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DEVELOPMENT AND CHARACTERISATION OF A CARBONATED FUNCTIONAL BEVERAGE FROM PEACH *(Prunus persica)*, STEVIA *(Stevia rebaudiana)*, LEMON *(Citrus limon)* AND MINT *(Mentha piperita)* FOR Diabetic And Health Conscious Individuals

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Carbonated beverage, stevia, peach, lemon, mint, diabetes, antioxidant, preservative-free, functional drink.

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Abstract

Background: *Rising consumption of sugar-sweetened beverages is associated with obesity, hyperglycemia, and diabetes, creating a need for natural, sugar-free functional beverages with health-promoting properties.*

Objective: *This study aimed to develop and evaluate a carbonated functional beverage prepared from peach* (Prunus persica)*, stevia* (Stevia rebaudiana)*, lemon* (Citrus limon)*, and mint* (Mentha piperita) *without added sugar or chemical preservatives.*

Methods: *Peach puree (20%) was used as the beverage base. Stevia concentration (0.5–1.5% v/v) was optimized by sensory evaluation, while lemon juice (0.5–2.0%) and mint extract (0.25–1.0%) were optimized using response surface methodology. The optimized formulation (T_2: 20% peach, 1.0% stevia, 1.0% lemon, and 0.5% mint) was pasteurized (85°C, 15 min), carbonated (35 psi; 3.2 volumes CO_2), and stored at 4 ± 1°C for 60 days. Physicochemical, antioxidant, sensory, microbiological, and storage stability analyses were performed.*

Results: *The optimized beverage exhibited a pH of 3.8, titratable acidity of 0.28%, total soluble solids of 4.5°Brix, total phenolic content of 162.5 mg GAE/L, and DPPH radical scavenging activity of 71.2%, all superior to the sucrose control. The beverage achieved an overall sensory acceptability score of 8.4/9 and remained microbiologically safe (<10 CFU/mL) throughout 60 days of refrigerated storage. Although total phenolic content and antioxidant activity decreased by approximately 20% and 26%, respectively, the beverage maintained acceptable physicochemical quality, color stability, and sensory acceptability.*

Conclusion: *The optimized formulation represents a sugar-free, preservative-free carbonated functional beverage with desirable physicochemical properties, high antioxidant potential, excellent consumer acceptability, and*

satisfactory refrigerated storage stability. The formulation provides a promising clean-label alternative to conventional sugar-sweetened carbonated beverages for diabetic and health-conscious consumers. Further in vivo and clinical studies are recommended to validate its potential health benefits.

Graphical Abstract

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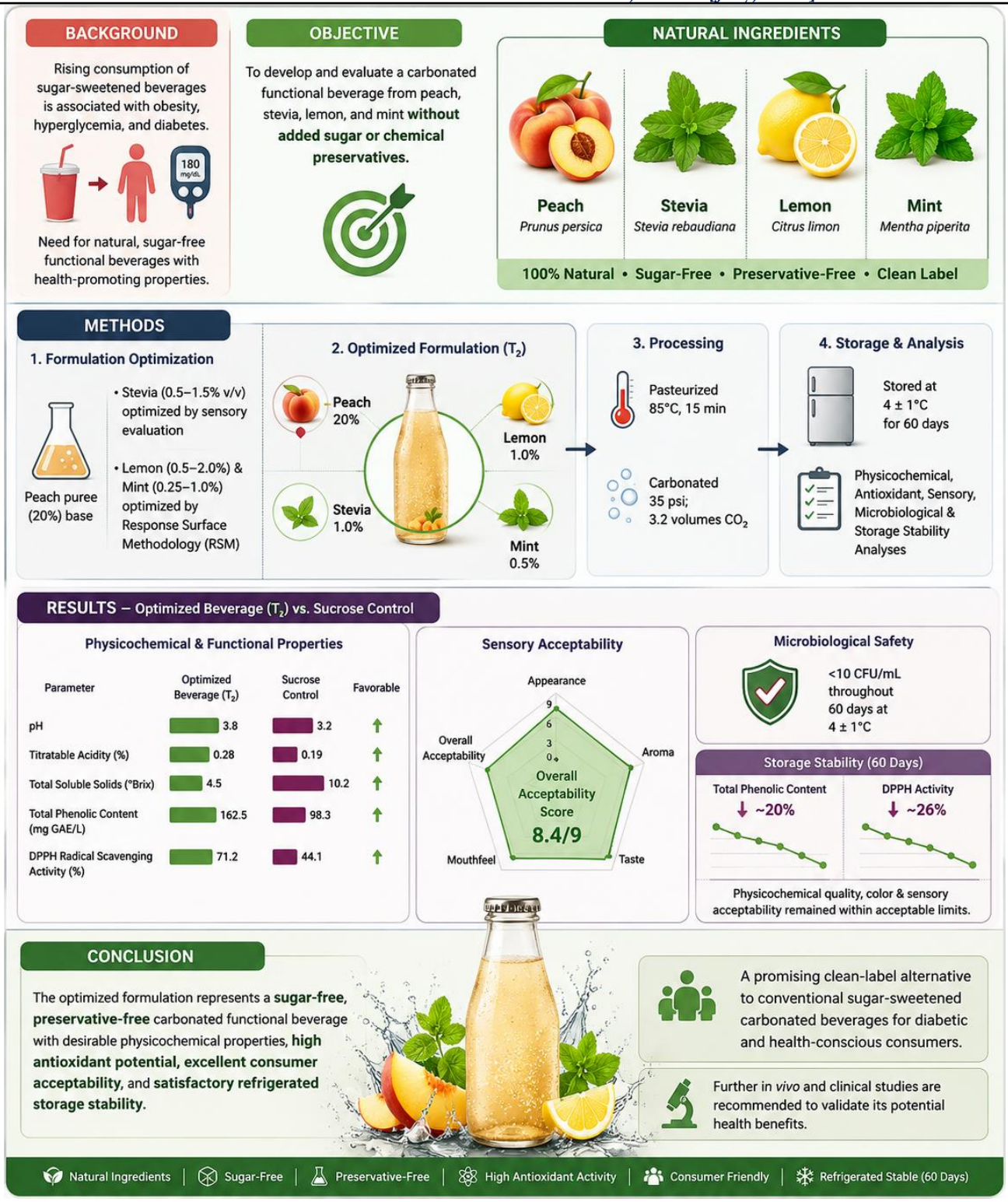


1. INTRODUCTION

One of the most important and quickly expanding metabolic diseases in the world is Diabetes mellitus. Banday et al. (2020) estimate that 463 million adults between the ages of 20 and 79 had diabetes in 2019; by 2045, this figure

is expected to rise to 700 million. Urbanization, sedentary lifestyles, and bad eating habits, especially frequent use of sugar-sweetened drinks (SSBs), are all strongly linked to the rising incidence of diabetes. The need for healthy nutritional options is highlighted by the fact that these drinks contribute to insulin resistance, obesity, hyperglycemia, and long-term diabetes problems (Sarkar et al., 2022; Malik et al., 2010). In order to prevent and treat diabetes, dietary management is essential. Because they may help control blood glucose while offering antioxidant and other health-promoting benefits, natural food products containing bioactive compounds have drawn a lot of attention (Golovinskaia & Wang, 2023). Because they offer hydration together with physiologically active substances including phenolics, vitamins, minerals, flavonoids, and other plant-derived components, functional beverages are one of the fastest-growing categories of functional foods (Carvalho et al., 2023). As a result, there is a growing demand from consumers for drinks made with natural ingredients free of artificial preservatives or added sugar (Raghavan et al., 2023).Polyphenols, vitamins, carotenoids, dietary fiber, and other phytochemicals with antioxidant and possibly hypoglycemic qualities are known to be abundant in peaches *(Prunus persica L.)* (Wu et al., 2022; Zhou et al., 2025; Bento et al., 2020). According to earlier research, peach bioactive chemicals may enhance glucose homeostasis, lipid metabolism, and oxidative stress, making peaches an appropriate basis ingredient for the creation of functional beverages (Petruccelli et al., 2023; Noratto et al., 2009). Soluble solids, titratable acidity, phenolic compounds, carotenoids, and antioxidant activity have all shown significant variation between peach cultivars, according to recent evaluations (Petruccelli et al., 2023). While variations in antioxidant capacity were linked to cultivar traits, yellow-fleshed cultivars often showed higher total phenolic content, underscoring the significance of cultivar selection in the creation of functional peach-based products (Gil et al., 2002). These results reinforce the suitability of peach as a raw material for the development of functional beverages with improved nutritional and antioxidant properties (Soares et al., 2022; Martin-Diana et al., 2025).Steviol glycosides found in stevia *(Stevia rebaudiana Bertoni)*, a naturally occurring non-caloric sweetener, give it a strong sweetness without raising blood sugar levels (Samuel et al., 2018; Ahmad & Ahmad, 2018). Stevia is especially good for diabetics since, in contrast to traditional sugars, it adds sweetness with very little calorie input (Chowdhury et al., 2022). Stevia's established safety profile and global regulatory approval for culinary uses have contributed to its growing popularity in addition to its sweetening qualities (Raghavan et al., 2023; Alhalawi & Ervita, 2025). Clinical studies have demonstrated that stevia consumption can improve glycemic control and reduce post-prandial glucose levels in diabetic patients (Anton et al., 2010; Gregersen et al., 2004).The nutritional and practical qualities of mint *(Mentha piperita)* and lemon *(Citrus limon)* are well known. While mint has essential oils and phenolic compounds that enhance scent, sensory acceptance, and digestive health, lemon offers citric acid, vitamin C, and flavonoids that support antioxidant activity and flavor enhancement (Gandhi et al., 2020; Soleimani et al., 2022). Combining these natural substances may increase overall consumer acceptance while offering additional functional benefits (Prieto-Santiago et al., 2025; Sinaga, 2026).In addition to the selection of ingredients, the processing techniques of beverages are important in order to preserve bioactive compounds, sensory quality and microbiological safety during storage (Peng et al., 2025; Bie et al., 2024). Thus, to develop successful functional beverages, the formulation and processing conditions need to be optimised.Although commercially prepared diet beverages are available, many of them have little functional health advantages and rely on artificial sweeteners and chemical preservatives (Carvalho et al., 2023). Furthermore, fruit juices frequently include significant levels of naturally occurring sugars, which, if consumed in excess, may not be appropriate for those with diabetes (Bass et al., 2016). Thus, the creation of a sugar-free, preservative-free, carbonated functional beverage

made with natural ingredients offers a significant chance to address present consumer and public health requirements (Han et al., 2025). Studies on peach processing have also shown that materials obtained from peaches maintain significant amounts of phenolic compounds with significant antioxidant capability, highlighting their potential use in functional food items with added value (Garcia-Aparicio et al., 2024; Ossowski et al., 2025).Many studies have been made on fruit-based functional beverages or phytochemicals of peach separately, but there is little information available on development of a carbonated peach beverage sweetened only with stevia and improved with lemon and mint without the addition of chemical preservatives (Parveen & Ain, 2023; Moreno et al., 2022). This is an important research gap in the development of naturally formulated functional beverages targeted to health-conscious consumers.The current study sought to fill this research gap by creating and assessing a carbonated functional beverage made without additional sugar or chemical preservatives using peach, stevia, lemon, and mint. The produced beverage's appropriateness as a functional beverage for diabetics and health-conscious people was assessed based on its physicochemical characteristics, antioxidant activity, sensory acceptability, microbiological quality, and storage stability.To our knowledge, the present study is one of the few studies that include peach, stevia, lemon, mint, and carbonation in one preservative-free functional beverage and is evaluated for its physicochemical, antioxidant, sensory, microbiological and storage properties under refrigerated conditions.

