ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF THAI LOCAL FRUIT EXTRACTS: APPLICATION OF A SELECTED FRUIT EXTRACT, *PHYLLANTHUS EMBLICA* LINN. AS A NATURAL PRESERVATIVE IN RAW GROUND PORK DURING REFRIGERATED STORAGE

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Abstract: Crude methanolic extracts of Thai local fruits including *Ardisia polycephala* Wall. (pirangasa), *Elaeocarpus hygrophilus* Kurz. (makoknum), *Limonia acidissima* Linn. (maquid or elephant apple), *Phyllanthus emblica* Linn. (makampom or Indian gooseberry), *Garcinia schomburgkiana* Pierre. (madan) and *Averrhoa carambola* Linn. (mafueng or star fruit) were tested for their antimicrobial and antioxidant activities. Fruit extracts of madan, makampom and makoknum had higher antimicrobial activity, while the extracts of makampom, pirangasa and makoknum had stronger antioxidant activity compared to the others. Then, the extract of makampom (0.25 –2.0%) was used as a natural preservative in raw ground pork during refrigerated storage at 4°C. This extract at 2.0% was the most effective to decrease the number of total viable counts and total *Pseudomonas* in raw ground pork. After 12-day storage at 4°C, total viable counts and total *Pseudomonas* in ground pork samples added with 2.0% makampom extract had low survival rate of 23.27% and 2.06%, respectively. These raw ground pork samples had acceptable appearance with 5.52 pH value. Moreover, addition of 2.0% makampom extract was the most effective to delay lipid oxidation by slowing down the increasing of thiobarbituric reactive substance (TBARS) value of raw ground pork. Then, the chilled raw ground pork added with 2.0% makampom extract was used to produce a seasoned ground pork product. After duo-trio testing, some of 12 taste panels could not detect flavor of this fruit extract in the product.

Keywords: fruit extract, *Phyllanthus emblica*, antimicrobial, antioxidant

INTRODUCTION

Fruit is important as a natural source of antioxidant. Many fruits are rich in some active compounds such as phenolic compounds, vitamins, β-carotene and others which have an important role in antioxidant activity. Some tropical fruits have been reported to have antioxidant activity such as star fruit (*Averrhoa carambola*) (Lim et al., 2007) and Indian gooseberry (*Phyllanthus emblica* or makampom) (Pinsuwan et al., 2007). Epidemiological studies have shown that many phytonutrients in fruits have potentially protective effects against many diseases including cancer, diabetes and cardiovascular diseases caused by oxygen radicals. Phytochemical antioxidants might prevent the oxidative damage (Blasa et al., 2010). In addition, some fruits was reported to have antimicrobial activitiy (Mayachiew and Devahastin , 2008). However, antioxidant and antimicrobial activities of some other Thai local fruits was unknown.

Fruit of *P. emblica* is available in several countries of Southeast Asia. Its extract has been reported to possess antioxidant, antimicrobial, antidiarrheal and spasmolytic activities (Pinsuwan et al., 2007; Mayachiew and Devahastin , 2008; Medmood et al., 2011). Therefore, it is interesting to study the use of *P. emblica* fruit extract as a source of natural preservatives in raw ground pork.

MATERIALS AND METHODS

Microorganisms

Fifteen microbial strains (6 species of bacteria, 4 species of yeasts and 5 species of molds) were used in this study. *Bacillus cereus* DMST 5040, *Escherichia coli* DMST 4212, *Listeria monocytogenes* DMST 11256, *Pseudomonas fluorescens* DMST 20076 and *Salmonella* Typhimurium DMST 0562 were obtained from the culture collection of the Department of Medical Sciences, Ministry of Public Health, Thailand. *Staphylococcus aureus* TISIR 118, *Candida lipolytica* TISTR 5655, *Pichia membranaefaciens* TISTR 5093, *Rhodotorula glutinis* TISTR 5159, *Zygosaccharomyces rouxii* TISTR 5044, *Aspergillus flavus* TISTR 3041, *Aspergillus parasiticus* TISTR 3276, *Aspergillus ochraceus* TISTR 3557, *Fusarium moniliforme* TISTR 3175 and *Rhizopus stolonifer* TISTR 3199 were obtained from the Microbiological Resources Centre for Southeast Asian Region (Bangkok MIRCEN).

