International Food Research Journal 24(4): 1667-1675 (August 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antioxidant properties of Karanda (*Carissa carandas* Linn.) extracts and its application in Thai traditional fermented pork sausage (Nham)

[1]Sueprasarn, J., [2]Reabroy, S. and [1*]Pirak, T.

[1]Department of Product Development, Faculty of Agro-Industry, Kasetsart University, 50 Ngamwongwan Road, Ladyao, Chatuchak, Bangkok, Thailand
[2]Department of Home Economics, Faculty of Agriculture, Kasetsart University, 50 Ngamwongwan Road, Ladyao, Chatuchak, Bangkok, Thailand

Article history

Received: 26 April 2016
Received in revised form: 2 July 2016
Accepted: 5 August 2016

Keywords

Anthocyanin
*Karanda (*Carissa carandas *Linn.) Extract*
Natural pigment
Antioxidant
Thai Traditional Fermented Pork Sausage (Nham)

Abstract

Anthocyanin is the natural pigment which can easily extract from plants such as blackberry, mulberry and Karanda. Karanda (*Carissa carandas* Linn.) had the fully ripe dark violet fruit contains the large amount of natural anthocyanin. The effect of extraction conditions on anthocyanin content, total phenolic content, and antioxidant activity in the extract was investigated. The extract with highest antioxidant activity was selected to use as natural pigment and antioxidant in Thai traditional fermented pork sausage (Nham). Under controlled extraction condition, type of solvent is the major factor that significantly affected the amount of anthocyanin and phenolic compounds in the extracts, and also antioxidant properties. Extraction of Karanda with 1% HCl in 95% ethanol at the weight ratio of 1:10 and extraction time of 90 minutes yielded the anthocyanin enriched extracts with the highest antioxidant activity. The extract was homogeneously mixed into the batter of Nham prior to stuff into natural casing and ferment at 30°C. The amount of lactic acid bacteria (LAB) and lactic acid content were increased during the fermentation and hence reduce the pH of Nham. At 36 hours of fermentation, the pH of Nham reached to 4.5 and then the reaction was ceased. Anthocyanin did not affect the fermentation of LAB. Nham with Sodium nitrite and 0.50% (w/w) of the extract had the shelf-life at 20 days, which was higher than that of the control (10 days). The consumer accepted the products and could not detect the difference between the sample with Sodium nitrite and 0.50% (w/w) extract.

Introduction

Karanda (*Carissa carandas* Linn.) is wild plants that can be categorized as herb in Apocynaceae family and commonly found in various parts of Thailand. All parts of this plant can be used as traditional medicine. The unripe fruit was reported as an astringent, while the ripe fruit was taken as an antiscorbutic and remedy for biliousness. The leaf was used in the case of intermittent fever, diarrhea, oral inflammation and earache (Devmurari *et al*., 2010). This plant was also reported as the treatment for intestinal worms, scabies, biliousness and pruritus. Moreover, it's biological property in treatment of cardiotonic analgesic, anti-inflammatory, antirheumatic, histamine releasing, anti-pyretic, free radical scavenging, hepato-protective, antibacterial, antiviral and anticonvulsant activity was also reported (Kumar *et al*., 2013). The fully ripe fruit of Karanda is the prominent source of vitamin C and antioxidant, especially anthocyanin and phenolic compounds. The types of anthocyanin commonly found in this fruit are cyanidin-3-O-rhamnoside, pelargonidin-3-O-glucoside and cyanidin-3-O-glucoside (Prasard, 2010). Anthocyanin is categorized in flavonoids group and soluble in water and other hydroxyl solvents. The suitable method for plant anthocyanin extraction was solvent extraction. The important parameters that must be controlled were the ratio of raw material to solvent, type of solvent, extraction time and temperature and concentration of acid in solvent (Castaneda-Ovando *et al*., 2009). The antioxidant property of anthocyanin is well documented. The antioxidation of flavonoids is due to the o-dihydroxyl (Catechol) group at benzopyran ring, that able to give hydrogen atom to free radicals. The induction of other free radicals was then terminated (Pedrielli *et al*., 2001). The utilization of anthocyanin in food is regulated and permitted in some cases. For example, anthocyanin was used as natural color, such as color from grape (*V. labrusca* and *V.vinifera*), in the form of dried powder and concentrated solution

*Corresponding author.
Email: *fagitwk@ku.ac.th, kratay111@gmail.com*
Tel: *+6625625004, +6625625009*
Fax: *+6625625005, +6625623456*