MATERIALS AND METHODS

2.1. Raw Materials

We purchased mature, disease-free fresh peaches *(Prunus persica L. cv. Hujingmilu)* from a fruit farm on Samundpur Road in Okara, Pakistan. We bought fresh lemons *(Citrus limon L.)* and mint leaves *(Mentha piperita L.)* at the Okara, Pakistan, local fruit and vegetable market. Fazaldeen Pharmacy in Okara, Pakistan, provided food-grade stevia powder (steviol glycosides, ≥95% purity, Rebaudioside A content 97%). We bought 99.9% pure food-grade CO2 gas from Micelles in Pakistan. Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA) provided all analytical-grade chemicals and reagents. During the production of the beverage, no artificial preservatives such as sodium benzoate, citric acid, or ascorbic acid were included. All raw materials were visually examined before processing for uniformity of ripening, free from physical damage and microbial spoilage. The fresh ingredients were processed immediately after procurement to minimise deterioration and preserve the physicochemical characteristics.

2.2. Beverage Development

Peach base preparation, stevia extraction, lemon juice extraction, mint extraction, formulation optimization, carbonation, pasteurization, packaging, and chilled storage were all used to create the carbonated functional beverage. Physicochemical traits, phytochemical qualities, antioxidant activity, sensory acceptability, microbiological quality, and storage stability of the final beverage were assessed. The overall experimental workflow was orientated to the development of a preservative-free functional beverage, ensuring desirable sensory quality, physicochemical stability and antioxidant potential during the refrigerated storage. The entire processing was carried out in sterile laboratory conditions to maintain the quality and reproducibility of the product.

2.3. Preparation of Peach Base

After being cleaned and pitted, fresh peaches were cut into little pieces. 500 g of peach slices were blanched in boiling water for two minutes, promptly cooled in ice water for one minute, and then blended for three minutes in a lab blender. Before being used, the puree was heated to 80°C for ten minutes while being continuously stirred, filtered through a cotton towel, cooled to room temperature, and kept at 4°C for no more than twenty-four hours.Blanching was performed to reduce enzymatic activity and minimize oxidative

browning prior to puree preparation. Cooling immediately after blanching helped preserve the natural color and quality of the peach puree before further processing.

2.4. Preparation of Stevia, Lemon, and Mint Extracts

10 g of stevia powder was dissolved in 100 mL of distilled water and stirred magnetically at 50°C for 30 minutes to create stevia extract. Whatman No. 1 filter paper was used to filter the mixture, which was then kept at 4°C. The concentration of the extract was changed to 10% (w/v). An Agilent 1260 Infinity II HPLC system was used to confirm the steviol glycoside level. Before being used, fresh lemon juice was manually squeezed, filtered through muslin cloth, and kept at 4°C. To make fresh mint extract, 100 g of mint leaves were homogenized with 200 mL of distilled water, heated to 60°C for 15 minutes, and then filtered using Whatman No. 1 filter paper.All extracts were prepared on the day of beverage formulation to minimize degradation of naturally occurring bioactive compounds and to maintain consistency among experimental treatments. The prepared extracts were kept under refrigerated conditions until incorporation into the beverage formulations.

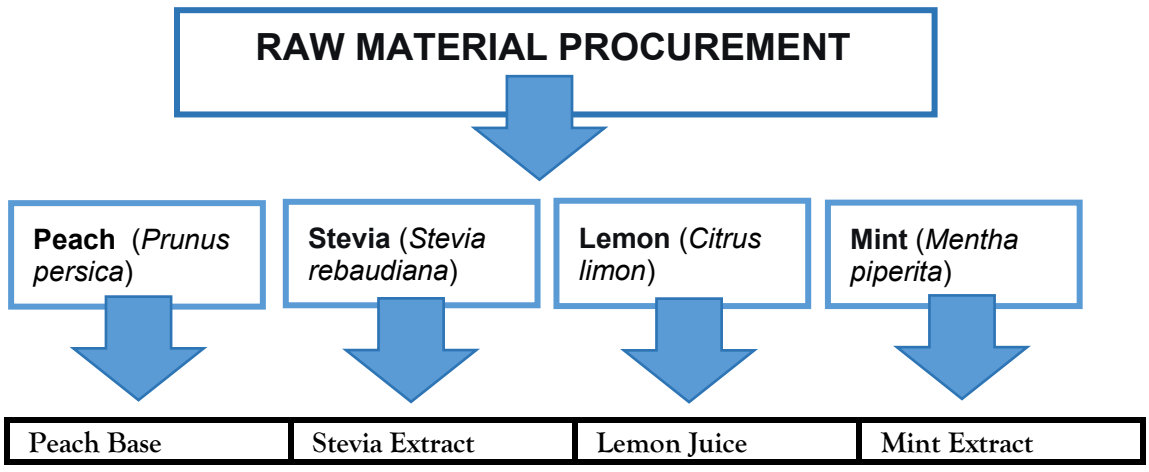
2.5. Beverage Formulation

There were four different beverage formulas made. 20% peach base and 8% sucrose made up the control treatment (T0). The experimental treatments included 1.0% lemon juice, 0.5% mint extract, and 20% peach base with different stevia concentrations (0.5–1.5% v/v). A final volume of 1 L was achieved by adding carbonated water.The formulations were developed to assess the physicochemical, antioxidant, microbiological and sensory properties of stevia sweetened beverages in comparison with the conventional sucrose sweetened control. All ingredients were thoroughly mixed before carbonation to obtain a homogeneous beverage formulation.

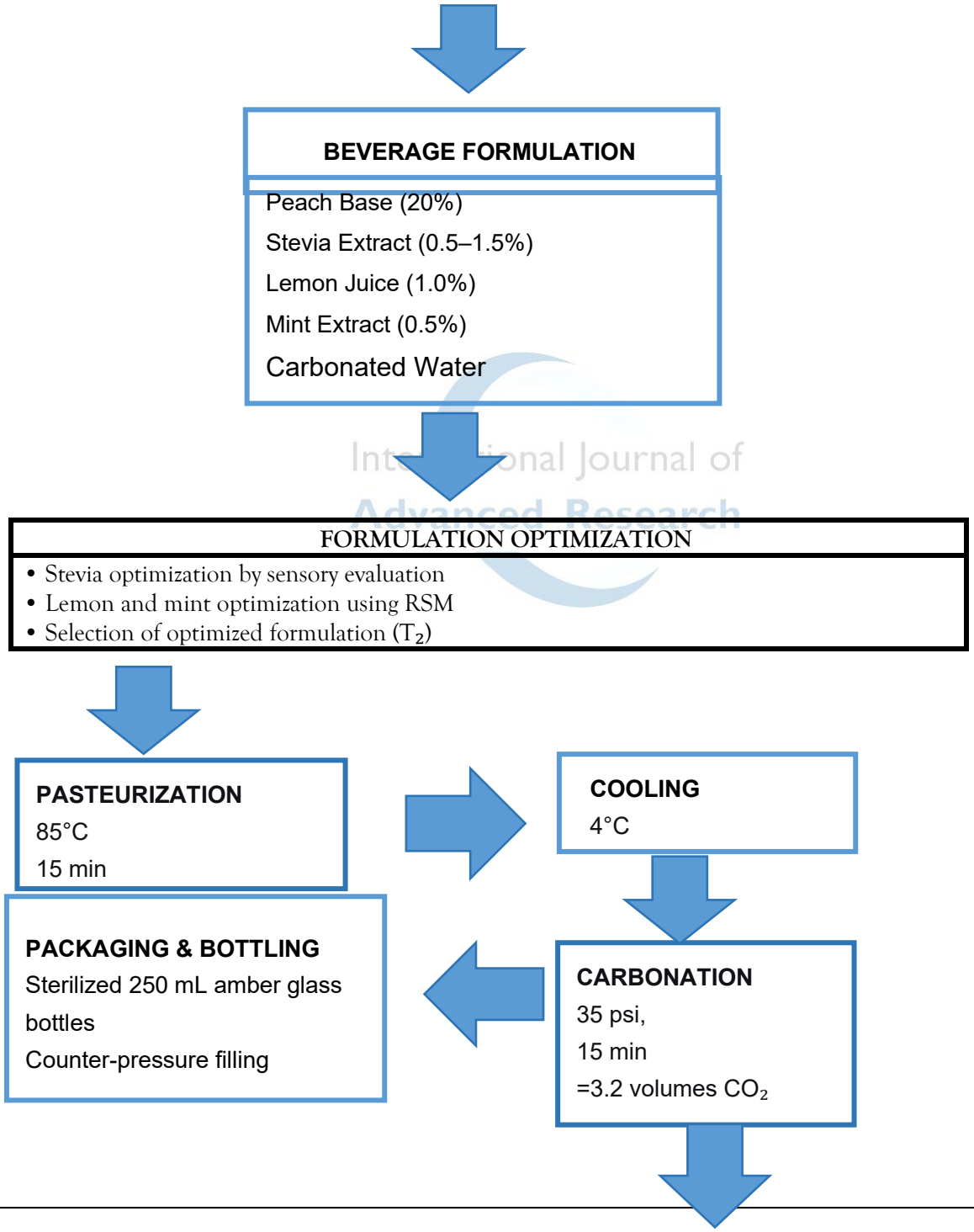
Table 1. Formulation of carbonated peach functional beverages

