar solvents (polarity index of water: 9 > polarity index of ethanol: 5.2) (Iloki-Assanga *et al.* 2015). Therefore, water was the best solvent for bioactive compounds in this study. However, in the cases of Do *et al.* (2014) and Singh *et al.* (2014), who found that the highest TPC and TFC was obtained in the 100% ethanol extract compared to the water solvent, and Sun *et al.* (2015), who suggested that 75% ethanol/water solvent can be the best extraction solvent for phenolics in propolis, could be explained by the differences about the presence and position of hydroxyl groups, and the molecular size and the length of constituent hydrocarbon chains (Hsu *et al.* 2006).

4.1.2.2 TPC and TFC of *C. carandas* crude extracts

The recovery of phenolics and flavonoid contents was shown in Table 4.2. The TPC ranged from 15.87 to 20.44 mg GAE/g crude. Absolute ethanol extract gave the highest TPC with the value of 20.44 mg GAE/g crude, whereas other extracts consist of 75% ethanol (16.95 mg GAE/g crude), 50% ethanol (16.19 mg GAE/g crude), 25% ethanol (15.96 mg GAE/g crude) and water (15.87 mg GAE/g crude) had no significant difference ($P<0.05$). The TFC ranged from 0.11 mg QE/g crude to 0.20 mg QE/g crude and other solvents had no significant difference ($P<0.05$) such as absolute ethanol extract (0.17 mg QE/g crude), 75% ethanol (0.20 mg QE/g crude), 50% ethanol (0.16 mg QE/g crude), 25% ethanol (0.14 mg QE/g crude) and water extract (0.11 mg QE/g crude). These findings were in agreement with the reports of Mehmood *et al.* (2014), who reported that phytochemical studies of *C. carandas* revealed the presence of flavonoids, phenolic,

tannins compounds. Singh *et al.* (2014) also found that pomegranate ethanol extract produced the highest extraction amount of phenolic compounds to be the most effective solvent for recovering polyphenols from these materials. These differences amount of bioactive compounds in different solvent extracts could be explained by the influence of the solvents on the extractability of phenolic content (Foo *et al.* 2015; Sun *et al.* 2015).

Table 4.1 Crude yield, total phenolic and flavonoid contents of the *P. acidus* leaf crude extracts by various solvent ratios

Solvent (% ethanol)	Crude yield (g/100 g DW)	TPC (mg GAE/g crude)	TFC (mg QE/g crude)
Water	2.80 ± 0.13^{a}	49.87 ± 0.23^{a}	0.70 ± 0.04^{a}
25%	1.93 ± 0.14^{b}	49.82 ± 0.10^{a}	0.66 ± 0.03^{ab}
50%	1.77 ± 0.05^{bc}	49.46 ± 0.17^{ab}	0.52 ± 0.11^{b}
75%	1.59 ± 0.15^{c}	48.54 ± 0.13^{bc}	0.50 ± 0.01^{b}
100%	0.53 ± 0.07^{d}	48.04 ± 0.92^{c}	0.51 ± 0.05^{b}

GAE = Gallic acid equipvalent

QE = Quercertin equipvalent.

$^{a\text{-}c}$ Means sharing different letters in the same column are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.2 Crude yield, total phenolic and flavonoid contents of the *C. carandas* fruit crude extracts by various solvent ratios

Solvents (% ethanol)	Crude yield (g/100 g DW)	TPC (mg GAE/g crude)	TFC (mg QE/g crude)
Water	5.28 ± 0.03 [a]	15.87 ± 0.08 [b]	0.11 ± 0.05 [a]
25%	4.81 ± 0.04 [a]	15.96 ± 0.06 [b]	0.14 ± 0.08 [a]
50%	4.58 ± 0.05 [ab]	16.19 ± 0.10 [b]	0.16 ± 0.12 [a]
75%	3.97 ± 0.05 [bc]	16.95 ± 0.08 [b]	0.20 ± 0.03 [a]
100%	3.34 ± 0.05 [c]	20.44 ± 0.08 [a]	0.17 ± 0.05 [a]

GAE = Gallic acid equipvalent.

QE = Quercertin equipvalent.

[a-c] Means sharing different letters in the same column are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

4.2 Evaluation of the effect of various solvent ratios of ethanol (0%, 25%, 50%, 75% and 100%) in water on *in vitro* antioxidant and antimicrobial properties of the crude extracts from *P. acidus* leaves and *C. carandas* fruits

4.2.1 *In vitro* antioxidant activity of the extracts from *P. acidus* leaves and *C. carandas* fruits

4.2.1.1 *In vitro* antioxidant activity of the extracts from *P. acidus* leaves

The *in vitro* antioxidant activity of the extracts from *P. acidus* leaves is presented in Table 4.3. The results are expressed as IC_{50} values, which are defined as the concentration of the sample necessary to cause 50% inhibition and are obtained by interpolation from linear regression analysis. A lower IC_{50} value is associated with higher activity. In this study, the antioxidant activity of the extracts from *P. acidus* leaves was increased with increasing water concentration in solvent. The highest antioxidant activity was found in water extract and the highest IC_{50} values were 716.32, 932.25, 88.68, and 1311.17 mg/l for lipid peroxidation, reducing power, DPPH scavenging, and metal chelating, respectively. Whereas, the lowest IC_{50} values were 1006.15, 1462.27, 95.32, and 6442.60 mg/l for lipid peroxidation, reducing power, DPPH scavenging, and metal chelating, respectively. These results are supported by studies of Kim (2012) and Kim *et al.* (2013), who reported that the scavenging activity of water extract was significantly higher than absolute ethanol extract from dried boxthorn (*Lycium chinensis*) fruit. Jia *et al.* (2012) demonstrated that absolute ethanol had lowest reducing power, DPPH and ABTS scavenging water in absolute ethanol solvent than among extraction ratio of ethanol such as water, 20%, 40%, 60% and 80% ethanol. The antioxidant activities of *P. acidus* leaf extracts could be estimated by the phenolic compounds present in the extracts played an important role in antioxidant activity directly through the reduction of oxidized intermediates in the chain reaction (Tachakittirungrod *et al.* 2007).

Table 4.3 Effect of various crude extracts from *P. acidus* leaves on *in vitro* antioxidant activities

Solvents (% ethanol)	IC_{50} (mg/l) Lipid peroxidation	Reducing power	DPPH	Metal chelating
Water	716.32 ± 0.95 [d]	932.25 ± 0.16 [e]	88.68 ± 0.04 [c]	1311.17 ± 0.70 [e]
25%	885.56 ± 0.68 [c]	988.21 ± 0.06 [d]	89.03 ± 0.87 [c]	2512.29 ± 0.31 [d]
50%	983.20 ± 0.91 [b]	1084.95 ± 0.87 [c]	92.47 ± 0.59 [b]	3677.13 ± 0.02 [c]
75%	1004.87 ± 0.25 [a]	1288.50 ± 0.16 [b]	94.66 ± 0.61 [a]	4599.27 ± 0.17 [b]
100%	1006.15 ± 0.22 [a]	1462.27 ± 0.03 [a]	95.32 ± 1.22 [a]	6442.60 ± 0.53 [a]

[a-e] Means sharing different letters in the same column are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

4.2.1.2 *In vitro* antioxidant activity of the extracts from *C. carandas* fruits

For most of the plant products, the compounds responsible for the antioxidant effects are phenolic and flavonoid compounds. Oxidative stresses which can be relieved by antioxidants are caused mainly by free radicals and lipid peroxidation, moreover, the ability of the antioxidant to produce reduction of ferric ions are necessitated thus there is important to measure the free radical scavenging activity using DPPH, reducing power metal chelating and lipid peroxidation in the *in vitro* antioxidant activity (Ortuño *et al.* 2016).

In vitro antioxidant properties of the extracts from *C. carandas* fruits expressed in Table 4.4. The results were shown that 50% ethanol extract revealed the highest activity in lipid peroxidation (IC_{50}: 715.15 mg/l), reducing power (IC_{50}: 1513.25 mg/l), and 75% ethanol extract showed the highest activity in DPPH radical scavenging (IC_{50}: 143.03 mg/l), metal chelating (IC_{50}: 1350.25 mg/l). These results were lower than the study of Sadek *et al.* (2013) who reported that antioxidant activities on DPPH free radical scavenging with the IC_{50} was 1.292 µg/ml in ethanolic extract. The antioxidant activities of *C. carandas* fruit extracts could be estimated that the presence of phenolic and flavonoid compounds, especially the antioxidant activity was enhanced with the increasing of water concentration in solvent, which may be directly correlated to the bioactive compounds of the extracts.

Table 4.4 Effect of various crude extracts from *C. carandas* fruits on *in vitro* antioxidant activities

Solvents (% ethanol)	IC_{50} (mg/l)			
	Lipid peroxidation	Reducing power	DPPH	Metal chelating
Water	796.40 ± 0.21 [c]	1845.92 ± 0.04 [c]	259.52 ± 0.81 [a]	4331.15 ± 0.10 [a]
25%	785.61 ± 0.11 [d]	1642.31 ± 0.78 [d]	236.56 ± 0.08 [c]	3512.10 ± 0.51 [d]
50%	715.15 ± 0.36 [e]	1513.25 ± 0.06 [e]	166.24 ± 0.03 [d]	3677.15 ± 0.02 [c]
75%	826.25 ± 0.18 [b]	2078.69 ± 0.02 [b]	143.03 ± 0.05 [e]	1350.25 ± 0.07 [e]
100%	855.22 ± 0.12 [a]	2349.85 ± 0.41 [a]	253.13 ± 0.05 [b]	3842.60 ± 0.03 [b]

[a-e] Means sharing different letters in the same column are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

4.2.2 *In vitro* antimicrobial activity of the extracts from *P. acidus* leaves and *C. carandas* fruits

4.2.2.1 *In vitro* antimicrobial properties of the extracts from *P. acidus* leaves

The antimicrobial activity of *P. acidus* leaf extracts using different solvents ethanol in water (0%, 25%, 50%, 75% and 100% (v/v)) was evaluated according to their clear zone of inhibition against pathogenic and spoilage bacteria Table 4.5. The results showed that tested bacteria were not inhibited by any extracts from *P. acidus* leaves. These findings were in agreement with report of Nascimento *et al.* (2000), who reported that sage and yarrow extracts did not present any antimicrobial activity, even they contained high bioactive compounds. These results could be caused by the present of bioactive compounds from *P. acidus* leaf extracts did not significantly affect on antimicrobial activity ($P<0.05$) and this finding can be explained by although *P. acidus* crude water extract contained high bioactive compounds but it did not act as an antimicrobial (Jain and Singhai. 2011; Sawhney *et al.* 2011).

Table 4.5 Effect of various crude extracts from *P. acidus* leaves on *in vitro* antimicrobial activity using agar well diffusion method

Cultures	The occurement zone of inhibition				
	Water	25% ethanol	50% ethanol	75% ethanol	100% ethanol
Pathogenic bacteria					
Salmonella Typhimurium TISTR 292	-	-	-	-	-
Staphylococcus aureus TISTR 118	-	-	-	-	-
Escherichia coli TISTR 780	-	-	-	-	-
Aeromonas hydrophila TISTR 1321	-	-	-	-	-
Spoilage bacteria	-	-	-	-	-
Pseudomonas fluorescens TISTR 358	-	-	-	-	-
Lactobacillus plantarum ATCC 14947^{T}	-	-	-	-	-
Lactobacillus sakei TISTR 890	-	-	-	-	-
Leuconostoc mesenteroides subsp. *mesenteroides* TISTR 942	-	-	-	-	-
Streptococcus sp. TISTR 1030	-	-	-	-	-
Lactococcus cremoris TISTR 1344	-	-	-	-	-
Bacillus coagulans TISTR 1447	-	-	-	-	-

- = No inhibition.

TISTR = Thailand Institute of Scientific and Technological Research, Thailand.

ATCC = American Type Culture Collection, Rockville, Md.

4.2.2.2 *In vitro* antimicrobial properties of the extracts from *C. carandas* fruits

The antimicrobial activity of *C. carandas* extracts using different solvents ethanol in water (0%, 25%, 50%, 75% and 100% (v/v)) was evaluated according to their clear zone of inhibition against pathogenic and spoilage bacteria Table 4.6 – 4.11. Among extract solvent ratio, the extract from absolute ethanol solvent showed more effective than other solvents and all extracts showed varying degrees of antimicrobial activity against all the tested bacteria. The highest inhibiting activity of absolute ethanol extract were 23.55 mm *(P. fluorescens)*, 28.78 mm (*S.* Typhimurium), 27.33 mm (*S. aureus*), and 25.44 mm (*E. coli*) (Figure 4.1). These results were in the agreement to many studies such as Salar and Dhall (2010), who reported that acetone,

methanol and ethanol extracts of *C. carandas* was the most effective against the tested microorganisms closely followed by *Tinospora cordifolia*, *Cordia dichotoma*, *Prosopis cineraria* and *Capparis decidua*; Rojas *et al.* (2006) reported that ethanol extract exhibited a higher degree of antimicrobial activity as compared to water and hexane extracts fractions in screening ten medicinal plants used in Colombian folkloric medicine against *S. aureus*; Israr *et al.* (2012) investigated that antimicrobial activities of ethanolic extract from *C. carandas* fruits have been reported against *S. aureus, S. epidermidis, S. pneumoniae, B. subtilis, E. coli.* Sokmen *et al.* (2004) and Taie *et al.* (2010) also found the phenolic compounds found in numerous plant species appear to protect against pathogenic bacteria.

Plants extracts are generally considered to contain antimicrobial compounds. Phenolic compounds are the major components of these antimi- crobial compounds and are responsible for the antimicrobial activities of most plant extracts (Jalosinska and Wilczak. 2009). Many early papers reported that the phenolic compounds found in numerous plant species appear to protect against pathogenic invasion (Medina *et al.* 2007). Additionally, phenolic compounds are regarded as a representative group of antioxidant substances. The potentially antimicrobial mechanisms of phenolic compounds include the interruption of function of bacterial cell membranes. The -OH groups in phenolic compounds are highly reactive under aqueous conditions and react with several biomolecules, causing deformation of these molecules, which results in retardation of growth and bacterial growth. Phenolic compounds are also involved in protein and cell wall binding, inactivation of bacterial enzymes, and intercalation into the bacterial DNA during replication (Fullerton *et al.* 2011).

Table 4.6 Effect of various crude extracts from *C. carandas* fruits on *in vitro* antimicrobial activity using agar well diffusion method

Cultures	The occurement zone of inhibition				
	Water	25% ethanol	50% ethanol	75% ethanol	100% ethanol
Pathogenic bacteria					
Salmonella Typhimurium TISTR 292	+	+	+	+	+
Staphylococcus aureus TISTR 118	+	+	+	+	+
Escherichia coli TISTR 780	+	+	+	+	+
Aeromonas hydrophila TISTR 1321	+	+	+	+	+
Spoilage bacteria					
Pseudomonas fluorescens TISTR 358	+	+	+	+	+
Lactobacillus plantarum ATCC 14947^{T}	-	-	+	+	+
Lactobacillus sakei TISTR 890	-	-	+	+	+
Leuconostoc mesenteroides subsp. *mesenteroides* TISTR 942	-	-	+	+	+
Streptococcus sp. TISTR 1030	-	-	+	+	+
Lactococcus cremoris TISTR 1344	-	-	+	+	+
Bacillus coagulans TISTR 1447	+	+	+	+	+

\+ = Inhibition.

\- = No inhibition.

TISTR = Thailand Institute of Scientific and Technological Research, Thailand.

ATCC = American Type Culture Collection, Rockville, Md.

Table 4.7 Effect of ethanol crude extract from *C. carandas* fruits on antimicrobial activity using agar well diffusion method

Cultures	Inhibiting zone of the extracts (mm)				
	6.25 mg/ml	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Pathogenic bacteria					
Salmonella Typhimurium TISTR 292	Ni	10.00 ± 0.00	14.00 ± 0.00	23.78 ± 0.66	28.78 ± 0.69
Staphylococcus aureus TISTR 118	Ni	10.00 ± 0.00	14.00 ± 0.00	22.00 ± 0.00	27.33 ± 1.20
Escherichia coli TISTR 780	Ni	11.00 ± 0.00	10.00 ± 1.00	20.28 ± 0.25	25.44 ± 0.51
Aeromonas hydrophila TISTR 1321	Ni	10.73 ± 0.23	15.00 ± 1.00	23.00 ± 1.00	28.11 ± 0.19
Spoilage bacteria					
Pseudomonas fluorescens TISTR 358	Ni	10.00 ± 0.00	14.33 ± 0.58	22.00 ± 0.00	23.55 ± 0.39
Lactobacillus plantarum ATCC 14947^{T}	Ni	Ni	Ni	10.66 ± 0.67	16.89 ± 0.77
Lactobacillus sakei TISTR 890	Ni	Ni	Ni	17.00 ± 0.00	20.78 ± 0.39
Leuconostoc mesenteroides subsp. *mesenteroides* TISTR 942	Ni	Ni	Ni	14.00 ± 0.00	19.00 ± 1.00
Streptococcus sp. TISTR 1030	Ni	Ni	Ni	Ni	12.78 ± 0.69
Lactococcus cremoris TISTR 1344	Ni	Ni	Ni	Ni	11.78 ± 0.69
Bacillus coagulans TISTR 1447	Ni	12.00 ± 0.00	11.00 ± 0.73	20.73 ± 0.23	26.55 ± 0.51

Ni = No inhibition.

All values were expressed as mean ± standard deviation.

Table 4.8 Effect of 75% ethanol crude extract from *C. carandas* fruits on antimicrobial activity using agar well diffusion method

Cultures	Inhibiting zone of the extracts (mm)				
	6.25 mg/ml	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Pathogenic bacteria					
Salmonella Typhimurium TISTR 292	Ni	Ni	14.00 ± 0.00	18.73 ± 0.23	28.33 ± 1.20
Staphylococcus aureus TISTR 118	Ni	Ni	15.00 ± 1.00	20.00 ± 0.00	26.11 ± 0.19
Escherichia coli TISTR 780	Ni	Ni	12.00 ± 0.00	17.00 ± 0.00	25.73 ± 0.23
Aeromonas hydrophila TISTR 1321	Ni	Ni	16.66 ± 0.00	22.00 ± 0.00	27.44 ± 0.51
Spoilage bacteria					
Pseudomonas fluorescens TISTR 358	Ni	Ni	12.66 ± 0.67	16.33 ± 1.20	21.66 ± 0.67
Lactobacillus plantarum ATCC 14947^{T}	Ni	Ni	Ni	10.00 ± 0.50	16.78 ± 0.69
Lactobacillus sakei TISTR 890	Ni	Ni	Ni	15.00 ± 0.00	20.00 ± 1.00
Leuconostoc mesenteroides subsp. *mesenteroides* TISTR 942	Ni	Ni	Ni	15.00 ± 0.00	18.66 ± 0.67
Streptococcus sp. TISTR 1030	Ni	Ni	Ni	Ni	12.55 ± 0.39
Lactococcus cremoris TISTR 1344	Ni	Ni	Ni	Ni	11.33 ± 0.34
Bacillus coagulans TISTR 1447	Ni	Ni	13.00 ± 1.00	17.00 ± 1.00	25.28 ± 0.25

Ni = No inhibition.

All values were expressed as mean ± standard deviation.

Table 4.9 Effect of 50% ethanol crude extract from *C. carandas* fruits on antimicrobial activity using agar well diffusion method

Cultures	Inhibiting zone of the extracts (mm)				
	6.25 mg/ml	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Pathogenic bacteria					
Salmonella Typhimurium TISTR 292	Ni	Ni	11.00 ± 0.00	15.00 ± 0.00	20.00 ± 1.00
Staphylococcus aureus TISTR 118	Ni	Ni	12.00 ± 0.00	16.33 ± 0.33	25.39 ± 0.10
Escherichia coli TISTR 780	Ni	Ni	10.00 ± 0.00	14.00 ± 0.00	20.33 ± 0.33
Aeromonas hydrophila TISTR 1321	Ni	Ni	12.33 ± 0.33	15.66 ± 0.34	21.00 ± 0.00
Spoilage bacteria					
Pseudomonas fluorescens TISTR 358	Ni	Ni	13.66 ± 0.34	18.00 ± 0.00	18.66 ± 0.34
Lactobacillus plantarum ATCC 14947^T	Ni	Ni	Ni	Ni	12.00 ± 1.00
Lactobacillus sakei TISTR 890	Ni	Ni	Ni	12.00 ± 1.00	15.00 ± 0.50
Leuconostoc mesenteroides subsp. *mesenteroides* TISTR 942	Ni	Ni	Ni	12.00 ± 1.00	15.66 ± 0.67
Streptococcus sp. TISTR 1030	Ni	Ni	Ni	12.33 ± 0.33	12.00 ± 1.00
Lactococcus cremoris TISTR 1344	Ni	Ni	Ni	15.33 ± 0.33	11.00 ± 1.00
Bacillus coagulans TISTR 1447	Ni	Ni	12.00 ± 1.00	15.66 ± 0.67	20.28 ± 0.25

Ni = No inhibition.

All values were expressed as mean ± standard deviation.

Table 4.10 Effect of 25% ethanol crude extract from *C. carandas* fruits on antimicrobial activity using agar well diffusion method

Cultures	Inhibiting zone of the extracts (mm)				
	6.25 mg/ml	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Pathogenic bacteria					
Salmonella Typhimurium TISTR 292	Ni	Ni	Ni	16.33 ± 0.33	20.00 ± 0.34
Staphylococcus aureus TISTR 118	Ni	Ni	Ni	17.00 ± 0.00	17.33 ± 0.33
Escherichia coli TISTR 780	Ni	Ni	Ni	14.00 ± 0.00	18.33 ± 0.67
Aeromonas hydrophila TISTR 1321	Ni	Ni	11.00 ± 0.00	17.00 ± 1.00	20.00 ± 0.00
Spoilage bacteria					
Pseudomonas fluorescens TISTR 358	Ni	Ni	Ni	15.89 ± 0.77	20.33 ± 0.33
Lactobacillus plantarum ATCC 14947^T	Ni	Ni	Ni	Ni	Ni
Lactobacillus sakei TISTR 890	Ni	Ni	Ni	Ni	Ni
Leuconostoc mesenteroides subsp. *mesenteroides* TISTR 942	Ni	Ni	Ni	Ni	Ni
Streptococcus sp. TISTR 1030	Ni	Ni	Ni	Ni	Ni
Lactococcus cremoris TISTR 1344	Ni	Ni	Ni	Ni	Ni
Bacillus coagulans TISTR 1447	Ni	Ni	Ni	15.33 ± 1.20	16.00 ± 1.00

Ni = No inhibition.