Culture preparation

The bacterial cultures (*B. cereus, E. coli, L. monocytogenes, P. fluorescens, S.* Typhimurium and *S. aureus*) were subcultured twice onto Nutrient Agar (NA, Difco) and incubated for 24 h at 37°C, except for *P. fluorescens* incubated at 30°C). Yeast cultures (*C. lipolytica, P. membranaefaciens, R. glutinis* and *Z. rouxii*) were subcultured twice onto Saboraud

Dextrose Agar (SDA, Difco) and incubated at 30°C for 48 h. A loopful of each bacteria and yeast in agar slant was inoculated into NB and SDB, respectively. After incubation, microbial cells were collected by centrifugation at 3000 ×g for 15 min, washed twice and resuspended in 0.1% peptone water. Turbidity was adjusted to match the turbidity of 5 McFarland standard to obtain an inoculum concentration of 10^7 CFU/ml.

To prepare spore suspension of mold, each mold culture was grown in Potato Dextrose Agar (PDA, Difco) at 30°C for 7 days. Conidia were harvested by adding with 5 µl of sterile 0.01% Tween 80 in culture tubes, and the agar surface was scraped. Conidial concentration was adjusted to 10^6 conidia/ml using haemacytometer.

Extraction of Thai local fruits

Six species of Thai local fruits including fruits of *Ardisia polycephala* Wall. (Thai name: pilungasa), *Averrhoa carambola* Linn. (common name: star fruit or Thai name: mafueng), *Elaeocarpus hygrophilus* Kurz. (common name: Spanish plum or Thai name: makoknum), *Garcinia schomburgkiana* Pierre. (Thai name: madan), *Limonia acidissima* Linn. (Thai name: makwit) and *Phyllanthus emblica* Linn. (common name: Indian gooseberry or Thai name: makampom) were purchased at retail in Bangkok, Thailand. These plants were extracted using methanol as a solvent.

To prepare crude methanolic extracts of Thai local fruits, all fruits were washed, cut into small pieces, freeze-dried and powdered. Then, 10 g of each dried fruit were soaked in 100 ml methanol, and shaked at 200 rpm for 24 h at ambient temperature. The mixtures were then filtered. The filtrates were evaporated using vacuum rotary evaporator (BÜCHI Rotavapor R-200/205, Model R205V800, Switzerland), and air dried. Stock solutions of crude methanolic extracts were prepared by diluting 0.2 g dried extracts with 1 ml of 10% dimethyl sulphoxide (DMSO) solution.

Screening of fruit extracts using disk diffusion test

The disk diffusion test was performed using the standard procedure as described by Jorgensen et al. (1999). The inoculum suspension (100 µl) of each microbial strain was added and swabbed onto the surface of Mueller-Hinton Agar (MHA, Difco) for bacteria, SDA for yeasts and PDA for molds. Sterile 6-mm filter paper discs (Whatman) were aseptically placed on MHA, SDA and PDA surfaces. Crude methanolic extracts (15 µl) were immediately added to discs. A 15-µl aliquot of 10% DMSO was also added to a sterile paper disc as a negative control. The plates were incubated at 37°C for 24 h for bacteria, except for *P. fluorescens* incubated at 30°C and at 30°C for 72 h for yeasts and molds. Antimicrobial activity was evaluated by measuring inhibition zone diameters. The experiment was done in triplicate.

