THE FINAL REPORT

Effect of *Carissa carandas* L. fruit extracts on quality and antioxidant properties in ground pork

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Abstract

The aim of this study was to investigate the antioxidant and antimicrobial activities of *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 and *C. carandas* fruit extracts. 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 and *C. carandas* fruit extracts were ranged from 15.87 to 20.44 mg GAE/g crude, while total flavonoid content from *C. carandas* fruit extracts were ranged from 0.11 to 0.17 mg QE/g crude. The *in vitro* antioxidant and antibacterial activities of *C. carandas* fruits of various crude extracts were determined. The results indicated that 50% and 75% ethanol extracts had the higher antioxidant activities compared to other extracts. In particular, extracts from *C. carandas* fruits showed the strong inhibition on the growth of all

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the tested bacteria. Thus, the water crude extract from *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 consisted of control, 0.2 g BHT/kg meat, 2.5 g CWCE/kg meat and 5 g CWCE/kg meat. The effect of 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 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 CWCE. The lightness (L*) and yellowness (b*) values of meat samples contained 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 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 *C. carandas* fruits are high potential to be used as natural ingredients to maintain the quality, antioxidant and antimicrobial activities and prolong the shelf life of ground pork as well as develop new functional food safety to satisfy consumers.

Keywords: *Carissa carandas* L., Antioxidant activity, Pork.

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CHAPTER 1
INTRODUCTION

1.1 The problem and its back ground

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 discolouration, off-flavours, 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

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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 (No. 2012). 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 1300 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,

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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.

Plants are a generous source 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 4oC for 15 days (Zhang et al. 2016).

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 foethnomedicine in the treatment of human diseases, such as diarrhea, stomachic,

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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).

Therefore, the objectives of this study were to focus on two main points. The first, evaluation of antioxidant and antimicrobial activities of *C. carandas* extracts using extract solvent system of water, 25, 50, 75 and 100% ethanol in water (v/v). The second, application of *C. carandas* extracts in ground pork for prolong the quality at chilled (4°C) and frozen (-20°C) storage time up to 8 days and 12 weeks, respectively. The quality of meat samples was evaluated on physical (pH, color), chemical (TBARS, DPPH radical scavenging activity, ABTS radical cation decolorization, reducing power), biologycal qualities (total plate count, yeast/molds, *E. coli*/Coliform, psychophilic bacteria).

1.2 The purpose of the research project

Effect of *Carissa carandas* L. fruit extracts on quality and antioxidant properties in ground pork

1.3 The scope of the research project

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

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

3. Evaluation of the effect of optimal crude extracts from *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.

4. Sensory evaluation.

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CHAPTER 2

LITERATURE REVIEW

2.1 *Carissa carandas*

Carissa carandas 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 aramda. 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 andperunkila 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).

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). Phytochemical studies revealed the presence of glycosides,

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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.

2.2. 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

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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. Microbes in meat

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. 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. 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.

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CHAPTER 3

RESEARCH METHODOLOGY

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

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

Fresh *C. carandas* fruits were collected from the local area in Ladkrabang and Samut Songkhram, Bangkok, Thailand. After washing under running tap water, they 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 systems (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.

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

— 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).

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

3.2.1 *In vitro* antioxidant activity of the extracts from *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 *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 1000, 750, 500, 250, and 100 mg/l (w/v) for the following antioxidant determination:

— 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 (1000, 750, 500, 250, and 100 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.

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— Reducing power ability

The reducing power of *C. carandas* extracts was measured by the method described by Kim (2012). In brief, one ml of each concentration (1000, 750, 500, 250, and 100 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.

— 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 (1000, 750, 500, 250, and 100 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.

— 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 (1000, 750, 500, 250, and 100 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.

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3.2.2 *In vitro* antimicrobial activity of the extracts from *C. carandas* fruits:

– 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.

– 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 100, 50, 25, 12.5, and 6.25 mg/ml (w/v).

– Agar well diffusion

Antibacterial property of the *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 petri plates 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 zone of inhibition (mm). The experiment was done three times and the mean values are presented.

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3.3 Evaluation of the effect of optimal crude extracts from *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

3.3.1 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), then the mixture was ground by a houlinette homogenator (DPA17, Tefal, Thailand). 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 and 0, 4, 8, 12 weeks at -20°C in refrigerator.