All values were expressed as mean ± standard deviation.

Table 4.11 Effect of water crude extract from *C. carandas* fruits on antimicrobial activity using agar well diffusion method

Cultures	Inhibiting zone of the extracts (mm)				
	6.25 mg/ml	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Pathogenic bacteria					
Salmonella Typhimurium TISTR 292	Ni	Ni	8.00 ± 0.00	16.00 ± 0.00	19.00 ± 0.34
Staphylococcus aureus TISTR 118	Ni	Ni	Ni	14.33 ± 0.67	20.33 ± 0.88
Escherichia coli TISTR 780	Ni	Ni	Ni	13.33 ± 0.00	17.00 ± 1.00
Aeromonas hydrophila TISTR 1321	Ni	Ni	11.00 ± 0.00	16.00 ± 1.00	19.33 ± 0.58
Spoilage bacteria					
Pseudomonas fluorescens TISTR 358	Ni	Ni	Ni	12.00 ± 0.00	18.28 ± 0.25
Lactobacillus plantarum ATCC 14947^T	Ni	Ni	Ni	Ni	Ni
Lactobacillus sakei TISTR 890	Ni	Ni	Ni	Ni	Ni
Leuconostoc mesenteroides subsp. *mesenteroides* TISTR 942	Ni	Ni	Ni	Ni	Ni
Streptococcus sp. TISTR 1030	Ni	Ni	Ni	Ni	Ni
Lactococcus cremoris TISTR 1344	Ni	Ni	Ni	Ni	Ni
Bacillus coagulans TISTR 1447	Ni	Ni	Ni	13.00 ± 0.00	19.00 ± 0.34

Ni = No inhibition.

All values were expressed as mean ± standard deviation.

4.2.3 Evaluation of the effect of optimal crude extracts from *P. acidus* leaves and *C. carandas* fruits on chemical, physical and biological quality in chilled at 4°C up to 8 days and frozen ground pork storaged at -20°C up to 12 weeks

4.2.3.1 Evaluation of the effect of optimal crude extracts from *P. acidus* leaves and *C. carandas* fruits on chemical, physical and biological quality in ground pork storaged at 4°C up to 8 days

(1) Evaluation of the effect of optimal crude extract from *P. acidus* leaves on chemical, physical and biological quality in ground pork storaged at 4°C up to 8 days

Chemical analysis

Free scavenging radical activity (DPPH)

DPPH radical scavenging activity: According to the DPPH assay the 2.5 g PWCE/kg and 5 g PWCE/kg treatments produced significantly ($P<0.05$) effective antioxidant activity in both raw and cooked samples compared to the control and BHT samples (Table 4.12). The overall radical scavenging activity was significantly ($P<0.05$) higher for the PWCE treatment in both raw and cooked samples. The results showed that percentage inhibition of DPPH radical scavenging activity was 20.31%, 29.20%, 58.56% and 74.66% at 0 day and slightly decreased to 19.41%, 29.23%, 55.77% and 72.70% at the end of the storage time for the control, BHT, 2.5 g PWCE/kg and 5g PWCE/kg treatments, respectively. In cooked ground pork, percentage inhibition of DPPH radical scavenging activity of all samples increased in comparison with raw samples and reached to 22.86%, 35.92%, 62.35% and 79.60% at 0 day and also decreased to 19.93%, 33.42%, 60.85% and 77.40% at the end of the storage time for the control, BHT, 2.5 g PWCE/kg and 5g PWCE/kg treatments, respectively.

Radical cation decolorization (ABTS)

The results of ABTS radical cation decolorization activity was shown in Table 4.13. The 2.5 g PWCE/kg and 5 g PWCE/kg treatments were higher significantly ($P<0.05$) effective ABTS activity in both raw and cooked samples than the control and BHT samples. In raw ground pork, percentage inhibition of ABTS activity was 37.75%, 66.85%, 69.14% and 82.71% at 0 day and was decreased to 36.39%, 63.79%, 66.80% and 81.22% at the end of the storage time for the control, BHT, 2.5 g PWCE/kg and 5 g PWCE/kg, respectively. In cooked ground pork, percentage inhibition of ABTS activity of all samples increased in comparison with raw samples and reached to 40.77%, 68.44%, 71.52% and 85.39% at 0 day and also decreased to 38.55%, 66.18%, 70.17% and 83.86% at the end of the storage time for the control, BHT, 2.5 g PWCE/kg

and 5g PWCE/kg treatments, respectively. In this study, DPPH and ABTS assays both showed that the addition of PWCE significantly increased radical scavenging and in agreement with the previous studies (Huang *et al.* 2011). Radical scavenging was also affected over time in all untreated meat samples, perhaps because oxidation of the meat leads to the creation of compounds that scavenge free radicals. The DPPH radical assay is only suitable for lipid-soluble antioxidants, while the ABTS radical cation decolorization assay is applicable for both water-soluble and lipid-soluble antioxidants (Re *et al.* 1999). According to some studies, free radical scavenging activity depends on the structural conformation of the phenolic compounds (Chumyam *et al.* 2013; Fernandes *et al.* 2012).

Reducing power ability

The reducing power ability was expressed as the absorbance at 700 nm (Table 4.14). The results showed that the meat samples treated with water extract from *P. acidus* leaves increased reducing power (higher absorbance) compared to the control and BHT at 0.2g/kg meat. In raw ground pork, the absorbance of reducing power of ground pork samples was 0.38, 0.45, 0.59 and 0.75 at 0 day and was slightly decreased to 0.42, 0.62, 0.75 and 0.88 at the end of the storage time for the control, BHT, 2.5 g PWCE/kg and 5 g PWCE/kg, respectively. In cooked ground pork; percentage inhibition of the absorbance of reducing power of all samples increased in comparison with raw samples and reached to 0.44, 0.61, 0.76 and 0.87 at 0 day and also decreased to 0.36, 0.44, 0.58 and 0.77 at the end of the storage time for the control, BHT, 2.5 g PWCE/kg and 5g PWCE/kg treatments, respectively. This finding could be explained by the presence of reductants (antioxidants) in meat causes the reduction of Fe^{3+}/Ferric cyanide complex to the ferrous form. Therefore, the Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Beyhan *et al.* 2010). *P. acidus* extract treatment gave a significantly higher absorbance than the control and BHT treatments due to the high polyphenolic content of the *P. acidus* extract. Antioxidant properties are known to be concomitant with the development of reducing power. Reductones can react directly with peroxides and can also prevent peroxide formation by reacting with certain precursors (Kim *et al.* 2013b).

Thiobarbituric acid reaction substances (TBARS)

Thiobarbituric acid reaction substances (TBARS) method has been widely used to determine the degree of lipid oxidation through malondialdehyde compound which is the organic compound with the formula $CH_2(CHO)_2$ and results from lipid peroxidation of polyunsaturated fatty acids (Kumar and Jain. 2015). Table 4.15 showed the effect of addition of crude water

extract from *P. acidus* leaves at 2.5 and 5 g/kg in ground pork during the storage time at 4°C. The results showed that level of lipid oxidation in raw ground pork (control) increased over 8 days of refrigerated storage. No statistical difference was observed between PWCE addition treatments (2.5 and 5 g/kg) and the control on all storage days. Level of lipid oxidation was lowest in BHT containing raw ground pork. According to Hernandez-Hernandez *et al.* (2009), this could be due to a reaction between malonaldehyde - its concentration increases with time and lysine e-amino and sulfhydryl groups in myosin subfragment 1, and the fact that the protein prevents hydroperoxide interactions with prooxidants. In addition, in cooked ground pork, lipid peroxidation increased as function of storage time. Ground pork contain PWCE at 2.5 and 5.0 g/kg had significantly lower level of lipid peroxidation compared to control and BHT treatments ($P<0.05$). The results showed that the MDA content of the meat samples were 4.61 mg MDA/kg meat (2.5 g PWCE/kg), 2.95 mg MDA/kg meat (5 g PWCE/kg), 13.03 mg MDA/kg meat (control) and 6.69 mg MDA/kg meat (0.2 g BHT/kg) in the 0 day. In particular, addition of 5 g PWCE/kg unaffected to total MDA content during the storage time up to 8 days. These findings are in agreement with other research studies that have investigated the effects of plant extract on lipid peroxidation in meat (Coutinho de Oliveira *et al.* 2012; Hernandez-Hernandez *et al.* 2009). In general, the mechanism of the protective effect of crude extract on lipid oxidation may be due to the presence of a number of oligomer procyanidins, such as catechin and epicatechin (Carpenter *et al.* 2007) which possess a greater antioxidant potential than monomer components (Llópiz *et al.* 2004). This finding can be explained by cooking decreased the ferric ion reducing capacity (FRC) but increased nonheme iron of meat, resulting in comparatively lower FRC in cooked meat than raw meat. Therefore, heat stable FRC may be primarily responsible for the regeneration of ferrous ion to increase total malondialdehyde in cooked meat during storage (Min *et al.* 2008). Moreover, according to Zhang *et al.* (2016), the phenolic compounds are associated with the hydroxyl group linked to the aromatic ring, which is capable of donating hydrogen atoms with electrons and neutralizing free radicals. This mechanism blocks further degradation to more active oxidizing forms, such as MDA. Therefore, the strong *in vitro* antioxidant activity shown by *P. acidus* extracts also had a protective role in raw meat products.

Table 4.12 Effect of water crude extract from *P. acidus* leaves on DPPH radical scavenging activity ground pork at 4°C

Storage time		% Inhibition of DPPH radical scavenging activity			
(days)		Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
Before cooking	0	$20.31 \pm 0.59^{d,AB}$	$29.20 \pm 0.92^{c,A}$	$58.56 \pm 0.78^{b,A}$	$74.66 \pm 0.71^{a,A}$
	2	$21.56 \pm 0.94^{d,A}$	$28.08 \pm 0.95^{c,A}$	$57.02 \pm 0.87^{b,AB}$	$73.94 \pm 0.40^{a,AB}$
	4	$19.78 \pm 0.58^{d,B}$	$29.53 \pm 0.72^{c,A}$	$56.99 \pm 0.96^{b,AB}$	$72.74 \pm 0.11^{a,B}$
	6	$20.50 \pm 0.57^{d,AB}$	$28.08 \pm 0.64^{c,A}$	$56.74 \pm 0.07^{b,AB}$	$72.94 \pm 0.65^{a,B}$
	8	$19.41 \pm 0.48^{d,B}$	$29.23 \pm 0.77^{c,A}$	$55.77 \pm 0.63^{b,B}$	$72.70 \pm 0.51^{a,B}$
After cooking	0	$22.86 \pm 0.76^{d,A}$	$35.92 \pm 0.99^{c,A}$	$62.35 \pm 0.63^{b,A}$	$79.60 \pm 0.79^{a,A}$
	2	$22.37 \pm 0.94^{d,A}$	$36.54 \pm 0.65^{c,A}$	$61.67 \pm 0.80^{b,B}$	$79.41 \pm 0.79^{a,A}$
	4	$22.25 \pm 0.98^{d,A}$	$36.74 \pm 0.64^{c,A}$	$62.57 \pm 0.67^{b,AB}$	$78.40 \pm 0.38^{a,AB}$
	6	$21.61 \pm 0.62^{d,AB}$	$35.54 \pm 0.55^{c,A}$	$64.32 \pm 0.52^{b,A}$	$78.23 \pm 0.49^{a,AB}$
	8	$19.93 \pm 0.41^{d,B}$	$36.42 \pm 0.65^{c,A}$	$60.85 \pm 0.89^{b,B}$	$77.40 \pm 0.60^{a,B}$

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-B}$ Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.13 Effect of water crude extract from *P. acidus* leaves on ABTS radical cation decolorization in ground pork at 4°C

Storage time		% Inhibition of ABTS radical cation decolorization			
(days)		Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
Before cooking	0	$37.75 \pm 0.15^{d,A}$	$66.85 \pm 0.03^{c,A}$	$69.14 \pm 0.05^{b,A}$	$82.71 \pm 0.15^{a,A}$
	2	$36.50 \pm 0.08^{d,B}$	$66.34 \pm 0.08^{c,A}$	$69.10 \pm 0.08^{b,A}$	$82.69 \pm 0.02^{a,A}$
	4	$36.96 \pm 0.02^{d,B}$	$65.15 \pm 0.14^{c,A}$	$67.82 \pm 0.11^{b,AB}$	$82.32 \pm 0.01^{a,AB}$
	6	$36.22 \pm 0.03^{d,B}$	$65.14 \pm 0.08^{c,AB}$	$66.65 \pm 0.26^{b,B}$	$81.55 \pm 0.21^{a,AB}$
	8	$36.39 \pm 0.01^{d,B}$	$63.79 \pm 0.12^{c,B}$	$66.80 \pm 0.12^{b,B}$	$81.22 \pm 0.11^{a,B}$
After cooking	0	$40.77 \pm 0.22^{d,A}$	$68.44 \pm 0.01^{c,A}$	$71.52 \pm 0.19^{b,A}$	$85.39 \pm 0.36^{a,A}$
	2	$40.82 \pm 0.08^{d,A}$	$68.38 \pm 0.03^{c,A}$	$71.30 \pm 0.01^{b,A}$	$85.24 \pm 0.14^{a,A}$
	4	$40.03 \pm 0.01^{d,A}$	$67.35 \pm 0.36^{c,B}$	$70.52 \pm 0.39^{b,B}$	$85.19 \pm 0.28^{a,A}$
	6	$40.22 \pm 0.02^{d,A}$	$67.25 \pm 0.04^{c,B}$	$70.24 \pm 0.15^{b,B}$	$84.72 \pm 0.17^{a,AB}$
	8	$38.55 \pm 0.02^{d,B}$	$66.18 \pm 0.11^{c,C}$	$70.17 \pm 0.06^{b,B}$	$83.86 \pm 0.02^{a,B}$

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-C}$ Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.14 Effect of water crude extract from *P. acidus* leaves on reducing power in ground pork at 4°C

Storage time		Absorbance at 700 nm			
(days)		Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
Before cooking	0	0.38 ± 0.36 ^{a,A}	0.45 ± 0.21 ^{a,A}	0.59 ± 0.11 ^{a,A}	0.75 ± 0.10 ^{a,A}
	2	0.36 ± 0.40 ^{a,A}	0.44 ± 0.18 ^{a,A}	0.58 ± 0.22 ^{a,A}	0.77 ± 0.03 ^{a,A}
	4	0.35 ± 0.22 ^{a,A}	0.43 ± 0.10 ^{a,A}	0.54 ± 0.16 ^{a,A}	0.72 ± 0.03 ^{a,A}
	6	0.30 ± 0.08 ^{a,A}	0.38 ± 0.09 ^{a,A}	0.55 ± 0.05 ^{a,A}	0.70 ± 0.05 ^{a,A}
	8	0.32 ± 0.15 ^{a,A}	0.32 ± 0.05 ^{a,A}	0.55 ± 0.05 ^{a,A}	0.68 ± 0.12 ^{a,A}
After cooking	0	0.44 ± 0.30 ^{a,A}	0.61 ± 0.02 ^{a,A}	0.76 ± 0.10 ^{a,A}	0.87 ± 0.23 ^{a,A}
	2	0.38 ± 0.11 ^{a,A}	0.60 ± 0.25 ^{a,A}	0.70 ± 0.10 ^{a,A}	0.80 ± 0.10 ^{a,A}
	4	0.38 ± 0.20 ^{a,A}	0.57 ± 0.23 ^{a,A}	0.67 ± 0.33 ^{a,A}	0.75 ± 0.05 ^{a,A}
	6	0.38 ± 0.36 ^{a,A}	0.45 ± 0.21 ^{a,A}	0.59 ± 0.11 ^{a,A}	0.75 ± 0.10 ^{a,A}
	8	0.36 ± 0.40 ^{a,A}	0.44 ± 0.18 ^{a,A}	0.58 ± 0.22 ^{a,A}	0.77 ± 0.03 ^{a,A}

[a] Means sharing different letters in the same row are significantly different ($P<0.05$).

[A] Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.15 Effect of water crude extract from *P. acidus* leaves on total MDA in ground pork at 4°C

Storage time		Total MDA (mg/kg meat)			
(days)		Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
Before cooking	0	$2.99 \pm 0.94^{a,B}$	$2.13 \pm 0.08^{a,B}$	$3.69 \pm 0.94^{a,A}$	$2.78 \pm 0.44^{a,A}$
	2	$3.21 \pm 0.14^{bc,AB}$	$2.55 \pm 0.37^{c,B}$	$4.74 \pm 0.88^{a,A}$	$4.03 \pm 0.30^{ab,A}$
	4	$3.28 \pm 0.35^{bc,AB}$	$2.65 \pm 0.22^{c,B}$	$4.85 \pm 0.46^{a,A}$	$4.25 \pm 0.67^{ab,A}$
	6	$4.08 \pm 0.18^{ab,AB}$	$2.74 \pm 0.17^{b,AB}$	$4.72 \pm 0.36^{a,A}$	$4.27 \pm 0.77^{a,A}$
	8	$4.56 \pm 0.34^{a,A}$	$3.35 \pm 0.24^{b,A}$	$4.83 \pm 0.13^{a,A}$	$4.33 \pm 0.61^{a,A}$
After cooking	0	$13.03 \pm 0.39^{a,C}$	$6.69 \pm 0.13^{b,B}$	$4.61 \pm 0.14^{c,B}$	$2.95 \pm 0.14^{d,A}$
	2	$13.34 \pm 0.34^{a,BC}$	$7.20 \pm 0.45^{b,B}$	$5.09 \pm 0.26^{c,B}$	$3.01 \pm 0.22^{d,A}$
	4	$14.47 \pm 0.59^{a,AB}$	$7.37 \pm 0.41^{b,B}$	$5.01 \pm 0.20^{c,AB}$	$2.91 \pm 0.45^{d,A}$
	6	$15.06 \pm 0.47^{a,A}$	$7.80 \pm 0.23^{b,B}$	$5.08 \pm 0.12^{c,AB}$	$3.14 \pm 0.27^{d,A}$
	8	$15.21 \pm 0.66^{a,A}$	$8.22 \pm 0.09^{b,A}$	$5.68 \pm 0.39^{c,A}$	$3.30 \pm 0.23^{d,A}$

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-C}$ Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Physical analysis

pH

Table 4.16 showed the effect of water extract from *P. acidus* leaves on the pH of ground pork samples during refrigerated storage at 4°C for 8 days. The initial pH value of the control and samples submitted to various treatments had no significant difference. The pH value ($P<0.05$) of the meat samples was found to increase from 5.85 to 6.25 (control), 5.89 to 6.18 (0.2 g BHT/kg), 5.58 to 6.12 (2.5 g PWCE/kg) and 5.84 to 6.11 (5 g PWCE/kg), at the end of the storage period. The pH increase slowly but still was not significant difference ($P<0.05$) of the samples probably due to the utilization of amino acids by bacteria, which are released during protein degradation because the stored glucose has been depleted. Accumulation of ammonia and the products of amino acid decomposition resulted in an increase in pH (Zhang *et al.* 2016). According to previous findings (Masniyom *et al.* 2002), the hypothetical greater availability of glycogen due to such mechanism could explain the lower value of the pH in PWCE. These results were in agreement with previous reports (Castellini *et al.* 2002; Castellini *et al.* 2006), which investigated that pH values increased in meat treated with plant during display 0, 24, 48, 72 and 96 hours of storage at 4°C.

Color

Color changes in raw ground pork were significantly affected by the 2.5 and 5 g PWCE/kg compared to the control and 0.2 g BHT/kg in Table 4.17. All treatments significantly increased lightness (L*), yellowness (b*) and decreased redness (a*) values during the storage period up to 8 days ($P<0.05$). All meat samples had no significant difference ($P<0.05$) from day 0 to day 2 for lightness (L*) values, to day 4 for redness (a*) and to day 6 for yellowness (b*). At the end of storage, meat samples contained PWCE were lower significantly lightness (L*) and yellowness (b*) values than the control. The a* value (redness) is the most important color parameter in evaluating meat oxidation, as a decrease in redness makes the meat product unacceptable to consumers (Kim *et al.* 2013b). In all samples, the redness (a* value) decreased as storage time progressed, but the redness of the control samples faded very rapidly. At the end of storage, the a* value of the control sample was significantly lower ($P<0.05$) than the control. Therefore, the natural plant extracts affected meat color, specifically red, and are therefore potentially useful in prolonging the shelf life of the meat product. Several researchers have examined the effects of different antioxidants on the color of meat and meat products, and have shown that meat oxidation decreases a* values (Dai *et al.* 2014; Radha Krishnan *et al.* 2014). We

observed a decrease in the a* value (due to myoglobin oxidation) after extract treatment despite the protective effects of the extracts against lipid oxidation. Meat color deterioration (or oxidation of oxymyoglobin) and lipid oxidation maybe dependent on the test system, and whether the sample is stored frozen or not. Studies on meat color have often concentrated on a* values because the redness of meat is an important factor that impacts visual attraction in consumers. Karakaya *et al.* (2011) indicated that color discoloration or oxidized pigments might promote lipid oxidation. Nevertheless, many researchers have tried to establish a correlation between lipid oxidation and discoloration in meat products (Kim *et al.* 2013b). Meanwhile, other studies have supported a lack of interaction between lipid oxidation and myoglobin oxidation, which means that the addition of certain natural extracts with polyphenolic compounds may retard lipid oxidation but showed no efficacy against meat discoloration (Karre *et al.* 2013). Recent studies have highlighted that secondary lipid oxidation products (e.g., unsaturated aldehydes) can accelerate the formation of metmyoglobin in meat products (Faustman *et al.* 2010). Phenolic compounds are mostly water-soluble compounds that would allow direct interaction with myoglobin, a water-soluble protein known to be present in the cytoplasm. It is expected that the reaction might retard myoglobin oxidation and discoloration (Kroll and Rawel. 2001). According to Coutinho de Oliveira *et al.* (2012); Dai *et al.* (2014), the loss of redness was due to the oxidation of myoglobin to metamyoglobin, and CWCE retarded this process to maintain meat redness longer. Therefore, we infer that bioactive compounds from PWCE retarded the discoloration by loss of myoglobin in ground pork during the storage time at 4°C at the level of 2.5 and 5 g/kg meat.

