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Changes in anthocyanins, total phenolics, total flavonoid, and antioxidant activity of Karanda fruit at different stages of maturity

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Abstract

Karanda (*Carissa carandas* L.) is mostly eaten raw and contains numerous phytochemicals and high antioxidant capacity. The objective of this study was to investigate the effects of the Karanda fruit's developmental stages on its phytochemical contents, antioxidant capacity, and volatile compounds. The study herein examined three developmental stages; unripe, semi-ripe, and ripe, based on their stage of maturity as observed in their color and texture. Phytochemical properties; including pH, titratable acidity, vitamin C contents, total soluble solid, total phenolic contents, total flavonoid content, anthocyanin contents, and antioxidant capacity (determined through FRAP, DPPH, and ABTS) were measured. Our results found significant differences in the phytochemical properties in each stage of development. Ripe fruits had the highest pH, total soluble solid, anthocyanin content, and antioxidant capacity determined by FRAP. Thirteen volatile compounds were identified in the fruit samples: eight compounds within the unripe fruits, nine within the semi-ripe fruits, and seven compounds were present in the fully ripe fruits. Alanine ethyl amide and acetic acid were major volatile compounds found in unripe and semi-ripe fruits, whereas tartronic acid was present in the fully ripe fruit. We may conclude that the ripe stage of development, having the highest phytochemical contents and antioxidant activity, is most suitable for harvest and most beneficial for human health. The information obtained in this study will be useful for the efficient utilization of Karanda fruit.

Keywords: volatile; chemical traits; ripening.
Abbreviations: TSS_ total soluble solid; TA_ titratable acidity; mL_ milliliters; FW_ fresh weight; GAE, gallic acid equivalents; QE_ quercetin equivalents; FRAP_ Ferric reducing/antioxidant power; DPPH_ di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium; TEAC_ trolox equivalent antioxidant capacity; ABTS_ 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid); LSD_ least significant difference.

Introduction

Plants are well recognized as a source of vitamins, minerals, and fibers; and, the consumption of fruits and vegetables has been associated with reduced mortality and morbidity for several non-communicable chronic diseases, such as heart disease and cancer. The protective effects exerted by these foods have been attributed to the presence of several bioactive compounds, including phytochemicals with a large variety of antioxidant actions (Maieves et al., 2015). Eating fruits and vegetables can also reduce blood pressure, boost the immune system, detoxify contaminants and pollutants, and reduce inflammation. The phytochemicals in plant tissues responsible for their antioxidant capacity can largely be attributed to phenolics, anthocyanins, and other flavonoid compounds (Wang et al., 2000; Tan et al., 2022). Phytochemicals may also confer health-protective benefits by alleviating oxidative stress; such as preventing free radical proteins, DNA, and lipids (Huang et al., 2005; Isabelle et al., 2010). The consumption and utilization of indigenous plants with important phytochemicals and health benefits are currently increasing (Sudjaroen and Suwannahong, 2017).

Karanda is a shrub plant with red fruit widely grown in many Asian countries (Verma et al., 2015). It is a hardy, evergreen, spiny and indigenous shrub; also referred to as Bengal currant, Christ's thorn, and carandas plum that thrives as a rainfed crop. Unripe fruit is sub-acidic, whereas ripe fruit is sweet and rich in carbohydrates, pectins, and minerals, particularly iron and vitamin C. Dry fruit contains roughly 67.1% carbohydrates, 9.6% fat, 2.3% pectin, and 3.91% iron. Ripe Karanda fruit may be eaten as a dessert, used for the preparation of jelly, sauce, carissa cream, and jello salad; or as a substitute for raisins. Karanda wine contains 14.5 to 15% alcohol and is also popular in tropical regions. Its fruit can also be used in the dyeing and tanning industries (Diengngan and Hasan, 2015). Fruit juice from ripe Karanda is high in vitamin C, DPPH scavenging activity, flavonoids, tannins, anthocyanins, and phenol; denoting that Karanda is a highly nutritional food source with numerous health benefits (Felice et al., 2017).