Determination of the minimum inhibitory concentrations using agar dilution test

The minimum inhibitory concentrations (MICs) of all fruit extracts against 15 microbial strains were determined using an agar dilution method (Collins et al., 2001). Each fruit extract was diluted to obtain 5 concentrations (200, 140, 102.4, 51.2 and 25.6 mg/ml). Then, 19 ml appropriate agar medium (MHA, SDA or PDA) was poured into each petridish to obtain final concentrations (10, 7, 5.12, 2.56 and 1.28 µg/ml). Negative control was performed using distilled water. Penicillin G (at final concentration of 2000, 1000, 500, 250, 125, 62.5 and 31.25 unit/ml) and fluconazole (at final concentration of 0.1, 0.08, 0.04, 0.02 and 0.01 mg/ml) were tested as positive controls. After surface drying, a loopful of each microbial suspension (spore suspension for molds) was inoculated at the centre of each agar plate. After incubation at appropriate temperature and time, the growth of each microbial strains at different concentrations of fruit extracts was recorded. The lowest concentration of a fruit extract that completely inhibited visible growth of each microbial strain was recorded as the MIC.

Determination of antioxidant activity

Free radical scavenging activity assay (DPPH method)

The free radical scavenging activity of fruit extracts was measured according to the method of Brand-Williams (1995). Each stock solution of extracts and α–tocopherol (10,000 µg/ml, a positive control) were prepared and diluted to the concentrations of 1,000, 500, 100, 10, and 1 µg/ml in methanol. Seventy five microliter of each diluted extract at five concentrations were added to 2.925 ml of a 0.025 g/L DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution in methanol. The reaction mixtures were then incubated in the dark for 30 min. The absorbance at 515 nm was measured at 0 and 30 min of incubation using a UV-Visible spectrophotometer (UNICO, 2800A). To prepare standard curve of DPPH, the absorbance of DPPH at different concentrations was measured at 515 nm. The remaining $DPPH^{\cdot}$ concentration in the reaction mixture was calculated from the DPPH standard curve, and the percentage of the remaining $DPPH^{\cdot}$ was calculated using the following equation:

$$\%DPPH^{\cdot}_{REM} = [DPPH^{\cdot}]_T / [DPPH^{\cdot}]_{T=0}$$

Where $[DPPH^{\cdot}]_T$ and $[DPPH^{\cdot}]_{T=0}$ were the concentration of $DPPH^{\cdot}$ at steady state and zero time, respectively. The percentages of the remaining $DPPH^{\cdot}$ in each reaction mixture of five different concentrations of all extracts were then plotted against µg of extract / mg of $DPPH^{\cdot}$ to obtain the amount of antioxidant or extract necessary to decrease the initial $DPPH^{\cdot}$ by 50% (EC_{50}). The EC_{50} values of all extracts were calculated by the following linear regression of plots, and the antiradical efficiency ($AE=1/EC_{50}$) values were also calculated.

$$[\%DPPH^{\cdot}_{REM}] = b\,[\mu g\ antioxidant/\ mg\ DPPH^{\cdot}] + a.$$

Ferric reducing antioxidant power (FRAP) assay

Antioxidant activity of fruit extracts was determined according to the FRAP method previously described by Lado et al. (2004). To do FRAP assay, 1 mg/ml fruit extract (50 µl) was mixed with 1.5 mL FRAP reagent (25 ml of 300 mM acetate buffer, 2.5 mL of 10 mM TPTZ (2,4,6-tri-2-pyridyl-2-triazine, Fluka, Sigma-Aldrich, Switzerland) in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$), and incubated at 37°C for 5 min. The absorbance was measured at 594 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia) against blank (FRAP reagent without the sample). The concentration of Fe^{2+}-TPTZ (reducing capacity) was calculated by comparing the absorbance at 594 nm with the standard curve of the Fe (II) standard solutions (ferrous sulfate heptahydrate). Alpha-tocopherol was used as a positive control.

