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Research Article

Investigating the Optimal Treatment to Improve Cashew Apple Juice Quality and Shelf Life

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An investigation was carried out to extend the shelf life of cashew apple juice (CAJ) by up to 90 days from its natural shelf life. CAJ was obtained by pressing apples. Then, extracted juice was clarified, pasteurized, and added with preservatives, citric acid (0.01%), and sodium benzoate (0.01%). The juice was analyzed for physicochemical qualities, sugars, microbial (total bacteria, yeast, and mould) and sensory evaluation tests for appearance (yellow and brown color), aroma, and taste (astringent, bitter, and sweet). CAJ was stored at refrigeration (4°C) and ambient temperature (22.6-32.5°C) for 90 days. Sensory and shelf life analyses were conducted at 0, 15, 30, 45, 60, 75, and 90 days during storage. The results showed that cashew apple juice had strong vitamin C content (256.5 mg/100 mL). At ambient storage, there was high decrease of vitamin C (6.2-59.8%) and low decrease at refrigeration storage (1.6-10.5%). pH was found to decrease (4.4-3.15) and TSS (11-10.6°Bx), while titratable acidity (0.4-0.59%) increases with time at refrigerating storage. Also, at ambient storage, CAJ showed the similar trend, having decrease in pH (4.4-3.06) and TSS (11-10.3°Bx), while titratable acidity increased (0.4-0.61%). Moreover, sugar content for juice had minimum and maximum decrease at refrigeration and ambient temperatures, respectively. Storage at ambient temperature resulted in growth of microbes which was observed after 15 days for juices without preservatives and 75 days for juices with preservatives, with no E. coli growth. Juice on refrigeration had higher intensity of yellow color (7.50) and sweetness (5.58) while low intensity for astringency (1.58) (p < 0.05). Sensory evaluation of the beverage was found to be satisfactory. Thus, shelf life of cashew apple juice was extended to 90 days satisfactorily, ensuring consumption-safe parameters and satisfactory sensory qualities.

1. Introduction

In recent years, there has been a growing interest in using fruit by-products to create nutritious and value-added food items. One such by-product is the cashew apple, which is typically discarded after extracting the cashew nut [1].

The cashew apple, belonging to the family Anacardiaceae and genus Anacardium [2–4], is a significant tropical fruit native to the Cerrados of central Brazil [5, 6]. It holds great potential for commercial processing and can be transformed into various products like juice, wine, syrup, fruit bars, vinegar, and pickle [2, 7].

It is evident that CAJ is highly nutritious, with genuine medicinal value, and they hold great potential in the developing market of beverages to become a popular, highquality beverage of commercial interest in Tanzania. Cashew apple products such as juice reported to be rich in nutrients such as vitamin C, minerals, antioxidants, and bioactive compounds, making it a potential source for developing healthy beverages [8].

However, for cashew apple juice to be commercially viable and appealing to consumers, it is crucial to examine its shelf life and its impact on physicochemical and sensory attributes. Shelf life refers to the period during which a food product maintains its quality, safety, and sensory properties under specified storage conditions [9].

Physicochemical attributes include parameters like pH, titratable acidity, total soluble solids (TSS), and sugar content,

which significantly influence the overall quality and stability of the juice. Changes in these attributes during storage can affect the taste, appearance, and nutritional value of the product [10, 11].

Sensory attributes refer to the organoleptic properties perceived by consumers, including taste, aroma, and color [12]. Understanding how these attributes change throughout the shelf life of cashew apple juice is crucial for predicting consumer acceptability and marketability [12, 13].

Various factors can affect the shelf life of cashew apple juice, such as microbial activity, enzymatic reactions, oxidation, and physical changes [14]. Spoilage-causing microorganisms like bacteria and yeasts can alter the product's sensory and physicochemical properties [15]. Enzymatic reactions may lead to browning and degradation of bioactive compounds, resulting in color changes and loss of nutritional value [16].

To ensure the preservation of cashew apple juice quality, various preservation techniques can be employed, including pasteurization and addition of preservatives. However, the effectiveness of these preservation methods and their impact on the juice's physicochemical and sensory attributes during storage need to be systematically evaluated [17, 18].

Therefore, this study is aimed at investigating how shelf life affects the physicochemical and sensory attributes of clarified cashew apple juice. The research will analyze changes in pH, titratable acidity, TSS, sugar, and vitamin C content, as well as conduct sensory evaluation tests to assess appearance (yellow and brown color), aroma, and taste (astringent, bitter, and sweet). Various storage conditions and preservation techniques will be tested to understand their effects on the juice's quality over time.

The findings of this research will provide valuable insights into the shelf life of cashew apple juice and contribute to the development of effective preservation strategies for maintaining its quality and consumer acceptability. Furthermore, this study will support the utilization of cashew apple by-products, promoting sustainability and reducing waste in the food industry.

2. Material and Methods

2.1. Preparation of Cashew Apple Juice. Cashew apple juice (CAJ) was produced by extracting the juice from cashew apples using a manually operated pressing machine. The extracted juice was then filtered using muslin cloth to remove any solid particles. To clarify the juice, a gelatine solution with a concentration of 0.2 mg/L was added. The mixture was thoroughly stirred for a short duration and allowed to settle for 2 hours at room temperature.

Afterward, the clarified juice was carefully separated from the sediment and transferred to clean containers. The juice was divided into different portions, with one portion receiving the addition of preservatives, specifically sodium benzoate (0.01%) and citric acid (0.01%).

The second portion of the juice was not treated with preservative. Both portions of the juices were subjected to pasteurization at 75°C for 5 minutes [19]. The temperature of the juice during the pasteurization was monitored using a thermocouple. Following pasteurization, the juice was fast cooled in potable cold water and then packaged in 250 mL plastic bottles that were sterilized using 3% hydrogen peroxide for 30 min, rinsed well with sterile water, and dried [20]. During packaging, 28 bottles containing preservative and 28 bottles without preservatives were obtained, respectively, of which bottles of unclarified CAJ were included.