Table 4.16 Effect of water extract from *P. acidus* leaves on pH in ground pork during storage at 4°C

Storage time (days)	pH values			
	Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
0	5.85 ± 0.42 ^{a,A}	5.89 ± 0.76 ^{a,A}	5.58 ± 0.44 ^{a,A}	5.84 ± 0.16 ^{a,A}
2	6.06 ± 0.71 ^{a,A}	5.99 ± 0.82 ^{a,A}	6.03 ± 0.75 ^{a,A}	5.99 ± 0.77 ^{a,A}
4	6.06 ± 0.82 ^{a,A}	6.09 ± 1.06 ^{a,A}	6.08 ± 0.95 ^{a,A}	6.06 ± 0.73 ^{a,A}
6	6.09 ± 0.12 ^{a,A}	6.11 ± 1.02 ^{a,A}	6.11 ± 0.32 ^{a,A}	6.07 ± 0.83 ^{a,A}
8	6.25 ± 0.79 ^{a,A}	6.18 ± 0.77 ^{a,A}	6.12 ± 0.02 ^{a,A}	6.11 ± 0.81 ^{a,A}

^{a} Means sharing different letters in the same row are significantly different ($P<0.05$).

^{A} Means sharing different letters in the same column are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.17 Effect of water extract from *P. acidus* leaves on color parameter in ground pork during storage at 4°C

Parameter	Storage time (days)	Color values			
		Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
Lightness (L*)	0	51.10 ± 0.88 ^{a,D}	51.87 ± 0.27 ^{a,AB}	51.55 ± 0.58 ^{a,B}	50.99 ± 0.42 ^{a,A}
	2	51.92 ± 0.37 ^{a,CD}	50.83 ± 0.32 ^{a,B}	50.61 ± 1.01 ^{a,B}	51.54 ± 0.03 ^{a,A}
	4	53.19 ± 0.27 ^{a,BC}	51.82 ± 0.62 ^{ab,AB}	50.12 ± 0.32 ^{ab,B}	51.03 ± 1.38 ^{b,A}
	6	54.19 ± 0.12 ^{a,AB}	52.17 ± 0.72 ^{ab,AB}	51.57 ± 0.19 ^{b,B}	50.95 ± 1.38 ^{b,A}
	8	55.34 ± 0.44 ^{a,A}	53.70 ± 0.64 ^{b,A}	53.40 ± 0.19 ^{b,A}	52.34 ± 0.42 ^{b,A}
Redness (a*)	0	5.95 ± 0.32 ^{a,A}	6.08 ± 0.02 ^{a,A}	5.98 ± 0.26 ^{a,A}	6.02 ± 0.16 ^{a,A}
	2	4.57 ± 0.15 ^{b,B}	5.81 ± 0.32 ^{a,A}	5.31 ± 0.26 ^{ab,AB}	5.45 ± 0.84 ^{a,A}
	4	4.24 ± 0.17 ^{a,B}	5.02 ± 0.36 ^{a,B}	5.28 ± 1.30 ^{a,ABC}	5.48 ± 0.99 ^{a,A}
	6	3.28 ± 0.04 ^{c,C}	4.56 ± 0.16 ^{b,B}	4.16 ± 0.07 ^{a,BC}	4.69 ± 0.09 ^{a,AB}
	8	2.54 ± 0.13 ^{c,D}	3.46 ± 0.09 ^{b,C}	3.60 ± 0.38 ^{ab,C}	4.06 ± 0.16 ^{a,AB}
Yellowness (b*)	0	14.15 ± 0.38 ^{a,B}	14.18 ± 0.37 ^{a,B}	14.22 ± 0.08 ^{a,AB}	14.09 ± 0.87 ^{a,B}
	2	14.68 ± 0.46 ^{a,B}	14.45 ± 0.57 ^{a,AB}	14.47 ± 0.84 ^{a,AB}	14.81 ± 0.76 ^{a,AB}
	4	14.23 ± 0.59 ^{a,B}	15.01 ± 0.12 ^{a,AB}	15.31 ± 1.38 ^{a,AB}	15.49 ± 0.00 ^{a,AB}
	6	16.12 ± 0.56 ^{a,A}	15.00 ± 0.53 ^{a,AB}	15.39 ± 0.48 ^{a,AB}	15.50 ± 0.09 ^{a,AB}
	8	16.95 ± 0.51 ^{a,A}	15.26 ± 0.30 ^{b,A}	15.73 ± 0.34 ^{b,A}	15.90 ± 0.50 ^{b,A}

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-D}$ Means sharing different letters in the same column in each parameter are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Biological analysis

The changes in TPC, Y/M, psychophilic bacteria, coliform bacteria of ground pork samples with or without PWCE during refrigerated storage at 4°C over 8 days were shown in Table 4.18. The addition of PWCE resulted no significant difference to the control and BHT treatments in total plate count, yeasts/molds, psychrophilic bacteria and coliform bacteria during the storage time up to 8 days at 4°C ($P<0.05$). The total plate count of meat samples was initially approximately 5.17, 5.03, 5.02, 4.94 log CFU/g meat, which increased steadily with storage time and reached close to 7.51, 7.50, 7.45, 7.34 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g PWCE/kg and 5 g PWCE/kg, respectively, at the end of the storage. The Y/M of meat samples was initially approximately 4.02, 3.99, 3.94, 3.92 log CFU/g meat, which increased steadily with storage time and reached close to 5.21, 5.16, 5.16, 5.14 log CFU/g meat for the control, 0.2 g BHT, 2.5 g PWCE and 5 g PWCE/kg, respectively, at the end of the storage. The psychrophilic bacteria of meat samples was initially approximately 4.17, 4.24, 4.14, 4.22 log CFU/g meat, which increased steadily with storage time and reached close to 8.18, 8.13, 8.04, 8.04 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g PWCE/kg and 5 g PWCE/kg, respectively, at the end of the storage. The coliforms of meat samples was initially approximately 4.28, 4.25, 4.13, 4.30 log CFU/g meat, which increased steadily with storage time and reached close to 5.23, 5.27, 5.22, 5.25 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g PWCE/kg and 5 g PWCE/kg, respectively, at the end of the storage. Therefore, the addition of CE did not significantly affect biological analysis ($P<0.05$). This results can be explained through the researches of Jain and Singhai. (2011) and Sawhney *et al.* (2011) suggesting that although PWCE containing high bio-active compounds but it did not act as an antimicrobial agent.

Table 4.18 Effect of water extract from *P. acidus* leaves on biological quality in ground pork during storage at 4°C

Storage time (days)		Log 10 CFU/g			
		Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
Total plate count	0	5.17 ± 0.30 ^{a,B}	5.03 ± 0.39 ^{a,B}	5.02 ± 0.18 ^{a,B}	4.94 ± 0.16 ^{a,B}
	2	5.18 ± 0.29 ^{a,B}	5.25 ± 0.25 ^{a,B}	5.17 ± 0.30 ^{a,B}	5.05 ± 0.09 ^{a,B}
	4	5.78 ± 0.49 ^{a,AB}	5.79 ± 0.55 ^{a,AB}	5.65 ± 0.82 ^{a,AB}	5.54 ± 0.83 ^{a,AB}
	6	6.54 ± 0.03 ^{a,AB}	6.58 ± 0.71 ^{a,AB}	6.61 ± 0.82 ^{a,AB}	6.49 ± 0.83 ^{a,AB}
	8	7.51 ± 0.12 ^{a,A}	7.50 ± 0.10 ^{a,A}	7.45 ± 0.92 ^{a,A}	7.34 ± 0.94 ^{a,A}
Yeasts/Molds	0	4.02 ± 0.08 ^{a,D}	3.99 ± 0.04 ^{a,D}	3.94 ± 0.06 ^{a,D}	3.92 ± 0.01 ^{a,D}
	2	4.26 ± 0.18 ^{a,CD}	4.28 ± 0.08 ^{a,CD}	4.47 ± 0.26 ^{a,CD}	4.22 ± 0.19 ^{a,CD}
	4	4.55 ± 0.12 ^{a,BC}	4.51 ± 0.10 ^{a,BC}	4.50 ± 0.14 ^{a,BC}	4.49 ± 0.14 ^{a,BC}
	6	4.91 ± 0.30 ^{a,AB}	4.91 ± 0.31 ^{a,AB}	4.87 ± 0.25 ^{a,AB}	4.92 ± 0.25 ^{a,AB}
	8	5.21 ± 0.15 ^{a,A}	5.16 ± 0.14 ^{a,A}	5.16 ± 0.13 ^{a,A}	5.14 ± 0.10 ^{a,A}
Psychophilic bacteria	0	4.17 ± 0.04 ^{a,D}	4.24 ± 0.18 ^{a,E}	4.14 ± 0.07 ^{a,D}	4.22 ± 0.21 ^{a,D}
	2	5.21 ± 0.14 ^{a,C}	5.15 ± 0.09 ^{a,D}	5.24 ± 0.02 ^{a,C}	5.03 ± 0.06 ^{a,C}
	4	5.68 ± 0.43 ^{a,C}	5.81 ± 0.36 ^{a,C}	5.51 ± 0.25 ^{a,C}	5.44 ± 0.39 ^{a,C}
	6	7.10 ± 0.07 ^{a,B}	7.03 ± 0.10 ^{a,B}	6.95 ± 0.12 ^{a,B}	7.00 ± 0.03 ^{a,B}
	8	8.18 ± 0.12 ^{a,A}	8.13 ± 0.14 ^{a,A}	8.04 ± 0.14 ^{a,A}	8.04 ± 0.12 ^{a,A}
Coliforms	0	4.28 ± 0.12 ^{a,C}	4.25 ± 0.01 ^{a,B}	4.13 ± 0.03 ^{a,C}	4.30 ± 0.05 ^{a,C}
	2	4.30 ± 0.08 ^{a,C}	4.23 ± 0.11 ^{a,B}	4.32 ± 0.11 ^{a,C}	4.34 ± 0.11 ^{a,C}
	4	4.42 ± 0.03 ^{a,BC}	4.40 ± 0.01 ^{a,B}	4.42 ± 0.04 ^{a,BC}	4.41 ± 0.04 ^{a,BC}
	6	4.78 ± 0.29 ^{a,B}	4.64 ± 0.38 ^{a,B}	4.66 ± 0.17 ^{a,B}	4.69 ± 0.25 ^{a,B}
	8	5.23 ± 0.13 ^{a,A}	5.27 ± 0.14 ^{a,A}	5.22 ± 0.16 ^{a,A}	5.25 ± 0.15 ^{a,A}

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-D}$ Means sharing different letters in the same column in each parameter are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

(2) Evaluation of the effect of optimal crude extract from *C. carandas* fruits on chemical, physical and biological quality in ground pork storaged at 4°C up to 8 days

Chemical analysis

Free scavenging radical activity (DPPH)

According to the DPPH assay the 2.5 g CWCE/kg and 5 g CWCE/kg treatments produced significantly ($P<0.05$) effective antioxidant activity in both raw and cooked samples compared to the control and BHT samples in Table 4.19. The overall radical scavenging activity was significantly ($P<0.05$) higher for the CWCE treatment in both raw and cooked samples. The results showed that percentage inhibition of DPPH radical scavenging activity was 18.95%, 29.43%, 38.76% and 58.40% at 0 day and slightly decreased to 16.13%, 27.63%, 37.09% and 57.42% at the end of the storage time for the control, BHT, 2.5 g CWCE/kg and 5g CWCE/kg treatments, respectively. In cooked ground pork, percentage inhibition of DPPH radical scavenging activity of all samples increased in comparison with raw samples and reached to 22.16%, 35.40%, 43.52% and 65.30% at 0 day and also decreased to 20.63%, 33.35%, 41.34% and 63.25% at the end of the storage time for the control, BHT, 2.5 g CWCE/kg and 5g CWCE/kg treatments, respectively.

Radical cation decolorization (ABTS)

The results of ABTS radical cation decolorization activity were shown in Table 4.20. The 2.5 g CWCE/kg and 5 g CWCE/kg treatments were higher significantly ($P<0.05$) effective ABTS activity in both raw and cooked samples than the control and BHT samples. In raw ground pork, percentage inhibition of ABTS activity was 36.81%, 64.13%, 67.24% and 74.04% at 0 day and was decreased to 35.09%, 63.24%, 65.38% and 73.49% at the end of the storage time for the control, BHT, 2.5 g CWCE/kg and 5 g CWCE/kg, respectively. In cooked ground pork, percentage inhibition of ABTS activity of all samples increased in comparison with raw samples and reached to 40.15%, 69.15%, 71.47% and 78.94% at 0 day and also decreased to 37.95%, 66.36%, 70.05% and 76.15% at the end of the storage time for the control, BHT, 2.5 g CWCE/kg and 5g CWCE/kg treatments, respectively. Therefore, DPPH and ABTS assays both showed that *P. acidus* water crude extract treatments significantly increased radical scavenging and in agreement with the previous studies (Huang *et al.* 2011). Radical scavenging was also affected over time in all untreated meat samples, perhaps because oxidation of the meat leads to the creation of compounds that scavenge free radicals. The DPPH radical assay is only suitable for lipid-soluble antioxidants, while the ABTS radical cation decolorization assay is applicable for

both water-soluble and lipid-soluble antioxidants (Re *et al.* 1999). According to other studies, free radical scavenging activity depends on the structural conformation of the phenolic compounds (Chumyam *et al.* 2013; Fernandes *et al.* 2012).

Reducing power ability

The reducing power ability was expressed as the absorbance at 700 nm in Table 4.21. The results showed that the meat samples treated with CWCE increased reducing power (higher absorbance) compared to the control and BHT at 0.2g/kg meat. In raw ground pork, the absorbance of reducing power of ground pork samples was 0.35, 0.48, 0.61 and 0.69 at 0 day and was slightly decreased to 0.30, 0.36, 0.50 and 0.54 at the end of the storage time for the control, BHT, 2.5 g CWCE/kg and 5 g CWCE/kg, respectively. In cooked ground pork, percentage inhibition of the absorbance of reducing power of all samples increased in comparison with raw samples and reached to 0.42, 0.58, 0.75 and 0.84 at 0 day and also decreased to 0.33, 0.46, 0.59 and 0.72 at the end of the storage time for the control, BHT, 2.5 g CWCE/kg and 5g CWCE/kg treatments, respectively. This finding could be explained by the presence of reductants (antioxidants) in meat causing the reduction of Fe^{3+}/Ferric cyanide complex to the ferrous form. Therefore, the Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Beyhan *et al.* 2010). CWCE treatment gave a significantly higher absorbance than the control and BHT treatments due to the high polyphenolic content of the *P. acidus* extract. Antioxidant properties are known to be concomitant with the development of reducing power. Reductones can react directly with peroxides and can also prevent peroxide formation by reacting with certain precursors (Kim *et al.* 2013b).

Thiobarbituric acid reaction substances (TBARS)

Table 4.22 showed the effect of addition of crude water extract from *C. carandas* fruits at 2.5 and 5 g/kg in ground pork during the storage time at 4°C. The results showed that level of lipid oxidation in raw ground pork (control) increased over 8 days of refrigerated storage. No statistical difference was observed between CWCE addition treatments (2.5 and 5 g/kg) and control on any of the storage days. Level of lipid oxidation was lowest in BHT containing raw ground pork. According to Hernandez-Hernandez *et al.* (2009), this could be due to a reaction between malonaldehyde - its concentration increased with time and lysine e-amino and sulfhydryl groups in myosin subfragment 1, and the fact that the protein prevents hydroperoxide interactions with prooxidants. According to Jain and Singhai. (2011) and Sawhney *et al.* (2011), although ethanol dried CWCE contained high bioactive compounds but it did not act as an antioxidant.

However, in cooked ground pork, lipid peroxidation increased as function of storage time. Ground pork contain CWCE at 2.5 and 5.0 g/kg had significantly lower level of lipid peroxidation compared to control and BHT treatments ($P<0.05$). The results showed that the MDA contents of the meat samples were 7.35 mg MDA/kg meat (2.5 g CWCE/kg), 6.85 mg MDA/kg meat (5 g CWCE/kg), 12.75 mg MDA/kg meat (control) and 5.80 mg MDA/kg meat (0.2 g BHT/kg) in the 0 day. In particular, addition of 5 g CWCE/kg maintained total MDA content during the storage time up to 8 days. These findings were in agreement with other research studies that investigated the effects of plant extract on lipid peroxidation in meat (Coutinho de Oliveira *et al.* 2012; Hernandez-Hernandez *et al.* 2009). In general, the mechanism of the protective effect of crude extract on lipid oxidation may be due to the presence of a number of oligomer procyanidins, such as catechin and epicatechin (Carpenter *et al.* 2007) which possess a greater antioxidant potential than monomer components (Llópiz *et al.* 2004). This finding can be explained by cooking that decreased the ferric ion reducing capacity (FRC) but increased nonheme iron of meat, resulting in comparatively lower FRC in cooked meat than raw meat. Therefore, heat stable FRC may be primarily responsible for the regeneration of ferrous ion to increase total malondialdehyde in cooked meat during storage (Min *et al.* 2008). Moreover, according to Zhang *et al.* (2016), the phenolic compounds are associated with the hydroxyl group linked to the aromatic ring, which is capable of donating hydrogen atoms with electrons and neutralizing free radicals. This mechanism blocks further degradation to more active oxidizing forms, such as MDA.

Table 4.19 Effect of water crude extract from *C. carandas* fruits on DPPH radical scavenging activity ground pork at 4°C

Storage time		% Inhibition of DPPH radical scavenging activity			
(days)		Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Before cooking	0	$18.95 \pm 0.18^{d,A}$	$29.43 \pm 0.73^{c,A}$	$38.76 \pm 0.11^{b,A}$	$58.40 \pm 0.10^{a,AB}$
	2	$18.58 \pm 0.05^{d,B}$	$29.65 \pm 0.55^{c,A}$	$38.59 \pm 0.73^{b,A}$	$58.06 \pm 0.13^{a,AB}$
	4	$18.78 \pm 0.30^{d,AB}$	$29.45 \pm 0.13^{c,A}$	$38.25 \pm 0.73^{b,A}$	$58.50 \pm 0.13^{a,A}$
	6	$16.35 \pm 0.18^{d,C}$	$28.54 \pm 0.11^{c,AB}$	$38.95 \pm 0.21^{b,AB}$	$57.77 \pm 0.49^{a,BC}$
	8	$16.13 \pm 0.02^{d,C}$	$27.63 \pm 0.05^{c,B}$	$37.09 \pm 0.04^{b,B}$	$57.42 \pm 0.10^{a,C}$
After cooking	0	$22.16 \pm 0.06^{d,A}$	$35.40 \pm 0.11^{c,A}$	$43.52 \pm 0.05^{b,A}$	$65.30 \pm 0.06^{a,A}$
	2	$22.18 \pm 0.16^{d,A}$	$35.06 \pm 0.02^{c,B}$	$43.65 \pm 0.20^{b,A}$	$64.22 \pm 0.21^{a,C}$
	4	$21.99 \pm 0.04^{d,A}$	$34.87 \pm 0.11^{c,B}$	$43.02 \pm 0.13^{b,B}$	$64.96 \pm 0.13^{a,B}$
	6	$21.76 \pm 0.21^{d,B}$	$34.23 \pm 0.12^{c,C}$	$41.83 \pm 0.04^{b,C}$	$63.93 \pm 0.06^{a,C}$
	8	$20.63 \pm 0.13^{d,C}$	$33.35 \pm 0.12^{c,D}$	$41.34 \pm 0.12^{b,D}$	$63.25 \pm 0.07^{a,D}$

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-D}$ Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.20 Effect of water crude extract from *C. carandas* fruits on ABTS radical cation decolorization in ground pork at 4°C

Storage time		% Inhibition of ABTS radical cation decolorization			
(days)		Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Before cooking	0	36.81 ± 0.13 ^{d,A}	64.13 ± 0.01 ^{c,A}	67.24 ± 0.17 ^{b,B}	74.04 ± 0.11 ^{a,A}
	2	36.25 ± 0.22 ^{d,B}	63.96 ± 0.09 ^{c,A}	67.74 ± 0.02 ^{b,A}	74.16 ± 0.01 ^{a,A}
	4	36.25 ± 0.01 ^{d,B}	64.15 ± 0.10 ^{c,A}	66.24 ± 0.04 ^{b,B}	73.77 ± 0.05 ^{a,B}
	6	35.01 ± 0.02 ^{d,C}	63.14 ± 0.06 ^{c,B}	66.32 ± 0.09 ^{b,B}	73.69 ± 0.06 ^{a,B}
	8	35.09 ± 0.11 ^{d,C}	63.24 ± 0.13 ^{c,B}	65.38 ± 0.10 ^{b,D}	73.49 ± 0.05 ^{a,C}
After cooking	0	40.15 ± 0.15 ^{d,A}	69.15 ± 0.14 ^{c,A}	71.47 ± 0.04 ^{b,A}	78.94 ± 0.13 ^{a,A}
	2	40.22 ± 0.13 ^{d,A}	68.70 ± 0.11 ^{c,B}	70.50 ± 0.13 ^{b,B}	78.37 ± 0.04 ^{a,B}
	4	39.58 ± 0.31 ^{d,B}	67.51 ± 0.10 ^{c,C}	70.59 ± 0.02 ^{b,B}	77.38 ± 0.14 ^{a,C}
	6	38.02 ± 0.09 ^{d,C}	66.71 ± 0.15 ^{c,D}	70.68 ± 0.10 ^{b,B}	76.29 ± 0.02 ^{a,D}
	8	37.95 ± 0.12 ^{d,C}	66.36 ± 0.03 ^{c,E}	70.05 ± 0.02 ^{b,C}	76.15 ± 0.13 ^{a,D}

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-E}$ Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.21 Effect of water crude extract from *C. carandas* fruits on reducing power in ground pork at 4°C

Storage time (days)		Absorbance at 700 nm			
		Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Before cooking	0	0.35 ± 0.66 ^{a,A}	0.48 ± 0.78 ^{a,A}	0.61 ± 0.75 ^{a,A}	0.69 ± 0.76 ^{a,A}
	2	0.34 ± 0.20 ^{a,A}	0.43 ± 0.15 ^{a,A}	0.59 ± 0.20 ^{a,A}	0.67 ± 0.86 ^{a,A}
	4	0.33 ± 0.78 ^{a,A}	0.40 ± 0.85 ^{a,A}	0.55 ± 0.75 ^{a,A}	0.62 ± 0.98 ^{a,A}
	6	0.29 ± 1.07 ^{a,A}	0.37 ± 0.08 ^{a,A}	0.54 ± 0.45 ^{a,A}	0.59 ± 0.48 ^{a,A}
	8	0.30 ± 0.70 ^{a,A}	0.36 ± 0.04 ^{a,A}	0.50 ± 0.94 ^{a,A}	0.54 ± 0.80 ^{a,A}
After cooking	0	0.42 ± 0.14 ^{a,A}	0.58 ± 0.70 ^{a,A}	0.75 ± 0.84 ^{a,A}	0.84 ± 0.74 ^{a,A}
	2	0.40 ± 0.30 ^{a,A}	0.55 ± 0.75 ^{a,A}	0.72 ± 0.08 ^{a,A}	0.88 ± 0.12 ^{a,A}
	4	0.39 ± 0.50 ^{a,A}	0.54 ± 0.45 ^{a,A}	0.68 ± 1.10 ^{a,A}	0.76 ± 0.42 ^{a,A}
	6	0.34 ± 0.73 ^{a,A}	0.48 ± 0.20 ^{a,A}	0.60 ± 0.40 ^{a,A}	0.75 ± 1.08 ^{a,A}
	8	0.33 ± 0.08 ^{a,A}	0.46 ± 0.12 ^{a,A}	0.59 ± 1.15 ^{a,A}	0.72 ± 1.05 ^{a,A}

^{a} Means sharing different letters in the same row are significantly different ($P<0.05$).

