

Comparative evaluation of physicochemical compositions, antioxidant activities and microbiological quality of three chili pastes from *Carissa carandas* L. under different conditions

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Abstract

The development of chili paste from karanda (*Carissa carandas* L.) was aimed to obtain a product which will benefit consumer health and promote the use of Thai herbs to become functional foods. This research was designed to conduct a comparative evaluation of physicochemical compositions, total phenolic content (TPC), antioxidant activities, microbiological qualities, and sensory testing of three karanda chili paste formulas (formula A, B and C). The physicochemical proximate compositions were analyzed by the AOAC method. The TPC of the extracts was determined by the Folin-Ciocalteu method. The antioxidant activities were tested by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging DPPH and ferric reducing antioxidant power (FRAP) assay. The results showed that the colour values of redness (a^*) and yellowness (b^*) of chili paste in formula B were lower than others. Formula B was significantly higher than others in protein content whereas formula C was significantly higher than others in fat, carbohydrate, ash and energy. The extract of steamed formula A contained the highest TPC (3.94 ± 0.11 milligram gallic acid equivalent per gram of fresh weight (mg GAE/g FW)). Similarly, this extract containing most karanda showed stronger antioxidant capacities, 11.55 ± 0.04 milligram trolox equivalent per gram of fresh weight (mg TE/g FW), and 11.93 ± 0.02 micromole trolox equivalent per gram of fresh weight ($\mu\text{mole TE/g FW}$) as determined by DPPH and FRAP assays, respectively. The DPPH and FRAP values of steamed chili paste were higher compared with unsteamed chili paste in all formulas. Moreover, the steamed chili paste did not have counts of the total aerobic bacteria, *Escherichia coli*, yeasts, and mold that exceeded the community product standard. Overall preference for all formulas of karanda chili paste was not significantly different, but formula A was the most acceptable. This study demonstrated that karanda chili paste could retain its antioxidant activity and help to reduce microbial contamination due to steaming and its spice phytochemicals; therefore, it could be valuable as a functional food for preventing oxidative stress-mediated human disorders.

1. Introduction

The change in lifestyle globally has led to the emergence of chronic non-communicable diseases related to food. Chronic diseases are a global health problem which results in the deaths of millions of people (Zhang *et al.*, 2015). It has been demonstrated that fruits and vegetables exert a protective effect against the development of these chronic diseases (Kruk, 2014; Mursu *et al.*, 2014). Fruits and vegetables in foods are an important source of phytonutrients that have been shown to be necessary for sustaining human consumption

(Zhang *et al.*, 2015). They are immensely valuable, not only for their nutritional value but also for their potential health functionality against various degenerative diseases (Dimitrios, 2006; Deepa *et al.*, 2007). The phytonutrients display antioxidant properties and can therefore act as free radical scavengers, resulting in the inhibition of the oxidative mechanisms which are responsible for many disorders and diseases in humans (Valko *et al.*, 2007; Painupong, 2017). Free radicals such as reactive oxygen species (ROS) are generally produced during the various metabolic processes in our body and are involved in the

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onset of many diseases such as cancer, diabetes, Alzheimer's disease and atherosclerosis as well as in degenerative processes associated with aging (Halliwell and Gutteridge, 1999; Patel *et al.*, 2010). Almost all organisms have various antioxidants, but these systems are not sufficient to prevent free radical damage entirely. Thus, organisms need antioxidants in their food as supplements to fulfil their needs (Siddhuraju and Becker, 2007). However, a healthy diet may improve or maintain optimal health by giving an individual proper nutrition and protecting them from various diseases.

Karanda (*Carissa carandas* L.) is a tropical plant that belongs to the Apocynaceae family and is commonly found in various parts of Thailand, locally known as Namdaeng, Manao ho, Naam kheehaet). It is a large dichotomously branched evergreen shrub or small crooked tree of up to 3 m in height with a short stem and strong thorns in pairs (Hegde *et al.*, 2009). The whole plant and its parts contain a huge amount of vitamins, minerals, and phenolics, so they have been used in traditional medicine for the treatment of various ailments (Sueprasarn *et al.*, 2017; Singh *et al.*, 2020). The prominent biological activities reported include antidiabetic, antimicrobial, hepatoprotective, antihyperlipidemic, cardiac depressant, anti-inflammatory and antiviral properties (Bhaskar and Balakrishnan, 2009; Hegde and Joshi, 2009; Itankar *et al.*, 2011; Agarwal *et al.*, 2012; Sumbul and Ahmed, 2012; Sudjaroen *et al.*, 2021). The unripe fruit of karanda is a rich source of flavonoids, flavanones, alkaloids, saponins, tannins, iron, vitamin C, and pectin, which are substances that have biological activity in antioxidants against cancer cells and are useful in the prevention of anemia (Kubola *et al.*, 2011; Gupta *et al.*, 2014; Debashis *et al.*, 2016; Kasturi *et al.*, 2018).

