

DEVELOPMENT OF LOW CALORIE READY TO SERVE (RTS) DRINK FROM GUAVA (*Psidium guajava* L.)

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ABSTRACT. *Psidium guajava* L. (guava), often referred to as the "poor man's apple," is a tropical fruit rich in vitamin C, minerals, natural sugars, and dietary fibre. This study aimed to develop a low-calorie ready-to-serve (RTS) guava beverage using various concentrations of *Stevia rebaudiana* Bertoni and sucrose. The formulations were evaluated for physicochemical properties, sensory attributes, and shelf-life stability over a 90-day storage period. Five treatments were prepared: one sucrose-based control, three sucrose–stevia blends, and one stevia-only formulation, all stored at ambient temperature (25 ± 2 °C). During storage, pH, ascorbic acid, and non-reducing sugars decreased, while total soluble solids, titratable acidity, and reducing sugars increased. Sensory scores for colour, taste, and flavour declined progressively. Statistical analysis was conducted using a completely randomized design with two-way factor analysis of variance (ANOVA), and treatment means were compared using the Least Significant Difference (LSD) test at 5% significance level. The statistical analysis demonstrated the significant differences ($p < 0.05$) among the treatments and storage duration. The stevia-based formulations demonstrated best physicochemical stability and enhanced shelf-life compared to the control treatment. These findings support the use of stevia–sugar blends in the development of low-calorie functional beverages, particularly for health-conscious consumers managing diabetes or obesity.

Keywords: *stevia rebaudiana*, low-calorie guava drink, physicochemical properties, sensory evaluation, shelf-life stability

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a natural, non-nutritive sweetener widely used as an alternative to sucrose in the food and beverage industries. It contains steviol glycosides, which are 200–300 times sweeter than sucrose [1]. Stevia-sweetened beverages have been shown to significantly reduce glycaemic response and caloric intake, making them suitable for individuals managing blood glucose levels [2]. Furthermore, stevia is globally recognized for its application in functional RTS (Ready-to-Serve) beverages due to its anti-inflammatory properties and favourable safety profile [3].

Guava (*Psidium guajava* L.) is a tropical fruit rich in vitamin C, dietary fibre, and antioxidants, making it ideal for functional food and beverages [4]. However, its climacteric nature and microbial susceptibility lead to rapid spoilage. Preservation strategies have shown to extend the shelf life by reducing enzymatic browning and microbial growth [5, 6].

Excessive sugar intake is associated with the health issues such as obesity, diabetes, and heart disease, gaining interest in developing the low-calorie functional beverages. *Stevia rebaudiana*, known for its high sweetness, zero calories, which is widely used as a sugar substitute. Studies on low-caloric mandarin nectar [7], orange drinks [8], peach beverages [9], and low-calorie jam [10] have shown the use of stevia with sucrose effectively maintaining

physicochemical and sensory quality. These findings support stevia's role in developing nutritious, shelf-stable, health-oriented RTS beverages

The development of guava and jamun-based Ready-to-Serve (RTS) beverages need proper formulation for physicochemical stability and sensory quality during storage. The parameters include TSS, titratable acidity, pH [11], and ascorbic acid significantly affect taste, nutrition, and consumer acceptance. [12]. Studies report an increase in TSS and acidity, while a decline in pH and vitamin C over time is due to microbial activity, [13] enzymatic reactions, [14] and oxidation [15]. Carboxymethyl cellulose (CMC) is a widely used food-grade stabilizer that enhances the texture, stability, sensory quality and shelf life of guava-based drinks, but it can also maintain the structural integrity. Similar trends are also observed in guava stored under both ambient and refrigerated conditions [16].

Postharvest treatments such as calcium chloride with aloe vera gel help preserve pH, TSS, and vitamin C in guava [5]. The reducing sugars and acidity increase while non-reducing sugars decrease due to hydrolysis and fermentation [17, 18]. Product formulation strongly influences the sensory quality and shelf life of RTS beverages. Higher pulp content (40%) enhances consumer acceptance [19], while pre-harvest foliar novel application for compounds like calcium nitrate improve overall acceptability [20]. Guava leather and value-added products like guava–pineapple jelly and guava–toffee retain sensory quality during storage [21, 22]. Similar improvements in flavour and stability have been reported in ginger-beetroot and blended-based RTS drinks [23, 24]. Proper packaging (Beeswax-LDPE packaging) and postharvest treatments can significantly enhance guava's shelf life [25, 26], while chitosan–carvacrol coatings maintain nutritional quality. Melatonin boosts antioxidant stability and reduces browning [27]. 1-MCP, essential oils, and ethylene-absorbing sheets delay ripening and spoilage [28]. Postharvest maturity and storage temperature also play critical roles in vitamin C retention and overall quality [29, 30].

The shift toward healthier lifestyles has increased demand for low-calorie, nutrient-rich functional beverages. Stevia-sweetened RTS drinks offer a natural alternative to traditional sugar-based beverages [4], although the traditional sugar-based beverages are still popular for its taste and shelf life [31]. However, research on stevia–sugar blends in RTS guava beverages remains limited, particularly regarding their effects on physicochemical, sensory, and shelf-life attributes [32]. The addition of antioxidants has also shown potential to enhance stability and overall quality [33].

Although stevia has been studied in various fruit beverages, its use in guava-based RTS formulations remains largely unexplored. This research is essential to examine stevia's role in developing low-calorie, health promoting drink alternatives to sugar-sweetened drinks and promoting the nutritional quality, shelf-life stability, and consumer satisfaction.

MATERIALS AND METHODS

Experimental Site and Design

The experiment was conducted at the Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan, located near Tarnab Farm. The research was performed in the Food Processing Laboratory and the analytical work was carried out in the Food Analysis Laboratory between January and June 2024. Five treatments (T_0 – T_4) were prepared, each representing different combinations of sucrose and stevia. All formulations were processed using sterilized polyethylene terephthalate (PET) bottles and handled under hygienically controlled laboratory conditions. Physicochemical and sensory evaluations were performed at an ambient temperature of $(25 \pm 2^\circ\text{C})$. The experimental design followed a Completely Randomized Design (CRD) with two-way Analysis of Variance (ANOVA). Treatment means were compared

using the Least Significant Difference (LSD) test at a 5% significance level. All measurements were conducted in triplicate ($n = 3$) for each treatment to ensure statistical reliability.

Selection, washing, sorting, peeling, and cutting of fruits

Fresh, ripe, medium-sized guava fruits (*Psidium guajava* L.), approximately 20 kg were procured from a local market in Peshawar, Pakistan. The fruits were selected based on uniformity in size, colour, and ripeness. Upon arrival at the laboratory, the guava fruits were thoroughly washed under running tap water for 5 minutes to remove surface contaminants, pesticide residues, and microbial load. The fruits were manually sorted to eliminate any overripe, bruised, or damaged fruits. Only clean, undamaged fruits were selected for further processing. The selected guava fruits were manually peeled using stainless steel knives under hygienic conditions to minimize enzymatic browning and oxidative reactions. Seeds and peels were removed, and the solid edible portion was cut into uniform slices (approximately 2–3 cm) to ensure consistency during the pulping process.

Pulp extraction

Guava slices were processed using a stainless-steel pulper (Model: Dawlance Juicer Blender DWTB-620, 2,500 RPM). The extracted pulp was filtered through stainless-steel sieves or mesh filters with a pore size of 0.5–1.0 mm to remove coarse particles and fibrous material. The average pulp yield was obtained as 480–510 g per kilogram of fresh fruit using a water-assisted extraction method.

Optimization of sugar content

To optimize the sugar concentration, eight formulations were prepared using various concentrations of guava pulp, sugar, water, and citric acid (Table 1). Among these recipes, Recipe 8, comprising 200 mL guava pulp, 700 mL water, 100 g sugar, and 1 g citric acid, demonstrated the highest mean overall acceptability score of 8.5 ± 0.1 . This formulation was selected for further use.

Table 1: Optimization of sugar content in RTS guava drink

Recipes	Sugar (g)	Citric acid (g)	Guava pulp (mL)	Water (mL)	Sensory score (Mean \pm SD)
1	50	2	500	450	8.0 ± 0.2
2	50	2	400	550	7.7 ± 0.3
3	80	2	100	820	6.0 ± 0.2
4	80	2	200	720	6.7 ± 0.3
5	80	2	300	620	7.5 ± 0.2
6	100	1	100	800	6.3 ± 0.3
7	100	1	150	750	6.5 ± 0.2
8	100	1	200	700	8.5 ± 0.1

Note: *Each formulation was prepared in triplicate ($n = 3$) and evaluated by 15 semi-trained panellists using a 9-point hedonic scale under controlled conditions (25 ± 2 °C). Sensory scores are presented as mean \pm standard deviation. Recipe 8, with a mean overall acceptability (8.5 ± 0.1) was selected.

Optimization of stabilizer concentration

Carboxymethylcellulose (CMC) was used as a stabilizing agent to minimize pulp sedimentation in the guava-based ready-to-serve (RTS) beverage. Four concentrations 0.0 g, 0.1 g, 0.5 g, and 1.0 g per litre, were added to examine the sedimentation behaviour and stabilizer effectiveness. Among the recipes, Recipe 3 (1.0 g CMC) exhibited no visible

sedimentation and demonstrated the highest overall acceptability score (8.9 ± 0.1) (Table 2), indicating the best sensory performance and enhanced physical stability.