^{A} Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.22 Effect of water crude extract from *C. carandas* fruits on total MDA in ground pork at 4°C

Storage time (days)		Total MDA (mg/kg meat)			
		Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Before cooking	0	$3.07 \pm 0.03^{a,B}$	$2.99 \pm 0.09^{a,C}$	$3.08 \pm 0.02^{a,D}$	$3.01 \pm 0.13^{a,D}$
	2	$3.15 \pm 0.06^{a,B}$	$3.18 \pm 0.08^{a,C}$	$3.19 \pm 0.07^{a,CD}$	$3.16 \pm 0.04^{a,CD}$
	4	$3.35 \pm 0.06^{a,B}$	$3.24 \pm 0.06^{a,B}$	$3.34 \pm 0.08^{a,C}$	$3.32 \pm 0.12^{a,C}$
	6	$4.06 \pm 0.18^{a,A}$	$4.04 \pm 0.08^{a,A}$	$4.04 \pm 0.05^{a,B}$	$4.17 \pm 0.05^{a,B}$
	8	$4.32 \pm 0.15^{a,A}$	$4.19 \pm 0.09^{a,A}$	$4.34 \pm 0.15^{a,A}$	$4.44 \pm 0.07^{a,A}$
After cooking	0	$12.75 \pm 0.11^{a,D}$	$5.80 \pm 0.09^{d,C}$	$7.35 \pm 0.05^{b,C}$	$6.85 \pm 0.06^{c,D}$
	2	$12.87 \pm 0.04^{a,D}$	$6.00 \pm 0.09^{d,C}$	$7.59 \pm 0.09^{b,B}$	$7.01 \pm 0.03^{c,D}$
	4	$13.39 \pm 0.11^{a,C}$	$6.27 \pm 0.09^{d,B}$	$8.02 \pm 0.13^{b,A}$	$7.22 \pm 0.03^{c,C}$
	6	$14.07 \pm 0.02^{a,B}$	$6.45 \pm 0.03^{d,B}$	$8.19 \pm 0.07^{b,A}$	$7.81 \pm 0.11^{c,B}$
	8	$14.34 \pm 0.08^{a,A}$	$7.15 \pm 0.12^{c,A}$	$8.14 \pm 0.04^{b,A}$	$7.99 \pm 0.08^{b,A}$

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-D}$ Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Physical analysis

pH

Effect of water extract from *C. carandas* fruits on pH values in ground pork during storage at 4°C up to 8 days was shown in Table 4.23. The results revealed that the initial pH values were 5.74, 5.76, 5.78 and 5.78 for the control, 0.2 g BHT/kg, 2.5 g CWCE/kg and 5 g CWCE/kg, respectively. The pH value increased slowly but still did not significant difference ($P<0.05$) of the samples may have been caused by the utilization of amino acids by bacteria, which are released during protein degradation because the stored glucose has been depleted. Overall, the pH values of all ground pork samples were increased to 6.36, 6.29, 6.08 and 6.01 for control, 0.2 g BHT/kg, 2.5 g CWCE/kg and 5 g CWCE/kg, respectively at 8 days. In addition, the pH value reflects the rate of post mortem glycolysis which is a key indicator of meat quality. Ground pork is mostly composed of red muscle fibers and post mortem glycolysis may be accompanied by rapid anaerobic carbohydrate degradation (Smiecinska *et al.* 2015).

Color

Color changes in raw ground pork were significantly affected by the 2.5 and 5 g CWCE/kg compared to the control and 0.2g BHT/kg in Table 4.24. All treatments significantly increased lightness (L*), yellowness (b*) and decreased redness (a*) values during the storage period up to 8 days ($P<0.05$). All meat samples had no significant difference ($P<0.05$) from day 0 to day 2 for lightness (L*) values, to day 4 for redness (a*) and to day 6 for yellowness (b*). At the end of storage, meat samples contained CWCE were lower significantly lightness (L*) and yellowness (b*) values than the control. The a* value (redness) is the most important color parameter in evaluating meat oxidation, as a decrease in redness makes the meat product unacceptable to consumers (Kim *et al.* 2013b). In all samples, the redness (a* value) decreased as storage time progressed, but the redness of the control samples faded very rapidly. At the end of storage, the a* value of the control sample was significantly lower ($P<0.05$) than the control. Therefore, the natural plant extracts affected meat color, specifically red, and are therefore potentially useful in prolonging the shelf life of the meat product. Several researchers have examined the effects of different antioxidants on the color of meat and meat products, and have shown that meat oxidation decreases a* values (Dai *et al.* 2014; Radha Krishnan *et al.* 2014). We observed a decrease in the a* value (due to myoglobin oxidation) after extract treatment despite the protective effects of the extracts against lipid oxidation. Meat color deterioration (or oxidation of oxymyoglobin) and lipid oxidation may dependent on the test system, and whether the sample

is stored frozen or not. Studies on meat color have often concentrated on a* values because the redness of meat is an important factor that impacts visual attraction in consumers. Karakaya *et al.* (2011) indicated that color discoloration or oxidized pigments might promote lipid oxidation. Nevertheless, many researchers have tried to establish a correlation between lipid oxidation and discoloration in meat products (Kim *et al.* 2013b). Meanwhile, other studies have supported a lack of interaction between lipid oxidation and myoglobin oxidation, which means that the addition of certain natural extracts with polyphenolic compounds may retard lipid oxidation but show no efficacy against meat discoloration (Karre *et al.* 2013).

Table 4.23 Effect of water extract from *C. carandas* fruits on pH in ground pork during storage at 4°C

Storage time	pH values			
(days)	Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
0	$5.74 \pm 0.89^{a,A}$	$5.76 \pm 0.05^{a,A}$	$5.78 \pm 0.44^{a,A}$	$5.78 \pm 0.71^{a,A}$
2	$6.09 \pm 0.75^{a,A}$	$5.96 \pm 0.87^{a,A}$	$6.06 \pm 0.07^{a,A}$	$5.96 \pm 0.13^{a,A}$
4	$6.06 \pm 0.22^{a,A}$	$6.09 \pm 0.06^{a,A}$	$6.06 \pm 0.45^{a,A}$	$5.96 \pm 0.43^{a,A}$
6	$6.19 \pm 0.72^{a,A}$	$6.11 \pm 1.01^{a,A}$	$6.07 \pm 0.83^{a,A}$	$5.97 \pm 0.02^{a,A}$
8	$6.36 \pm 0.11^{a,A}$	$6.29 \pm 0.08^{a,A}$	$6.08 \pm 0.04^{a,A}$	$6.01 \pm 0.51^{a,A}$

[a] Means sharing different letters in the same row are significantly different ($P<0.05$).

[A] Means sharing different letters in the same column are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

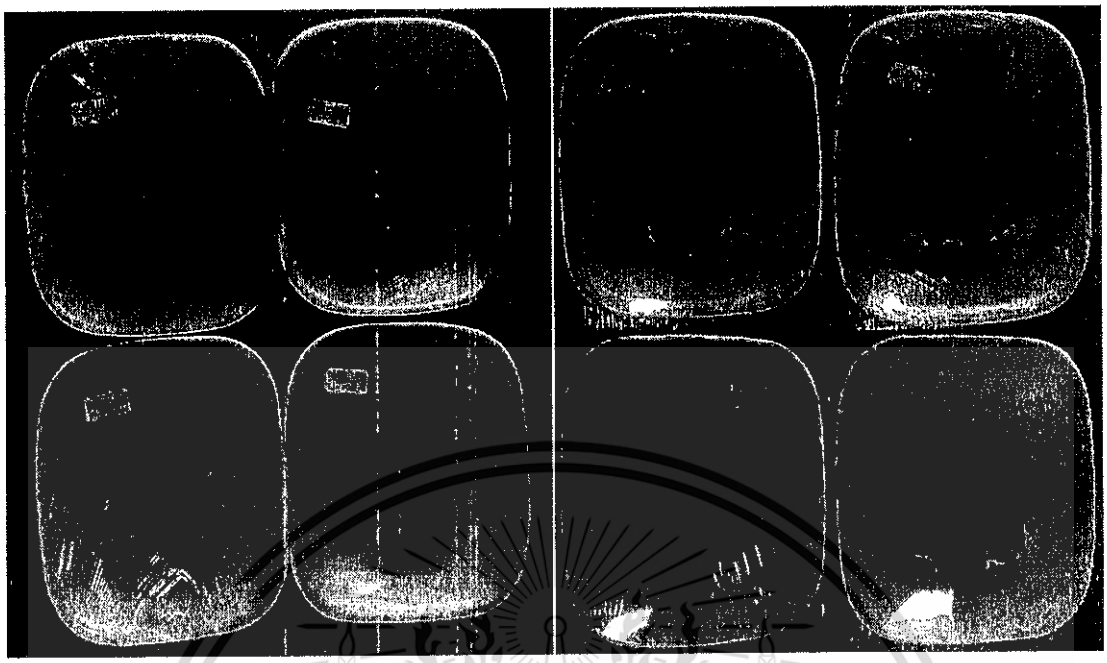
Table 4.24 Effect of water extract from *C. carandas* fruits on color parameter in ground pork during storage at 4°C

Parameter	Storage time (days)	Color values			
		Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Lightness (L*)	0	$51.54 \pm 0.84^{a,C}$	$51.87 \pm 0.17^{a,AB}$	$51.55 \pm 0.92^{a,B}$	$51.78 \pm 0.22^{a,B}$
	2	$51.94 \pm 0.05^{a,C}$	$50.85 \pm 0.22^{a,B}$	$51.44 \pm 0.95^{a,B}$	$51.55 \pm 1.05^{a,B}$
	4	$53.19 \pm 0.27^{a,BC}$	$51.71 \pm 0.11^{b,AB}$	$51.09 \pm 0.72^{b,B}$	$51.04 \pm 1.38^{b,B}$
	6	$54.20 \pm 0.13^{a,AB}$	$52.25 \pm 0.02^{ab,AB}$	$51.55 \pm 0.79^{b,B}$	$51.95 \pm 0.39^{b,B}$
	8	$55.34 \pm 0.14^{a,A}$	$53.58 \pm 0.10^{b,A}$	$53.35 \pm 0.11^{b,A}$	$53.51 \pm 0.22^{b,A}$
Redness (a*)	0	$6.06 \pm 0.07^{a,A}$	$6.19 \pm 0.20^{a,A}$	$6.02 \pm 0.26^{a,A}$	$6.33 \pm 0.15^{a,A}$
	2	$4.58 \pm 0.14^{b,B}$	$5.82 \pm 0.34^{a,A}$	$5.33 \pm 0.26^{ab,AB}$	$5.49 \pm 0.45^{a,A}$
	4	$4.22 \pm 0.13^{a,B}$	$5.01 \pm 0.34^{a,B}$	$5.29 \pm 1.30^{a,ABC}$	$5.47 \pm 0.18^{a,A}$
	6	$3.29 \pm 0.04^{b,C}$	$4.55 \pm 0.15^{a,B}$	$4.17 \pm 0.07^{a,BC}$	$4.71 \pm 0.15^{a,AB}$
	8	$2.58 \pm 0.11^{c,D}$	$3.45 \pm 0.10^{b,C}$	$3.59 \pm 0.37^{ab,C}$	$4.07 \pm 0.17^{a,AB}$
Yellowness (b*)	0	$14.37 \pm 0.97^{a,B}$	$14.18 \pm 0.37^{a,B}$	$14.12 \pm 0.05^{a,B}$	$14.14 \pm 0.17^{a,B}$
	2	$14.67 \pm 0.86^{a,B}$	$14.41 \pm 0.57^{a,AB}$	$14.45 \pm 0.24^{a,AB}$	$14.84 \pm 0.26^{a,AB}$
	4	$14.23 \pm 0.89^{a,B}$	$15.01 \pm 0.12^{a,AB}$	$15.29 \pm 0.08^{a,AB}$	$15.50 \pm 0.01^{a,AB}$
	6	$16.12 \pm 0.26^{a,A}$	$14.99 \pm 0.53^{a,AB}$	$15.36 \pm 0.48^{a,AB}$	$15.48 \pm 0.06^{a,AB}$
	8	$16.96 \pm 0.50^{a,A}$	$15.38 \pm 0.30^{b,A}$	$15.77 \pm 0.34^{b,A}$	$15.89 \pm 0.20^{b,A}$

$^{a-c}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-D}$ Means sharing different letters in the same column in each parameter are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.



(a) (b)

Figure 4.1 Effect of water crude extract from (a) *P. acidus* leaves and (b) *C. carandas* fruits on color quality of ground pork samples

Biological analysis

The changes in TPC, Y/M, psychophilic bacteria, coliform of ground pork samples with or without CWCE during refrigerated storage at -20°C over 12 weeks were shown in Table 4.25. The addition of CWCE resulted in a reduction in the growth of TPC and coliform, but it had no effect on Y/M and psychophilic bacteria to the control and BHT treatments during the storage time up to 8 days at 4°C ($P<0.05$). The total plate count of meat samples was initially approximately 4.83, 4.86, 4.79, 4.76 log CFU/g meat, which increased steadily with storage time and reached close to 7.41, 7.30, 7.05, 7.03 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g CWCE and 5 g CWCE/kg , respectively, at the end of the storage. The Y/M of meat samples was initially approximately 4.03, 4.02, 4.02, 4.03 log CFU/g meat, which increased steadily with storage time and reached close to 5.16, 5.15, 5.16, 5.14 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g CWCE and 5 g CWCE/kg, respectively, at the end of the storage. The psychrophilic bacteria of meat samples was initially approximately 4.20, 4.19, 4.20, 4.13 log CFU/g meat, which increased steadily with storage time and reached close to 8.17, 8.09, 8.01, 8.04 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g CWCE and 5 g CWCE/kg, respectively, at the end of the storage. The coliforms of meat samples was initially approximately 3.87, 3.88, 3.83, 3.81 log CFU/g meat, which increased steadily with storage time and reached close to 6.01, 6.20, 5.01, 5.02 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g CWCE and 5 g CWCE/kg, respectively, at the end of the storage. Therefore, the addition of CWCE did not significantly affect biological analysis ($P<0.05$). This results can be explained through the researches of Jain and Singhai. (2011) and Sawhney *et al.* (2011) suggesting that although PWCE containing high bio-active compounds but it did not act as an antimicrobial agent.

Table 4.25 Effect of water extract from *C. carandas* fruits on biological quality in ground pork during storage at 4°C

Storage time (days)		Log 10 CFU/g			
		Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Total plate count	0	4.83 ± 0.12 ^{a,D}	4.86 ± 0.19 ^{a,D}	4.79 ± 0.14 ^{a,C}	4.76 ± 0.18 ^{a,D}
	2	5.12 ± 0.14 ^{a,D}	5.12 ± 0.15 ^{a,D}	5.06 ± 0.17 ^{a,C}	5.02 ± 0.06 ^{a,D}
	4	5.84 ± 0.17 ^{a,C}	5.85 ± 0.15 ^{a,C}	5.77 ± 0.20 ^{a,B}	5.68 ± 0.13 ^{a,C}
	6	6.65 ± 0.05 ^{a,B}	6.64 ± 0.21 ^{a,B}	6.20 ± 0.19 ^{b,B}	6.19 ± 0.13 ^{b,B}
	8	7.41 ± 0.08 ^{a,A}	7.30 ± 0.17 ^{ab,A}	7.04 ± 0.17 ^{b,A}	7.04 ± 0.06 ^{b,A}
Yeasts/Molds	0	4.03 ± 0.03 ^{a,B}	4.02 ± 0.12 ^{a,D}	4.02 ± 0.04 ^{a,D}	4.03 ± 0.02 ^{a,C}
	2	4.32 ± 0.01 ^{a,B}	4.34 ± 0.06 ^{a,C}	4.33 ± 0.14 ^{a,C}	4.25 ± 0.22 ^{a,C}
	4	4.81 ± 0.23 ^{a,A}	4.79 ± 0.09 ^{a,B}	4.80 ± 0.04 ^{a,B}	4.76 ± 0.07 ^{a,B}
	6	5.02 ± 0.12 ^{a,A}	5.00 ± 0.15 ^{a,AB}	4.99 ± 0.06 ^{a,AB}	4.98 ± 0.02 ^{a,AB}
	8	5.16 ± 0.20 ^{a,A}	5.15 ± 0.15 ^{a,A}	5.16 ± 0.07 ^{a,A}	5.14 ± 0.02 ^{a,A}
Psychophilic bacteria	0	4.20 ± 0.10 ^{a,E}	4.19 ± 0.18 ^{a,E}	4.20 ± 0.13 ^{a,E}	4.12 ± 0.04 ^{a,E}
	2	5.22 ± 0.18 ^{a,D}	5.19 ± 0.19 ^{a,D}	5.12 ± 0.17 ^{a,D}	5.10 ± 0.06 ^{a,D}
	4	6.46 ± 0.02 ^{a,C}	6.45 ± 0.01 ^{a,C}	6.40 ± 0.05 ^{a,C}	6.39 ± 0.05 ^{a,C}
	6	7.10 ± 0.07 ^{a,B}	7.06 ± 0.15 ^{a,B}	6.91 ± 0.12 ^{a,B}	6.90 ± 0.02 ^{a,B}
	8	8.17 ± 0.11 ^{a,A}	8.09 ± 0.11 ^{a,A}	8.01 ± 0.09 ^{a,A}	8.04 ± 0.12 ^{a,A}
Coliforms	0	3.87 ± 0.16 ^{a,C}	3.88 ± 0.19 ^{a,C}	3.83 ± 0.16 ^{a,C}	3.81 ± 0.24 ^{a,C}
	2	3.95 ± 0.14 ^{a,C}	3.97 ± 0.05 ^{a,C}	3.90 ± 0.03 ^{a,C}	3.90 ± 0.05 ^{a,C}
	4	4.13 ± 0.17 ^{a,C}	4.10 ± 0.07 ^{a,C}	3.99 ± 0.05 ^{a,C}	3.95 ± 0.10 ^{a,C}
	6	5.01 ± 0.14 ^{a,B}	4.99 ± 0.21 ^{a,B}	4.71 ± 0.13 ^{b,B}	4.57 ± 0.05 ^{b,B}
	8	6.19 ± 0.19 ^{a,A}	6.20 ± 0.23 ^{a,A}	5.01 ± 0.04 ^{b,A}	5.02 ± 0.02 ^{b,A}

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-E}$ Means sharing different letters in the same column in each parameter are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

4.2.3.2 Evaluation of the effect of optimal crude extracts from *P. acidus* leaves and *C. carandas* fruits on chemical, physical and biological qualities in ground pork storaged at -20°C up to 12 weeks

(1) Evaluation of the effect of optimal crude extract from *P. acidus* leaves on chemical, physical and biological quality in ground pork storaged at -20°C up to 12 weeks

Chemical analysis

Free scavenging radical activity (DPPH)

Generally, the antioxidant activity of bio-active compounds is due to their radical scavenging effects. The DPPH assay is one of the most widely employed and preferred methods for measuring the radical scavenging activity of samples. DPPH is a stable nitrogen-centered free radical that produces a violet color in methanol solution. When DPPH radicals react with suitable reducing agent as antioxidants, the solution loses its color depending on the number of electrons absorbed (Umamaheswari and Chatterjee. 2007). The DPPH radical scavenging activities of the meat samples were shown in Table 4.26. The meat samples containing PWCE at the concentration 2.5 g PWCE/kg meat (raw sample: 57.56%, cooked sample: 61.15%) and 5 g PWCE/kg meat (raw sample: 71.66%, cooked sample: 78.20%) crude extracts had significantly ($P<0.05$) higher DPPH scavenging activity than the control (raw sample: 16.33%, cooked sample: 22.16%) and 0.2 g PWCE/kg meat (raw sample: 27.20%, cooked sample: 38.72%). This result could be explained by the increasing phenolic and flavonoid compounds in the meat samples. Bakoush *et al.* (2015) demonstrated that flavonoids are highly scavengers of most oxidizing molecules, including singlet oxygen and various other free radicals implicated in several diseases. Flavonoids suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses (Baba and Malik. 2014). Similarly, phenolics confer oxidative stress tolerance on plants. Moreover, following cooking of meat, the overall DPPH scavenging activity of the samples increased significantly ($P<0.05$). This finding was similar to that found by Fasseas *et al.* (2008), who reported that the DPPH scavenging activity of meat substitute was enhanced with increasing heating temperature and time. This could be due to reactions between reducing sugars and free amino acids or free amino groups in proteins - the Maillard reaction. As in previous studies, the Maillard reaction products have antioxidant activity by scavenging oxygen radicals or chelating metals (Baba and Malik. 2014).