(Henry, 1996). In addition, some researchers reported the utilization of anthocyanin in foods such as soft drinks, clear drinks, dried fruits, sugar confectionary (Henry, 1996), yoghurt and ice cream (Jitpisoot, 2007), ground pork (Jia *et al*., 2012). The extraction method of anthocyanin can be performed by solvent extraction. There are some researchers reported the production method of natural anthocyanin color from various plants; for example, mangosteen peels (Palakajornsak, 2004), black sticky rice (Luemchan, 2007), red cabbage (McDougall *et al*., 2007), roselle (Duangmal *et al*., 2008), berries and grape (Tunde *et al*., 2009; Oancea *et al*., 2012). Ethanol was reported as the proper solvent for anthocyanin extraction (Jia *et al*., 2012). The anthocyanin color is already used in many types of food; however, the utilization in meat products is still lacked. Due to the formation of "nitrosohemochrome", the pigment from nitrate or nitrite and myoglobin in meat product, the stable pink-red color was resulted. Even though the color from nitrate or nitrite is stable, some uncertainty was realized from the excess nitrate or nitrite that can form the carcinogen named "nitrosamine". Hence, the utilization of natural color instead of chemical nitrate or nitrite compounds is the alternative method for producing healthy and safety meat products. There are some researchers reported that the utilization of plants extract in meat product was successfully done in term of natural antioxidant (Shah *et al*., 2014) and natural preservatives (Nowak *et al*., 2016). However, there is still lacked of information of using natural extract as nitrite substitute. The reduction of nitrite in meat products directly affected the attitude and perception of consumer (Hung *et al*., 2016). Karanda, the plant with natural anthocyanin, was selected to be used in this study. The aim of this study was to investigate the optimal anthocyanin extraction condition for producing anthocyanin extract and using as nitrite substitute in Thai traditional fermented pork sausage or Nham. The resulted extract was determined for quality characteristics and antioxidant properties and the extract with the highest antioxidant property was selected to be used as nitrite substitute in Nham. The effect of anthocycanin color on quality of Nham during fermentation and storage period was also investigated.

Materials and Methods

Materials

Fully ripe Karanda fruits were purchased from Samutsongkram Province, Thailand. The quality of raw material was controlled by measuring total soluble solid, titratable acidity and pH and kept under -18°C for further experiments.

Solvent extraction of Karanda

In this experiment, the two factors including the type of solvents (distilled water, 95% ethanol, and 1% HCl in 95% ethanol) and the extraction time (30, 60, 90, 120, 150, and 180) was studied using factorial experiment in CRD. The solvent extraction was performed using the method of Li *et al*. (2011). Karanda was thawed, ground and mixed with solvent at the ratio of 1:10. During extraction, mixture solutions of Karanda and each solvent were stirred homogeneously with magnetic stirrer, and the sample was collected at the time mentioned previously. The resulted extract was then stored and tightly sealed in black glass jar; consequently, the pH, total anthocyanin content (AOAC, 2000), total phenolic content (Kim and Lee, 2002), the antioxidant properties, including DPPH Antioxidant Activity Assay (Stoilova *et al*., 2007) and ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996) of the extracts was performed within the same day of extraction. The experiment was done in duplicate. The best treatment was selected from the antioxidant activity and the selected sample was concentrated with rotary evaporator under high pressure (Cui *et al*., 2013). Concentrated anthocyanin extracts was determined for all parameters similar to that of the extract solution. The yield (%) of concentrated extracts was also calculated (Maisuthisakul *et al*., 2007). The black glass jar was used to keep the concentrated anthocyanin extracts from lights and the jar was then stored at 4°C and used within 3 days. The experimental design in this experiment was a 3x6 factorial experiment in CRD.

Production of Thai traditional fermented pork sausage (Nham)

Nham is the famous Thai traditional meat product and it has been sole on shelf of minimart, open-market, super-market, and hyper-market. The formulation of Nham was obtained from previous work of Rotsachakul (2002) and consisted of 52.59% ground pork, 35.06% chopped pork skin, 4.38% ground garlic, 4.38% cooked rice, 1.84% salt, 0.88% fresh chili, 0.53% sugar, 0.18% sodium tripolyphosphate, 0.18% sodium erythrobate and 125 ppm sodium nitrite. Process for making Nham was performed using the method of Rotsachakul (2002) with a slight modification. Ground pork was put into the mixing bowl and then mixed with dried ingredients, cooked rice, ground garlic, chopped pork skin, and chili. The batter was homogenous mixed by mixer at medium speed for 2 minutes, stuffed into 2 inch plastic casing,

tightly sealed and then placed into an incubator at 30°C for continue fermentation. The change of pH (AOAC, 2000), lactic acid content (AOAC, 2000), total plate count (TPC) (BAM, 1998), total lactic bacteria count (BAM, 1998) was recorded. In addition, the pH of Nham was continually decreased as the increase of lactic acid content which occurred from the fermentation of Lactic acid bacteria. The fermentation was stopped at 36 hours, since the pH of Nham was reached to 4.5. Nham with an appropriate sour taste was obtained.

Effect of Karanda extracts on fermentation and quality characteristics of Nham

In this experiment, the effect of the concentration of anthocyanin from Karanda for using as natural color and Sodium nitrite substitute was studied. Furthermore, its effect of fermentation and quality characteristics of Nham was also studied. This natural pigment was also expected to have the strong antioxidant activity similar to nitrite.

Application of Karanda extracts as natural pigment and antioxidant in Nham and its effect during fermentation

Nham was produced using the similar formulation and method of the previous section (2.3). The concentrated extract was added into the batter of Nham at 0.00, 0.25, 0.50 and 1.00% (w/w). The control sample was Nham with 125 ppm Sodium nitrite. During the fermentation period, the change of pH, lactic acid content, total plate count (TPC), total lactic bacteria count was carefully recorded using the similar method of 2.3. After 36 hours of fermentation, Nham with and without the concentrated extract was picked up, packed into plastic bag, tightly sealed and kept at 4°C for further experiment. The experimental design in this experiment was CRD with 5 treatments.