Table 2: Optimization of CMC stabilizer to prevent pulp sedimentation

Recipes	CMC (g)	Guava Pulp (mL)	Water (mL)	Sugar (g)	Citric Acid (g)	Sediment Score	Sensory Score (Mean \pm SD) *
Control (0g)	0.0	200	700	100	1	1 (Poor)	7.8 ± 0.3
1	0.1	200	700	100	1	2 (Fair)	8.1 ± 0.2
2	0.5	200	700	100	1	3 (Good)	8.5 ± 0.2
3	1.0	200	700	100	1	4 (Excellent)	8.9 ± 0.1

Note: *Values are means \pm standard deviation ($n = 3$). Sensory evaluation was conducted using a 9-point hedonic scale by 15 semi-trained panellists after 7 days of storage at 25 ± 2 °C. Sedimentation scored was based on clarity and visible pulp settling on a 4-point scale (1 = poor, 4 = excellent). Recipe 3 with mean overall acceptability (8.9 ± 0.1) and a sedimentation score 4 (Excellent) was selected.

Optimization of stevia concentration

Seven formulations were developed using stevia at various concentrations ranging from (0.010% to 0.040%), while maintaining constant levels of guava pulp (20.00%), water content (80%), citric acid (0.10%), carboxymethylcellulose (CMC, 0.10%) presented in Table 3. Recipe 5, containing 0.030% stevia, demonstrated the highest overall acceptability score (7.0 ± 0.11), attributed to its pleasant sweetness with no detectable bitterness aftertaste, and was selected for further use.

Table 3: Optimization of stevia concentration in RTS guava drink

Recipe	Stevia (%)	Guava Pulp (%)	Water (%)	Citric acid (%)	CMC (%)	Sensory Score* (Mean \pm SD)
1	0.010	20	80	0.10	0.10	6.6 ± 0.05
2	0.015	20	80	0.10	0.10	6.7 ± 0.05
3	0.020	20	80	0.10	0.10	6.7 ± 0.15
4	0.025	20	80	0.10	0.10	6.9 ± 0.11
5	0.030	20	80	0.10	0.10	7.0 ± 0.11
6	0.035	20	80	0.10	0.10	7.1 ± 0.05
7	0.040	20	80	0.10	0.10	7.1 ± 0.11

Note: *Sensory evaluation was performed on a 9-point hedonic scale by 15 semi-trained panellists. Each recipe was evaluated in triplicate ($n = 3$) and the Sensory scores are expressed as mean \pm standard deviation (SD). Recipe 5 demonstrated optimal sweetness with no detectable bitterness aftertaste with the overall acceptability score (7.0 ± 0.11) was selected.

Formulation of Low-Caloric RTS Drink using Sugar-Stevia Blends

Five treatments (T_0 to T_4) of low-calorie ready-to-serve (RTS) guava beverages were developed using various concentrations of sucrose and stevia, based on previously optimized recipes. A 10% sucrose solution was equivalent to 100 g, while 10% stevia was standardized at 0.3 g. The treatments were formulated as follows: T_0 : 10% sucrose (control), T_1 : 7.5% sucrose + 2.5% stevia, T_2 : 5.0% sucrose + 5.0% stevia, T_3 : 2.5% sucrose + 7.5% stevia, T_4 : 10% stevia (no sucrose). The guava pulp (20.00%), citric acid (0.10%), carboxymethyl cellulose (CMC; 0.10%), and potassium metabisulfite (KMS; 0.06%) were used at a constant level across all treatments (Table 4). The sugar and stevia concentrations were adjusted to achieve equivalent sweetness levels, based on their relative sweetness values (sucrose = 1.0; stevia \approx 250–300). The formulation of a low-calorie mandarin nectar using stevia in an RTS beverage was also reported by [7].

Table 4: Followed Study plan

Treatments	Guava Pulp (%)	Sugar (%)	Stevia (%)	Water (%)	Citric Acid (%)	CMC (%)	KMS (%)
T ₀	20	10	—	70.0	0.1	0.1	0.06
T ₁	20	7.5	2.5	72.5	0.1	0.1	0.06
T ₂	20	5.0	5.0	75.0	0.1	0.1	0.06
T ₃	20	2.5	7.5	77.5	0.1	0.1	0.06
T ₄	20	—	10	80.0	0.1	0.1	0.06

Note: The reference values for sugar and stevia were taken from previously optimized formulations specifically 200 g sugar = (100%) and 0.30 g stevia = (100%). The treatments were formulated based on followed study plan as follows: T₀ = 10% sugar (control), T₁ = 7.5% sugar + 2.5% stevia, T₂ = 5.0% sugar + 5.0% stevia, T₃ = 2.5% sugar + 7.5% stevia, and T₄ = 10% stevia only. All other ingredients were kept constant across all treatments. A dash (—) indicates the absence of the corresponding ingredient in the formulation.

Preparation, packaging and storage of RTS guava drink

After the preparation of treatments, the blended beverage was filled into sterilized transparent food-grade polyethylene terephthalate (PET) bottles filled in 250 mL under aseptic conditions. The bottles were stored at ambient temperature (25 ± 2 °C) for a period of 90 days. The physicochemical and sensory analyses were conducted at 15-day intervals throughout the storage duration.

Physicochemical Analysis

Analytical methods

All analytical-grade reagents were procured from Merck® or Sigma-Aldrich®, and the analytical procedures were performed with the following standard methods outlined by [35]. Results were expressed as mean \pm standard deviation and subjected to statistical analysis to evaluate significant differences among treatments. All experiments were conducted in triplicate ($n = 3$) to ensure accuracy. The RTS beverage samples were analysed for physicochemical and nutritional parameters using standard methods. pH was measured with a digital pH meter, while TSS (°Brix) was determined using a refractometer. Titratable acidity and ascorbic acid content were estimated via titration using analytical reagents, including sodium hydroxide (NaOH) and 2,6-dichlorophenolindophenol (DCPIP) dye were freshly prepared and standardized prior to use. Sensory evaluation of color, taste, flavours, and overall acceptability was performed using a 9-point hedonic scale by a panel of 15 semi-trained panellists.

pH

The pH of the RTS guava drink samples was measured using a calibrated digital pH meter (Model: Hanna HI 2210). The instrument was calibrated prior to each measurement using standard buffer solutions at pH 4.0, 7.0, and 10.0. For each treatment, a 10 mL aliquot was transferred into a 50 mL glass beaker for pH determination. The electrode was rinsed with distilled water and gently dried using lint-free tissue to prevent cross-contamination. The probe was then immersed into the sample, and the readings were recorded after stabilization. All measurements were performed in triplicate ($n = 3$) following the method described by [34].

Total soluble solids (TSS)

Total soluble solids (TSS) of the RTS guava drink samples were measured using a handheld refractometer (Model: MASTER-80H, ATAGO; range: 0–80 °Brix). The instrument was calibrated with distilled water prior to use for each treatment. A few drops of the homogenized sample were placed on the clean prism surface and the cover lid was gently closed. The °Brix value was recorded once the reading stabilized. After each measurement, the prism was thoroughly rinsed with distilled water and dried using a lint-free tissue to prevent cross-contamination. All measurements were performed in triplicate ($n = 3$), following the method described by [34].

Titrateable acidity (%)

Titrateable acidity (TA) of the RTS guava drink samples was determined using the acid-base titration method described by the [34].

Reagents and chemicals used

Phenolphthalein indicator (1% solution)

0.1 N Sodium hydroxide (NaOH) solution

0.1 N Oxalic acid solution (for standardization of NaOH)

Standardization of NaOH solution

To ensure accurate titration 0.1 N sodium hydroxide (NaOH) solution was standardized using 0.1 N oxalic acid dihydrate ($C_2H_2O_4 \cdot 2H_2O$). For preparation of the standard solution, 6.3 g of oxalic acid dihydrate was accurately weighed using a digital analytical balance and dissolved in distilled water to make a final volume of 1 L. Approximately 4.5 g of NaOH pellets were dissolved in distilled water and diluted to 1 L to prepare an approximate 0.1 N NaOH solution. For standardization, 10 mL of the oxalic acid solution was pipetted into a clean 250 mL conical flask. Two to three drops of phenolphthalein indicator were added, and the solution was titrated with NaOH from a burette. The titrant was added dropwise with continuous swirling until a persistent pale pink endpoint was observed lasting for 10–15 seconds. The procedure was repeated until final readings were obtained and the average volume was used to calculate the exact normality of the NaOH solution.

The exact normality of the NaOH was calculated using the formula:

$$N_1 V_1 = N_2 V_2$$

Eqn. 1

Here

N_1 = Normality of NaOH solution

N_2 = Normality of Oxalic acid solution

V_1 = Volume of NaOH solution

V_2 = Volume of Oxalic acid solution

The NaOH solution was adjusted to achieve precise 0.1 N for subsequent titrations.