Treatment	Peach Base (%)	Stevia (% v/v)	Lemon (%)	Mint (%)	Sucrose (%)
T0	20	0	0	0	8.0
T1	20	0.5	1.0	0.5	0
T2	20	1.0	1.0	0.5	0
T3	20	1.5	1.0	0.5	0

All beverage formulations were prepared under identical processing conditions to ensure that differences among treatments were attributable only to formulation composition



Washing Peeling Pitting Blanching Homogenization 80°C, 10 min	Extraction 10% (w/v) 50°C 30 min Filtration	Washing Cutting Juicing Filtration	Washing Blot Dry Homogenization 60°C, 15 min Filtration



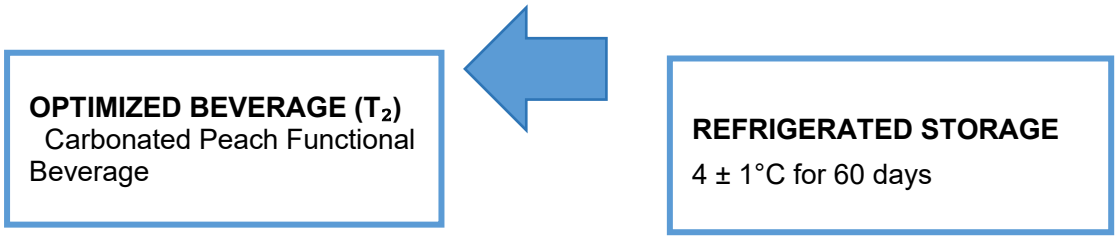


Figure.1.1. flow chart for the preparation of Majito beverage

2.6. Optimization of Formulation

By comparing sweetness intensity with an 8% sucrose control, stevia content was optimized by sensory evaluation. Response surface methodology (RSM) was used to adjust the quantities of lemon juice and mint extract in order to achieve optimal overall acceptance with balanced acidity and pH. The optimisation procedure was done sequentially. The optimum stevia concentration was first selected according to sensory acceptability. The selected level of stevia was then used to optimise the concentrations of lemon juice and mint extract for the most desirable balance of sweetness, acidity, aroma and overall acceptability. The optimised formulation (T2) was chosen for further physicochemical, antioxidant, microbiological, sensory and storage stability analyses.

2.7. Carbonation and Storage

The optimum beverage (T2) was put into sterile 250 mL amber glass bottles under counter pressure, sealed with crown caps, and kept at 4 ± 1°C for 60 days after being pasteurized at 85°C for 15 minutes, chilled to 4°C, and carbonated at 35 psi for 15 minutes to produce about 3.2 liters of CO2. Pasteurisation was done before carbonation to reduce the microbial load while maintaining the product quality. The carbonation was done after the desired cooling temperature was attained so that the dissolution and retention of the carbon dioxide would be efficient. Amber glass bottles were selected to minimise the possible deterioration of naturally occurring bioactive compounds due to light during refrigerated storage. Samples were taken at predetermined time intervals for physicochemical, antioxidant, microbiological, colour and sensory evaluation during the storage period.

2.8. Physicochemical Analysis

Using conventional AOAC (2016) techniques and calibrated laboratory equipment, the beverage was examined for pH, titratable acidity, total soluble solids (TSS), density, and color (L*, a*, b*). Three separate measurements were made. Titratable acidity was represented as a percentage of citric acid, and pH was measured with a calibrated digital pH meter. A digital refractometer was used to measure total soluble solids, which were then reported as °Brix. While color measurements (L, a, and b*) were collected using a calibrated colorimeter in accordance with the manufacturer's operating instructions, beverage density was calculated using normal laboratory procedures. To improve the dependability and repeatability of the experimental results, all physicochemical studies were conducted in triplicate, and statistical analysis was conducted using the means.

2.9. Phytochemical and Antioxidant Analysis

The Folin-Ciocalteu technique was used to calculate the total phenolic content (TPC), which was then represented as mg GAE/L. The DPPH radical scavenging assay was used to measure antioxidant activity, and the dose-response curve was used to calculate IC•¹ values. Every analysis was carried out three times. The total phenolic content was expressed as mg of gallic acid equivalents per litre (mg GAE/L) and the antioxidant activity as percentage of inhibition of DPPH free radicals. To compare the antioxidant efficiency of the different formulations, IC_{50} values were calculated, a lower IC_{50} value

indicates higher radical scavenging activity. All phytochemical and antioxidant analyses were performed under the same laboratory conditions to minimise analytical variation and ensure comparability among treatments.

2.10. Microbiological Analysis

Total plate count and yeast and mold count were used to assess microbiological quality in accordance with ISO 4833-1:2013 and ISO 21527-1:2008, respectively. After being refrigerated for 0, 15, 30, 45, and 60 days, the samples were examined. Microbiological evaluation was done for the whole refrigerated storage period for the evaluation of microbial safety and shelf stability of the developed beverage. The product safety during storage was assessed by the absence or presence of microbial growth.

2.11. Sensory Evaluation

A 9-point hedonic scale was used by thirty semi-trained panelists (15 males and 15 females, ages 20 to 45) to rate the beverage's color, scent, taste, mouthfeel, aftertaste, and general acceptability. Peach flavor, lemon flavor, mint flavor, sweetness, sourness, bitterness, and carbonation feeling were also assessed using a five-point intensity scale. Sensory evaluations were carried out in a controlled laboratory environment. Samples were coded and presented in random order to minimise evaluation bias. Panellists were provided with potable water to rinse their mouths between successive samples to minimise carry-over effects. The means of sensory scores of all panellists were used for statistical analysis and comparison of beverage formulations.

2.12. Statistical Analysis

Every experiment was carried out in triplicate, and the results were reported as mean ± standard deviation. SPSS version 26.0 was used for statistical analysis. Treatment means were compared at $p < 0.05$ using a one-way ANOVA and Tukey's HSD test. Data from sensory evaluations were subjected to Friedman's test, and correlations between physicochemical and sensory characteristics were ascertained using Pearson correlation analysis. Prior to the application of parametric statistical tests, the assumptions of normality and homogeneity of variances were verified. All analyses were considered statistically significant if $p < 0.05$.

RESULTS

3.1 Proximate Composition of Raw Ingredients

The proximate composition of the raw materials used for beverage preparation is presented in Table 2. Fresh peach contained the highest moisture content (86.2%), while lemon juice exhibited an even higher moisture level (91.5%) and the lowest fat content (0.2%). In contrast, stevia powder showed the highest carbohydrate (73.4%) and protein (10.5%) contents due to its concentrated dry composition. Fresh mint contributed moderate protein (3.2%) and crude fiber (2.1%), supporting its nutritional value.These variations in composition show that each component added special functional and nutritional qualities to the final product.The proximate composition of fresh peach (*Prunus persica L. cv. Hujingmilu*), stevia powder, lemon juice, and fresh mint leaves is presented in Table 2

Table 2. Proximate composition of raw ingredie

Parameter (%)	Fresh Peach	Stevia Powder	Lemon Juice	Fresh Mint
Moisture	86.2 ± 1.1	5.3 ± 0.2	91.5 ± 0.8	84.6 ± 1.3
Ash	0.6 ± 0.1	8.2 ± 0.3	0.3 ± 0.1	1.8 ± 0.2
Crude protein	0.9 ± 0.1	10.5 ± 0.5*	0.5 ± 0.1	3.2 ± 0.3
Crude fat	0.2 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.6 ± 0.1
Crude fiber	1.5 ± 0.2	2.1 ± 0.2	0.2 ± 0.0	2.1 ± 0.2
Carbohydrates	10.6 ± 0.5	73.4 ± 1.2	7.3 ± 0.4	7.7 ± 0.5

3.2 Optimization of Stevia Concentration

Table 3 displays the sensory responses acquired during stevia optimisation. From 2.1 at 0.25% stevia to 8.7 at 2.0% stevia, sweetness intensity gradually rose as stevia concentration increased. At increasing concentrations, however, aftertaste and harshness also increased. The 1.0% stevia concentration was chosen for later beverage preparation because it produced a sweetness score (7.1) that was equivalent to the sucrose control while retaining a comparatively low bitterness (1.5).With little bitterness and aftertaste, the 1.0% (v/v) stevia extract yielded sweetness comparable to 8% sucrose. Bitterness, metallic aftertaste, and licorice aftertaste were all markedly enhanced at higher concentrations (>1.25%). For all ensuing tests, 1.0% stevia extract (Treatment T2) was chosen.

Table 3. Sweetness intensity and off-taste perception of stevia-sweetened peach base (9-point scale, n = 20).

Stevia (% v/v)	Sweetness intensity	Bitterness	Metallic aftertaste	Licorice aftertaste	Sucrose equivalence
0.25	2.1 ± 0.5 (weak)	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	Below 4% sucrose
0.50	3.8 ± 0.6	1.1 ± 0.3	1.0 ± 0.0	1.0 ± 0.0	~5% sucrose
0.75	5.2 ± 0.7	1.3 ± 0.5	1.2 ± 0.4	1.1 ± 0.3	~6.5% sucrose
1.00	7.1 ± 0.8	1.5 ± 0.5	1.3 ± 0.5	1.2 ± 0.4	~8% sucrose
1.25	7.8 ± 0.6	2.3 ± 0.6	2.0 ± 0.6	1.8 ± 0.5	>8% sucrose (too sweet)
1.50	8.2 ± 0.5	3.1 ± 0.7	2.8 ± 0.7	2.5 ± 0.6	Excessively sweet & bitter
1.75	8.5 ± 0.4	3.9 ± 0.8	3.5 ± 0.8	3.2 ± 0.7	Unacceptable off-taste
2.00	8.7 ± 0.3	4.5 ± 0.9	4.2 ± 0.8	4.0 ± 0.8	Unacceptable

3.3 Optimization of Lemon and Mint Concentrations

The optimization results are presented in Table 4. Response surface methodology identified 1.0% lemon juice and 0.5% mint extract as the optimum combination, producing an overall acceptability of approximately 8.2, with a pH of 3.8 and titratable acidity of 0.28%. These findings show that without overpowering the inherent peach flavour, the chosen combination offered a favourable balance between acidity and sensory quality.