Radical cation decolorization (ABTS)

The ABTS radical cation decolorization of the meat samples was shown in Table 4.27. The meat samples containing water extract from *P. acidus* leaves at the concentration of 2.5 g PWCE/kg meat (raw sample: 68.24%, cooked sample: 73.52%), and 5 g PWCE/kg meat (raw sample: 85.39%, cooked sample: 88.41%), crude extracts had significantly ($P<0.05$) higher DPPH scavenging activity than the control (raw sample: 36.75%, cooked sample: 38.58%) and 0.2 g BHT/kg meat (raw sample: 59.44%, cooked sample: 65.13%). This finding also could be explained as the DPPH scavenging activity phenomenon causing the difference of phenolic and flavonoid compounds in the meat samples (Huang *et al.* 2011). Radical scavenging was also affected over time in all untreated meat samples, perhaps because oxidation of the meat leads to the creation of compounds that scavenge free radicals. Moreover, following cooking of the meat, the overall ABTS radical cation decolorization activity of the samples increased significantly ($P<0.05$). This finding could be due to reactions between reducing sugars and free amino acids or free amino groups in proteins, the Maillard reaction.

Reducing power ability

The reducing power ability was expressed as the absorbance at 700 nm in Table 4.28. The results showed that the meat samples treated with water extract from *P. acidus* leaves at concentration of 2.5 g/kg meat (raw sample: 0.57, cooked sample: 0.72), 5 g/kg meat (raw sample: 0.77, cooked sample: 0.84) significantly ($P<0.05$) increased reducing power (higher absorbance) compared to the control (raw sample: 0.32, cooked sample: 0.42) and 0.2 g BHT/kg meat (raw sample: 0.47, cooked sample: 0.60) in both raw and cooked samples. This finding could be explained by the presence of reductants (antioxidants) in meat causes the reduction of Fe^{3+}/Ferric cyanide complex to the ferrous form. Therefore, the Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Beyhan *et al.* 2010). *P. acidus* extract treatment gave a significantly higher absorbance than the control and BHT treatments due to the high polyphenolic content of the *P. acidus* extract.

Thiobarbituric acid reaction substances (TBARS)

Thiobarbituric acid reaction substances (TBARS) method has been widely used to determine the degree of lipid oxidation through malondialdehyde compound, which results from lipid peroxidation of polyunsaturated fatty acids. Table 4.29 presented the influence of adding PWCE on the lipid oxidation of raw and cooked ground pork. The results revealed that total malondialdehyde content was total MDA content was lower in raw samples of the addition of

PWCE when compared to control and BHT treatments (control: 2.89, 0.2 g BHT/kg: 2.71, 2.5g/kg meat: 2.68 and 5 g/kg meat: 2.76 mg MDA/kg meat sample). This result could be explained by pre-oxidation in the PWCE which affected to total MDA in raw samples. Whereas, in cooked meat samples, total malondialdehyde content in the control (13.03 mg MDA/kg meat) and 0.2 g BHT/kg (6.69 mg MDA/kg meat) was higher than samples contained *P. acidus* extracts (2.5 g PWCE/kg: 4.61, 5 g PWCE/kg: 2.95 mg MDA/ kg meat). These findings were in agreement with other studies that have investigated the effects of plant extract on lipid peroxidation in meat (Fasseas *et al.* 2008; Samouris *et al.* 2007). The results of the present study showed that adding phenolic-rich extracts protected ground pork samples against lipid peroxidation. Other previous studies have reported on the relationship between phenolic content and antioxidant activity (Maisarah *et al.* 2013). The phenolic compounds are associated with the hydroxyl group linked to the aromatic ring, which is capable of donating hydrogen atoms with electrons and neutralizing free radicals. This mechanism blocks further degradation to more active oxidizing forms, such as MDA. Therefore, the strong *in vitro* antioxidant activity shown by *P. acidus* extracts also had a protective role in real meat products.

Moreover, heat treatment of meat revealed significant differences between raw and corresponding cooked samples with much higher malondialdehyde values were noted for the cooked meat samples than for the corresponding raw ones. These findings could be explained by the production of pre-oxidized myoglobin that is susceptible to further oxidation (Fasseas *et al.* 2008) or by cooking's decreased ferric ion reducing capacity, but increases non-heme iron of meat, resulting in comparatively lower ferric ion reducing capacity in cooked than raw meat. Therefore, heat stable ferric ion capacity may be primarily responsible for the regeneration of ferrous ion to increase total malondialdehyde in cooked meat.

Table 4.26 Effect of water crude extract from *P. acidus* leaves on DPPH radical scavenging activity ground pork at -20°C

Storage time (weeks)		% Inhibition of DPPH radical scavenging activity			
		Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
Before cooking	0	$16.33 \pm 0.15^{d,B}$	$27.20 \pm 0.19^{c,A}$	$57.56 \pm 0.08^{b,A}$	$71.66 \pm 0.01^{a,A}$
	4	$16.99 \pm 0.24^{d,A}$	$27.70 \pm 0.43^{c,A}$	$57.66 \pm 0.77^{b,A}$	$71.26 \pm 0.22^{a,A}$
	8	$17.05 \pm 0.11^{d,A}$	$26.71 \pm 0.22^{c,A}$	$56.35 \pm 0.42^{b,A}$	$70.32 \pm 0.13^{a,AB}$
	12	$16.67 \pm 0.02^{d,AB}$	$26.52 \pm 0.22^{c,A}$	$56.06 \pm 0.84^{b,A}$	$69.11 \pm 0.91^{a,B}$
After cooking	0	$22.16 \pm 0.06^{d,A}$	$38.72 \pm 0.09^{c,A}$	$61.15 \pm 0.03^{b,A}$	$78.20 \pm 0.08^{a,A}$
	4	$22.16 \pm 0.15^{d,A}$	$38.02 \pm 0.11^{c,AB}$	$61.25 \pm 0.13^{b,A}$	$78.22 \pm 0.11^{a,A}$
	8	$22.00 \pm 0.22^{d,A}$	$37.12 \pm 0.72^{c,B}$	$60.45 \pm 0.13^{b,B}$	$77.23 \pm 0.11^{a,B}$
	12	$21.06 \pm 0.41^{d,B}$	$37.21 \pm 0.03^{c,B}$	$60.05 \pm 0.23^{b,C}$	$77.37 \pm 0.13^{a,B}$

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-C}$ Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.27 Effect of water crude extract from *P. acidus* leaves on ABTS radical cation decolorization in ground pork at -20°C

Storage time (weeks)		% Inhibition of ABTS radical cation decolorization			
		Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
Before cooking	0	$36.75 \pm 0.07^{d,A}$	$59.44 \pm 0.02^{c,A}$	$68.24 \pm 0.01^{b,A}$	$85.39 \pm 0.36^{a,A}$
	4	$36.25 \pm 0.22^{d,A}$	$58.84 \pm 0.23^{c,B}$	$68.04 \pm 0.25^{b,AB}$	$85.92 \pm 0.16^{a,A}$
	8	$36.25 \pm 0.41^{d,A}$	$58.94 \pm 0.27^{c,B}$	$67.24 \pm 0.17^{b,C}$	$84.55 \pm 0.05^{a,B}$
	12	$35.01 \pm 0.22^{d,B}$	$58.99 \pm 0.11^{c,B}$	$67.74 \pm 0.02^{b,B}$	$84.11 \pm 0.31^{a,B}$
After cooking	0	$38.58 \pm 0.12^{d,A}$	$65.13 \pm 0.01^{c,B}$	$73.52 \pm 0.18^{b,A}$	$88.41 \pm 0.01^{a,A}$
	4	$38.77 \pm 0.02^{d,A}$	$65.65 \pm 0.21^{c,A}$	$72.09 \pm 0.05^{b,B}$	$87.08 \pm 0.21^{a,B}$
	8	$37.97 \pm 0.16^{d,B}$	$64.77 \pm 0.16^{c,B}$	$71.72 \pm 0.41^{b,B}$	$87.20 \pm 0.35^{a,B}$
	12	$38.02 \pm 0.09^{d,B}$	$64.05 \pm 0.08^{c,C}$	$71.92 \pm 0.73^{b,B}$	$87.09 \pm 0.11^{a,B}$

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-C}$ Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.28 Effect of water crude extract from *P. acidus* leaves on reducing power in ground pork at -20°C

Storage time (weeks)		Absorbance at 700 nm			
		Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
Before cooking	0	0.32 ± 0.17 ^{a,A}	0.47 ± 0.12 ^{a,A}	0.57 ± 0.04 ^{a,A}	0.77 ± 0.36 ^{a,A}
	4	0.30 ± 0.45 ^{a,A}	0.46 ± 0.38 ^{a,A}	0.55 ± 1.07 ^{a,A}	0.77 ± 0.22 ^{a,A}
	8	0.27 ± 0.40 ^{a,A}	0.44 ± 0.58 ^{a,A}	0.55 ± 0.23 ^{a,A}	0.72 ± 0.22 ^{a,A}
	12	0.25 ± 0.22 ^{a,A}	0.38 ± 0.22 ^{a,A}	0.48 ± 0.11 ^{a,A}	0.65 ± 1.12 ^{a,A}
After cooking	0	0.42 ± 0.09 ^{a,A}	0.60 ± 0.31 ^{a,A}	0.72 ± 0.17 ^{a,A}	0.84 ± 0.32 ^{a,A}
	4	0.44 ± 1.12 ^{a,A}	0.62 ± 0.22 ^{a,A}	0.70 ± 0.11 ^{a,A}	0.83 ± 0.22 ^{a,A}
	8	0.40 ± 0.22 ^{a,A}	0.58 ± 0.18 ^{a,A}	0.65 ± 0.28 ^{a,A}	0.80 ± 1.18 ^{a,A}
	12	0.32 ± 0.77 ^{a,A}	0.47 ± 0.82 ^{a,A}	0.57 ± 0.04 ^{a,A}	0.77 ± 0.36 ^{a,A}

^{a} Means sharing different letters in the same row are significantly different ($P<0.05$).

^{A} Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.29 Effect of water crude extract from *P. acidus* leaves on total MDA in ground pork at -20°C

Storage time		Total MDA (mg/kg meat)			
(weeks)		Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
Before cooking	0	$2.89 \pm 0.17^{a,A}$	$2.71 \pm 0.01^{a,A}$	$2.68 \pm 0.24^{a,A}$	$2.76 \pm 0.44^{a,A}$
	4	$2.87 \pm 0.04^{a,A}$	$2.72 \pm 0.04^{ab,A}$	$2.68 \pm 0.11^{b,A}$	$2.77 \pm 0.02^{ab,A}$
	8	$2.95 \pm 0.07^{a,A}$	$2.74 \pm 0.07^{b,A}$	$2.69 \pm 0.07^{b,A}$	$2.77 \pm 0.08^{ab,A}$
	12	$2.97 \pm 0.04^{a,A}$	$2.74 \pm 0.09^{a,A}$	$2.70 \pm 0.08^{a,A}$	$2.77 \pm 0.18^{a,A}$
After cooking	0	$13.03 \pm 0.39^{a,A}$	$6.69 \pm 0.13^{b,A}$	$4.61 \pm 0.14^{c,A}$	$2.95 \pm 0.14^{d,A}$
	4	$13.08 \pm 0.05^{a,A}$	$6.69 \pm 0.51^{b,A}$	$4.62 \pm 0.71^{c,A}$	$2.99 \pm 0.73^{d,A}$
	8	$13.11 \pm 0.09^{a,A}$	$6.71 \pm 0.23^{b,A}$	$4.75 \pm 0.12^{c,A}$	$3.01 \pm 0.25^{d,A}$
	12	$13.15 \pm 0.47^{a,A}$	$6.74 \pm 0.73^{b,A}$	$4.79 \pm 0.07^{c,A}$	$3.05 \pm 0.14^{d,A}$

[a] Means sharing different letters in the same row are significantly different ($P<0.05$).

[A] Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Physical analysis

pH: Table 4.30 shows the effect of water extract from *P. acidus* leaves on the pH of ground pork samples during refrigerated storage at -20°C for 12 weeks. The initial pH value of the control and samples submitted to various treatments had no significant difference during the storage time up to 12 weeks. The pH values of the meat samples was 6.08 (control), 6.03 (0.2 g BHT/kg), 5.98 (2.5 g PWCE/kg) and 6.03 (5 g PWCE/kg) at 0 day and was not significant difference until the end of the storage ($P<0.05$). The final pH value of the stored products was similar to their initial pH value can be explained by the similar total number of microorganisms between storage days from 0 to 12 weeks at -20°C (Jolosinska and Wilczak. 2009). This finding was in agreement with the study of Jalosinska and Wilczak. (2009), who reported that in the sample with an addition of plant extract, pH level was the most stable for storage time at -20°C to fall to the value of 5.91 at the end storage. In the course of this study, during the entire storage cycle, major changes in pH values were not observed, and at the end of the storage, the pH of raw ground pork samples were unaffected by the addition of PWCE.

Table 4.30 Effect of water extract from *P. acidus* leaves on pH in ground pork during storage at -20°C

Storage time (weeks)	pH values			
	Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
0	$6.08 \pm 0.41^{a,A}$	$6.03 \pm 0.72^{a,A}$	$5.98 \pm 0.47^{a,A}$	$6.03 \pm 0.12^{a,A}$
4	$6.06 \pm 0.21^{a,A}$	$5.99 \pm 0.42^{a,A}$	$6.03 \pm 0.55^{a,A}$	$5.99 \pm 0.07^{a,A}$
8	$6.10 \pm 0.44^{a,A}$	$6.09 \pm 0.66^{a,A}$	$6.04 \pm 0.37^{a,A}$	$6.07 \pm 0.75^{a,A}$
12	$6.08 \pm 0.81^{a,A}$	$6.02 \pm 0.97^{a,A}$	$6.03 \pm 0.27^{a,A}$	$6.07 \pm 0.73^{a,A}$

[a] Means sharing different letters in the same row are significantly different ($P<0.05$).

[A] Means sharing different letters in the same column are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Biological analysis

The changes in TPC, Y/M, psychophilic bacteria, coliform bacteria of ground pork samples with or without PWCE during refrigerated storage at 4°C over 8 days are shown in Table 4.18. The addition PWCE resulted no significant difference to the control and BHT treatments in total plate count, yeasts/molds, psychrophilic bacteria and coliform bacteria during the storage time up to 8 days at 4°C ($P<0.05$). The total plate count of meat samples was initially approximately 5.17, 5.03, 5.02, 4.94 log CFU/g meat, which increased steadily with storage time and reached close to 7.51, 7.50, 7.45, 7.34 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g PWCE/kg and 5 g PWCE/kg, respectively, at the end of the storage. The Y/M of meat samples was initially approximately 4.02, 3.99, 3.94, 3.92 log CFU/g meat, which increased steadily with storage time and reached close to 5.21, 5.16, 5.16, 5.14 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g PWCE and 5 g PWCE/kg, respectively, at the end of the storage. The psychrophilic bacteria of meat samples was initially approximately 4.17, 4.24, 4.14, 4.22 log CFU/g meat, which increased steadily with storage time and reached close to 8.18, 8.13, 8.04, 8.04 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g PWCE/kg and 5 g PWCE/kg, respectively, at the end of the storage. The coliforms of meat samples was initially approximately 4.28, 4.25, 4.13, 4.30 log CFU/g meat, which increased steadily with storage time and reached close to 5.23, 5.27, 5.22, 5.25 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g PWCE/kg and 5 g PWCE/kg, respectively, at the end of the storage. Therefore, the addition of CE did not significantly affect biological analysis ($P<0.05$). This results can be explained by although PWCE contained high bioactive compounds but it did not act as an antimicrobial (Jain and Singhai. 2011; Sawhney *et al.* 2011).

Table 4.31 Effect of water extract from *P. acidus* leaves on biological quality in ground pork during storage at -20°C

Storage time (weeks)		Log 10 CFU/g			
		Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
Total plate count	0	5.26 ± 0.03 ^{a,B}	5.28 ± 0.24 ^{a,B}	5.30 ± 0.30 ^{a,B}	5.28 ± 0.31 ^{a,B}
	4	5.31 ± 0.05 ^{a,B}	5.37 ± 0.09 ^{a,B}	5.35 ± 0.10 ^{a,AB}	5.35 ± 0.05 ^{a,B}
	8	5.84 ± 0.28 ^{a,A}	5.86 ± 0.34 ^{a,AB}	5.86 ± 0.29 ^{a,AB}	5.87 ± 0.04 ^{a,AB}
	12	5.96 ± 0.13 ^{a,A}	5.97 ± 0.16 ^{a,A}	5.97 ± 0.20 ^{a,A}	5.97 ± 0.33 ^{a,A}
Yeasts/Molds	0	3.80 ± 0.29 ^{a,A}	3.79 ± 0.26 ^{a,A}	3.82 ± 0.09 ^{a,A}	3.78 ± 0.35 ^{a,A}
	4	3.79 ± 0.26 ^{a,A}	3.78 ± 0.25 ^{a,A}	3.75 ± 0.04 ^{a,A}	3.77 ± 0.28 ^{a,A}
	8	3.81 ± 0.28 ^{a,A}	3.83 ± 0.07 ^{a,A}	3.82 ± 0.07 ^{a,A}	3.88 ± 0.24 ^{a,A}
	12	4.08 ± 0.22 ^{a,A}	4.08 ± 0.12 ^{a,A}	4.08 ± 0.13 ^{a,A}	4.06 ± 0.10 ^{a,A}
Psychophilic bacteria	0	5.86 ± 0.13 ^{a,B}	5.86 ± 0.14 ^{a,A}	5.85 ± 0.21 ^{a,A}	5.88 ± 0.06 ^{a,B}
	4	5.94 ± 0.09 ^{a,AB}	5.96 ± 0.18 ^{a,A}	5.96 ± 0.13 ^{a,A}	5.90 ± 0.16 ^{a,B}
	8	6.07 ± 0.17 ^{a,AB}	6.06 ± 0.19 ^{a,A}	6.08 ± 0.22 ^{a,A}	6.09 ± 0.11 ^{a,A}
	12	6.20 ± 0.02 ^{a,A}	6.20 ± 0.18 ^{a,A}	6.23 ± 0.18 ^{a,A}	6.24 ± 0.06 ^{a,A}
Coliforms	0	3.90 ± 0.02 ^{a,A}	3.91 ± 0.22 ^{a,B}	3.91 ± 0.08 ^{a,B}	3.88 ± 0.20 ^{a,B}
	4	3.97 ± 0.40 ^{a,A}	3.97 ± 0.32 ^{a,AB}	3.96 ± 0.12 ^{a,AB}	3.96 ± 0.10 ^{a,B}
	8	4.01 ± 0.09 ^{a,A}	4.05 ± 0.07 ^{a,AB}	4.01 ± 0.53 ^{a,AB}	3.90 ± 0.07 ^{a,B}
	12	4.58 ± 0.10 ^{a,A}	4.57 ± 0.08 ^{a,A}	4.56 ± 0.44 ^{a,A}	4.58 ± 0.09 ^{a,A}

$^{a-b}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-B}$ Means sharing different letters in the same column in each parameter are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

(2) Evaluation of the effect of optimal crude extract from *C. carandas* fruits on chemical, physical and biological quality in ground pork storaged at -20°C up to 12 weeks

Chemical analysis

Free scavenging radical activity (DPPH)

DPPH Radical scavenging activity: DPPH radical scavenging activity was analyzed in each sample in terms of inhibition ability of a preformed free radical by antioxidants. There was significant variation in the radical scavenging activity of the addition of CWCE in ground pork in Table 4.32. The meat samples containing 2.5 g CWCE/kg meat (raw sample: 38.06%, cooked sample: 43.45%) and 5 g CWCE/kg meat (raw sample: 58.40%, cooked sample: 65.25%) were significantly ($P<0.05$) higher DPPH scavenging activity than the control (raw sample: 18.98%, cooked sample: 22.11%) and 0.2 g BHT/kg meat (raw sample: 29.43%, cooked sample: 36.10%). This result could be explained by the increasing phenolic and flavonoid compounds in the meat samples. Bakoush *et al.* (2015) demonstrated that flavonoids are highly scavengers of most oxidizing molecules, including singlet oxygen and various other free radicals implicated in several diseases. Flavonoids suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses (Baba and Malik. 2014). Similarly, phenolics confer oxidative stress tolerance on plants. Moreover, following cooking of meat, the overall DPPH scavenging activity of the samples increased significantly ($P<0.05$). This finding was similar to that found by Fasseas *et al.* (2008), who reported that the DPPH scavenging activity of meat substitute was enhanced with increasing heating temperature and time. This could be due to reactions between reducing sugars and free amino acids or free amino groups in proteins - the Maillard reaction. As in previous studies, the Maillard reaction products have antioxidant activity by scavenging oxygen radicals or chelating metals (Baba and Malik. 2014).

Radical cation decolorization (ABTS)

The ABTS radical cation decolorization of the meat samples are shown in Table 4.33. The meat samples containing 2.5 g CWCE/kg meat (raw sample: 67.34%, cooked sample: 71.74%), and 5 g CWCE/kg meat (raw sample: 74.11%, cooked sample: 78.86%), crude extracts had significantly ($P<0.05$) higher DPPH scavenging activity than the control (raw sample: 36.71%, cooked sample: 39.17%) and 0.2 g BHT/kg meat (raw sample: 65.11%, cooked sample: 69.35%). This finding also could be explained as the DPPH scavenging activity phenomenon cause the difference of phenolic and flavonoid compounds in the meat samples (Huang *et al.*

2011). Radical scavenging was also affected over time in all untreated meat samples, perhaps because oxidation of the meat leads to the creation of compounds that scavenge free radicals. Moreover, following cooking of the meat, the overall ABTS radical cation decolorization activity of the samples increased significantly ($P<0.05$). This finding could be due to reactions between reducing sugars and free amino acids or free amino groups in proteins, the Maillard reaction.

