

## Antibacterial and antioxidant effects of tropical citrus peel extracts to improve the shelf life of raw chicken drumettes

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### Abstract

The antibacterial and antioxidant effects of different tropical citrus peel extracts (Kaffir lime, Lime and Pomelo) obtained from ethanol and ethyl acetate extraction in raw chicken drumettes during storage at 4°C were studied. All citrus peels showed antibacterial activities against pathogenic bacteria with minimum inhibitory concentration (MIC) and minimum bactericidal concentration ranged from 0.4 to 50.0 mg/ml. The ethyl acetate-extracted Kaffir lime peel (KEa) indicated the highest antimicrobial, antioxidant activities and total phenolic contents. The total viable counts, 2-thiobarbituric acid reactive substances values of KEa-treated chicken wing samples were lower than those of control samples while the sensory properties maintained significantly ( $p < 0.05$ ) higher values during 14 days of storage. These results suggested that the tropical citrus peel extracts, especially Kaffir lime, are very effective against microbial growth, lipid oxidation and has potential as a natural antimicrobial and antioxidant for shelf life extension of chicken drumettes.

### Keywords

Citrus  
Antimicrobial agents  
Antioxidant activity  
Shelf life  
Poultry

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### Introduction

Consumer demand for minimally processed foods preserved with natural ingredients has increased, whereas the safety aspect of chemical additives has been questioned. Many plant extracts containing phenolic compounds have recently gained a great popularity and scientific interest. The phenolic compounds presenting in the extracts have been recognized as the bioactive components for the antimicrobial and antioxidant activity. The extracts, especially from many herbal plants were exhibited antimicrobial activity against many pathogenic microorganisms (Melendez and Capriles, 2006). Most plant phenolic compounds are classified as Generally Recognized as Safe (GRAS) substances, therefore they could be good candidates to improve the shelf life and the safety of certain food by preventing growth of many food-borne and food spoilage microorganisms and the oxidation in foods.

Citrus fruits, which are native to the tropical and subtropical regions of Asia, belong to six genera (*Fortunella*, *Eremocitrus*, *Clymendia*, *Poncirus*, *Microcitrus* and *Citrus*). The major commercial fruits belong to the genus *Citrus*, including several important fruits serving as fruits themselves and

also as spices and seasonings such as oranges, mandarins, lime, kaffir lime, lemons and grape fruits (Chanthaphon *et al.*, 2008). Therefore, in daily life and in processing line using citrus fruit as the major raw materials such as beverage, canning and minimally processing, citrus peels remain, for the major part, unutilized and disposed as wastes. Since it is well known that the extracts from citrus peels are rich source of antioxidant and antimicrobial against various microorganisms, another way to valorize these byproducts could be their application in food. The peels are an interesting source of phenolic compounds which include phenolic acids and flavonoids (flavanones, flavones, flavonols, anthocyanins and coumarins) and monoterpene hydrocarbons, exhibiting antimicrobial activity in several studies (Cushnie and Lamb, 2005). There are many studies focus on the bioactivities of essential oils from citrus peels. For instance, Kaffir lime and Lime exhibited antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhi*, both in the form of liquid and vapour (Chaisawadi *et al.*, 2003), in model systems and fruit product (Fisher, *et al.*, 2009). However, the ethyl acetate extracts of the citrus peels exhibited more effect against food-borne bacteria compared to the essential oil obtained

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from hydrodistillation (Chanthaphon *et al.*, 2008).

Minimally processed chicken products are gaining high popularity worldwide over the last decade due to low production cost, high nutritional value and distinct flavor. However, controlling the cross-contamination of microorganisms during slaughtering, processing, storage, handling, and preparation is a complex challenge (Mandrell and Wachtel, 1999). Quartered chicken products to obtain chicken drumettes resulting in an increased surface to weight ratio, and the skin covering the wing with rough surface may further enhance product quality deterioration due to additional microbial contamination, difficulty of microbial elimination and high risk for lipid oxidation. Consequently, seeking the technology to prolong the shelf life and overall safety and quality of highly perishable chicken wing products represents a major concern of the poultry processing industry. Edible coating containing approved antimicrobials and antioxidants could be applied on carcass washing process. The agents would gradually diffuse from the coating material into skin irregularities.

The main goal of this research was the evaluation of the bioactivity of the extracts of various tropical citrus cultivars available in Thailand obtained by ethanol and ethyl acetate, 2 kinds of solvents with different in polarity index and water solubility, towards the antioxidant activity and the inhibition of pathogenic bacteria regarding Gram positives (*S. aureus* and *B. cereus*) and Gram negatives (*E. coli* and *S. typhimurium*). In the application part, the bioactivity of the most effective citrus peel extract in prolonging the shelf life of raw chicken drumettes was also investigated.

## Materials and Methods

### Plant materials

Fruits of three citrus cultivars of Kaffir lime (*Citrus hystrix* DC.), Lime (*Citrus aurantifolia* Swingle) and Pomelo (*Citrus maxima* Merr.) were obtained at the mature stage from local market around Phitsanulok province area during July to September, 2014.