Karanda has been used in traditional medicine, having anthelmintic and antidiarrheal properties. Its fruit has also been used to cure skin infections, and its leaves are used to relieve fever and syphilitic pain. Alcoholic extract from its roots reduces blood pressure, and the aqueous extract from

its roots exhibits various pharmacological activities; like antihelmintic, spasmolytic, cardiotonic, and the release of histamines. Karanda has further been reported for its analgesic, anti-inflammatory, and lipase activities (Rasool et al., 2011). Its root extract has been found to have small quantities of alkaloids, flavonoids, and saponins; whereas large amounts of cardiac glycosides, triterpenoids, phenolic compounds, tannins, carisone, carindone, carinol, lignin, oderoside and 2-acetylphenol were present in its crude extract. Karanda leaves contain triterpenoid constitutes, as well as tannins and a new isomer of urosolic acid; namely carissic acid triterpene carandinol, betulinic acid, β-sitosterol-3-O-β-d-glucopyranoside, oleanolic acid, ursolic acid, and 4-hydroxybenzoic acid. Karanda contains carisol, epimer of α-amyrin, linalool, β-caryophyllene, carissone, carissic acid, carindone, ursolic acid, carinol, ascorbic acid, lupeol, and β-sitosterol. The volatile flavor constituents of Karanda fruit include isoamyl alcohol, isobutanol, and, predominantly, β-caryophyllene (Sudjaroen and Suwannahong, 2017; Rasool et al., 2011; Begum et al., 2013; Singh and Uppal, 2015). Rich in vitamin C, thiamine, riboflavin, and nicotinic acid (Felice et al., 2017), its ripe fruit also possesses significant antibacterial activities (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumanii and Enterococcus faecalis*) with no cytotoxicity to human dermal fibroblasts-neonatal dermal fibroblast nor the Vero cells that are generally used as normal cells (Sudjaroen and Suwannahong, 2017).

Karanda is an indigenous fruit in Thailand, recognized for its health benefits. Karanda bestows preventive properties for non-communicable diseases, has the potential for use as a health and/or medicinal food, which is beneficial to consumers and farmers, and enjoys increasing consumer demand. The popularity and acceptability of Karanda among consumers are not only due to its high nutritional value and characteristic taste but also due to its health-promoting properties (as a source of bioactive compounds). Ripeness and maturity are the key factors affecting the taste of karanda fruit (Alós et al., 2019). Fruit ripening is a complex process influenced by several factors. The changes in the composition of sugar, organic acids, and volatile compounds during the ripening process play a key role in flavor development, and affect both chemical and sensory characteristics; like pH, total acidity, microbial stability, and sweetness (Mahmood et al., 2012).

Because fruits undergo important changes during the maturation process; such as coloring, softening, (reduced) acidity, astringency, (increased) sweetness, and volatile emanation. Knowledge of the changes in fruit compositions is important for the selection of the optimum maturity stage in which to harvest Karanda as a functional food ingredient. Few studies have focused on the effects of maturity on the bioactive compounds in Karanda. Fruit for consumption is usually harvested at the optimum maturity stage determined by sensory characteristics. However, unripe Karanda fruit is usually rich in secondary metabolites with known bioactive roles in the human body and may have the potential as a significant source of bioactive compounds for other products (Maieves et al., 2015; King et al., 2021).

To the best of our knowledge, the research herein may be the first report on the variations of phytochemicals and volatile compounds during the maturation process. Our objective, therefore, was to evaluate the effects of maturity (in stages) on the phenolic content, flavonoid content, anthocyanins content, antioxidant activity, and volatile compounds. We intend that the information obtained in this study will be useful in determining Karanda as an alternative source of bioactive compounds for functional foods, dietary supplements, and nutraceutical formulations.