Effect of Karanda extracts on quality characteristics of Nham

The 5 samples of Nham, which obtained from previous experiment (2.4.2), were brought from the refrigerator and placed into sealed plastic box at normal room temperature for 60 minutes. The final product was then subjected to measure for pH, inside color (CIE$L^*a^*b^*$) (Visessanguan *et al*., 2004), lactic acid content (AOAC, 2000), total plate count (TPC) (BAM, 1998), total lactic bacteria count (BAM, 1998), texture profile using texture profile analysis or TPA (Bourne, 1978), and sensory characteristics using 9-point hedonic scaling test (Chompreeda, 2007).

Effect of Karanda extracts on shelf-life of Nham

Consequently, the shelf-life of Nham with and without concentrated Karanda extract was investigated. The 3 samples (control, Nham with 125 ppm Sodium nitrite and Nham with 0.5% concentrated Karanda extract) were produced using the method described above, fermented at 30°C for 36 hours, and then kept at 4°C for 30 days. The change of pH, lactic acid content, internal color (CIE $L^*a^*b^*$), total plate count (TPC) (BAM, 1998), total lactic bacteria count (BAM, 1998), Thiobarbituric acid number (TBA) (Mansour and Khalil, 2000) and sensory characteristics using 9-point hedonic scaling test (Chompreeda, 2007), was methodically recorded at day 0, 5 10, 15, 20, 25 and 30.

Statistical analysis

All experiments were carried out in duplicate and average values with standard errors were reported. The data were analyzed statistically using the Statistical Package for Social Sciences (SPSS), version 12.0. Analysis of variance (ANOVA) and Duncan's new multiple range test ($p<0.05$) was used to detect the differences among treatment means.

Results and Discussion

Effect of extraction condition on properties of Karanda extracts

According to solvent extraction, there are many significant factors that affected the yield and purity of extracts. Type of solvent, extraction time and temperature are the important factors. In this study, the effect of solvent type and extraction time was investigated. Extracts was collected after extracting in DI water, 95% ethanol or 1% HCl in 95% ethanol for 30, 60, 90, 120, 150 and 180 min and then determined for total anthocyanins, total phenolic compounds and antioxidant properties. The solvent used for Karanda extraction significantly affected pH, total anthocyanins content, total phenolic content and the antioxidant activities (Table 1). With DI water, the resulted extracts possessed pH in the range of 2.97 to 3.10. The extracts obtained from 95% ethanol had higher pH (4.59-4.69), while the lowest pH was found in the extracts with 1% HCl in 95% ethanol (0.82-1.05). The color of the extracts was varied from light red to dark red, depending of the amount of anthocyanin and pH. At pH lower than 7, anthocyanin had the red pigment color. The total anthocyanin found in the extracts was significantly different ($p<0.05$), due to the extraction capability of each solvent. An extraction with DI water yielded the extract with the lowest anthocyanins content (15.16-

Table 1. The pH and antioxidant activity including total anthocyanins (mg cyanidin-3-glucoside/100g), total phenolic content (mg GAE/100g), DPPH (% scavenging effect) and FRAP (mmol $FeSO_4$/g) of Karanda extracts and concentrated Karanda extract