### Chemicals and reagents

Ethanol and ethyl acetate and dimethyl sulfoxide (DMSO) were obtained from Labscan, Poland. Folin-Ciocalteu reagent was purchased from Loba Chemie, India. Tween 80, sodium carbonate and peptone were purchased from Merck, Germany. Gallic acid and 2, 2-diphenyl-1-picrylhydrazyl (DPPH), chloramphenicol, thiobarbituric acid (TBA),

butylated hydroxytoluene (BHT) and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich Chemicals, Germany. Tryptic Soy Broth (TSB), Tryptic Soy Agar (TSA) and Plate Count Agar (PCA) were purchased from Himedia Laboratories, India.

### Bacterial cultures

*Bacillus cereus* TISTR 687, *Staphylococcus aureus* TISTR 118, *Escherichia coli* TISTR 780, *Salmonella* Typhimurium TISTR 292 from Microbiological Resources Center of Thailand Institute of Scientific and Technological Research, were kept at -20 °C. The cultures were grown on TSA and subcultured in TSB at 35 °C for 24 h before used. Each of the bacterial cell concentration was then adjusted to obtain final concentration of 10<sup>7</sup> CFU/ml.

### Preparation of citrus peel extracts

The grinded citrus peels were extracted by soaking with ethanol and ethyl acetate in the ratio of 1:4 with shaking at room temperature for 3 days. The extracts were filtered through Whatman No.4. filter paper (Whatman, Kent, UK). The filtrates were evaporated to dryness by rotary-vacuum evaporator (Buchi RTE-221, Flawil, Switzerland) and weighed to determine the yields.

### Analysis of citrus peel extracts

#### Determination of total phenolic content (TPC)

TPC of the extracts were measured according to the Folin–Ciocalteu method of Singleton and Rossi (1965) with slight modification. Briefly, 100 µL of the extract made into 5 mg/ml with methanol was mixed with Folin–Ciocalteu reagent (1:9 of Folin–Ciocalteu reagent :distilled water). The mixture was added with 1.0 ml of sodium carbonate solution 15 % (w/v) and vigorously vortexed for 2 minutes. The final volume was made up to 5.0 ml with methanol and left in darkness at room temperature for 2 hours. Then the absorbance of the mixture was recorded at 750 nm using a spectrophotometer (Thermo Scientific, Genesys, USA). Gallic acid was used as the standard for the calibration curve. The amount of total phenolic was calculated as mg gallic acid equivalents (GAE) per gram of sample.

#### Determination of DPPH radical scavenging activity

Free radical scavenging activity was determined by a DPPH radical scavenging assay, according to the modified method of Brand-Williams *et al.* (1995). Briefly, 100 µM of DPPH in methanol was prepared and 2 ml of this solution was added to 1 ml of 5 mg/ml of sample solution. The reaction mixture was shaken

well and left in the darkness for 30 min at room temperature. The absorbance of the resulting solution was recorded at 517 nm using a spectrophotometer. Each sample was measured in triplicate and averaged. The percentage radical scavenging activity (RSA) was calculated using the following formula:

$$\% \text{ RSA} = [(A_0 - A_1)/A_0] \times 100$$

where  $A_0$  is the absorbance of the control, and  $A_1$  is the absorbance of samples after reaction. The free radical scavenging activities of the extracts were expressed as Inhibition Concentration 50 value ( $IC_{50}$ ). The  $IC_{50}$  value was defined as concentration in mg/ml of sample that inhibits 50% of the formation of DPPH radical.

#### *Assay for antibacterial activity*

Antibacterial activity of citrus peel extracts was determined using agar disc diffusion method (Oke *et al.*, 2009). A 100  $\mu\text{L}$  of bacterial suspension ( $10^7$  CFU/ml) was spread homogeneously onto the surface of TSA. Sterile filter paper discs (6 mm diameter) were placed on the agar surface and dropped with 6  $\mu\text{L}$  of the 50 mg/mL of the extract in DMSO. DMSO and 1 mg/ml of chloramphenicol were used as negative and positive control, respectively. After 15 min of diffusion time at room temperature, the plates were incubated at 35°C for 24 h. The inhibition zone diameters were measured in millimeter.

A broth micro-dilution method was performed to determine the minimum inhibitory concentration (MIC) of each citrus peel extract according to the method of Jorgensen and Turnidge (2007). The extract was added at two-fold dilution manner, ranging from 0.2 to 50 mg/ml in 2 ml of each bacterial suspension ( $10^7$  CFU/ml). After incubation at 35°C for 24 h, the lowest concentration of the extract required to inhibit visible growth of the tested microorganism was designated as the MIC. A 100  $\mu\text{L}$  of mixture of bacterial suspension and the extract culture from MIC test were taken from each of the broth tubes that showed no growth and introduced onto fresh TSA plates. After incubation at 35°C for 24 h, the plates were observed for growth. The concentration of the extracts that showed no visible growth was recorded as the minimum bactericidal concentration (MBC).

