beverages

MDPI

Article

The Impact of Sweetener Type on Physicochemical Properties, Antioxidant Activity and Rheology of Guava Nectar during Storage Time

Napassorn Peasura * and Pornrat Sinchaipanit

Institute of Nutrition, Mahidol University Salaya, 999 Phutthamonthon 4 Rd., Nakhon Pathom 73170, Thailand; pornrat.sin@mahidol.ac.th

* Correspondence: napassorn.pea@mahidol.ac.th

Abstract: This study aimed to evaluate the differences in physicochemical properties, antioxidant properties, and rheology between guava nectar with sucrose and guava nectar with neotame and stevia addition (0.01% and 0.05%). All parameters were investigated during refrigerated storage at 4 °C for 15 days. The result showed that all sweetened guava juice samples led to the juice with the greatest presence of overall appearance and antioxidant property. The flow behavior of sweetened guava juice was shear-thinning which was not different from guava juice without sweetener. However, the major volatile flavor compounds identified in all guava juice samples were β-caryophyllene, α-caryophyllene, bisabolene, aromadendrene, α-humulene, and nerolidol, which is not different from guava juice without sweeteners during storage. It indicated that stevia and neotame are good alternative sweeteners to produce low caloric juice production.

Keywords: guava nectar; stevia; neotame; physicochemical property; rheology

Citation: Peasura, N.; Sinchaipanit, P. The Impact of Sweetener Type on Physicochemical Properties, Antioxidant Activity and Rheology of Guava Nectar during Storage Time. *Beverages* **2022**, *8*, 24. https://doi.org/10.3390/beverages8020024

Academic Editors: Antonio Morata, António Manuel Jordão, Fernanda Cosme and Ileana Vigentini

Received: 22 February 2022
Accepted: 6 April 2022
Published: 12 April 2022

1. Introduction

Guava (*Psidium guajava*) is tropical fruit cultivated in tropical and subtropical regions. It contains high levels of antioxidants such as beta-carotene, lycopene which played an important role in combating free radicals. It is mostly consumed in fresh or processed form (puree, concentrated, and juice); guava juice is favored in tropical regions due to its sweet and refreshing flavor. Moreover, it is an excellent source of several important vitamins and minerals [1].

Nowadays, fruit juice consumption is increased due to its relief of thirst, nutrition, and easy digestibility [2]. Moreover, a large amount of refined sugar is used to sweeten fruit juice for preferable flavor, taste, and color [3]. They also play a crucial role in the appearance, physicochemical, and rheology of fruit juices [4]. However, it affected health problems, including obesity, diabetes, and hyperlipidemia [5]. Therefore, sweeteners are alternative compounds used to partly or completely replace sugar in fruit juice [6].

A sweetener is a substance used to replace sucrose which can provide the same sweet taste as sucrose. The type of sweetener used can influence the physicochemical properties which may limit its addition to a product [6] using each sweetener in situations for which it is best suited [7]. In addition, six sweeteners, including saccharin, aspartame, sucralose, neotame, acesulfame-K, and stevia have been classified under the generally recognized as safe (GRAS) category [8]. Several sweeteners are allowed for use in food and beverages [9].

Neotame is an artificial sweetener that has the same characteristics as aspartame. The sweetness is 5000 times that of sucrose, and is sweeter than aspartame. It also has a desirable flavor more than aspartame [10]. Derta and Bolini [11] reported that there was no significant difference in appearance and aroma of acerola nectar sweetened with sucrose, neotame, and stevia. In terms of vitamin C content and total titratable acidity, there was also no significant difference ($p > 0.05$).

Stevia is produced from the leaf of Stevia rebaudiana (Bertoni, The Netherlands) which has a relative sweetness of 200 times of sucrose [12]. Rebaudioside A, one of the major sweet components of the plant Stevia rebaudiana [13] is sweeter and stable, and less bitter than stevioside [14]. Additionally, stevia is used to replace sugar in many beverage products such as juice and nectar [15]. Mango nectar mixed with stevia had significant effects on the total soluble solids (°Brix), viscosity and pH which were affected by concentration. In addition flow behavior was not different from mango containing sucrose [15]. Moreover, the physicochemical properties of mango nectar were evaluated, including color, pH, titratable acidity, and soluble solids in mango nectar sweetened during storage time. The sample nectar blended with stevia showed similar physicochemical properties to sucrose in 0 days. However, sweeteners such as acesulfame-K, sucralose, neotame, and stevia in nectar displayed good stability in mango nectar [16]. Furthermore, the rheological properties of juice are also affected by sugar content. Sweetened products with sugar substitutes should have similar rheology to the original product. According to Miele et al. [17] yogurt with different sweeteners replacing sugar did not affect the yogurt's rheological properties.

However, sucrose replacement by different sweeteners is a challenging alternative that can affect the physicochemical property and rheology of guava nectar. In this study, guava nectar was sweetened with sucrose and two different sweeteners, including neotame and stevia. While stevia is a natural sweetener from a plant that has been used as a sweetener in beverages for decades, neotame is an artificial sweetener with a taste profile similar to sucrose. It promoted desirable flavors, including sweet taste and mouthfeel [18]. Therefore, the objective of this study was to determine physicochemical property, antioxidant activity, and rheological properties in guava nectar with different sweeteners during refrigerated storage. This will be a positive approach to the reduction of sugar consumption in a low calories drink.

2. Materials and Methods

2.1. Raw Materials

Fresh guava (*Psidium guajava*) of the Kimju variety was purchased from the local market in Bangkok, Thailand. The sweetener used for the sweetened guava juice was from Chemipan Corporation Co., Ltd., Bangkok, Thailand. The other ingredients such as salt (Prungthip) and sugar (Mitr-Phol) were from a supermarket.

2.2. Preparation Guava Nectar Formulation

The raw material was washed with running water and then cut into pieces. The pulp was mixed with distilled water in a ratio of 1:1 (*w/v*) by a blender. The mixture was transferred to a nylon bag which was applied pressure to separate the fruit juice from the pulp. Citric acid (0.15%, *w/v*) and NaCl (0.25%, *w/v*) were added to juice. The soluble solid was adjusted to 8 °Brix. After mixing, the beverage was homogenized in a rotor-stator type homogenizer (Walita®, São Paulo, Brazil) for 5 min.

2.3. Preparation of Sweetened Guava Nectar

The guava nectar was mixed with neotame and stevia (0.01% and 0.05%) with 0.1% guar gum. Briefly, intense sweetener and guar gum were gradually mixed with guava nectar. For guava nectar without sweetener, sucrose was added to nectar for 4% (*w/v*), 0.15% (*w/v*) citric acid and 0.25% (*w/v*) NaCl as control. The mixture was homogenized and heated to 80 °C for 30 s, then cooled in the ice bath to 40 °C. Finally, guava nectar and sweetened guava nectar were filled in a pasteurized clear bottle (80 °C for 5 s), sealed, and stored refrigerator (4 °C) for 15 days. All juice samples were analyzed immediately after preparation. The shelf life of guava nectar and sweetened guava nectar were determined every 3 days for physicochemical properties, antioxidant activity, and rheology.

