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Development of a High-Protein Sports Drink Using Banana Peel Protein-Fortified Guava Juice

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Abstract

This study aimed to develop a high-protein sports drink by incorporating banana peel protein (BPP) into guava juice and to evaluate its nutritional, functional, physicochemical, and sensory qualities during storage. Four formulations (T_0–T_3) were prepared with increasing levels of BPP. The results showed that protein content increased significantly across treatments, with T3 exhibiting the greatest improvement, confirming the effectiveness of BPP as a plant-based protein enhancer. Antioxidant activity and total phenolic content also increased with BPP fortification, along with notable rises in minerals such as calcium and magnesium. Although vitamin C levels decreased with higher BPP inclusion, the overall nutritional value improved. During 30 days of storage, all samples showed slight but expected changes in pH, acidity, total soluble solids, and color; however, these remained within acceptable limits. Sensory evaluation revealed that all fortified drinks maintained good acceptability, with T_3 receiving the most favorable overall response among the fortified treatments. The study demonstrates that banana peel, an underutilized agro-waste resource, can be transformed into a valuable functional ingredient for sports beverages. Incorporating BPP not only enhances nutritional properties but also supports sustainability through waste reduction. Overall, BPP-fortified guava juice shows strong potential as an affordable, nutritious, and consumer-friendly sports drink option for health-conscious individuals.

1. Introduction

The term "banana" refers to herbaceous plants belonging to the genus Musa, known for the fruit they yield. It is (*Musa sp*.) the world's most popular tropical fruit, enjoyed by people of all ages (Vu *et al*., 2019). It is one of the oldest fruits and most important food crops that are rich in nutrients. It can be utilized fresh or in the form of different snacks. Its worldwide production in 2020 was 155 million metric tons (Kema *et al*., 2021). With a global production share of 16%, it is the second abundant fruit in the world. The antacid properties of bananas have long been known to prevent stomach ulcers and ulcer damage. Consequently, it serves as a fundamental resource with significant potential for applications in the nutraceutical and medicinal fields. It helps the body retain calcium, nitrogen, and phosphorus; all of these work to build healthy, regenerated tissues.

Moreover, bananas are highly digestible and hardly induce any kind of allergic response. Bananas are also part of the BRAT (bananas, rice, applesauce, and toast) diet, a diet many physicians recommend for children recovering from gastrointestinal problems (Sampath Kumar *et al*.,

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2012). Powdered banana peel, when blended with the powder of banana fruit or many other agricultural products, is utilized to develop innovative products. Banana peel itself is a valuable source, containing about 6–9% crude protein, 3.8–11% crude fat, 43.2–49.7% dietary fiber, along with a variety of minerals and natural sugars. (Mohapatra *et al*., 2010). As bananas grow, the concentration of soluble sugars, proteins, and lipids increases, but hemicellulose declines. Banana peel waste (BPW) is the solid remains generated during banana processing, accounting for around 40% of the fresh fruit's weight. Biodegradable garbage is predominantly produced in residential settings, dining establishments, food service outlets, and processing enterprises, with the latter being the most significant local contributor to this waste (Essien *et al*., 2005).

Guava, scientifically named *Psidium guajava*, is a well-known tropical tree grown widely for its fruit. It belongs to the phylum Magnoliophyta, class Magnoliopsida, and family Myrtaceae, which includes about 133 genera and over 3,800 species. The guava plant thrives in tropical climates, displaying a high adaptability to different soil types. Ascorbic acid is the major component of guava that plays a crucial role against cancer. Guava is widely utilized globally for the treatment of several ailments, including diarrhea, fever, dysentery, stomach problems, high blood pressure, along with diabetes, dental issues, pain alleviation, and wounds. Guava possesses a significant proportion of chemical and inorganic compounds, secondary metabolites such as antioxidants, polyphenols, antiviral agents, and anti-infectious substances. Guava has several chemicals with anticancer properties. Lycopene, along with flavonoids, is a significant antioxidant. They assist in the eradication of malignant tissues. (Naseer *et al*., 2018).

