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Physico-Chemical Properties, Phytochemical Components, Vitamin C Content, Antioxidant Activity, Sensory Characteristics and Acceptability of Karanda (*Carissa carandas*) Fruit Nectar

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Abstract

This study was conducted to examine the sensory characteristics, acceptability, physico-chemical properties, phytochemical components, vitamin C content and antioxidant activity of Karanda (*carissa carandas*) fruit nectar. Four different brix nectar samples were evaluated. The fruit nectar with 20° Brix was observed to be the most acceptable. It had a pH of 2.567, 1.993 titratable acidity and 10.106 Brix to acid ratio. The Karanda fruit nectar had Vitamin C content of 2642.1 mg/100 ml and DPPH scavenging activity of 84.967%. It contained 44.49 mg/100 ml flavonoids, 1.05 mg/100 ml tannins, 3.73 mg/100 ml anthocyanidins and 633.68 mg/100 ml phenol. Results showed that he 20° brix Karanda fruit nectar can be a potential food source that can provide nutritional and other health benefits.

Keywords: *Karanda; Fruit Nectar; Phytochemicals; Vitamin C*

Introduction

According to International Agency for Research on Cancer [1], fruit is "the edible part of a plant, tree, bush or vine that contains the seeds and pulpy surrounding tissue and has a sweet or tart taste". Fruits can be eaten as is when it is ripe. However, different products such as jam, juice, preserves, pickles, and jellies can be made from fruits. One of the products that can be made from fruits is nectar. Nectar is made from the juice with or without the pulp of the fruit wherein water and sugar are added. The fruit juice content of the nectar vary between 25 and 99 percent but Codex Alimentarius states that the minimum fruit juice or pulp content for fruit nectars should be 25 to 50 percent [2-4]. One can say a fruit beverage is nectar if its total soluble solids are not more than 20° Brix [5]. "In accordance with the EU Council Directive 93/77, only the following ingredients can be added to nectars: water, fruit juice concentrate, sugar, fruit acid, natural flavourings and fruit pulp from juice" [6].

Karanda (*Carissa carandas*) is "a rank-growing, straggly, woody, climbing shrub, usually growing to 10 or 15 ft (3-5 m) high, sometimes ascending to the tops of tall trees; and rich in white, gummy latex" [7]. It is common and native in India and it usually thrives in tropical and subtropical climates. The fruit of Karanda when ripe is red to dark purple and it can be used as puddings, tarts, jam, while the unripe fruit can be used to make pickles and chutneys [8] Not only it is used as a main ingredient in dishes, it also has medicinal uses especially in the ayurveda system of medicine in India. According to The Ayurvedic Pharmacopoeia of India as cited by Khare [9], the stem bark is used to treat in obstinate skin disease, and the root is used for urinary disorders; alcoholic extract of roots shows hypotensive activity. Morton [7] reported that "the unripe fruit is used medicinally as an astringent. The ripe fruit is taken as an antiscorbutic and remedy for biliousness. The leaf decoction is valued in cases of intermittent fever, diarrhea, oral inflammation and earache. The root is employed as a bitter

stomachic and vermifuge and it is an ingredient in a remedy for itches. The roots contain salicylic acid and cardiac glycosides causing a slight decrease in blood pressure". The fruit is rich in different vitamins such as Vitamin C, thiamine, riboflavin, and nicotinic acid [10].

Fruit juice and fruit drinks are more common in the Philippine supermarkets than nectars. Difference between fruit juice and fruit drinks are not of interest to some as it is thought to be just the same. Nectars can be mistakenly thought by people as the sweet fluid from plants, but it is not; it is a fruit juice with water, sugar, and acid added. Mango, guava, calamansi, and four seasons are the common flavors of fruit juices and nectars in the Philippines. Globally, the consumption of the natural fruit products is expanding due to their various health benefits. Therefore, understanding sensory characteristics, acceptability and health qualities of Karanda nectar are of importance. The finding of this study can also be applied to researchers who intend to develop other fruit based products.