2.4. Analytical Methods for Shelf Life Evaluation

The pasteurized guava nectar shelf life was evaluated at 5 °C ± 2 °C to determine physicochemical properties and antioxidant activity which were performed on days 0, 3, 6, 9, 12, and 15.

2.5. Physico-Chemical Property

The cloud value was evaluated using a UV-1601 spectrophotometer (Shimadzu, Japan) that was calibrated with distilled water according to Abid et al. [19]. The samples were centrifuged at 618× *g* for 20 min, then absorbance at 660 nm of the supernatant was recorded. The transmittance rate was calculated as the following equation:

$$T = In\left(\frac{I}{I_0}\right)$$

where

I is the light density that passes through a sample solution.
I_0 is the light intensity.

The color of guava nectar samples was measured according to the method described by Wang et al. [20]. The colorimeter (ColorFlex EZ spectrophotometer, Horsham, PA, USA) was used to evaluate the color during storage.

The parameters including L*, a*, and b* of each sample were recorded. The results were expressed as total color difference (ΔE) values which were calculated as follows

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL, Δa* and Δb* are a difference of lightness, redness, and yellowness, respectively, for 0, 3, 6, 9, 12 and 15 days of storage.

The pH of guava nectar and sweetened guava nectar was measured at 25 °C by using a digital pH meter (Denver Instrument, Denver, CO, USA) with a glass electrode.

The acidity of nectar was evaluated by using an auto titrator (Titriline 700, Columbus, OH, USA). Fruit juice (10 mL) mixed with 20 mL of distilled water that was titrated with 0.1 NaOH to a pH value of 8.3 as previously described by Xiang et al., (2018). The results were expressed as g citric acid/100 mL of guava nectar.

$$\text{Acidity } (\%) = \frac{\text{mL of NaOH } \times 0.1 \times 64 \times 100}{\text{mL of juice} \times 1000}$$

2.6. Total Carotenoid Content

Total carotenoid was determined by spectrophotometer (UV-1601 spectrophotometer, Shimadzu, Japan) as proposed by Kotíková et al. [21]. The nectar samples (2 mL) were added to 15 mL of acetone and incubated for 2 h. The excess acetone was removed by centrifuge at 4000× *g* for 5 min, then adjusted volume to 25 with acetone. The samples were measured absorbance at 662 nm, 645 nm, and 470 nm and calculated with the equation.

$$\text{Total carotenoid } (\mu g/mL) = 4.405\ A_{470} - 6.65\ A_{645} + 1.303\ A_{662}$$

2.7. Antioxidant Activity

2.7.1. $ABTS^{\bullet+}$ Radical Scavenging Activity Assay

The $ABTS^{\bullet+}$ scavenging activity was performed according to Re et al. [22] as follows: The $ABTS^{\bullet+}$ solution was prepared by mixing two solutions of $ABTS^{\bullet+}$ (7 mM) and potassium persulfate (2.45 mM). The mixture was allowed to stand in the dark condition at room temperature for 16 h before use. The $ABTS^{\bullet+}$ solution was diluted to the absorbance of 0.700 ± 0.02 at 734 nm. Then, guava juice (1 mL) was added with 3 mL of $ABTS^{\bullet+}$ solution,

mixed, and kept in the dark for 6 min. The absorbance was measured at 734 nm. The scavenging activity was calculated as:

$$\text{scavenging activity} = \left(\frac{1 - A_{sample}}{A_{control}}\right) \times 100$$

where

$A_{control}$ is the absorbance of $ABTS^{\bullet+}$ solution without sample.
A_{sample} is the absorbance of $ABTS^{\bullet+}$ mixed with a sample.

2.7.2. DPPH$^{\bullet}$ Radical Scavenging Activity Assay

Antioxidant activity was assessed as DPPH$^{\bullet}$ radical scavenging activity according to Bhat and Goh. [23]. Briefly, 100 µL of the nectar was added to 2 mL of 0.1 mM DPPH$^{\bullet}$ in 95% ethanol. The mixture was incubated at room temperature (dark condition) for 30 min. The absorbance was recorded at 517 nm with a spectrophotometer (UV-1601 spectrophotometer, Shimadzu, Japan). The antioxidant activity was expressed as the scavenging activity according to the equation

$$\%\ \text{Scavenging activity} = \left(\frac{1 - A_{sample}}{A_{control}}\right) \times 100$$

where

$A_{control}$ is the absorbance of DPPH$^{\bullet}$ solution without sample.
A_{sample} is the absorbance of DPPH$^{\bullet}$ mixed with a sample.

2.8. Flow Behavior and Apparent Viscosity of Guava Juice

The apparent viscosity of nectar samples was determined by using a rheometer (Haake Mars 40 model, Thermo Fisher Scientific, Waltham, MA, USA) equipped with cup and coaxial cylinder geometry. The shear rate was varied from 0.01 s^{-1} to 10 s^{-1} at 540 s^{-1} at 5 °C and 37 °C with a gap of 0.01 mm. The nectar samples (17 mL) were placed into a cup and allowed to rest at 5 °C and 37 °C for 120 s^{-1} [24]. A total of 30 data points were recorded at 15 s^{-1} during shearing.

2.9. Volatile Compound Gas Chromatography

The volatile compound of all guava nectar samples was performed by chromatography-mass spectrometry (Agilent DB-WAX column, 60 m × 0.25 mm × 0.25 µm film thickness dimensions, Beijing, China) with flame ionization detector (FID). The sample (3 µL) was extracted by using headspace mode at 30 °C for 30 min. After that, volatile compounds were subjected to solid-phase microextraction (SPME fiber 50/30/µm Divinylbenzene/Carbon wide range/Polydimethylsiloxane stableflex 2 cm, Suppleco) and injection was performed in a splitless mode at 250 °C that helium was the carrier gas. The gas chromatography (GC) temperature was 50 °C for 2.5 min up to 200 °C in 4 min. The volatile compound was identified by comparing their GC retention time (RT) as proposed in NIST 2005 v 2.0 (Natl. Inst. of Standards and Technology, Gaithersburg, MD, USA) according to Guo et al. [25] with slight modification.