Traditional Thai foods have very distinctive characteristics because of the special combinations of herbs and spices used in their preparation. Thai native herbs provide essential nutrition and medicinal properties in Thai foods (Chaisawadi *et al.*, 2005). Chili paste is a traditional food in Thailand and is popularly consumed across Southeast Asia because it can be produced easily, is low in calories, and has a unique taste. However, its flavor depends on the local ingredients that have been added. In general, the ingredients used in the paste are chili, shallot, and garlic. Chili (*Capsicum annuum* L.) is a rich source of phenolics and flavonoids which provide antioxidant activities which could inhibit the growth of *Helicobacter pylori* (Jones *et al.*, 1997). Shallots (*Allium ascalonicum* L.) and garlic (*Allium sativum* L.) have mainly been observed to exhibit antibacterial activity and can inhibit both lipid peroxidation and radical scavenging activity (Ferrerres *et al.*, 1996; Nuutila *et al.*,

2003).

To the best of our knowledge, the physicochemical and antioxidant potential of karanda chili paste of different formulas has never been evaluated and compared. Given the high nutritional profile of karanda, it may serve as a potential source of plant dietary nutrients in industrial food. Therefore, this research aimed to develop chili paste made with karanda and was designed to evaluate the proximate compositions, antioxidant activity, microbiological quality, and sensory evaluation in three formulas of chili paste in order to improve the potential health benefits of the product.

2. Materials and methods

2.1 Plant materials and reagents

The unripe karanda fruits (pink-red colour), with the sour taste of chili paste, were purchased from Samut Songkram province, Thailand. The taxonomic identification of plant species was authenticated by Bangkok Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Thailand. The samples were selected to include only good quality fruit, washed thoroughly, and placed in a sieve to dry at room temperature. After that, the fruit was stored at $4\pm 1^\circ\text{C}$ until it was used for analysis or other processes. All chemicals, reagents, and media were of analytical grade obtained from Sigma Chemical Co. (St. Louis, MQ, USA) and Merck (Darmstadt, Germany).

2.2 Preparation and production of karanda chili paste

The formulations for producing karanda chili paste are shown in Table 1. In this experiment, karanda was used instead of tamarind. For the preparation of the karanda chili paste, all spices were sorted, trimmed and washed thoroughly with distilled water to remove dust

Table 1. Formulations of the ingredients used for karanda chili paste.

Ingredients	Amount (% w/w)		
	Formula A	Formula B	Formula C
Shrimp paste	10.48	9.91	13.80
Garlic	2.81	4.70	5.65
Dried chili	4.66	7.50	3.50
Karanda	40.20	29.51	23.05
Palm sugar	7.50	10.00	15.00
Pickled fish	22.04	13.36	20.60
Dried shrimp	-	8.91	5.20
Oil	3.19	-	3.50
Shallot	3.12	8.91	-
Salt	-	1.20	-
Fish sauce	-	-	3.70
Lemon grass	2.50	2.50	2.50
Galangal	2.50	2.50	2.50
Bergamot	1.00	1.00	1.00

and dirt, and then weighed according to the formula. Based on household preparations, the pickled fish, shrimp paste, dried chili, garlic, shallot, dried shrimp, oil, lemon grass, galangal, and bergamot were mixed and pounded together to make the karanda chili paste. The seasonings were adjusted and mixed well with palm sugar, fish sauce, or salt. After that, they were mixed in a blender at a level 3 speed for 5 mins. Then, karanda was added to the mixture and the mixture was blended for 5 mins more to make a fine paste. Lastly, some chili paste was steamed in the pot at 80°C for 5 mins, and packed in sterilized containers (Muninnopamas *et al.*, 2021).