Protein energy malnutrition (PEM) is one of the critical food-related (protein) deficiencies affecting newly born and younger children, contributing to more than 50% of child death rates in developing countries (Walker *et al*., 1990). PEM accounts for 54% of the 10.8 million deaths each year among children below five is linked to 53% of deaths related to contagious diseases in this age group within developed nations (Brundtland *et al*., 2000). Key risk factors for PEM include lack of awareness, large family size, place of residence, insufficient financial resources to purchase food, low parental education, scarcity, neglect of nutritional needs, cultural along religious dietary norms, inadequate access to effective healthcare, long-term infections, the gender of the child, and inappropriate vaccination (Nova *et al*., 2002). Malnutrition impacts several areas, such as physical development, death rates, reproductive well-being, incidence of diseases, cognitive growth, and ability to perform physical tasks (Mamiro *et al*., 2005). In more affluent nations, malnutrition and infectious illnesses are significant issues affecting infants and young children. (Ouedraogo *et al*., 2008).

Considering the growing interest in functional, nutrient-enriched formulations, the present study focuses on developing a high-protein sports drink derived from an unconventional, sustainable source. Specifically, this work aims to extract protein from banana peel using the Osborne fractionation method, incorporate the extracted protein into a novel high-protein sports drink, and evaluate the physicochemical and quality attributes of the resulting formulation.

2. Materials and Methods

2.1 Procurement of raw materials

Fresh ripe banana (*Musa acuminate)* and guava *(Psidium Guajava)* were procured from the local market in Bahawalpur, Pakistan. All analytical reagents and chemicals utilized in this study were sourced from the chemical repository located at the Department of Food Science and Technology, The Islamia University of Bahawalpur.

2.2. Preparation of banana peel powder (BPP)

About 1-1.5kg of raw bananas that were matured and sharp yellow in color without any dark spots were sorted out and peeled. The banana peel was first washed with a 1.25% H_2SO_4 solution, followed by rinsing with 1.25% NaOH, and lastly it was washed with distilled water. Banana peels were dipped in a 0.5% (weight/volume) solution of citric acid for about 10-15 minutes to avoid enzymatic browning. Later, the banana peel was oven-dried at 60°**C for 24 hours**. After drying, the dry peel was ground by using a lab scale grinder (Bhagwati-1, SS136, India) until it became a fine powder. The powder was further filtered through a 60-mesh sieve to achieve a consistent particle size of 250 µm. The processed banana peel powder was kept in a sealed plastic bag for subsequent protein extraction (Deb *et al*., 2022).

2.3. Protein extraction from banana peel powder (BPP)

The banana peel powder (BPP) was defatted by using the solvent extraction method (methanol) as delineated by Osborne and Voorhees (1894). The BPP was divided into four equal portions for protein extraction based on solubility. BPP was mixed with deionized water at a 1:10 (weight/volume) ratio at a pH of 7. The sample was dissolved in an Erlenmeyer flask and covered with foil. All four samples were kept on a mechanical shaker (Scilogex SK-O330-Pro, Orbitek, USA) overnight. After stirring, the sample was centrifuged (SIGMA 1-14K, Germany) at 8000rpm for 15 minutes. For the extraction of globulin, 0.5 N NaCl was used. For prolamin, 70% aqueous ethanol was used. Similarly, for glutelin, 0.2% NaOH was used. The rest of the procedure was like that for albumin. The supernatant was collected as Albumin, prolamin, glutelin, and globulin. After the extraction, proteins were kept at 4°C for subsequent analysis. Protein powder was formed by freeze-drying the supernatants extracted from albumin, globulin,

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prolamin, and glutelin, and the resultant protein powder was stored under refrigerated conditions at 25°C in airtight plastic packets for the subsequent formulation of a high-protein sports drink. (Du et al., 2018).

2.4. Development of high-protein sports drink

Raw guava and overripe fruits were separated from ripe, fresh guava. Subsequently, it was cleaned, weighed, cut, and the juice was extracted. The extracted juice was strained through muslin fabric (Hemanth *et al*., 2020). Albumin fraction was used at 0, 50, 70, and 90 g/300 mL to develop a high-protein sports drink because the albumin extraction rate was more than 60% and its ability to dissolve efficiently to develop a drink. Guava juice (300mL) was taken along with 10 grams of refined sugar. It was then blended for 1min. The beverage was then poured into glass bottles. These sports drink was then pasteurized at 90°C for 30 sec and cooled to 20°C, stored at 3°C for further analysis (Seifert *et al*., 2006).