3.3.2 Quality of ground pork samples

– Chemical analysis:

+ Free scaveging radical activity (DPPH)

The radical scavenging activities of the meat samples were measured according to the method of Qwele *et al.* (2013). Briefly, two 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

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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 Gallego *et al.* (2015). 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 Ortuño *et al.* (2016). 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).

– 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).

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+ Color

The color of the samples was determined using a color measurement spectrophotometers (HunterLab MiniScan 4500S, The Stothard Group Ltd., United Kingdom). Colour was described in terms of the L^* (lightness), a^* (redness), and b^* (yellowness) colour 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 colourimetric value.

– Biological analysis:

Brief, 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 were 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.

+ 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.

+ Psychrophilic bacteria

0.1 ml the sample solutions of each dilution as described above were speared 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.

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+ Coliforms/*E. coli* (Chromocult)

0.1 ml the sample solutions of each dilution as described above were speared 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.

3.4 Evaluation of the effect of optimal crude extracts from *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 panels from the Department of Animal Production Technology and Fisheries, 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: 7 = extremely like; 6 = very like; 5 = like; 4 = neither like nor dislike; 3 = dislike; 2 = very dislike; 1 = extremely dislike) was used to score the samples.

3.5 Statistical analysis

The experimental design was carried out as a Randomized Completely Block Design (RCBD) with three replications and was repeated three times using one-way analysis of variance (ANOVA). 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.

3.6 Duration and location of research work

The research was carried out to study during 1 year (9/ 2016 – 9/ 2017) in laboratory and greenhouse experiments, Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok10520, Thailand.

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CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of solvent system on extraction yield and bioactive compounds from *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 *C. carandas* fruits. The extraction yields of various solvents decreased in the following order: 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). 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)

The recovery of phenolics and flavonoid contents was shown in Table 4.1. 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%

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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 are in agreement with the reports of Mehmood *et al.* (2014), who reported that phytochemical studies of *C. carandas* revealed the presence of flavonoids, phenolic, tanins 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 *C. carandas* fruit crude extracts by various solvent systems

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

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

All values were expressed as mean ± standard deviation

4.1.3 *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 4.2. The results were shown that 50% ethanol extract revealed the highest activity in lipid peroxidation (IC_{50}: 715.15 mg/l), reducing power

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

	IC_{50} (mg/l)			
Solvents (% ethanol)	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}

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

All values were expressed as mean ± standard deviation

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

The antimicrobial activity of *C. carandas* extracts using different solvents was evaluated according to their clear zone of inhibition against pathogenic and spoilage bacteria Table 4.3 – 4.8. Among extract solvent system, 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 *T. cordifolia*, *C. dichotoma*, *P. cineraria* and *C. 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

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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).

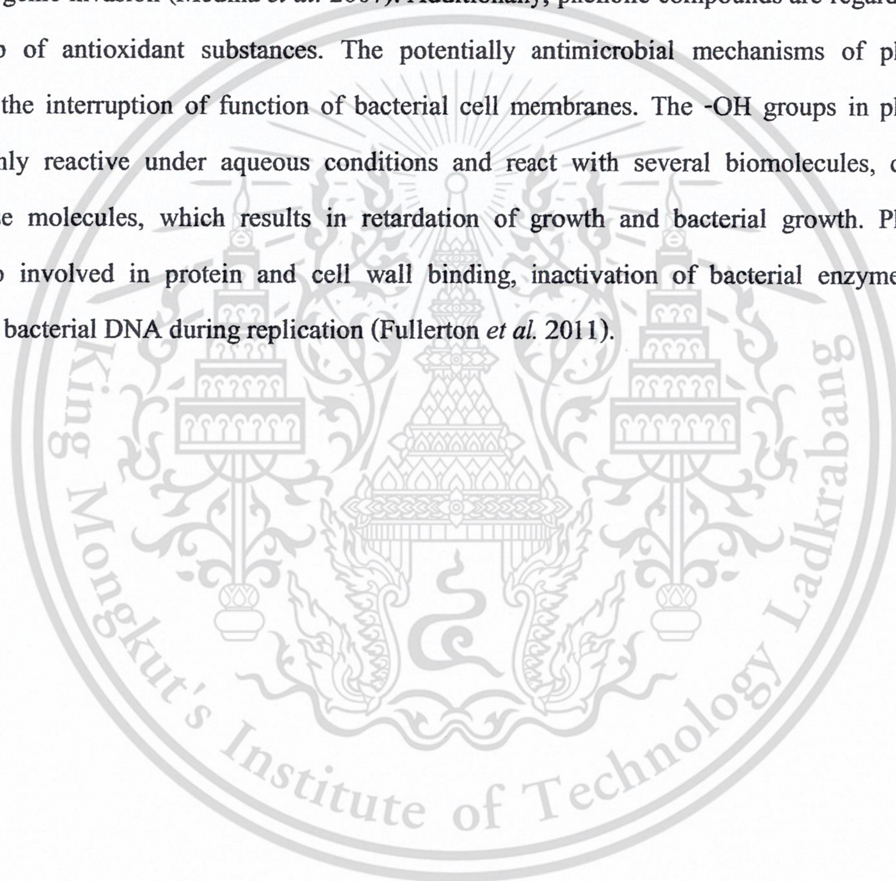
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Table 4.3 Effect of various crude extracts from *C. carandas* fruits on *in vitro* antimicrobial activity using agar well diffusion method