2.2. Determination of Shelf Life Stability of the Juice. Shelf life study of CAJ was studied at ambient and refrigerating temperatures. At each temperature, 4 bottles with and without preservative were stored. Therefore, a total of 8 bottles were collected for analysis of each interval. Juice quality from two storage temperatures was checked at different time points: 0, 15, 30, 45, 60, 75, and 90 days. The quality parameters of the juices monitored during the 90 days storage were microbial and physicochemical parameters, sugars, and sensory attributes.

2.3. Determination of Vitamin C. The determination of the vitamin C content was conducted following the procedures outlined by Desai and Desai [21] and Dimoso et al. [22]. In this method, 5 mL of the sample was mixed with 25 mL of a metaphosphoric acid-acetic acid solution (LOBA Chemie, India). The mixture was then subjected to centrifugation at 4000 rpm for 15 minutes using an Eppendorf (5810 centrifuge, Germany). After centrifugation, 4 mL of the resulting extract was combined with 0.23 mL of 3% bromine water (LOBA Chemie, India). Subsequently, 0.13 mL of a 10% thiourea solution (LOBA Chemie, India) was added, followed by the addition of 1 mL of a 2,4-dinitrophenylhy-drazine solution (LOBA Chemie, India).

The mixture obtained from the previous step was placed in a thermostatic water bath set at a temperature of 37°C for duration of three hours. Afterward, it was allowed to cool for 30 minutes. To this cooled mixture, 6 mL of chilled (4°C) 85% sulphuric acid (LOBA Chemie, India) was added. The resulting solution exhibited a red color, and its absorbance was measured at a wavelength of 521 nm using a UV/visible spectrophotometer (specifically, the UV-Vis multimode reader model SYNERGY/HTX, BioTek, USA). The estimation of the total ascorbic acid content was performed using a standard curve of ascorbic acid, which involved concentrations of 10, 20, 30, 40, and 50 mg/100 mL. The equation *y* = 0.0098*x* + 0.0857, with an R^2 value of 0.9872, was employed to determine the ascorbic acid content, which was expressed in mg/100 mL.

2.4. Sugar Analysis. To determine the total sugar content, a volume of 10 mL of cashew apple juice was subjected to centrifugation at a speed of 7,500 rpm for duration of five minutes. From the resulting mixture, 1 mL of the supernatant was diluted by a factor of 100 using distilled water. The determination of the total sugar content was carried out using the phenol sulphuric acid method [23]. In this method, 2 mL of the diluted sample or standard was pipetted into a 15 mL test tube. Subsequently, 50 μ L of an 80% w/v phenol solution (LOBA Chemie, India) was added to both the standards and the samples. After allowing 15 minutes

Attribute	Definition	Reference
Appearance		
Yellow	CAJ at ideal conditions of consumption (nonoxidized)	Strong: CAJ added by a 5% solution of citric acid followed by refrigerating Weak: CAJ stored at room temperature for 24 h and exposed to oxygen
Brown	CAJ stored at room temperature and exposed to the action of oxygen	Strong: CAJ stored at room temperature for 24 h and exposed to the action of oxygen Weak: CAJ added by a 5% solution of citric acid followed by refrigerating
Aroma		
	Like aroma of fresh cashew apple	Fresh cashew apple
Taste		
Astringent	CAJ with taste that induces the tongue's constriction	Strong: CAJ added by 0.5% tannin Weak: CAJ added by 50% of water
Bitter	CAJ with a taste that is sharp, acrid, and unpleasant	Strong: CAJ added by 0.1% of caffeine solution Weak: CAJ added by 50% of water
Sweet	CAJ with taste of honey or sugar	Strong: CAJ added by 20% sucrose Weak: CAJ pulp plus 50% water

TABLE 1: Definition of sensory attributes used in qualitative descriptive sensory analysis.

to elapse, 5 mL of concentrated H_2SO_4 (LOBA Chemie, India) was added to both the sample and the standard.

The mixtures of both the samples and the standards were gently shaken and allowed to stand at room temperature for duration of 10 minutes. Following this, both the samples and the standards were placed in a water bath set at a temperature of 27°C for 15 minutes. Subsequently, $200 \,\mu$ L of the samples and standards was transferred into wells of microplates. The absorbance of the mixture was measured at a wavelength of 485 nm using a multimode reader called SYN-ERGY/HTX (BioTek, USA). To estimate the concentration of the sample in mg/100 mL, a calibration curve of the standard glucose was employed. The calibration curve consisted of concentrations of 20, 40, 60, 80, and 100 mg/100 mL. The equation y = 0.0086x + 0.1164, with an R^2 value of 0.9892, was used to calculate the concentration of the sample based on the absorbance readings.

Sucrose, fructose, and glucose were determined using three standards and were prepared with concentration of 5 mg/mL, respectively, in distilled water and diluted to 1, 2, 3, and 4 mg/mL as standards. Starting with sucrose, 2 mL of each standard solution and samples was pipetted into different test tubes. Same amount of deionized (DI) water was used as blank.

Then, 2 mL of 6 M hydrochloric acid (HCl) solution was added to each test tube and placed in boiling water for 10 minutes. Then, 8 mL of 2.5 M sodium hydroxide (NaOH) solution and 2 mL of 3,5-dinitrosalicylic acid (DNSA) solution were introduced before the tubes were covered by parafilm and mixed well.

The mixtures were then placed in boiling water for another 5 minutes followed by 10 minutes in ice water. The absorbance of standards, blank, and samples was measured at 580 nm, and the concentrations were estimated from the standard calibration curve. For glucose and fructose, the addition of HCl solution and a stand time of 10 minutes in boiling water were skipped because the prior mixture can react readily with DNSA reagent (Perkin, 2015) without the presence of HCI. The absorbance of glucose and fructose was measured at 540 nm and 490 nm, respectively.

2.5. *Physicochemical Analysis.* The pH of CAJ was measured using a pH meter (Orion Star A214 from Indonesia). The pH meter was calibrated using three buffer solutions with pH values of 4, 7, and 10.

