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Formulation and Evaluation of Antioxidant, Physicochemical, Microbial, and Sensory Properties of Instant Functional Drink Powders Developed from Pumpkin (Cucurbita maxima) By-Products

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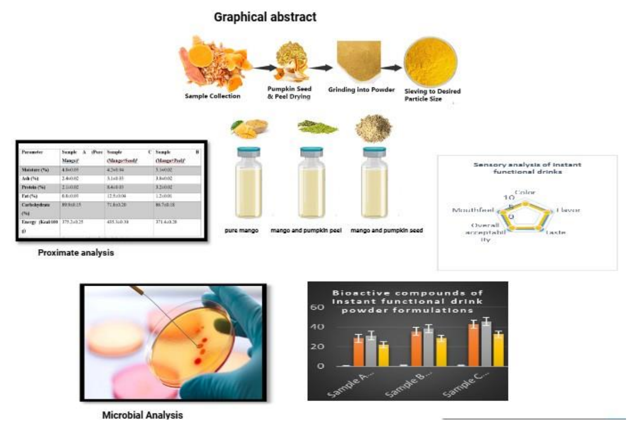
Graphical abstract



Abstract

Pumpkin (*Cucurbita maxima*) by-products, particularly seeds and peels, are rich in nutrients and possess high antioxidant potential. However, their utilization in functional beverages remains underexplored. This study addresses this gap by formulating and evaluating instant functional drink powders incorporating pumpkin by-products with mango. The research assesses these formulations' physicochemical, antioxidant, microbiological, and sensory attributes to enhance the nutritional profile of traditional fruit-based beverages. Three formulations were developed: pure mango, pumpkin seed powder, and pumpkin peel powder. Proximate analysis revealed that the pumpkin seed-enriched formulation had the highest protein (8.4%), fat (12.5%), and energy (435.3 kcal/100g) content, whereas the peel-based variant provided enhanced fiber and mineral content. Antioxidant profiling revealed significant increases in total flavonoid (42.8 mg QE/100 g) and phenolic content (45.6 mg GAE/100 g) in the pumpkin seed-enriched formulation, enhancing its free radical scavenging potential. Microbiological assessment confirmed that all formulations remained within safe consumption limits over a 90-day storage period. The sensory evaluation identified the pumpkin seed-enriched formulation as the most preferred due to its superior taste and acceptability. These findings highlight the potential of pumpkin by-products in functional beverage innovation, offering improved nutritional benefits while promoting sustainability in food production. In conclusion, pumpkin by-products serve as a viable functional ingredient in instant beverages, offering nutritional benefits, sustainability advantages, and commercial potential.

Keywords: pumpkin waste, functional drink powders, nutritional enhancement, sustainability

1. Introduction

Cucurbita maxima, commonly known as a pumpkin, is one of the principal vegetables in the family and is extensively cultivated in tropical and subtropical regions. This species is also known under several standard designations, such as squash, auyama, or sambo (Hagos *et al.* 2022). It belongs to the genus Cucurbita of the Cucurbitaceae family. Among the genera of economically important vegetable crops, Cucurbita stands out the most. The principal cultivated species are *C. maxima*, *C. moschata*, and *C. pepo*. Thus, it can be said that pumpkin's agricultural importance is reflected in its many uses- not only its fractions ready for human consumption but also its by-products such as syrups, purees, and preserves (Gavril *et al.* 2024). In Pakistan, it holds agricultural significance not only for its economic value but also due to its high nutritional content. Fresh pumpkin is rich in various nutrients, while the compositional analysis shows impressive nutritional value: 0.6–1.8% proteins, 0.0–0.2% lipids, 80–96% moisture content, 4.6–6.5% sugars, 0.5–1.3% fiber, and relatively low carbohydrate content at about 8.8% (Batool *et al.* 2022). Pumpkin's high antioxidant potential and health-promoting properties make it a valuable ingredient for functional products. However, its nutrient-rich composition is offset by significant post-harvest challenges, including high moisture content (>80%), a short storage life of 1–3 months after a 3–4-month growth period (Yo'lchiev *et al.* 2022). These factors lead to transportation difficulties and unfavorable market pricing, posing financial challenges for agricultural producers (Wanjiku Kiharason 2019).