Titration procedure for samples

A 10 mL aliquot of the RTS guava beverage sample was transferred into a 100 mL volumetric flask and diluted to volume with distilled water. An aliquot of the diluted solution was then pipetted into a clean conical flask, and 2–3 drops of phenolphthalein indicator were added. The sample was titrated against standardized 0.1 N sodium hydroxide (NaOH) solution until a faint pink endpoint persisted for 10–15 seconds, indicating neutralization. The initial

and final burette readings were recorded to determine the volume of NaOH used. Each sample was titrated in triplicate ($n = 3$) to ensure analytical precision.

The titratable acidity was calculated using the volume of NaOH used and expressed as:

$$\text{Titrable Acidity (\%)} = \frac{C.F \times T \times N}{A \times D} \times 100$$

Eqn. 2

D = Dilution of the sample

A = Aliquot sample

C.F= Conversion Factor

N = Normality of NaOH

T = Volume of NaOH used

Ascorbic acid (mg/100 g)

The Ascorbic acid content of the RTS guava drink was determined using the 2,6-dichlorophenol-indophenol (DCPIP) dye titration method as described by [34].

Reagents and chemicals used

Oxalic acid (0.4%)

Sodium bicarbonate

2,6-dichlorophenol-indophenol (DCPIP) dye

Ascorbic acid standard (analytical grade)

Preparation of dye solution

Exactly 50 mg of 2,6-dichlorophenolindophenol (DCPIP) and 42 mg of sodium bicarbonate were accurately weighed using a digital analytical balance and transferred to a 250 mL beaker. Distilled water was added to filled the volume up to 250 mL and the solution was stirred thoroughly until completely dissolved. The solution was filtered through a double-layered muslin cloth to remove undissolved substance. It was then transferred to an amber-coloured volumetric flask to protect the dye from photodegradation. The flask was sealed and stored in a cool, dry environment to ensure dye stability during storage.

Preparation of 0.4% oxalic acid solution

Exactly 4.0 g of oxalic acid ($C_2H_2O_4 \cdot 2H_2O$) was accurately weighed using a digital analytical balance and dissolved in distilled water. The solution was made up to 1 L in a volumetric flask and then filtered to remove any undissolved particles and stored in a tightly sealed, labelled container for later use in titration and dilution process.

Preparation of ascorbic acid standard solution

To prepare the standard solution a 50 mg of pure L-ascorbic acid was accurately weighed using a digital analytical balance and transferred to a 50 mL volumetric flask. Approximately 20–30 mL of 0.4% oxalic acid solution was added to dissolve the ascorbic acid completely. The solution was then diluted to volume with 0.4% oxalic acid, sealed, and gently shaken to ensure homogeneity. The standard solution was freshly prepared for analysis to maintain stability.

Standardization of dye solution

A 5 mL aliquot of the ascorbic acid standard solution was accurately pipetted into a clean conical flask. The burette was filled with the freshly prepared DCPIP dye solution, and titration was carried out by adding the dye (dropwise) with continuous swirling. The endpoint was identified by the appearance of a light pink color that persisted for 10–15 seconds, indicating the complete oxidation of ascorbic acid. The volume of DCPIP was recorded for standardization calculations.

Titration of samples

A 10 mL aliquot of the RTS guava beverage was pipetted into a 50 mL volumetric flask, diluted with 0.4% oxalic acid, and mixed thoroughly. A 10 mL aliquot was transferred into a clean conical flask and titrated with the standardized DCPIP dye solution. Titration was continued until a faint pink colour persisted for 10–15 seconds, indicating the endpoint. All titrations were performed in triplicate ($n = 3$) to ensure analytical accuracy and for statistical analysis.

Finally, the ascorbic acid content was calculated using the appropriate formula given below.

$$\text{Ascorbic Acid (mg/100g) \%} = \frac{D.F \times T \times A}{S \times D} \times 100$$

Eqn. 3

D.F= Dye Factor

S = Volume of the sample taken

D =Volume of sample taken for dilution

T =Volume of dye solution used

A =Standard ascorbic acid solution taken

Reducing sugar (%)

The reducing sugar content was determined using the Lane and Eynon titrimetric method as described [34].

Reagents and chemicals used

Fehling's Solution A: Fehling's A solution was prepared by dissolving 34.639 g of copper (II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in a distilled water and diluting with the volume of 500 mL in a volumetric flask. The solution was allowed to stand for 48 hours and then filtered to remove any impurities.

Fehling's Solution B: Fehling's B solution was prepared by dissolving 173 g of potassium sodium tartrate tetrahydrate ($\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$) and 50 g of sodium hydroxide (NaOH) in distilled water and diluting to volume of 500 mL in a volumetric flask. The solution was allowed to stand for 48 hours and then filtered to remove any insoluble impurities.

Methylene Blue Indicator: The methylene blue was prepared by dissolving 200 mg of methylene blue in distilled water and diluting to a final volume of 100 mL in a volumetric flask.

Sample preparation

A 10 mL aliquot of the guava drink sample was transferred into a 100 mL volumetric flask and diluted with distilled water to prepare the test solution. The solution was thoroughly mixed to ensure homogeneity for titration use.

Standardization of the titration

To accurately determine the concentration of reducing sugars, a standard invert sugar solution (glucose–fructose mixture) was titrated under the same conditions as the test samples. This procedure facilitated the establishment of a conversion factor, enabling the titrated volume of the sample to be correlated with the corresponding mass (mg) of reducing sugars.

Titration procedure

In a clean conical flask, 5 mL each of Fehling's Solution A and Fehling's Solution B were mixed with 10 mL of distilled water. The mixture was placed on a preheated hot plate and brought to a gentle boil. The diluted sample solution was titrated from a burette into the boiling Fehling's solution. As the blue color began to fade, 2–3 drops of methylene blue indicator were added and the titration was continued until the blue color disappeared completely and a brick-red precipitate formed, indicating the endpoint. All titrations were performed in triplicate ($n = 3$) to ensure analytical accuracy and enable statistical reliability.

Calculation

5ml of Fehling A + 5ml of Fehling B = Xml of 10% sample solution = 0.05g of Reducing sugar

$$100\text{ml of 10\% sample solution contain} = \frac{0.05 \times 100}{X\text{ml}} = Y\text{g of Reducing sugar}$$

Eqn. 4

$$\text{Reducing sugar \%} = \frac{Y \times 100}{10}$$

Eqn. 5

Non-reducing sugar (%)

The non-reducing sugar content of the samples was determined using the Lane and Eynon method as outlined by [34].

Reagents and chemicals used

Fehling's Solution A

Fehling's Solution B

Methylene Blue Indicator

1N Hydrochloric Acid (HCl)

1N Sodium Hydroxide (NaOH)

Procedure

A 10 mL aliquot of the sample was transferred into a 100 mL volumetric flask and diluted to the mark with distilled water. A 20 mL aliquot was transferred to a 250 mL conical flask for hydrolysis and the 10 mL of 1 N hydrochloric acid (HCl) was added, and the mixture was heated for 5–6 minutes to promote sucrose inversion (hydrolysis). After hydrolysis, the solution was cooled to room temperature followed by the addition of 10 mL 1 N sodium hydroxide (NaOH) to neutralize the acid. The volume was then filled up to 250 mL with distilled water and the neutralized solution was transferred to a burette for titration. In a separate clean conical flask, 5 mL each of Fehling's Solution A and Fehling's Solution B, along with 10 mL of distilled water were added. The mixture was brought to a gentle boil on a preheated hot plate and the hydrolysed sample was titrated into the boiling Fehling's solution. As the blue color began to fade, 2–3 drops of methylene blue indicator were added. The titration was continued until the blue color disappeared completely, leaving a persistent brick-red precipitate indicating the endpoint. The concentration of non-reducing sugars was calculated by subtracting the

reducing sugar content (before hydrolysis) from the total sugar content (after hydrolysis). All titrations were performed in triplicate ($n = 3$) to ensure analytical and statistical accuracy.

Calculation

$$\begin{aligned} \text{Yml of sample solution} &= 0.05\text{g of reducing sugar} \\ 250\text{ml of sample} &= \frac{250 \times 0.05}{\text{Yml}} = \text{Zg of reducing sugar} \\ \text{Eqn. 6} \end{aligned}$$

$$\begin{aligned} 250\text{ml of sample solution} &\text{ prepared from } 20\text{ml diluted of } 10\% \text{ solution} \\ 20\text{ml of } 10\% \text{ sample solution} &= \text{Zg of reducing sugar} \\ 100 \text{ ml of } 10\% \text{ solution} &= \frac{\text{Z} \times 100}{20} = \text{Qg of Reducing sugar} \\ \text{Eqn. 7} \end{aligned}$$

$$\begin{aligned} 100\text{ml prepared from } 10\text{ml of the sample} \\ 10\text{ml of the sample} &= \text{Qg of reducing sugar} \\ 100\text{g of sample} &= \frac{\text{Q} \times 100}{10} = \text{Xml of reducing sugar} \\ \text{Eqn. 8} \end{aligned}$$

$$\text{Xml of Reducing sugar} = \text{Invert sugar} - \text{Free reducing sugar}$$

Organoleptic Evaluation

The sensory evaluation of the Ready-to-Serve (RTS) guava beverage was conducted to evaluate the taste, color, flavours, and overall acceptability. A panel of 15 semi-trained panellists (aged 21–45 years), comprising students and faculty members from the Food and Nutrition Division at NIFA, participated in the sensory evaluation. The evaluations were carried out under controlled hygienic conditions at 25 ± 2 °C in a neutral lighting and odor-free environment. Each sample was coded with a randomly assigned three-digit number to eliminate identification bias and served in identical, transparent glass cups (20 mL per sample) to prevent visual and cross-sample interference. Panellists were instructed to rinse their mouths with water between testing the samples to minimize after effects. The 9-point hedonic scale indicates (1 = “dislike extremely,” 9 = “like extremely”). All samples were evaluated independently and the mean sensory scores were calculated described by [35].