For determining the titratable acidity (TA) expressed as a percentage of citric acid, 6 mL of the cashew apple juice was placed in a beaker and then diluted with 50 mL of distilled water. The diluted solution was titrated against a 0.1 M sodium hydroxide solution (LOBA Chemie, India) until reaching the endpoint at pH 8.2. This method for determining titratable acidity follows the procedures described by [24, 25]. The titratable acidity was calculated using the appropriate formula:

$$\text{\%acid}\left(\frac{\text{wt}}{\text{wt}}\right) = \frac{N * V * \text{Eqwt}}{W * 1000} * 100, \tag{1}$$

where *N* represents the normality of the titrant, typically NaOH, expressed in milliequivalents per milliliter (mEq/mL). *V* denotes the volume of the titrant in milliliters (mL). Eq. wt. represents the equivalent weight of the predominant acid, expressed in milligrams per milliequivalent (mg/mEq). *W* indicates the mass of the sample in grams (g). The factor 1000 is used to convert milligrams (mg) to grams (g) (i.e., 1/10 = 100/1000).

The determination of total soluble solids (TSS) was carried out using a portable handheld refractometer (Griff-Chem model). The refractometer was equipped with a digital



FIGURE 1: Vitamin C content of different juice samples under ambient and refrigeration storage at different storage times (0-90 days). Mean value (n = 3) ± SD wet basis. UPJP: unclarified and pasteurized juice with preservatives; UPJwP: unclarified and pasteurized juice without preservatives; CPJP: clarified and pasteurized juice with preservatives; CPwP: clarified and pasteurized.

display, allowing for easy and accurate readings. The TSS values were expressed in degrees Brix (°Bx) [25].

2.6. Microbial Analysis. Total bacteria, moulds and coliform, were analyzed separately by spreading technique. 1 mL of homogenized cashew apple juice was mixed with 9 mL of sterile peptone salt (LOBA Chemie, India) and was shaken uniformly by rotation and tilting the tube. Then, 1 mL of the suspension was transferred into the peptone salt diluents (9 mL) up to 10^{-10} dilution series. From each dilution, 1 mL of diluted sample was inoculated into the respective growth medium: total bacteria (plate count agar), yeast and mould (potato dextrose agar), and coliform (violet red bile agar) (HiMedia Laboratories, Mumbai, India). The incubation conditions were bacteria (30° C, 24h), mould and yeast (37° C, 72h), and coliform (30° C, 48h).

2.7. Sensory Evaluation

2.7.1. Panelist Selection and Training. A quantitative descriptive analysis was used to carry out the sensory evaluation. Twelve panelists were recruited based on interest, availability, health, and ability to discriminate appearance (yellow and brown color), aroma, and taste (astringent, bitter, and sweet). Panelists were trained before the testing session in order to develop a common understanding of terminologies and procedures during sensory evaluation.

During the training phase, panelists were given samples of fresh CAJ as well as juice that had been stored for different periods of time. Their task was to identify and describe the sensory changes observed in the juices. Through consensus among the panelists, a set of sensory attributes, as shown in Table 1, was selected to evaluate these changes. To establish consensus, a comparison was made between freshly made juice and stored samples. A 9-point hedonic scale was utilized to rate the intensity of the sensory attribute changes observed in the samples.

2.7.2. Sensory Test. The sensory evaluation took place in a well-ventilated room, ensuring a suitable environment for accurate assessments. Panelists were instructed to refrain from smoking, snacking, or chewing gum for at least 20 minutes prior to the evaluation session. The samples were served at ambient temperature and presented in transparent cups. Each sample was assigned a three-digit random code to prevent any bias in evaluation. To avoid order effects, the samples were presented to the panelists in a randomized sequence.

Panelists were provided with portable water to rinse their mouths before and between testing different samples. A designated sheet was given to the panelists to record their evaluation results. The sensory attributes were evaluated at 15-day intervals for each storage time point up to 90 days.

2.8. Statistical Analysis. Analysis of variance (ANOVA) was applied using a factorial design with two factors including ambient and refrigerating storage temperatures and storage time (0, 15, 30, 45, 60, 7 5, and 90 days). The effect of each factor on the response variable (total soluble solids, pH, titratable acidity, vitamin C, sugars, and microbial and sensory quality) and values were reported as the mean values along with their corresponding standard deviations. Statistical analysis was conducted using version 21 of the SPSS statistical software. A one-way analysis of variance (ANOVA) was employed to perform the statistical comparisons, with *p* values < 0.05 considered significant. To further analyze the mean differences, the least significance difference (LSD) test was utilized.

TABLE 2

Days of storage at refrigerating temperature pН 0 15 30 45 60 75 90 UPIP 3.7 ± 0.0^{a} 3.50 ± 0.0^{a} 3.35 ± 0.0^{a} 3.29 ± 0.0^{a} 3.47 ± 0.0^{a} 3.44 ± 0.0^{a} 3.38 ± 0.0^{a} UPJwP 4.4 ± 0.0^{b} 4.19 ± 0.0^{b} 4.05 ± 0.0^{b} 3.95 ± 0.0^{a} 3.88 ± 0.0^{a} х х CPJP 3.8 ± 0.0^{a} 3.65 ± 0.0^{a} 3.53 ± 0.0^{a} 3.47 ± 0.0^{a} 3.34 ± 0.0^{a} 3.26 ± 0.0^{a} 3.15 ± 0.1^{a} 4.3 ± 0.0^{b} 4.22 ± 0.0^{b} 4.13 ± 0.0^{b} 4.01 ± 0.0^{b} CPwP 3.97 ± 0.0^{a} х х ΤA UPJP 0.4 ± 0.0^{a} 0.4 ± 0.0^a 0.4 ± 0.0^{a} 0.5 ± 0.0^a 0.51 ± 0.0^{a} 0.56 ± 0.0^{a} 0.59 ± 0.0^{a} 0.3 ± 0.0^{b} 0.3 ± 0.0^{b} 0.3 ± 0.0^{b} 0.42 ± 0.0^{b} 0.44 ± 0.0^{b} UPJwP x х CPJP 0.4 ± 0.0^{a} 0.4 ± 0.0^{a} 0.4 ± 0.0^{a} 0.4 ± 0.0^{b} 0.42 ± 0.0^{b} 0.43 ± 0.1^{b} 0.43 ± 0.0^{b} 0.3 ± 0.0^{b} 0.3 ± 0.0^{b} 0.3 ± 0.0^{b} $0.35 \pm 0.0^{\circ}$ CPwP 0.33 ± 0.0^{c} х х TSS 11.7 ± 0.0^{b} 11.7 ± 0.0^{b} UPJP 12 ± 0.0^{b} 12 ± 0.0^{b} 12 ± 0.0^{b} 11.8 ± 0.0^{b} $12 \pm 0.0^{\circ}$ 12 ± 0.0^{b} 12 ± 0.0^{b} 12 ± 0.0^{c} 11.8 ± 0.0^{b} UPJwP 12 ± 0.0^{b} х х CPJP 12 ± 0.0^{b} 12 ± 0.0^{b} 12 ± 0.0^{b} 11.8 ± 0.0^{b} 11.7 ± 0.0^{b} 11.7 ± 0.0^{b} 11.7 ± 0.0^{b} 12 ± 0.0^{b} 12 ± 0.0^{b} 12 ± 0.0^{b} 11.9 ± 0.0^{b} 11.8 ± 0.0^{b} CPwP х x