Today, different food products are introduced in the market---therefore, the production of nectar made from a Karanda fruit, which has potential health benefits could offer another variety of food product.

The aims of the study were to evaluate the sensory characteristics, acceptability, physico-chemical properties, phytochemical components, and antioxidant activity of Karanda (*Carissa carandas*) fruit nectar.

Methodology

Preparation of Karanda fruit nectar

The procedure for the nectar production was adopted from the method developed by International Centre for under utilised Crops, UK (2004). The amount of sugar, citric acid, water, and the length of cooking time were adjusted according to the weight of the sample harvested. Four hundred ten grams of Karanda fruit were harvested, 0.4 grams of citric acid and 152 grams of sugar, and 1025 mL of distilled water was added. The mixture was boiled for one and a half minutes at 70°C. Four nectar samples with 5°, 10°, 15° and 20° brix were prepared. Dilution with water and addition of sugar was done to adjust the total soluble solids (TSS) of each sample.

Sensory Evaluation

The sensory evaluation was conducted at the Institute of Human Nutrition of Food, College of Human Ecology, University of the Philippines Los Baños Laguna. Thirty-five (35) young adults were randomly selected as the respondents for the study. Each respondent tasted 5 mL of the four Brix samples of Karanda fruit nectar. Standard sucrose solution (2%) and standard citric acid solution (0.04%) were provided as the basis for sweetness and sourness while Pantone Colour Chart was also provided as basis for evaluating the color of the samples. The samples were provided to the panelist cups coded with random three-digit numbers at a temperature of 6 ± 2°C in individual booths under white light. A 7-scale hedonic scale was used.

Chemical Analysis

The most acceptable Karanda fruit nectar were subjected to chemical analysis to see its physico-chemical properties (pH, titratable acidity, total soluble solids, and brix to acid ratio), phytochemical components (flavonoids, tannins, phenols, and anthocyanin), Vitamin C content, and antioxidant activity.

Phytochemical components

Analysis of Flavonoids

0.20 mL Karanda fruit nectar was obtained and 2.00 mL distilled water was added. 0.30 mL of 5% $NaNO_2$ was also added to the mixture. It was mixed using the shaker and was allowed to stand for 5 minutes. After standing, 0.30 mL of 10% $AlCl_3$ was added and the solution was allowed to stand again for one minute. One mL 1.0 M NaOH was added and solution was mixed well. Absorbance reading was obtained at 710 nm using the spectrophotometer. Analysis of flavonoids of the Karanda fruit nectar was done in triplicates. Absorbance readings were plotted on the standard curve.

Analysis of Tannins

One mL of the Karanda fruit nectar was obtained and 5.0 mL vanillin reagent was added. It was incubated at 30°C for 20 minutes. Absorbance was read at 500 nm using the spectrophotometer. Analysis of tannins of the Karanda fruit nectar was done in triplicates. Absorbance readings were plotted on the standard curve.

Analysis of Anthocyanidins

0.20 mL of Karanda fruit nectar was obtained. 1.00 mL of 1% vanillin in methanol was added; followed by the addition of 1.00 mL of 9.0 N HCL. 1.80 mL distilled water was added and it was mixed well. It was allowed to stand for 20 minutes and the absorbance was obtained at 700 nm using the spectrophotometer. Analysis of anthocyanidins of the Karanda fruit nectar was done in triplicates. Absorbance readings were plotted on the standard curve.

Analysis of Total Phenols

One mL of the Karanda fruit extract was diluted with 9 mL 50% methanol. From this mixture, one mL was obtained and was again diluted with 9 mL of 50% methanol. From the diluted mixture, 0.2 mL of Karanda fruit nectar was obtained and was added with 2.8 mL of distilled water; it was mixed thoroughly using the shaker. 1.0 mL of 0.2M Na_2CO_3 was added followed by the addition of 0.2 mL Folin-Ciocalteus Phenol reagent. After mixing the solution using the shaker, it was placed in boiling water bath for 15 minutes; it was then cooled to room temperature. Absorbance reading was obtained at 710 nm using the spectrophotometer. Analysis of total phenols of the Karanda fruit nectar was done in triplicates. Absorbance readings were plotted on the standard curve.