Reducing power ability

The reducing power ability was expressed as the absorbance at 700 nm in Table 4.34. The results showed that the meat samples treated 2.5 g CWCE/kg meat (raw sample: 0.60, cooked sample: 0.75), 5 g CWCE/kg meat (raw sample: 0.74, cooked sample: 0.86) significantly ($P<0.05$) increased reducing power (higher absorbance) compared to the control (raw sample: 0.38, cooked sample: 0.45) and 0.2 g BHT/kg meat (raw sample: 0.52, cooked sample: 0.58) during the storage at -20°C over 12 weeks. This finding could be explained by the presence of reductants (antioxidants) in meat causes the reduction of Fe^{3+}/Ferric cyanide complex to the ferrous form. Therefore, the Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Beyhan *et al.* 2010). CWCE treatment gave a significantly higher absorbance than the control and BHT treatments due to the high polyphenolic content of CWCE.

Thiobarbituric acid reaction substances (TBARS)

Thiobarbituric acid reaction substances (TBARS) method has been widely used to determine the degree of lipid oxidation through malondialdehyde compound, which results from lipid peroxidation of polyunsaturated fatty acids. Table 4.35 represented the influence of adding CWCE on the lipid oxidation of raw and cooked ground pork. The results revealed that MDA content was similar for raw samples (control: 3.05, 0.2 g BHT/kg: 2.99, 2.5g CWCE/kg meat: 3.05 and 5 g CWCE/kg meat: 3.04 mg MDA/kg meat sample). Whereas, in cooked meat samples, total MDA in the control (13.75 mg MDA/kg meat) and 0.2 g BHT/kg (5.54 mg MDA/kg meat) were higher than samples contained CWCE (2.5 g CWCE/kg: 6.75, 5 g CWCE/kg: 6.65 mg MDA/kg meat). This is generally in agreement with other research studies that have investigated the effects of plant extract on lipid peroxidation in meat (Fasseas *et al.* 2008; Samouris *et al.* 2007). In both raw and cooked samples, the addition of CWCE in meat was lower total MDA content than control treatments ($p<0.05$). The results of the present study show that adding phenolic-rich extracts protects ground pork samples against lipid peroxidation. Other previous studies have reported on the relationship between phenolic content and antioxidant activity (Maisarah *et al.* 2013). The phenolic compounds are associated with the hydroxyl group linked to

the aromatic ring, which is capable of donating hydrogen atoms with electrons and neutralizing free radicals. This mechanism blocks further degradation to more active oxidizing forms, such as MDA. Therefore, the strong *in vitro* antioxidant activity shown by CWCE also had a protective role in real meat products. Moreover, heat treatment of meat revealed significant differences between raw and corresponding cooked samples with much higher malondialdehyde values were noted for the cooked meat samples than for the corresponding raw ones. These findings could be explained by the production of pre-oxidized myoglobin that is susceptible to further oxidation (Fasseas *et al.* 2008) or by cooking's decreased ferric ion reducing capacity, but increases non-heme iron of meat, resulting in comparatively lower ferric ion reducing capacity in cooked than raw meat.

Table 4.32 Effect of water crude extract from *C. carandas* fruits on DPPH radical scavenging activity ground pork at -20°C

Storage time (weeks)		% Inhibition of DPPH radical scavenging activity			
		Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Before cooking	0	$18.98 \pm 0.18^{d,A}$	$29.43 \pm 0.03^{c,A}$	$38.06 \pm 0.21^{b,A}$	$58.40 \pm 0.10^{a,A}$
	4	$18.99 \pm 0.04^{d,A}$	$29.70 \pm 0.08^{c,A}$	$38.06 \pm 0.07^{b,A}$	$58.22 \pm 0.91^{a,A}$
	8	$18.75 \pm 0.14^{d,A}$	$29.81 \pm 0.01^{c,A}$	$38.05 \pm 0.12^{b,A}$	$58.22 \pm 0.03^{a,A}$
	12	$18.07 \pm 0.42^{d,B}$	$28.92 \pm 0.22^{c,A}$	$37.86 \pm 0.04^{b,A}$	$58.21 \pm 0.11^{a,A}$
After cooking	0	$22.11 \pm 0.01^{d,A}$	$36.10 \pm 0.02^{c,B}$	$43.45 \pm 0.03^{b,A}$	$65.25 \pm 0.74^{a,A}$
	4	$22.23 \pm 0.05^{d,A}$	$36.02 \pm 0.21^{c,B}$	$43.25 \pm 0.33^{b,A}$	$65.22 \pm 0.01^{a,A}$
	8	$22.01 \pm 0.71^{d,A}$	$36.42 \pm 0.08^{c,A}$	$43.15 \pm 0.13^{b,A}$	$65.23 \pm 0.21^{a,A}$
	12	$21.94 \pm 0.07^{d,A}$	$35.95 \pm 0.83^{c,B}$	$43.05 \pm 0.05^{b,A}$	$64.37 \pm 0.07^{a,A}$

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-B}$ Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.33 Effect of water crude extract from *C. carandas* fruits on ABTS radical cation decolorization in ground pork at -20°C

Storage time		% Inhibition of ABTS radical cation decolorization			
(weeks)		Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Before cooking	0	$36.71 \pm 0.70^{d,A}$	$65.11 \pm 0.82^{c,A}$	$67.34 \pm 0.06^{b,A}$	$74.11 \pm 0.04^{a,A}$
	4	$36.75 \pm 0.52^{d,A}$	$66.15 \pm 0.12^{c,A}$	$68.04 \pm 0.45^{b,A}$	$73.98 \pm 0.31^{a,A}$
	8	$35.95 \pm 0.21^{d,A}$	$66.27 \pm 0.11^{c,A}$	$67.28 \pm 0.11^{b,A}$	$74.20 \pm 0.03^{a,A}$
	12	$36.81 \pm 0.24^{d,A}$	$65.25 \pm 0.57^{c,A}$	$68.09 \pm 0.02^{b,A}$	$74.31 \pm 0.21^{a,A}$
After cooking	0	$39.17 \pm 0.84^{d,A}$	$69.35 \pm 0.72^{c,A}$	$71.74 \pm 0.75^{b,A}$	$78.86 \pm 0.03^{a,A}$
	4	$39.67 \pm 0.23^{d,A}$	$70.04 \pm 0.03^{c,A}$	$72.89 \pm 0.07^{b,A}$	$77.92 \pm 0.12^{a,A}$
	8	$38.87 \pm 0.06^{d,A}$	$69.94 \pm 0.07^{c,A}$	$72.75 \pm 0.42^{b,A}$	$77.95 \pm 0.05^{a,A}$
	12	$39.06 \pm 0.03^{d,A}$	$69.87 \pm 0.21^{c,A}$	$72.02 \pm 1.11^{b,A}$	$77.61 \pm 0.51^{a,A}$

[a-d] Means sharing different letters in the same row are significantly different ($P<0.05$).

[A] Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.34 Effect of water crude extract from *C. carandas* fruits on reducing power in ground pork at -20°C

Storage time (weeks)		Absorbance at 700 nm			
		Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Before cooking	0	$0.38 \pm 0.85^{a,A}$	$0.52 \pm 0.20^{a,A}$	$0.60 \pm 0.70^{a,A}$	$0.74 \pm 0.20^{a,A}$
	4	$0.37 \pm 0.09^{a,A}$	$0.51 \pm 0.10^{a,A}$	$0.61 \pm 0.25^{a,A}$	$0.70 \pm 0.95^{a,A}$
	8	$0.37 \pm 1.25^{a,A}$	$0.50 \pm 0.40^{a,A}$	$0.57 \pm 0.18^{a,A}$	$0.69 \pm 0.16^{a,A}$
	12	$0.36 \pm 0.14^{a,A}$	$0.48 \pm 0.75^{a,A}$	$0.55 \pm 0.15^{a,A}$	$0.68 \pm 0.96^{a,A}$
After cooking	0	$0.45 \pm 0.30^{a,A}$	$0.58 \pm 0.15^{a,A}$	$0.75 \pm 0.05^{a,A}$	$0.86 \pm 0.28^{a,A}$
	4	$0.44 \pm 0.22^{a,A}$	$0.56 \pm 0.78^{a,A'}$	$0.74 \pm 0.24^{a,A}$	$0.83 \pm 0.30^{a,A}$
	8	$0.42 \pm 0.78^{a,A}$	$0.55 \pm 0.85^{a,A}$	$0.71 \pm 0.17^{a,A}$	$0.81 \pm 0.84^{a,A}$
	12	$0.40 \pm 0.08^{a,A}$	$0.52 \pm 0.28^{a,A}$	$0.68 \pm 0.18^{a,A}$	$0.78 \pm 0.77^{a,A}$

[a] Means sharing different letters in the same row are significantly different ($P<0.05$).

[A] Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.35 Effect of water crude extract from *C. carandas* fruits on total MDA in ground pork at -20°C

Storage time		Total MDA (mg/kg meat)			
(weeks)		Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Before cooking	0	$3.05 \pm 0.71^{a,A}$	$2.99 \pm 0.11^{a,A}$	$3.05 \pm 0.03^{a,A}$	$3.04 \pm 0.81^{a,A}$
	4	$2.87 \pm 0.84^{a,A}$	$2.22 \pm 0.84^{a,A}$	$2.68 \pm 0.11^{a,A}$	$2.67 \pm 0.32^{a,A}$
	8	$2.95 \pm 0.07^{a,A}$	$2.24 \pm 0.77^{a,A}$	$2.69 \pm 0.37^{a,A}$	$2.67 \pm 0.08^{a,A}$
	12	$2.97 \pm 0.04^{a,A}$	$2.24 \pm 0.09^{b,A}$	$2.70 \pm 0.08^{a,A}$	$2.69 \pm 0.18^{a,A}$
After cooking	0	$13.75 \pm 0.05^{a,A}$	$5.54 \pm 0.10^{c,BC}$	$6.75 \pm 0.21^{b,A}$	$6.65 \pm 0.04^{b,A}$
	4	$13.18 \pm 0.41^{a,B}$	$5.70 \pm 0.02^{c,AB}$	$6.99 \pm 0.07^{b,A}$	$6.55 \pm 0.73^{bc,A}$
	8	$13.21 \pm 0.08^{a,AB}$	$5.52 \pm 0.02^{d,C}$	$7.01 \pm 0.04^{b,A}$	$6.61 \pm 0.02^{c,A}$
	12	$13.18 \pm 0.06^{a,B}$	$5.80 \pm 0.09^{d,A}$	$7.03 \pm 0.03^{b,A}$	$6.69 \pm 0.08^{c,A}$

$^{a-d}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

$^{A-C}$ Means sharing different letters in the same column in each group are significantly different ($P<0.05$).

Physical analysis

pH

The effect of CWCE on pH values in ground pork during storage at -20°C over 12 weeks storage period throughout ripening were summarized in Table 4.36. The results demonstrated that the pH of raw ground pork samples were unaffected by the addition of CWCE during the storage time. At the beginning, pH amounted to 6.01 for the control, 6.07 for the sample with BHT treatment, 6.03 for the product with 2.5 g CWCE/kg and 5.97 for the 5 g CWCE/kg. At the end of storage it reached the value of 6.09 for the sample with control, 6.01 for the product with BHT treatment, and 6.05 for the product with 2.5 g and 5 g CWCE/kg. The final pH value of the stored products was similar to their initial pH value can be explained by the similar total number of microorganisms between storage days from 0 to 12 weeks at -20°C (Jalosinska and Wilczak. 2009). This finding was in agreement with the study of Jalosinska and Wilczak (2009), who reported that In the sample with an addition of plant extract, pH level was the most stable for 12 days of storage to fall to the value of 5.91 at the end thereof. In the course of this study, during the entire storage cycle, major changes in pH values were not observed, and at the end of the storage, the pH of raw ground pork samples were unaffected by the addition of CWCE. It might have resulted from the influence of the addition of CWCE or low temperature condition on directions of metabolic transformations of microorganisms and enzymes in the ground pork samples.

Table 4.36 Effect of water extract from *C. carandas* fruits on pH in ground pork during storage at -20°C

Storage time	pH values			
(weeks)	Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
0	6.01 ± 0.72 ^{a,A}	6.07 ± 0.77 ^{a,A}	6.03 ± 0.71 ^{a,A}	5.97 ± 0.24 ^{a,A}
2	6.01 ± 0.25 ^{a,A}	5.97 ± 0.24 ^{a,A}	6.01 ± 0.24 ^{a,A}	5.99 ± 1.01 ^{a,A}
4	5.99 ± 0.02 ^{a,A}	6.05 ± 0.24 ^{a,A}	6.01 ± 0.41 ^{a,A}	6.01 ± 0.10 ^{a,A}
8	6.09 ± 1.08 ^{a,A}	6.01 ± 0.31 ^{a,A}	6.05 ± 1.09 ^{a,A}	6.05 ± 0.91 ^{a,A}

^{a} Means sharing different letters in the same row are significantly different ($P<0.05$).

^{A} Means sharing different letters in the same column are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Biological analysis

The changes in TPC, Y/M, psychophilic bacteria, coliform of ground pork samples with or without CWCE during refrigerated storage at -20°C over 12 weeks are shown in Table 4.37. The addition CWCE resulted in a reduction in the growth of TPC and coliform, but it was no effect on Y/M and psychophilic bacteria to the control and BHT treatments during the storage time up to 12 weeks at -20°C ($P<0.05$). The total plate count of meat samples was initially approximately 4.53, 4.56, 4.51, 4.50 log CFU/g meat, which increased significantly with storage time and reached close to 5.96, 5.84, 5.16, 5.08 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g CWCE and 5 g CWCE/kg, respectively, at the end of the storage. However, the addition of 0.25 and 5 g CWCE/kg meat inhibited the development of TPC better than the control and BHT treatment at 4, 8 and 12 weeks ($P<0.05$). The Y/M of meat samples was initially approximately 3.81, 3.80, 3.80, 3.79 log CFU/g meat, which increased with storage time and reached close to 4.15, 4.12, 4.08, 4.06 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g CWCE and 5 g CWCE/kg, respectively, at the end of the storage. The psychrophilic bacteria of meat samples was initially approximately 4.19, 4.15, 4.12, 4.10 log CFU/g meat, which increased steadily with storage time and reached close to 5.82, 5.80, 5.63, 5.60 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g CWCE and 5 g CWCE/kg, respectively, at the end of the storage. The coliforms of meat samples was initially approximately 3.27, 3.28, 3.23, 3.21log CFU/g meat, which increased steadily with storage time and reached close to 4.86, 4.87, 4.65, 4.57 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g CWCE and 5 g CWCE/kg, respectively, at the end of the storage. However, the addition of 0.25 and 5 g CWCE/kg meat inhibited the development of coliforms better than the control and BHT treatment at 8 and 12 weeks. Therefore, the addition of CWCE was positive to significantly affect biological analysis ($P<0.05$). The potentially antimicrobial mechanisms of phenolic compounds include the interruption of function of bacterial cell membranes. The -OH groups in phenolic compounds are highly reactive under aqueous conditions and react with several biomolecules, causing deformation of these molecules, which results in retardation of growth and bacterial growth. Phenolic compounds are also involved in protein and cell wall binding, inactivation of bacterial enzymes, and intercalation into the bacterial DNA during replication (Fullerton *et al.* 2011).

Table 4.37 Effect of water extract from *C. carandas* fruits on biological quality in ground pork during storage at -20°C

Storage time (weeks)		Log 10 CFU/g			
		Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Total plate count	0	$4.53 \pm 0.42^{a,B}$	$4.56 \pm 0.42^{a,B}$	$4.51 \pm 0.54^{a,C}$	$4.50 \pm 0.36^{a,B}$
	4	$4.95 \pm 0.15^{a,B}$	$4.95 \pm 0.52^{a,B}$	$4.47 \pm 0.56^{b,C}$	$4.46 \pm 0.29^{b,B}$
	8	$5.36 \pm 0.05^{a,A}$	$5.37 \pm 0.09^{a,A}$	$4.77 \pm 0.19^{b,B}$	$4.93 \pm 0.19^{b,A}$
	12	$5.96 \pm 0.12^{a,A}$	$5.84 \pm 0.19^{a,A}$	$5.16 \pm 0.18^{b,A}$	$5.08 \pm 0.18^{b,A}$
Yeasts/Molds	0	$3.81 \pm 0.23^{a,B}$	$3.80 \pm 0.13^{a,B}$	$3.80 \pm 0.10^{a,B}$	$3.79 \pm 0.24^{a,B}$
	4	$3.85 \pm 0.25^{a,B}$	$3.86 \pm 0.11^{a,B}$	$3.83 \pm 0.07^{a,B}$	$3.81 \pm 0.14^{a,B}$
	8	$3.88 \pm 0.13^{a,B}$	$3.88 \pm 0.14^{a,B}$	$3.86 \pm 0.11^{a,B}$	$3.84 \pm 0.09^{a,B}$
	12	$4.15 \pm 0.13^{a,A}$	$4.12 \pm 0.06^{a,A}$	$4.08 \pm 0.14^{a,A}$	$4.06 \pm 0.09^{a,A}$
Psychophilic bacteria	0	$4.19 \pm 0.24^{a,C}$	$4.15 \pm 0.23^{a,C}$	$4.12 \pm 0.27^{a,C}$	$4.10 \pm 0.25^{a,C}$
	4	$4.98 \pm 0.17^{a,B}$	$4.90 \pm 0.10^{a,B}$	$4.96 \pm 0.02^{a,B}$	$4.92 \pm 0.11^{a,B}$
	8	$5.48 \pm 0.17^{a,AB}$	$5.36 \pm 0.09^{a,AB}$	$5.19 \pm 0.15^{a,AB}$	$5.22 \pm 0.12^{a,AB}$
	12	$5.82 \pm 0.23^{a,A}$	$5.80 \pm 0.21^{a,A}$	$5.63 \pm 0.12^{a,A}$	$5.60 \pm 0.09^{a,A}$
Coliforms	0	$3.27 \pm 0.04^{a,C}$	$3.28 \pm 0.05^{a,B}$	$3.23 \pm 0.06^{a,B}$	$3.21 \pm 0.09^{a,C}$
	4	$3.31 \pm 0.11^{a,C}$	$3.40 \pm 0.05^{a,B}$	$3.35 \pm 0.11^{a,B}$	$3.36 \pm 0.09^{a,C}$
	8	$4.14 \pm 0.06^{a,B}$	$4.16 \pm 0.05^{a,AB}$	$3.86 \pm 0.12^{b,AB}$	$3.86 \pm 0.12^{b,B}$
	12	$4.86 \pm 0.05^{a,A}$	$4.87 \pm 0.04^{a,A}$	$4.65 \pm 0.17^{b,A}$	$4.57 \pm 0.27^{c,A}$

[a-b] Means sharing different letters in the same row are significantly different ($P<0.05$).

[A-C] Means sharing different letters in the same column in each parameter are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

4.2.4 Evaluation of the effect of crude extracts from *P. acidus* leaves and *C. carandas* fruits on sensory evaluation in ground pork products

Sensory scores assessed for overall acceptance, color, odor, texture, flavor, overall quality of ground pork treated with PWCE and CWCE were presented in Table 4.38 and 4.39. In overall, addition of the extracts in the ground pork samples had no significantly affected the sensory scores of overall appearance, color, odor, texture, flavor and overall quality compared to the control. This result suggested that PWCE and CWCE could be applied as a natural product in ground pork.

Table 4.38 Effect of water extract from *P. acidus* leaves on sensory evaluation of ground pork

Parameters	Control	0.2 g BHT/kg	2.5 g PWCE/kg	5 g PWCE/kg
Overall appearance	6.20 ± 0.70^{a}	5.05 ± 1.19^{b}	6.00 ± 0.92^{a}	5.90 ± 1.11^{a}
Color	6.05 ± 0.94^{a}	5.60 ± 0.82^{a}	5.95 ± 0.82^{a}	6.00 ± 0.79^{a}
Odor	6.25 ± 0.91^{a}	6.25 ± 1.02^{a}	6.30 ± 0.80^{a}	6.25 ± 0.72^{a}
Texture	6.00 ± 0.73^{a}	5.85 ± 0.99^{a}	5.95 ± 0.76^{a}	5.90 ± 0.85^{a}
Flavor	5.90 ± 1.17^{a}	4.40 ± 0.99^{b}	5.35 ± 1.39^{ab}	5.55 ± 1.32^{a}
Overall quality	6.15 ± 0.74^{a}	4.65 ± 1.31^{b}	5.65 ± 1.04^{a}	5.75 ± 0.91^{a}

$^{a-b}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

Table 4.39 Effect of water extract from *C. carandas* fruits on sensory evaluation of ground pork

Parameters	Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Overall appearance	6.15 ± 0.75^{a}	5.25 ± 1.12^{b}	5.95 ± 0.89^{ab}	5.90 ± 1.02^{ab}
Color	6.15 ± 0.99^{a}	5.70 ± 1.13^{a}	6.10 ± 0.91^{a}	6.15 ± 0.86^{a}
Odor	6.20 ± 0.77^{a}	6.15 ± 0.81^{a}	6.10 ± 0.64^{a}	6.20 ± 0.62^{a}
Texture	5.95 ± 0.76^{a}	6.00 ± 0.73^{a}	5.85 ± 1.18^{a}	6.05 ± 0.83^{a}
Flavor	5.90 ± 0.72^{a}	5.05 ± 0.76^{a}	5.70 ± 0.80^{a}	5.60 ± 0.94^{a}
Overall quality	5.95 ± 0.76^{a}	4.90 ± 1.02^{a}	5.75 ± 0.91^{a}	5.60 ± 1.23^{a}

$^{a-b}$ Means sharing different letters in the same row are significantly different ($P<0.05$).

All values were expressed as mean ± standard deviation.