Cultures	Inhibiting zone of the extracts at 100 mg/ml (mm)				
	100% ethanol	75% ethanol	50% ethanol	25% ethanol	Water extract
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

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Table 4.4 Effect of ethanol crude extract from *C. carandas* fruits on antimicrobial activity using agar well diffusion method

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

Ni = No inhibition

All values were expressed as mean ± standard deviation

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Table 4.5 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)				
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Pathogenic bacteria					
Salmonella Typhimurium TISTR 292	28.33 ± 1.20	18.73 ± 0.23	14.00 ± 0.00	Ni	Ni
Staphylococcus aureus TISTR 118	26.11 ± 0.19	20.00 ± 0.00	15.00 ± 1.00	Ni	Ni
Escherichia coli TISTR 780	25.73 ± 0.23	17.00 ± 0.00	12.00 ± 0.00	Ni	Ni
Aeromonas hydrophila TISTR 1321	27.44 ± 0.51	22.00 ± 0.00	16.66 ± 0.00	Ni	Ni
Spoilage bacteria					
Pseudomonas fluorescens TISTR 358	21.66 ± 0.67	16.33 ± 1.20	12.66 ± 0.67	Ni	Ni
Lactobacillus plantarum ATCC 14947^{T}	16.78 ± 0.69	10.00 ± 0.50	Ni	Ni	Ni
Lactobacillus sakei TISTR 890	20.00 ± 1.00	15.00 ± 0.00	Ni	Ni	Ni
Leuconostoc mesenteroides subsp. *mesenteroides* TISTR 942	18.66 ± 0.67	15.00 ± 0.00	Ni	Ni	Ni
Streptococcus sp. TISTR 1030	12.55 ± 0.39	Ni	Ni	Ni	Ni
Lactococcus cremoris TISTR 1344	11.33 ± 0.34	Ni	Ni	Ni	Ni
Bacillus coagulans TISTR 1447	25.28 ± 0.25	17.00 ± 1.00	13.00 ± 1.00	Ni	Ni

Ni = No inhibition

All values were expressed as mean ± standard deviation

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Table 4.6 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)				
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Pathogenic bacteria					
Salmonella Typhimurium TISTR 292	20.00 ± 1.00	15.00 ± 0.00	11.00 ± 0.00	Ni	Ni
Staphylococcus aureus TISTR 118	25.39 ± 0.10	16.33 ± 0.33	12.00 ± 0.00	Ni	Ni
Escherichia coli TISTR 780	20.33 ± 0.33	14.00 ± 0.00	10.00 ± 0.00	Ni	Ni
Aeromonas hydrophila TISTR 1321	21.00 ± 0.00	15.66 ± 0.34	12.33 ± 0.33	Ni	Ni
Spoilage bacteria					
Pseudomonas fluorescens TISTR 358	18.66 ± 0.34	18.00 ± 0.00	13.66 ± 0.34	Ni	Ni
Lactobacillus plantarum ATCC 14947^T	12.00 ± 1.00	Ni	Ni	Ni	Ni
Lactobacillus sakei TISTR 890	15.00 ± 0.50	12.00 ± 1.00	Ni	Ni	Ni
Leuconostoc mesenteroides subsp. *mesenteroides* TISTR 942	15.66 ± 0.67	12.00 ± 1.00	Ni	Ni	Ni
Streptococcus sp. TISTR 1030	12.00 ± 1.00	12.33 ± 0.33	Ni	Ni	Ni
Lactococcus cremoris TISTR 1344	11.00 ± 1.00	15.33 ± 0.33	Ni	Ni	Ni
Bacillus coagulans TISTR 1447	20.28 ± 0.25	15.66 ± 0.67	12.00 ± 1.00	Ni	Ni