Analysis of Vitamin C

One mL of the Karanda fruit nectar was diluted with 9mL 0.4% oxalic acid solution. From this mixture, 1 mL of Karanda fruit nectar was obtained and was added with 0.2 mL of 0.01% methylene blue solution. One mL of acetate buffer with pH 4.2 was added into the solution; the solution was made up to 5 mL mark by addition of distilled water. Absorbance of the solution was read at 585 nm using the spectrophotometer. Analysis of Vitamin C of the Karanda fruit nectar was done in triplicates. The Vitamin C content was calculated using the formula:

$$Vitamin\ C\ (\frac{mg}{100\ ml}) = \frac{Mean\ absorbance\ x\ dilution\ \ factor}{Slope}$$

where:
dilution factor= weight of sample/volume of juice x dilution x volume used in analysis
slope= 0.05 from ascorbic acid absorbance

Analysis of Antioxidant Capacity

0.05 mL of the Karanda fruit nectar was obtained and was added with 4 mL of distilled water. One mL of freshly prepared 1 mM DPPH methanolic solution was added and was left to stand for 30 minutes. Absorbance reading of the solution was read at 517 nm. Analysis of the antioxidant capacity of the Karanda fruit nectar was done in triplicates. The % DPPH scavenging activity was calculated using the formula:

$$\%\ DPPH\ \ scavenging\ \ activity = \left[1 - \left(\frac{test\ sample\ absorbance}{absorbance\ of\ \ pure\ DPPH}\right)\right] \times 100$$

where:
absorbance of pure DPPH= 1.2

Physico-chemical properties

pH

The pH of the nectar was measured using a pH meter. The measurement of pH was done in triplicates.

Titratable acidity (%TA)

One mL of Karanda fruit nectar was diluted with enough water until the solution turned to a clear one. Three drops of phenolphthalein, an indicator if the end point is reached, were dropped into the mixture. 0.2 N NaOH was the chemical used titrate the fruit nectar. The endpoint of the mixture was reached when the color of the mixture turned to pale pink. Determination of %TA was done in triplicates. Percent TA was calculated using the formula:

$$\%\,TA = \frac{(N\ NaOH)\,(Vol\ of\ NaOH)\left(\frac{MW\ of\ ascorbic\ acid}{1000}\right)}{Volume\ of\ Sample} \times 100$$

where:
MW of ascorbic acid= 172.16 g/mol

Total Soluble Solids (TSS)

A handheld refractometer that can read up to 32° brix was used in determining the total soluble solids of the Karanda fruit nectar. Three drops of the fruit nectar were dropped on to the daylight plate of the refractometer and the degree brix was read. Determination of the TSS was done in triplicates.

Statistical Analysis

Results from the sensory evaluation of the four samples was analyzed using One Way Analysis of Variance (ANOVA) and Tukey's HSD test from which the most acceptable fruit nectar was determined. The correlation of general acceptability with the sensory characteristics namely color, aroma, sweetness, and sourness were analyzed using Pearson's correlation test. The results of chemical analysis were also statistically analyzed and correlated using independent samples t-test.

Results and Discussion

Sensory evaluation and acceptability

The Karanda fruit nectar with 20° brix was noted to be the most acceptable garnering the highest mean of 5.59 ± 1.559, followed by Karanda fruit nectars with 15° brix (5.38 ± 1.280), 10° brix (3.91 ± 1.640), and 5o brix (3.18 ± 1.507). Karanda fruit nectars with 5o brix and 10° brix were not significantly different from each other, same as Karanda fruit nectars with 15° brix and 20° brix. However these two groups were observed to be significantly different from each other. The Karanda fruit nectar with 5° and 10° brix readings were disliked slightly while the Karanda nectar with 15° brix reading was liked slightly and Karanda fruit nectar with 20° brix reading was liked moderately (Table 1).