#### *Application of extracts in raw chicken drumettes and analysis of the samples*

##### *Sample preparation*

Raw chicken wing drumettes (30 $\pm$ 5 g each) were provided by a local poultry processing plant in

Sukhothai province, Thailand. They were quartered within 1 h after slaughtering and after the meat had achieved 4°C after being stored in a chill chamber. They were placed in polystyrene boxes on ice and transferred to the laboratory within 1 h of slaughtering and stored under refrigeration at 4°C. All the chicken drumettes were collected from the same batch at the plant. The surface decontamination was conducted by dipping the chicken wing into one of the following treatments for 1 min at room temperature (25°C): no dipping, dipping in water and dipping in the solution of the most effective citrus peel extract in different concentration. After dipping, the samples were aseptically air-dried and packed in polystyrene tray and wrapped with plastic film under aerobic conditions, before being kept at 4°C. Chicken wing samples were removed on 0, 2, 4, 6, 8, 10, 12, 14 days of storage and analyzed for pH, TBA reactive substances (TBARS), total viable count (TVC), and sensory attributes (appearance and odour) at each storage interval.

##### *Measurement of pH*

pH was determined using the method of AOAC (1995) after appropriate modification. A deboned chicken wing sample was homogenized in 100 ml of sterile distilled water and the mixture was filtered. The pH of the filtrate was measured using a Docu-pH meter (Sartorius, Goettingen, Germany).

##### *Measurement of TBARS*

TBARS produced from lipid peroxidation were determined using the method of Alasnier *et al.* (2000). A 4 g portion of each sample was blended with 16 ml of 5% trichloroacetic TCA and BHT (10  $\mu\text{g}$  BHT/g of lipids). It was then filtered through Whatman No. 4 filter. Equal amount of the filtrate and TBA was heated in a boiling water bath for 30 min, cooled and the absorbance was measured at 532 nm. The amount of TBARS was expressed as mg malonaldehyde (MDA) per kg of sample.

##### *Microbiological analysis*

A deboned chicken wing sample was aseptically homogenized in 100 ml of 0.1% sterile peptone water in a stomacher bag. A 100  $\mu\text{L}$  of an appropriate serially diluted homogenate was spread on the surface of PCA. The TVC was enumerated after incubation at 30°C for 2 days.

##### *Sensory analysis*

Thirty seven panelists selected from the students and staffs of the Agro-Industry Department of Naresuan university, carried out the sensory evaluations of the

Table 1. Extraction yield, TPC (mg GAE/g) and IC<sub>50</sub> of citrus peel extracts

Activity	Ethanol			Ethyl acetate		
	Kaffir lime	Lime	Pomelo	Kaffir lime	Lime	Pomelo
Extraction yield (%)	8.0±1.3 <sup>b</sup>	1.3±0.3 <sup>c</sup>	2.7±0.5 <sup>c</sup>	24.5±1.0 <sup>a</sup>	11.0±1.0 <sup>b</sup>	28.0±1.6 <sup>a</sup>
TPC (mg GAE/g)	39.6±1.9 <sup>a</sup>	26.0±2.5 <sup>b</sup>	18.0±1.9 <sup>b</sup>	46.6±1.9 <sup>a</sup>	35.1±3.0 <sup>b</sup>	22.4±2.0 <sup>b</sup>
DPPH IC <sub>50</sub> (mg/ml)	1.5±0.0 <sup>ab</sup>	4.0±0.0 <sup>b</sup>	4.4±0.1 <sup>b</sup>	0.5±0.0 <sup>a</sup>	3.2±0.1 <sup>b</sup>	4.5±0.2 <sup>b</sup>

a, b, c with different lowercase letters in the same row are significantly different ( $p < 0.05$ )

Table 2. Inhibition zone (mm), MIC and MBC (mg/ml) of citrus peel extracts against pathogenic bacteria

Bacterial strain	Antibacterial analysis	Ethanol			Ethyl acetate		
		Kaffir lime	Lime	Pomelo	Kaffir lime	Lime	Pomelo
<i>E. coli</i>	Inhibition zone (mm)	NI	NI	NI	22.3±0.2 <sup>a</sup>	9.7±0.1 <sup>b</sup>	NI
	MIC (mg/ml)	>50.0	>50.0	>50.0	0.4	3.1	>50.0
	MBC (mg/ml)	>50.0	>50.0	>50.0	0.4	6.3	>50.0
<i>S. Typhimurium</i>	Inhibition zone (mm)	7.3±0.1 <sup>c</sup>	NI	NI	19.0±0.0 <sup>a</sup>	8.3±0.1 <sup>b</sup>	7.3±0.1 <sup>c</sup>
	MIC (mg/ml)	6.3	>50.0	>50.0	0.8	6.3	12.5
	MBC (mg/ml)	12.5	>50.0	>50.0	0.8	6.3	12.5
<i>B. cereus</i>	Inhibition zone (mm)	7.3±0.1 <sup>b</sup>	NI	6.7±0.6 <sup>b</sup>	10.3±0.1 <sup>a</sup>	9.7±0.1 <sup>b</sup>	7.0±0.0 <sup>b</sup>
	MIC (mg/ml)	6.3	>50.0	12.5	3.1	3.1	12.5
	MBC (mg/ml)	6.3	>50.0	12.5	3.1	6.3	12.5
<i>S. aureus</i>	Inhibition zone (mm)	9.0±0.1 <sup>b</sup>	NI	NI	18.0±0.1 <sup>a</sup>	8.7±0.6 <sup>b</sup>	8.3±0.1 <sup>b</sup>
	MIC (mg/ml)	3.1	>50.0	>50.0	6.3	3.1	6.3
	MBC (mg/ml)	6.3	>50.0	>50.0	6.3	6.3	12.5