2.5. Quality evaluation of high-protein sports drink

The quality attributes of the developed high-protein sports drink were evaluated through a series of standardized analytical procedures. Protein content was determined using the Kjeldahl method as described in AOAC (2016), which quantifies total nitrogen to estimate protein concentration. Reducing sugars were assessed following AOAC Method 920.183, based on titration with Fehling's solution to measure fermentable carbohydrates. Ascorbic acid content was analyzed according to the AACC (2000) dye-titration method, which relies on the reduction of 2,6-dichlorophenol-indophenol by vitamin C. Antioxidant activity was measured using the DPPH free-radical scavenging assay, enabling estimation of the beverage's radical-quenching capacity. Total phenolic content was determined following the Folin–Ciocalteu procedure described by Stratil *et al*. (2007), which evaluates the reducing capacity of phenolic compounds. Mineral composition was quantified using atomic absorption spectrophotometry (SP 9 Pye Unicam), following wet digestion of samples with HCl and HNO_3 to extract mineral elements for instrumental detection.

2.6. Storage study

The stability of all formulations was evaluated at room temperature for one month, with analyses conducted at 10-day intervals to assess physicochemical changes during storage. Acidity was determined by the standard AOAC (2016) titration method, where samples were titrated with 0.1N NaOH using phenolphthalein to indicate the endpoint for calculating titratable acidity. pH was measured according to AOAC guidelines using a calibrated digital pH meter, which quantifies hydrogen ion activity to determine acidity or alkalinity. Total soluble solids (TSS) were assessed following AOAC (2015) using a handheld refractometer, providing an estimation of dissolved sugars and soluble constituents based on refractive index. Sensory evaluation was performed by a trained panel of 30 members using a nine-point hedonic scale (Larmond, 1977) to assess color, aroma, flavor, texture, and overall acceptability of the beverages over the storage period. Color parameters (L*, a*, b*) were analyzed using a CR-400 chromameter after white-plate calibration, enabling instrumental assessment of lightness and chromatic attributes during storage.

2.7. Statistical analysis

All experiments were performed in triplicate, and results are presented as mean ± standard deviation. Statistical differences among treatments were evaluated using one-way analysis of variance (ANOVA) in Statistix 8.1.

3. Results and Discussion

3.1. Nutritional composition of BPP-fortified guava drinks

Banana peel protein (BPP) incorporation into guava

Table 1. Nutritional quality parameters of fortified guava drinks

Treatments	Protein (g/100mL)	Reducing sugar (g/100mL)	Ascorbic acid (mg/L)
T_0	1.21±0.00^d	4.12±0.05^a	653.67±1.3^a
T_1	4.64±0.00^c	3.85±0.07^b	206.30±1.0^d
T_2	6.46±0.01^b	3.42±0.06^c	287.98±1.1^c
T_3	9.15±0.01^a	2.95±0.08^d	418.27±1.1^b

T_0 (Control) = 0 g/300 mL Albumin; T_1= 50 g/300 mL Albumin; T_2= 70 g/300 mL Albumin; T_3= 90 g/300 mL Albumin

Table 2. Antioxidant and mineral content of fortified guava drinks

Treatments	DPPH (%)	TPC (mg GAE/L)	Magnesium (mg/L)	Potassium (mg/L)	Calcium (mg/L)
T_0	42.3±0.5^d	210.5±1.2^d	12.4±0.2^d	145.3±1.5^a	18.2±0.3^d
T_1	51.8±0.4^c	265.7±1.4^c	18.7±0.3^c	131.2±1.3^b	22.4±0.2^c
T_2	58.9±0.6^b	298.4±1.5^b	22.5±0.4^b	118.9±1.6^c	26.7±0.4^b
T_3	63.1±0.7^a	335.2±1.8^a	27.9±0.5^a	105.6±1.2^d	31.3±0.5^a