Emerging food science research has increasingly focused on innovative processing methodologies to preserve pumpkin's nutritional and bioactive components. Converting pumpkin by-products into powder offers several advantages, including enhanced shelf life, reduced transportation costs, and simplified handling (Tran and Nguyen 2024). Contemporary research initiatives are exploring novel applications, particularly in the beverage sector, where pumpkin powder could potentially enhance the nutritional profile of conventional beverages. Incorporating pumpkin by-product powder in instant functional drink powder formulations represents an innovative approach to maximizing its nutritional potential (A. Hussain *et al.* 2022). However, using pumpkin by-product powder as a beverage ingredient remains relatively unexplored, presenting opportunities for novel research directions. This emerging application promises to address waste reduction and environmental sustainability objectives and expand consumption patterns. The study aimed to develop mango-based instant functional drink powders enriched with pumpkin by-products and characterize their composition, bioactive compounds, antioxidant activity, and microbiological safety. Moreover, based on the developed instant functional drink powders, a functional beverage was prepared, and its sensory properties were evaluated. Moreover, these novel mango–pumpkin functional drinks were designed to offer rapid hydration and improved sensory appeal. Designed for the summer beverage market, it integrates natural ingredients with functional additives to offer nutritional value, convenience, and refreshing qualities, catering to time-conscious consumers. Emphasising convenience and nutrition, the study also highlights the need for further optimisation of pumpkin powder levels to enhance physicochemical, sensory, and storage characteristics. From a market point of view, pumpkin's by-products valorization aligns with the circular economy framework along with food waste mitigation, leading to ecological as well as economic advantages. The demand for functional beverages is expected to exceed $208.13 billion by 2024 globally, with a significant consumer preference for plant-based products (Gupta *et al.* 2023). In comparison with traditional fruit juices or caffeine-based drinks, pumpkin delivers a more comprehensive nutritional profile characterized by high dietary fiber, low sugar content, promoting sustained energy release along with supporting the gastrointestinal health without a glycemic spike.

This research excels in the use of pumpkin seeds and peels to make instant mango-based functional drink powders; this research has not been thoroughly studied in previous works. Unlike previous research based on bakery or dairy uses, this research delivers a shelf-stable, rehydratable drink with increased nutritional and antioxidant contents, with substantiation by rigorous sensory and microbiological analysis. Although previous works such as (Atencio *et al.* 2021) who studied the impact of processing on the functionalization of pumpkin pomace. Similarly, (Asaduzzaman *et al.* 2025) enhance the nutritional value of cookies by fortifying with pumpkin and (Gavril *et al.* 2024) Formulated the yogurt based on pumpkin peel as a bioactive powder. But the formulation of mango-based functional drinks employing pumpkin seeds and peels together has not yet been studied in depth.

2. Materials and methods

The current research project was conducted at the Department of Food Sciences and Technology, Government College University, Faisalabad. Fresh mangoes and pumpkins were purchased from the Al-Fatah Supermarket in Faisalabad, Pakistan, and chemicals were purchased from a scientific store located in Faisalabad, Pakistan. All the chemicals used were high-quality and research grade. The protocol used for the current project is illustrated below.

2.1. Preparation of mango drink powder

Fresh mangoes were procured from Al-Fatah Supermarket in Faisalabad, Pakistan, and and carefully examined to ensure the absence of insect infestation and contamination. The selected mangoes were thoroughly washed to remove any surface impurities, and excess moisture was carefully absorbed using blotting paper. unwanted portions were trimmed, and the fruits were manually peeled. The pulp was separated from the seeds and homogenized using a household blender to achieve a uniform consistency. To enhance the drying process, the pulp was sieved to eliminate large particles and insoluble fibers before being transferred to a tray dryer. Drying was performed at 70°C for three days under controlled conditions. Once the drying process was complete, the dehydrated mango content was collected and finely ground. The resulting powder was further sieved to ensure uniformity and remove any residual large fragments. The final mango powder was then weighed accurately and stored in air-tight bags at room temperature for later analysis.

2.2. Processing of Pumpkin By-products

Mature and Fresh pumpkins were washed to remove any dust and dirt. The peel and seeds were separated from the pumpkin pulp. Both parts were separately washed to ensure cleanliness.