Ni = No inhibition

All values were expressed as mean ± standard deviation

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Table 4.7 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)				
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Pathogenic bacteria					
Salmonella Typhimurium TISTR 292	20.00 ± 0.34	16.33 ± 0.33	Ni	Ni	Ni
Staphylococcus aureus TISTR 118	17.33 ± 0.33	17.00 ± 0.00	Ni	Ni	Ni
Escherichia coli TISTR 780	18.33 ± 0.67	14.00 ± 0.00	Ni	Ni	Ni
Aeromonas hydrophila TISTR 1321	20.00 ± 0.00	17.00 ± 1.00	11.00 ± 0.00	Ni	Ni
Spoilage bacteria					
Pseudomonas fluorescens TISTR 358	20.33 ± 0.33	15.89 ± 0.77	Ni	Ni	Ni
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	16.00 ± 1.00	15.33 ± 1.20	Ni	Ni	Ni

Ni = No inhibition

All values were expressed as mean ± standard deviation

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

Cultures	Inhibiting zone of the extracts (mm)				
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Pathogenic bacteria					
Salmonella Typhimurium TISTR 292	19.00 ± 0.34	16.00 ± 0.00	8.00 ± 0.00	Ni	Ni
Staphylococcus aureus TISTR 118	20.33 ± 0.88	14.33 ± 0.67	Ni	Ni	Ni
Escherichia coli TISTR 780	17.00 ± 1.00	13.33 ± 0.00	Ni	Ni	Ni
Aeromonas hydrophila TISTR 1321	19.33 ± 0.58	16.00 ± 1.00	11.00 ± 0.00	Ni	Ni
Spoilage bacteria					
Pseudomonas fluorescens TISTR 358	18.28 ± 0.25	12.00 ± 0.00	Ni	Ni	Ni
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	19.00 ± 0.34	13.00 ± 0.00	Ni	Ni	Ni

Ni = No inhibition

All values were expressed as mean ± standard deviation

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(a) (b)

(c) (d)

Figure 4.1 Effect of 100% ethanol crude extract from *C. carandas* fruits on (a) *S. aureus* TISTR 118, (b) *S.*Typhimurium TISTR 292, (c) *E coli* TISTR 780 and (d) *P. fluorescens* TISTR 358 using agar well diffusion method

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4.2 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

4.2.1 Chemical analysis

+ Free scaveging 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 4.9. 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 comparation with raw sanples 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 was showed in Table 4.10. 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 comparation with raw sanples 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. 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

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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 4.11. 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 comparation with raw sanples 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 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).

+ Thiobarbituric acid reaction substances (TBARS)

Table 4.12 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 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. Although ethanol dried CWCE contained high bioactive compounds (Jain and Singhai. 2011; Sawhney *et al.* 2011), 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 content of the meat samples were 7.35 mg MDA/kg meat (2.5 g CWCE/kg), 6.85 mg

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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. This is generally 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.

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Table 4.9 Effect of water crude extract from *C. carandas* fruits on DPPH radical scavenging activity ground pork at 4°C

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

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

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

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Table 4.10 Effect of water crude extract from *C. carandas* fruits on ABTS radical cation decolorization in ground pork at 4°C