(a) Physicochemical of cashew apple juice stored 0-90 days at refrigerating conditions

x: the product spoiled on that time. Mean value (n = 3) ± SD. UPJP: unclarified and pasteurized juice with preservatives; UPJwP: unclarified and pasteurized juice without preservatives; CPJP: clarified and pasteurized juice with preservatives; CPwP: clarified and pasteurized juice without preservatives. The same superscript letter within the column has no significant difference (p > 0.05).

(b) Physicochemical of cashew apple juice stored 0-90 days at ambient temperature condition

	Days of storage at ambient temperature												
рп	0	15	30	45	60	75	90						
UPJP	3.7 ± 0.0^a	3.48 ± 0.1^a	3.43 ± 0.1^a	3.31 ± 0.0^a	3.28 ± 0.0^a	3.22 ± 0.0^{a}	3.19 ± 0.0^{a}						
UPJwP	4.4 ± 0.0^{b}	x	x	x	x	x	x						
СРЈР	$3.8\pm0.0^{\rm b}$	3.52 ± 0.0^a	3.48 ± 0.0^{a}	3.32 ± 0.0^a	3.27 ± 0.0^{a}	3.20 ± 0.0^{a}	3.06 ± 0.0^a						
CPwP	4.3 ± 0.0^{b}	x	x	x	x	x	x						
ТА													
UPJP	0.4 ± 0.0^{a}	0.4 ± 0.0^a	0.51 ± 0.0^a	0.54 ± 0.0^a	0.57 ± 0.0^a	0.58 ± 0.0^a	0.61 ± 0.0^{a}						
UPJwP	$0.3\pm0.0^{\rm b}$	x	x	x	x	x	x						
СРЈР	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	$0.43\pm0.0^{\rm b}$	$0.43\pm0.0^{\rm b}$	0.44 ± 0.0^{b}	$0.46\pm0.0^{\rm b}$	$0.48\pm0.0^{\rm b}$						
CPwP	0.3 ± 0.0^{b}	x	x	x	x	x	x						
TSS													
UPJP	$12\pm0.0^{\rm b}$	11 ± 0.0^{a}	10.8 ± 0.0^{a}	10.6 ± 0.0^{a}	10.6 ± 0.0^a	10.5 ± 0.0^{a}	10.3 ± 0.0^{a}						
UPJwP	$12\pm0.0^{\mathrm{b}}$	x	x	x	x	x	x						
СРЈР	12 ± 0.0^{b}	11 ± 0.0^{a}	10.9 ± 0.0^a	$10.7\pm0.0^{\rm a}$	10.6 ± 0.0^a	10.5 ± 0.0^{a}	$10.5\pm0.0^{\rm a}$						
CPwP	12 ± 0.0^{b}	x	x	x	x	x	x						

x: the product spoiled on that time. Mean value (n = 3) ± SD. UPJP: unclarified and pasteurized juice with preservatives; UPJwP: unclarified and pasteurized juice without preservatives; CPJP: clarified and pasteurized juice with preservatives; CPwP: clarified and pasteurized juice without preservatives. The same superscript letter within the column has no significant difference (p > 0).

3. Results and Discussion

3.1. Effect of Time and Temperature of Storage on Vitamin C Content. The study's findings demonstrated that the vitamin C content in cashew apple juice was significantly influenced by both storage time and temperature. As the storage time increased, all storage temperatures experienced a gradual reduction in vitamin C concentration. Furthermore, higher storage temperatures led to a faster degradation of vitamin C in comparison to lower temperatures.

Initially, the vitamin C levels in various juice types were as follows: unclarified and pasteurized juice with preservatives (UPJP) contained 256.5 mg/100 mL, unclarified and pasteurized juice without preservatives (UPJwP) contained







(d) Sucrose content at refrigerating temperature





FIGURE 2: Continued.



FIGURE 2: Sugar contents of different juice samples under ambient and refrigeration storage at different storage times (0-90 days). Mean value (n = 3) ± SD wet basis. UPJP: unclarified and pasteurized juice with preservatives; UPJwP: unclarified and pasteurized juice without preservatives; CPJP: clarified and pasteurized juice with preservatives; CPwP: clarified and pasteurized.

255.3 mg/100 mL, clarified and pasteurized juice with preservatives (CPJP) contained 244.7 mg/100 mL, and clarified and pasteurized juice without preservatives (CPwP) contained 244.6 mg/100 mL. Over the course of 90 days, the vitamin C content decreased to 103.1 mg/100 mL and 100.7 mg/100 mL when stored at ambient temperature for UPJP and UPJwP, respectively. For CPJP and CPwP, the vitamin C content decreased to 230.6 mg/100 mL and 215.1 mg/100 mL, respectively, when refrigerated (Figure 1).