Statistical Analysis

All physicochemical parameters (e.g., pH, total soluble solids, titratable acidity, vitamin C, reducing sugar, non-reducing sugar) and sensory attributes (e.g., taste, color, flavor, and overall acceptability) were statistically analysed using a Completely Randomized Design (CRD) in a two-factor factorial arrangement (treatment type and storage duration). Each treatment was replicated three times ($n = 3$) to ensure statistical reliability. Treatments were randomly assigned to experimental units using a computer-generated randomization sequence to minimize bias. Data were analyzed using STATISTIX® 8.1 statistical software, and a two-way Analysis of Variance (ANOVA) was performed to evaluate the effects of treatment and storage time as well as their interaction. The assumptions of normality and homogeneity of variance were tested using the Shapiro–Wilk test and descriptive statistics. Mean comparisons were conducted using the Least Significant Difference (LSD) test at a 5% significance level ($p \leq 0.05$) followed by the methods described by [36].

RESULTS AND DISCUSSION

Physicochemical and sensory analyses were conducted at 15-day intervals over a 90-day storage period at ambient temperature (25 ± 2 °C). The parameters include pH, total soluble solids (TSS), titratable acidity, ascorbic acid content, reducing sugars, and non-reducing sugars. All analysis were carried out under hygienic laboratory conditions using standardized formulations. Each measurement was performed in triplicate ($n=3$) to ensure statistical reliability.

pH

The pH values of all treatments formulated with various combinations of sugar and stevia are presented in Table 5. A progressive decline in pH was observed across all treatments during the 90-day storage period. The lowest value (3.00) was recorded in (T_0), while the highest value (3.85) was observed in (T_4) treatment. The overall mean value decreased significantly from 3.73^a to 3.21^g , among treatments, the mean overall value ranged from 3.32^e to 3.64^a in T_0 to T_4 . The percent decrease in pH value varied from 16.67% in T_0 to 11.43% in T_4 . Statistical analysis revealed significant differences ($p < 0.05$) among treatments.

The findings of the present study were consistent with those of [7], who observed a decrease in pH from 3.74 to 3.44 in mandarin nectar formulated with a stevia–sucrose blend over a 60-day storage period. Similarly, the results were also similar to those [10], who reported a decline in pH from 3.50 to 3.26 in a low-calorie jam prepared with stevia and sugar during 28 days of storage. A comparable trend was also noted by [8] and [9], who documented pH reduction from 3.68 to 3.27 and 3.03 to 2.90, respectively, in low-calorie orange and apricot beverages containing a stevia–sugar blend of during the 90 and 60-day storage periods. The decrease in pH over 90 days storage can be attributed to a combination of biochemical and chemical reactions, including the enzymatic hydrolysis of pectin and the oxidative degradation of sugars. [15]. These processes lead to the formation of organic acids such as galacturonic, oxalic, quinic, and carboxylic acids, which contribute to increased titratable acidity and decreased pH values. enzymatic reactions, [14]. Furthermore, the use of stevia in the formulations enhances pH stability, as steviol glycosides are chemically stable under acidic conditions and are resistant to hydrolysis and sugar degradation [2].

Total soluble solids (TSS)

The total soluble solids (TSS) values of all treatments formulated with various combinations of sugar and stevia are presented in Table 6. An increase in total soluble solids (TSS) was observed across all treatments during the 90-day storage period. The lowest value (2.26 °Brix) was recorded in (T_4), while the highest value was observed (12.51 °Brix) in (T_0) treatment. The overall mean value increased significantly from 6.72^g to 7.34^a °Brix. Among treatments, the mean overall value ranged from 2.34^e to 13.11^a °Brix in T_4 and T_0 . The percent increase in TSS value varied from 9.22% to 7.00% in T_0 and T_4 . Statistical analysis revealed significant differences ($p < 0.05$) among treatments.

The findings of the present study are similar with the of [12], who reported an increase in total soluble solids (TSS) from 7.88 to 8.21 in a Jamun-based ready-to-serve (RTS) beverage formulated with stevia over a 90-day storage period. Similarly, similar results were also in resemblance with the [10], who observed a rise in TSS from 45.00 to 48.50 in a low-calorie jam prepared with a stevia–sugar blend during 28 days of storage. These results are also in agreement with [7], who documented an increase in TSS from 8.88 to 9.33 in mandarin nectar sweetened with a stevia–sucrose blend over 60 days. [8], also observed an increase in TSS from 3.07 to 3.21 in a low-calorie peach RTS beverage sweetened with stevia during a 60-day

storage period. The increase in TSS during storage can be attributed to a physicochemical process, notably the hydrolysis of polysaccharides such as pectin and starch into simple sugars under acidic conditions [17, 18]. Additionally, the inversion of sucrose can occur either enzymatically via invertase or through acid-catalysed hydrolysis of sucrose into glucose and fructose [16]. Furthermore, stevia-based treatments exhibited lower TSS values due to the absence of sucrose, which does not contribute to hydrolysis or sugar breakdown. Moreover, the packaging type and storage duration may also influence TSS levels, particularly under prolonged storage conditions [32].

Titrateable acidity (%)

The titrateable acidity (%) of all treatments formulated with various combinations of sugar and stevia are presented in Table 7. An increase in titrateable acidity (%) was observed across all treatments during the 90-day storage period. The lowest value (0.35%) was recorded in (T₄), while the highest value (0.70%) was observed in (T₀). The overall mean value increased significantly from 0.40^g to 0.60^a, while the overall mean value across the treatment ranged from 0.42^e to 0.56^a in T₄ and T₀. The increase in percent in titrateable acidity ranged from 30.00% in T₄ to 35.71% in T₀. Statistical analysis revealed significant differences ($p < 0.05$) among the treatments.

The findings of the present study are similar to those [10], who reported an increase in titrateable acidity (%) from 1.10 to 1.22 of a gourd and kiwifruit low-calorie beverage formulated with stevia during the storage of 6 months. A similar trend was also followed by [7], who observed a rise in titrateable acidity from 0.59% to 0.68% in mandarin nectar prepared with low-calorie stevia over 60 days. The results were also in agreement with [8], who reported an increase in titrateable acidity from 0.20% to 0.30% in low-calorie orange beverages formulated with a stevia–sugar blend with a total duration of 60 days. The rise in titrateable acidity during storage can be attributed to a combination of chemical and enzymatic reactions. Specifically, the degradation of ascorbic acid results in the production of organic acids such as dehydroascorbic, oxalic, and tartaric acids, and the sugar degradation into acids [13]. Additionally, enzymatic activity may catalyse the hydrolysis of pectin and sugars, which promotes the pectin breakdown and the release of galacturonic acid, leading to the formation of formic, citric, and acetic acids, contributing to the overall increase in titrateable acidity. This can adversely affect product quality and accelerate the degradation of sensitive bioactive compounds such as ascorbic acid, flavonoids, and anthocyanins [17, 18]. In contrast, the stevia-based treatment demonstrated greater stability due to the presence of steviol glycosides, which are chemically inert under acidic conditions and non-fermentable [3]. Moreover, the oxygen permeability of polyethylene terephthalate (PET) bottles facilitates oxidative reactions during storage, which can accelerate acid accumulation. There exists an inverse relationship between pH and titrateable acidity, wherein a decline in pH corresponds to an increase in titrateable acidity [28].

Vitamin C (mg/100 g)

The vitamin C (mg 100 g) content of all treatments formulated with various combinations of sugar and Stevia is presented in Table 8. A decline in vitamin C concentration was observed in all the treatments during the 90-day storage period. The lowest value (18.12 mg 100 g) was recorded in (T₀), while the highest value (26.38 mg 100 g) was observed in (T₄). The overall mean value decreased significantly from 25.51^a to 19.36^g, while the mean overall value across all the treatments ranged from 21.51^e to 23.31^a in T₀ and T₄. The decrease in vitamin C content ranged from 26.90% to 21.97% in T₀ and T₃. Statistical analysis revealed significant differences ($p < 0.05$) among the treatments.