Results and Discussion

Changes in antioxidants and chemical compounds

The designated fruit maturity stages proved significantly different ($P \leq 0.05$) for chemical properties; including pH, titratable acidity, vitamin C content, total soluble solids, total phenolic content, total flavonoid content, and total anthocyanin content (Tables 2 and 3). In this study, fully ripe fruit had a significantly higher pH (3.32) than unripe fruit (3.15), indicating the lower acidic properties of the fully ripe fruit (Table 2). Patel and Rao, 2013; and King et al., 2021 reported similar results of pH changes in the ripening of both Karanda and *Aronia mitschurinii* berries, respectively.

The titratable acidity of a solution is determined by the approximation of the solution's total acidity measured by the reaction of an acid solution with a basic solution, such as sodium hydroxide, to a chosen endpoint, close to neutrality, as indicated by an acid-sensitive color indicator. Fruit acidity is used to indicate a fruit's flavor characteristics (Ernest et al., 2017); where a high level of acidity in unripe fruit causes a sour taste. Titratable acidity in unripe fruit (28.04%) was significantly higher than that in fully ripe fruit (6.55%). The titratable acidity, closely related to pH, confirmed that unripe Karanda fruit was sourer than fully ripe fruit. The dramatical decrease of titratable acids from the unripe to fully ripe stages may be caused by the high respiration rate in the ripened phase of Karanda fruit (Patel and Rao, 2013). Generally, organic acids in fruit decrease from the earlier to later stages of ripening (Alós et al., 2019).

Vitamin C, also known as L-ascorbic acid or simply ascorbate (the anion of ascorbic acid), is an essential nutrient for humans and certain animal species. Vitamin C is an essential requirement for a healthy diet, as well as being a highly effective antioxidant, a substrate for ascorbate peroxidase in plants (APX is plant specific enzyme), and an enzyme cofactor for the biosynthesis of many important biochemicals. Vitamin C also acts as an electron donor for important enzymes. In this study, the vitamin C contents in unripe fruit (52.20 mg 100 g^{-1} of FW) and semi-ripe fruit (53.77 mg 100 g^{-1} of FW) were significantly higher than that found in fully ripe fruit (45.27 mg 100 g^{-1} of FW). According to Ernest et al., 2017; unripe and semi-ripe fruit are good sources of vitamin C, recommended at 100-120 mg of vitamin C within a daily diet.

Total soluble solid content (ºBrix) is determined by the index of refraction, measured via a refractometer, and is widely used in fruit and vegetable processing to determine the concentration of sugar in various products. Increasing the soluble solid content during ripening may cause sugar importation or starch degradation during the ripening process (Batista-Silva et al., 2018). In this study, fully ripe fruit displayed the highest total soluble solid of 11.90 ºBrix, followed significantly by that of the semi-ripe and unripe fruit, each at 10.50 and 10.05 ºBrix, respectively. The results supported previous findings that fully ripe fruit were sweeter than unripe and half-ripe fruit.

Phenolic compounds in plants are beneficial to human health, due to their antioxidant properties (Mark et al., 2019). In this study, fully ripe fruit had a significantly higher

total phenolic content (7,692.20 mg GAE 100 g^{-1} of FW) than that of unripe fruit (4,099.50 mg GAE 100 g^{-1} of FW) and semi-ripe fruit (4,269.30 mg GAE 100 g^{-1} of FW), which, in comparison, were not significantly different.

Anthocyanins are water-soluble vacuolar pigments that may appear red, purple, or blue; depending on their pH. Anthocyanins occur in all tissues of higher plants within the leaves, stems, roots, flowers, and fruit. Anthocyanins can, therefore, be employed as a natural coloring compound in food. Anthocyanin contains nutraceutical and antioxidant agent properties (Panche et al., 2016). In this study, fully ripe fruit had the highest total anthocyanin (2.80 mg CGE 100 g^{-1} of FW) followed by semi-ripe fruit (0.43 mg CGE 100 g^{-1} of FW), and unripe fruit (0.18 mg CGE 100 g^{-1} of FW), which was 15.56 times lower than that in the fully ripe fruit. Miletić et al., 2012; reported similar changes in anthocyanin contents during fruit ripening in the plum.