NI = No inhibition.

a, b, c with different lowercase letters in the same row are significantly different ( $p < 0.05$ )

chicken wing samples. Acceptability of appearance and odour was scored on a 9-point hedonic scale with 9 corresponding to the most liked sample and 1 corresponding to the least liked sample. The lower acceptable score of 6 was taken as the lower limit of acceptability (Mexis *et al.*, 2009).

#### Statistical analysis

All experiments were carried out in triplicate and average values with standard errors were reported. Mean values of various parameters were computed and compared by analysis of variance (ANOVA) using the SPSS software (version 13.0). Microbiological data were transformed into logarithms of the number of colony forming units (CFU/g). Means and standard errors were calculated. Significance was defined at  $p < 0.05$ .

## Results and Discussion

#### Effect of cultivars and extraction solvents on production yield, TPC, DPPH radical scavenging activity and antimicrobial activity of citrus peel extracts

##### Production yields

Effect of cultivars and extraction solvents on production yield of citrus peel extracts has been investigated. From the differences in polarity index (ethanol 5.2, ethyl acetate 4.4) and water solubility (ethanol 100%, ethyl acetate 8.7%) of the solvents, the extraction yields were compared. The extraction

yields of ethanol and ethyl acetate extracts from fresh peels of citrus fruit varied depending on cultivars (Table 1). For each cultivar, the production yields of the ethanol extract were much lower than that of the ethyl acetate extract. Ethyl acetate extraction of Kaffir lime (KEa), Lime (LEa) and Pomelo (PEa) peels provided the production yields of 24.5±1.0, 11.0±1.0 and 28.0±0.6 %, whereas only 8.0±1.3, 1.3±0.3 and 2.7±0.5% yields, respectively were obtained from ethanol extraction. Pomelo peel yielded the highest amount of ethyl acetate extract. The lowest yields of both solvents were obtained from lime peel.

##### TPC

Phenolic compounds, secondary metabolites produced by most plants, are generally responsible for the antioxidant activity of many fruits and vegetables. The Folin–Ciocalteu procedure has been proposed to rapidly estimate the level of TPC in foods and supplements (Prior *et al.*, 2005). The results showed that both solvents showed no significant difference in the ability to extract phenolic compounds (Table 1). The significant difference of the ability appeared between different citrus cultivars. Kaffir lime exhibited the highest TPC of 46.6±1.9 and 39.6±1.9 mg GAE/g for ethyl acetate and ethanol extract, respectively, while Pomelo showed the lowest with no significant difference with Lime.

##### DPPH radical scavenging activity

As shown in Table 1, in correlation with TPC, it was evident that the solvent type showed no significant

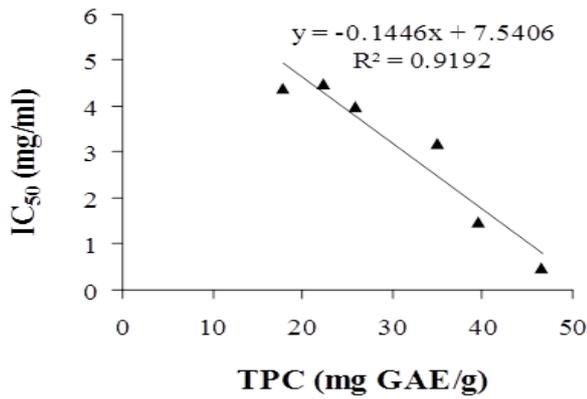


Figure 1. Correlation between TPC and IC<sub>50</sub> of citrus peel extracts

difference in DPPH radical scavenging activity of the extracts. However, by far the most potent radical scavenging activity was displayed by KEa with IC<sub>50</sub> of 0.5±0.0 mg/ml. KEa scavenged the DPPH radical more efficiently than PEa as IC<sub>50</sub> of KEa was nine times higher than that of PEa, indicating that PEa lacked hydrogen donating capacity.

Several studies have revealed positive relationship between TPC and antioxidant activity in many parts of plants and fruits. It is considered that the antioxidant activity of phenolic compounds is due to their high redox potentials, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Radha *et al.*, 2014). Our results support the above earlier findings (Figure 1). Therefore, the TPC could be used as an indicator, due to the presence of the antioxidant capacity of citrus peel extracts.