T_0 (Control) = 0 g/300 mL Albumin; T_1= 50 g/300 mL Albumin; T_2= 70 g/300 mL Albumin; T_3= 90 g/300 mL Albumin

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Table 3. Effect of treatments and storage on physicochemical attributes of fortified guava drink

Treatments	Storage (days)	pH	Acidity (%)	TSS (°Brix)	L*	a*	b*
T_0	0	4.53±0.02^{a}	0.08±0.01^{b}	14.31±0.31^{a}	52.1±1.5^{b}	-1.2±0.02^{c}	18.4±0.02^{a}
	10	4.51±0.01^{ab}	0.09±0.02^{ab}	14.41±0.24^{a}	51.8±2.3^{ab}	-1.1±0.03^{b}	18.1±0.03^{ab}
	20	4.48±0.03^{b}	0.11±0.03^{ab}	14.51±0.34^{a}	51.4±1.2^{a}	-1.0±0.01^{ab}	17.8±0.02^{ab}
	30	4.44±0.02^{c}	0.12±0.01^{a}	14.61±0.12^{ab}	51.0±2.4^{a}	-0.9±0.01^{a}	17.5±0.03^{b}
T_1	0	4.16±0.01^{a}	0.46±0.02^{a}	5.33±0.44^{d}	49.3±2.2^{a}	-1.5±0.02^{b}	16.9±0.04^{a}
	10	4.14±0.02^{a}	0.47±0.02^{a}	5.45±0.34^{c}	48.9±1.5^{a}	-1.4±0.03^{ab}	16.6±0.03^{ab}
	20	4.11±0.03^{b}	0.49±0.03^{a}	5.56±0.22^{b}	48.5±2.3^{a}	-1.3±0.01^{ab}	16.3±0.04^{ab}
	30	4.08±0.02^{c}	0.51±0.01^{a}	5.67±0.45^{a}	48.2±1.5^{b}	-1.2±0.02^{a}	16.0±0.02^{b}
T_2	0	4.23±0.01^{a}	0.47±0.01^{a}	7.47±0.56^{bc}	50.2±1.6^{a}	-1.4±0.02^{b}	17.2±0.03^{a}
	10	4.22±0.03^{ab}	0.49±0.02^{a}	7.55±0.27^{c}	49.8±2.3^{ab}	-1.3±0.02^{b}	17.0±0.04^{a}
	20	4.21±0.02^{b}	0.51±0.03^{a}	7.67±0.44^{b}	49.4±1.2^{ab}	-1.2±0.03^{ab}	16.8±0.03^{ab}
	30	4.17±0.01^{b}	0.53±0.01^{a}	7.78±0.26^{a}	49.0±3.1^{ab}	-1.1±0.01^{a}	16.5±0.01^{b}
T_3	0	4.34±0.03^{a}	0.49±0.02^{b}	10.66±0.43^{b}	53.0±1.4^{a}	-1.0±0.03^{b}	19.1±0.02^{a}
	10	4.33±0.02^{a}	0.52±0.01^{ab}	10.77±0.25^{ab}	52.6±2.5^{ab}	-0.9±0.01^{a}	18.8±0.03^{ab}
	20	4.32±0.01^{a}	0.53±0.01^{ab}	10.88±0.47^{ab}	52.2±1.3^{ab}	-0.8±0.02^{a}	18.5±0.02^{ab}
	30	4.27±0.02^{b}	0.55±0.03^{a}	10.99±0.23^{a}	51.8±3.6^{b}	-0.7±0.01^{a}	18.2±0.01^{b}

T_0 (Control) = 0 g/300 mL Albumin; T_1= 50 g/300 mL Albumin; T_2= 70 g/300 mL Albumin; T_3= 90 g/300 mL Albumin

juice resulted in huge improvements in the beverage's nutritional value. As expected, protein content shows increasing trends with higher levels of BPP fortification. The control (T_0) contained 1.21±0.00 g/100 mL of protein, whereas the treatment with the most abundant BPP (T_3) reached 9.15±0.01 g/100 mL, marking a substantial enhancement (Table 1). This trend is comparable with findings from earlier works indicating that banana peel flour or extracts can significantly increase protein levels when used as a functional ingredient in foods (Adejumo *et al*., 2021; Romelle *et al*., 2016).