Flavonoids (or bioflavonoids) belong to a class of plant secondary metabolites, which are widely distributed in plants and fulfill many plant functions. Flavonoids produce the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinating insects. In higher plants, flavonoids protect plants from different biotic and abiotic stresses and are involved in UV filtration. They may also act as chemical messengers, physiological regulators, and cell cycle inhibitors (Panche et al., 2016). In the study herein, unripe fruit had the lowest total flavonoid contents (252.63 mg QE 100 g^{-1} of FW), which was not significantly different from the semi-ripe fruit (259.62 mg QE 100 g^{-1} of FW); whereas the fully ripe fruit produced the highest total flavonoid contents (452.14 mg QE 100 g^{-1} of FW), which proved a significant difference from the other groups ($P \leq 0.05$).

Quantification of phenolic contents

An analysis of the phenolic compounds of Karanda fruit at three maturity stages was performed using reversed-phase high-performance liquid chromatography (HPLC). The major groups of phenolic compounds were hydroxybenzoic and hydroxycinnamic acids. The hydroxybenzoic acids consisted of five phenolic compounds; gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, and syringic acid. The hydroxycinnamic acids consisted of ferulic acid, chlorogenic acid, caffeic acid, p-coumaric acid, and sinapic acid.

Differences in maturity stages were significant ($P \leq 0.05$) for all phenolic compounds (Table 4). Unripe fruits had the highest total phenolic content [160.56 mg 100 g^{-1} of dry weight (DW)] followed by semi-ripe fruits (114.50 mg 100 g^{-1} of DW) and fully ripe fruits (68.05 mg 100 g^{-1} of DW). Similar results were previously reported by Çelik et al., 2008; revealing that the phenolic contents in cranberries began to decrease when the fruit ripened. Fruit maturity stages were also different for major phenolic compounds. Protocatechuic acid (50.13 mg 100 g^{-1} of DW), ferulic acid (63.20 mg 100 g^{-1} of DW), and chlorogenic acid (3.11 mg 100 g^{-1} of DW) were the major phenolic compounds in the unripe fruit. Caffeic acid (30.16 mg 100 g^{-1} of DW) and p-coumaric acid (43.70 mg 100 g^{-1} of DW) were the major phenolic compounds in semi-ripe fruit, whereas gallic acid (34.37 mg 100 g^{-1} of DW), p-hydroxybenzoic acid (7.44 mg 100 g^{-1} of DW), vanillic acid (1.52 mg 100 g^{-1} of DW), and sinapic acid (12.30 mg 100 g^{-1} of DW) were the major phenolic compounds in fully ripe fruit. Notably, protocatechuic and vanillic acids were founded only in fully ripe fruit, whereas ferulic acid and caffeic acid were founded only in unripe and semi-ripe fruit, respectively.

Quantification of anthocyanin contents

Anthocyanins are members of a group of naturally occurring phenolic compounds responsible for the color of fruit and have potential pharmacological properties; including reducing the risk of cardiovascular diseases and cancers with antioxidant, anti-inflammatory, and chemopreventive properties (Yang and Zhai, 2010; Mark et al., 2019). The anthocyanin contents at different maturity stages are shown in Table 5. Maturity stages were significantly different ($p<0.01$) for all individual compounds of anthocyanins. The major anthocyanin found in karanda fruit was keracyanin. Only the fully ripe fruit produced the highest keracyanin content (61.13 mg 100 g^{-1} of DW), Cyanindin (3.09 mg/100 g^{-1} of DW), and kuromanin (2.71 mg/100 g^{-1} of DW). These findings were different from that of the previous study by Sarkar et al., 2018; which reported that cyanidin-3-glycoside was the major anthocyanin in Karanda fruit. In a previous study by Ribera et al., 2010; and Oszmiański et al., 2018; the anthocyanin contents in berries varied greatly with fruit type, cultivar, maturity, production area, yield, harvesting time, and the time in which the experiments were performed.