2.10. Statistical Analysis

The physicochemical and antioxidant properties were performed in triplicate in a Randomized Complete Block Design. The result was subjected to a one-way analysis of variance (ANOVA) with Statistical Analysis System (SAS, version 6.0) (SAS Institute, Cary, NC, USA). Duncan's Multiple Range Test was used to determine significant differences between means ($p < 0.05$). All data were presented as average values with the standard deviation.

3. Results and Discussion

3.1. Cloud Value

Turbidity is the cloudiness of nectar samples that occurred due to the colloid polysaccharides particles, including starch, pectin, and hemicellulose components [26]. Moreover, turbidity is an important quality criterion in fruit juice [27]. The cloud value was evaluated in guava nectar with sucrose as a reference control and guava nectar with neotame and stevia at different concentration levels (0.01% and 0.05%) as sweetened guava nectar. The result showed that guava nectar with 0.05% neotame had the highest cloud value. It was a significant difference with reference control, guava nectar with 0.01% neotame and guava nectar with stevia (0.01% and 0.05%). Moreover, turbidity was decreased with increasing storage time which was extremely significant in cold storage at 5 °C for 15 days (Table 1).

Table 1. Effect of different sweetener on turbidity of guava nectar during 15th of storage day at 5 °C.

Storage Day	Guava Nectar with Sucrose	Sweetened Guava Nectar			
		0.01% Neotame	0.01% Stevia	0.05% Neotame	0.05% Stevia
0	$30.42 \pm 0.25^{C,a}$	$31.01 \pm 0.55^{B,a}$	$30.93 \pm 1.35^{C,a}$	$32.49 \pm 0.21^{A,a}$	$30.94 \pm 0.57^{C,a}$
3	$26.43 \pm 0.4^{A,b}$	$20.46 \pm 0.6^{B,b}$	$19.53 \pm 0.64^{C,b}$	$20.48 \pm 1.30^{B,b}$	$19.67 \pm 0.52^{C,b}$
6	$25.29 \pm 0.08^{A,c}$	$19.80 \pm 0.19^{C,c}$	$17.88 \pm 0.88^{D,c}$	$20.35 \pm 012^{B,b}$	$18.46 \pm 0.15^{C,c}$
9	$24.13 \pm 0.04^{A,d}$	$19.10 \pm 0.06^{B,d}$	$18.38 \pm 0.02^{C,d}$	$18.73 \pm 0.55^{C,c}$	$17.57 \pm 0.41^{D,d}$
12	$22.09 \pm 0.42^{A,e}$	$18.74 \pm 0.30^{B,e}$	$17.90 \pm 0.06^{C,e}$	$17.29 \pm 0.06^{D,d}$	$17.18 \pm 0.14^{D,e}$
15	$13.84 \pm 0.04^{D,f}$	$18.10 \pm 0.08^{A,f}$	$17.36 \pm 0.12^{C,e}$	$17.51 \pm 0.11^{B,e}$	$17.71 \pm 0.30^{B,d}$

$^{A–D}$ Different capital letters in the same row show significant differences between the guava nectar with sucrose and sweetened guava nectar for the same day. $^{a–f}$ Different small letters in the same column show significant differences between storage days.

Although in 3 days of storage reference control had the lowest cloudiness, guava nectar with 0.01% stevia had the highest cloud value. It implied that fruit particles in the nectar aggregated and degraded by oxidation-reduction reaction resulted in the decline of turbidity which was observed in reference control [28].

Therefore, the nectar had a high precipitate which allowed light more to pass through the nectar than nectar with less precipitate. Moreover, the appearance of reference control and sweetened guava nectar precipitate were recorded by observation. It was confirmed that storage time had a significant effect on turbidity. Pulp was separated from nectar at the start of storage (0 days) in reference control, while pulp-sweetened guava nectar precipitated at 6 days of storage and settled at bottom of the bottle (Table 2). The cloudy appearance displayed the nectar homogenization. As a result, low cloudiness in all samples was observed during storage. It indicated an undesirable quality.

3.2. Color Attributes

Color attributes are an important standard to estimate the quality of fruit nectar that was related to consumers' satisfaction and requirements [29]. The color analyses were performed as color differences that were represented by ΔE at 0, 3, and 6, 9, 12 and 15 days of storage. Although the total difference was observed in the color of reference control and guava nectar with 0.01% and 0.05% stevia, there was no significant difference between guava nectar containing 0.01% and 0.05% neotame (Table 3).

Moreover, the color had a significant difference ($p \leq 0.05$) during the storage period in reference control and sweetened guava nectar. It might be the destruction of the cell membrane which would lead to the loss of functional cell compartmentalization. Thus, it accelerates enzyme-substrate contact which promotes tissue browning [30]. However, the color variation was larger in reference control and guava nectar with neotame. The result demonstrated that guava nectar with 0.01% stevia showed the least color changes after 15 days. The addition of stevia might cause the inactivation of enzymes which prevent enzyme–substrate contact [30].

Table 2. The appearance of guava nectar and sweetened guava nectar during 15th of storage day at 5 °C.

Storage Day	Guava Nectar with Sucrose	Sweetened Guava Nectar			
		0.01% Neotame	0.01% Stevia	0.05% Neotame	0.05% Stevia
0	Pulp separated from the juice	Pulp uniformly distributed	Pulp does not precipitate	Pulp uniformly distributed	Pulp does not precipitate
3	Pulp separated from the juice and settled at the bottom of the	Pulp did not precipitate	Pulp does not precipitate	Pulp does not precipitate	Pulp does not precipitate
6	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle	Pulp does not precipitate	Pulp does not precipitate
9	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle
12	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle
15	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle	Pulp separated from the juice and settled at the bottom of the bottle

Table 3. The color change of guava nectar and sweetened guava nectar during 15th of storage day at 5 °C.

Storage Day	Guava Nectar with Sucrose	Sweetened Guava Nectar			
		0.01% Neotame	0.01% Stevia	0.05% Neotame	0.05% Stevia
ΔE (0 day)	$0 \pm 0^{A,f}$	$0 \pm 0^{A,f}$	$0 \pm 0^{A,e}$	$0 \pm 0^{A,e}$	$0 \pm 0^{A,f}$
ΔE (3 days)	$6.72 \pm 0.03^{A,e}$	$6.08 \pm 0.15^{B,e}$	$5.18 \pm 0.07^{D,d}$	$6.22 \pm 0.04^{A,d}$	$5.81 \pm 0.10^{C,e}$
ΔE 6 days)	$7.11 \pm 0.15^{A,d}$	$6.22 \pm 0.05^{B,d}$	$5.20 \pm 0.01^{D,d}$	$6.24 \pm 0.06^{B,d}$	$5.92 \pm 0.07^{C,d}$
ΔE (9 days)	$8.09 \pm 0.08^{A,c}$	$6.41 \pm 0.09^{B,c}$	$5.48 \pm 0.03^{D,c}$	$6.41 \pm 0.02^{B,c}$	$6.22 \pm 0.05^{C,c}$
ΔE (12 days)	$8.55 \pm 0.04^{A,b}$	$6.68 \pm 0.07^{B,b}$	$5.71 \pm 0.04^{D,b}$	$6.68 \pm 0.03^{B,b}$	$6.35 \pm 0.05^{C,b}$
ΔE (15 days)	$9.03 \pm 0.05^{A,a}$	$6.88 \pm 0.07^{B,a}$	$6.08 \pm 0.08^{D,a}$	$6.82 \pm 0.02^{B,a}$	$6.51 \pm 0.02^{C,a}$

$^{A–D}$ Different capital letters in the same row show significant differences between the guava nectar with sucrose and sweetened guava nectar for the same day. $^{a–f}$ Different small letters in the same column show significant differences between storage days.