Table 3. Optimized lemon and mint concentrations and predicted responses.

Variable	Low	High	Optimum	Predicted OAA	Predicted pH	Predicted TA (%)
Lemon (% v/v)	0.5	2.0	1.0	8.2 ± 0.3	3.8 ± 0.1	0.28 ± 0.02
Mint (% v/v)	0.25	1.0	0.5	8.1 ± 0.4	3.9 ± 0.1	0.26 ± 0.02

3.4 Carbonation Level Optimization

The influence of carbonation pressure on sensory quality is presented in Table 5. Increasing carbonation pressure improved mouthfeel and overall acceptability up to 35 psi, where the beverage achieved the highest overall acceptability

(8.3/9) and mouthfeel score (8.0/9). A further increase to 45 psi reduced acceptability (5.8/9), suggesting that excessive carbonation negatively affected sensory quality.Consequently, the ideal carbonation pressure was determined to be 35 psi.

Table 5. Effect of carbonation pressure on sensory attributes.

Pressure (psi)	CO_2 volumes	Tingling sensation	Mouthfeel	Overall acceptability
25	2.5 ± 0.2	5.2 ± 0.6 (moderate)	5.5 ± 0.5	6.1 ± 0.7
35	3.2 ± 0.2	7.8 ± 0.5 (strong)	8.0 ± 0.4	8.3 ± 0.5
45	4.1 ± 0.3	8.5 ± 0.4 (very strong)	6.2 ± 0.7 (excessive bite)	5.8 ± 0.8

3.5 Physicochemical Properties

The physicochemical characteristics of the beverage formulations are presented in Table 6. The highest pH (4.2 ± 0.1), TSS (9.5 ± 0.2 °Brix) and density (1.015 ± 0.002 g/mL) were found in the sucrose control (T0) and the lowest titratable acidity (0.15 ± 0.01%) was observed in this sample. The TSS values were lower in the formulations containing stevia (4.2 ± 0.1 to 4.8 ± 0.1 °Brix) with T_2 showing 4.5 ± 0.1 °Brix. Both T_2 and T_3 had a pH of 3.8 ± 0.1 and a titratable acidity of 0.28 ± 0.02%. No significant differences were found in density values among the stevia treatments, which varied from 1.004 ± 0.001 to 1.006 ± 0.001 g/mL.

Table 6. Physicochemical properties of carbonated peach beverages.

Treatment	pH	TA (% citric acid)	TSS (°Brix)	Density (g/mL)
T0 (control, 8% sucrose)	4.2 ± 0.1[a]	0.15 ± 0.01[a]	9.5 ± 0.2[a]	1.015 ± 0.002[a]
T1 (0.5% stevia)	3.9 ± 0.1[b]	0.26 ± 0.02[b]	4.2 ± 0.1[b]	1.004 ± 0.001[b]
T2 (1.0% stevia)	3.8 ± 0.1[b]	0.28 ± 0.02[b]	4.5 ± 0.1[c]	1.005 ± 0.001[b]
T3 (1.5% stevia)	3.8 ± 0.1[b]	0.28 ± 0.02[b]	4.8 ± 0.1[c]	1.006 ± 0.001[b]

3.6 Total Phenolic Content and Antioxidant Activity

The antioxidant characteristics of the beverage formulations are summarized in Table 7. Treatment T_2 exhibited the highest total phenolic content (162.5 mg GAE/L) compared with the control (85.3 mg GAE/L). Similarly, DPPH radical scavenging activity increased from 42.1% in T_0 to 71.2% in T_2, whereas the IC_{50} value decreased to 26.9 mg/mL, indicating stronger antioxidant activity. These findings support the optimised beverage formulation's high functional qualities

Table 7. Total phenolic content (TPC), DPPH radical scavenging activity, and IC_{50} values of carbonated peach beverages (mean ± SD, n = 3).

Treatment	TPC (mg GAE/L)	DPPH Scavenging (%)	IC_{50} (mg/mL)	Treatment
T0 (control)	85.3 ± 4.2[a]	42.1 ± 2.3[a]	48.2 ± 2.5[a]	T0 (control)
T1 (0.5% stevia)	156.2 ± 5.8[b]	68.5 ± 3.1[b]	28.4 ± 1.8[b]	T1 (0.5% stevia)
T2 (1.0% stevia)	162.5 ± 6.1[b]	71.2 ± 3.4[b]	26.9 ± 1.6[b]	T2 (1.0% stevia)
T3 (1.5% stevia)	3.8 ± 0.1[b]	0.28 ± 0.02[b]	4.8 ± 0.1[c]	1.006 ± 0.001[b]

3.7 Color Characteristics

The instrumental color values are presented in Table 8. The optimized treatment (T_2) recorded L*, a*, and b* values of 37.9, 6.2, and 26.0, respectively, showing only minor variation compared with the control treatment. The very minor variations across treatments show that adding the natural ingredients preserved the beverage's acceptable appearance. The low ΔE values indicate that the visual differences between the formulations and the control treatment were minor.

Table 8. Color characteristics of carbonated peach beverages (mean ± SD, n = 3).

Treatment	L (lightness)*	a* (redness)*	b* (yellowness)*	ΔE (vs control)
T0 (control)	42.3 ± 1.2[a]	8.2 ± 0.4[a]	22.5 ± 0.8[a]	-
T1	38.6 ± 1.1[b]	6.5 ± 0.3[b]	25.1 ± 0.9[b]	5.2
T2	37.9 ± 1.0[b]	6.2 ± 0.3[b]	26.0 ± 0.9[b]	5.9
T3	37.5 ± 1.0[b]	6.0 ± 0.3[b]	26.3 ± 0.9[b]	6.3

3.8 Sensory Evaluation

The sensory evaluation results are presented in Table 9. Treatment T_2 consistently achieved the highest scores for taste (8.5), aroma (8.2), aftertaste (8.3), and overall acceptability (8.4/9). In comparison, T_3 showed a lower overall acceptability (6.9/9), suggesting that increasing the stevia concentration beyond the optimum level adversely affected sensory perception. Out of all the treatments, T_2 was found to be the most tolerable formulation.

Table 9. Sensory attributes of carbonated peach beverages (mean ± SD, n = 30).

Attribute	T0 (control)	T1	T2	T3
Color/appearance	7.2 ± 0.8[a]	6.9 ± 0.7[a]	7.1 ± 0.7[a]	6.8 ± 0.8[a]
Aroma	7.0 ± 0.9[a]	7.5 ± 0.8[ab]	8.2 ± 0.6[b]	7.8 ± 0.7[ab]
Taste	7.4 ± 0.8[a]	7.3 ± 0.9[a]	8.5 ± 0.5[b]	7.0 ± 0.9[a]
Mouthfeel (carbonation)	7.8 ± 0.7[a]	7.9 ± 0.6[a]	8.1 ± 0.5[a]	7.6 ± 0.8[a]
Aftertaste	7.0 ± 0.9[a]	7.2 ± 0.8[a]	8.3 ± 0.6[b]	6.5 ± 1.0[c]
Overall acceptability	7.3 ± 0.7[a]	7.4 ± 0.8[a]	8.4 ± 0.5[b]	6.9 ± 0.9[c]

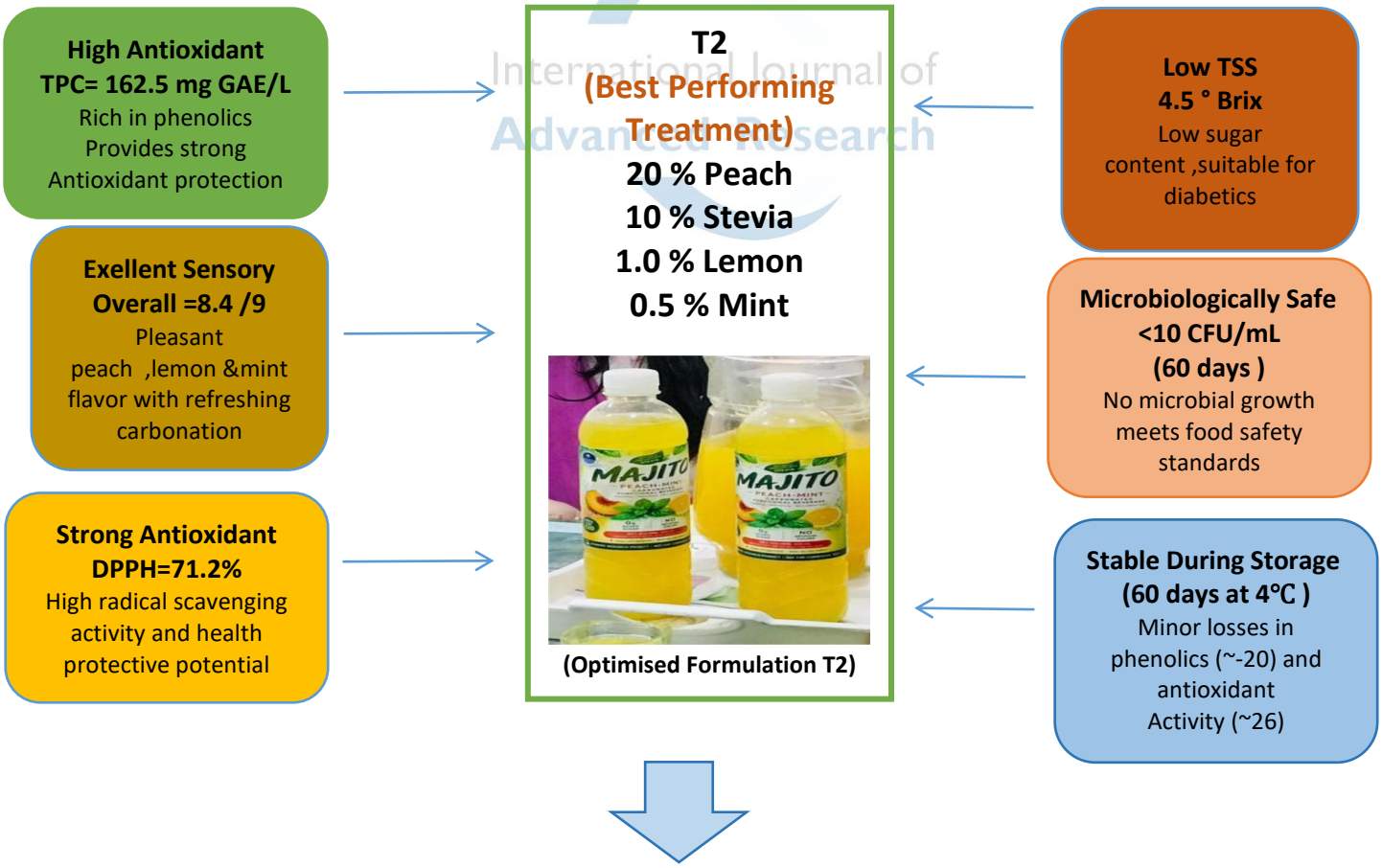
3.9 Storage Stability

Table 10 shows the optimised beverage's storage stability under refrigeration. Titratable acidity rose from 0.28% to 0.32% throughout the 60-day storage period, whereas pH marginally dropped from 3.81 to 3.68. DPPH radical scavenging activity dropped from 71.2% to 52.4%, and total phenolic content fell from 162.5 to 130.8 mg

GAE/L. Despite these gradual changes, microbial counts remained below the detectable limit (<10 CFU/mL) throughout storage. These findings indicate that the beverage maintained acceptable physicochemical quality, antioxidant activity, and microbiological safety during refrigerated storage without the addition of chemical preservatives.