Determination of total phenolic content

Total phenolic content was determined according to the method of Tepe et al. (2005). Each fruit extract (0.1 ml of 10,000 µg/ml crude methanolic extract) was transferred to a flask containing 46 ml distilled water. Folin-Ciocalteu's phenol reagent (Fluka, Sigma-Aldrich, Switzerland) (1 ml) was added, shaken thoroughly, and allowed to stand for 3 min. Then, 3 ml of 2% Na_2CO_3 was added, and allowed to stand for 2 h with intermittent shaking. Then, the absorbance was measured at 760 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia). Standard curve of gallic acid (Fluka, Sigma-Aldrich, Spain) was prepared using the similar procedure. The results were expressed as µg GAE (gallic acid equivalents) /mg extract.

Application of a selected fruit extract for extending shelf-life of chilled ground pork

A fruit extract with high antimicrobial and antioxidant activities was selected for use as a natural preservative in ground pork during refrigerated storage. Six treatments of ground pork (80% lean meat mixed with 20% pork fat) were prepared. These were treatment 1 (ground pork without fruit extract added, control), treatment 2 (ground pork added with 0.02% BHT, butylated hydroxytoluene) and treatment 3-6 (ground pork added with 0.25, 1.0, 1.5 and 2.0% of a selected fruit extract, respectively). All ground pork samples were added with cell suspension (10^7 cells/ml) of *P. fluorescens* DMST 20076 (10 µl/ 25 g ground pork) to get a final cell concentration of 10^7 cells/ g ground pork. Then, all samples were stored at 4°C for 12 days. At day 0, 1, 3, 7 and 12 days of storage, total viable counts, total psychrotrophic bacteria and total *Pseudomonas* in chilled ground pork samples were analysed by spiral plating technique using the Spiral Plater (Autoplate 4000, Spiral Biotech company, USA) onto Plate Count agar (PCA, incubated at 37°C for 24 h), PCA (incubated at 4°C for 10 days) and *Pseudomonas* isolation agar (PIA, incubated at 30°C for 24 h), respectively. Measurement of pH and color values were also performed using pH meter (Testo 205, Germany) and color value meter (Konica Minolta model CR – 300, Japan), respectively, while TBARS (thiobarbituric reactive substances) values were analysed by using a method of Kirk and Sawyer (1991). Three replicates of experiments were performed.

Statistical analysis

Data were analysed by using analysis of variance to determine if significant differences ($P<0.05$) existed between mean values and using Duncan multiple range test to compare between treatment means.

Use of chilled ground pork for production of a seasoned pork product

This study was aimed at comparing a seasoned pork product made from fresh ground pork without a fruit extract (a control sample) and those made from chilled ground pork added with a selected fruit extract and stored at 4°C for 0, 3 and 7 days. This product contained 87.97% ground pork (with or without the fruit extract), 0.44% sucrose, 4.1% soy sauce, 0.88% tasty sauce, 0.15% salt, 1.47% milk, 2.35% oyster sauce, 1.47% chopped garlic with white pepper, 0.95% olive oil and 0.59% tapioca flour. All ingredients were mixed together. Then, a sensory analysis (a duo-trio test)l was performed in triplicate by 12 untrained taste panels to establish differences between the control sample and the seasoned pork samples made from chilled ground pork stored at different period of time.

To do duo-trio test, the seasoned pork samples including the control sample and the sample made from ground pork added with a fruit extract were divided to small pieces (15 g/piece), and each piece was rounded to a circular shape with 1 inch thick. All samples were fried in palm oil at 160-170°C for 10 min. Then, the cooked samples were served to the 12 untrained panelists. Each panel needed to evaluate 3 samples independently. One sample was coded as "R" and the other two samples were coded with 3 digit numbers. These panels were asked to evaluate "which sample of these two samples was similar to the "R" sample?". The number of the taste panels giving the correct answer was evaluated if the significant differences existed by using a statistical table for duo-trio test (Roessler et al., 1978).