2.3 Effect of temperature on activities of karanda chili paste

The samples of karanda chili paste were developed to test the effect of temperature on their biological activities. This experiment was divided into four groups: group 1 was steamed for 5 mins and kept at 4°C; group 2 was steamed for 5 mins and kept at room temperature; group 3 was not steamed and was kept at 4°C, and group 4 was not steamed and was kept at room temperature. The two factors, including the cooking processes (steamed, and not steamed) and storage conditions (4°C, and room temperature) were studied using a completely randomized design (CRD) in the experiments.

2.4 Sample extraction

The sample extraction method used by Leong and Shui (2002) was modified. A total of 100 g sample of the paste was extracted with 300 mL of distilled water twice, and the extracts were combined. The extraction was done using a vortex mixer for 1 min and the mixture was filtered through a Whatman filter paper no. 1. The filtrate was then centrifuged at 3000 rpm for 20 mins, and the supernatant decanted. The supernatant was adjusted to 10 mL with distilled water and was then used for all assays including physicochemical and proximate compositions, antioxidant activities, and microbiological examination.

2.5 Physicochemical analysis

The samples in each formula were diluted by distilled water and mixed together. The pH of karanda chili paste was measured using a pH meter (PH211 model). The colour value of karanda chili paste was determined at the surface in L*, a*, and b* mode of CIE (angle 10o, illuminant D65) using HunterLab (ColorFlex, Hunter Associates Lab, USA). The L*, a*, and b* values indicate lightness, greenness/redness, and blueness/yellowness, respectively. Water activity (a_w) of the karanda chili paste was determined by following AOAC (2000). The water activity meter set aw (Model ms1, Novasina, Switzerland) was used in this study. Triplicate samples were measured at 24±1°C.

2.6 Proximate composition

The protein concentration in the karanda chili paste was determined by the Kjeldahl method in accordance with the Association of Official Analytical Chemists (AOAC), method number 984.13 (AOAC, 2000). Crude protein content was estimated by multiplying the total nitrogen content by a factor of 6.25. Crude fat content was determined gravimetrically after the Soxhlet extraction of dried samples with hexane. The moisture and ash contents were evaluated according to the AOAC standard methods 930.15 and 942.05 (AOAC, 1999), respectively. Carbohydrate levels were calculated by subtracting the total sum of protein, fat, ash, and moisture from 100% of the dry weight sample (Judprasong *et al.*, 2013). The chili paste energy value (expressed in kcal) was estimated by multiplying the percentages of protein, fat, and carbohydrate by the factors 16.7, 37.7, and 16.7, respectively (AOAC, 1990).

2.7 Determination of total phenolic content

The total phenolic content of the karanda chili paste extracts was determined by the Folin-Ciocalteu colorimetric method with slight modification (Tibuhwa, 2014). Each 100 µL of extract was mixed thoroughly with 900 µL of 10% Folin-Ciocalteu reagent and left to stand for 8 mins at room temperature. Then, 400 µL of 7.5% (w/v) sodium carbonate was added to the mixture and was left at room temperature for a further 30 mins, before a reading of the absorbance at 765 nm was taken for blue coloured complex using a UV-Vis spectrophotometer. A calibration curve was constructed with different concentrations of gallic acid ($y = 63.643x$). The results were expressed as mg of gallic acid equivalents (GAE) per gram of fresh weight (mg GAE/g FW).

2.8 Antioxidant activity assays

2.8.1 DPPH radical scavenging activity

This assay was used to evaluate the antioxidant activity of the extracts based on the reduction of the DPPH radical solution in the presence of hydrogen-donating antioxidants from purple to yellow through a DPPH scavenging system and determined using the method outlined by Seephonkai *et al.* (2011). Initially, a 0.1 mM DPPH radical solution in 95% ethanol was prepared. An aliquot (1 mL) of the serially diluted extract samples was thoroughly mixed to which 1 mL of 0.1 mM DPPH solution was added. The mixture was thoroughly mixed using a vortex and kept in the dark for 30 mins. The absorbance, using a UV-Vis spectrophotometer, was then measured at 517 nm against a blank of ethanol without DPPH. The results were expressed on a dry weight basis as mg of trolox

equivalents (TE) per gram of fresh weight (mg TE/g FW) using a standard curve prepared by different concentrations of trolox ($y = 5.8258x + 5.431$).