3.3. pH

The pH of reference control and sweetened guava nectar are presented in Table 4. The initial pH degrees of guava nectar were controlled with citric acid. The result showed that pH in reference control and sweetened guava nectar increased with extended storage time at 5 °C.

Table 4. The pH of guava nectar and sweetened guava nectar during 15th of storage day at 5 °C.

Storage Day	Guava Nectar with Sucrose	Sweetened Guava Nectar			
		0.01% Neotame	0.01% Stevia	0.05% Neotame	0.05% Stevia
0	$3.51 \pm 0.01^{A,d}$	$3.52 \pm 0.02^{A,d}$	$3.18 \pm 0.09^{B,e}$	$3.51 \pm 0.02^{A,e}$	$3.56 \pm 0.02^{A,b}$
3	$3.55 \pm 0.01^{A,c}$	$3.52 \pm 0.02^{B,d}$	$3.53 \pm 0.02^{B,d}$	$3.52 \pm 0.02^{B,d}$	$3.54 \pm 0.01^{A,d}$
6	$3.60 \pm 0.02^{A,b}$	$3.55 \pm 0.01^{B,c}$	$3.56 \pm 0.02^{B,c}$	$3.50 \pm 0.11^{C,c}$	$3.55 \pm 0.01^{B,c}$
9	$3.61 \pm 0.01^{A,b}$	$3.55 \pm 0.04^{B,c}$	$3.57 \pm 0.02^{B,b}$	$3.56 \pm 0.01^{B,b}$	$3.53 \pm 0.03^{C,d}$
12	$3.66 \pm 0.01^{A,a}$	$3.56 \pm 0.01^{B,b}$	$3.57 \pm 0.02^{B,b}$	$3.57 \pm 0.01^{B,a}$	$3.41 \pm 0.25^{C,e}$
15	$3.67 \pm 0.02^{A,a}$	$3.57 \pm 0.02^{C,a}$	$3.58 \pm 0.03^{B,a}$	$3.57 \pm 0.01^{C,a}$	$3.57 \pm 0.01^{C,a}$

$^{A–C}$ Different capital letters in the same row show significant differences between the guava nectar with sucrose and sweetened guava nectar for the same day. $^{a–e}$ Different small letters in the same column show significant differences between storage days.

It was indicated that poly-saccharides were hydrolyzed by acid which is responsible for the increase in pH [8]. However, reference control and guava nectar with different levels of sweetener (0.01%, 0.05% neotame and 0.01%, 0.05% stevia) had pH values ranged from 3.50 to 3.60 which were not significantly different ($p > 0.05$) as a function of storage time. It indicated that the substitution of sucrose with neotame and stevia did not affect the pH of the guava nectar. It was observed that the pH of guava nectar with stevia increased slightly with the increasing level of stevia at 0 days of storage due to the high pH of stevia [31]. In the beverage industry, the stability of sweeteners will be influenced by pH, temperature, and storage time. Stevia and neotame are also stable in acidic pH conditions at 2.9–4.5 [32]. Consequently, it is allowed for soft drinks and fruit nectar at acid pH [32].

3.4. Titratable Acidity (TA)

Titratable acidity in fruit nectar includes all the substances of an acid in the fruit nectar, of which the most acidic component is an organic acid. Titratable acidity was expressed as a percentage of the predominant acid which was citric acid (g/100 g). The decreasing trend of titratable acidity was presented from the initial to the 6th day of storage in both reference control and sweetened guava nectar (Table 5).

Table 5. The acidity of guava nectar and sweetened guava nectar during 15th of storage day at 5 °C.

Storage Day	Guava Nectar with Sucrose	Sweetened Guava Nectar			
		0.01% Neotame	0.01% Stevia	0.05% Neotame	0.05% Stevia
0	$0.59 \pm 0.01^{C,a}$	$0.60 \pm 0.01^{B,a}$	$0.61 \pm 0.01^{A,a}$	$0.59 \pm 0.01^{C,a}$	$0.61 \pm 0.01^{A,a}$
3	$0.59 \pm 0^{A,a}$	$0.59 \pm 0.01^{B,b}$	$0.56 \pm 0.01^{D,b}$	$0.58 \pm 0.02^{C,b}$	$0.59 \pm 0.01^{B,b}$
6	$0.57 \pm 0.01^{A,b}$	$0.57 \pm 0.02^{A,c}$	$0.55 \pm 0.01^{C,c}$	$0.55 \pm 0.01^{C,c}$	$0.56 \pm 0.01^{B,c}$
9	$0.55 \pm 0.01^{B,c}$	$0.56 \pm 0.01^{A,d}$	$0.55 \pm 0.01^{B,c}$	$0.56 \pm 0.01^{A,d}$	$0.56 \pm 0.01^{A,c}$
12	$0.55 \pm 0.01^{B,c}$	$0.56 \pm 0.01^{A,d}$	$0.53 \pm 0.01^{D,d}$	$0.53 \pm 0.01^{D,e}$	$0.54 \pm 0.01^{C,d}$
15	$0.52 \pm 0.01^{D,d}$	$0.55 \pm 0.01^{A,e}$	$0.53 \pm 0.01^{C,d}$	$0.53 \pm 0.01^{C,e}$	$0.54 \pm 0.01^{B,d}$

$^{A–D}$ Different capital letters in the same row show significant differences between the guava nectar with sucrose and sweetened guava nectar for the same day. $^{a–e}$ Different small letters in the same column show significant differences between storage days.

Although pH was increased from 0 to 15 storage days, the acidity of all samples non-significantly decreased from 0 days to 15 days. Generally, the pH is always inversely proportional to acidity which acidity has decreased during the storage period so pH has increased [33]. This was also found in kinnow juice storage in the refrigerator [34] and mandarin juice storage at low temperatures [35]. The storage conditions of low temperatures and high humidity might be reduce the conversion of acids into sugar and salt by invertase enzymes in guava nectar [36]. Additionally, guava nectar with 0.01% neotame had the highest percent titratable acidity during storage. However, the different types and levels of sweeteners did not affect the pH and total acidity in guava juice.