RESULTS AND DISCUSSION

Antimicrobial activity

Extracts of madan, makoknum and makampom exhibited wider inhibition zone against most of bacteria tested by disk diffusion test. However, all fruit extracts could not inhibit growth of all mold species tested, but makoknum and madan extracts could produce inhibition zone only yeast species, *R. glutinis* (Table 1). Madan, makoknum and makampom extracts were selected for MICs determination. Among all fruit extracts tested, fruit extract of madan showed the broadest antimicrobial action to all bacterial species tested (Table 2). Interestingly, it could effectively inhibit foodborne bacterial pathogens (*L. monocytogenes* and *S.* Typhimurium) with the MIC of 2.56 mg/ml. The only yeast species susceptible to madan extract was *R. glutinis*. However, its antimicrobial activity may be due its organic acids. Suntornsuk et al. (2002) reported that the amount of vitamin C in madan fruit juice was 4.6 mg/100 g.

Fruit extract of makampom also exhibited strong inhibitory to some bacteria, especially *P. fluorescens* and *S. aureus* with 2.56 mg/ml MIC (Table 2). Mayachiew and Devahastin (2008) also found that *P. emblica* fruit extract inhibited *S. aureus* with MIC of 13.97 mg/ml. Several components of *P. emblica* fruits may act as antimicrobial agents. They reported that this fruit extract contained gallic acid, hydrolysable tannin and ascorbic acid (11.21%).

Table 1 Antimicrobial activity of Thai local fruit extracts using disk diffusion test

Microbial species	Diameter of inhibition zone (mm)[a] ± SD					
	Ardisia polycephala (pilungasa)	*Averrhoa carambola* (mafueng)	*Elaeocarpus hygrophilus* (makoknum)	*Garcinia schomburgkiana* (madan)	*Phyllanthus emblica* (Imakampom)	*Limonia acidissima* (makwit)
Bacteria						
Bacillus cereus	-[b]	-	11.7± 0.3	11.0 ± 0.5	9.4 ± 0.5	-
Escherichia coli	-	-	-	8.5 ± 0.5	-	-
Listeria monocytogenes	-	-	9.4 ± 0.1	-	-	-
Pseudomonas fluorescens	-	-	11.5± 1.2	10.0 ± 0.3	9.8 ± 0.6	-
Salmonella Typhimurium	-	-	9.0 ± 0.0	11.3 ± 0.2	-	-
Staphylococcus aureus	-	-	8.7 ± 0.3	10.8 ± 0.3	10.3 ± 0.6	-
Yeasts						
Candida lipolytica	-	-	-	-	-	-
Pichia membranaefaciens	-	-	-	-	-	-
Rhodotorula glutinis	-	-	14.7 ± 1.1	21.7 ± 1.1	-	-
Zygosaccharomyces rouxii	-	-	-	-	-	-

[a]Data are mean of three replications.
[b]No inhibition was observed.

Table 2 Minimum inhibitory concentrations of Thai local fruit extracts

Microbial species	Minimum inhibitory concentrations of Thai local fruit extracts (mg/ml)				
	Elaeocarpus hygrophilus (makoknum)	*Garcinia schomburgkiana* (madan)	*Phyllanthus emblica* (makampom)	Pennicillin G*	Fluconazole
Bacteria					
Bacillus cereus	>10	5.12	5.12	250	>0.10
Escherichia coli	>10	5.12	>10	125	>0.10
Listeria monocytogenes	>10	2.56	>10	62.50	>0.10
Pseudomonas fluorescens	7	5.12	2.56	> 2000	>0.10
Salmonella Typhimurium	5.12	2.56	>10	250	>0.10
Staphylococcus aureus	>10	5.12	2.56	31.25	>0.10
Yeasts					
Candida lipolytica	>10	>10	>10	> 2000	0.02
Pichia membranaefaciens	>10	>10	>10	> 2000	0.08
Rhodotorula glutinis	>10	5.12	>10	> 2000	>0.10
Zygosaccharomyces rouxii	>10	>10	>10	> 2000	0.10