2.8.2 Ferric reducing antioxidant power

A ferric reducing antioxidant power (FRAP) assay was performed using a modified method (Benzie and Strain, 1996). This assay uses antioxidants as reductants in the redox-linked colorimetric method. The reduction of ferric (Fe^{3+}) (colorless) to ferrous (Fe^{2+}) (blue) can be monitored. Briefly, a 150 μ L aliquot of properly diluted extract was thoroughly mixed with 2,850 μ L FRAP reagent and incubated at 37°C for 4 mins. The absorbance was then determined at 593 nm against a blank that was prepared using distilled water. FRAP was freshly prepared by mixing 2.5 mL of a 10 mM 2,4,6-tris(1-pyridyl)-5-triazine (TPTZ) solution in 40 mM HCl with 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 M acetate buffer at a pH of 3.6. A calibration curve was prepared using different concentrations of trolox ($y = 0.5828x - 0.0007$). FRAP values were expressed on a dry weight basis as micromole of trolox equivalents (TE) per gram of fresh weight (μ mole TE/g FW).

2.9 Microbiological quality analysis

In this study, the microbiological evaluation of karanda chili paste was carried out immediately after storage treatment within two weeks according to the standard recommended by the Department of Medical Science, Ministry of Public Health, as follows. Total aerobic count < 106 CFU/g, MPN *E. coli* < 3 MPN/g, and yeasts and mold < 100 CFU/g. The method used to test microbiological quality was modified from that proposed in the Bacteriological Analytical Manual (BAM) (Feng et al., 2020). All tests were carried out in triplicate, and mean values were reported.

2.10 Sensory evaluation

The procedure for the sensory evaluation was modified by Meilgaard et al. (2006). All three formulas of karanda chili paste were subjected to sensory testing by 40 untrained panelists at Suan Sunandha Rajabhat University, Bangkok, Thailand. A questionnaire was given to determine which was the most acceptable product using a 9-point hedonic scale which varied from “1 = dislike extremely” to “9 = like extremely”. Approximately 1.5 g of karanda chili paste was served on 15 g of steamed rice and presented to the evaluation panelists. A 3-digit number was coded on each formula. The sensory quality was assessed in order to select the chili recipe that was most acceptable to consumers in order to then produce a standard recipe of karanda chili paste.

2.11 Statistical analysis

A completely randomized design (CRD) was used throughout the study. All experiments were carried out in triplicate and average values with standard deviations were reported. The data were analyzed statistically using the Statistical Package for Social Sciences (SPSS), version 26.0. Analysis of variance (ANOVA) and Duncan's new multiple range test ($p < 0.05$) were used to detect the differences among treatment means.

3. Results and discussion

3.1 Physicochemical analysis

The results obtained for the physicochemical composition and appearance of karanda chili paste in each formula were revealed in Table 2 and Figure 1, respectively. The karanda chili pastes had a pH range of 4.55 - 5.63. However, the pH of karanda chili paste decreased compared to the pH of shrimp paste which had pH 7.62 for Indonesian dried shrimp paste, and pH 6.83 - 7.23 for Korean dried shrimp paste (Kim et al., 2014). Karanda, as the acid source of chili paste, had dominant organic acid which imparts a tangy citrus flavour (Berry, 2001), which might decrease the pH of the product. The water activity (a_w) of karanda chili paste was in the range of 0.79 - 0.84, and showed no significance in all three formulas, even though formula A had a water activity of 0.84 ± 0.00 , which was the highest. This chili paste is classified as close to the group of high a_w value, as the approximate value is more than 0.85. However, the water activity of all formulas was higher than the Thai community standard level ($a_w = 0.6$). Water activity is an important value for the control of the deterioration of food products, so it has a direct effect on their shelf life (Muninnopamas et al., 2021). The result showed that when there was more karanda, the lightness (L^*), redness (a^*), and yellowness (b^*) increased.

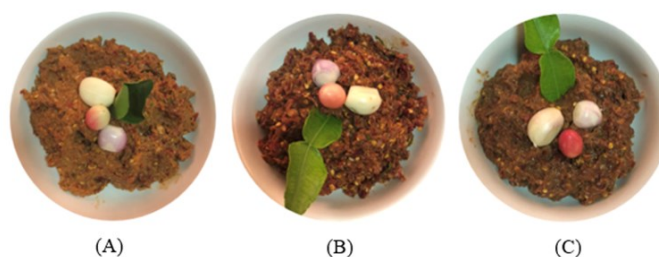


Figure 1. Appearance of karanda chili paste in formulas A, B, and C, respectively.