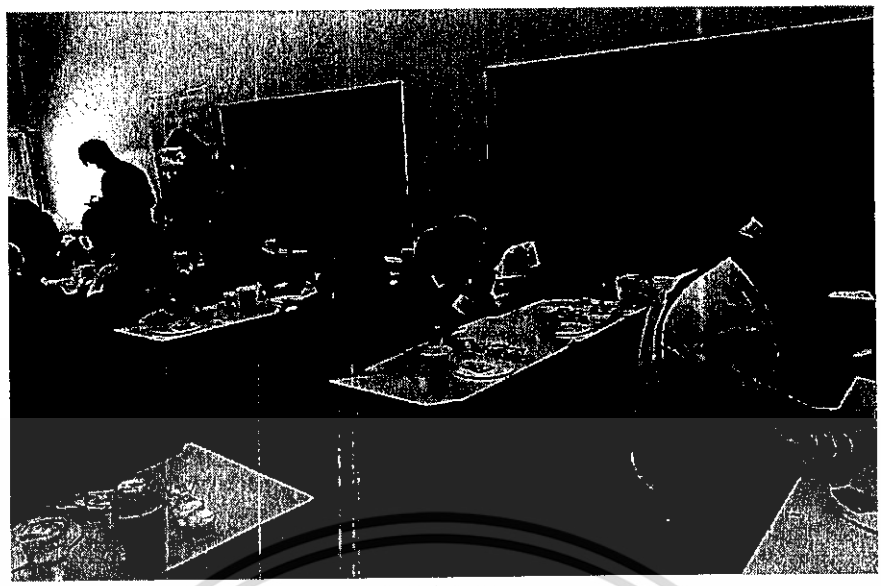


Figure 4.2 The consumer panel are evaluating the ground pork samples treated with water crude extracts from *P. acidus* leaves and *C. carandas* fruits

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Experiment 1: Evaluation of the effect of various solvent ratios of ethanol (0%, 25%, 50%, 75% and 100%) in water on the extraction crude yield, TPC and TFC of *P. acidus* leaves and *C. carandas* fruits

1. The highest crude yield was obtained from water solvent for both *P. acidus* leaf and *C. carandas* fruit extracts.

2. The highest recovery yield of TPC and TFC from *P. acidus* leaf extracts was found in water extract.

3. The highest total phenolic and total flavonoid contents were found in water extract.

4. The highest total phenolic content from *C. carandas* fruit extracts was found in 100% ethanol extract and no significant difference on total flavonoid content among different solvent ratios.

Experiment 2: Evaluation of the effect of various solvent ratios of ethanol (0%, 25%, 50%, 75% and 100%) in water on *in vitro* antioxidant and antimicrobial properties of the crude extracts from *P. acidus* leaves and *C. carandas* fruits.

1. The highest antioxidant activity was found in water extract for lipid peroxidation, reducing power, DPPH scavenging, and metal chelating.

2. The extracts from *C. carandas* fruits showed that 50% ethanol extract revealed the highest activity in lipid peroxidation, reducing power and 75% ethanol extract showed the highest activity in DPPH radical scavenging, metal chelating.

3. The extracts from *P. acidus* leaves had no inhibition effect on the tested bacteria.

4. 100% ethanol solvent showed more effective than other solvents and all extracts showed varying degrees of antimicrobial activity against all the tested bacteria.

Experiment 3: Evaluation of the effect of optimal crude extracts from *P. acidus* leaves and *C. carandas* fruits on chemical, physical and biological quality in ground pork storaged at 4°C up to 8 days.

1. According to the DPPH, ABTS and reducing power assays, the ground pork samples treated with PWCE and CWCE exhibited significantly ($P<0.05$) higher activity in both raw and cooked samples compared to the control and BHT samples. Besides, the addition of PWCE and

CWCE in ground pork showed significantly lower level of lipid peroxidation compared to control and BHT treatments ($P<0.05$) during the storage time up to 8 days.

2. Addition of PWCE and CWCE at 2.5 and 5 g/kg had no effect on the pH values during the storage time over 8 days at 4°C.

3. The lightness (L*) and yellowness (b*) values of meat samples contained PWCE and CWCE were significantly lower while the redness (a*) values higher than the control samples at the end of the storage ($P<0.05$).

4. The addition of PWCE resulted in no significant difference from the control and BHT treatments in total plate count, yeasts/molds, psychrophilic bacteria and coliforms during the storage time up to 8 days at 4°C. Whereas, total plate count and coliform of the ground pork samples decreased significantly with addition of CWCE ($P<0.05$).

Experiment 4: Evaluation of the effect of optimal crude extracts from *P. acidus* leaves and *C. carandas* fruits on chemical, physical and biological quality in ground pork storaged at -20°C up to 12 weeks.

1. The results revealed that the DPPH, ABTS and reducing power assays of the ground pork samples treated with PWCE and CWCE were significantly higher activity in both raw and cooked samples compared to the control and BHT samples ($P<0.05$). Moreover, total MDA content was lower in both raw and cooked samples in the addition of PWCE and CWCE when compared to control and BHT ($P<0.05$).

2. Addition of PWCE and CWCE at 2.5 and 5 g/kg had no an effect on the pH values during the storage time over 12 weeks at -20°C.

3. The addition of PWCE resulted in no significant difference on biological analysis but the addition of CWCE resulted on a reduction in the growth of TPC and coliform, but it was no effect on Y/M and psychophilic bacteria compared to the control and BHT treatments during the storage time up to 8 days at 4°C ($P<0.05$).

Experiment 5: Evaluation of the effect of optimal crude extracts from *P. acidus* leaves and *C. carandas* fruits on sensory evaluation of ground pork products.

The sensory properties of the cooked ground pork supplemented with 2.5 and 5 g/kg meat of PWCE and CWCE were also evaluated. Sensory evaluation indicated that the addition of PWCE and CWCE did not adversely affect on the sensory properties of the pork samples.

5.2 Recommendation

P. acidus leaf and *C. carandas* fruit extracts contain a large amount of bio-active compounds that can help control foodborne pathogenic and spoilage bacteria, as well as high antioxidant activities. In particular, the addition of PWCE and CWCE was effective against microbial growth and oxidative reactions as synthetic additives and higher antioxidant activities than synthetic antioxidant (BHT). Moreover, these extracts had no negative effect on the sensory evaluation of meat samples, more acceptable to consumers, and also potentially good for human health as a functional food. Therefore, extracts from *P. acidus* leaves and *C. carandas* fruits could be used as multifunctional preservatives in meat products. Further studies should be conducted on the chemical compositions and mode of active of antioxidant and antimicrobial activities from these extracts.

REFERENCES

Abdulmumeen, H.A, Risikat, A.N. and Sururah, A.R. 2012. "Food: Its preservatives, additives and applications." **Int. J. Chem. Bio. Sci.** 1 : 36–47.

Addis, M. 2015. "Major causes of meat spoilage and preservation techniques: A review." **Food Sci. Qual. Manage.** 41 : 101–115.

Angiolillo, L., Conte, A. and Del Nobile, M.A. 2015. "Technological strategies to produce functional meat burgers." **LWT - Food Sci. Technol.** 62 : 697–703.

Baba, S.A. and Malik, S.A. 2014. "Evaluation of antioxidant and antibacterial activity of methanolic extracts of *Gentiana kurroo* royle." **Saudi J. Biol. Sci.** 21 : 493–498.

Bakoush, S.M.M., Yaacob, W.A., Adam, J.H. and Ibrahim, N. 2015. "Antioxidant activities, total phenolic and flavonoid contents of the aqueous extracts from *Rafflesia cantleyi* bud." **Parts. Res. J. Med. Plant** 9 : 347–353.

Beinner, M.A., Soares, A.D.N., Barros, A.L.A. and Monteiro, M.A.M. 2010. "Sensory evaluation of rice fortified with iron." **Ciência e Tecnol. Aliment** 30 : 516–519.

Beyhan, Ö., Elmastas, M. and Gedikli, F. 2010. "Total phenolic compounds and antioxidant capacity of leaf, dry fruit and fresh fruit of feijoa (*Acca sellowiana*, Myrtaceae)." **J. Med. Plants Res.** 4 : 1065–1072.

Birt, D.F., Hendrich, S. and Wang, W. 2001. "Dietary agents in cancer prevention: Flavonoids and isoflavonoids." **Pharmacol. Ther.** 90 : 157–177.

Biswas, B., Rogers, K., Mclaughlin, F., Daniels, D. and Yadav, A. 2013. "Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two gram-negative and gram-positive bacteria. **Int. J. Microbiol.** 2013 : 1–7.

Biswas, S.K., Chowdhury, A., Das, J., Karmakar, U.K., Shill, M.C. and Raihan, S.Z. 2011. "Assessment of cytotoxicity, antibacterial activity and phytochemical screening of ethanol extract of *Phyllanthus acidus* L. (family: Euphorbiacceae) bark." **J. Appl. Pharm. Sci.** 1 : 112–114.

Böhme, K., Fernández-No, I.C., Barros-Velázquez, J., Gallardo, J.M., Cañas, B. and Calo-Mata, P. 2012. "Species identification of food spoilage and pathogenic bacteria by MALDI-TOF mass fingerprinting." **Food Qual.** 32 : 29–46.

Boskovic, M., Zdravkovic, N., Ivanovic, J., Janjic, J., Djordjevic, J., Starcevic, M. and Baltic,

M.Z. 2015. "Antimicrobial activity of thyme (*Tymus vulgaris*) and oregano (*Origanum vulgare*) essential oils against some food-borne microorganisms." **Procedia Food Sci.** 5 : 18–21.

Bozoglu, F., Alpas, H. and Kaletunç, G. 2004. "Injury recovery of foodborne pathogens in high hydrostatic pressure treated milk during storage." **FEMS Immunol. Med. Microbiol.** 40 : 243–247.

Brewer, M.S. 2011. "Natural antioxidants: sources, compounds, mechanisms of action, and potential applications." **Compr. Rev. Food Sci. Food Saf.** 10 : 221–247.

Buege, J.A and Aust, S.D. 1987. "Microsomal lipid peroxidation." Methods Enzymol. 52 : 302–310.

Carpenter, R., O'Grady, M.N., O'Callaghan, Y.C., O'Brien, N.M. and Kerry, J.P. 2007. "Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork." **Meat Sci.** 76 : 604–610.

Castellano, P., Belfiore, C., Fadda, S. and Vignolo, G. 2008. "A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina." **Meat Sci.** 79 : 483–499.

Castellini, C., Dal Bosco, A., Mugnai, C. and Pedrazzoli, M. 2006. "Comparison of two chicken genotypes organically reared: Oxidative stability and other qualitative traits of the meat." **Ital. J. Anim. Sci.** 5 : 29–42.

Castellini, C., Muginai, C. and Dal Bosco, A. 2002. "Effect of organic production system on broiler carcass and meat quality." **Meat Sci.** 60 : 219–225.

Chakraborty, R., Biplab, D., Devanna, N. and Sen, S. 2012. "Antiinflammatory, antinociceptive and antioxidant activities of *Phyllanthus acidus* L. extracts." **Asian Pac. J. Trop. Biomed.** 2 : 953–961.

Chang, H.Y., Ho, Y.L., Sheu, M.J., Lin, Y.H., Tseng, M.C., Wu, S.H., Huang, G.J. and Chang, Y.S. 2007. "Antioxidant and free radical scavenging activities of *Phellinus merrillii* extracts." **Bot. Stud.** 48, 407–417.

Chumyam, A., Whangchai, K., Jungklang, J., Faiyue, B. and Saengnil, K. 2013. "Effects of heat treatments on antioxidant capacity and total phenolic content of four cultivars of purple skin eggplants." **Science Asia.** 39 : 246–251.

Coutinho de Oliveira, T.L., Malfitano de Carvalho, S., de Araújo Soares, R., Andrade, M.A., Cardoso, M.D.G., Ramos, E.M. and Piccoli, R.H. 2012. "Antioxidant effects of *Satureja*

montana L. essential oil on TBARS and color of mortadella-type sausages formulated with different levels of sodium nitrite." **LWT - Food Sci. Technol.** 45 : 204–212.

Dai, Y., Lu, Y., Wu, W., Lu, X.M., Han, Z.P., Liu, Y., Li, X.M. and Dai, R.T. 2014. "Changes in oxidation, color and texture deteriorations during refrigerated storage of ohmically and water bath-cooked pork meat." **Innov. Food Sci. Emerg. Technol.** 26 : 341–346.

Dailey, A. and Vuong, Q.V. 2015. "Effect of extraction solvents on recovery of bioactive compounds and antioxidant properties from macadamia (*Macadamia tetraphylla*) skin waste." **Food Sci. Technol.** 2015 : 1–10.

Dent, M., Dragovic-Uzelac, V., Penic, M., Brnc, M., Bosiljkov, T. and Levaj, B. 2013. "The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in dalmatian wild sage (*Salvia officinalis* L.) extracts." **Food Technol. Biotechnol.** 51 : 84–91.

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S. and Ju, Y.H. 2014. "Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*." **J. Food Drug Anal.** 22 : 296–302.

Ebrahimabadi, A.H., Mazoochi, A., Kashi, F.J., Djafari-Bidgoli, Z. and Batooli, H. 2010. "Essential oil composition and antioxidant and antimicrobial properties of the aerial parts of *Salvia eremophila* Boiss." **Iran. Food Chem. Toxicol.** 48 : 1371–1376.

Ebrahimzadeh, M.., Nabavi, S., Nabavi, S., Bahramian, F. and Bekhradnia, R. 2008. "Antioxidant and free radical scavenging activity." **Pak. J. Pharm. Sci.** 23 : 29–34.

Eldeen, I.M.S., Seow, E.M., Abdullah, R. and Sulaiman, S.F. 2011. "*In vitro* antibacterial, antioxidant, total phenolic contents and anti-HIV-1 reverse transcriptase activities of extracts of seven *Phyllanthus* sp." **South African J. Bot.** 77 : 75–79.

Falowo, A.B., Fayemi, P.O. and Muchenje, V. 2014. "Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: A review." **Food Res. Int.** 64 : 171–181.

Fartyal, M. and Kumar, P. 2014. "Bioactivity of crude extracts of *Carissa carandas* Linn. extracted in polar and non polar solvents." **Int. J. Eng. Sci. Innov. Technol.** 3 : 186–191.

Fasseas, M.K., Mountzouris, K.C., Tarantilis, P.A., Polissiou, M. and Zervas, G. 2008. "Antioxidant activity in meat treated with oregano and sage essential oils." **Food Chem.** 106 : 1188–1194.

Faustman, C., Sun, Q., Mancini, R. and Suman, S.P. 2010. "Myoglobin and lipid oxidation

interactions: Mechanistic bases and control." **Meat Sci.** 86 : 86–94.

Fernandes, A., Sousa Pinheiro, L., Souto Pereira, C., Neves Matias, W., Albuquerque Gomes, R., Souza Chaves, O., Vanderlei de Souza, M., Nóbrega de Almeida, R. and Simões de Assis, T. 2012. "Total phenolic content and antioxidant activity of some malvaceae family species." **Antioxidants** 1 : 33–43.

Filgueras, R.S., Gatellier, P., Aubry, L., Thomas, A., Bauchart, D., Durand, D., Zambiazi, R.C. and Sant-Lhoutellier, V. 2010. "Colour, lipid and protein stability of Rhea americana meat during air- and vacuum-packaged storage: Influence of muscle on oxidative processes." **Meat Sci.** 86 : 665–673.

Foo, S.C., Yusoff, F.M., Ismail, M., Basri, M., Khong, N.M.H., Chan, K.W. and Yau, S.K. 2015. "Efficient solvent extraction of antioxidant-rich extract from a tropical diatom, Chaetoceros calcitrans (Paulsen) Takano 1968." **Asian Pac. J. Trop. Biomed.** 5 : 834–840.

Fullerton, M., Khatiwada, J., Johnson, J.U., Davis, S. and Williams, L.L. 2011. "Determination of antimicrobial activity of sorrel (*Hibiscus sabdariffa*) on *Escherichia coli* O157:H7 isolated from food, veterinary, and clinical samples." **J. Med. Food.** 14 : 950–956.

Gallego, M.G., Gordon, M.H., Segovia, F.J. and Almajano, M.P. 2015. "*Caesalpinia decapetala* extracts as inhibitors of lipid oxidation in beef patties." **Molecules** 20 : 13913–13926.

Gheldof, N. and Engeseth, N.J. 2002. "Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of *in vitro* lipoprotein oxidation in human serum samples." **J. Agric. Food Chem.** 50 : 3050–3055.

Habib, R., Rahman, M., Mannan, A., Zulfiker, A.H., Uddin, M.E. and Sayeed, M.A. 2011. "Evaluation of antioxidant, cytotoxic, antibacterial potential and phytochemical screening of chloroform extract of *Phyllanthus acidus*." **Int. J. Appl. Biol. Pharm. Technol.** 2 : 420–427.

Hernandez-Hernandez, E., Ponce-Alquicira, E., Jaramillo-Flores, M.E. and Guerrero Legarreta, I. 2009. "Antioxidant effect of rosemary (*Rosmarinus officinalis* L.) and oregano (*Origanum vulgare* L.) extracts on TBARS and colour of model raw pork batters." **Meat Sci.** 81 : 410–417.

Ho, C. 1992. "Phenolic compounds in food: An overview." **ACS Symp. Ser. Washington, DC** 2–7.

Hsu, B., Coupar, I.M. and Ng, K., 2006. "Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*." **Food Chem.** 98 : 317–328.

Huang, B., He, J., Ban, X., Zeng, H., Yao, X. and Wang, Y. 2011. "Antioxidant activity of bovine and porcine meat treated with extracts from edible lotus (*Nelumbo nucifera*) rhizome knot and leaf." **Meat Sci.** 87 : 46–53.

Iloki-Assanga, S.B., Lewis-Luján, L.M., Lara-Espinoza, C.L., Gil-Salido, A. A., Fernandez-Angulo, D., Rubio-Pino, J.L. and Haines, D.D. 2015. "Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*." **BMC Res. Notes** 8 : 396–409.

Israr, F., Hassan, F., Naqvi, B.S., Azhar, I., Jabeen, S. and Hasan, S.M.F. 2012. "Report: Studies on antibacterial activity of some traditional medicinal plants used in folk medicine." **Pak. J. Pharm. Sci.** 25 : 669–674.

Itankar, P.R., Lokhande, S.J., Verma, P.R., Arora, S.K., Sahu, R.A. and Patil, A.T. 2011. "Antidiabetic potential of unripe *Carissa carandas* Linn. fruit extract." **J. Ethnopharmacol.** 135 : 430–433.

Jagajothi, A., Manimekalai, G., Evanjelene, V.K. and Nirmala, A. 2013. "Antimicrobial activity and phytochemical analysis of *Phyllanthus acidus*." **J. Today's Biogical Sci. Res. Rev.** 2 : 55–62.

Jagessar, R., Mars, A. and Gomes, G. 2008. "Selective antimicrobial properties of *Phyllanthus acidus* leaf extract against *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* using stokes disc diffusion, well diffusion, streak plate and a dilution method." **Nat. Sci.** 6 : 24–38.

Jain, N.K., Lodhi, S., Jain, A., Nahata, A. and Singhai, A.K. 2010. "Protective effects of *Phyllanthus acidus* L. skeels extract on acetaminophen mediated hepatic injury and oxidative stress in Wistar rats." **J. Complement Integr. Med.** 7 : 1–16.

Jain, N.K. and Singhai, A.K. 2011. "Protective effects of *Phyllanthus acidus* L. Skeels leaf extracts on acetaminophen and thioacetamide induced hepatic injuries in Wistar rats." **Asian Pac. J. Trop. Med.** 4 : 470–474.

Jalosinska, M. and Wilczak, J. 2009. "Influence of plant extracts on the microbiological shelf life of meat products." **Polish J. Food Nutition Sci.** 59 : 303–308.

Jamuna, S., Paulsamy, S. and Karthika, K. 2012. "Screening of *in vitro* antioxidant activity of methanolic leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae)." **J. Appl.**

Pharm. Sci. 2 : 149–154.

Jayasena, D.D. and Jo, C. 2013. "Essential oils as potential antimicrobial agents in meat and meat products: A review." **Trends Food Sci. Technol.** 34 : 96–108.

Jia, N., Kong, B., Liu, Q., Diao, X. and Xia, X. 2012. "Antioxidant activity of black currant (*Ribes nigrum* L.) extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage." **Meat Sci.** 91 : 533–539.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S. and Heinonen, M., 1999. "Antioxidant activity of plant extracts containing phenolic compounds." **J. Agric. Food Chem.** 47 : 3954–3962.

Karakaya, M., Bayrak, E. and Ulusoy, K. 2011. "Use of natural antioxidants in meat and meat products." **J. food Sci. Eng.** 1 : 1–10.

Karre, L., Lopez, K. and Getty, K.J.K. 2013. "Natural antioxidants in meat and poultry products." **Meat Sci.** 94 : 220–227.

Khoddami, A., Wilkes, M.A. and Roberts, T.H. 2013. "Techniques for analysis of plant phenolic compounds. **Molecules** 18 : 2328–2375.

Kim, J.S. 2012. "Comparison of antioxidant properties of water and ethanol extracts obtained from dried boxthorn (*Lycium chinensis*) fruit." **Food Nutr. Sci.** 3 : 1307–1320.

Kim, S.J., Cho, A.R. and Han, J. 2013a. "Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation." **Food Control.** 29 : 112–120.

Kim, S.J., Min, S.C., Shin, H.J., Lee, Y.J., Cho, A.R., Kim, S.Y. and Han, J. 2013b. "Evaluation of the antioxidant activities and nutritional properties of ten edible plant extracts and their application to fresh ground beef." **Meat Sci.** 93 : 715–722.

Kiokias, S. and Gordon, M.H. 2003. "Antioxidant properties of annatto carotenoids." **Food Chem.** 83 : 523–529.

Kroll, J. and Rawel, H. 2001. "Reactions of plant phenols with myoglobin: Influence of chemical structure of the phenolic compounds." **JFS Food Chemistry Toxicol.** 66 : 48–58.