Sample		Time (min)	pH	L* values	a* values	b* values	Total monomeric anthocyanins (mg cyanidin-3-glucoside/100g	Total phenolic (mg GAE/100g)	FRAP (mmol $FeSO_4$/g))	DPPH (% scavenging effect)
Karanda extracts	DI water	30	3.08^{B}±0.00	65.33±0.03	47.39±0.06	25.99±0.10	15.16^{Cc}±0.23	82.04^{Ce}±0.55	118.83^{Ca}±1.93	19.39^{Ce} ± 0.16
		60	3.10^{B}±0.00	62.02±0.01	47.58±0.02	29.45±0.04	15.70^{Cc}±0.12	92.69^{Cc}±1.45	120.40^{Ca}±2.29	23.36^{Cd}± 0.24
		90	3.02^{B}±0.00	71.80±0.01	43.54±0.01	18.32±0.02	27.44^{Ca}±0.66	114.50^{Cb}±1.90	114.88^{Cab}±6.07	26.08^{Ca} ± 0.24
		120	2.97^{B}±0.01	64.80±0.00	44.06±0.01	27.09±0.05	25.08^{Cb}±0.59	114.86^{Cb}±0.33	114.78^{Cab}±3.50	23.12^{Cb} ± 0.00
		150	3.09^{B}±0.00	63.46±0.02	44.36±0.02	26.36±0.06	24.89^{Cb}±1.07	118.41^{Ca}±164	112.02^{Cb}±1.80	22.70^{Cc} ± 0.28
		180	3.04^{B}±0.00	59.36±0.04	45.75±0.02	29.68±0.04	24.89^{Cb}±0.33	118.49^{Ca}±1.82	100.88^{Cc}±3.11	22.14^{Cd}± 0.26
	95% Ethanol	30	4.64^{A}±0.00	60.62±0.24	49.83±0.25	-6.78±0.06	55.17^{Ba}±1.28	144.79^{Bc}±5.72	177.50^{Be}±1.81	34.66^{Bd} ± 0.06
		60	4.64^{A}±0.00	60.34±0.06	50.05±0.07	-6.73±0.07	60.37^{Ba}±5.49	153.56^{Bc}±2.68	179.45^{Be}±2.01	36.09^{Be}± 0.21
		90	4.61^{A}±0.02	58.33±0.01	52.40±0.04	-6.91±0.03	65.58^{Ba}±2.71	172.83^{Be}±4.32	192.92^{Da}±3.24	38.42^{Bb} ± 0.30
		120	4.59^{A}±0.01	66.23±0.10	42.43±0.11	-6.12±0.05	45.42^{Ba}±2.27	151.38^{Bb}±1.07	155.35^{Bc}±3.90	39.19^{Ba}± 0.66
		150	4.66^{A}±0.01	64.99±0.05	44.73±0.04	-6.84±0.01	48.83^{Bd}±0.49	155.88^{Bb}±1.45	154.54^{Bc}±3.55	31.40^{Be} ± 0.16
		180	4.69^{A}±0.01	69.11±0.06	38.90±0.07	-5.48±0.04	40.50^{Be}±0.43	141.09^{Bc}±4.02	127.40^{Be}±3.91	34.69^{Bd} ± 0.42
	1% HCl in 95% Ethanol	30	1.08^{C}±0.00	43.46±0.01	73.33±0.02	59.00±0.17	76.74^{Aa}±2.29	202.69^{Abc}±2.77	231.02^{Aa}±3.24	77.34^{Aa} ± 0.12
		60	1.03^{C}±0.00	43.39±0.13	73.24±0.17	58.73±0.16	76.74^{Aa}±1.00	206.89^{Ab}±7.78	226.07^{Abc}±3.84	77.96^{Aa} ± 0.06
		90	0.97^{C}±0.02	42.96±0.01	72.90±0.01	63.57±0.03	81.00^{Aa}±1.80	216.53^{Aa}±6.64	259.11^{Aa}±3.64	78.28^{Aa} ± 0.12
		120	0.82^{C}±0.01	44.24±0.01	73.97±0.03	54.57±0.11	67.09^{Ac}±2.91	198.78^{Abc}±2.18	220.40^{Abc}±9.36	78.24^{Aa} ± 0.00
		150	1.01^{C}±0.01	44.22±0.02	73.85±0.02	54.07±0.03	65.96^{Acd}±1.61	196.75^{Ac}±1.54	215.11^{Acd}±4.22	77.44^{Ad} ± 0.06
		180	1.05^{C}±0.00	44.55±0.01	73.89±0.02	51.92±0.10	63.21^{Ad}±0.43	186.31^{Ac}±4.02	202.69^{Ac}±9.38	77.79^{Ac} ± 0.12
Concentrated Karanda extract			0.86±0.00	2.48±0.04	6.46±0.27	1.62±0.12	1,202.65±30.16	4,123.96±64.20	44.14±1.55	66.37± 0.12

$^{A-F}$ means within the same column with different letters are significantly different ($p<0.05$).
$^{a-e}$ means within the same column with different letters are significantly different ($p<0.05$) on time in each solution.

Table 2. Microbial content of Nham during fermentation and physical properties and sensory characteristics of Nham after fermenting for 36 hours

Properties		Sample				
		$NaNO_2$	Control	0.25%	0.50%	1.00%
Microbial properties						
TPC (log cfu/g)	Time (h)					
	0	6.59	6.55	6.56	6.55	6.25
	12	7.46	7.53	7.46	7.53	7.52
	24	7.71	7.81	7.77	7.78	7.90
	36	7.79	7.89	7.73	7.80	7.85
LAB (log cfu/g)	Time (h)					
	0	6.49	6.57	6.56	6.55	6.25
	12	7.46	7.53	7.46	7.53	7.52
	24	7.81	7.87	7.72	7.81	7.79
	36	7.57	7.79	7.62	7.66	7.73
Physical properties at 36 hours of fermentation						
Texture	Hardness	154.92^{a} ± 1.62	142.35^{b} ± 0.01	117.38^{c} ± 0.01	100.35^{d} ± 1.70	49.91^{e} ± 2.11
	Cohesiveness	0.59^{a} ± 8.00	0.55^{b} ± 0.00	0.57^{a} ± 0.00	0.55^{b} ± 2.56	0.34^{c} ± 1.91
	Springiness	0.90^{a} ± 0.83	0.91^{a} ±0.01	0.88^{b} ± 0.00	0.88^{b} ± 0.76	0.73^{c} ± 0.47
	Gumminess	92.78^{a} ± 2.07	79.11^{b} ± 0.02	64.86^{c} ± 0.01	54.19^{d} ± 2.06	16.96^{e} ± 1.14
	Chewiness	83.09^{a} ± 0.34	71.57^{b} ± 0.01	56.78^{c} ± 0.01	42.58^{d} ± 0.31	12.79^{e} ± 0.13
Sensory characteristis at 36 hours of fermentation						
Attribute	Appearance	6.87^{a} ± 1.04	6.53^{b} ± 1.11	6.77^{ab} ± 1.07	6.83^{ab} ± 1.05	5.53^{c} ± 1.70
	Color	6.77^{a} ± 1.48	6.17^{a} ± 1.29	6.27^{a} ± 1.41	6.80^{a} ± 1.13	5.37^{b} ± 1.65
	Odor	6.83^{ab} ± 1.05	6.30^{b} ± 1.49	7.03^{a} ± 0.93	6.63^{ab} ± 1.27	6.27^{b} ± 1.60
	Flavor	6.50^{a} ± 1.04	6.07^{a} ± 1.55	6.47^{a} ± 1.22	6.30^{a} ± 1.44	5.20^{b} ± 1.49
	Firmness	6.97^{a} ± 0.93	6.47^{a} ± 1.36	6.77^{a} ± 1.36	6.53^{a} ± 1.17	4.60^{b} ± 1.57
	Taste	6.63^{a} ± 0.89	6.00^{a} ± 1.39	6.43^{a} ± 1.28	6.33^{a} ± 1.42	5.03^{b} ± 1.54
	Overall liking	6.70^{a} ± 0.95	5.93^{b} ± 1.31	6.53^{ab} ± 1.25	6.50^{ab} ± 1.20	4.73^{c} ± 1.46

$^{a-e}$ means within the same column with different letters are significantly different ($p<0.05$).