3.5. Carotenoid Content

Carotenoids are natural pigments that contribute to both the appearance and attractiveness of beverages as well as additional nutritional value in the form of powerful dietary antioxidants. The result showed that the lowest carotenoid was found in the reference control during storage. However, total carotenoid content decreased significantly ($p < 0.05$) after storage in reference control and sweetened guava nectar (Figure 1).

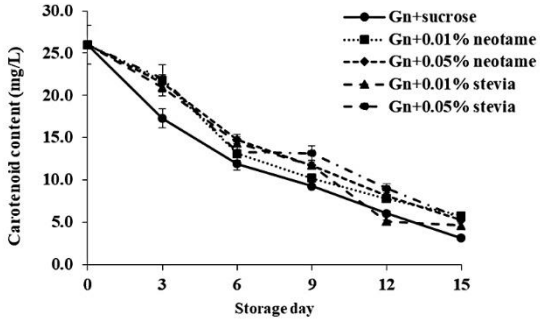
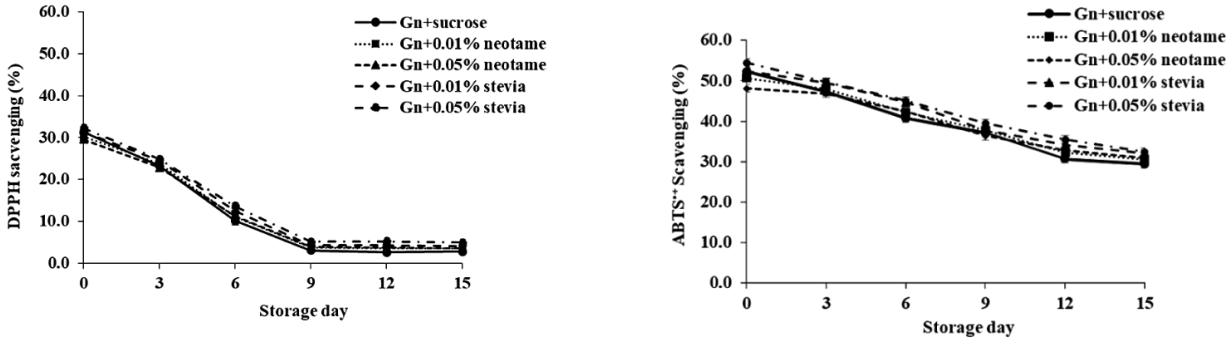


Figure 1. The effect of different sweetener on carotenoid content in guava nectar (Gn) with sucrose and sweetened guava nectar during storage time at 5 °C.

Although total carotenoids in sweetened guava nectar decreased non-significantly in 3 days of storage, total carotenoids were significantly reduced after 3 days of storage. It suggested that sweeteners including, stevia and neotame as protective carotenoids in model systems [37]. Ordóñez-Santos and Vázquez-Riascos [38] reported that the reduction of the total carotenoid content was found in guava nectar during storage at 10 °C for 240 days. It indicated that oxidative degradation was the major cause of carotenoid loss. Additionally, a total carotenoid in guava nectar containing 0.01% and 0.05% neotame and stevia during storage days was no different. Thus, sweetener addition does not influence carotenoid content in guava nectar.

3.6. ABTS$^{\bullet+}$ Radical Scavenging

Total antioxidant capacity was measured by ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ method which were evaluated in vitro. As shown in Figure 2a,b, the trend of radical scavenging was decreased during the storage period in the reference control and sweetened guava nectar. The result showed that the ABTS$^{\bullet+}$ radical scavenging activity ranged from 54.55 to 29.43%. Guava nectar with 0.05% stevia had the highest ABTS$^{\bullet+}$ scavenging followed by 0.05% neotame, control, 0.01% stevia and 0.05% neotame exhibited the lowest radical scavenging by ABTS$^{\bullet+}$ assay. However, at day 0, the antioxidant of guava nectar with sucrose was not significant with sweetened guava nectar (0.01% neotame). All samples were observed with low antioxidant activity at the end of the storage. The ABTS$^{\bullet+}$ scavenging activity of sweetened guava nectar was higher compared to the control. It might be from carotenoid content and sweetener as well as the synergistic effect.



(**a**) (**b**)

Figure 2. The radical scavenging activity of guava nectar (Gn) with sucrose and sweetened guava nectar during storage time at 5 °C by (**a**) ABTS$^{\bullet+}$ radical scavenging assay and (**b**) DPPH$^{\bullet}$ radical scavenging assay.

3.7. DPPH• Radical Scavenging

The antioxidant activity ranged from 3.01 to 32.26% which guava nectar with 0.05% stevia had the highest DPPH• scavenging value in 0 days of storage. However, guava nectar with different levels of neotame was no significant difference in DPPH• radical scavenging activity. It was expected that stevia powder contained a high amount of ascorbic acid which powerfully affects the antioxidant properties. Moreover, antioxidant capacity was related to carotenoid in guava nectar. In this study, it showed a decrease in DPPH• scavenging values in the presence of different carotenoid content. Although the antioxidant activity of reference control and sweetened guava nectar (0.1% stevia, 0.001% and 0.005% neotame) at the initial storage time decreased significantly different from 0, 3 and 6 days of storage, the sweetened guava nectar decreased not significantly DPPH• scavenging activity between 3 and after 6 days of storage. Moreover, the higher scavenging $ABTS^{\bullet+}$ activity than DPPH• might be related to the different structures of the radical and different molar ratios of the antioxidant to the radical molecules.

3.8. Volatile Compound

The volatile flavor of guava nectar was identified by gas chromatography at 0 and 15 days of storage which was reported as peak area percentages. As a result, the nectar contained aromatic volatile with low molecular weight, particularly alcohol and aldehyde. The major volatile in reference control and sweetened guava nectar was the sesquiterpenes, including β-caryophyllene, α-caryophyllene, bisabolene, aromadendrene, α- humulene and nerolidol (Table 6). These compounds were biogenetic, which was a response to the sweet and fruity aroma in guava [39].

Table 6. Description of the volatile compound in fresh and sweetened guava juice at 0 and 15 days of storage at 5 °C.