However, the decrease in vitamin C content of samples appears to be greater in samples stored at ambient temperature than in samples at refrigerating temperature. The statistical analysis showed that the vitamin C content of refrigeration and ambient storage temperature-exposed juices decreases significantly (p < 0.05) throughout the storage time (Figure 1). The degradation of vitamin C was also observed by Marc et al. [26] and Emelike and Ebere [27] who did on cashew apple juice and also in other fruits juices by other studies [19, 28, 29]. Moreover, samples with added preservative had a lower decrease compared to the ones without preservative. In addition, storage time also has an impact on vitamin C content, as samples stored for 90 days show the largest decreases. Juice exposed at refrigerating storage temperature retained higher vitamin C content than ambient temperature-exposed juice throughout the storage period.

The decline in vitamin C content during storage is primarily caused by exposure to atmospheric oxygen [30, 31]. Moreover, oxidative processes triggered by factors like light, heat (during pasteurization), and enzymes such as ascorbate oxidase and peroxide also contribute to vitamin C loss [30]. These degradation factors affect the vitamin C content regardless of the presence of preservatives [31]. Throughout the storage period, all samples of cashew apple juice exhibited a statistically significant decrease (p < 0.05) in vitamin C content.

These findings indicate that storing the juice at ambient temperatures negatively impacts its vitamin C concentration, leading to potential nutritional losses. To maintain the nutritional quality of cashew apple juice, it is advisable to store it at lower temperatures, such as refrigeration temperature, and consume it within a reasonable time frame.

3.2. Effect of Storage Conditions on *Physicochemical Parameters*

3.2.1. *pH*. The pH of cashew apple juice, both at refrigerating and ambient temperatures, exhibited a decrease over time (p < 0.05). For instance, in juice with added preservatives, the pH decreased from 3.8 to 3.15 during refrigeration and from 3.8 to 3.06 during ambient temperature storage (Tables 2(a) and 2(b)). In the case of juices without preservatives, the pH ranged from 4.4 to 3.8 during refrigeration, while at ambient temperature, the unclarified juice had a value of 4.4 and the clarified juice had a value of 4.3 (Tables 2(a) and 2(b)). There were no significant differences observed in the pH changes at different storage time points between the two storage temperatures (Tables 2(a) and 2(b)).

Significant reductions in pH were noted during storage at both ambient and refrigerating temperatures, specifically between day 0 and day 15. The decrease in pH during storage might be attributed to an increase in titratable acidity, as acidity and pH have an inverse relationship. Several factors can influence the rate of acidification and pH decline in the juice during storage, including temperature, oxygen availability, sugar content, and the presence of preservatives. Higher temperatures generally promote microbial growth and metabolic activity, leading to a faster acidification process. Oxygen exposure can also accelerate the growth of certain spoilage microorganisms, further contributing to the acidification. This observation is consistent with the findings of Touati et al. [32] in thermally treated grape, orange, and pear nectars, as well as in soursop juice observed by Abbo et al. [33].

Juice formulation							Days o	f storage						
Total hastoria		0	1	5	3	30	. 4	45	6	50	7	5	9	00
Total Dacteria	RT	AT	RT	AT	RT	AT	RT	AT	RT	AT	RT	AT	RT	AT
UPJP	_	_		_	_	_	_			_	_		_	_
UPJwP	_	_	_	10^{6}	_	10^{6}	_	10^{6}	_	10^{7}	10^{7}	10^{7}	10^{6}	10^{8}
СРЈР	_	_	_	—	_	—	_	_	_	_	—	_	—	_
CPwP	_	_	_	10^{5}	_	10^{6}	_	10^{8}	_	10^{7}	10^{8}	10^{8}	10^{8}	10^{8}
Moulds														
UPJP	_	_	_	_	_	_	_	_	_	_	_	_	_	_
UPJwP	_	_	_	10^{4}	_	10^{6}	_	10^{5}	_	10^{5}	10^{5}	10^{6}	10 ⁶	10 ⁶
СРЈР	_	_	_	—	_	—	_	_	_	_	—	_	—	_
CPwP	_	_	_	10^{5}	_	10^{6}		10^{6}	_	10^{6}	10^{6}	10^{6}	10^{7}	10^{7}
E. coli														
UPJP	_	_	_	_	_	_	_	_	_	_	_	_	_	_
UPJwP	_	_	_	_	_	_	_	_	_	_	_	_	_	_
СРЈР	_	_	_	_	_	—	_	_	_	_	_	_	_	_
CPwP	_	_	_	_	_	_	_	_	_	_	_	_	_	_

TABLE 3: Effect of ambient and refrigeration storage on total bacteria count, moulds, and *E. coli* (colony forming unit/mL) in cashew apple juice.

UPJP: unclarified and pasteurized juice with preservatives; UPJwP: unclarified and pasteurized juice without preservatives; CPJP: clarified and pasteurized juice with preservatives; CPwP: clarified and pasteurized juice without preservatives; RT: refrigerating storage; AT: ambient storage. The same superscript letter within the column has no significant difference (p > 0.05).

3.2.2. Titratable Acidity. Before storage, the titratable acidity (TA) value ranged from 0.3 to 0.4. Over the course of 90 days of storage, there was a significant gradual increase (p < 0.05) in TA at ambient temperature from 0.4 to 0.59, while at ambient temperature, it increased from 0.4 to 0.61 (Tables 2(a) and 2(b)).

Cashew apple juice (CAJ) with added preservatives showed a continuous increase in TA up to day 45 at refrigerating temperature, and then, it started increasing from day 60, while at ambient temperature, the increase began from day 45. CAJ without preservatives deteriorated visibly and reached a TA of 0.60 after 60 days at refrigerating temperature and 0.61 after 15 days at ambient temperature (Table 2) due to the growth of mould on the juice serving as an indicator of deterioration.

These results suggest that higher temperatures (ambient temperature) lead to an increased production of acids compared to lower temperatures (refrigerating temperature) during storage. The elevated temperatures at ambient temperature facilitate higher metabolic activity, resulting in more acid production. Similar increases in titratable acidity have been observed in other fruit juices during storage [30, 34, 35].