Reducing sugars were inversely associated with BPP incorporation. T0 observed the highest value (4.12±0.05 g/100 mL), while T_3 noted the lowest (2.95±0.08 g/100 mL). A plausible explanation is the dilution effect caused by the addition of protein concentrate, which replaced part of the naturally sweeter fruit matrix. Similar trends in sugar concentration have been reported when plant-based proteins or fibers are incorporated into juice systems (Ali *et al*., 2019).

Guava is inherently rich in vitamin C, so the observed reduction may result from oxidative interactions between the protein fractions in BPP and the ascorbic acid molecules, which accelerate vitamin degradation. Ascorbic acid exhibited a non-linear pattern. The control sample showed the highest vitamin C content (653.67±1.3 mg/L), whereas T_1 had the lowest (206.30±1.0 mg/L). Although treatments T_2 and T_3 showed partial recovery (287.98±1.1 – 418.27±1.1 mg/L), they remained below the control. Prior studies also highlight protein-phenolic interactions that can influence vitamin stability in beverages (Papoutsis *et al*., 2019).

3.2 Minerals and antioxidant properties

Mineral content, particularly magnesium, calcium, and potassium, also increased steadily with higher BPP levels. Banana peel is known to be mineral-dense, which explains the increased levels in fortified samples (Anhwange, 2008). Interestingly, potassium levels showed a declining trend from T_0 to T_3, possibly due to compositional alteration caused by the addition of the BPP mixture, which has relatively lower potassium compared to fresh guava pulp. Nonetheless, magnesium and calcium levels increased consistently, further strengthening the nutritional appeal of the beverage.

Fortification with BPP significantly increased antioxidant activity. DPPH scavenging ability increased from 42.3±0.5 in T0 to 63.1±0.7 in T_3 (Table 2). This increase is attributable to the polyphenolic and flavonoid compounds naturally present in banana peel, which have been widely reported to possess strong antioxidant potential (Sulaiman *et al*., 2011). Similarly, total phenolic content (TPC) increased significantly across treatments, with T_3 exhibiting the highest value (335.2±1.8 mg GAE/L). This confirms that BPP produces valuable bioactive molecules that enhance the drink's functional quality.

3.3. Physicochemical attributes during storage

Across a 30-day storage period, the pH of all samples displayed a slight but consistent decline (Table 3). Total soluble solids (TSS) showed a gradual increase during storage for all treatments. This may be attributed to acid

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Table 4. Effect of treatments and storage on sensory attributes of fortified guava drink

Treatments	Storage (days)	Color	Taste	Aroma	Overall Acceptability
T_0	0	8.51±0.04^{a}	8.72±0.03^{a}	8.61±0.03^{a}	8.71±0.04^{a}
	10	8.43±0.05^{b}	8.64±0.04^{ab}	8.53±0.01^{ab}	8.63±0.02^{ab}
	20	8.35±0.03^{c}	8.55±0.03^{b}	8.45±0.02^{b}	8.56±0.01^{b}
	30	8.26±0.02^{d}	8.46±0.05^{c}	8.33±0.04^{c}	8.43±0.01^{c}
T_1	0	8.04±0.05^{a}	7.93±0.03^{a}	7.85±0.02^{a}	8.03±0.06^{a}
	10	7.97±0.04^{ab}	7.85±0.05^{ab}	7.76±0.05^{ab}	7.94±0.07^{ab}
	20	7.89±0.03^{b}	7.76±0.03^{ab}	7.64±0.05^{ab}	7.86±0.03^{b}
	30	7.70±0.02^{c}	7.67±0.05^{b}	7.57±0.06^{b}	7.73±0.05^{c}
T_2	0	8.10±0.05^{a}	8.05±0.03^{a}	7.97±0.04^{a}	8.16±0.06^{a}
	10	8.06±0.05^{ab}	7.93±0.05^{ab}	7.83±0.06^{ab}	8.08±0.03^{ab}
	20	7.94±0.03^{ab}	7.86±0.06^{ab}	7.76±0.03^{b}	7.94±0.06^{ab}
	30	7.83±0.05^{b}	7.78±0.07^{b}	7.64±0.02^{b}	7.84±0.03^{b}
T_3	0	8.36±0.03^{a}	8.13±0.03^{a}	8.08±0.05^{a}	8.23±0.05^{a}
	10	8.2±0.05^{ab}	8.05±0.05^{ab}	7.94±0.06^{a}	8.15±0.03^{b}
	20	8.18±0.03^{ab}	7.97±0.03^{b}	7.87±0.06^{ab}	8.06±0.01^{c}
	30	8.08±0.05^{b}	7.83±0.05^{c}	7.78±0.07^{b}	7.96±0.02^{d}