#### Antimicrobial activity

The antibacterial activity of citrus peel extracts was measured using disc diffusion assay followed by broth dilution assay in order to determine MIC. Various degrees of inhibition against pathogenic bacterial strains were shown and the results were given in Table 2. It was evident that solvent type affects antibacterial activity. Ethyl acetate showed antibacterial effect against all strains while ethanol showed no effect against *E. coli*. KEa inhibited *E. coli* showing the greatest inhibition zone of 22.3±0.2 mm with the lowest MIC/MBC determined at 0.4 mg/ml, while PEa showed no inhibition zone against *E. coli*. Moreover, KEa exhibited no significant difference compared with the positive control, chloramphenicol showing inhibition zone of 25.0±0.3 mm against *E. coli* (data not shown). *S. typhimurium* and *S. aureus* were also found to be susceptible to KEa, showing inhibition zone of 19.0±0.0 and 18.0±0.1 mm, with MIC/MBC of 0.8 and 6.3 mg/ml, respectively. The

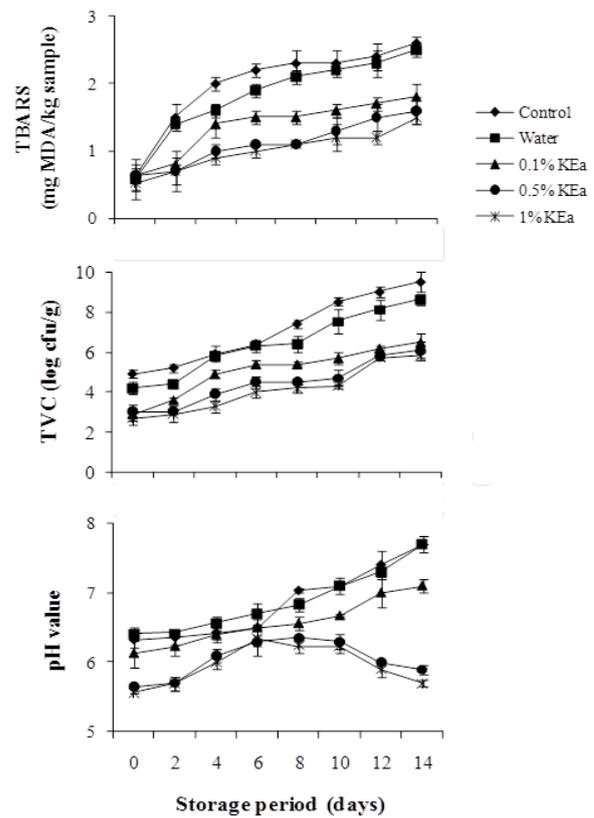


Figure 2. Effect of KEa on TBARS, TVC and pH value of raw chicken drumette during storage at 4°C

antibacterial activity of citrus peel extracts was in the following order: Kaffir lime > Lime > Pomelo. Base on KEa data, the susceptibility of the test bacteria was in the following order: *E. coli* > *S. Typhimurium* > *S. aureus* > *B. cereus*. These results were consistent with the previous studies on antibacterial effects of other plant extracts generally showing higher ability to inhibit Gram positive rather than Gram negative bacteria (Oke *et al.*, 2009; Melendez and Capriles, 2006). Therefore, KEa is an expectable candidate as a natural antibacterial agent effective against broad spectrum of both Gram positive and Gram negative bacteria. Furthermore, KEa obtained in this study exhibited more effective against Gram negative bacteria than Kaffir lime essential oil reported in previous studies having MIC against *E. coli* and *S. Typhimurium* at > 2.5 mg/ml (Chanthaphon *et al.*, 2008).

Base on ethyl acetate extraction, KEa exhibited the highest antioxidant along with antimicrobial activities while PEa exhibited the lowest. Hence, there may be the correlation of these two parameters, antioxidant and antimicrobial activities. Some researchers have also reported that phenolic compounds from different plant sources could inhibit various food-borne pathogens, and the TPC was highly correlated with antibacterial activity (Radha

Table 3. Effect of KEa on pH values and sensory attributes of chicken drumettes during 4°C storage