Volatile Compound	% Area					
	Guava Nectar with Sucrose		Guava Nectar with Neotame		Guava Nectar with Stevia	
			0.01%	0.05%	0.01%	0.05%
	0 Day	15 Days	0 Day	15 Days	0 Day	15 Days
1. β-Caryophyllene	26.71	10.15	26.71	13.55	26.71	12.95
2. α-Caryophyllene	2.84	1.58	2.84	1.92	2.84	1.95
3. Bisabolene	2.89	-	2.89	-	2.89	-
4. Aromadendrene	-	-	-	2.98	-	3.87
5. α- Humulene	2.84	1.52	2.84	1.93	2.84	1.54
6. Nerolidol	0.44	-	0.44	0.39	0.44	0.33
7. β-ocimene	0.07	ND	0.07	ND	0.07	ND
8. Acetic acid	1.37	0.02	1.05	0.03	1.05	0.02
9. octanoic acid	1.00	ND	1.00	ND	1.00	ND
10. ethyl acetate	0.12	ND	0.12	ND	0.12	ND
11. 2-methyl-1-propanol	0.53	ND	0.53	0.02	0.53	ND
12. 2-pentanone	0.35	0.02	0.35	0.01	0.35	ND
13. ethyl propanoate	0.22	ND	0.22	ND	0.22	ND
14. n-propyl acetate	0.13	ND	0.13	ND	0.13	ND
15. ethyl butyrate	0.11	ND	0.11	ND	0.11	ND
16. methyl butyrate	0.20	0.02	0.20	ND	0.20	0.01
17. pentanol	0.18	ND	0.18	ND	0.18	0.01
18. 1-penten-3-ol	0.09	ND	0.09	ND	0.09	ND
19. ethyl-2-butenoate	0.18	ND	0.18	ND	0.18	ND
20. 2-hexenal	0.26	0.01	0.26	0.02	0.26	0.01

ND, not detected.

Changes in the volatile flavor of the reference control and sweetened guava nectar during storage were observed. The result showed that all volatile flavors declined during the storage period due to chemical reactions and oxidation of free volatile compounds [40]. Among the volatiles present in all samples, guava nectar was observed to occur in various guava species [41]. β-caryophyllene, γ-caryophyllene and nerolidol are important in the characterization of tropical fruit flavor. Moreover, β-caryophyllene is often found together with α-humulene which is present in many aromatic plants [42]. In addition, the aroma

of nerolidol is woody and reminiscent of fresh bark [43]. However, the major volatile flavor was detected during storage of bisabolene and aromadendrene. Although bisabolene was only detected as volatile at 0 days storage, and at 15 days, aromadendrene was only detected in 6 days.

3.9. Rheology Properties

Rheology is the deformation and flow behavior of fluid which was important for food quality. The apparent viscosity of reference control and sweetened guava nectar was obtained from the flow curve (shear rate versus viscosity). The results are expressed as the apparent viscosity of the reference control and sweetened guava nectar at 5 °C and 37 °C. It can be seen that viscosity decreased with increasing shear rate which presents the non-Newtonian behavior of a shear-thinning fluid [44]. As shown in Figure 3a–e, the viscosity was varied slightly different between reference control and guava nectar with 0.01% and 0.05% neotame.

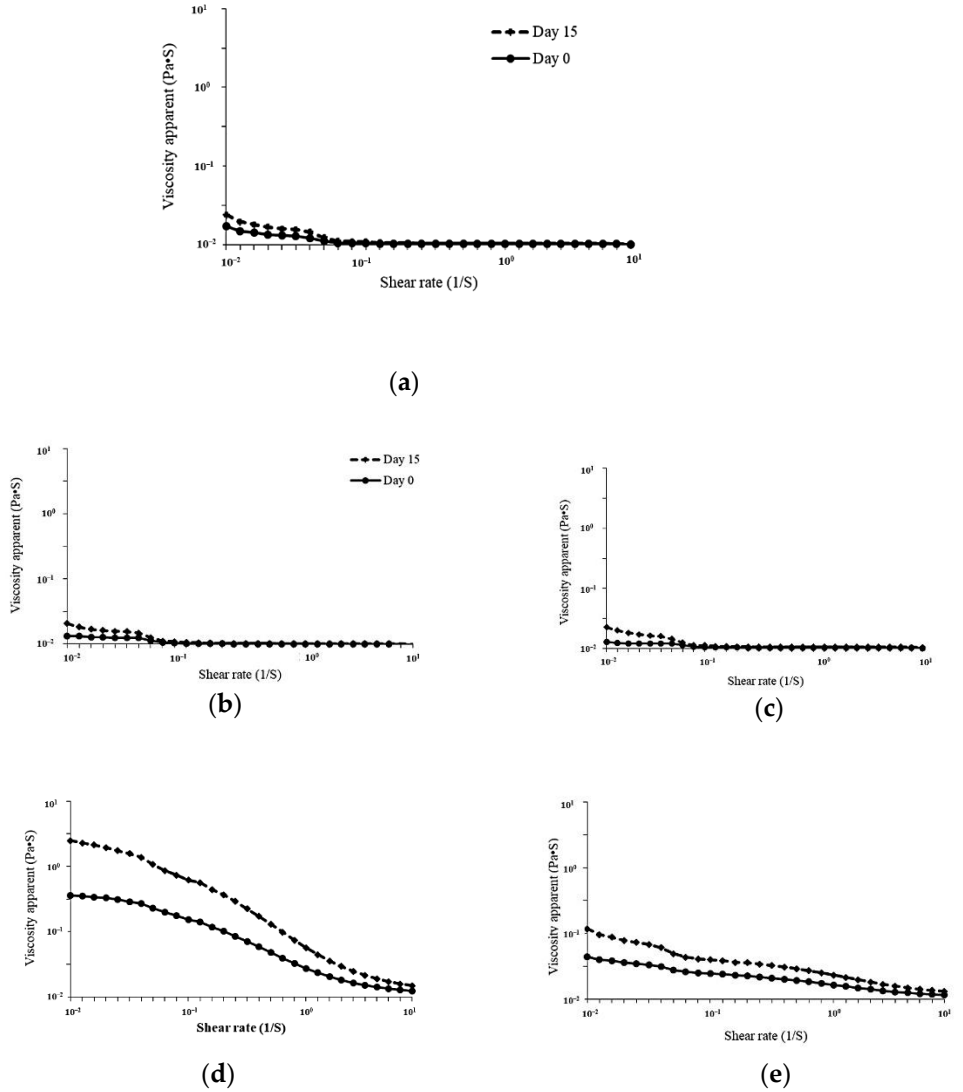


Figure 3. The viscosity of (**a**) guava nectar with sucrose, (**b**) guava nectar with 0.01% neotame, (**c**) guava nectar with 0.01% stevia, (**d**) guava nectar with 0.05% neotam€nd (**e**) guava nectar with 0.05% stevia during storage at 5 °C for 0 and 15 days.

The guava juice with 0.01% and 0.05% stevia had a higher viscosity than the reference control and guava nectar with neotame, respectively. It might be caused by a decrease in the guava nectar water activities due to the replacement of sucrose with stevia [45].