Table 5: Effect of pH on low caloric RTS guava drink using stevia-sugar blend

Treatments	Storage interval in days							Decrease (%)	Mean
	0	15	30	45	60	75	90		
T ₀	3.60 ± 0.03 ^{IJ}	3.51 ± 0.01 ^L	3.42 ± 0.02 ^M	3.34 ± 0.02 ^N	3.25 ± 0.03 ^O	3.13 ± 0.03 ^Q	3.00 ± 0.04 ^R	16.67	3.32 ^e
T ₁	3.68 ± 0.02 ^{DEf}	3.58 ± 0.02 ^{JK}	3.49 ± 0.01 ^L	3.41 ± 0.01 ^M	3.32 ± 0.03 ^N	3.20 ± 0.02 ^P	3.12 ± 0.02 ^Q	15.22	3.40 ^d
T ₂	3.73 ± 0.03 ^c	3.67 ± 0.03 ^{EFg}	3.62 ± 0.01 ^{HI}	3.55 ± 0.03 ^K	3.43 ± 0.02 ^M	3.35 ± 0.01 ^N	3.20 ± 0.02 ^P	14.21	3.51 ^c
T ₃	3.79 ± 0.04 ^B	3.70 ± 0.04 ^{cDE}	3.64 ± 0.03 ^{GH}	3.57 ± 0.02 ^{JK}	3.49 ± 0.01 ^L	3.42 ± 0.03 ^M	3.33 ± 0.02 ^N	12.14	3.56 ^b
T ₄	3.85 ± 0.05 ^A	3.78 ± 0.03 ^B	3.71 ± 0.02 ^{cD}	3.65 ± 0.02 ^{fGH}	3.59 ± 0.02 ^{IJ}	3.48 ± 0.02 ^L	3.41 ± 0.02 ^M	11.43	3.64 ^a
Mean	3.73 ^a	3.65 ^b	3.58 ^c	3.50 ^d	3.42 ^e	3.32 ^f	3.21 ^g		

Note: Values are presented as mean ± standard deviation. Means with different superscript letters are significantly different from one another at 0.05 significance level by the Least Significant Difference (LSD) test. The LSD (0.05) value used for mean comparison was ≈ 0.035. Grand Mean = 3.4860 and CV = 0.62

Table 6: Effect of Total soluble solid (TSS) on low caloric RTS guava drink using stevia-sugar blend

Treatments	Storage interval in days							Increase (%)	Mean
	0	15	30	45	60	75	90		
T ₀	12.51 ± 0.04 ^G	12.70 ± 0.05 ^f	12.89 ± 0.04 ^E	13.09 ± 0.05 ^D	13.30 ± 0.05 ^c	13.51 ± 0.05 ^B	13.78 ± 0.05 ^A	9.22	13.11 ^a
T ₁	9.38 ± 0.03 ^N	9.52 ± 0.03 ^M	9.66 ± 0.03 ^L	9.81 ± 0.03 ^K	9.96 ± 0.03 ^J	10.12 ± 0.02 ^I	10.28 ± 0.04 ^H	8.75	9.82 ^b
T ₂	6.30 ± 0.03 ^U	6.38 ± 0.02 ^T	6.46 ± 0.03 ^s	6.55 ± 0.03 ^R	6.64 ± 0.04 ^Q	6.74 ± 0.02 ^P	6.84 ± 0.04 ^O	7.89	6.56 ^c
T ₃	3.13 ± 0.03 ^z	3.16 ± 0.03 ^{yz}	3.20 ± 0.02 ^{xy}	3.24 ± 0.03 ^{wx}	3.28 ± 0.02 ^w	3.34 ± 0.03 ^v	3.39 ± 0.03 ^v	7.67	3.25 ^d
T ₄	2.26 ± 0.02 ^e	2.28 ± 0.03 ^{de}	2.30 ± 0.03 ^{de}	2.33 ± 0.02 ^{cd}	2.36 ± 0.03 ^{bc}	2.40 ± 0.03 ^{ab}	2.43 ± 0.02 ^a	7.00	2.34 ^e
Mean	6.72 ^g	6.81 ^f	6.90 ^e	7.00 ^d	7.11 ^c	7.22 ^b	7.34 ^a		

Note: Values are presented as mean ± standard deviation. Means with different superscript letters are significantly different from one another at 0.05 significance level by the Least Significant Difference (LSD) test. The LSD (0.05) value used for mean comparison was ≈ 0.052. Grand Mean = 7.0149 and CV = 0.45

Table 7: Effect of titratable acidity (%) on low caloric RTS guava drink using stevia

Treatments	Storage interval in days							Increase (%)	Mean
	0	15	30	45	60	75	90		
T₀	0.45 ± 0.12 ^L	0.48 ± 0.10 ^I	0.51 ± 0.10 ^f	0.55 ± 0.10 ^D	0.59 ± 0.10 ^e	0.64 ± 0.17 ^B	0.70 ± 0.02 ^A	35.71	0.56 ^a
T₁	0.42 ± 0.11 ^o	0.45 ± 0.18 ^L	0.48 ± 0.27 ^I	0.51 ± 0.15 ^f	0.54 ± 0.19 ^e	0.59 ± 0.15 ^e	0.64 ± 0.02 ^B	34.38	0.52 ^b
T₂	0.39 ± 0.13 ^p	0.41 ± 0.11 ^p	0.43 ± 0.15 ⁿ	0.46 ± 0.15 ^k	0.49 ± 0.15 ^h	0.54 ± 0.14 ^e	0.59 ± 0.03 ^e	33.90	0.47 ^c
T₃	0.37 ± 0.11 ^r	0.39 ± 0.09 ^q	0.41 ± 0.19 ^p	0.44 ± 0.08 ^m	0.47 ± 0.17 ^j	0.51 ± 0.14 ^f	0.55 ± 0.03 ^D	32.73	0.45 ^d
T₄	0.35 ± 0.13 ^s	0.37 ± 0.08 ^r	0.39 ± 0.16 ^q	0.41 ± 0.10 ^p	0.44 ± 0.15 ^m	0.47 ± 0.14 ^j	0.50 ± 0.03 ^g	30.00	0.42 ^e
Mean	0.40 ^g	0.42 ^f	0.44 ^c	0.47 ^d	0.51 ^c	0.55 ^b	0.60 ^a		

Note: Values are presented as mean ± standard deviation. Means with different superscript letters are significantly different from one another at 0.05 significance level by the Least Significant Difference (LSD) test. The LSD value (0.05) used for mean comparison was ≈ 0.0073 . Grand Mean 0.4837 and CV 1.00

Table 8: Effect of ascorbic acid content (mg/100 g) on low caloric RTS guava drink using stevia

Treatments	Storage interval in days							Decrease (%)	Mean
	0	15	30	45	60	75	90		
T₀	23.77 ± 0.12 ^{GH}	23.77 ± 0.12 ^H	22.75 ± 0.10 ^K	21.59 ± 0.10 ^P	20.43 ± 0.10 ^R	19.13 ± 0.10 ^y	18.12 ± 0.17 ^z	26.90	21.51 ^d
T₁	25.07 ± 0.11 ^B	25.07 ± 0.12 ^B	24.06 ± 0.18 ^E	23.04 ± 0.27 ^J	22.03 ± 0.15 ^m	20.29 ± 0.19 ^s	19.42 ± 0.15 ^w	25.97	22.88 ^b
T₂	24.93 ± 0.13 ^c	24.93 ± 0.11 ^c	23.91 ± 0.11 ^f	22.75 ± 0.15 ^K	21.74 ± 0.15 ^o	20.14 ± 0.15 ^t	19.28 ± 0.14 ^x	23.12	22.55 ^c
T₃	24.93 ± 0.11 ^c	24.93 ± 0.11 ^c	23.91 ± 0.09 ^{IG}	23.04 ± 0.19 ^J	21.88 ± 0.08 ⁿ	20.00 ± 0.17 ^u	19.57 ± 0.14 ^v	21.97	22.63 ^c
T₄	25.51 ± 0.13 ^A	25.51 ± 0.13 ^A	24.20 ± 0.08 ^D	23.33 ± 0.16 ^I	22.32 ± 0.10 ^L	21.01 ± 0.15 ^φ	20.43 ± 0.14 ^R	22.53	23.31 ^a
Mean	25.51 ^a	24.84 ^b	23.77 ^c	22.75 ^d	21.68 ^e	20.11 ^f	19.36 ^g		

Note: Values are presented as mean ± standard deviation. Means with different superscript letters are significantly different from one another at 0.05 significance level by the Least Significant Difference (LSD) test. The LSD value (0.05) used for mean comparison was ≈ 0.140 . Grand Mean 22.481 and CV 0.38

The findings of the present study are in agreement with [12], who reported a decrease in vitamin C content from 12.37 to 11.65 mg/100 g during the storage of a Jamun-based ready-to-serve (RTS) beverage formulated with an alternative sweetener (stevia) during the storage of 90 days. The results are also aligned with the findings of [7], who observed a reduction in vitamin C from 42.67 to 40.62 mg/100 g in a stevia–sucrose sweetened mandarin nectar over the 60 days storage period. Similarly, the results are also in agreement with the [8], who recorded a decrease in vitamin C content from 8.34 to 4.54 mg/100 g in low-calorie orange beverages containing a stevia–sugar blend with the 90 days storage. The decline in vitamin C content during storage was primarily attributed due to oxidative degradation reaction, as the ascorbic acid is highly sensitive to oxidation thus conversion of ascorbic acid into dehydroascorbic acid and subsequently to diketogulonic acid in the presence of oxygen and light [33]. Even minor temperature fluctuations and light exposure under ambient conditions can accelerate the degradation process. Additionally, high sucrose content may facilitate Maillard reactions, through the conversion of sucrose into glucose and fructose and further into reactive carbonyl compounds [5] and [13]. Notably, vitamin C retention was observed in stevia-based formulations due to the chemical stability of steviol glycosides. Moreover, the stevia does not produce free radicals, thereby reducing the degradation process. Packaging also plays a crucial role; polyethylene terephthalate (PET) bottles are permeable to oxygen, allowing oxidative reactions. The loss of ascorbic acid directly affects the nutritional value of the beverage and may compromise flavours stability [29-31].