3.2 Proximate compositions

The proximate compositions of the samples differed significantly from each other ($p < 0.05$). Moisture (63.26 ± 0.31 g/100 g) and protein (12.61 ± 0.01 g/100 g) content were significantly higher in formula A and formula B respectively, whereas formula C exhibited the

Table 2. Physicochemical compositions of karanda chili paste in each formula per 100 g under steaming and kept at a temperature of 4°C.

Composition	Formula A	Formula B	Formula C
pH	4.55 ^a	5.63 ^b	5.42 ^b
Color			
L*	36.65±0.47 ^b	36.17±0.42 ^b	33.82±0.24 ^a
a*	+19.27±0.68 ^c	+14.01±0.48 ^a	+15.50±0.29 ^b
b*	+28.97±1.01 ^c	+21.71±0.26 ^a	+25.24±0.54 ^b
Water activity (a _w)	0.84±0.00 ^a	0.83±0.00 ^a	0.79±0.00 ^a
Moisture (g)	63.26±0.31 ^c	56.14±0.32 ^b	49.34±0.44 ^a
Protein (g)	7.72±0.03 ^a	12.61±0.01 ^c	11.63±0.27 ^b
Fat (g)	5.52±0.04 ^b	2.49±0.06 ^a	7.23±0.04 ^c
Carbohydrate (g)	17.25±0.38 ^a	21.53±0.39 ^b	23.22±0.79 ^c
Ash (g)	6.25±0.01 ^a	7.23±0.02 ^b	8.58±0.05 ^c
Energy (kcal)	149.56±1.06 ^a	158.97±1.07 ^b	204.47±1.72 ^c

Values are presented as mean±SD (n = 3) of triplicate measurements. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$).

Moisture based on 100 g fresh weight and all other parameters based on 100 g dry weight.

highest content of fat, carbohydrate (23.22±0.79 g/100 g), ash (8.58±0.05 g/100 g), and energy (204.47±1.72 kcal/100 g). This result showed that the protein content in chili paste increased with the addition of dried shrimp. This is correlated with the findings of Munir *et al.* (2016), who observed that protein contents were significantly increased with the addition of skimmed milk powder in fruit bars. The developed karanda chili paste contains higher protein levels when compared to other studies (Ruangchai and Tantakasem, 2011; Punaaterkoon *et al.*, 2016). Moreover, the protein, fat, carbohydrate, ash, and energy of Karanda chili paste in this study appeared higher than the reported values for dried chili paste with grilled fish (Chamchan *et al.*, 2019). The increase in moisture content may be attributed to the denaturation of proteins that turn to retain more moisture (Fennema, 1996). The results suggested that the proximate compositions were influenced by the different ingredients in each formula similar to the study of Ruangchai and Tantakasem (2011). The variations in the proximate compositions could be due to the variety, growing conditions, season, differences in production and processing, and storage practices or analytical methods used (Bhandari *et al.*, 2016).

3.3 Total phenolic content of the karanda chili pastes

Phenolic compounds present in dietary herbs have been shown to be responsible for the antioxidant activity of plant materials (Rice-Evans *et al.*, 1996; Boulanouar *et al.*, 2013). Total phenolic content of Karanda chili paste in all three formulas kept at 4°C varied from 3.02±0.06 to 3.94±0.11 mg GAE/g FW (Table 3) by the steaming process of chili paste which had a higher TPC than in the case of no steaming. The steamed chili paste

extract of formula A showed the highest phenolic content (3.94±0.11 mg GAE/g FW). When compared to other findings, it found that TPC of karanda chili pastes was higher than that of Keang-hleung paste or turmeric-chili paste made of garlic, turmeric, galangal and red chili pepper (Seah *et al.*, 2010), but lower than that of spiced red chili paste (Engeda, 2015), and green spiced chili paste made of green chili, red onion and garlic (Ruanma *et al.*, 2010). Many other studies have shown that the temperature is one of the factors that affect the total phenolic content of plant materials in both positive and negative ways, depending on the type of raw materials and the groups of compounds present (Zhang and Hamazu, 2004; Lombard *et al.*, 2005; Turkmen *et al.*, 2005; Odriozola-Serrano *et al.*, 2007; Randhir *et al.*, 2008). An increase in total phenolic content in some plants after heating may be due to the disruption of the plant cell wall, and therefore bound polyphenolic compounds may be released more easily than those in fresh plants (Peleg *et al.*, 1991). This is consistent with the research of Tagouelbe *et al.* (2017) who recommended that a higher temperature enhances the total phenolic content. Steaming at the right temperature can increase the total phenolic content. In addition, when comparing the fresh and dried condition of the extract, it was found that the extract in the dried condition in the unripe stage exhibited an increase in the total phenolic content. This may be because the heating process can change some polyphenol in *C. carandas* extracts (Khuanekaphan *et al.*, 2021). Therefore, thermal processing may enhance the phenolic release in the extracts, a fact which can be explained by considering that the extraction of intracellular contents is improved by thermal processing. Moreover, Wang *et al.* (2014) found that high temperatures enhanced the phenolic