Furthermore, this result agrees with the low apparent viscosity found in chocolate milk. In contrast, the storage time increased, and the viscosity decreased which was found in all guava nectar formulated [46]. However, all guava nectar formulated at 37 °C exhibited lower viscosity than guava nectar at 5 °C. This phenomenon might be caused by some compound precipitates during storage at low temperatures.

4. Conclusions

The sweetened guava nectar with neotame and stevia at two levels could be effective to reduce the sugar content. Neotame and stevia improved the appearance of guava nectar during storage. Furthermore, carotenoid and antioxidant activity in sweetened guava nectar were higher than the reference control. Moreover, the physicochemical, including turbidity, color, pH and %TA of sweetened guava nectar provided resemble level compared to reference control. Therefore, stevia and neotame are good substitutes for sucrose which may keep nectar quality without affecting the physicochemical properties.

Author Contributions: Conceptualization: N.P. and P.S.; methodology: N.P.; writing—original draft preparation: N.P.; writing—review and editing: P.S.; All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work was supported by a New Researcher Grant from Mahidol University, Thailand.

Conflicts of Interest: The authors declare that there is no conflict of interest.

References

1. Bal, L.M.; Ahmad, T.; Senapati, A.K.; Pandit, P.S. Evaluation of quality attributes during storage of guava nectar cv. Lalit from different pulp and TSS ratio. *Int. J. Food Sci.* **2014**, *5*, 1–5.
2. Sethi, V.; Sethi, S. *Processing of Fruits and Vegetables for Value Addition*; Indus Publishing: Delhi, India, 2006.
3. Ogden, C.L.; Carroll, M.D.; Flegal, K.M. High body mass index for age among US children and adolescents, 2003–2006. *JAMA* **2008**, *299*, 2401–2405. [CrossRef] [PubMed]
4. Pareyt, B.; Talhaoui, F.; Kerckhofs, G.; Brijs, K.; Goesaert, H.; Wevers, M.; Delcour, J.A. The role of sugar and fat in sugar-snap cookies: Structural and textural properties. *J. Food Eng.* **2009**, *90*, 400–408. [CrossRef]
5. Ahmad, U.; Ahmad, R.S.; Imran, A.; Mushtaqq, Z.; Hussian, S.M. Characterization of low calorie ready-to-serve peach beverage using natural sweetener, Stevia (*Stevia rebaudiana* Bertoni). *Prog. Nutr.* **2019**, *21*, 435–444.
6. Pinheiro, M.V.S.; Oliveira, M.N.; Penna, A.L.B.; Tamime, A.Y. The effect of different sweeteners in low-calorie yogurts e a review. *Int. J. Dairy Technol.* **2005**, *58*, 193–199. [CrossRef]
7. Nabors, L.O. Sweet choices: Sugar replacements for foods and beverages. *J. Food Technol.* **2002**, *56*, 28–34.
8. Dhaka, A.; Sharma, M.; Singh, S.K. Use of additives to reduce browning, microbial load and quality loss of Kinnow Juice under ambient storage. *Indian J. Sci. Technol.* **2016**, *9*, 1–11. [CrossRef]
9. Malik, V.S.; Matthias, B.; Schulze, M.B.; Hu, F.B. Intake of sugar-sweetened beverages and weight gain: A systematic revie. *Am. J. Clin. Nutr.* **2006**, *84*, 274–288. [CrossRef]
10. Prakash, I.; Corliss, G.; Ponakala, R.; Ishikawa, G. Neotame: The next-generation sweetener. *Food Technol.* **2002**, *56*, 36–40.
11. Dutra, M.B.D.L.; Bolini, H.M.A. Sensory and physicochemical evaluation of acerola nectar sweetened with sucrose and different sweeteners. *J. Food Sci. Technol.* **2013**, *33*, 612–618. [CrossRef]
12. Boileau, A.; Fry, J.C.; Murray, R.A. New calorie-free sugar substitute from the leaf of the stevia plant arrives in the UK. *Nutr. Bull.* **2012**, *37*, 47–50. [CrossRef]
13. Majchrzak, D.; Ipsen, A.; Koenig, J. Sucrose-replacement by rebaudioside a in a model beverage. *J. Food Sci. Technol.* **2015**, *52*, 6031–6036. [CrossRef] [PubMed]
14. Goyal, S.K.; Samsher, R.K. Goyal, Stevia (*Stevia rebaudiana*) a bio-sweetener: A review. *Int. J. Food Sci.* **2010**, *61*, 1–10. [CrossRef] [PubMed]
15. Alizadeh, A.; Oskuyi, A.S.; Amjadi, S. The optimization of prebiotic sucrose-free mango nectar by response surface methodology: The effect of stevia and inulin on physicochemical and rheological properties. *Int. J. Food Sci. Technol.* **2019**, *25*, 243–251. [CrossRef]