3.2.3. Total Soluble Solids. The changes in total soluble solids (TSS) of cashew apple juice during the storage period are presented in Tables 2(a) and 2(b). Initially, the TSS for cashew apple juice ranged from 10.6 to 11.0 Bx at refrigerating temperature and from 10.3 to 11.0 Bx at ambient temperature over the 90-day storage period. Significant decreases in TSS were observed after 15 days of storage at ambient temperature compared to the control.

However, at refrigerating temperature, there was no significant effect on the TSS of the juice, and a similar trend was observed by Anaya-Esparza et al. [34]. The results indicated that the TSS content remained fairly stable and retained at refrigerating temperature storage, which can be attributed to the combined effect of low temperature during storage and the heat treatment given prior to storage. Consequently, storing the juice at a low temperature can help to retain the total soluble solid content of cashew apple juice.

According to Bhardwaj and Pandey [36], retaining TSS in fruit juices during storage is desirable for quality preservation, as it extends the product's shelf life and maintains its overall quality over time. Therefore, storing cashew apple juice at a low temperature can be beneficial in retaining its TSS content and ensuring better product quality.

3.2.4. Sugars. The total sugars, fructose, glucose, and sucrose contents of CAJ exhibited significant differences (p < 0.05) across various treatments during the entire storage period. There was a significant decrease from day 0 to day 90 of storage, regardless of the treatments and storage conditions (Figure 2). Initially, at day 0, the storage conditions did not have a significant effect, but significant effects were observed at 15, 30, 45, 60, 75, and 90 days of the storage period.

Juice stored at refrigerating temperature showed the least decrease in fructose, glucose, and sucrose, in comparison to juice stored at ambient temperature, which experienced the most significant decrease. Similar findings were reported by Ma et al. [37], and it was revealed that the decrease might be attributed to the hydrolysis of nonreducing sugars, leading to the production of sugars like fructose and glucose. An increase in storage temperature accelerates the hydrolysis reaction, contributing to continuous nonenzymatic browning. When