Changes in antioxidant activity

Polyphenols are scavengers of a wide variety of reactive species; such as superoxide, hydroxyl radical, peroxyl radical, hypochlorous acid, and peroxynitrous acid, resulting in fewer reactive radicals. Different methods exist to evaluate antioxidant activity, either in vitro or in vivo. Fruit maturity stages were significantly different ($P \leq 0.05$) in all three methods of antioxidant assay; including FRAP radical scavenging ability (FRAP), DPPH radical scavenging ability (DPPH), and Trolox equivalent antioxidant inhibition (TEAC), as illustrated in Table 6.

The FRAP method uses Trolox as a standard and is often used to measure the antioxidant capacity of foods, beverages, and nutritional supplements containing polyphenols. This assay measures the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in the presence of antioxidants. In this study, antioxidant activity determined by the FRAP method showed similar behavior to total phenolic contents (Table 6), as antioxidant activity increased during the maturity stages. Nevertheless, the highest FRAP values were observed in fully ripe fruit [19,047.00 mM Fe(II) 100 g^{-1} of FW] followed by semi-ripe fruit [7,571.90 mM Fe(II) 100 g^{-1} of FW] and unripe fruit [6,895.80 mM Fe(II) 100 g^{-1} of FW].

Scavenging DPPH free radicals is the basis of a common antioxidant assay. Several protocols have been followed for this assay resulting in variations in the results of different laboratories. In this study, fruit maturity stages were significantly different ($P \leq 0.01$) for DPPH inhibition values (Table 6). The highest inhibition activity was observed in the semi-ripe fruit (91.62%), however, there was no significant difference from that of the fully ripe fruit (90.44%). The lowest antioxidant activity was founded in the unripe fruit (31.48%), which agreed with the total phenolic contents and FRAP antioxidant activity values.

TEAC measures the antioxidant capacity of a given substance compared to the standard: Trolox. Most commonly, antioxidant capacity is measured using the ABTS Decolorization Assay. In the study herein, TEAC inhibition values varied depending on the stage of maturity (Table 6).

Table 1. Changes in fruit skin color and firmness of Karanda fruit at three different stages of maturity.

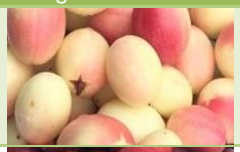
Maturity stage	Color	Firmness	Figure
Unripe	White, red	Hard	
Semi-ripe	Red	Fairly hard	
Fully ripe	Dark purple	Soft	

Table 2. pH, titratable acid, vitamin C content, and total soluble solids of Karanda fruit at three different stages of maturity.

Maturity stage	pH	Titratable acid (%)	Vitamin C content (mg **100** g^{-1} FW)	Total soluble solids (ºBrix)
Unripe	3.15 b	28.04 a	52.20 a	10.05 b
Semi-ripe	3.11 b	19.17 b	53.77 a	10.50 b
Fully ripe	3.32 a	6.55 c	45.27 b	11.90 a
F-test	**	**	**	**
CV (%)	1.03	6.28	5.39	4.22

**Significant at P ≤ 0.01. Means in the same column with different letters are significantly different (P < 0.05) as determined by LSD.

Table 3. Total phenolic content (TPC), total flavonoid content (TFC), and total anthocyanin content (TAC) of Karanda fruit at three different stages of maturity.