Table 3. Total phenolic content and antioxidant activities of the karanda chili paste in each formula when steamed/not steamed and kept at a temperature of 4°C.

Formula	Conditions	Antioxidant activities		Total phenolic content (mg GAE/g FW)
		DPPH (mg TE/g FW)	FRAP (μmole TE/g FW)	
A	Steamed	11.55±0.04 ^c	11.93±0.02 ^d	3.94±0.11 ^c
	Not steamed	11.24±0.10 ^c	10.91±0.04 ^c	3.64±0.16 ^c
B	Steamed	11.39±0.43 ^c	10.64±0.10 ^{bc}	3.30±0.14 ^b
	Not steamed	10.73±0.07 ^b	10.49±0.25 ^b	3.28±0.15 ^b
C	Steamed	8.99±0.15 ^a	10.90±0.25 ^c	3.46±0.06 ^{bc}
	Not steamed	8.90±0.13 ^a	7.63±0.26 ^a	3.02±0.10 ^a

Values are expressed as mean±SD (n = 3) of triplicate measurements. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$). TE: trolox equivalent, GAE: gallic acid equivalent, FW: fresh weight.

content due to the hydrolysis of polysaccharides. On the other hand, if an excessive temperature is used, the cell walls and the bound phenolic compounds are destroyed, causing the loss of phenolic. Li *et al.* (2007) reported that heat treatment caused a reduction in total phenolic content during the processing of purple wheat bran. Zhang and Hamauzu (2004) also reported that antioxidant components in broccoli are significantly lost during cooking.

3.4 Antioxidant activities of the karanda chili pastes

3.4.1 DPPH scavenging activity

The antioxidant capacity of karanda chili pastes was measured by the DPPH and this result of the antioxidant study in the samples is presented in Table 3. Different values of each antioxidant assay from the same sample could be caused by the unique mechanism of each assay and the different antioxidant capacity of the compounds in the samples (Pellegrini *et al.*, 2003; Yang *et al.*, 2006). The DPPH scavenging activity, as well as the total phenolic content of chili paste kept at 4°C was the highest in the steamed chili paste of formula A with values of 11.55±0.04 mg TE/g FW. The steaming process exhibited a significantly higher ($p < 0.05$) DPPH scavenging value in formula B as compared to unsteamed samples. The increase in antioxidant activities of steamed samples showed that the antioxidant activity might be enhanced by the phenolic compounds that increased during the thermal treatment. A previous study reported that the major sources of antioxidant activity of karanda were both ascorbic acid and phenolic compounds, which are strong hydrogen ion donors (Sarma *et al.*, 2015; Debashis *et al.*, 2016). Moreover, the unripe extract of karanda was efficient in resisting free radicals, which may be due to the unripe extract from *C. carandas* fruit being a good source of ascorbic acid (Singh *et al.*, 2020). The findings of various research studies suggest that phenolic compounds and ascorbic acid contribute greatly to the antioxidant activity of fruit (Wang *et al.*, 1996; Prior *et al.*, 1998; Luximon-Ramma *et al.*, 2003; Sarma *et al.*, 2014). Even if ascorbic acid was decomposed by thermal treatment

the phenolic compounds found in the chili paste were heat resistant and highly effective in reducing DPPH radicals. In addition, a release of bound antioxidants during the heating process or the formation of new active compounds may occur (Shobana and Naidu, 2000; Kim *et al.*, 2002). This finding is in agreement with the results of Dewanto *et al.* (2002), who reported that the total antioxidant activity of sweet corn was elevated by 44% after thermal processing despite the loss of ascorbic acid. In this regard, it is clear that the antioxidant properties may not only come from phenolic compounds; substances such as capsaicin and sulphur compounds may also be associated with the antioxidant properties of chili and garlic (Kogure *et al.*, 2002). Our results suggested that greater total phenolic content can be increased DPPH scavenging activity following the thermal processing of chili paste in spite of the decline in vitamin C content.