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	Sensory attributes/juice Periods (days)										
Storage condition	samples	0	15	30	45	60	75	90			
				Yellow colo	or						
	UPJP	6.50 ± 1.38^a	$7.50\pm1.00^{\rm a}$	6.50 ± 0.79^a	6.50 ± 1.24^a	7.00 ± 0.85^a	$7.08\pm0.90^{\rm a}$	6.75 ± 0.97^a			
	UPJwP	5.42 ± 2.39^b	7.42 ± 0.90^{a}	$7.58\pm0.99^{\rm b}$	6.92 ± 0.90^a	6.75 ± 0.96^b	_	_			
	СРЈР	$7.00\pm1.48^{\rm c}$	$6.83\pm1.12^{\rm b}$	6.75 ± 1.29^a	6.58 ± 0.99^a	6.92 ± 0.99^b	6.67 ± 0.78^{b}	$7.08\pm0.99^{\rm b}$			
	CPwP	$7.33 \pm 1.07^{\rm c}$	$7.08\pm1.08^{\rm a}$	7.08 ± 0.90^a	6.75 ± 0.75^a	6.58 ± 0.99^b	_	_			
				Brown colo	r						
	UPJP	1.00 ± 0.00^a	1.17 ± 0.39^a	1.25 ± 0.45^a	1.50 ± 0.52^a	1.50 ± 0.52^a	1.75 ± 0.45^a	1.50 ± 0.52^a			
	UPJwP	1.00 ± 0.00^a	1.33 ± 0.49^a	1.33 ± 0.49^a	1.25 ± 0.45^a	1.50 ± 0.52^a	_	_			
	СРЈР	1.00 ± 0.00^a	1.42 ± 0.52^a	1.17 ± 0.39^{a}	1.67 ± 0.49^a	1.75 ± 0.45^a	1.50 ± 0.52^a	1.83 ± 0.39^a			
	CPwP	1.00 ± 0.00^a	1.33 ± 0.49^a	1.50 ± 0.52^a	1.50 ± 0.52^a	1.42 ± 0.51^a	_	_			
				Aroma							
	UPJP	4.58 ± 1.73^a	$4.00\pm1.47^{\rm a}$	4.00 ± 2.33^a	4.58 ± 2.35^a	4.25 ± 1.91^a	3.83 ± 1.34^a	4.92 ± 2.07^a			
	UPJwP	4.75 ± 2.09^a	4.08 ± 2.31^a	$2.92\pm1.97^{\rm b}$	$3.33\pm2.15^{\rm b}$	$2.00\pm1.13^{\rm b}$	—	_			
	СРЈР	4.08 ± 2.28^a	4.42 ± 1.93^a	4.58 ± 1.83^a	$3.92 \pm 1.88^{\rm b}$	$3.17\pm2.08^{\rm c}$	$4.92\pm2.28^{\rm b}$	4.67 ± 1.83^a			
Defrigoration tomporature	CPwP	4.00 ± 1.65^a	3.08 ± 1.78^{b}	$3.67\pm2.27^{\rm c}$	4.17 ± 2.04^a	4.50 ± 2.19^a	—	—			
Reingeration temperature		Astringency									
	UPJP	4.42 ± 1.78^a	3.67 ± 2.39^a	2.42 ± 1.98^a	2.17 ± 2.04^a	4.67 ± 2.10^a	2.50 ± 1.57^a	3.67 ± 2.31^a			
	UPJwP	4.33 ± 2.46^a	3.75 ± 2.45^a	$3.33\pm2.10^{\rm b}$	2.75 ± 1.77^a	2.33 ± 1.49^{b}	—	—			
	СРЈР	$2.25\pm1.96^{\rm b}$	$2.42\pm1.62^{\rm b}$	$3.83\pm2.48^{\rm b}$	$3.08\pm2.02^{\rm b}$	$2.25\pm1.36^{\rm b}$	4.25 ± 2.38^b	$5.58\pm2.58^{\rm b}$			
	CPwP	$1.58\pm0.96^{\rm c}$	$2.17\pm1.85^{\rm b}$	2.25 ± 2.01^a	$2.17 \pm 1.53^{\rm b}$	$2.00\pm1.48^{\rm b}$	—	—			
				Bitterness							
	UPJP	3.58 ± 1.88^a	3.67 ± 2.15^a	3.25 ± 2.67^a	2.58 ± 1.73^a	3.75 ± 2.49^a	2.17 ± 1.59^a	3.08 ± 2.61^a			
	UPJwP	3.92 ± 2.07^a	3.50 ± 2.11^a	$2.08 \pm 1.68^{\rm b}$	2.42 ± 2.07^a	3.42 ± 2.50^a	—	—			
	СРЈР	$1.58\pm0.90^{\rm b}$	$2.25\pm1.60^{\rm b}$	$2.92\pm2.02^{\rm b}$	2.75 ± 1.66^a	$2.83 \pm 1.99^{\rm b}$	$4.17\pm2.33^{\rm b}$	$6.58\pm2.35^{\rm b}$			
	CPwP	2.00 ± 1.04^{c}	$2.08\pm1.31^{\rm b}$	$2.00\pm1.28^{\rm b}$	2.33 ± 1.49^a	2.58 ± 2.19^{b}	_	_			
		Sweetness									
	UPJP	4.17 ± 2.21^a	4.92 ± 1.62^a	3.42 ± 1.44^a	4.50 ± 1.83^a	5.08 ± 1.78^a	3.25 ± 1.96^a	4.08 ± 2.23^a			
	UPJwP	4.83 ± 2.21^a	4.42 ± 2.11^a	4.58 ± 1.98^{b}	$5.08 \pm 1.56^{\rm b}$	3.92 ± 0.99^{b}	_	_			
	СРЈР	5.17 ± 1.99^{b}	4.50 ± 2.07^a	$5.00\pm2.37^{\rm c}$	4.25 ± 1.77^{b}	5.33 ± 1.83^a	$5.00 \pm 2.17^{\rm b}$	$5.33\pm2.67^{\rm b}$			
	CPwP	$5.58 \pm 1.88^{\rm b}$	$3.83\pm2.04^{\rm b}$	4.92 ± 2.43^{b}	5.58 ± 2.71^{b}	5.08 ± 1.98^{a}	—	—			
				Yellow colo	r						
	UPJP	6.50 ± 1.38^a	$1.17\pm0.39^{\rm a}$	1.25 ± 0.45^a	$1.50\pm0.52^{\rm a}$	$1.50\pm0.52^{\rm a}$	1.75 ± 0.45^{a}	$1.50\pm0.52^{\rm a}$			
	UPJwP	$5.42\pm0.69^{\rm b}$	_	_	_	_	_	_			
	СРЈР	6.83 ± 1.34^a	$1.42\pm0.52^{\rm a}$	1.25 ± 0.45^a	$1.67\pm0.49^{\rm a}$	$1.75\pm0.45^{\rm a}$	$1.58\pm0.52^{\rm a}$	1.83 ± 0.39^{a}			
	CPwP	$7.33 \pm 1.07^{\rm c}$	_	_	_	_	_	_			
				Brown colo	r						
	UPJP	$1.00\pm0.00^{\rm a}$	$7.50\pm1.00^{\rm a}$	6.50 ± 0.79^{a}	6.50 ± 1.24^a	$7.00\pm0.85^{\rm a}$	$7.08\pm0.90^{\rm a}$	6.75 ± 0.97^a			
	UPJwP	$1.00\pm0.00^{\rm a}$	_	_	_	_	_	_			
	СРЈР	$1.00\pm0.00^{\rm a}$	6.83 ± 1.12^{b}	6.75 ± 1.29^a	$6.58\pm0.99^{\rm a}$	6.92 ± 0.99^{b}	$6.67\pm0.78^{\rm b}$	$7.00\pm1.04^{\rm b}$			
A	CPwP	1.00 ± 0.00^a	_	_	_	_	_	_			
Ambient temperature				Aroma							
	UPJP	4.58 ± 1.73^a	$4.00\pm1.48^{\rm a}$	4.00 ± 2.34^a	4.58 ± 2.35^a	4.25 ± 1.91^a	3.83 ± 1.34^a	4.92 ± 2.07^a			
	UPJwP	4.75 ± 0.61^a	_	_	_	_	_	_			
	СРЈР	3.83 ± 1.80^{b}	4.42 ± 1.93^a	4.58 ± 1.83^a	3.92 ± 1.88^{b}	3.17 ± 2.08^b	$4.92\pm2.28^{\rm b}$	4.58 ± 1.83^a			
	CPwP	4.00 ± 1.65^a	_	_	_	_	_	_			
				Astringenc	у						
	UPJP	4.42 ± 1.78^a	3.67 ± 2.39^a	2.42 ± 1.98^a	2.17 ± 2.04^a	$4.67\pm2.10^{\rm a}$	2.50 ± 1.57^a	3.67 ± 2.31^a			
	UPJwP	4.33 ± 0.71^a	_	_	_	_	_	_			
	СРЈР	$1.67\pm0.78^{\rm b}$	$2.42\pm1.62^{\rm b}$	3.83 ± 2.48^{b}	3.08 ± 2.02^{b}	2.25 ± 1.36^b	4.25 ± 2.38^b	$5.17\pm2.89^{\rm b}$			
	CPwP	1.58 ± 0.99^{b}	—	—	—	—	—	—			

Stores on dition	Sensory attributes/juice	Periods (days)							
storage condition	samples	0	15	30	45	60	75	90	
			Bit	terness					
	UPJP	3.58 ± 1.89^a	3.67 ± 2.15^a	3.25 ± 2.67^a	2.58 ± 1.73^a	3.75 ± 2.49^a	2.17 ± 1.59^a	3.08 ± 2.61^a	
	UPJwP	3.92 ± 0.59^a	_	_	_	_	_	_	
	СРЈР	$1.58\pm0.90^{\rm b}$	$2.25\pm1.60^{\rm b}$	$2.92\pm2.02^{\rm b}$	2.75 ± 1.66^a	$2.83 \pm 1.99^{\rm b}$	$4.50\pm2.11^{\rm b}$	$6.58\pm2.35^{\rm b}$	
	CPwP	$2.00\pm1.04^{\rm c}$	_	_	_	_	_	_	
				Sweetness					
	UPJP	4.17 ± 2.21^a	4.92 ± 1.62^a	3.42 ± 1.44^a	4.50 ± 1.83^a	5.08 ± 1.78^a	3.25 ± 1.96^a	4.08 ± 2.23^a	
	UPJwP	4.83 ± 0.64^a	_	_	_	_	_	_	
	CPJP	$5.17 \pm 1.99^{\rm b}$	4.50 ± 2.07^a	5.00 ± 2.37^{b}	4.25 ± 1.77^a	5.33 ± 1.83^a	5.25 ± 2.09^{b}	$5.00\pm2.95^{\rm b}$	
	CPwP	$5.58 \pm 1.88^{\rm b}$	_	_	_	_	_	_	

TABLE 4: Continued.