Maturity stage	TPC (mg GAE **100** g^{-1} FW)	TFC (mg QE**100** g^{-1} FW)	TAC (mg CGE **100** g^{-1} FW)
Unripe	4,099.50 b	252.63 b	0.18 c
Semi-ripe	4,269.30 b	259.62 b	0.43 b
Fully ripe	7,692.20 a	452.14 a	2.80 a
F-test	**	**	**
CV (%)	13.37	8.82	15.44

**Significant at P ≤ 0.01.Means in the same column with different letters are significantly different (P < 0.05) as determined by LSD.

Table 4. Phenolic contents (mg 100 g^{-1} DW) of Karanda fruit at three different stages of maturity.

Maturity stage	Hydrobenzoic acids (mg/**100** g DW)									
	GA		PCCA		p-OH		VA		SyA	
Unripe	14.41	b	50.13	a	0.00	b	0.00	b	13.12	a
Semi-ripe	16.34	b	0.00	b	0.00	b	0.00	b	12.20	b
Fully ripe	34.37	a	6.29	b	7.44	a	1.52	a	0.00	c
F-test	**		**		**		**		**	
CV (%)	11.22		24.45		2.96		20.31		3.89	
Maturity stage	Hydrocinnamic acids (mg/100 g DW)									
	FA		ChA		CFA		p-CA		SNA		Total (mg/100 g DW)	
Unripe	63.20	a	3.11	a	0.00	b	12.64	b	3.94	c	160.56	a
Semi-ripe	0.00	b	1.64	b	30.16	a	43.70	a	10.47	b	114.50	b
Fully ripe	0.00	b	0.00	c	0.00	b	5.51	c	12.30	a	68.05	c
F-test	**		**		**		**		**		**	
CV (%)	9.55		11.67		16.95		2.39		9.82		5.31	

GA, gallic acid; PCCA, Protocatechuic acid; p-OH, *p*-Hydroxybenzoic acid; VA, vanillic acid; SyA, syringic acid; FA, ferulic acid; Ch A, chlorogenic acid; CFA, caffeic acid; p-CA, *p*-coumaric acid and SNA, sinapic acid.

** Significant at P ≤ 0.01.

Means in the same column with different letters are significantly different (P < 0.05) determined by LSD.

Table 5. Anthocyanin content (mg/100 g DW) of Karanda fruit at three different stages of maturity.

Maturity stage	Kuromanin		Keracyanin		Malvin	Delphinidin	Cyanidin		Pelargonidin	Malvidin
Unripe	0	b	4.20	b	nd	nd	0	b	nd	nd
Semi-ripe	0	b	5.10	b	nd	nd	0	b	nd	nd
Fully ripe	2.71	a	61.13	a	nd	nd	3.09	a	nd	nd
F-test	**		**		-	-	**		-	-
CV (%)	12.98		6.35		-	-	9.62		-	-

** Significant at $P \leq 0.01$. Means in the same column with different letters are significantly different ($P < 0.05$) determined by LSD.nd: not detected.

Table 6. Antioxidant activity of Karanda fruit at three different stages of maturity.

Maturity stage	FRAP (mM Fe(II)/**100**g FW)		DPPH (% inhibition)		TEAC (% inhibition)	
Unripe	6,895.80	b	31.48	b	8.69	a
Semi-ripe	7,571.90	b	91.62	a	6.19	b
Fully ripe	19,047.00	a	90.44	a	8.16	a
F-test	**		**		**	
CV (%)	14.05		1.29		13.39	

** significant at $P \leq 0.01$. Means in the same column with different letters are significantly different ($P < 0.05$) determined by LSD.

Table 7. Volatile compounds of Karanda fruit at three different stages of maturity.

