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Antimicrobial properties of extracts from *Carissa carandas* L. fruits and its application in chilled and frozen ground pork

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Abstract *In vitro* antimicrobial activities of *Carissa carandas* L. fruit extracts dissolved in water and their effect on biological qualities in ground pork during storage period for 8 days at 4 °C and at -20 °C for 12 weeks were investigated. The antimicrobial avctivity was carried out using agar well diffusion method against food pathogenic and spoilage bacteria. For antimicrobial testing, water extracts of *C. carandas* inhibited both pathogenic and spoilage bacteria including *Salmonella* Typhimurium TISTR 292, *Staphylococcus aureus* TISTR 118, *Escherichia coli* TISTR 780, *Aeromonas hydrophila* TISTR 1321, *Pseudomonas fluorescens* TISTR 358 and *Bacillus coagulans* TISTR 1447, at the concentration of 50 mg/ml. The water extract of *C. carandas* fruit was applied in ground pork. The ground pork samples were subjected to four treatments including control (non-treated), 0.2 g BHT/kg meat, 2.5 g and 5 g extracts /kg meat. Total microbial count, yeast/mold, psychrophilic bacteria and coliforms were determined in ground pork during storage time for 0, 2, 4, 6 and 8 days at 4 °C and frozen ground pork storage at -20 °C for 0, 4, 8, and 12 weeks. The results showed that pH was not different in each treatments. Total plate count and coliforms bacteria was decreased after adding 5 g extracts /kg meat at 6-8 days in chilled ground pork and up to 8 and 12 weeks in frozen ground pork. This finding indicated that *C. carandas* fruit extracts could be used for natural antimicrobial in ground pork for prolong shelf-life.

Keywords: Antimicrobial. *Carissa carandas*, extract

Introduction

Consumers demand for safe and high-quality meat which can be attributed in part to the widespread availability and accessibility of quality health data and information. There is also new concern about food safety due to increasing occurrence of new foodborne disease outbreaks caused by

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pathogenic microorganisms. This raises considerable challenges, particularly since there is increasing the use of chemical preservatives and artificial antimicrobials to inactivate or inhibit growth of spoilage and pathogenic microorganisms (Munuswamy *et al.*, 2013; Obeidat *et al.*, 2012; Sokmen *et al.*, 2004). As a consequence, natural antimicrobials are receiving a good deal of attention for a number of microorganism control issues. Reducing the need for antibiotics, controlling microbial contamination in food, improving shelf-life extension technologies to eliminate undesirable pathogens and/or delay microbial spoilage and decrease the development of antibiotic resistance by pathogenic microorganisms or strengthening immune cells in humans are some of the benefits (Böhme *et al.*, 2012; Mor-Mur and Yuste, 2010).

At present, meat industries use chemical additives in several meat processes to prevent the growth of food-borne pathogens and extend the shelf life of refrigerated storage. Since concern over the safety of chemical additives has arisen in recent years, consumers increasingly demand the use of natural products as alternative preservatives in foods (Negi, 2012). The use of natural compounds such as plant extracts has been identified for decontamination of meat and meat products against *Salmonella* spp., *E. coli, S. aureus* (Boskovic *et al.*, 2015; Vashist and Jindal, 2012). *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 caramda. It is called kerenda in Malaya, karaunda in Malaya and India; Bengal currant or Christ's thorn in South India; nam phrom, or namdaeng in Thailand; and caramba, caranda, caraunda and perunkila in the Philippines. Karja tenga in Assam and Koromcha in Bengali. 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 (Kumar *et al.,* 2013). Antimicrobial activity against *S. aureus, S. epidermidis, E. coli, A. niger, Candida. albican*s was observed in aqueous, ethanol, methanol, chloroform and acetone extract of *C. carandas* (Salar and Dhall, 2010). Another report about methanol and acetone extract of *C. carandas* showed antibacterial activity against both Gram-positive strains (*S. aureus, B. cereus and B. subtilis*) and Gram-negative strains (*Klebsiella pneumonia* and *Proteus mirablis*) and *C. albicans* and *Cryptococcus luteolus* (Vaghasiya and Chanda, 2007).

Therefore, the objectives of this study was to investigate *in vitro* studies antibacterial properties of water extracts from *C. carandas* fruits for new natural ingredients that can be further used as food safety in ground pork and prolong shelf-life.

Materials and Methods

Preparation of water extracts from C. carandas fruits (WEC)

Fresh *C. carandas* fruits were obtained from Samut Songkhram Province, Thailand. After washing, the seeds were removed and dried in a hot-air oven at 45^{o}C for 3 days. Then dry fruits were ground to small pieces. The powder was extracted in water using 1 / 9 parts (w/v) for 72 h at 4°C. Extraction was repeated three times and mixed 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, until it becomes a sticky and stored at 4°C until use. The sticky crude extracts were dissolved in water at concentrations of 100, 50, 25, 12.5, and 6.25 mg/ml (w/v) before use.