24.89 mg cyanidin-3-glucoside/100g) and phenolic content (82.04-118.49 mg GAE/100g), hence, the lowest antioxidant activity was also resulted (Table 1). The increase of total anthocyanins content and total phenolic content was resulted as the increase of extraction time with DI water. Moreover, it was found that the 95% ethanol was the better solvent that able to extract the anthocyanin and phenolic compounds. The resulted extracts had total anthocyanin content at 40.50-55.17 mg cyanidin-3-glucoside/100g, total phenolic content at 141.09-172.83 mg GAE/100g, FRAP at 127.40-192.92 mmol $FeSO_4$/g, and DPPH at 31.40-39.19% scarvenging effect. The best solvent was resulted as 1% HCl in 95% ethanol solution. The

amount of total anthocyanin content (63.21-81.00 mg cyanidin-3-glucoside/100g) and total phenolic content (186.31-216.53 mg GAE/100g), were significantly higher than that of the other solvents ($p<0.05$). As a result, the highest antioxidant activity was also received with this solvent. The result revealed that the extracts possessed FRAP at 202.69-259.11 mmol $FeSO_4$/g, and DPPH at 77.34-78.28% scavenging effect.

In this experiment, the optimal condition for extraction of anthocyanin from Karanda was obtained (Table 1). The extracts with the highest antioxidant activity was obtained by extracting Karanda with 1% HCl in 95% ethanol for 90 minutes at the ratio of ground Karanda to solvent of 1:10. This treatment was characterized for total anthocyanin content, total phenolic content, and antioxidant acitivity (FRAP and % DPPH) and resulted in the highest amount in all parameters. It possessed total anthocyanin content at 81.00 mg cyanidin-3-glucoside/100g, total phenolic content at 216.53 mg GAE/100g, FRAP at 259.11 mmol $FeSO_4$/g, and DPPH at 78.28% scarvenging effect. This treatment was finally prepared in large scale and then concentrated for using as concentrated Karanda extract in the remained experiment. Furthermore, the very dark red-viscous solution was resulted after finishing concentration. This concentrated Karanda extract had the high content of total anthocyanin (1,202.65 mg cyanidin-3-glucoside/100g) and total phenolic compound (4,123.96 mgGAE/100g). This result revealed that rotary evaporator at mild condition was the suitable method for the evaporation of the excess solvent out of the Karanda extracts. The concentrated Karanda extracts was also shown the high antioxidant activity (44.14 mmol $FeSO_4$/ 0.0001g and 66.37% scarvenging effect at the dilution of 1 to 10,000). This sample was kept in black glass jar and tightly sealed until further use in the next experiment.

Effect of concentrated Karanda extracts on fermentation and quality characteristics of Nham during fermentation and after fermentation for 36 hours

The effect of concentrated anthocyanin extracts from Karanda on fermentation and quality characteristics of Nham was resulted. The optimum concentration of the concentrated extract for using as natural color and Sodium nitrite substitute in Nham was revealed at 0.50% (w/w). During fermentation, the amount of lactic acid bacteria (LAB) was recorded at 0, 12, 24 and 36 hours (Table 2). As a result, the addition either Sodium nitrite or the concentrated extract did not affect the growth of LAB (Table 2).

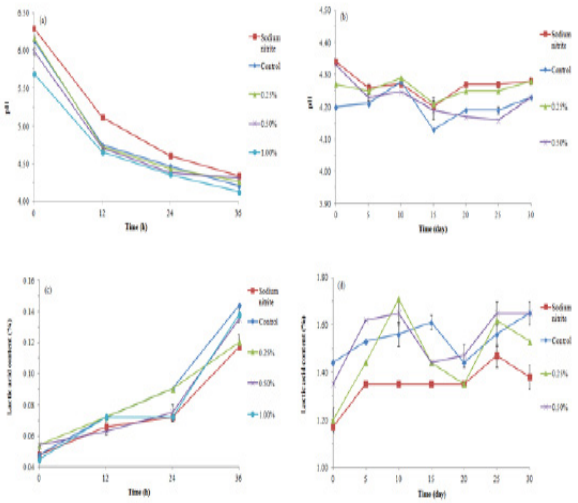

Figure 1. The changes of pH of Nham during fermentation (a) and Nham during storage for 30 days (b) and the changes of lactic acid content of Nham during fermentation (c) and Nham during storage for 30 days (d)

The numbers of lactic acid bacteria in Nham with and without the extracts were almost similar. Lactic acid bacteria can growth under fermentation condition with the extract. Moreover, the pH of Nham was reduced gradually (Figure 1a), which was resulted from lactic acid produced by LAB (Figure 1c). The pH of Nham was lower than 4.5 after 36 hours of fermentation (Figure 1a), and this condition was selected for the following experiment.