* Units/ml for penicillin G

Antioxidant activity

Among all fruit extracts tested, makampom (*P. emblica*) fruit extract had the highest antioxidant activity by DPPH method (EC_{50} of 501.71 μg extract /mg DPPH) and strongest reducing capacity (4.86 mmol/L) by FRAP method, followed by pilangasa, makoknum, madan, mafueng and makwit (Table 3). The lower EC_{50} value of the extract indicates its higher antioxidant activity (Brand-Williams et al., 1995). Their antioxidant activities were related to their phenolic contents. Makampom contained the highest phenolic content (4,220 μg GAE/mg dry extract). Pinsuwan et al. (2007) reported that the alcoholic extract of *P. emblica* possessed high antioxidant capacity with EC_{50} of 1.55 μg/ml and high phenolic content (454.7 mg gallic acid equivalent/g). In addition, Lou et al. (2009) isolated six compounds from *P. emblica* fruit and identified as cinnamic acid, quercetin, 5-hydroxymethylfurfural, gallic acid, β-daucosterol and ellagic acid.

Fruit of pilungasa (*A. polycephala*) was found to possess high antioxidant activity and high phenolic contents. Ahamad et al. (1977) reported that phytochemical constituents in leaves of pirangasa were bauerenol, α-amyrin and β-amyrin. Ruangchakpet and Sajjaanantakul (2007) reported that *E. hygrophilus* (makoknum) fruit at 6 month maturity had high total

phenolics (345.8 mg gallic acid/ 100 g fresh weight) and flavonoid content (49.0 mg catechin/100 g fresh weight). The highest gallic acid content (103.6 mg/100 g fresh weight) was found at 6 month maturity.

Compared to other fruits, mafueng had not much antioxidant activity and total phenolics. Samee et al. (2006) also reported that star fruit (mafueng) had lower antioxidant activity, compared to other fruits tested. Lim et al. (2007) report that star fruit contained antioxidant activity (IC_{50} of 3.8 mg/ml by DPPH method) and total phenolics of 131 mg/100 g fresh fruit.

Table 3 Antioxidant activity and total phenolic content of Thai local fruit extracts

Fruit extracts	DPPH method	FRAP method	Total phenolic content
	EC_{50} (µg extract / mg DPPH)[a] ± SD	Reducing ability (mmol/L)[a] ± SD	(µg Gallic Acid Equivalents (GAE)/mg dry extract) [a] ± SD
Ardisia polycephala (pilungasa)	739.38 ± 15.61	4.72 ± 0.12	1,270 ± 208.71
Averrhoa carambola (mafueng)	17,308.33 ± 339.25	1.27 ± 0.11	116.67 ± 48.21
Elaeocarpus hygrophilus (makoknum)	2,082.49 ± 46.91	2.79 ± 0.08	263.33 ± 108.74
Garcinia schomburgkiana (madan*)*	6,952.48 ± 638.97	2.60 ± 0.05	210 ± 80.32
Phyllanthus emblica (makampom)	501.71 ± 16.61	4.86 ± 0.14	4,220 ± 121.62
Limonia acidissima (makuit)	27,773.4 ± 846.18	0.50 ± 0.01	166.67 ± 39.80
∝-tocopherol	467.55 ± 16.79	4.89 ± 0.08	-[b]

[a] Data are mean of three replications.
[b] Data are not determined.