Table 10. Changes in physicochemical properties, antioxidant activity, and microbiological quality of Treatment T2 during refrigerated storage (mean ± SD, n = 3).

Parameter	Day 0	Day 15	Day 30	Day 45	Day 60
pH	3.81 ± 0.05^{a}	3.79 ± 0.05^{a}	3.76 ± 0.06^{a}	3.72 ± 0.07^{ab}	3.68 ± 0.08^{b}
TA (% citric)	0.28 ± 0.01^{a}	0.29 ± 0.01^{a}	0.30 ± 0.01^{ab}	0.31 ± 0.01^{b}	0.32 ± 0.01^{b}
TSS (°Brix)	4.5 ± 0.1^{a}	4.5 ± 0.1^{a}	4.4 ± 0.1^{a}	4.4 ± 0.1^{a}	4.3 ± 0.1^{a}
TPC (mg GAE/L)	162.5 ± 6.1^{a}	158.2 ± 5.8^{a}	151.4 ± 6.0^{ab}	142.3 ± 5.5^{b}	130.8 ± 6.2^{c}
DPPH scavenging (%)	71.2 ± 3.4^{a}	69.5 ± 3.2^{a}	65.8 ± 3.5^{ab}	60.1 ± 3.0^{b}	52.4 ± 3.1^{c}
L* (lightness)	37.9 ± 1.0^{a}	37.2 ± 1.1^{a}	36.5 ± 1.0^{ab}	35.4 ± 1.2^{b}	34.1 ± 1.3^{c}
Microbial count (CFU/mL)	<10	<10	<10	<10	<10



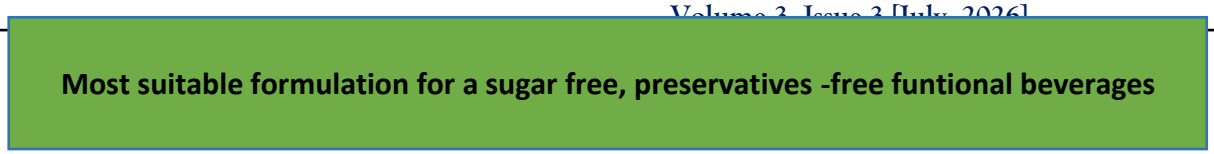


Figure.1.2.Summary of Best Performing Treatment

4. Discussion

Without the use of refined sugar or artificial preservatives, the current study successfully created a carbonated functional beverage made from peach *(Prunus persica)*, stevia *(Stevia rebaudiana)*, lemon *(Citrus limon)*, and mint *(Mentha piperita)*. During refrigerated storage, the improved formulation (T2) showed high sensory acceptability, outstanding antioxidant activity, desirable physicochemical qualities, and satisfactory microbiological stability. These results indicate that the chosen combination of natural ingredients and processing parameters was effective in producing a functional beverage with desirable attributes for a health-conscious consumer, while maintaining the quality of the product during storage.

4.1 Physicochemical Properties

Treatment T2 had a density of 1.005 g/mL, total soluble solids of 4.5°Brix, titratable acidity of 0.28%, and pH of 3.8. The stevia-based drinks had far fewer total soluble solids than the sucrose control while still having the proper acidity and pH for beverage stability (Raghavan et al., 2023). The lower total soluble solids of the stevia-sweetened formulations was expected because stevia provides intense sweetness without contributing significant soluble carbohydrates as does sucrose (Samuel et al., 2018). Therefore, the replacement of sucrose with stevia significantly reduced the °Brix value, without affecting the sweetness, and improved the nutritional profile of the beverage.Carbonated fruit-based beverages favour an acidic pH (about 3.8) as it enhances microbial stability, improves flavour perception and maintains product retention in refrigerated storage (Petruzzi et al., 2017). Similar pH values (3.7-4.0) have been reported for other fruit-based functional beverages (Prieto-Santiago et al., 2025). Moreover, the titratable acidity values obtained in the optimized formulation show an appropriate balance of acidity and sweetness, which is important for consumer acceptability (Stampanoni, 1993). These findings are consistent with earlier research showing that fruit-based drinks sweetened with natural non-caloric sweeteners typically have lower total soluble solids than drinks sweetened with sucrose while still having acceptable physicochemical quality (Raghavan et al., 2023). Similar results have also been documented for peach-based functional drinks, where the addition of natural fruit acids helped to achieve the right acidity and stability during storage (Papachristoudis et al., 2025). The addition of lemon juice increased the acidity and improved the flavor balance (Gandhi et al., 2020), while the addition of mint extract improved the overall freshness of the beverage without affecting the measured physicochemical parameters (Soleimani et al., 2022). This optimized formulation achieved a desirable balance of sweetness, acidity and carbonation which is essential for a commercially acceptable carbonated functional beverage.

4.2 Total Phenolic Content and Antioxidant Activity

In comparison to the sucrose control, treatment T2 exhibited the highest total phenolic content (162.5 mg GAE/L), DPPH radical scavenging activity (71.2%), and the lowest IC_{50} value (26.9 mg/mL), indicating greater antioxidant capability. The enhanced antioxidant activity for the optimized formulation is attributed mainly to the naturally occurring phenolic compounds in peach, lemon and mint and not the sweetening agent itself (Bento et al., 2020; Wu et al., 2022). The combined effect of such ingredients probably led to a synergistic effect of the antioxidants, which resulted in higher total phenolic content and free radical scavenging activity in Treatment T2 (Zhou et al., 2025; Soares et al., 2022).The

inverse correlation between total phenolic content and IC_{50} values obtained in the present study provided further support for the contribution of phenolic compounds to antioxidant capacity (Bie et al., 2024). The lower IC_{50} values indicated the better efficacy to neutralize the free radicals, thus confirming the optimized formulation with higher antioxidant performance. Beverages with higher phenolic contents typically showed better DPPH radical scavenging efficacy, according to similar findings from earlier research assessing fruit-based functional beverages (Peng et al., 2025; Ossowski et al., 2025). These results provide credence to the idea that naturally occurring phytochemicals significantly increase fruit beverages' functional value (Carvalho et al., 2023).Oxidative stress is considered to be an important factor in the development of diabetes mellitus and its complications (Sarkar et al., 2022). Therefore, beverages with higher levels of natural antioxidant compounds may offer additional health benefits by assisting in the reduction of oxidative damage (Golovinskaia & Wang, 2023). Studies have demonstrated that phenolic compounds from peach and other fruits can effectively scavenge free radicals and reduce oxidative stress markers (Petruccelli et al., 2023; Bento et al., 2020). The high antioxidant capacity of the optimized beverage indicates promising potential as a functional beverage for diabetic and health-conscious consumers, although biological activity in vivo was not tested in the present study.

Relationship Between Physicochemical Properties and Antioxidant Activity

Another interesting observation in the present study was that the optimized formulation showed good physicochemical properties and the highest antioxidant activity. This implies that the optimization of the ingredient composition successfully balanced the beverage stability and the functional quality (Garcia-Aparicio et al., 2024). The acidic environment resulting from the formulation might have also contributed to improved stability of some phenolic compounds during refrigerated storage and thus helped maintain antioxidant activity during the experimental period (Ossowski et al., 2025).In conclusion, the physicochemical and antioxidant results showed that the substitution of sucrose with stevia, combined with peach, lemon and mint, resulted in a beverage with improved functional properties without affecting the product quality (Raghavan et al., 2023). These findings are in favor of the potential use of natural ingredients in the formulation of healthier carbonated beverages with improved antioxidant potential.

4.3. Color Characteristics

With very slight variations in the L, a, and b* values, the improved formulation retained desired color properties during refrigerated storage, demonstrating acceptable color stability (Moreno et al., 2022). Treatment T2 maintained the characteristic appearance expected of a peach-based beverage while displaying a suitable balance of lightness, redness, and yellowness. Colour is one of the main quality attributes evaluated by consumers and greatly affects product acceptance (Pathare et al., 2013). Color changes in fruit beverages are often linked to enzymatic and non-enzymatic browning, pigment degradation, oxidation of phenolic compounds and storage conditions (Wibowo et al., 2015). Therefore, color stability during storage is an important indicator of product quality (Han et al., 2025).The relatively stable color in the optimized formulation suggests that the selection of processing conditions such as blanching, pasteurization, carbonation and refrigerated storage were effective on minimizing the pigment degradation during storage (Papachristoudis et al., 2025). Also, the acidic nature of the beverage may have contributed to the stability of naturally occurring pigments by minimizing oxidative reactions that normally occur during prolonged storage (Bie et al., 2024). Similar findings have been documented in research on fruit-based functional drinks, where proper processing conditions and chilled storage helped maintain color quality while reducing unwanted visual alterations (Prieto-Santiago et al., 2025). The relatively small ΔE values obtained in the present study indicate that the differences in color among

the formulations remained within an acceptable range corroborating the visual stability of the developed beverage throughout the storage period.The obtained color stability in this study is especially relevant because the beverage was formulated without the addition of artificial color stabilizers or chemical preservatives, thus demonstrating that the desirable visual quality can be achieved using only natural ingredients and appropriate processing conditions (Martin-Diana et al., 2025).