No.	Volatile Compound	Retention Time	Base peak (m/z)	Area (%)			Chemical Formula
				Unripe	Semi-ripe	Fully ripe	
1	Silanediol	409	76.95	8.24	5.97	5.57	$C_2H_8O_2Si$
2	n-Hexane	618	43.00	-	11.98	-	C_6H_{14}
3	Methyl Isobutyl Ketone	690	43.00	1.65	1.85	-	$C_6H_{12}O$
4	Acetic acid	785	43.00	19.32	-	16.74	$C_6H_{12}O_2$
5	Hexanal	806	43.95	-	1.22	-	$C_6H_{12}O$
6	Decane	1086	43.00	-	1.61	-	C12H26
7	Alanine ethyl amide	1097	43.95	39.80	32.98	-	$C_5H_{12}N_2O$
8	Undecane	1115	43.00	2.85	-	-	$C_{13}H_{28}$
9	Tartronic acid	1223	45.05	-	10.90	16.35	$C_4H_6O_5$
10	Tridecane	1313	43.00	4.54	-	1.09	$C_{13}H_{28}$
11	Oxalic acid	1385	43.05	0.96	2.93	2.12	$C_7H_{10}O_4$
12	Farnesene	1458	41.00	-	3.19	1.57	$C_{15}H_{24}$
13	Carbonic acid	1507	70.00	1.17	-	0.86	$C_{14}H_{28}O_3$
	Total			78.53	72.63	44.30	

The highest inhibition value was observed in unripe fruit (8.69% inhibition), which was not significantly different from that in fully ripe fruit (8.16% inhibition). Semi-ripe fruit produced the lowest inhibition value (6.19% inhibition).

Volatile compounds

The extraction of the volatile compounds from the Karanda fruit was carried out through headspace sampling, and the samples were analyzed using the coupled GC–MS method. Thirteen volatile compounds were identified in the fruit samples (Table 7). Unripe fruits presented eight compounds; silanediol (8.24% area), methyl isobutyl ketone (1.65% area), acetic acid (19.32% area), alanine ethyl amide (39.80% area), undecane (2.85% area), tridecane (4.54% area), oxalic acid (0.96% area), and carbonic acid (1.17% area). Semi-ripe fruits evidenced nine volatile compounds; silanediol (5.97% area), n-hexane (11.98% area), methyl isobutyl ketone (1.85% area), hexanal (1.22% area), decane (1.61% area), alanine ethyl amide (32.98% area), tartronic acid (10.90% area), oxalic acid (2.93% area), and farnesene (3.19% area). The major volatile compound in both the unripe and semi-ripe fruit was alanine ethyl amide. Seven volatile compounds were recorded in fully ripe fruits; namely silanediol (5.57% area), acetic acid (16.74% area), tartronic acid (16.35% area), tridecane (1.09% area), oxalic acid (2.12% area), farnesene (1.57% area), and carbonic acid (0.86% area). Acetic and tartronic acids were major volatile compounds in the fully ripe fruit. The different types of volatile compounds found at different stages of maturity may be due to the changes in the various tastes of fruit during the ripening process, as volatile compounds are responsible for plant flavor and aroma (Sánchez-Rodríguez et al., 2019).

Materials and Methods

Collection of Karanda fruit

The Karanda fruit utilized herein was harvested from the research field at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Maha Sarakham province, Thailand (Latitude:16.24155, Longitude: 103.24655 and 150 meters above mean sea level) from May to July 2016. The fruit was divided into three groups according to their stage of maturity based upon their color and texture (Table 1). The fruit was separated into pulp and

seeds. The pulp was blended and then dipped in liquid nitrogen (-196 ^{o}C) to stop the enzymatic activity and stored at -20 ^{o}C for further analysis.

pH measurement

The 1 g blended samples were added to a 50 mL beaker with 25 mL of distilled water, stirred until they were homogenous, and set aside for 30 minutes. The samples were then filtered through filter paper, and the pH was measured via a pH meter. The measurement was repeated two times per sample.

Total soluble solid (TSS)

The analysis of TSS readings was performed by using a hand-held portable refractometer. As the refractive index of a sugar-containing solution is also temperature-dependent, the refractometer was calibrated at 20 ^{o}C with deionized water (refraction index = 1.3330 and 0°Brix at 20°C) and the measurement of TSS was performed after calibration. The measurement was repeated two times per sample.