3.4.2 Ferric reducing antioxidant power

The FRAP assay determines the antioxidant effect of plant materials in the reaction as its reducing ability (Siddhuraju and Becker, 2007; Alothman *et al.*, 2009). The reducing power of karanda chili pastes performed as ferric reducing antioxidant power (FRAP value) is presented in Table 3. The FRAP value obtained from steamed chili paste of formula A was significantly ($p < 0.05$) the highest with value of 11.93±0.02 μmole TE/g FW. In addition, the result showed that the steamed samples had a significantly higher FRAP value in formulas A and C compared with unsteamed samples. An interesting observation in this study was that the steaming process in the chili paste affected reducing power. It is possible that the antioxidant activity of chili paste tended to increase after steaming because of increasing the total phenolic content. The antioxidant activities of phenolic compounds are mainly reducing agents because of their ability to donate a single electron or hydrogen atom for reduction (Dini *et al.*, 2008). Additionally, the active compounds of various ingredients in chili paste are derived from the enzymatic

system such as polyphenols during the blending process used in the paste making (Romson *et al.*, 2011). This indicated that compounds present in the karanda chili paste have some reducing ability.

3.5 Microbiological quality analysis

The results of total aerobic plate count, MPN *E. coli*, and yeasts and mold of each chili paste formula under different conditions after storage treatment within two weeks are shown in Table 4. Comparing the total aerobic count of the chili paste between conditions, such as steamed or not, and storage temperature showed that the total aerobic count of samples was mostly lower than 10^6 CFU/g, except for formula C which was not steamed and was kept at 4°C. Moreover, this result indicated that steamed chili paste had a lower total aerobic count than unsteamed paste in all formulas, but storage temperature was revealed as the variation. The MPN *E. coli* test revealed that the MPN *E. coli* of all samples was less than 3.0 MPN/g. The total yeasts and mold count of steamed samples in all formulas was no more than 100 CFU/g. The previous study found that the total plate count of chicken drumsticks cooked for 2 mins at 70°C reduced from 10^7 CFU/g to less than 10 CFU/g (Can and Haran, 2015). Moreover, the microbial content of steaming Hae-Kuen was decreased as compared with the control sample (without steaming). While the initial microbial content of the control sample was 6.4×10^7 CFU/g, there were steamed at 80°C for 5 and 10 mins the microbial levels of 5.3×10^2 and 2.3×10^2 CFU/g, respectively (Kolakul *et al.*, 2019). The heat of steam causes microbial cells to change their condition, resulting in breakage and degradation of genetic material as well as destroying the membrane of microbes causing

microbial cells to be destroyed (Kolakul *et al.*, 2019). Therefore, it could be concluded that the steamed condition could decrease microbial contents, so it had better food preservative properties.

Microorganisms grow under the value of water activity (a_w) which is limited, resulting in food often having the a_w value lower than the point at which microorganisms can grow. For example, almost no bacteria can grow at the a_w value lower than 0.9 (Food Crumbles, 2022). However, the number and type of microorganisms in some food products are dependent on several factors such as composition and concentration of spices, sanitation during processing, harvesting, storage, transport, and packaging (Siripongvutikorn *et al.*, 2005). Additionally, the herbal ingredients in the samples such as garlic contained sulphur compounds (Bakri and Douglas, 2005), the capsaicin in chili (Jones *et al.*, 1997), and galangal (Oonmetta-aree *et al.*, 2006) lead to the inhibition of microbial growth. In addition, this study suggested that the karanda fruit can be applied as the active ingredient for the inhibition of microbiological organisms because of various active phytochemicals in karanda including polyphenols, tannins, glycosides, alkaloids, saponin and vitamin C (Debashis *et al.*, 2016; Kumar *et al.*, 2017).

3.6 Sensory evaluation

Sensory evaluation of karanda chili paste was carried out by forty panelists on a nine-point hedonic scale for different parameters such as appearance, color, flavor, taste, spiciness, texture and overall preference. All of these sensory attributes were not significantly different ($p > 0.05$) in terms of mean scores of all formulas (Table

Table 4. Microbiological quality of karanda chili paste in all three formulas under different conditions after storage treatment within two weeks.