Sensory attributes	Treatment	Storage period (days)							
		0	2	4	6	8	10	12	14
Appearance	Control	9.0±0.0 <sup>Aa</sup>	8.2±0.4 <sup>ABa</sup>	7.0±0.1 <sup>Bb</sup>	5.6±0.4 <sup>Bc</sup>	5.4±0.1 <sup>Bc</sup>	4.3±0.4 <sup>ABc</sup>	4.2±0.2 <sup>Bd</sup>	3.3±0.2 <sup>Bd</sup>
	Water	8.7±0.3 <sup>Aa</sup>	8.0±0.3 <sup>ABa</sup>	7.0±0.6 <sup>Bb</sup>	5.5±0.3 <sup>Bc</sup>	4.9±0.1 <sup>Bc</sup>	4.2±0.5 <sup>Bc</sup>	4.0±0.3 <sup>Bc</sup>	3.4±0.1 <sup>Bd</sup>
	0.1%	8.8±0.1 <sup>Aa</sup>	8.7±0.1 <sup>Aa</sup>	8.8±0.1 <sup>Aa</sup>	8.3±0.3 <sup>Aa</sup>	6.3±0.5 <sup>Bb</sup>	4.9±0.2 <sup>Ab</sup>	4.6±0.1 <sup>Ac</sup>	4.1±0.1 <sup>Ac</sup>
	0.5%	8.9±0.4 <sup>Aa</sup>	8.6±0.4 <sup>Aa</sup>	8.7±0.2 <sup>Aa</sup>	8.2±0.2 <sup>Aa</sup>	7.5±0.2 <sup>Ab</sup>	4.8±0.3 <sup>Ab</sup>	4.0±0.1 <sup>Bc</sup>	4.0±0.4 <sup>Ac</sup>
	1.0%	8.5±0.2 <sup>Aa</sup>	8.6±0.4 <sup>Aa</sup>	8.8±0.3 <sup>Aa</sup>	8.4±0.4 <sup>Aa</sup>	7.6±0.3 <sup>Ab</sup>	4.3±0.1 <sup>ABc</sup>	4.3±0.2 <sup>ABc</sup>	4.0±0.2 <sup>Ac</sup>
Odour	Control	9.0±0.0 <sup>Aa</sup>	7.7±0.1 <sup>Bb</sup>	4.8±0.1 <sup>Bc</sup>	4.2±0.2 <sup>Cc</sup>	4.4±0.2 <sup>Bc</sup>	2.3±0.4 <sup>Bd</sup>	2.1±0.0 <sup>Bd</sup>	1.7±0.2 <sup>Bd</sup>
	Water	9.0±0.0 <sup>Aa</sup>	7.7±0.2 <sup>Bb</sup>	4.7±0.4 <sup>Bc</sup>	4.2±0.1 <sup>Cc</sup>	4.1±0.3 <sup>Bc</sup>	3.1±0.2 <sup>Bd</sup>	2.0±0.4 <sup>Bd</sup>	2.1±0.1 <sup>Bd</sup>
	0.1%	8.1±0.3 <sup>ABa</sup>	8.6±0.2 <sup>Aa</sup>	8.8±0.1 <sup>Aa</sup>	6.2±0.1 <sup>Bab</sup>	4.9±0.3 <sup>Bc</sup>	4.8±0.3 <sup>Ac</sup>	4.4±0.3 <sup>Ac</sup>	3.1±0.1 <sup>Ac</sup>
	0.5%	7.3±0.4 <sup>Bb</sup>	8.2±0.4 <sup>Aa</sup>	8.5±0.2 <sup>Aa</sup>	7.1±0.4 <sup>Ab</sup>	6.1±0.5 <sup>Ab</sup>	4.7±0.4 <sup>Ac</sup>	4.7±0.0 <sup>Ac</sup>	3.3±0.2 <sup>Ac</sup>
	1.0%	7.0±0.4 <sup>Bb</sup>	8.3±0.5 <sup>Aa</sup>	8.5±0.3 <sup>Aa</sup>	7.2±0.4 <sup>Ab</sup>	6.2±0.2 <sup>Ab</sup>	4.9±0.2 <sup>Abc</sup>	4.7±0.2 <sup>Ac</sup>	3.2±0.2 <sup>Ac</sup>

a, b, c with different lowercase letters in the same row are significantly different ( $p < 0.05$ ).

A, B, C with different uppercase letters in the same column are significantly different ( $p < 0.05$ ).

*et al.*, 2014). The antimicrobial activities of phenolic compounds may involve multiple modes of action. For example, phenolic compounds can degrade the cell wall, disrupt the cytoplasmic membrane, cause leakage of cellular components, change fatty acid and phospholipid constituents, influence the synthesis of DNA and RNA and destroy protein translocation (Shan *et al.*, 2007). Chanthaphon *et al.* (2008) revealed that the major hydrocarbon components of the ethyl acetate extract from Kaffir lime were limonene (31.64%), citronellal (25.96%) and b-pinene (6.83%). A positive correlation between the monoterpene content of the plant extract and the Gram positive and Gram negative pathogenic bacterial inhibition was observed and reported. Limonene, citronella and b-pinene exert microbial membrane damaging effects inhibiting the growth of *Salmonella* spp., *S. aureus* and *Bacillus* spp. (Trombetta *et al.*, 2005).

#### *Effect of citrus peel extracts on the shelf life of raw chicken drumettes*

As investigated in this study to be the most effective antioxidant and antibacterial (MBC of 0.4-6.3 mg/ml), KEa was chosen for shelf life study of raw chicken drumettes. The effects were evaluated and compared between the sample treated with KEa (0.1%, 0.5% and 1.0%), without KEa (Water-dipping) and Control (no dipping).