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

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

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

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Table 4.11 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	0.48 ± 0.78	0.61 ± 0.75	0.69 ± 0.76
	2	0.34 ± 0.20	0.43 ± 0.15	0.59 ± 0.20	0.67 ± 0.86
	4	0.33 ± 0.78	0.40 ± 0.85	0.55 ± 0.75	0.62 ± 0.98
	6	0.29 ± 1.07	0.37 ± 0.08	0.54 ± 0.45	0.59 ± 0.48
	8	0.30 ± 0.70	0.36 ± 0.04	0.50 ± 0.94	0.54 ± 0.80
After cooking	0	0.42 ± 0.14	0.58 ± 0.70	0.75 ± 0.84	0.84 ± 0.74
	2	0.40 ± 0.30	0.55 ± 0.75	0.72 ± 0.08	0.88 ± 0.12
	4	0.39 ± 0.50	0.54 ± 0.45	0.68 ± 1.10	0.76 ± 0.42
	6	0.34 ± 0.73	0.48 ± 0.20	0.60 ± 0.40	0.75 ± 1.08
	8	0.33 ± 0.08	0.46 ± 0.12	0.59 ± 1.15	0.72 ± 1.05

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Table 4.12 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 ± 0.03 ^{a,B}	2.99 ± 0.09 ^{a,C}	3.08 ± 0.02 ^{a,D}	3.01 ± 0.13 ^{a,D}
	2	3.15 ± 0.06 ^{a,B}	3.18 ± 0.08 ^{a,C}	3.19 ± 0.07 ^{a,CD}	3.16 ± 0.04 ^{a,CD}
	4	3.35 ± 0.06 ^{a,B}	3.24 ± 0.06 ^{a,B}	3.34 ± 0.08 ^{a,C}	3.32 ± 0.12 ^{a,C}
	6	4.06 ± 0.18 ^{a,A}	4.04 ± 0.08 ^{a,A}	4.04 ± 0.05 ^{a,B}	4.17 ± 0.05 ^{a,B}
	8	4.32 ± 0.15 ^{a,A}	4.19 ± 0.09 ^{a,A}	4.34 ± 0.15 ^{a,A}	4.44 ± 0.07 ^{a,A}
After cooking	0	12.75 ± 0.11 ^{a,D}	5.80 ± 0.09 ^{d,C}	7.35 ± 0.05 ^{b,C}	6.85 ± 0.06 ^{c,D}
	2	12.87 ± 0.04 ^{a,D}	6.00 ± 0.09 ^{d,C}	7.59 ± 0.09 ^{b,B}	7.01 ± 0.03 ^{c,D}
	4	13.39 ± 0.11 ^{a,C}	6.27 ± 0.09 ^{d,B}	8.02 ± 0.13 ^{b,A}	7.22 ± 0.03 ^{c,C}
	6	14.07 ± 0.02 ^{a,B}	6.45 ± 0.03 ^{d,B}	8.19 ± 0.07 ^{b,A}	7.81 ± 0.11 ^{c,B}
	8	14.34 ± 0.08 ^{a,A}	7.15 ± 0.12 ^{c,A}	8.14 ± 0.04 ^{b,A}	7.99 ± 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 are significantly different (P<0.05)

All values were expressed as mean ± standard deviation

4.2.2 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 4.13. 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, and the pH increase 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, which reflects the rate of post mortem glycolysis, is a key indicator of meat quality. In ground pork, which is mostly composed of red muscle fibers, post mortem glycolysis may be accompanied by rapid anaerobic carbohydrate degradation (Smiecinska *et al.* 2015).

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— 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 4.14. 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 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 show 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, awater-soluble protein known to be present in the cytoplasm. It is expected that the reaction might retard myoglobin oxidation and discoloration (Kroll and Rawel.

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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 retard this process to maintain meat redness longer. Therefore, we infer that bioactive compounds from CWCE retarted 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 4.13 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 ± 0.89^{a,A}	5.76 ± 0.05^{a,A}	5.78 ± 0.44^{a,A}	5.78 ± 0.71^{a,A}
2	6.09 ± 0.75^{a,A}	5.96 ± 0.87^{a,A}	6.06 ± 0.07^{a,A}	5.96 ± 0.13^{a,A}
4	6.06 ± 0.22^{a,A}	6.09 ± 0.06^{a,A}	6.06 ± 0.45^{a,A}	5.96 ± 0.43^{a,A}
6	6.19 ± 0.72^{a,A}	6.11 ± 1.01^{a,A}	6.07 ± 0.83^{a,A}	5.97 ± 0.02^{a,A}
8	6.36 ± 0.11^{a,A}	6.29 ± 0.08^{a,A}	6.08 ± 0.04^{a,A}	6.01 ± 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 ($P<0.05$)