Kumar, S., Gupta, P. and Gupta, K.L.V. 2013. "A critical review on Karamarda (*Carissa carandas* Linn.)." **Int. J. Pharm. Biol. Arch.** 4 : 637–642.

Kumar, T. and Jain, V. 2015. "Appraisal of total phenol, flavonoid contents, and antioxidant potential of folkloric *Lannea coromandelica* using *in vitro* and *in vivo* assays." **Scientifica**

(Cairo). 2015 : 1–13.

Lee, M.A., Choi, J.H., Choi, Y.S., Kim, H.Y., Kim, H.W., Hwang, K.E., Chung, H.K. and Kim, C.J. 2011. "Effects of kimchi ethanolic extracts on oxidative stability of refrigerated cooked pork." **Meat Sci.** 89 : 405–411.

Llópiz, N., Puiggròs, F., Céspedes, E., Arola, L., Ardévol, A., Bladé, C. and Salvadó, M.J. 2004. "Antigenotoxic effect of grape seed procyanidin extract in Fao cells submitted to oxidative stress." **J. Agric. Food Chem.** 52 : 1083–1087.

Maisarah, A., Nurul Amira, B., Asmah, R. and Fauziah, O. 2013. "Antioxidant analysis of different parts of *Carica papaya*." **Int. Food Res. J.** 20 : 1043–1048.

Manju, K., Jat, R.K. and Anju, G. 2012. "A review on medicinal plants used as a source of anticancer agents." **Int. J. Drug Res. Tech.** 177–183.

Maqsood, S., Abushelaibi, A., Manheem, K., Al Rashedi, A. and Kadim, I.T. 2015. "Lipid oxidation, protein degradation, microbial and sensorial quality of camel meat as influenced by phenolic compounds." **LWT - Food Sci. Technol.** 63 : 953–959.

Mariutti, L.R.B., Nogueira, G.C. and Bragagnolo, N. 2011. "Lipid and cholesterol oxidation in chicken meat are inhibited by sage but not by garlic." **J. Food Sci.** 76 : 909–915.

Masniyom, P., Benjakul, S. and Visessanguan, W. 2002. "Shelf-life extension of refrigerated seabass slices under modified atmosphere packaging." **J. Sci. Food Agric.** 82 : 873–880.

Medina, I., Gallardo, J.M., Gonzlez, M.J., Lois, S. and Hedges, N. 2007. "Effect of molecular structure of phenolic families as hydroxycinnamic acids and catechins on their antioxidant effectiveness in minced fish muscle." **J. Agric. Food Chem.** 55 : 3889–3895.

Mehmood, M.H., Anila, N., Begum, S., Syed, S.A., Siddiqui, B.S. and Gilani, A.H. 2014. "Pharmacological basis for the medicinal use of *Carissa carandas* in constipation and diarrhea." **J. Ethnopharmacol.** 153 : 359–367.

Mhalla, D., Bouaziz, A., Ennouri, K., Chawech, R., Smaoui, S., Jarraya, R., Tounsi, S. and Trigui, M. 2017. "Antimicrobial activity and bioguided fractionation of rumex tingitanus extracts for meat preservation." **Meat Sci.** 125 : 22–29.

Min, B. and Ahn, D.U. 2005. "Mechanism of lipid peroxidation in meat and meat products-A Review." **Food Sci. Biotechnol.** 14 : 152–163.

Min, B.R., Nam, K.C., Cordray, J.C. and Ahn, D.U. 2008. "Factors affecting oxidative stability of pork, beef, and chicken meat." **Iowa State Univ. Anim. Ind. Rep.** 654 : 1–5.

Mith, H., Duré, R., Delcenserie, V., Zhiri, A., Daube, G. and Clinquart, A. 2014. "Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria." **Food Sci. Nutr.** 2 : 403–16.

Mor-Mur, M. and Yuste, J. 2010. "Emerging bacterial pathogens in meat and poultry: An overview." **Food Bioprocess Technol.** 3 : 24–35.

Munuswamy, H., Thirunavukkarasu, T., Rajamani, S., Elumalai, E.K. and Ernest, D. 2013. "A review on antimicrobial efficacy of some traditional medicinal plants in Tamilnadu." **J. Acute Dis.** 2 : 99–105.

Nascimento, G.G.F., Locatelli, J., Freitas, P.C. and Silva, G.L. 2000. "Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria." **Brazilian J. Microbiol.** 31 : 247–256.

Negi, P.S. 2012. "Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application." **Int. J. Food Microbiol.** 156 : 7–17.

Ngoci, N.S., Ramadhan, M., Ngari, M.S. and Leonard, O.P. 2014. "Screening for antimicrobial activity of *Cissampelos pareira* L methanol root extract." **European J.Med. Plants** 4 : 45–51.

AOAC. 2005. Chapter 17 AOAC Official Method 940. p.2. In Horwitz, W. and Latimer, G.W. "Official methods of analysis of AOAC international." Maryland.

Obeidat, M., Shatnawi, M., Al-alawi, M., Al-Zubi, E., Al-Dmoor, H., Al-Qudah, M., El-Qudah, J. and Otri, I. 2012. "Antimicrobial activity of crude extracts of some plant leaves." **Res. J. Microbiol.** 7 : 59–67.

Olmedilla-Alonso, B., Jimnez-Colmenero, F. and Snchez-Muniz, F.J. 2013. "Development and assessment of healthy properties of meat and meat products designed as functional foods." **Meat Sci.** 95 : 919–930.

Omojate, G.C., Enwa, F.O., Jewo, A.O. and Eze, C.O. 2014. "Mechanisms of antimicrobial actions of phytochemicals against enteric pathogens – A review." **J. Pharm. Chem. Biol. Sci.** 2 : 77–85.

Ortuño, J., Serrano, R., Jordán, M.J. and Bañón, S. 2016. "Relationship between antioxidant status and oxidative stability in lamb meat reinforced with dietary rosemary diterpenes." **Food Chem.** 190 : 1056–1063.

Parsaeimehr, A., Sargsyan, E. and Javidnia, K. 2010. "A comparative study of the antibacterial,

antifungal and antioxidant activity and total content of phenolic compounds of cell cultures and wild plants of three endemic species of Ephedra." **Molecules** 15 : 1668–1678.

Patra, J.K. and Baek, K.H. 2016. "Antibacterial activity and action mechanism of the essential oil from *Enteromorpha linza* L. against foodborne pathogenic bacteria." **Molecules** 21 : 1–11.

Popova, T. and Marinova, P. 2013. "Lipid and protein oxidation during cooking in meat of lambs reared indoors and on pasture." **Bulg. J. Agric. Sci.** 19 : 590–594.

Qi, S., Huang, H., Huang, J., Wang, Q. and Wei, Q. 2015. "Lychee (*Litchi chinensis* Sonn.) seed water extract as potential antioxidant and anti-obese natural additive in meat products." **Food Control** 50 : 195–201.

Qwele, K., Hugo, A., Oyedemi, S.O., Moyo, B., Masika, P.J. and Muchenje, V. 2013. "Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay." **Meat Sci.** 93 : 455–462.

Radha Krishnan, K., Babuskin, S., Azhagu Saravana Babu, P., Sasikala, M., Sabina, K., Archana, G., Sivarajan, M. and Sukumar, M. 2014. "Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat." **Int. J. Food Microbiol.** 171 : 32–40.

Rahman, M.H., Hossain, M.M., Mohammad, S. and Rahman, E. 2014. "Effect of repeated freeze-thaw cycles on beef quality and safety." **Korean J. Food Sci. An.** 34 : 482–495.

Rajneesh, C.P., Manimaran, A., Sasikala, K.R. and Adaikappan, P. 2008. "Lipid peroxidation and antioxidant status in patients with breast cancer." **Singapore Med. J.** 49 : 640–643.

Rawat, S. 2015. "Food spoilage : Microorganisms and their prevention." **Asian J.Plant Sci. Res.** 5 : 47–56.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. "Antioxidant activity applying an improved ABTS radical cation decolorization assay." **Free Radic. Biol. Med.** 26 : 1231–1237.

Reddy, G.V.B., Sen, A.R., Nair, P.N., Reddy, K.S., Reddy, K.K. and Kondaiah, N. 2013. "Effects of grape seed extract on the oxidative and microbial stability of restructured mutton slices." **Meat Sci.** 95 : 288–294.

Richheimer, S.L., Bernart, M.W., King, G.A., Kent, M.C. and Beiley, D.T. 1996. "Antioxidant activity of lipid-soluble phenolic diterpenes from rosemary." **J. Am. Oil Chem. Soc.** 73 :

507–514.

Rojas, J.J., Ochoa, V.J., Ocampo, S.A. and Muñoz, J.F. 2006. "Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections." **BMC Complement Altern. Med.** 6 : 1–6.

Sadek, Y., Choudhury, N. and Shahriar, M. 2013. "Biological investigations of the leaf extracts of *Carissa carandas*." **European J.Med. Plants.** 5 : 97–105.

Salar, R.K. and Dhall, A. 2010. "Antimicrobial and free radical scavenging activity of extracts of some Indian medicinal plants." **J. Med. Plants Res.** 4 : 2313–2320.

Samouris, G.I., Bampidis, V.A., Sossidou, E. and Zantopoulos, N. 2007. "Lipid oxidation of raw and cooked turkey breast meat during refrigerated storage." **Arch. fur Geflugelkd.** 71 : 41–44.

Sawhney, S.S., Painuli, R.M. and Singh, D. 2011. "Antioxidant and antimicrobial activity of *Phyllanthus emblica* for its application in treatment of ophthalmic disorders." **Int. J. Pharm. Pharm. Sci.** 3 : 129–132.

Sebranek, J.G., Sewalt, V.J.H., Robbins, K.L. and Houser, T.A. 2005. "Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage." **Meat Sci.** 69 : 289–296.

Shilali, K., Ramachandra, Y.L., Rajesh, K.P. and Kumara Swamy, B.E. 2014. "Assessing the antioxidant potential of *Phyllanthus acidus* bark extracts." **Int. J. Pharm. Pharm. Sci.** 6 : 522–531.

Singh, A. and Uppal, G.K. 2015. "A review on *Carissa carandas* - phytochemistry, ethno-pharmacology, and micropropagation as conservation strategy." **Asian J. Pharm. Clin. Res.** 8 : 26–30.

Singh, M., Jha, A., Kumar, A., Hettiarachchy, N., Rai, A.K. and Sharma, D. 2014. "Influence of the solvents on the extraction of major phenolic compounds (Punicalagin, ellagic acid and gallic acid) and their antioxidant activities in pomegranate aril." **J. Food Sci. Technol.** 51 : 2070–2077.

Smiecinska, K., Hnatyk, N., Daszkiewicz, T., Kubiak, D. and Matusevicius, P. 2015. "The effect of frozen storage on the quality of vacuum-packaged Turkey meat." **Vet. ir Zootech.** 71 : 61–66.

Sokmen, A., Gulluce, M., Askin Akpulat, H., Daferera, D., Tepe, B., Polissiou, M., Sokmen, M.

and Sahin, F. 2004. "The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*." **Food Control** 15 : 627–634.

Sousa, M., Ousingsawat, J., Seitz, R., Puntheeranurak, S., Regalado, A., Grego, T., Jansakul, C., Amaral, M.D., Schreiber, R. and Kunzelmann, K. 2007. "An extract from the medicinal Plant." **Mol. Pharmacol.** 71 : 366–376.

Sumbul, S. and Ahmed, S.I. 2012. "Anti-hyperlipidemic activity of *Carissa carandas* (Auct.) leaves extract in egg yolk induced hyperlipidemic rats." **J. Basic Appl. Sci.** 8 : 124–134.

Sun, C., Wu, Z., Wang, Z. and Zhang, H. 2015. "Effect of ethanol/water solvents on phenolic profiles and antioxidant properties of Beijing propolis extracts." **Evidence-based Complement Altern. Med.** 2015 : 1–9.

Tachakittirungrod, S., Okonogi, S. and Chowwanapoonpohn, S. 2007. "Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract." **Food Chem.** 103 : 381–388.

Tagne, R.S., Telefo, B.P., Nyemb, J.N., Yemele, D.M., Njina, S.N., Goka, S.M.C., Lienou, L.L., Kamdje, A.H.N., Moundipa, P.F. and Farooq, A.D. 2014. "Anticancer and antioxidant activities of methanol extracts and fractions of some Cameroonian medicinal plants." **Asian Pacific J. Trop. Med.** 442–447.

Taie, H.A.A., Salama, Z.A.E.R. and Radwan, S. 2010. "Potential activity of basil plants as a source of antioxidants and anticancer agents as affected by organic and bio-organic fertilization." **Notulae Botanicae Horti Agrobotanici Cluj-Napoca** 119–127.

Tajkarimi, M.M., Ibrahim, S.A. and Cliver, D.O. 2010. "Antimicrobial herb and spice compounds in food. **Food Control** 21 : 1199–1218.

Tan, M.C., Tan, C.P. and Ho, C.W. 2013. "Effects of extraction solvent system, time and temperature on total phenolic content of henna (*Lawsonia inermis*) stems." **Int. Food Res. J.** 20 : 3117–3123.

Tinoi, J., Rakariyatham, N. and Deming, R. 2006. "Determination of major carotenoid constituents in petal extracts of eight selected flowering plants in the north of Thailand, Chiang Mai." **Chiang Mai J. Sci.** 33 : 327–334.

Umamaheswari, M. and Chatterjee, T.K. 2007. "*In vitro* antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract." **Afr. J. Tradit. Complement. Altern. Med.** 5 : 61–73.

Vashist, H. and Jindal, A. 2012. "Antimicrobial activities of medicinal plants – review." **Int. J.**

Res. Pharm. Biomed. Sci. 3 : 222–230.

Velasco, V. and Williams, P. 2011. "Improving meat quality through natural antioxidants." **Chil. J. Agric. Res.** 71 : 313–322.

Vijayalakshmi, M and Ruckmani, K. 2016. "Ferric reducing anti-oxidant power assay in plant extract." Bangladesh J. Pharmacol. 11: 570-572.

Zhang, H., Wu, J. and Guo, X. 2016a. "Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality." **Food Sci. Hum. Wellness** 5 : 1--10.

Zhang, H., Wu, J. and Guo, X. 2016b. "Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality." **Food Sci. Hum. Wellness** 5 : 39–48.

Zhang, W., Xiao, S., Samaraweera, H., Lee, E.J. and Ahn, D.U. 2010. "Improving functional value of meat products." **Meat Sci.** 86 : 15–31.

Zielinski, H. and Kozlowska, H. 2000. "Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions." **J. Agric. Food Chem.** 48 : 2008–2016.

APPENDIX

CHEMICAL PREPERATION

1. Thiobarbituric Acid Reactive Substances (TBARS)

   0.069 2M Thiobarbituric acid (1000 ml)

2-Thiobarbituric acid	10.00 g
Conc. HCl	20.00 mg
90% Acetic acid	1000 ml

Dissolve 2-Thiobarbituric acid 10.00 g in 90% Acetic acid 600 ml mixed by heating with the rod magnetic to aid in dispersion of the filler conc. Then, disolve 20 ml HCl and adjust the volume by 90% acetic acid to 1000 ml bottles with adjustable volume. The solution stored in a brown bottle at a temperature of 4°C.

2. Butylated hydroxytoluene (BHT)

   0.2% Butylated hydroxytoluene (BHT) (100 ml)

Butylated hydroxytoluene	0.2 g
Ethanol	100 ml

Dissolve 0.2 g butylated hydroxytoluene in ethanol until reaching 100 ml. Keep Butylated hydroxytoluene at 4°C for further using.

3. Stock MDA 100 µg/ml 1,1,3,3-Tetraethoxypropane (TEP)	100 ml
1,1,3,3-Tetraethoxypropane	10.88 g
95% ethanol	100 ml

Dissolve 10.88 g of 1,1,3,3-Tetraethoxypropane in 100 ml of distilled water. Stock 100 µg/ml 1,1,3,3-Tetraethoxypropane stored at 4°C for further using.

4. ABTS Solution

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)	38.42 g
Potassium persulfate	66.17 g
Distilled water	10 ml

Dissolve 38.41 g of ABTS in 5 ml of distlled water and 66.17 g of potassium persulfate in 5 ml of distilled water. The ABTS stock solution incubated in dark room at room temperature for 12-16 h berfore using.

5. 4N Hydrochloric acid (HCl)

Hydrochloric acid	82.81 ml
Distilled water	250 ml

82.81 ml HCl is poured into bottle and then add distlled wauntil 250 ml. 4N Hydrochloric acid is stored in a brown bottle at a temperature of 4°C.

APPENDIX

MEDIA PREPERATION

1. Total plate count agar

Peptone from casein	5 g
Yeast extract	2.5 g
D(+)Glucose	1 g
Agar-agar	14 g

Dissolve MRS agar in 1000 ml distilled water (pH 5.7 ± 0.2), and then autoclave at 121°C in 15 mins.

2. Chromocult agar

Chromocult	26.5 g
Distilled water	1000 ml

Dissolve 26.5 g of Chromocult agar in 1000 ml of distlled water that autoclaved at 121°C in 15 mins.

3. Malt agar

Malt	30 g
Agar	15 g

Dissolve 30 g of Malt and 15 g of agar in 1000 ml distilled water, and then autoclave at 121°C in 15 mins. Adjust pH value to reach 3.5 before using.

4. MRS broth

Peptone from casein	10 g
Meat extract	8 g
Yeast extract	4 g
D(+)Glucose	20 g
di-Potassium hydrogen phosphate	2 g
Tween 80	1 g
di-Ammonium hydrogen citrate	2 g

Sodium acetate	5 g
Magnesium sulfate	0.2 g
Manganese sulfate	0.04 g

Dissolve MRS agar in 1000 ml distilled water (pH 5.7 ± 0.2), and then autoclave at 121°C in 15 mins.

5. MRS agar + 0.5% $CaCO_3$

Peptone from casein	10 g
Meat extract	8 g
Yeast extract	4 g
D(+)Glucose	20 g
di-Potassium hydrogen phosphate	2 g
Tween 80	1 g
di-Ammonium hydrogen citrate	2 g
Sodium acetate	5 g
Magnesium sulfate	0.2 g
Manganese sulfate	0.04 g
Calcium carbonate	5 g

Dissolve MRS agar in 1000 ml distilled water (pH 5.7 ± 0.2), and then autoclave at 121°C in 15 mins.

6. Tryptic Soy Broth (TSB)

Peptone from casein	17 g
Peptone from soymeal	3 g
D(+)Glucose	2.5 g
Sodium chloride	5 g
di-Potassium hydrogen phosphate	2.5 g

Dissolve TSB in 1000 ml distilled water (pH 7.3 ± 0.2), and then autoclave at 121°C in 15 mins.

7. Tryptic Soy Agar (TSA)

Peptone from casein	17 g
Peptone from soymeal	3 g

D(+)Glucose	2.5 g
Sodium chloride	5 g
di-Potassium hydrogen phosphate	2.5 g
Agar	15 g

Dissolve TSA in 1000 ml distilled water (pH 7.3 ± 0.2), and then autoclave at 121°C in 15 mins.

Sensory Evaluation Division of ground pork products using the water extracts from *P. acidus* leaves and *C. carandas* fruits as preservation

Date: .....

Name: .....

The first details of the survey:

1.1 Gender

☐ Male ☐ Female

1.2 Please specify your age

☐ Under 20 years old ☐ 20 - 35 years old

☐ 36 - 50 years old ☐ over 50 years old

1.3 Profession

☐ Students ☐ Government officials

☐ Private companies ☐ Own business

☐ Other, please tell detaily.....

1.4 Do you eat porcine meat?

☐ Like very much ☐ Quite like

☐ Quite dislike ☐ Dislike very much

☞ Please wash your mouth with water before come to start first sample of the sensory evaluation.

☞ Before evaluate next sample, please eat a cracker and drink a little purified water. Moreover, you can spit into the cup which prepared for each panellist.

Please give the level of preferences (from 1 to 7 points) on the nature of the samples which you are testing, one by one. The levels are used consist of 7 points in the table below:

Point		The preference
7	=	extremely like
6	=	very like
5	=	like
4	=	neither like nor dislike
3	=	dislike
2	=	dislike very much
1	=	extremely dislike

Product appearance	**Product code**					
Overall appearance						
Color						
Odor						
Texture						
Flavor						
Overall quality						

Additional comments: .....

.....

.....

.....

.....

Thank you very much!

AUTHOR BIOGRAPHY

NAME	Miss NGUYEN THI KIM TUYEN
DATE OF BIRTH	FEB 21st, 1993
PLACE OF BIRTH	Hon Dat District, Kien Giang Province, Vietnam
ADDRESS	8-112 Student Dormitory, King Mongkut's Institude Technology Ladkrabang. 1 Chalong Krung, Chalong Krung Road, Lat Krabang, Bangkok 10520, Thailand.
EDUCATION	2014 B.Sc of Food Technology at Can Tho University, Vietnam. 2017 M.Sc of Agriculture at King Mongkut's Institute Technology Ladkrabang, Bangkok, Thailand.
PUBLICATIONS	T.K.T. Nguyen, C. Laosinwattana, M. Teerarak and K. Pilasombut. 2017. Potential antioxidant and lipid peroxidation inhibition of *Phyllanthus acidus* leaf extract in minced pork. Asian-Australasian Journal of Animal Sciences (AJAS). (IF = 0.756). T.K.T. Nguyen, K. Pilasombut and C. Laosinwattana. 2016. Preliminary study of ethanol extract of *Phyllanthus acidus* l. (skeels) leaves as natural antioxidant in raw and cooked ground pork. The 17th International Congress of Meat Science and Technology, August 22-25. 2016, Kyushu University, Fukuoka, Japan. T.K.T. Nguyen, C. Laosinwattana, M. Teerarak and K. Pilasombut. 2017. Effect of *Phyllanthus acidus* L. water extract on TBARS and color in ground pork storage at 4°C. International Interdisciplinary Conference on Science, Technology, Engineering, Management, Pharmacy and Humanities, April 22-23. 2017, Nanyang Technological University, Singapore.
EXPERIENCES	10/2015 – 5/2017 Research Assistant at Faculty of Agricultural Technology, King Mongkut's Institute Technology Ladkrabang, Bangkok 10520, Thailand.