#### *Lipid oxidation*

Together with microbial spoilage, chemical deterioration in particular lipid oxidation is a main factor limiting the shelf-life of muscle foods. The TBARS method has been widely used to determine the degree of lipid oxidation. The effect of KEa on the lipid oxidation of the chicken drumettes was represented in Figure 2. At day 0, the TBARS values were found to be the same at 0.6 mg MDA/kg sample for all samples. Over the storage period,

TBARS values increased considerably in Control and Water samples (2.5 mg MDA/kg sample) but remained relatively low in KEa-treated samples with no significant differences at all concentration (1.5 - 1.8 mg MDA/kg sample). At the end of storage, KEa reduced the lipid oxidation for more than 40%.

Although lipid oxidation is well known as one of the major causes of the progressive deterioration in the quality of meat products, limiting their storage shelf life, the deterioration in organoleptic characteristics, and the associated loss of nutritional value induced by the oxidative process, can be delayed by the addition of antioxidants. Natural antioxidants have been widely studied as alternative food preservatives including flavonoids, phenolic acids, organic acids and carotenoids, which can reduce lipid oxidation by scavenging free radicals, chelating metal ions or quenching oxygen radicals (Brewer, 2011). For instance, beneficial effects on the oxidative stability and organoleptic properties of food have been found for grape seed extract in cooked ground beef (Ahn *et al.*, 2002), green tea and grape seed extract in beef patties (Bañón *et al.*, 2007), oregano essential oil in chicken breast meat (Chouliara *et al.*, 2007) and rosemary extract in beef (McBride *et al.*, 2007). Moreover, the addition of the natural extracts in the packaging material can protect food from lipid oxidation, thus increasing its shelf-life (Siripatrawan and Harte, 2010).

The results of the present study show that although containing great amount of unsaturated fatty acid, adding phenolic-rich extracts, KEa protects chicken drumette samples against lipid oxidation. Furthermore, KEa reduced the lipid oxidation for 40%, showing higher activity than spice extracts previously study (Radha *et al.*, 2014). Therefore, the strong in-vitro antioxidant activity shown by KEa also had a protective role in real meat products.

### Microbiological properties

The effect of KEa on microbiological quality of chicken drumette samples during storage is shown in Figure 2. The TVC of all samples increased during storage. KEa reduced TVC soon after dipping, to be lower than Control and Water for 2 and 1.2 log-orders. Interestingly, during 6 to 10 days of storage, the TVC of KEa-treated samples at all concentrations was stable, exhibiting bacteriostatic effect, whereas those of Control and Water remained steadily increasing to 8.5 and 7.5 log-orders, showing over microbial perceptible level of raw chicken product (5.7 log-orders) from day 4. KEa at 0.5 and 1% prolonged the microbiological shelf life of chicken drumettes for 10 days (4.7 and 4.3 log-orders), while 0.1% can prolong for 8 days (5.4 log-orders). It was clear that KEa at a concentration of 0.5% was able to increase the microbiological shelf life of the chicken drumettes by 10 days and therefore higher concentrations are not required in the decontamination treatment.

### pH value

Figure 2 also showed the effect of KEa on the pH of raw chicken drumette samples in refrigerated storage at 4°C for 14 days. The pH values of both Control and Water were found to rise significantly to 7.7 at the end of storage. However, they were found to be lower in KEa-treated samples, especially at 0.5 and 1% exhibiting in the range of 5.7 to 5.9 at the end of the storage, showing no significant difference compared to day 0.

Interestingly, during initial stage of storage, pH values of all samples had increased with increasing TVC, but in KEa-treated samples, both TVC and pH values remain stable during 6 to 10 days of storage. The pH rise of control samples may be due to *Pseudomonas* spp. that induced proteolysis resulting in production of ammonia, dimethylamine, trimethylamine and the use of free amino acids when glucose is exhausted by growing bacteria (Alasnier *et al.*, 2000). KEa at 0.5 and 1.0% was possibly moderate these reactions until the storage at day 10. On the contrary, a decrease in pH during chicken spoilage is due to the growth of Lactic acid bacteria.

### Sensory analysis

Finally, the effect of KEa on the appearance and odour acceptances of the chicken samples was exhibited in Table 3. The decrease in odour acceptance of all samples was found earlier compared to appearance attribute. The sensory scores of Control and Water were under acceptable level at day 6 for appearance and day 4 for odour attributes. However, in KEa-treated samples, the lower acceptability score

for appearance was reached after 10 days for all concentrations. The decreasing of odour acceptability of KEa was observed earlier at day 8 for 0.1% and day 10 for 0.5 and 1.0%. Although the unique odour of KEa significantly affected the acceptability at initial stage, the odour was disappear during further storage, making the odour acceptable score risen at day 2 and 4.