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Table 4.14 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 ± 0.84 ^{a,C}	51.87 ± 0.17 ^{a,AB}	51.55 ± 0.92 ^{a,B}	51.78 ± 0.22 ^{a,B}
	2	51.94 ± 0.05 ^{a,C}	50.85 ± 0.22 ^{a,B}	51.44 ± 0.95 ^{a,B}	51.55 ± 1.05 ^{a,B}
	4	53.19 ± 0.27 ^{a,BC}	51.71 ± 0.11 ^{b,AB}	51.09 ± 0.72 ^{b,B}	51.04 ± 1.38 ^{b,B}
	6	54.20 ± 0.13 ^{a,AB}	52.25 ± 0.02 ^{ab,AB}	51.55 ± 0.79 ^{b,B}	51.95 ± 0.39 ^{b,B}
	8	55.34 ± 0.14 ^{a,A}	53.58 ± 0.10 ^{b,A}	53.35 ± 0.11 ^{b,A}	53.51 ± 0.22 ^{b,A}
Redness (a*)	0	6.06 ± 0.07 ^{a,A}	6.19 ± 0.20 ^{a,A}	6.02 ± 0.26 ^{a,A}	6.33 ± 0.15 ^{a,A}
	2	4.58 ± 0.14 ^{b,B}	5.82 ± 0.34 ^{a,A}	5.33 ± 0.26 ^{ab,AB}	5.49 ± 0.45 ^{a,A}
	4	4.22 ± 0.13 ^{a,B}	5.01 ± 0.34 ^{a,B}	5.29 ± 1.30 ^{a,ABC}	5.47 ± 0.18 ^{a,A}
	6	3.29 ± 0.04 ^{b,C}	4.55 ± 0.15 ^{a,B}	4.17 ± 0.07 ^{a,BC}	4.71 ± 0.15 ^{a,AB}
	8	2.58 ± 0.11 ^{c,D}	3.45 ± 0.10 ^{b,C}	3.59 ± 0.37 ^{ab,C}	4.07 ± 0.17 ^{a,AB}
Yellowness (b*)	0	14.37 ± 0.97 ^{a,B}	14.18 ± 0.37 ^{a,B}	14.12 ± 0.05 ^{a,B}	14.14 ± 0.17 ^{a,B}
	2	14.67 ± 0.86 ^{a,B}	14.41 ± 0.57 ^{a,AB}	14.45 ± 0.24 ^{a,AB}	14.84 ± 0.26 ^{a,AB}
	4	14.23 ± 0.89 ^{a,B}	15.01 ± 0.12 ^{a,AB}	15.29 ± 0.08 ^{a,AB}	15.50 ± 0.01 ^{a,AB}
	6	16.12 ± 0.26 ^{a,A}	14.99 ± 0.53 ^{a,AB}	15.36 ± 0.48 ^{a,AB}	15.48 ± 0.06 ^{a,AB}
	8	16.96 ± 0.50 ^{a,A}	15.38 ± 0.30 ^{b,A}	15.77 ± 0.34 ^{b,A}	15.89 ± 0.20 ^{b,A}

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

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

All values were expressed as mean ± standard deviation

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4.2.3 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 4.15. 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 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 by although CWCE contained high bioactive compounds but it did not act as an antimicrobial (Jain and Singhai. 2011; Sawhney *et al.* 2011).

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Table 4.15 Effect of water extract from *C. carandas* fruits on biological quality in ground pork during storage at 4°C

Storage time (days)		Treatments			
		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 are significantly different (P<0.05)

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4.3 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

4.3.1 Chemical analysis

— Free scaveging radical activity (DPPH)

DPPH Radical scavenging activity: DPPH radical scavenging activitywas 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 4.16. 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 4.17. 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

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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 4.18. 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 4.19 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

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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 4.16 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 ($P<0.05$)