4.4. Sensory Evaluation

With an overall acceptance score of 8.4 on the nine-point hedonic scale, treatment T2 received the greatest ratings for scent, taste, aftertaste, and overall acceptability. Conversely, worse sensory scores were obtained when the stevia content was increased over the optimal level, especially for aftertaste and general acceptability (Parveen & Ain, 2023). The sensory quality of functional beverages is a major determinant of consumer acceptance, as nutritional value alone is not enough to ensure market success (Verbeke, 2006). Thus, it is important to achieve a proper balance of sweetness, acidity, aroma, carbonation and mouthfeel in beverage formulation (Moreno et al., 2022).The superior sensory performance of Treatment T2 suggested that the optimum concentration of stevia could replace sucrose without the unpleasant bitterness generally associated with high concentrations of steviol glycosides (Samuel et al., 2018). The peach, lemon and mint blend was optimized to enhance flavor complexity with a balance of sweetness with natural acidity and refreshing herbal notes (Soleimani et al., 2022). Treatment T3's lower sensory scores are in line with stevia's well-established sensory traits at greater concentrations, where excessive sweetness is sometimes accompanied by bitter, metallic, or licorice-like aftertastes (Anton et al., 2010; Alhalawi & Ervita, 2025). These undesirable organoleptic properties are primarily associated with specific steviol glycosides, which are more pronounced at higher concentrations, thereby reducing overall consumer acceptance (Raghavan et al., 2023).Carbonation also had a positive influence on the sensory perception by improving the mouthfeel and increasing the perception of freshness (Moreno et al., 2022). The carbonation pressure chosen (35 psi) provided a proper amount of effervescence to enhance the fruit flavor without being too sharp or irritating (Prieto-Santiago et al., 2025). This led to an optimized formulation with a good balance of all the main sensory parameters measured in this study. The overall acceptability score of 8.4 indicates that the consumers accepted the optimized formulation positively, and the study shows the potential of natural sweetening with stevia to replace sucrose successfully, as long as the formulation is optimized properly. These results support the feasibility of developing sugar-free carbonated beverages with high consumer acceptance (Han et al., 2025).

4.5. Storage Stability

During the 60-day refrigerated storage period, the optimized formulation (T_2) exhibited only minor changes in its physicochemical, antioxidant, and microbiological characteristics, indicating satisfactory overall storage stability (Ossowski et al., 2025). The pH decreased slightly from 3.81 to 3.68, while titratable acidity increased from 0.28% to 0.32%, reflecting the expected gradual changes in organic acid composition during storage (Papachristoudis et al., 2025). Likewise, only small variations were observed in total soluble solids and instrumental color values, demonstrating that the beverage maintained its physicochemical integrity throughout refrigerated storage (Bie et al., 2024).The antioxidant properties of the optimized beverage gradually declined during storage. Total phenolic content decreased from 162.5 to 130.8 mg GAE/L, representing an approximately 20% reduction, while DPPH radical scavenging activity decreased from 71.2% to 52.4%, corresponding to an approximately 26% reduction after 60 days (Peng et al., 2025). These decreases are expected in fruit-based functional beverages and are primarily attributed to the gradual oxidation, polymerization, and degradation of naturally occurring phenolic compounds during storage (Zhou et al., 2025). Nevertheless, despite these

reductions, the beverage retained considerable antioxidant activity at the end of the storage period, indicating that a substantial proportion of the bioactive compounds remained stable under refrigerated conditions (Garcia-Aparicio et al., 2024).Throughout the storage study, no detectable microbial growth (<10 CFU/mL) was observed, confirming the microbiological safety of the beverage without the addition of chemical preservatives (Parveen & Ain, 2023). The absence of microbial proliferation demonstrates that the selected formulation and processing conditions were sufficient to ensure product safety during refrigerated storage (Prieto-Santiago et al., 2025). The combination of several preservation factors, such as pasteurisation (85°C for 15 minutes), acidic pH (about 3.8), carbonation (about 3.2 volumes of CO2), hygienic processing techniques, and continuous refrigerated storage (4 ± 1°C), can explain this microbiological stability. When combined, these preservation elements serve as a prime illustration of hurdle technology, where a number of gentle preservation techniques work in concert to prevent the growth of microorganisms without the need for artificial preservatives like sodium benzoate (Petruzzi et al., 2017).Previous studies on acidic fruit beverages have similarly reported that the combination of mild heat treatment, low pH, dissolved carbon dioxide, and refrigerated storage effectively suppresses spoilage microorganisms while preserving product quality during storage (Moreno et al., 2022; Han et al., 2025). The present findings therefore support the effectiveness of this preservation strategy in maintaining microbiological safety using only natural ingredients and appropriate processing conditions.Overall, although gradual reductions in antioxidant compounds occurred during refrigerated storage, the optimized beverage maintained acceptable physicochemical quality, substantial antioxidant capacity, desirable sensory characteristics, and complete microbiological safety throughout the 60-day storage period (Ossowski et al., 2025). These findings demonstrate that the selected formulation and processing conditions successfully produced a sugar-free, preservative-free carbonated functional beverage with satisfactory shelf stability, supporting its potential for future commercial development as a clean-label functional beverage for health-conscious and diabetic consumers.

4.6. Practical Significance and Novelty of the Study

The results of the present study demonstrate the feasibility of developing a naturally formulated carbonated functional beverage without the addition of refined sugar or chemical preservatives while maintaining desirable physicochemical, antioxidant, sensory and microbiological characteristics. The optimized formulation successfully combined the nutritional benefits of peach with the natural sweetening properties of stevia and the functional contributions of lemon and mint to produce a beverage with high consumer acceptability and satisfactory storage stability (Raghavan et al., 2023; Prieto-Santiago et al., 2025). Unlike many commercially available diet beverages that use artificial sweeteners and synthetic preservatives, the beverage developed was formulated with 100% natural ingredients. The present approach is in response to the increasing consumer demand for clean-label products that can deliver nutritional and functional benefits while minimizing the use of synthetic food additives (Carvalho et al., 2023).This study is novel for the formulation of a carbonated beverage with peach (Prunus persica), stevia (Stevia rebaudiana), lemon (Citrus limon) and mint (Mentha piperita), which was optimized by sensory evaluation and response surface methodology, and a complete evaluation of its physicochemical properties, antioxidant activity, sensory characteristics, microbiological quality and refrigerated storage stability (Soares et al., 2022; Martin-Diana et al., 2025). Only a few studies have explored the association of these ingredients in the preservative free state to the best of our knowledge (Parveen & Ain, 2023; Moreno et al., 2022). Thus, the optimized beverage is a promising prototype for the development of healthier carbonated beverages targeted for diabetic and health-conscious consumers while supporting current trends

toward natural, functional, and clean label food products.

4.7. Study Limitations

Despite the positive outcomes of the current study, a number of caveats should be noted. First, more research is needed to evaluate process performance under pilot-scale and commercial manufacturing circumstances because the beverage was made and assessed under laboratory-scale conditions. Second, the behaviour of the beverage under ambient storage settings was not examined; storage stability was only assessed under refrigeration for 60 days. Third, despite the beverage's strong antioxidant activity and sugar-free formulation, its possible health advantages for diabetics were deduced from its composition and functional characteristics rather than verified by in vivo or clinical studies (Chowdhury et al., 2022). Lastly, only a small number of semi-trained panellists participated in the consumer sensory evaluation; bigger consumer research with a wider range of demographics would support the results.

4.8. Future Perspectives

Future research should include carefully planned human clinical trials and animal tests to assess the produced beverage's glycaemic response and long-term health consequences (Sarkar et al., 2022). The bioavailability of the phenolic compounds and other bioactive components after ingestion should be further studied (Peng et al., 2025). Antioxidant chemicals may be better preserved and product shelf life may be increased with more carbonation, packaging, and storage condition optimisation (Ossowski et al., 2025). Additionally, economic feasibility studies and pilot-scale production would offer important insights into the created beverage's commercial viability (Garcia-Aparicio et al., 2024). In order to further improve the nutritional and functional qualities of the beverage while preserving the desired sensory quality, future research may potentially examine the addition of extra functional ingredients, such as dietary fibre, probiotics, or plant-derived bioactive compounds (Han et al., 2025; Prieto-Santiago et al., 2025). Future investigations should also focus on establishing the microbiological safety, stability, and quality consistency of the beverage throughout its intended storage period. Comprehensive assessments of microbial contamination, spoilage organisms, and potential food-safety hazards would be valuable, particularly because the beverage is formulated as a low-pH functional product. Such investigations could incorporate systematic microbiological monitoring and risk-based safety assessment approaches, similar to those emphasized in studies addressing microbial safety and foodborne hazards in food products (Butt et al., 2024; Riaz et al., 2026; Farrukh et al., 2026). Further work should also investigate how formulation, processing, and storage conditions influence microbial stability and the retention of functional constituents.

The interaction between the beverage's bioactive compounds and the gastrointestinal environment represents another important research direction. Future studies should characterize the digestion, absorption, metabolism, and biological activity of phenolic and other functional constituents following consumption. Particular attention could be given to the relationship between functional-food consumption and gut microbiota, as emerging evidence indicates that dietary bioactive compounds may influence microbial composition and inflammation-related metabolic processes (Nawaz et al., 2026). In this context, in-vitro gastrointestinal digestion models followed by cellular, animal, and ultimately human investigations could determine whether the antioxidant and metabolic benefits observed at the product level translate into meaningful physiological effects. Research examining gut microbiome-mediated responses could further clarify whether the beverage contributes to improved metabolic health through interactions between dietary bioactives and intestinal microorganisms.

Moreover, the future research should further strengthen the safety and nutritional validation of the developed beverage through integrated preclinical and clinical investigations. Animal studies could initially be used to investigate dose-

dependent effects, organ safety, oxidative-stress biomarkers, and metabolic responses before progression to controlled human trials. Nutritional and biosafety assessment of novel protein-based food systems in animal models provides a useful framework for evaluating both nutritional performance and potential biological risks before human application (Khan et al., 2024; Butt et al., 2025). In addition, comparative evaluation of food products using physicochemical, microbial, cooking, textural, and sensory parameters demonstrates the importance of multidimensional product characterization rather than relying on a single quality indicator (Butt et al., 2025). Future beverage studies should therefore combine nutritional, biochemical, microbiological, and sensory endpoints to establish a comprehensive evidence base.