Application of a selected fruit extract as a natural preservative in chilled ground pork

Makampom extract was selected for use as a natural preservative to extend shelf-life of chilled ground pork. Total microbial counts in ground pork added with 1.5 and 2.0% makampom extract decreased as storage time increased. After 7 days of storage at 4°C, ground pork added with 1.5-2.0% of this fruit extract had lower survival populations of total microorganisms (29-36% survival) than the samples added with lower concentrations of this extract (0.25-1.0% survival) (Figure 1a). Of all, samples added with 2.0% makampom extract had the lowest percentage of *Pseudomonas* survival cells (Figure 1b). However, the number of total psychrotrophs in all treatments of chilled ground pork increased by 0.13-0.51 log unit after 12-day storage. Unlike other treatments, the number of total psychrotrophs in ground pork samples added with 1.5-2.0% makampom extract slightly decreased after 1 day of storage, then gradually increased as the storage time increased (Figure 1c).

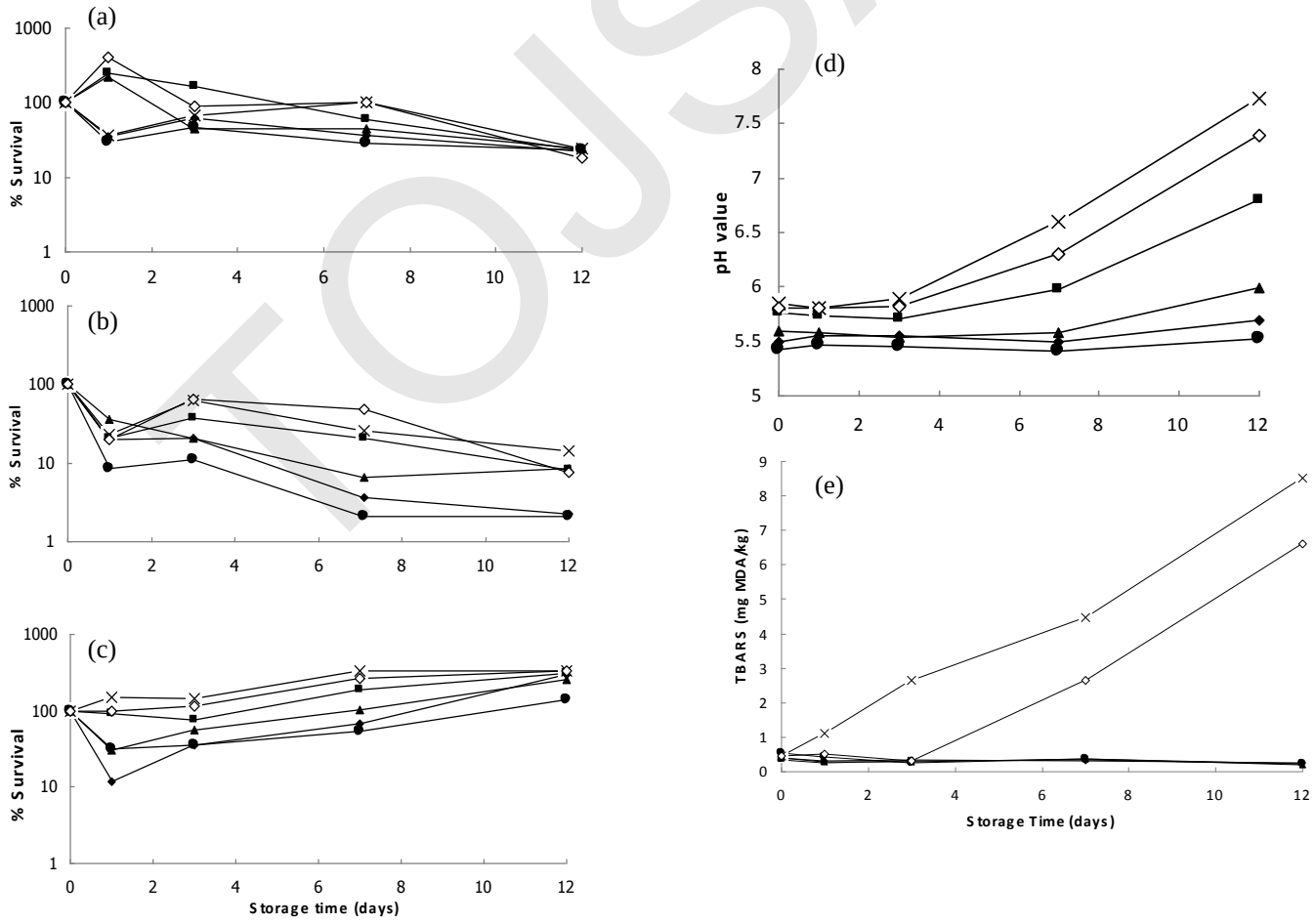

Figure 1 Survival of total microorganisms (a), total *Pseudomonas* (b) and total psychrotroph (c), pH values (d), and thiobarbituric reactive substances (TBARS) values (e) in ground pork added with some preservatives during refrigerated

storage (Symbol: ×, control (no preservative added); ■, 0.25% makampom extract; ▲, 1.0% makampom extract; ♦, 1.5% makampom extract; ●, 2.0% makampom extract, and ◊, 0.02% BHT)

Decreasing of total microbial counts and *Pseudomonas* counts in the samples added with 1.5-2.0% makampom extract was probably due to its active compounds. This indicated that this extract could delay spoilage of chilled ground pork up to 7 days, while the control samples could be kept at 4°C for only 3 days before changing appearance. Medmood et al. (2011) found that crude extract of *P. emblica* fruit contained alkaloids, tannins, terpenes, flavonoids, sterols and saponins.

At the beginning of storage, pH values of ground pork samples without any preservative (control, pH 5.84) and the samples added with BHT (pH 5.81) were higher than the pH values of the samples added with 0.25-2.0% makampom extract (pH 5.76-5.43). The pH values of all pork samples slightly changed or remained constant until 3 days of storage, then increased until the end of storage. The pH values