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

All values were expressed as mean ± standard deviation

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Table 4.17 Effect of water crude extract from *C. carandas* fruits 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 CWCE/kg	5 g CWCE/kg
Before cooking	0	36.71 ± 0.70 ^{d,A}	65.11 ± 0.82 ^{c,A}	67.34 ± 0.06 ^{b,A}	74.11 ± 0.04 ^{a,A}
	4	36.75 ± 0.52 ^{c,A}	66.15 ± 0.12 ^{b,A}	68.04 ± 0.45 ^{b,A}	73.98 ± 0.31 ^{a,A}
	8	35.95 ± 0.21 ^{d,A}	66.27 ± 0.11 ^{c,A}	67.28 ± 0.11 ^{b,A}	74.20 ± 0.03 ^{a,A}
	12	36.81 ± 0.24 ^{d,A}	65.25 ± 0.57 ^{c,A}	68.09 ± 0.02 ^{b,A}	74.31 ± 0.21 ^{a,A}
After cooking	0	39.17 ± 0.84 ^{d,A}	69.35 ± 0.72 ^{c,A}	71.74 ± 0.75 ^{b,A}	78.86 ± 0.03 ^{a,A}
	4	39.67 ± 0.23 ^{d,A}	70.04 ± 0.03 ^{c,A}	72.89 ± 0.07 ^{b,A}	77.92 ± 0.12 ^{a,A}
	8	38.87 ± 0.06 ^{d,A}	69.94 ± 0.07 ^{c,A}	72.75 ± 0.42 ^{b,A}	77.95 ± 0.05 ^{a,A}
	12	39.06 ± 0.03 ^{d,A}	69.87 ± 0.21 ^{c,A}	72.02 ± 1.11 ^{b,A}	77.61 ± 0.51 ^{a,A}

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

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

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Table 4.18 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 ± 0.85	0.52 ± 0.20	0.60 ± 0.70	0.74 ± 0.20
	4	0.37 ± 0.09	0.51 ± 0.10	0.61 ± 0.25	0.70 ± 0.95
	8	0.37 ± 1.25	0.50 ± 0.40	0.57 ± 0.18	0.69 ± 0.16
	12	0.36 ± 0.14	0.48 ± 0.75	0.55 ± 0.15	0.68 ± 0.96
After cooking	0	0.45 ± 0.30	0.58 ± 0.15	0.75 ± 0.05	0.86 ± 0.28
	4	0.44 ± 0.22	0.56 ± 0.78	0.74 ± 0.24	0.83 ± 0.30
	8	0.42 ± 0.78	0.55 ± 0.85	0.71 ± 0.17	0.81 ± 0.84
	12	0.40 ± 0.08	0.52 ± 0.28	0.68 ± 0.18	0.78 ± 0.77

All values were expressed as mean ± standard deviation

Table 4.19 Effect of water crude extract from *C. carandas* fruits on total MDA in ground pork at -20°C

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

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4.3.2 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 are summarized in Table 4.20. 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 4.20 Effect of water extract from *C. carandas* fruits on pH in ground pork during storage at -20°C

Storage time (weeks)	pH values			
	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

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4.3.3 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 4.21. 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 devolopment 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 devolopment 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).

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Table 4.21 Effect of water extract from *C. carandas* fruits on biological quality in ground pork during storage at -20°C

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

All values were expressed as mean ± standard deviation

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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 *C. carandas* fruits

1. The highest crude yield was obtained from water solvent for *C. carandas* fruit extracts.

2. 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 *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. 100% ethanol solvent showed more effective than other solvents and extracts showed varying degrees of antimicrobial activity against all the tested bacteria.

Experiment 3: Evaluation of the effect of optimal crude extracts from *C. carandas* fruits on chemical, physical and biological quality in ground pork storage at $4^{o}C$ up to 8 days.

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1. According to the DPPH, ABTS and reducing power assays, the ground pork samples treated with 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 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 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 CWCE were significantly lower while the redness (a*) values higher than the control samples at the end of the storage ($P<0.05$).

4. 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 *C. carandas* fruits on chemical, physical and biological quality in ground pork stored 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 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 CWCE when compared to control and BHT ($P<0.05$).

2. Addition of 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.

Experiment 5: Evaluation of the effect of optimal crude extracts from *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 CWCE did not adversely effect on the sensory properties of the pork samples.

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REFERENCES

Baba, S.A., 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., 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., Monteiro, M.A.M., 2010. Sensory evaluation of rice fortified with iron. Ciência e Tecnol. Aliment. 30, 516–519.