### Conclusion

Citrus peel extracts are promising sources of antioxidant and antibacterial against broad spectrum of food borne pathogens. Also, the extracts can be used as a natural preservative in high-perishable meat product. Comparison of control and KEa-treated chicken drumette samples during storage at 4°C for 14 days showed that the addition of the extract was effective as antioxidant and antibacterial agents for improving the properties of the samples from a quality view point. It can be concluded that 0.5% was the optimum concentration of the investigated extract, effective in reducing TBARS, the TVC as well as gaining a high acceptability when incorporated into chicken drumettes. On basis of microbiological, oxidative and sensory studies, a shelf life extension of 8 days was obtained in 0.5% KEa-treated chicken drumette products, indicating 6 days longer than that of the controls. The results of this work indicated the potential of the citrus peel extract, applied to prolong the shelf life of chicken drumette, for their antibacterial ability towards a wide range of microorganisms and antioxidant ability, the economically sustainable extraction, and health benefits.

### References

- Ahn, J., Grün, I. U. and Fernando, L. N. 2002. Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *Journal of Food Science* 67: 1364–1369.
- Alasnier, C., Meynier, A., Viau, M. and Gandmer, G. 2000. Hydrolytic and oxidative changes in the lipids of chicken breast and thigh muscles during refrigerated storage. *Journal of Food Science* 65: 9–14.
- Association of Official Analytical Chemistry - AOAC. 1995. *Official Methods of Analysis*. 16<sup>th</sup> ed. Washington: AOAC 1141.
- Bañón, S., Díaz, P., Rodríguez, M., Garrido, M.D. and Price, A. 2007. Ascorbate, green tea and grape seed extracts increase the shelf life of low sulphite beef patties. *Meat Science* 77: 626–633.
- Brand-Williams W, Cuvelier, M.E. and Berset, C. 1995. Use of free radical method to evaluate antioxidant activity. *Lebensm Wiss Technology* 28: 25–30.

- Brewer, M.S. 2011. Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. *Comprehensive Reviews in Food Science and Food Safety* 10: 221–247.
- Chaisawadi, S., Thongbute, D., Methawiriyaslip, W., Pitakworarat, N., Chaisawadi, A., Jaturonrasamee, K., Khemkhaw, J. and Thnuthumchareon, W. 2003. Preliminary study of antimicrobial activities on medicinal herbs of Thai food ingredients. *Acta Horticulturae* 675: 111–114.
- Chanthaphon, S., Chanthachum, S. and Hongpattarakere, T. 2008. Antimicrobial activities of essential oils and crude extracts from tropical *Citrus* spp. against food related microorganisms. *Songklanakarin Journal of Science and Technology* 30: 125–131.
- Chouliara, E., Karatapanis, A., Savvaidis, I.N. and Kontominas, M.G. 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 °C. *Food Microbiology* 24: 607–617.
- Cushnie, T.P. and Lamb, A.J. 2005. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents* 5: 343–356.
- Fisher, K., Phillips, C. and Mcwatt, L. 2009. The use of an antimicrobial citrus vapour to reduce *Enterococcus* sp. on salad products. *International Journal of Food Science and Technology* 44: 1748–1754.
- Jorgensen, J.H. and Turnidge, J.D. 2007. Susceptibility test methods: dilution and disk diffusion methods, In P. R. Murray, E. J. Baron, J.H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.). *Manual of clinical microbiology*, 9th ed. Pp. 1152–1172. Washington, D.C., ASM Press.
- Mandrell, R.E. and Wachtel, M.R. 1999. Novel detection techniques for human pathogens that contaminate poultry. *Current Opinion in Biotechnology* 10: 273–278.
- McBride, N.T.M., Hogan, S.A. and Kerry, J.P. 2007. Comparative addition of rosemary extract and additives on sensory and antioxidant properties of retail packaged beef. *International Journal of Food Science and Technology* 42: 1201–1207.
- Melendez, P.A. and Capriles, V.A. 2006. Antibacterial properties of tropical plants from Puerto Rico. *Phytomedicine* 13: 272–276.
- Mexis, S.F., Chouliara, E., and Kontominas, M.G. 2009. Combined effect of an O<sub>2</sub> absorber and oregano essential oil on shelf-life extension of Greek cod roe paste (tarama salad) stored at 4°C. *Innovative Food Science Emerging Technologies* 10: 572–579.
- Oke, F., Aslim, B., Ozturk, S. and Altundag, S. 2009. Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. *Food Chemistry* 112: 874–879.
- Prior, R.L., Wu, X., Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* 53: 4290–4302.
- Radha, K.K., Babuskin, S, Azhagu, S.B.P., Sasikala, M., Sivarajan, M. and Sukumar, M. 2014. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *International Journal of food microbiology* 171: 32–40.
- Shan, B., Cai, Y.Z., Brooks, J.D. and Corke, H. 2007. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. *International Journal of food microbiology* 117: 112–119.
- Singleton, V. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16: 144–158.
- Siripatrawan, U. and Harte, B.R. 2010. Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids* 24: 770-775.
- Trombetta, D., Castelli, F., Sarpietro, M.G., Venuti, V., Cristani, M. and Daniele, C. 2005. Mechanisms of antibacterial action of three monoterpenes. *Antimicrobial Agents and Chemotherapy* 49: 2474–2478.