Reducing sugar (%)

The reducing sugar (%) content of all treatments formulated with various combinations of sugar and stevia is presented in Table 9. An increase in reducing sugar content (%) was observed across all the treatments during the 90-day storage period. The lowest value was recorded as 6.01% in (T₄), while the highest value was observed in 7.23% (T₀). The overall mean value increased significantly from 6.04^g to 7.05^a during the storage, while the overall mean value across the treatments ranged from 6.42^d to 6.57^a in T₄ and T₀. The increase percent in reducing sugar content varied from 16.83% to 12.50% in T₀ to T₄. Statistical analysis revealed significant differences ($p < 0.05$) among the treatments.

Similar trends are also observed in guava-based and protein-enriched beverages stored under both ambient and refrigerated conditions. Carboxymethyl cellulose (CMC) is a widely used food-grade stabilizer that enhances the texture, stability, sensory quality and shelf life of guava-based drinks, but it can also maintain the structural integrity [15]. The findings of the present study are consistent with [11], who reported an increase in reducing sugar content from 6.31% to 6.88% of a low-calorie bitter melon and kiwifruit beverage prepared with stevia during the storage of 6 months. A similar trend was also observed by [12], who noted an increase in reducing sugars from 2.94% to 3.33% in a Jamun-based ready-to-serve (RTS) beverage formulated with an alternative sweetener (stevia) during the storage of 90 days. Similar results were also reported by [9], who documented an increase in reducing sugar content from 1.94% to 2.08% in low-calorie apricot beverages sweetened with a stevia–sugar blend with 60 days storage. The increase in reducing sugars in guava juice is primarily due to the inversion of sucrose (hydrolysis), where it breaks it down into glucose and fructose [14]. Additionally, naturally occurring enzymes in guava facilitate the breakdown of complex carbohydrates, such as polysaccharides and starches, into simpler sugars like disaccharides and monosaccharides, including glucose [16]. Additionally, antioxidant compounds such as polyphenols, naturally present in fruits like guava, may facilitate the oxidative degradation of polysaccharides. In stevia-based formulations the steviol glycosides are chemically stable under acidic and thermal conditions and resist enzymatic hydrolysis. [17, 18]. Although

packaging materials like polyethylene terephthalate (PET) bottles are can promote non-enzymatic browning processes, such as the Maillard reaction [4].

Non-reducing sugar (%)

The non-reducing sugar (%) content of all treatments formulated with various combinations of sugar and stevia is presented in Table 10. A decrease in non-reducing sugar (%) was observed in all the treatment during the 90-day storage period. The lowest value 1.00% was observed in (T₄), while the highest value 4.59% was recorded in (T₀). The overall mean value declined significantly from 2.55^a to 2.24^g during storage, while the overall mean value among treatments ranged from 1.09^e to 4.32^a in T₄ to T₀. The decrease in non-reducing sugar content varied from 12.64% in T₀ to 11.79% in T₂. Statistical analysis revealed significant differences ($p < 0.05$) among treatments.

The results of the present study are in agreement with [12], who reported a decrease in non-reducing sugar content from 10.45% to 10.25% during the storage of a Jamun-based ready-to-serve (RTS) beverage formulated with an alternative sweetener (stevia) during the storage of 90 days. Similar trends were also observed by [9], who documented a reduction in non-reducing sugar levels from 2.97% to 2.82% in low-calorie apricot beverages prepared with a stevia–sugar blend with the duration of 60 days. The decrease in non-reducing sugar content during storage is primarily attributed to the acid-catalysed inversion of sucrose. Moreover, the enzymatic activity, such as invertase and pectinase, may enhance the hydrolysis of sucrose can contribute to the increase in reducing sugars (see section reducing sugars), while simultaneously decreasing non-reducing sugars, as there is an inverse relationship between the reducing sugar and non-reducing sugars [13]. In contrast, steviol glycosides are diterpenoid compounds, thereby preserving their structural integrity under storage conditions. [16]. The increase in reducing sugars (glucose and fructose) alongside the decline in non-reducing sugars strongly suggests that sucrose hydrolysis is the dominant mechanism. Packaging materials such as polyethylene terephthalate (PET) allow oxygen and light exposure, which promote oxidative and hydrolytic reactions [22, 23].

Organoleptic Analysis

Color

The color score of all the treatments formulated with sugar and stevia is presented in Table 11. A decrease in color score was observed in all the treatments during the 90-day storage period. The lowest score, 6.30, was observed in (T₄), while the highest score, 8.90, was recorded in (T₀). The overall mean score declined significantly from 8.42^a to 6.66^g. during storage, while the overall mean value among treatments ranged from 8.10^a to 7.27^e in in T₀ and T₄. The decrease percent in color score varied from 20.22% in T₀ to 21.95% in T₃. Statistical analysis revealed significant differences ($p < 0.05$) among treatments and storage duration.

The findings of the present study are consistent with [12], who reported a decrease in color score from 7.75 to 7.38 during the storage of a Jamun-based ready-to-serve (RTS) beverage formulated with an alternative sweetener (stevia) during the 90-day storage. A similar decline was noted by [7], who observed a reduction in color score from 8.73 to 6.64 in a stevia–sucrose sweetened mandarin nectar over the 60-day storage period. Similar results were also reported by [11], who documented a decrease in color score from 7.62 to 7.41 in a low-calorie bitter gourd and kiwifruit beverage sweetened with stevia with the 6 months duration. The reduction in color score was primarily attributed to the natural pigments present in guava juice, which mainly consists of carotenoids such as lycopene and β -carotene and flavonoids. During storage these pigments can degrades due to the high exposure to oxygen, light, and high temperatures, resulting in a visible decline in color intensity. In stevia-based formulations, the absence of

Table 9: Effect of reducing sugar (%) on low caloric RTS guava drink using stevia

Treatments	Storage interval in days							Increase (%)	Mean
	0	15	30	45	60	75	90		
T ₀	6.01 ± 0.06 ^z	6.16 ± 0.06 ^v	6.31 ± 0.06 ^s	6.54 ± 0.08 ⁿ	6.76 ± 0.07 ⁱ	6.98 ± 0.07 ^d	7.23 ± 0.08 ^A	16.83	6.57 ^a
T ₁	6.07 ± 0.06 ^x	6.19 ± 0.07 ^u	6.35 ± 0.09 ^r	6.61 ± 0.09 ^j	6.76 ± 0.08 ⁱ	6.94 ± 0.09 ^e	7.14 ± 0.08 ^B	15.05	6.58 ^a
T ₂	6.04 ± 0.06 ^y	6.19 ± 0.06 ^u	6.35 ± 0.06 ^r	6.51 ± 0.08 ^o	6.65 ± 0.08 ^k	6.91 ± 0.09 ^f	7.05 ± 0.08 ^c	14.33	6.53 ^b
T ₃	6.07 ± 0.04 ^x	6.13 ± 0.06 ^w	6.28 ± 0.09 ^t	6.48 ± 0.07 ^p	6.61 ± 0.09 ^j	6.79 ± 0.09 ^h	6.98 ± 0.09 ^g	13.11	6.48 ^c
T ₄	6.01 ± 0.06 ^z	6.13 ± 0.07 ^w	6.28 ± 0.06 ^t	6.41 ± 0.09 ^q	6.58 ± 0.09 ^m	6.68 ± 0.09 ^j	6.87 ± 0.09 ⁱ	12.50	6.42 ^d
Mean	6.04 ^g	6.16 ^f	6.31 ^c	6.51 ^d	6.67 ^c	6.86 ^b	7.05 ^a		

Note: Values are presented as mean ± standard deviation. Means with different superscript letters are significantly different from one another at 0.05 significance level by the Least Significant Difference (LSD) test. The LSD value (0.05) used for mean comparison was ≈ 0.0225 . Grand Mean 6.5155 and CV 0.21

Table 10: Effect of non-reducing sugar (%) on low caloric RTS guava drink using stevia