Color of Nham was slightly changed during fermentation (Figure 2a, 2b and 2c). Lightness (L^*) and yellowness (b^*) of Nham with Sodium nitrite was lower than the other samples with the extract, while the redness (a^*) of such sample was increased sharply. When considering the concentration of the concentrated Karanda extract, the color of Nham with 0.00 (control) and 0.25% (w/w) Karanda extract was differed from that of the Nham with 0.50 and 1.00% (w/w) Karanda extract. Nham after fermenting for 36 hours had red color; however, the bright red was found only in Nham with Sodium Nitrite. Nham with the extracts had lower a^* and higher L^* and b^*, when comparing to that of the Sodium nitrite sample.

After fermentation for 36 hours, Nham with the concentrated extract showed the lower value of hardness, cohesiveness, springiness, gumminess and chewiness, than that of the Nham with Sodium nitrite. The textural property was almost changed from that of the Nham with Sodium nitrite, when adding the concentrated Karanda extract at 1.00% (w/w). The textural change was occurred from the pH of the concentrated extract. When considering the sensory characteristic of Nham, the concentrated

extract also affected the liking of consumer (Table 2), especially in Nham with 1% (w/w) extract. Undesirable texture and sensory properties were found in treatment with 1.00% (w/w) extract. This treatment also possessed the lowest score of overall liking (4.73). Unexpectedly, the sensory characteristic of Nham with the concentrated extract at 0.25 and 0.05% (w/w) were not significantly different from that of the treatment with Sodium nitrite ($p<0.05$). This result revealed that even though the textural property of Nham was changed from the addition of the extracts, consumer still accepted the product. They could not detect the change of texture of Nham with 0.25 and 0.50% extracts. Moreover, Nham without the concentrated extract (control) had the overall liking score at 5.93, which was lower than Nham with the concentrated extract at 0.25% (6.53) and 0.50% (6.50). This result revealed that consumer could detect the difference of Nham with Sodium nitrite and Nham without Sodium nitrite and extract (control). This result also suggested that the concentrated extract had the potential to be used as Sodium Nitrite substitute in Nham. The optimum concentration of Karanda extract was 0.50% (w/w).

Effect of concentrated Karanda extracts on shelf-life of Nham (4°C, 30 days)

Nham was kept at 4°C for 30 days in order to investigate the product shelf-life. The pH and lactic acid content (%) of Nham during storage were recorded (Figure 1b and 1d). The change of pH occurred during the storage period from the production of lactic acid by LAB (Figure 1b). These results were agreed with the change of lactic content (Figure 1d). Moreover, the pH and lactic acid content of Nham stored for 20 days, was not significantly different from that of the initial sample (day 0). This result implied that the quality of Nham was not differed from the initial sample when stored at 4°C for 20 days. The color of Nham during storage was shown in Figure 2d, 2e and 2f. $L^*$and b^* of all samples tended to increase, while a^* was gradually reduced ($p<0.05$). The different color among samples was occurred due to the different properties of Sodium nitrite and the extract.

Nham has high water activity and protein content; hence it is easily spoiled. The discoloration and rancid odor were occurred after spoilage. This is unacceptable and unsafe for consumer. The addition of Sodium nitrite into Nham resulted in lowering rancidity; however, the discoloration was still occurred. The cure pigment was faded due to the expose with light. This result was shown in Table 3. The TBA value of Nham with Sodium nitrite was

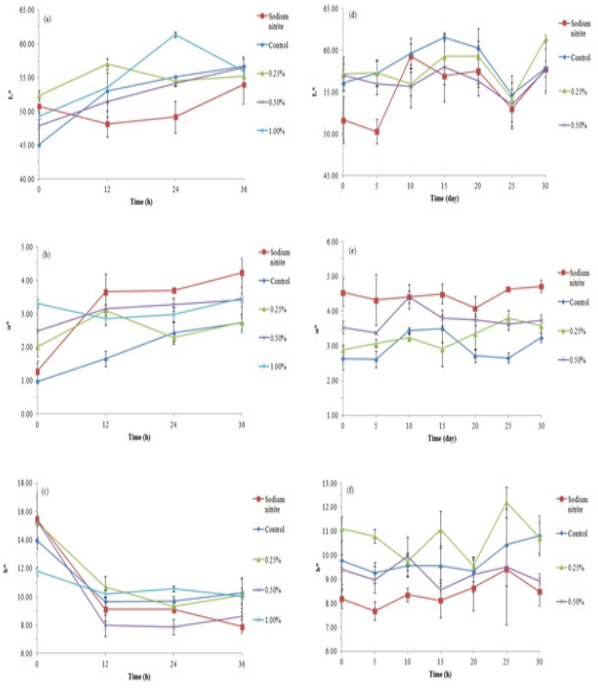

Figure 2. The color changes (CIE $L^*a^*b^*$) of Nham during fermentation: (a) = lightness (L^*), (b) = redness (a^*) and (c) = yellowness (b^*), and the color changes of Nham during storage for 30 days: (d) = lightness (L^*), (e) = redness (a^*) and (f) = yellowness (b^*)