T_0 (Control) = 0 g/300 mL Albumin; T_1= 50 g/300 mL Albumin; T_2= 70 g/300 mL Albumin; T_3= 90 g/300 mL Albumin

hydrolysis of polysaccharides or concentration of solids due to minimal evaporation. The fortified samples naturally exhibited lower baseline TSS values than the control, likely due to protein addition reducing the fruit sugar proportion.

The reduction was modest (e.g., T_3 decreased from 4.34±0.03 to 4.27±0.02) and is a common trend in fruit-based beverages as organic acids accumulate due to natural biochemical reactions. The increase in titratable acidity throughout storage corroborates this pattern. Such changes are consistent with observations made in fruit juices subjected to ambient storage (Kumar *et al*., 2020).

Color values (L*, a*, b*) revealed minor shifts, indicating slight darkening over time. Samples containing BPP (T_1–T_3) showed slightly lower L* values compared to T_0, which is consistent with the darker hue of banana peel extracts. Similar observations have been reported in fortified beverages using plant-derived proteins or fibers (Bhardwaj & Pandey, 2011). Overall, color changes were minimal and remained within acceptable sensory limits.

3.4. Sensory evaluation

Sensory scores declined gradually across all attributes during storage; however, the beverages remained within acceptable limits even after 30 days (Table 4). The control (T_0) consistently scored the highest for color, taste, aroma, and overall acceptability, reflecting consumers' general preference for unmodified guava juice.

Among fortified samples, T_3 exhibited relatively higher acceptability than T_1 and T_2 across most parameters. The higher protein level in T_3 may have contributed to a smoother mouthfeel, which some panelists preferred, despite the slight darkening observed in color. Previous research shows that moderate incorporation of plant proteins can maintain or even improve the sensory profile of fruit beverages if well balanced (Singh *et al*., 2020).

Taste scores showed the most noticeable decline with increasing BPP levels. This could be due to the characteristic earthy notes or slight bitterness associated with phenolic-rich banana peel extracts. Nonetheless, mean overall acceptability values for all fortified beverages remained above 7.5 on a 9-point hedonic scale, indicating that BPP incorporation did not compromise palatability to unacceptable levels.

Conclusion

This study showed that banana peel protein (BPP) can be successfully added to guava juice to create a nutritious, high-protein sports drink. Fortification significantly increased protein, antioxidant activity, and key minerals, making the beverage more functional without causing major changes to its stability during storage. Sensory results confirmed that all fortified samples remained acceptable, with the highest BPP level (T_3) performing better than the lower-level treatments. Although vitamin C decreased with BPP addition, the overall nutritional gain was substantial. The findings highlight the potential of banana peel often treated as waste as a valuable ingredient for developing affordable, sustainable, and health-focused beverages. Overall, BPP-fortified guava juice offers a promising option for consumers seeking natural sports drinks while supporting food waste reduction and improved nutritional intake.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

Manahil Zafar contributed to the conceptualization of the study, conducted the primary research, and drafted the initial manuscript, including data collection, analysis, and interpretation of findings, while Hammad Hafeez contributed to the design of the methodology, provided critical revisions, supervised the overall project, ensured the accuracy of results, and approved the final version of the manuscript for submission.

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