Treatments	Storage interval in days							Decrease (%)	Mean
	0	15	30	45	60	75	90		
T ₀	4.59 ± 0.04 ^A	4.51 ± 0.04 ^B	4.42 ± 0.03 ^c	4.33 ± 0.04 ^D	4.23 ± 0.03 ^E	4.12 ± 0.03 ^f	4.01 ± 0.04 ^G	12.64	4.32 ^a
T ₁	3.44 ± 0.03 ^H	3.39 ± 0.02 ^I	3.33 ± 0.02 ^J	3.26 ± 0.03 ^K	3.19 ± 0.02 ^L	3.11 ± 0.02 ^M	3.03 ± 0.02 ^N	11.92	3.25 ^b
T ₂	2.29 ± 0.03 ^O	2.26 ± 0.02 ^P	2.22 ± 0.02 ^Q	2.18 ± 0.02 ^R	2.13 ± 0.02 ^s	2.08 ± 0.02 ^t	2.02 ± 0.03 ^u	11.79	2.17 ^c
T ₃	1.30 ± 0.03 ^V	1.29 ± 0.02 ^V	1.27 ± 0.02 ^W	1.25 ± 0.02 ^x	1.22 ± 0.02 ^y	1.18 ± 0.02 ^z	1.14 ± 0.02 ^a	12.31	1.24 ^d
T ₄	1.14 ± 0.02 ^a	1.13 ± 0.02 ^{ab}	1.12 ± 0.02 ^b	1.10 ± 0.02 ^c	1.07 ± 0.02 ^d	1.04 ± 0.02 ^e	1.00 ± 0.02 ^f	12.28	1.09 ^e
Mean	2.55 ^a	2.52 ^b	2.47 ^c	2.42 ^d	2.37 ^e	2.31 ^f	2.24 ^g		

Note: Values are presented as mean ± standard deviation. Means with different superscript letters are significantly different from one another at 0.05 significance level by the Least Significant Difference (LSD) test. The LSD value (0.05) used for mean comparison was ≈ 0.0114 . Grand Mean 2.4111 and CV 0.29

sucrose may reduce pigment stability and visibility, potentially accelerating color loss [29-31]. Moreover, color degradation is also influenced by polyphenol oxidase (PPO) activity, which catalyzes the oxidation of phenolic compounds into quinones that polymerize into pigments. The carotenoid degradation is further accelerated under oxidative and thermal stress. Furthermore, sucrose may contribute to color stability by lowering water activity, thereby retarding pigment degradation, highlighting the critical role of sucrose for preserving the color intensity. [17, 18].

Taste

The taste score of all the treatments formulated with sugar and stevia is presented in Table 12. A decrease in taste score was observed in all the treatments during the 90-day storage period. The lowest score 6.10 was observed in (T₄), while the highest score, 8.90 was recorded in (T₀). The overall mean score declined significantly from 8.32^a to 6.56^g during storage, while the overall mean value among treatments ranged from 8.00^a to 7.13^e in T₀ and T₄. The decrease percent in taste score varied from 21.79% in T₄ to 20.48% in T₂. Statistical analysis revealed significant differences ($p < 0.05$) among treatments and storage.

The findings of the present study are consistent with the [12], who reported a decrease in taste score from 7.73 to 7.27 during the storage of a Jamun-based RTS beverage formulated with stevia as an alternative sweetener during the storage of 90 days. Similarly, the results were also similar with the [7], who documented a reduction in taste from 8.47 to 6.58 in stevia–sucrose sweetened mandarin nectar over the storage period of 60 days. Comparable trends were also observed by [9], who recorded a decline in taste score from 6.74 to 6.45 in low-calorie apricot beverages formulated with stevia and sugar with the duration of 90 days. The reduction in taste score during storage can be attributed to both the intrinsic chemical properties of sweeteners and various physicochemical changes that occur over time. The acid-catalysed hydrolysis of sucrose into glucose and fructose and the loss of volatile aroma compounds such as esters and aldehydes that can contribute to change in taste and aroma, which can further change the flavour during the storage [16, 18]. Non-enzymatic browning reactions, particularly the Maillard reaction, may produce flavour compounds, while the oxidation of polyphenolic compounds, especially under aerobic conditions, can further lead to the formation of astringent or bitter off-flavors, that can also affect the sweetness intensity and changes the sensory profile. [32]. Additionally, steviol glycosides, the primary sweetening agent in stevia-based formulations, are known to impart a bitter or metallic aftertaste [3].

Flavor

The flavour score of all the treatments formulated with sugar and stevia are presented in Table 13. A decrease in flavour score was observed in all the treatments during the 90-day storage period. The lowest score 5.90 was observed in (T₄), while the highest score 8.80 was recorded in (T₀). The overall mean score declined significantly from 8.22^a to 6.44^g during storage, while the overall mean value among treatments ranged from 7.84^a in T₀ to 7.00^e in T₄. The decrease percent in flavour score varied from 23.38% in T₄ to 20.45% in T₀. Statistical analysis revealed significant differences ($p < 0.05$) among treatments and storage.

The findings of the present study are in line with Ahmad et al. (2019), who reported a decrease in flavour score from 6.73 to 6.36 in low-calorie apricot beverages formulated with a stevia–sugar blend. A similar decline was observed by [10], who recorded a reduction in flavour score from 7.50 to 7.15 in a low-calorie jam prepared with stevia and sugar during the 28 days. The similar results are also reported by [11], a decrease in flavour score from 8.04 to 7.74 during the storage of a bitter melon and kiwifruit low-calorie beverage formulated with stevia during the 6 months. The reduction in flavour quality during storage is primarily attributed to the degradation of volatile compounds such as ethyl butanoate, β -caryophyllene, and hexanal.

These volatile compounds are highly sensitive to oxidative stress, light, and temperature fluctuations during the storage. The loss of esters and aldehydes is highly susceptible to oxidative degradation. Furthermore, the decline in vitamin C content reduces the antioxidant activity of the beverage, accelerating the breakdown of volatile compounds easily [20, 33]. Furthermore, the development of organic acids such as citric, formic, and acetic acids contributes to the development of undesirable off-flavours and the acid-catalysed hydrolysis of sucrose into glucose and fructose further promotes through non-enzymatic browning reactions, leading to the formation of Maillard products that affects both flavour and aroma characteristics [29-31].

Overall acceptability

The overall acceptability score of all the treatments formulated with sugar and stevia are presented in Table 14. A decrease in overall acceptability score was observed in all the treatment during the 90-day storage period. The lowest score 6.00 was observed in (T₄), while the highest score 8.90 was recorded in (T₀). The overall mean score declined significantly from 8.32^a to 6.56^g during storage, while the overall mean value among treatments ranged from 8.01^a to 7.13^e in T₀ and T₄. The decrease percent in overall acceptability score varied from 23.08% in T₄ to 20.22% in T₀. Statistical analysis revealed significant differences ($p < 0.05$) among treatments and storage

The findings of the present study are resembling with the [12], who reported a decrease in overall acceptability score from 7.63 to 7.26 during the storage of a Jamun-based ready-to-serve (RTS) beverage formulated with an alternative sweetener (stevia) during the storage of 90 days. Similar results were reported by [11], who observed a reduction in overall acceptability from 7.96 to 7.77 in a gourd and kiwifruit low-calorie beverage containing stevia with the 6 months storage. Similarly, [9], also documented a decline from 7.49 to 7.21 in low-calorie apricot beverages formulated with a stevia–sugar blend during the 90 days storage. The reduction in overall acceptability of guava-based beverages during storage is primarily associated with the progressive deterioration of different sensory attributes, notably color, taste, and flavour. These sensory degradations collectively affect the quality and consumer appeal of the product. A significant decline in pH due to increased acid formation alters the organoleptic profile, contributing to a sharper and less desirable taste. Concurrently, the hydrolysis of sucrose into glucose and fructose elevates the concentration of reducing sugars, which further promotes acid generation, exacerbating the sourness. Moreover, the degradation and volatilization of aromatic compounds, with a reduction in antioxidant compounds negatively affect the flavour and freshness perception [16, 17]. The breakdown of visual pigments, particularly carotenoids and flavonoids, leads to a noticeable decline in color intensity, reducing the visual freshness of the beverage. As the balance between sweetness and acidity shifts due to sugar degradation and organic acid accumulation, affecting the overall quality. The presence of stevia can also contribute to the taste and stability of the drink [8, 9, 31].

To enhance sensory stability and consumer acceptance, several advanced formulation and packaging strategies can be employed. The use of masking agents such as erythritol, inulin, or glycyrrhizin has been shown to improve mouthfeel and bitter aftertaste associated with the use of higher concentrations of steviol glycosides. Techniques like spray-drying and microencapsulation can effectively protect volatile aroma compounds from oxidative degradation during storage. Additionally, antioxidant fortification using ascorbic acid, tocopherols, or natural extracts such as rosemary can delay the breakdown of both flavour and bioactive compounds. Therefore, the adoption of advanced packaging technologies such as multilayer PET with enhanced oxygen barrier properties or glass containers can significantly reduce oxygen entrance and help to preserve flavour integrity and shelf-life stability [23-25].