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

Microbial preparation

The antimicrobial activities of fruit extracts were studied using eleven strains of pathogenic and spoilage bacteria which often found in meat. 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 were obtained from Thailand institute of scientific and technological research, Thailand; and American Type Culture Collection, Rockville, Md. The bacteria strains were grown in de Man, Rogosa and Sharpe (MRS; Merck, Germany) broth for lactic acid bacteria and Trypticase Soy Broth (TSB; Merck, Germany) with 0.6% Yeast Extract (YE; Merck, Germany) for pathogenic bacteria. All the stock bacteria strains were stored at -80^{o}C for further use.

Agar well diffusion

Agar well diffusion method of Biswas *et al.,* (2013) was modified for antimicrobial activities testing of WEC against both pathogenic and spoilage bacteria. Bacterial strains were cultured on MRS agar petri plates 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 with 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, while 10% of ethanol was used as negative control. The plates were incubated overnight at proper conditions for each strain. Microbial growth was determined by measuring the diameter of inhibition zone (mm). The experiment was repeated three times and the mean values were presented.

Evaluation of the effect of optimal crude extracts from C. carandas fruits on pH and biological quality in chilled and frozen ground pork

Preparation of meat samples

Raw pork and back fat were obtained from supermarket in Bangkok, Thailand. Ground pork was mixed with 30% ground back fat (w/w). Four treatments: none added (control); 0.2 g BHT/kg meat (positive lipid peroxidation); ground pork plus 2.5 and 5.0 g WEC/kg meat; were mixed vigorously. Cooked meat samples were prepared by boiling at 95 ^{o}C for 20 mins. Chilled meat samples were packed in polyethylene bags and kept for 0, 2, 4, 6, 8 days at 4^{o}C. Frozen meat stored at -20 ^{o}C for 0, 4, 8 and 12 weeks. All sample were taken for analysis during storage periods.

Microbial determination of ground pork

Meat samples (25 g) were homogenized 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 the 10-fold dilution was prepared in 0.85% NaCl to reach 10^{-5} dilutions.

The sample solutions were used for microbial parameters. For total microbial count (TPC) and psychrophilic bacteria, one ml of the sample solutions of each dilution as described above were poured into sterilized plates which contained 20 ml of plate count agar (Merck, Germany) to determine total viable counts. Malt agar (Merck, Germany) was used to determine total yeast and mold count. To determine coliforms/*E. coli,* 0.1 ml the sample solutions of each were speared on Chromocult agar (Merck, Germany). The incubation period for TPC, coliforms and *E. coli* was at 37 ^{o}C for 24-48 hr, while yeast/mold at 26 ^{o}C (3-5 days) and psychrophilic bacteria at 7 ^{o}C for 10 days. All plates were examined visually for colony count. 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.

Determination of pH in ground pork

The pH values were performed 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 by a pH meter (Mettler Toledo, Greifensee, Switzerland).

Statistical analyses

The experimental design was carried out in 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.

Results

In vitro antimicrobial properties of C. carandas fruits extracts using agar well diffusion method

The antimicrobial activity of *C. carandas* extracts dissolved in water was evaluated according to their clear zone inhibition against pathogenic and spoilage bacteria. The results displayed that WEC showed antimicrobial activities against both pathogenic and spoilage bacteria. The inhibition activity of WEC at 100 mg/ml showed against *S. aureus* (20.33 mm), *Aeomonas hydrophila* (19.33 mm), *P. fluorescens* (18.28 mm) and *E. coli* (17.00 mm). However, the inhibition of *L. plantarum, L. sakei, Leu. mesenteroides* subsp. *mesenteroides, Streptococcus* spp. and *L. cremoris* were not observed (Table 1).

Determination of microbial in ground pork

The changes of TPC, yeast/mold, psychophilic bacteria, coliforms in chilled ground pork samples with or without WEC during refrigerated storage at 4 ºC for 8 days were shown in Table 2. The WEC addition 2.5 g and 5 resulted in a reduction of TPC and coliform growth, but it was no effect on yeast/mold and psychrophilic bacteria to the control and BHT treatments during the storage time up to 8 days at 4ºC ($p<0.05$). The TPC of meat samples was initially approximately 4.83, 4.86, 4.79 and 4.76 log CFU/g meat, which increased steadily with storage time and reached 7.41, 7.30, 7.05 and 7.03 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g WEC and 5 g WEC/kg, respectively, at the end of the storage. The coliforms of meat samples were initially approximately 3.87, 3.88, 3.83, 3.81 log CFU/g meat, which increased steadily with storage time and reached to 6.01, 6.20, 5.01 and 5.02 log CFU/g

meat for the control, 0.2 g BHT/kg, 2.5 g WEC and 5 g WEC/kg, respectively, at the end of the storage. However, *E.coli* was not observed in ground pork as the number of *E. coli* was lower that limit of detection (<1 log cfu/g). Therefore, the addition of WEC showed significantly effect on TPC and coliforms.