Karanda fruit nectar treatments	Sensory characteristics				General acceptability
	Color	Aroma	Sweetness	Sourness	
5° Brix	2.65±0.691[c]	2.41±1.500[b]	2.12±1.472[b]	3.12±1.805[b]	3.18±1.507[b]
10° Brix	3.82±0.797[b]	4.24±1.986[a]	2.88±1.387[b]	3.24±1.793[b]	3.91±1.640[b]
15° Brix	4.56±0.504[a]	4.85±1.877[a]	4.59±1.861[a]	4.56±2.077[a]	5.38±1.280[a]
20° Brix	4.62±0.604[a]	5.12±1.610[a]	5.32±1.408[a]	4.85±1.635[a]	5.59±1.559[a]

Table 1: *Sensory characteristics and acceptability of Karanda fruit nectars.*

**Values are expressed as mean ± standard deviation.*

***Mean bearing different superscripts in columns are significantly different based on Tukey's HSD test at 5% level of significance.*

The evaluation of color of the four Karanda fruit nectar samples ranged from 2.65 ± 0.691 to 4.62 ± 0.604. Karanda fruit nectar with 15° brix and 20° brix readings were found to be not significantly different from each other, unlike the 5° and 10° brix samples which were noted to be significantly different. In terms of color, the nectar that had the highest mean is the one with 20° Brix reading (4.62 ± 0.604a), followed by 15° brix (4.56 ± 0.504), 10° brix (3.82 ± 0.797) and 5° Brix (4.62 ± 0.604). Colors of the fruit nectars were bright pink for 5°brix (Pantone 184 U), wild watermelon for 10° brix (Pantone 185 U) and torch red for 15° and 20° brix (Pantone 186 U). Torch red is the darkest color of red among the colors of the four treatments.

The fruit nectar with 20° brix has the highest mean for aroma which was 5.12 ± 1.610 (liked). The aroma of the three fruit nectar with 10° brix (4.24 ± 1.986), 15° brix (4.85 ± 1.877) and 20° Brix (5.12 ± 1.610) were not significantly different from each other but they are significantly different compared with the fruit nectar with 5° brix (2.41 ± 1.500). The Karanda fruit nectar with 5° Brix reading had the least liked aroma; 10° and 15° brix fruit nectars were rated as neither liked nor disliked.

For the sweetness, one of the five basic tastes that would mean having a taste of sugar, the 20° brix fruit nectar had the highest mean of 5.32 ± 1.408 followed by fruit nectars with 15° Brix, 10° brix, and 5° brix readings with means 4.59 ± 1.861, 2.88 ± 1.387, and 2.12 ± 1.472, respectively. Karanda fruit nectars with 15° brix and 20° brix readings were not significantly different from each other as well as Karanda fruit nectars with 5° brix and 10° brix readings. However significant difference in terms of sweetness was found between 5° brix and 10° brix nectar samples. The Karanda fruit nectar with 5° brix and 10° brix readings were not sweet thus disliked by the panelists while the Karanda fruit nectars with 15° brix and 20° brix readings were near to the very sweet characteristic hence liked by the panelists.

For the sourness, or the taste that is similar to an acid, the Karanda fruit nectar with 20o brix reading had the highest mean of 4.85 ± 1.635 followed by Karanda fruit nectars with 15° brix (4.56 ± 2.077), 10° brix (3.24 ± 1.793) and 5° brix (3.12 ± 1.805). Karanda fruit nectars with 15° brix and 20° brix were not significantly different from each other but were significantly different from Karanda fruit nectars of 5° brix and 10° brix. The fruit nectars with 5° and 10° brix readings were also not significantly different from each other. The 5° brix and 10° brix Karanda fruit nectars were not sour while 15° brix and 20° brix Karanda fruit nectars were near to the very sour characteristic.