Mean value $(n = 12) \pm$ SD. UPJP: unclarified and pasteurized juice with preservatives; UPJwP: unclarified and pasteurized juice without preservatives; CPJP: clarified and pasteurized juice without preservatives. The same superscript letter within the column has no significant difference (p > 0.05). 0, 15, 30, 45, 60, 75, and 90 are days of storage. The same superscript letter within the column has no significant difference (p > 0.05).

the hydrolysis rate surpasses the consumption rate of reducing sugar in the browning reaction, the reducing sugar content in the juice increases. However, with the occurrence of the nonenzymatic browning reaction, the content of reducing sugar stabilizes or even decreases, as noted by Ma et al. [37].

3.3. Microbial Results of CAJ. Table 3 results show the effect of storage temperature on the growth of total bacteria, yeast/ moulds, and *E. coli*, respectively. At refrigerating temperature, there was no growth of microorganism, as juice was subjected to pasteurization and refrigeration temperature hinders growth of microorganisms hence making the juice quality to be stable.

Storage at ambient temperature resulted in growth of microbes (total bacteria, yeast/mould), which was observed after 15 days for juices without preservatives and 75 days for juices with preservatives, but there was no growth for *E. coli*.

The inhibition of microbial growth at refrigerating temperature means that the storage of cashew apple juice can be extended beyond 90 days. This was also observed in pasteurized pineapple juice; the microbial population remained unchanged during refrigerated temperature storage for 12 weeks [30].

These findings indicate that storage conditions can impact the growth and proliferation of microorganisms in cashew apple juice, potentially leading to spoilage and compromising the quality and safety of the juice.

3.4. Sensory Evaluation. The sensory evaluation scores for cashew apple juice are presented in Table 4. The panel of 12 people evaluated five attributes, which included appearance (yellow and brown color), aroma (astringent, bitter, and sweet), and taste.

At refrigerating temperature in terms of appearance attribute, the scores obtained ranged from 5.42 to 7.50 for yellow color and from 1.00 to 1.75 for brown color. The aroma scores varied from 2.00 to 4.92. Regarding taste, the scores ranged from 1.58 to 5.58 for astringency, 1.58 to 6.58 for bitterness, and 3.25 to 5.58 for sweetness. At ambient temperature, for the appearance attribute, the scores obtained varied from 1.17 to 7.33 for yellow color and from 1.00 to 7.50 for brown color. The aroma scores ranged from 3.17 to 4.92. As for taste, the scores varied from 1.58 to 5.17 for astringency, 1.58 to 6.58 for bitterness, and 3.25 to 5.58 for sweetness.

Samples stored under ambient temperature, both with and without preservatives, initially had higher intensity scores for yellow color on day zero (p > 0.05) as shown in Table 4. However, as storage time prolonged, these scores decreased, while the intensity score for brown color increased over time (p > 0.05) (Table 4). This observation aligns with the findings of Parfait et al. [38], who reported that one of the main issues with cashew apple juice is its tendency to undergo a color change from yellow to brown during storage, which is attributed to the formation of brown pigments resulting from enzymatic and/or nonenzymatic reactions.

The sensory evaluation scores showed statistically significant differences (p < 0.05) between samples at different storage times and between the stored samples and the control samples. The heat treatment and varying storage times had notable effects on the attributes of the juice samples, as indicated by the scores obtained. According to the presented scale, the cashew apple juice in this study received a moderate level of appreciation.

The results highlight that storage significantly influenced the sensory attributes of the juice. Notably, changes in appearance, particularly the color, were observed during storage, with the juice gradually transitioning from a vibrant yellow to a darker brown. The aroma intensity was also affected, and in terms of taste, alterations were noted in the perceived astringency, bitterness, and sweetness of the juice.

4. Conclusions

The storage temperature variation significantly influenced the nutritional content of cashew apple juice. Vitamin C, a key nutrient in the juice, exhibited a gradual decrease over the storage period, emphasizing the importance of proper temperature control to maintain its nutritional value.

The physicochemical parameters of cashew apple juice, including pH, titratable acidity, total soluble solids (TSS), and sugar content, were influenced by storage temperature variation. Moreover, pasteurization ($75^{\circ}C$ for 5 min) and treatment with citric acid (0.01%) and sodium benzoate (0.01%) were found to be more efficient in retaining the physicochemical properties, inhibiting microbial growth, and sensory attributes of cashew apple juice hence effectively extend the shelf life of cashew apple juice by up to 90 days. Understanding these changes can aid in determining the optimal storage temperature (4°C) to maintain the desired physicochemical characteristics of cashew apple juice.

The sensory attributes, including appearance, aroma, and taste, were affected by storage temperature variation. Changes in color, aroma intensity, and taste profile, such as astringency, bitterness, and sweetness, were observed during storage. These variations can impact consumer acceptance and satisfaction. Hence, controlling storage temperature is crucial to preserve the sensory quality of cashew apple juice.

Overall, clarification, addition of preservatives, and temperature control during storage are essential for maintaining the nutritional content, physicochemical parameters, and sensory attributes of cashew apple juice. These findings provide valuable guidance for juice processors and consumers, highlighting the significance of optimized processing and storage conditions to ensure the high quality and prolonged shelf life of cashew apple juice.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

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