Table 11: Effect of judges for color score on low caloric RTS guava drink using stevia

Treatments	Storage interval in days							Decrease (%)	Mean
	0	15	30	45	60	75	90		
T ₀	8.90 ± 0.12 ^A	8.70 ± 0.12 ^B	8.60 ± 0.12 ^c	8.20 ± 0.12 ^G	7.80 ± 0.12 ^{JK}	7.40 ± 0.12 ^O	7.10 ± 0.12 ^{QR}	20.22	8.10 ^a
T ₁	8.60 ± 0.06 ^c	8.50 ± 0.06 ^D	8.20 ± 0.06 ^G	8.00 ± 0.06 ^I	7.50 ± 0.06 ^N	7.20 ± 0.06 ^{PQ}	6.80 ± 0.08 ^T	20.93	7.83 ^b
T ₂	8.40 ± 0.10 ^E	8.30 ± 0.06 ^f	8.00 ± 0.06 ^I	7.80 ± 0.06 ^{JK}	7.40 ± 0.06 ^O	7.00 ± 0.08 ^s	6.70 ± 0.08 ^U	20.24	7.66 ^c
T ₃	8.20 ± 0.06 ^G	8.10 ± 0.08 ^H	7.80 ± 0.11 ^K	7.60 ± 0.06 ^M	7.20 ± 0.08 ^P	6.80 ± 0.08 ^T	6.40 ± 0.10 ^W	21.95	7.44 ^d
T ₄	8.00 ± 0.08 ^{HI}	7.90 ± 0.12 ^J	7.70 ± 0.04 ^L	7.40 ± 0.08 ^O	7.00 ± 0.08 ^s	6.60 ± 0.08 ^V	6.30 ± 0.08 ^W	21.25	7.27 ^e
Mean	8.42 ^a	8.30 ^b	8.06 ^c	7.80 ^d	7.38 ^e	7.00 ^f	6.66 ^g		

Note: Values are presented as mean ± standard deviation. Means with different superscript letters are significantly different from one another at 0.05 significance level by the Least Significant Difference (LSD) test. The LSD value (0.05) used for mean comparison was ≈ 0.0886. Grand Mean 7.6591 and CV 0.71

Table 12: Effect of judges for taste score on low caloric RTS guava drink using stevia

Treatments	Storage interval in days							Decrease (%)	Mean
	0	15	30	45	60	75	90		
T ₀	8.90 ± 0.05 ^A	8.60 ± 0.05 ^B	8.40 ± 0.06 ^D	8.10 ± 0.08 ^f	7.70 ± 0.08 ^J	7.30 ± 0.08 ^M	7.00 ± 0.06 ^O	21.35	8.00 ^a
T ₁	8.50 ± 0.06 ^c	8.30 ± 0.06 ^E	8.10 ± 0.04 ^f	7.80 ± 0.04 ^I	7.40 ± 0.04 ^L	7.00 ± 0.06 ^O	6.70 ± 0.06 ^ϕ	21.18	7.69 ^b
T ₂	8.30 ± 0.08 ^E	8.10 ± 0.04 ^f	8.00 ± 0.08 ^G	7.60 ± 0.04 ^K	7.30 ± 0.06 ^M	6.90 ± 0.04 ^P	6.60 ± 0.06 ^r	20.48	7.54 ^c
T ₃	8.10 ± 0.06 ^f	7.90 ± 0.06 ^H	7.80 ± 0.04 ^I	7.40 ± 0.08 ^L	7.10 ± 0.06 ^N	6.70 ± 0.08 ^ϕ	6.40 ± 0.04 ^t	20.99	7.34 ^d
T ₄	7.80 ± 0.06 ^I	7.70 ± 0.08 ^J	7.60 ± 0.06 ^K	7.30 ± 0.08 ^M	6.90 ± 0.06 ^P	6.50 ± 0.08 ^s	6.10 ± 0.08 ^u	21.79	7.13 ^e
Mean	8.32 ^a	8.12 ^b	7.98 ^c	7.64 ^d	7.28 ^e	6.88 ^f	6.56 ^g		

Note: Values are presented as mean ± standard deviation. Means with different superscript letters are significantly different from one another at 0.05 significance level by the Least Significant Difference (LSD) test. The LSD value (0.05) used for mean comparison was ≈ 0.0318. Grand Mean 7.5400 and CV 0.26

Table 13: Effect of judges for flavour score on low caloric RTS guava drink using stevia

Treatments	Storage interval in days							Decrease (%)	Mean
	0	15	30	45	60	75	90		
T₀	8.80 ± 0.14 ^A	8.40 ± 0.14 ^B	8.20 ± 0.15 ^c	7.90 ± 0.20 ^{DE}	7.50 ± 0.10 ^H	7.10 ± 0.10 ^{JK}	7.00 ± 0.20 ^{KL}	20.45	7.84 ^a
T₁	8.40 ± 0.04 ^B	8.20 ± 0.02 ^c	8.00 ± 0.10 ^D	7.70 ± 0.20 ^{FG}	7.30 ± 0.15 ^I	6.80 ± 0.18 ^{MN}	6.60 ± 0.10 ^{OP}	21.43	7.57 ^b
T₂	8.20 ± 0.08 ^c	8.00 ± 0.10 ^D	7.90 ± 0.05 ^{DE}	7.50 ± 0.15 ^H	7.10 ± 0.05 ^{JK}	6.70 ± 0.09 ^{NO}	6.40 ± 0.20 ^{QR}	21.95	7.41 ^c
T₃	8.00 ± 0.12 ^D	7.80 ± 0.02 ^{Ef}	7.70 ± 0.05 ^{FG}	7.30 ± 0.05 ^I	6.93 ± 0.15 ^{LM}	6.50 ± 0.20 ^{PQ}	6.30 ± 0.20 ^R	21.25	7.23 ^d
T₄	7.70 ± 0.04 ^{FG}	7.60 ± 0.04 ^{GH}	7.50 ± 0.15 ^H	7.20 ± 0.15 ^{IJ}	6.70 ± 0.15 ^{NO}	6.30 ± 0.10 ^R	5.90 ± 0.20 ^s	23.38	7.00 ^e
Mean	8.22 ^a	8.00 ^b	7.86 ^c	7.52 ^d	7.16 ^e	6.68 ^f	6.44 ^g		

Note: Values are presented as mean ± standard deviation. Means with different superscript letters are significantly different from one another at 0.05 significance level by the Least Significant Difference (LSD) test. The LSD value (0.05) used for mean comparison was ≈ 0.155 . Grand Mean 7.4038 and CV 1.28

Table 14: Effect of judges for overall acceptability score on low caloric RTS guava drink using stevia

Treatments	Storage interval in days							Decrease (%)	Mean
	0	15	30	45	60	75	90		
T₀	8.90 ± 0.04 ^A	8.60 ± 0.04 ^B	8.40 ± 0.02 ^{cD}	8.10 ± 0.09 ^{Ef}	7.70 ± 0.10 ^{GH}	7.30 ± 0.04 ^I	7.10 ± 0.06 ^J	20.22	8.01 ^a
T₁	8.50 ± 0.06 ^{Bc}	8.30 ± 0.03 ^D	8.13 ± 0.05 ^E	7.80 ± 0.09 ^G	7.40 ± 0.06 ^I	7.00 ± 0.12 ^{JK}	6.70 ± 0.10 ^L	21.18	7.69 ^b
T₂	8.30 ± 0.09 ^D	8.10 ± 0.08 ^{Ef}	8.00 ± 0.07 ^f	7.60 ± 0.03 ^H	7.30 ± 0.06 ^I	6.90 ± 0.05 ^K	6.60 ± 0.06 ^{LM}	20.48	7.54 ^c
T₃	8.10 ± 0.08 ^{Ef}	8.00 ± 0.11 ^f	7.80 ± 0.06 ^G	7.40 ± 0.05 ^I	7.10 ± 0.11 ^J	6.70 ± 0.11 ^L	6.40 ± 0.12 ^N	20.99	7.36 ^d
T₄	7.80 ± 0.10 ^G	7.80 ± 0.13 ^G	7.60 ± 0.06 ^H	7.30 ± 0.02 ^I	6.90 ± 0.06 ^K	6.50 ± 0.11 ^{MN}	6.00 ± 0.10 ^O	23.08	7.13 ^e
Mean	8.32 ^a	8.16 ^b	7.98 ^c	7.64 ^d	7.28 ^e	6.88 ^f	6.56 ^g		

Note: Values are presented as mean ± standard deviation. Means with different superscript letters are significantly different from one another at 0.05 significance level by the Least Significant Difference (LSD) test. The LSD value (0.05) used for mean comparison was ≈ 0.121 . Grand Mean 7.5465 and CV 0.98

CONCLUSION

This study demonstrated the development of a low-calorie ready-to-serve (RTS) guava beverage using stevia, which was found to be satisfactory in both physicochemical and sensory analyses. The formulation based on a stevia–sucrose blend, was evaluated for its physicochemical properties, sensory acceptability, and shelf-life stability. The stevia-based formulations exhibited best physicochemical stability, while the sucrose-based control treatment showed the highest sensory acceptability over a 90-day storage period. These findings support the use of stevia as a best alternative to sucrose in the development of health-oriented RTS beverages, particularly for consumers managing obesity or diabetes. However, the slightly bitter or metallic aftertaste commonly associated with steviol glycosides may reduce consumer acceptance. To address this challenge, advanced formulation strategies and emerging food technologies can be employed to enhance physicochemical characteristics, sensory properties, and shelf-life stability. Overall, the use of stevia in low-calorie beverages presents significant potential for innovation in the food and beverage industry. Further research is recommended to evaluate microbiological safety, assess the effect of different sweetener combinations, and evaluate the influence of various packaging materials on product shelf-life.

Conflict of interest statement. The authors declare no conflicts of interest related to the publication of this research.

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