In addition, TPC and population of coliforms in frozen ground pork adding WCE was lower than control BHT treatments (p<0.05). However, WCE was no effect on yeat/mold and psychrophilic bacteria. The total plate count of meat samples was initially approximately 4.53, 4.56, 4.51 and 4.50 log CFU/g meat, which increased significantly with storage time and reached close to 5.96, 5.84, 5.16 and 5.08 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g WCE and 5 g WCE/kg, respectively, at the end of the storage. However, the addition of 0.25 and 5 g WCE/kg meat inhibited the development of TPC better than the control and BHT treatment at 4, 8 and 12 weeks (p<0.05). The coliforms of meat samples was initially approximately 3.27, 3.28 and 3.23 and 3.21log CFU/g meat, which increased steadily with storage time and reached close to 4.86, 4.87, 4.65 and 4.57 log CFU/g meat for the control, 0.2 g BHT/kg, 2.5 g WCE and 5 g WCE/kg, respectively, at the end of the storage. However, the addition of 0.25 and 5 g WCE/kg meat inhibited the development of coliforms better than the control and BHT treatment at 8 and 12 weeks. Therefore, the addition of WCE was positive to significantly affect biological analysis (p<0.05) as shown in Table 3.

Determination of physical quality of ground pork

The pH values and color were performed to analyze physical quality in ground pork. Effect of extracts from *C. carandas* fruits on pH values in ground pork during storage at 4^{o}C up to 8 days was shown in Table 4. 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 WEC/kg and 5 g WEC/kg, respectively, and the pH increased slowly with non-significant difference (P>0.05) of the samples. 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 WEC/kg and 5 g WEC/kg, respectively at 8 days. From the results, WEC showed no effect on pH in ground pork.

The effect of WCE on pH values in ground pork during storage at -20^{o}C over 12 weeks storage period throughout ripening were summarized in Table 4. The results demonstrated that the pH of raw ground pork samples were unaffected by the addition of WCE 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 WCE/kg and 5.97 for the 5 g WCE/kg.

Table 1. Effect of extracts from *C. carandas* fruits on antimicrobial activity using agar well diffusion method

	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

Table 2. Effect of extracts from *C. carandas* fruits on microbiological quality in ground pork during storage at 4°C

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

[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)

All values were expressed as mean ± standard deviation

E.coli was not observed in ground pork as the number of *E. coli* was lower that limit of detection (<1 log cfu/g)

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 WCE/kg.

Table 3. Effect of water extract from C. carandas fruits on biological quality in ground pork during storage at -20oC

Storage time (weeks)		Log 10 CFU/g			
		Control	0.2 g BHT/kg	2.5 g CWCE/kg	5 g CWCE/kg
Total plate count	0	4.53 ± 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}

$^{a-b}$ Means sharing different letters in the same row are significantly different (p <0.05).
$^{A-C}$ Means sharing different letters in the same column in each parameter are significantly different (p <0.05).
All values were expressed as mean ± standard deviation.

Table 4. Effect of extracts from *C. carandas* fruits on pH in ground pork during storage

Storage time (days at 4 ℃)	pH values			
	Control	0.2 g BHT/kg	2.5 g WEC/kg	5 g WEC/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}
(weeks at -20 ℃)	Control	0.2 g BHT/kg	2.5 g WEC/kg	5 g WEC/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

Discussion

This research was studied on antimicrobial activities of WEC and its effect on microbiological and physical qualities in ground pork. WEC showed antimicrobial activities againt both pathogenic and spoilage bacteria which often found in meat such as *S.* Typhimurium*, S. aureus, E. coli, A. hydrophila, P. fluorescens* and *B. coagulans*. In addition, TPC and coliforms in ground pork adding WEC also decreased at the end of storage time. These results were in the agreement to many previous studies. It has been reported that the phenolic compounds found in numerous plant species appear to inhibit pathogenic bacteria. Plants extracts are generally considered to contain antimicrobial compounds (Sokmen *et al.,* 2004; Taie *et al.,* 2010). 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). Additionally, phenolic compounds are regarded as a representative group of antioxidant substances. The potential 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 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). 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. albican*s 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).

It was demonstrated that WEC showed no effect on pH in ground pork. The pH value, which reflects the rate of post mortem glycolysis, is a key indicator of meat quality (Smiecinska *et al.*, 2015). The final pH value of the stored products was similar to their initial pH value during storage days in both chilled and frozen ground pork. 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 WCE. It might have resulted from the influence of the addition of

WCE or low temperature condition on directions of metabolic transformations of microorganisms and enzymes in the ground pork samples.

It is concluded thast *in vitro* antimicrobial activities of *C. carandas* fruit extracts dissolved in water found that the extracts inhibited both pathogenic bacteria and spoilage bacteria at concentration of 100 mg/ml. In addition, number of total microbial count and coliforms with WEC slightly decreased during storage at 6-8 days of chilled and 8-12 weeks of frozen ground pork. After applied WEC in ground pork, WEC had no effect on pH in chilled and frozen ground pork. This finding indicated that *C. carandas* fruit extract could be used as natural antimicrobial substance in ground pork for prolong shelf-life.

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