of the control samples and the samples added with 0.02% BHT increased more rapidly (to neutral pH level) than the samples of other treatments. Among all treatments of ground pork, the pH values of ground pork samples added with 1.5 and 2.0% makampom extract were the lowest (5.68 and 5.52, respectively) after 12-day storage (Figure 1d). In the control samples, increasing of pH to neutral level was related with the high number of total viable counts which indicated their spoilage. At 12-day storage, the control samples had green surface with stink odor, while the appearance of the samples added with 1.5-2.0% makampom extract was almost similar to fresh ground pork.

TBARS values of the control samples and the samples added with 0.02% BHT significantly increased more rapidly as the storage time increased ($P<0.05$), compared to other samples. The TBARS values of the samples added with 0.25-2.0% makampom extract slightly changed at each storage time, but no significant different was found between those of the samples with each concentration of the extract ($P>0.05$). Among all treatment samples, the TBARS values of the samples added with 0.25-2.0% makampom extract were the lowest (0.20 – 0.26 mg MDA/kg) at the end of the storage (Figure 1e).

Use of chilled ground pork added with a selected fruit extract to produce a seasoned pork product

Makampom extract at 2.0% was the most suitable to extend shelf-life of chilled ground pork. Therefore, the ground pork added with 2.0% makampom extract and stored for 0, 3 and 7 days at 4°C was used to produce the seasoned pork product (a_w 0.97). After duo-trio test, only 5, 4 and 8 panels (out of 12 panelists) could discriminate between the control samples (pH 5.67) and the samples made from ground pork added with 2.0% makampom extract and stored for 0, 3 and 7 days at 4°C (pH 5.58), respectively. Based on the table of Roessler et al. (1978), it can be concluded that the taste panels could not discriminate between the control samples and the treated samples, when using probability level at 0.05. This indicates that the taste panels could not detect the flavor of 2.0% makampom extract added. Thus, the flavor of this extract should not cause product unacceptability. It is possible to use makampom extract as a natural preservative to extend shelf-life of chilled ground pork.

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