The functional composition of the beverage could also be expanded through the incorporation of carefully selected protein, plant-based, and bioactive ingredients. Research on sustainable whey–corn hybrid protein systems has demonstrated the potential for combining nutritional enhancement with functionality and environmental considerations (Butt et al., 2025). Similarly, comparative evaluation of oat flour and soy protein incorporation highlights the importance of simultaneously examining nutritional, textural, antioxidant, and sensory properties when developing functional food products (Adnan et al., 2026). Such approaches could be adapted to the present beverage to determine whether protein or plant-derived components can increase nutritional density without adversely affecting solubility, mouthfeel, colour, flavour, carbonation, or consumer acceptance.

Probiotic fortification represents another promising avenue. The successful utilization of *Lactobacillus rhamnosus* as a probiotic adjunct culture demonstrates the feasibility of incorporating beneficial microorganisms into functional food systems (Ahmed et al., 2024). Future investigations should determine whether suitable probiotic strains can remain viable under the beverage's low-pH and carbonated conditions and whether processing, packaging, and storage affect their survival. Research on functional probiotic consumption in relation to metabolic health further supports investigating functional beverages within broader nutritional and metabolic-health frameworks (Rashid et al., 2026). However, probiotic inclusion should be supported by strain-specific stability testing and appropriately designed clinical trials rather than assumed to confer health benefits solely from the presence of live microorganisms.

The beverage could also be investigated as part of a broader precision-nutrition strategy. Research examining dietary zinc supplementation and IGF-1 expression illustrates how nutritional interventions can be evaluated in relation to specific physiological and biochemical biomarkers (Butt et al., 2026). Similarly, work on phytochemical-rich functional diets and epigenetic markers associated with obesity and insulin resistance suggests that bioactive-rich dietary interventions may warrant investigation at molecular as well as physiological levels (Butt et al., 2026). Future studies could therefore assess whether consumption of the developed beverage influences glucose regulation, insulin sensitivity, inflammatory markers, oxidative-stress indicators, and selected epigenetic or metabolic biomarkers. Such investigations would help determine whether the beverage has measurable effects beyond its immediate antioxidant capacity.

The interaction between beverage-derived bioactive compounds and the gut microbiome should receive particular attention. Functional foods have increasingly been investigated as potential modulators of gut microbial composition and low-grade chronic inflammation (Nawaz et al., 2026). In parallel, research linking gut microbiome diversity with severe acute malnutrition illustrates the importance of microbial composition in nutritional health (Shazif et al., 2026). Future studies could therefore employ simulated gastrointestinal digestion, fecal fermentation models, microbiome sequencing, metabolomics, and controlled human interventions to determine whether the beverage influences microbial diversity or the production of beneficial metabolites. Individual

differences in baseline microbiota should also be considered when assessing variability in response.

A particularly advanced direction would involve AI-assisted nutrigenomic modelling. Computational investigation of blueberry anthocyanin–gut microbiome interactions provides an example of how predictive models can be used to explore relationships between dietary bioactives, microbial metabolites, and metabolic outcomes (Butt et al., 2026). Similarly, the development of polyphenol-rich pomegranate peel nutraceuticals illustrates the potential of phenolic compounds for investigating glycaemic regulation, insulin sensitivity, and oxidative-stress reduction (Butt et al., 2026). Future beverage research could use comparable modelling strategies to predict how combinations of phenolic compounds, fibre, probiotics, and other functional ingredients may influence metabolic pathways. These models should primarily be regarded as hypothesis-generating tools and should subsequently be validated through experimental and clinical research.

AI could also contribute to formulation optimization and quality prediction. AI-guided precision fermentation has been proposed as a framework for developing personalized functional foods and optimizing nutritional characteristics (Butt et al., 2026). More broadly, integration of laboratory biomarkers, imaging, and molecular data in AI-assisted cardiovascular diagnostics illustrates the potential of combining heterogeneous datasets within predictive systems (Arif et al., 2026). Future beverage research could similarly integrate formulation composition, pH, carbonation, antioxidant activity, phenolic retention, microbial stability, sensory responses, and storage data into machine-learning models. Such systems could identify promising formulations and processing conditions before extensive experimental optimization is undertaken.

The application of adaptive and intelligent digital systems may be extended to consumer and research-management dimensions. Studies of AI tools in language acquisition and teaching demonstrate the potential of intelligent systems to support adaptive, data-driven decision-making (Kamal et al., 2026), while research on AI-assisted learning highlights their potential for personalized information delivery and improved learning outcomes (Faizi et al., 2026). In the context of functional beverages, similar approaches could be explored for personalized consumer education, dietary guidance, and interpretation of nutritional information. Adaptive digital platforms could potentially provide consumers with evidence-based information according to their nutritional characteristics, although future studies should carefully assess the accuracy, transparency, and ethical implications of algorithm-generated health information.

Advanced processing technologies should also be explored to improve functional stability and product quality. Ultrasound-assisted modification of soy protein isolate demonstrates the potential of ultrasound processing to alter functional and nutritional properties (Butt et al., 2026). Likewise, ultrasonication combined with microbial transglutaminase crosslinking has been investigated as an advanced strategy for modifying proteins in sustainable food systems (Butt et al., 2026). Although these studies concern protein systems rather than beverages, their technological principles could inform future investigations into non-conventional processing methods for improving ingredient dispersion, stability, or functional-component retention. Any application to the beverage should be evaluated for possible effects on phenolic compounds, carbonation, sensory properties, microbial safety, and storage stability.

Future studies should additionally investigate sustainable sources of functional ingredients. Research on plant-based functional foods highlights the expanding nutritional potential of plant-derived ingredients (Riaz et al., 2026), while the valorization of watermelon-peel flavonoids demonstrates the possibility of recovering kaempferol, quercetin, and rutin as natural antioxidant compounds for functional-food applications (Butt et al., 2026). These findings suggest that agricultural and food-processing by-products could potentially serve as economical sources of bioactive compounds for future

beverage formulations. Such an approach could simultaneously increase functional value and support food-waste reduction, provided that contaminant levels, bioavailability, sensory effects, and regulatory requirements are appropriately addressed.

Agricultural biotechnology could provide an additional long-term pathway for improving the consistency of raw materials used in functional beverages. CRISPR-Cas-mediated genome editing for crop disease resistance demonstrates the potential of biotechnology to improve crop resilience and agricultural productivity (Jabeen et al., 2025). Similarly, CRISPR-Cas12a-mediated epigenome editing has been investigated to enhance drought survival in *Zea mays* (Fatima et al., 2026), while foliar trehalose application has been explored to alleviate salinity stress in cauliflower (Shafeeq et al., 2026). Although these technologies are not directly part of beverage formulation, future interdisciplinary research could investigate whether improved crop resilience and more consistent phytochemical profiles can contribute to reliable supplies of functional raw materials. Such applications would require separate evaluation of food safety, regulatory status, nutritional composition, and consumer acceptance.

Food-safety research should be expanded beyond conventional microbial analysis to include adulteration, heavy metals, antimicrobial resistance, and emerging contaminants. Research on raw cow and buffalo milk has demonstrated the importance of simultaneously evaluating microbiological safety, adulteration, and heavy-metal-associated health risks (Riaz et al., 2026). Similarly, research on antimicrobial resistance and virulence determinants in *Staphylococcus aureus* highlights the broader importance of monitoring pathogenic microorganisms and their resistance characteristics (Farrukh et al., 2026). Future beverage investigations should therefore establish comprehensive hazard-monitoring protocols covering relevant pathogens, spoilage organisms, contaminants, and adulterants. Where rapid screening methods are employed, their results should be confirmed using validated analytical techniques, consistent with the distinction between screening and confirmatory diagnostic approaches demonstrated in comparative HCV testing research (Nadeem et al., 2026).

Emerging environmental contaminants should also be considered where relevant to raw materials and processing environments. Multi-omics investigation of aquatic organisms exposed to environmental contaminants demonstrates the value of integrating molecular information when evaluating complex toxicological responses (Ullah et al., 2026). Similarly, toxicological evaluation of microplastic and nanoplastic exposure highlights the importance of monitoring oxidative stress and histopathological effects when assessing potential environmental contaminants (Ullah et al., 2026). Future beverage research could therefore incorporate appropriate contaminant screening, particularly where ingredients, water sources, packaging materials, or processing environments create potential exposure pathways. Such assessments would strengthen the overall safety profile of the product.

The biological evaluation of the beverage could also be broadened through multidisciplinary health-outcome research. Studies examining long-term alterations in knee joint loading following ACL reconstruction demonstrate how nutritional and physical-health research can benefit from objective physiological and functional measurements (Mahmood et al., 2026). Similarly, research on post-exercise low-carbohydrate recovery diets demonstrates the value of evaluating dietary interventions in relation to bone mineral turnover, hormonal stability, and lean-mass preservation (Butt et al., 2026). Future beverage trials could therefore examine whether specific consumer groups, including physically active individuals, respond differently to the beverage in terms of glycaemic response, recovery-related biomarkers, oxidative stress, or nutritional status. These investigations should remain exploratory until supported by appropriately powered clinical studies.

Long-term nutritional research could also examine the beverage within different dietary and socioeconomic contexts. Research investigating

dietary habits, nutritional status, and socioeconomic factors among university students in Punjab demonstrates that dietary behaviour is influenced by broader social and economic circumstances (Riaz et al., 2026). Likewise, research on diet quality and health-associated outcomes among young adults illustrates the value of considering overall dietary patterns rather than evaluating a single food or beverage in isolation (Gulzar et al., 2026). Future studies should therefore examine how the beverage fits within habitual diets, whether it displaces less nutritious alternatives, and whether its potential benefits remain meaningful under real-world consumption conditions.

Accessibility and equity should similarly be considered during future commercialization. Research examining migration, displacement, and health inequities emphasizes that access to health-related resources can vary substantially between populations (Riaz et al., 2026). Future development should consequently consider affordability, availability, cultural acceptability, and accessibility across different socioeconomic groups. In addition, research addressing wellbeing-oriented human-resource management and workforce sustainability highlights the importance of considering human and organizational dimensions when evaluating sustainability (Rehman et al., 2026). Therefore, future commercial development should assess not only environmental sustainability but also workforce practices, supply-chain equity, and social responsibility.