Formula	Conditions	Microbiological quality		
		Total aerobic count (CFU/g)	<i>E. coli</i> (MPN/g)	Yeasts and mold (CFU/g)
A	Steamed, 4°C	4.6×10^3	< 3	< 10
	Steamed, RT	2.9×10^3	< 3	< 10
	Not steamed, 4°C	6.6×10^3	< 3	2.5×10
	Not steamed, RT	3.4×10^5	< 3	< 10
B	Steamed, 4°C	2.3×10^3	< 3	< 10
	Steamed, RT	4.8×10^3	< 3	< 10
	Not steamed, 4°C	4.5×10^3	< 3	1.2×10^2
	Not steamed, RT	3.4×10^5	< 3	< 10
C	Steamed, 4°C	5.4×10^3	< 3	< 10
	Steamed, RT	4.4×10^3	< 3	< 10
	Not steamed, 4°C	6.7×10^3	< 3	1.1×10^2
	Not steamed, RT	5.6×10^7	< 3	1.4×10^4

Values are expressed as mean±SD (n = 3) of triplicate measurements. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$). RT: room temperature, CFU: colony forming unit, MPN: most probable number.

5). The average scores of all sensory attributes were in the range of 7.37 – 8.03, matching the level of “like very much”. The results of the sensory evaluation indicate that karanda chili paste in formulas A, B, and C recorded overall preference scores of 7.67 ± 1.06 , 7.63 ± 1.19 and 7.67 ± 1.18 , respectively. The result indicated that the addition of different amounts of karanda in the chili paste had not influenced the evaluators’ overall preference. Moreover, the product from formula A got high rating scores in appearance, flavour, taste, texture, and overall preference because it had a smooth texture, was reddish brown with mellow taste, had a good flavour, and was moderately spicy, so it was the most acceptable formula as shown in Figure 1. This result was similar to the findings of Muninnopamas *et al.* (2021) who revealed that 37.5% of consumers accepted the fried chili paste seasoned sauce of Betong instant noodle products in the level of “like very much” with an average score of 7.64 ± 1.34 . Also, the sensory scores in soymilk residue or okara chili paste (Praphunthatewa *et al.*, 2015), and tamarind chili paste mixed roselle (Ruangchai and Tantakasem, 2011) were at this level. Furthermore, it was also similar to Chamchan *et al.* (2019) reported that there was no significant difference in terms of perceptions between dried chili paste with formula A and B and the acceptability level was quite high. This might be due to the amount of ingredients used in both formulas being the same, except the amount of chili.

Table 5. Sensory evaluation of karanda chili paste in all three formulas by 9-point hedonic scale test.

Sensory attributes	Average scores		
	Formula A	Formula B	Formula C
Appearance	7.93 ± 1.01^a	7.63 ± 1.03^a	7.87 ± 0.97^a
Colour	7.97 ± 1.13^a	7.70 ± 1.09^a	8.03 ± 0.93^a
Flavour	7.67 ± 1.03^a	7.67 ± 1.09^a	7.60 ± 1.30^a
Taste	7.57 ± 1.30^a	7.57 ± 1.22^a	7.37 ± 1.47^a
Spiciness	7.70 ± 1.06^a	7.90 ± 1.06^a	7.50 ± 1.25^a
Texture	7.80 ± 0.89^a	7.37 ± 1.27^a	7.57 ± 1.22^a
Overall preference	7.67 ± 1.06^a	7.63 ± 1.19^a	7.67 ± 1.18^a

Values are expressed as mean \pm SD (n = 3) of triplicate measurements. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$).

4. Conclusion

The results of this study concluded that the total phenolic content and antioxidant activities of karanda chili paste with its ingredients were increased slightly after steaming. The chili paste retained its antioxidant activity even after cooking and was also rich in nutritive constituents. In addition, unripe karanda fruit plays a key role in the chili paste in terms of its ability to inhibit microorganisms. In summary, karanda chili paste has the potential for both its antioxidant and antimicrobial

activities which could allow it to serve as a functional food for protecting our body from various diseases when ingested as a supplement to a balanced diet. Therefore, the study provides that the karanda chili paste can be used as natural antioxidants and antimicrobials in preventing various oxidative stresses and as food preservatives.

Conflict of interest

The authors herewith declare no conflict of interest.

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