2.3. Preparation of pumpkin peel powder

The pumpkin peel was dried in a drying oven for 3-4 hours at 60°C. After drying, grinding is carried out using a mechanical grinder, and the powder is then passed to a fine, homogenized powder through a 0.35mm mesh sieve. The resultant powder was stored in a clean, dry, food-grade bag at 4°C for further experiments.

2.4. Preparation of pumpkin seed powder

The cleaned pumpkin seeds were dried at 60°C in a hot air oven dryer for approximately 5–6 hours. Following the drying process, grinding was carried out using a mechanical grinder. After grinding, the seeds were manually dehulled The resulting powder was then passed through a 300-mesh sieve to ensure uniform particle size. To preserve its quality, the seed powder was stored in a clean, dry, food-grade bag at 4°C until further experimentation. Pre-treatment of pumpkin by-products, such as washing, drying at 60 °C, grinding, and sieving, directly affected the quality and stability of the final drink powders. Effective washing reduced microbial load, and controlled drying helped to preserve the heat-sensitive nutrients such as polyphenols and carotenoids. Fine sieving improved mouthfeel and solubility, enhancing the product's dispersibility and bioactive release. In addition, combining pumpkin by-products with functional additives significantly improved nutritional quality and antioxidant stability.

2.5. Preparation of instant functional drinks powder

The formulation of the functional drink powder was standardized using a carefully selected combination of ingredients. These included sugar, citric acid, trisodium citrate, tricalcium phosphate, ascorbic acid, aspartame, salt, titanium dioxide, CMC (carboxymethyl cellulose), silicon dioxide, mango powder, and food colorants (E110, E102). This optimized formulation was designed to ensure the desired physicochemical properties, stability, and functional benefits in the final product (**Table 1**).

Table 1. Formulation of instant pumpkin seed and peel-based drink powder

Ingredients	Pure mango drink powder	Mango and pumpkin seed powder	Mango and pumpkin peel powder
Sugar	680 g	680 g	680 g
Mango Powder	220 g	160 g	160 g
Pumpkin Seed Powder	-	60 g	-
Pumpkin Peel Powder	-	-	60 g
Salt	15 g	15 g	15 g
Citric Acid	40 g	40 g	40 g
Trisodium citrate	8 g	8 g	8 g
Titanium Dioxide	1.5 g	1.5 g	1.5 g
Tricalcium Phosphate	4 g	4 g	4 g
Color (E110, E102)	1 g	1 g	1 g
Aspartame	8 g	8 g	8 g
Ascorbic Acid	8 g	8 g	8 g
CMC	4 g	6 g	6 g
Silicon dioxide	0.5 g	0.5 g	0.5 g
Flavor	10 g	8 g	8 g

2.6. Compositional analysis

2.6.1. Proximate analysis

By applying the procedure of (Kaur *et al.* 2014), moisture, protein, fat, and fiber of the samples (pumpkin seeds and peels) were evaluated, the NFE nitrogen-free extract was determined using the (AOAC 2000) method. The total carbohydrate was calculated as

% CHO = 100% - (% Protein + % Fat + % Fiber + % Ash + % Moisture content).

The energy value of the samples was determined by multiplying the protein content by 4, the carbohydrate content by 4, and the fat content by 9, according to the James formula (James 1995).

Energy Value = (Crude protein × 4) + (Total carbohydrate × 4) + (Crude fat × 9).

2.6.2. Mineral analysis

Following (Costa *et al.* 2002) protocol, Na, K, levels were analyzed using a Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge), and Mg, Ca, Zn, and Fe levels were evaluated using an atomic absorption spectrophotometer (Varian AA240, Australia).

2.7. Bioactive compounds of functional drink powder

2.7.1. Preparation of extracts

Phenolic compounds were extracted from the samples using the Soxhlet extraction method. Ethanol was employed as the extraction solvent. The samples were placed in the Soxhlet apparatus and subjected to continuous extraction at room temperature for seven hours. Following the extraction process, the supernatant was filtered through a muslin cloth to remove any solid residues. The solvent was then evaporated using a rotary vacuum evaporator (Eyela, N-N series, Japan) at 50°C, leaving behind the crude extract. Each extract was weighed and subsequently stored at 4°C for further analyses.