gradually increased from 0.73 mg malonaldehyde/kg at day 0 to 1.25 mg malonaldehyde/kg at day 30. In contrast, the TBA value of Nham with the concentrated Karanda extract was drastically increased to 7.81 mg malonaldehyde/kg at day 30. The highest TBA value (9.56 mg malonaldehyde/kg) was found in control sample after storage for 30 days ($p<0.05$). However, the sensory characteristics of Nham with Sodium nitrite and Nham with the extract were not significantly different ($p>0.05$). The change of TBA value along the storage period was not detected by consumer. Moreover, the TPC and LAB counts of all samples were performed with the almost similar amount of TPC and LAB were resulted. This result implied that the almost bacteria found in Nham were LAB. The percentage of acceptance of Nham with Sodium nitrite, control and the extract was gradually decreased from 100% to 66.67, 43.33, and 56.67, respectively. At day 20, the percentage of acceptance and overall liking score of Nham with sodium nitrite and the extract were not significantly different ($p<0.05$) (Table 3). This result revealed that consumer could not detect the different of color, odor and texture of these two samples and also accepted the product with the extract similar to sodium nitrite. At the end of storage period (30 days), all samples were unacceptable and had overall liking lower than 6.0. Hence, Nham with sodium nitrite and the extract had the shelf-life at 20 days, which was higher than

Table 3. TBA value, microbial analysis and sensory evaluation of Nham during storage for 30 days

Sample	Days	TBA (mg malonaldehyde/kg)	Microbial content		Sensory characteristics					
			TPC (log cfu/g)	LAB (log cfu/g)	Appearance	Color	Odor	Firmness	Overall linking	Acceptance (%)
$NaNO_2$	0	0.73^{g}±0.04	7.79	7.57	6.87^{abc} ± 1.04	6.77^{ab} ± 1.48	6.83^{ab}± 1.05	6.97^{ab} ± 0.93	6.70^{abcde} ± 0.95	100
	5	0.74^{g}±0.07	7.92	7.70	7.20^{a} ± 1.30	7.10^{a} ± 1.42	6.57^{abcde} ± 1.59	6.97^{ab} ± 1.35	6.83^{abc} ± 1.32	86.67
	10	0.76^{g}±0.04	7.83	7.55	$6.60^{abcdefg}$± 1.69	6.77^{ab}± 1.14	6.77^{abc} ± 1.59	6.77^{abcde} ± 1.43	6.97^{ab} ± 1.13	93.33
	15	1.00^{fg}±0.03	7.68	7.64	7.00^{ab} ± 1.20	7.03^{a} ± 1.07	6.93^{a} ± 1.14	7.03^{a} ± 1.00	7.00^{a} ± 1.20	83.33
	20	1.07^{fg}±0.05	7.41	7.10	$6.60^{abcdefg}$ ± 1.22	6.30^{abcd} ± 1.56	6.83^{ab} ± 1.02	6.93^{abc} ± 1.05	6.73^{abcd} ± 1.17	86.67
	25	1.14^{fg}±0.02	7.61	7.54	6.20^{bcdefg} ± 1.81	6.40^{abcd} ± 1.63	5.90^{cdefg} ± 1.84	$6.17^{abcdefg}$ ± 1.58	6.10^{cdefgh} ± 2.01	73.33
	30	1.25^{fg}±0.03	7.35	7.07	6.10^{bcdefg} ±1.49	6.30^{abcd} ±1.66	5.43^{fg} ±1.59	$6.27^{abcdefg}$ ±1.41	5.93^{defgh} ±1.36	66.67
Control	0	2.25^{cd}±0.06	7.99	7.79	$6.53^{abcdefg}$ ± 1.11	6.17^{abcde} ± 1.29	$6.30^{abcdefg}$ ±1.49	$6.47^{abcdefg}$ ± 1.36	5.93^{defgh} ± 1.31	100
	5	2.58^{cd}±0.07	7.92	7.78	$6.53^{abcdefg}$± 1.22	6.37^{abcd} ± 1.35	$6.27^{abcdefg}$ ± 1.51	6.67abcd ± 0.99	$6.47^{abcdefg}$ ± 1.20	86.67
	10	3.25^{bc}±0.08	7.62	7.64	6.20^{bcdefg} ± 1.32	5.97^{bcdefg} ± 1.61	$6.00^{abcdefg}$ ± 1.76	$6.40^{abcdefg}$ ± 1.75	$6.37^{abcdefg}$ ± 1.25	86.67
	15	4.36^{b}±0.05	7.45	7.23	5.43^{g} ± 1.63	5.07^{efg}± 1.46	5.67^{defg} ± 1.71	$6.27^{abcdefg}$ ± 1.23	5.57^{efgh} ± 1.55	56.67
	20	5.47^{b}±0.04	7.83	7.69	5.87^{cdefg} ± 1.20	5.53^{defg} ± 1.43	5.90^{cdefg} ± 1.75	$6.23^{abcdefg}$ ± 1.65	5.93^{defgh}± 1.46	56.67
	25	5.60^{b}±0.06	7.60	7.64	5.83^{cdefg} ± 1.68	5.33^{efg} ± 1.75	5.40^{fg} ± 1.75	$5.97^{abcdefg}$ ± 1.52	5.53^{efgh} ± 1.72	53.33
	30	9.56^{a}±0.03	7.39	6.98	5.43^{g} ± 1.74	4.87^{g} ± 1.80	4.33^{g} ± 1.83	5.87^{bcdefg} ± 1.78	4.80^{h} ±1.65	43.33
0.50%	0	1.91^{ef}±0.10	7.80	7.66	6.83^{abcd} ± 1.05	6.80^{ab} ± 1.13	6.63^{abcd} ± 1.27	$6.53^{abcdefg}$± 1.17	$6.50^{abcdefg}$ ± 1.20	100
	5	2.13^{de}±0.02	7.96	8.00	6.77^{abcd} ± 0.94	6.60^{ab} ± 1.13	$6.43^{abcdefg}$ ± 1.33	$6.43^{abcdefg}$± 0.94	$6.53^{abcdefg}$ ± 1.17	93.33
	10	2.66^{de}±0.06	7.68	7.63	6.67^{abcde} ± 1.18	6.27^{abcd} ± 1.41	$6.07^{abcdefg}$± 1.66	$6.33^{abcdefg}$ ± 1.35	$6.33^{abcdefgh}$± 1.37	86.67
	15	3.25^{bc}±0.04	7.57	7.57	$6.27^{abcdefg}$ ± 1.23	6.07^{abcde}±1.72	5.87^{cdefg} ± 1.59	$5.93^{abcdefg}$ ± 1.20	5.90^{defgh}± 1.35	63.33
	20	4.03^{b}±0.07	7.54	7.40	$6.37^{abcdefg}$ ± 1.13	6.00^{abcde} ± 1.17	5.93^{cdefg} ± 1.48	$6.57^{abcdefg}$ ± 1.01	$6.17^{abcdefg}$± 1.12	83.33
	25	5.00^{b}±0.07	7.63	7.64	6.20^{bcdefg} ± 1.24	6.00^{abcde} ± 1.36	5.60^{defg}± 1.85	$6.10^{abcdefg}$ ± 1.37	5.73^{defgh}± 1.64	56.67
	30	7.81^{a}±0.04	7.39	7.16	5.87^{cdefg}± 1.46	6.00^{abcde}± 1.34	5.23^{efg}± 1.61	5.67^{cdefg}±1.49	5.33^{gh}± 1.47	56.67