Beyhan, Ö., Elmasta, M., 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.

Biswas, B., Rogers, K., Mclaughlin, F., Daniels, D., 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.

Bozoglu, F., Alpas, H., 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.

Carpenter, R., O'Grady, M.N., O'Callaghan, Y.C., O'Brien, N.M., 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.

Chumyam, A., Whangchai, K., Jungklang, J., Faiyue, B., Saengnil, K., 2013. Effects of heat treatments on antioxidant capacity and total phenolic content of four cultivars of purple skin eggplants. ScienceAsia 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., 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., 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.

Dent, M., Dragovic-Uzelac, V., Penic, M., Brnc, M., Bosiljkov, T., Levaj, B., 2013. The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in

This material is reserved for educational use only, not allowed for commercial use.
Forbidden to modify the content, and cite the document when use.

dalmatian wild sage (*Salvia officinalis* L.) extracts. Food Technol. Biotechnol. 51, 84–91.

Ebrahimzadeh, M.., Nabavi, S.., Nabavi, S.., Bahramian, F., Bekhradnia, R., 2008. Antioxidant and Free Radical Scavenging Activity. Pak. J. Pharm. Sci. 23, 29–34.

Fasseas, M.K., Mountzouris, K.C., Tarantilis, P.A., Polissiou, M., 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., 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., Simões de Assis, T., 2012. Total Phenolic Content and Antioxidant Activity of Some Malvaceae Family Species. Antioxidants 1, 33–43.

Foo, S.C., Yusoff, F.M., Ismail, M., Basri, M., Khong, N.M.H., Chan, K.W., 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., Williams, L.L., 2011. Determination of antimicrobial activity of sorrel (*Hibiscus sabdariffa*) on *Esherichia 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., Almajano, M.P., 2015. *Caesalpinia decapetala* extracts as inhibitors of lipid oxidation in beef patties. Molecules 20, 13913–13926.

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

Huang, B., He, J., Ban, X., Zeng, H., Yao, X., 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., 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., 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.

This material is reserved for educational use only, not allowed for commercial use.
Forbidden to modify the content, and cite the document when use.

Jain, N.K., 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.

Jałosińska, M., 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., 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.

Karakaya, M., Bayrak, E., Ulusoy, K., 2011. Use of Natural Antioxidants in Meat and Meat Products. J. food Sci. Eng. 1, 1–10.

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

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., Min, S.C., Shin, H.J., Lee, Y.J., Cho, A.R., Kim, S.Y., Han, J., 2013. 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.

Kroll, J., Rawel, H., 2001. Reactions of plant phenols with myoglobin : influence of chemical structure of the phenolic compounds. JFS Food Chemiistry Toxicol. 66, 48–58.

Llópiz, N., Puiggròs, F., Céspedes, E., Arola, L., Ardévol, A., Bladé, C., 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., Fauziah, O., 2013. Antioxidant analysis of different parts of *Carica papaya*. Int. Food Res. J. 20, 1043–1048.

Medina, I., Gallardo, J.M., Gonzlez, M.J., Lois, S., 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., Gilani, A.H., 2014. Pharmacological basis for the medicinal use of *Carissa carandas* in constipation and diarrhea. J. Ethnopharmacol. 153, 359–367.

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

Ortuño, J., Serrano, R., Jordán, M.J., Bañón, S., 2016. Relationship between antioxidant status and

Forbidden to modify the content, and cite the document when use.

oxidative stability in lamb meat reinforced with dietary rosemary diterpenes. Food Chem. 190, 1056–1063.

Qwele, K., Hugo, A., Oyedemi, S.O., Moyo, B., Masika, P.J., 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., 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.

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

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

Rojas, J.J., Ochoa, V.J., Ocampo, S.A., 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.

Salar, R.K., 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., 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., 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.

Singh, A., 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., 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.

Śmiecińska, K., Hnatyk, N., Daszkiewicz, T., Kubiak, D., Matusevičius, 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., Sahin, F., 2004. The in vitro antimicrobial and antioxidant activities of the essential oils and methanol

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extracts of endemic *Thymus spathulifolius*. Food Control 15, 627–634.

Sun, C., Wu, Z., Wang, Z., 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.

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

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

Tan, M.C., Tan, C.P., 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.

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

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

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