Titratable acidity (TA)

TA was determined by titration, according to the method previously described in AOAC, 1990.

Vitamin C

Vitamin C content was determined according to the method previously described in AOAC, 1990 against a standard curve of ascorbic acid (0, 0.05, 0.1, 0.5, and 1.0 mg 100 mL^{-1}) and expressed as mg 100 g^{-1} of FW.

Sample extraction for phytochemical content and antioxidant activity

Chemical compositions were analyzed according to the method previously described by Krasaetep et al., 2011. The samples were kept at -20^{o}C until phytochemical analysis was conducted.

Total phenolic content (TPC)

Phenolic content was determined using the Folin-Ciocalteu (F-C) method previously described by Hu and Xu, 2011.

Total anthocyanin content (TAC)

TAC was measured by the pH differential method previously described by Giusti and Wrolstad, 2001.

Total flavonoid content (TFC)

Total flavonoid content was determined using the colorimetric method described by Kubola and Siriamornpun, 2011.

HPLC Analysis for phenolic compounds

HPLC was performed for the analysis of phenolic compounds using Shimadzu LC-20AC pumps. SPD-M20A with diode array detector and chromatographic separation was performed on a LUNA C-18 column (4.6 × 250 mm i.d., 5 μm). The composition of the solvents, as well as the gradient elution conditions used, were based on the previous work of Kubola et al., 2011.

HPLC Analysis for anthocyanin compounds

Anthocyanin compound analysis was conducted according to Jorjong et al., 2015; using the HPLC–DAD system (Shimadzu, Japan) consisting of Shimadzu LC-20AC pumps, an SPD-M20A diode array detector, and an Apollo C-18 column (Alltech Associates, Deerfield, IL, USA) (4.6 mm × 250 mm, 5 μm) protected with guard column Inertsil ODS-3 (4.0 mm × 10 mm, 5 μm; GL Science Inc., Tokyo, Japan). Anthocyanin compound concentrations were calculated using a corresponding external standard.

Measurements of antioxidant activity

Ferric reducing/antioxidant power (FRAP) assay, following the protocol determined by Kubola and Siriamornpun, 2008; was based on the reduction of Fe3+–TPTZ to a blue-colored Fe2+–TPTZ (Benzie and Strain, 1996). The ability for scavenging di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) radicals was assessed by measuring the bleaching of a black-colored methanol solution of DPPH radicals as previously described by Yang and Zhai, 2010. The Trolox equivalent antioxidant capacity (TEAC) assay, which measures the reduction of radical cations of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) by antioxidants, was carried out through the procedure previously described by Jorjong et al., 2015.

Determination of volatile compounds

Volatile compounds were determined according to Siriamornpun et al., 2014. Identification of the components was performed by comparing the mass spectra with those on record in the NIST11 library.

Statistical analysis

The data of antioxidants and phytochemical compounds were analyzed according to a completely randomized design with four replications (Hoshmand, 2006). The least significant difference (LSD) was used to compare the mean difference between groups at a 0.05 probability level.

Conclusion

The different developmental stages of Karanda fruit had a significant effect on the fruit's phytochemicals properties; such as pH, titratable acidity, vitamin C content, total soluble solid, total phenolic content, total flavonoid content, anthocyanin content, and antioxidant capacity; determined by FRAP, DPPH, and ABTS. Ripe fruit had the highest pH, total soluble solid, anthocyanin content, and antioxidant capacity, determined by FRAP. Thirteen volatile compounds were identified in the fruit samples: eight within the unripe fruit; nine in the semi-ripe fruit; and seven in the fully ripe fruit. Alanine ethyl amide was the major volatile compound found in the unripe and semi-ripe fruit, whereas acetic and tartronic acids were the major volatile compounds in the fully ripe fruit.

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