Consumer communication should be designed carefully to avoid exaggerated functional or therapeutic claims. Research on orthodontic treatment and patient-specific risk factors demonstrates the importance of recognizing individual variability when interpreting health-related outcomes (Khan et al., 2026). Similarly, clinical evidence concerning silver diamine fluoride and caries arrest illustrates the importance of distinguishing between an intervention's demonstrated clinical application and broader assumptions about health benefits (Khan et al., 2026). These principles are relevant to functional beverages because consumers may interpret terms such as "antioxidant," "functional," or "metabolic health" as evidence of therapeutic efficacy. Future research should therefore evaluate consumer understanding of health claims and establish communication strategies based strictly on experimentally and clinically validated evidence.

Packaging, fortification, and stability studies should also be expanded. The development and stability assessment of calcium- and vitamin-D3-fortified chocolate spread demonstrates that fortification must be evaluated alongside physicochemical stability and product quality (Zaidi and Randhawa, 2025). Future beverage studies could similarly investigate selected micronutrient fortification strategies where a clear nutritional rationale exists, while assessing interactions with acidity, phenolic compounds, carbonation, packaging, and storage. Nutritional biomarkers may additionally be incorporated into clinical investigations, as research on biomarkers and rehabilitation outcomes demonstrates the potential value of linking nutritional status with measurable physiological outcomes (Zaidi et al., 2026).

Sustainability should ultimately be evaluated as a multidimensional concept rather than being restricted to carbon emissions. Research examining the social dimensions of sustainability performance in emerging-market firms provides a broader framework for considering environmental, social, and organizational outcomes simultaneously (Khurshid et al., 2026). Future beverage development should therefore incorporate life-cycle assessment, water and energy consumption, packaging waste, ingredient sourcing, food-by-product utilization, transportation requirements, and social sustainability. Sustainable protein research similarly demonstrates that nutritional functionality and environmental stewardship can be considered together during product development (Butt et al., 2025).

Commercial scale-up will require systematic economic and operational modelling. Simulation-based analysis of AI-enhanced venture decision systems provides a useful conceptual basis for examining complex business decisions under

uncertainty (Naeem et al., 2026). Future research could apply comparable modelling approaches to ingredient costs, production capacity, market demand, packaging alternatives, storage requirements, and sensitivity to changes in raw-material prices. Risk governance frameworks can further provide a structured approach for identifying, prioritizing, and controlling technical, financial, regulatory, and operational risks during commercialization (Butt et al., 2026). Such approaches would complement conventional pilot-scale production and techno-economic analysis.

Digital automation could additionally support future manufacturing and quality-control systems. Research examining AI automation and its influence on productivity and job redesign illustrates the potential organizational implications of implementing intelligent technologies (Naeem et al., 2026). Machine-learning approaches developed for fraud detection similarly demonstrate the ability of algorithms to identify abnormal patterns within complex datasets (Naeem et al., 2026). Future beverage manufacturing could potentially adapt these principles for automated detection of deviations in pH, carbonation, ingredient concentration, microbial indicators, packaging integrity, and storage conditions. Such systems could improve consistency and early identification of quality failures, although their performance would need to be validated against established laboratory methods.

Advanced computational modelling may also support the broader systems-level optimization of the beverage. Multi-scale computational research in complex physical systems demonstrates how modelling can be used to understand interactions occurring across different scales (Shah and Butt, 2026). While the specific application differs substantially from food science, the methodological principle could be adapted to beverage research by integrating molecular, formulation, processing, sensory, and consumer-level information within a unified modelling framework. Similarly, AI-based adaptive protection systems demonstrate how intelligent systems can respond dynamically to changing conditions (Awais and Butt, 2026). Future beverage-processing systems could explore analogous adaptive approaches for real-time adjustment of processing or quality-control parameters in response to monitored product conditions.

Finally, future research should increasingly adopt a systems-based and interdisciplinary perspective. The development of a next-generation functional beverage should not be restricted to antioxidant activity, pH, sensory evaluation, or short-term glycaemic response. Instead, future work should integrate food chemistry, microbiology, nutrition, clinical science, gut microbiome research, nutrigenomics, agricultural biotechnology, AI, computational modelling, sustainability, consumer science, and health economics. Even research from apparently distant domains can contribute methodological perspectives: for example, interdisciplinary approaches to AI, sustainability, personalized learning, and complex-system governance demonstrate the value of integrating heterogeneous information and adapting interventions to specific contexts (Kamal et al., 2026; Amanat et al., 2026; Butt et al., 2026). In addition, research on food formulation, functional ingredients, protein systems, and plant-based foods provides directly transferable approaches for improving nutritional and technological performance (Butt et al., 2025; Adnan et al., 2026; Riaz et al., 2026).

Overall, the future development of this beverage should progress from laboratory-scale optimization toward a comprehensive translational pathway involving safety validation, bioavailability assessment, animal studies, controlled human trials, microbiome characterization, nutrigenomic modelling, AI-assisted formulation, sustainable ingredient sourcing, advanced processing, consumer research, pilot-scale production, economic evaluation, and responsible commercialization. Importantly, computational and predictive studies should be used to generate hypotheses and guide experimental design, whereas definitive claims concerning health benefits, safety, or therapeutic potential should be established through appropriately designed experimental and

clinical investigations. This integrated strategy could facilitate the development of a functional beverage that is not only nutritionally and technologically optimized but also biologically validated, socially accessible, environmentally responsible, and commercially feasible.

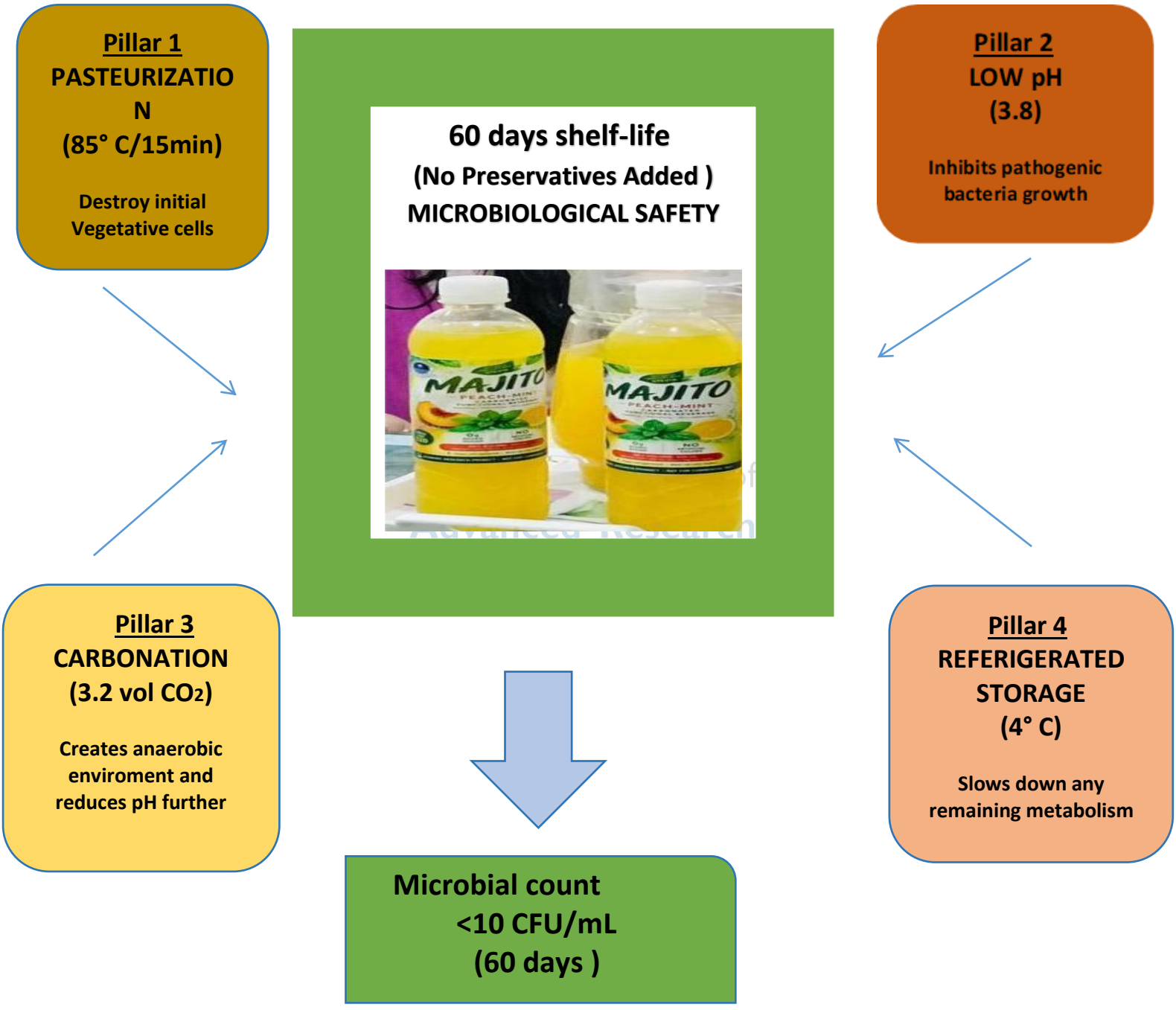


Figure.1.3.Natural Preservation without chemical preservatives

Conclusion

The present study successfully developed a carbonated functional beverage prepared from peach (*Prunus persica*), stevia (*Stevia rebaudiana*), lemon (*Citrus limon*), and mint (*Mentha piperita*) without the addition of refined sugar or chemical preservatives. The optimized formulation (T_2) demonstrated desirable physicochemical characteristics, high antioxidant activity, excellent sensory acceptability, and complete microbiological safety throughout 60 days of refrigerated storage. The successful replacement of sucrose with stevia substantially reduced total soluble solids while maintaining consumer acceptance, whereas the combination of peach, lemon, and mint enhanced the beverage's functional and sensory properties.The absence of detectable microbial growth during storage confirmed that pasteurization, carbonation, low pH, and refrigerated storage were sufficient to maintain product safety without synthetic preservatives. Although total phenolic content and antioxidant activity declined gradually during storage, the beverage retained considerable functional quality and remained microbiologically stable.Overall, the optimized formulation represents a promising sugar-free, preservative-free functional beverage for diabetic and health-conscious consumers while supporting the growing demand for clean-label products. Future studies should focus on pilot-scale production, shelf-life evaluation under different storage conditions, and clinical investigations to validate its potential health benefits and commercial applicability.

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