	Color	Aroma	Sweetness	Sourness
General Acceptability				
Pearson correlation	0.465**	0.296**	0.588**	-0.250**
Sig. (2-tailed)	0.000	0.000	0.000	0.003
N	136	136	136	136

Table 2: *Correlation of general acceptability to the sensory characteristics (color, aroma, sweetness, and sourness).*

***. Correlation is significant at the 0.01 level (2-tailed).*

It can be seen that all of the sensory characteristics has a relationship with the general acceptability. The correlation coefficient value of color and general acceptability is 0.465. This value means that there is statistically significant correlation between color and general acceptability and this value is in the moderate positive type of relationship. It can be concluded that as the color of the fruit nectar intensifies, the general acceptability increases.

For the aroma and general acceptability, the correlation coefficient value is 0.296. This value means that there is statistically significant correlation between aroma and general acceptability and they have weak positive relationship. As the aroma of the nectar intensifies, the general acceptability increases.

In terms of the sweetness and general acceptability, there was moderate positive significant correlation between sweetness and general acceptability and that they have a moderate positive relationship. As the sweetness of the fruit nectar increases, the general acceptability also increases.

The value of correlation coefficient of sourness and general acceptability is -0.250. There was a significantly weak and negative difference between sourness and general acceptability. As the sourness of the fruit nectar decreases, the general acceptability increases.

It can be concluded that as color and aroma intensifies, and as the sweetness of the nectar increases and the sourness decreases, the general acceptability of the Karanda fruit nectar increases.

Physico-chemical Properties

Consumption of various fruit and fruit products as refreshments are often recommended in the diet for nutritional and health purposes. Most fruits and fruit products contain varying amounts of vitamins, minerals, fibers and other biologically active phytochemicals such as phenols, tannins, anthocyanidins which are reported to exhibit various biological activities such as antioxidants, antimicrobial and anti-inflammatory agents [11-12].

The 20° brix Karanda fruit nectar had 2.567 pH comparable to lemon (2.81) and lime (2.79) juices [12], 1.993 %titratable acidity and 10.106 Brix to acid ratio (Table 3).

Sample	Physico-chemical properties			
	pH	Total Soluble Solids (° Brix)	Titratable acidity (%)	Brix to Acid Ratio
Karanda fruit nectar	2.567 ± 0.0577	20.00 ± 0.0000	1.9933 ± 0.20207	10.1067 ± 1.08542

Table 3: *Physico-chemical characteristics of Karanda fruit nectar.*
**Values are expressed as mean ± standard deviation.*

Phytochemical Contents

The flavonoids, tannins, anthocyanidins and phenol contents of the 20° Brix Karanda fruit nectar were 33.49 mg/100 ml, 1.05 mg/100 ml, 3.73 mg/100 ml and 633.68 mg/100 ml, respectively. These phytochemical readings are comparable with other fruits such as raw pink grapefruit in terms of tannins [13] and orange juice in terms of phenol contents [12,15].

The Karanda fruit nectar had Vitamin C content of 2642.1 mg/100 ml and DPPH scavenging activity of 84.967%. Antioxidant is the collective term for the phytochemical components as well as the Vitamin C content. Since it has been observed that that fruit nectar had

high phytochemical components as well as Vitamin C content, it resulted to high DPPH scavenging activity. The obtained results in this study showed substantial health merit of the Karanda fruit nectar.

Sample	Phytochemical components, Vitamin C Content, and Antioxidant Activity					
	Flavonoids (mg/100 ml)	Tannins (mg/100 ml)	Anthocyanidins (mg/100 ml)	Phenols (mg/100 ml)	Vitamin C Content (mg/100 ml)	Antioxidant Activity (%)
Karanda fruit nectar	33.49 ± 2.03	1.05 ± 5.54	3.73±0.47	633.68 ± 67.10	2642.1 ± 1767.60	84.967 ± 0.3512

Table 4: *Phytochemical components, Vitamin C Content, and Antioxidant Activity of 20° Brix Karanda fruit nectar.*
**Values are expressed as mean ± standard deviation.*

Conclusion

The Karanda fruit nectar upon evaluation was found to be liked at 20° brix compared with 5°, 10° and 15° brix. It had torch red color, liked aroma, sweetness and sourness. It was observed to contain potential health benefits such flavonoids, tannins, anthocyanidins, phenol, vitamin C and antioxidant property.

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