[a-j] means within the same column with different letters are significantly different ($p<0.05$).

that of the control (10 days).

The results from extraction part revealed that anthocyanin can easily dissolve in ionic solvents, such as ethanol, since it has the dipole-dipole force under the rules of "like dissolve like" (Amelia *et al*., 2013). The addition of hydrochloric acid can increase the solubility of anthocyanin by hydrolyzing the Karanda tissue and hence the higher amount of anthocyanin content was resulted Barnes *et al*. (2009). The phenolic content of Karanda extract was increased at 30, 60 and 90 minutes of extraction and then decreased after 90 minutes of extraction. The highest phenolic content was found in treatment with extraction time of 90 minutes. This result might state from the oxidation reaction which occurred during the long extraction period (Naczk and Shahidi, 2004). Moreover, the excess extraction time could destroy the phenolic compound in the tissue (Kuljarachanan *et al*., 2009) and hence resulted in lowering the phenolic content in the extract. The antioxidant property (DPPH and FRAP) of the extracts was depended on the content of anthocyanin and phenolic compounds. The sample which showed the highest antioxidant activity also possessed the highest anthocyanin and phenolic content. This result was agreed with the report of Chew *et al*. (2011), who studied the antioxidant activity of Asiatic pennywort extract.

The concentrated Karanda extract was added in Nham before fermentation. The extract did not affect the growth of LAB because the fermentation was performed under the optimal condition enriched for LAB (30°C, 36 hours). This result was agreed with the study of Visessanguan *et al*. (2006), who found the similar condition for fermentation of Nham. The concentration of Karanda extract was varied and the increase of its concentration resulted in lowering all texture parameters. This result might cause by protein denaturation occurred due to low pH of the extract (Quinton *et al*., 1997). When stored Nham at 4°C for 30 days, Sodium nitrite showed the best antioxidant property from the ability to interact with free radical, retard the formation of peroxide and terminate the oxidation reaction (Kanner *et al*., 1984). Anthocyanin and phenolic compound in the concentrated Karanda extract also showed the similar mechanism; however, their antioxidant ability was lowered due to the degradation from heat and light exposition (Dufosse *et al*., 2005). It easily destroyed and could not act as antioxidant. The sharp increase of TBA was then resulted.

Conclusion

Extraction of Karanda with 1% HCl in 95% ethanol at the weight ratio of 1:10 and extraction time of 90 minutes yielded the extracts enriched with anthocyanin. It had the highest antioxidant activity and possessed total anthocyanin content at 81.00 mg cyanidin-3-glucoside/100g, total phenolic

content at 216.53 mg GAE/100g, FRAP at 259.11 mmol $FeSO_4$/g, and DPPH at 78.28% scavenging effect. Anthocyanin did not affect the fermentation of LAB. Nham with Sodium nitrite and the extract (0.50% w/w) had the shelf-life at 20 days, which was higher than that of the control (10 days). The consumer accepted the products and could not detect the difference between these two samples. Hence, the concentrated Karanda extract can be used as antioxidant and natural pigment in fermented meat products with the quality characteristics similar to the sample with Sodium nitrite.

Acknowledgement

This research was financially supported by Research Grant for Graduated student, Graduate School of Kasetsart University, Bangkok, Thailand.

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