16. Cadena, R.S.; Cruz, A.G.; Netto, R.R.; Castro, W.F.; Faria, J.D.A.F.; Bolini, H.M.A. Sensory profile and physicochemical characteristics of mango nectar sweetened with high intensity sweeteners throughout storage time. *Food Res. Int.* **2013**, *54*, 1670–1679. [CrossRef]
17. Miele, N.A.; Cabisidan, E.K.; Blaiotta, G.; Leone, S.; Masi, P.; Di Monaco, R.; Cavella, S. Rheological and sensory performance of a protein-based sweetener (MNEI), sucrose, and aspartame in yogurt. *Int. J. Dairy Sci.* **2017**, *100*, 9539–9550. [CrossRef]
18. Chattopadhyay, S.; Utpal, R.; Runu, C. Artificial sweeteners–a review. *J. Food Sci. Technol.* **2014**, *51*, 611–621. [CrossRef]
19. Abid, M.; Jabbar, S.; Wu, T.; Hashim, M.M.; Hu, B.; Lei, S.; Zhang, X.; Zeng, X. Effect of ultrasound on different quality parameters of apple juice. *Ultrason. Sonochem.* **2013**, *20*, 1182–1187. [CrossRef]
20. Wang, J.; Vanga, S.K.; Raghavan, V. High-intensity ultrasound processing of kiwifruit juice: Effects on the ascorbic acid, total phenolics, flavonoids and antioxidant capacity. *LWT* **2019**, *107*, 299–307. [CrossRef]
21. Kotíková, Z.; Lachman, J.; Hejtmánková, A.; Hejtmánková, K. Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. *LWT* **2011**, *44*, 1703–1710. [CrossRef]
22. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
23. Bhat, R.; Goh, K.M. Sonication treatment convalesce the overall quality of hand-pressed strawberry juice. *Food Chem.* **2017**, *215*, 470–476. [CrossRef] [PubMed]
24. Silveira, M.R.; Coutinho, N.M.; Rocha, R.S.; Moraes, J.; Esmerino, E.A.; Pimentel, T.C.; Freitas, M.Q.; Silva, M.C.; Raices, R.S.; Ranadheera, C.S.; et al. Guava flavored whey-beverage processed by cold plasma: Physical characteristics, thermal behavior and microstructure. *Food Res. Int.* **2019**, *119*, 564–570. [CrossRef] [PubMed]
25. Guo, H.; Lee, S.C.; Chan, L.Y.; Li, W.M. Risk assessment of exposure to volatile organic compounds in different indoor environments. *Environ. Res.* **2004**, *94*, 57–66. [CrossRef]
26. Pinelo, M.; Zeuner, B.; Meyer, A.S. Juice clarification by protease and pectinase treatments indicates new roles of pectin and protein in cherry juice turbidity. *Food Bioprod. Process.* **2010**, *88*, 259–265. [CrossRef]
27. Ertan, K.; Türkyılmaz, M.; Özkan, M. Effects of natural copigment sources in combination with sweeteners on the stability of anthocyanins in sour cherry nectars. *Food Chem.* **2019**, *294*, 423–432. [CrossRef]
28. Ma, Y.H.; Ma, F.W.; Wang, Y.H.; Zhang, J.K. The responses of the enzymes related with ascorbate–glutathione cycle during drought stress in apple leaves. *Acta Physiol. Plant.* **2011**, *33*, 173–180. [CrossRef]
29. Aadil, R.M.; Zeng, X.A.; Han, Z.; Sun, D.W. Effects of ultrasound treatments on quality of grapefruit juice. *Food Chem.* **2013**, *141*, 3201–3206. [CrossRef]
30. Carbonell-Capella, J.M.; Buniowska, M.; Cortes, C.; Zulueta, A.; Frigola, A.; Esteve, M.J. Influence of pulsed electric field processing on the quality of fruit juice beverages sweetened with Stevia rebaudiana. *Food Bioprod. Process.* **2017**, *101*, 214–222. [CrossRef]
31. Jabeen, F.; Wahab, S.; Hashmi, M.S.; Mehmood, Z.; Riaz, A.; Ayub, M.; Muneeb, M. Liquid stevia extract as a substitute of sucrose in the preparation of guava drink. *Fresenius Environ. Bull.* **2019**, *28*, 233–243.
32. Satyavathi, K.; Raju, P.B.; Bupesh, K.V.; Kiran, T.N.R. Neotame: High intensity low caloric sweetener. *Asian J. Chem.* **2010**, *22*, 5792–5796.
33. Phillips, K.M.; Council-Troche, M.R.; McGinty, C.; Rasor, A.S.; Tarrago-Trani, M.T. Stability of vitamin C in fruit and vegetable homogenates stored at different temperatures. *J. Food Compos. Anal.* **2016**, *45*, 147–162. [CrossRef]
34. Sethi, V.; Anand, J.C.; Saxena, S.K. Kinnow orange in juice and beverage making. *Indian J. Hortic.* **1980**, *25*, 13.
35. Pareek, S.; Paliwal, R.; Mukherjee, S. Effect of juice extraction methods, potassium meta-bi-sulphite concentration and storage temperature on the extent of degradation and reactivity of chemical constituents in mandarin (Citrus reticulata Blanco) juice. *J. Food Agric. Environ.* **2015**, *13*, 39–44.
36. Galani Yamdeu, J.H.; Gupta, P.H.; Patel, N.J.; Shah, A.K.; Talati, J.G. Effect of storage temperature on carbohydrate metabolism and development of cold-induced sweetening in indian potato (*Solanum tuberosum* L.) Varieties. *J. Food Biochem.* **2016**, *40*, 71–83. [CrossRef]
37. Lemus-Mondaca, R.; Vega-Gálvez, A.; Zura-Bravo, L.; Ah-Hen, K. Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. *Food Chem.* **2012**, *132*, 1121–1132. [CrossRef]
38. Ordóñez-Santos, L.E.; Vázquez-Riascos, A. Effect of processing and storage time on the vitamin C and lycopene contents of nectar of pink guava (*Psidium guajava* L.). *Arch. Latinoam. Nutr.* **2010**, *60*, 280.
39. Yen, G.C.; Lin, H.T.; Yang, P. Volatile constituents of guava fruits (*Psidium guajava* L.) and canned puree. *J. Food Sci.* **1992**, *57*, 679–681. [CrossRef]
40. Ryo, S.; Barringer, S.A. Effect of storage, oxygen and concentration on the levels of key volatiles in processed orange juice. *J. Food Process. Preserv.* **2015**, *39*, 495–507. [CrossRef]
41. Bodini, R.B.; Montini, E.M.; de Carvalho, C.C.; de Moraes, L.A.; Meirelles, A.J.D.A.; de Oliveira, A.L. Sensory and composition analyses of the aqueous phases from the concentration of guava (*Psidium guava* L.) and Mango (*Mangifera indica* L.) Juices and the process-induced losses of vitamin C. *Open Food Sci. J.* **2019**, *28*, 44–55. [CrossRef]

42. Helmig, D.; Ortega, J.; Duhl, T.; Tanner, D.; Guenther, A.; Harley, P.; Wiedinmyer, C.; Milford, J.; Sakulyanontvittaya, T. Sesquiterpene emissions from pine trees—Identifications, emission rates and flux estimates for the contiguous United States. *Environ. Sci. Technol.* **2007**, *41*, 1545–1553. [CrossRef] [PubMed]
43. Chan, W.K.; Tan, L.T.H.; Chan, K.G.; Lee, L.H.; Goh, B.H. Nerolidol: A sesquiterpene alcohol with multi-faceted pharmacological and biological activities. *Molecules* **2016**, *21*, 529. [CrossRef] [PubMed]
44. Huang, B.; Zhao, K.; Zhang, Z.; Liu, F.; Hu, H.; Pan, S. Changes on the rheological properties of pectin-enriched mango nectar by high intensity ultrasound. *LWT* **2016**, *91*, 414–422. [CrossRef]
45. Al-Dabbas, M.M.; Al-Qudsi, J.M. Effect of partial replacement of sucrose with the artificial sweetener sucralose on the physico-chemical, sensory, microbial characteristics, and final cost saving of orange nectar. *Int. Food Res. J.* **2012**, *19*, 1–6.
46. Rad, A.H.; Delshadian, Z.; Arefhosseini, S.R.; Alipour, B.; Jafarabadi, M.A. Effect of inulin and stevia on some physical properties of chocolate milk. *Health Promot. Perspect.* **2012**, *2*, 42–47.