2.7.2. Total anthocyanin content (TAC)

The TAC of the instant mango drink powder extracts was quantified using a colorimetric analysis, following the method outlined by (Selim 2008), with minor modifications. The TAC values were calculated and expressed in milligrams per 100 grams (mg/100 g) using the following equation:

TAC= Absorbance of sample×DF×100/m×E

- where: DF represents the dilution factor, m denotes the weight of the sample used in the preparation of the stock solution, and E refers to the extinction coefficient (55.9).

2.7.3. Total phenolic content (TPC)

Following S. Hussain *et al.* (2019). Each sample was mixed with the Follin-Ciocalteu reagent and 7% Na_2CO_3. The mixture stood for approximately 90 minutes, and the absorbance was calculated at 760nm using a UV-visible spectrometer. The TPC was expressed in mg of gallic acid equivalents per gram (mg GAE/g).

2.7.4. Total Flavonoid Concentration

TFC was determined on the modified method by (Rahman *et al.* 2024). Every extract (100 µL) was brought up to 1 mL with ethanol (Chemsense (M) SDN BHD., Selangor). A chromogen reagent was prepared by dissolving 1.00 g of PDAC from Sigma-Aldrich, Portland Oregon, USA in a cool solution of 750 mL of ethanol and 250 mL of concentrated hydrochloric acid from Sigma-Aldrich, Portland, Oregon, USA, and another 100 mL of solvent were further added; to this solution, one milliliter of this reagent was added to every diluted extract with possible reaction leaving for 10 minutes. The absorption at 640 nm was measured against the water blank.

2.8. Antioxidant assays

2.8.1. DPPH radical scavenging assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity is primarily used to assess the antioxidant potential of the desired compounds. Purposefully, 4 mL of the sample (pumpkin peel powder and pumpkin seed powder extract) and 1 mL of the DPPH solution (0.12 mM) were added to a test tube. Afterward, these were placed in a dark place for approximately 30 minutes. Finally, the absorbance was measured at 520 nm and recorded using a UV/visible spectrophotometer, along with controls and blanks, following the procedure mentioned by Madhujith and Shahidi (2006) with slight modifications.

2.8.2. Inhibition of β-Carotene Bleaching

The antioxidant potential of the extracted samples was evaluated using a method described by (Heimler *et al.* 2005) based on the oxidation of β-carotene. To prepare the assay solution, 20 mg of β-carotene was dissolved in 20 mL of chloroform, along with 40 mL of linoleic acid and 400 mg of Tween 20. A 3 mL aliquot of the resulting emulsion was then mixed with 10 mL of the test sample. To facilitate chloroform removal, the mixture was subjected to water bath heating, allowing the solvent to evaporate as it dissolved in water and subsequently volatilized into the air. The extent of β-carotene oxidation was then measured spectrophotometrically at 470 nm. The antioxidant activity was quantified using the following formula:

Bleaching (%) = (Absorbance after 2 hours of assay /Initial absorbance)×100

2.9. Microbiological Quality

The total viable count was assessed using the plate count agar method and quantified as colony-forming units per milliliter (CFU/mL). The enumeration of total coliforms and *Escherichia coli* was performed following established standard protocols described by (Nazir *et al.* 2024). Additionally, the yeast and mold count was determined following standardized microbiological procedures described by (Seiler, 1985). These microbiological tests help assess the overall quality and safety of the instant functional drink powder.

2.10. Functional beverage preparation and sensory evaluation

To formulate the functional beverage, 5 grams of each powdered sample were precisely weighed and dissolved in 250 mL of hot water maintained at 90 ± 5°C. The prepared beverages were cooled to a temperature range of 40-50°C . A nine-point hedonic scale was employed for sensory evaluation, with scores ranging from 9 ("like extremely") to 1 ("dislike extremely"), following Que *et al.* (2008). The sensory assessment of the novel powdered beverage samples was conducted by a panel of thirty trained evaluators, comprising students and faculty members from the Department of Food Science and Technology at Government College University, Faisalabad. The evaluation focused on key sensory attributes, including taste, color, flavor, and overall acceptability.

2.11. Statistical analysis

Experiments were performed in triplicate, and the results are presented as mean values ± standard deviation (SD). Statistical analysis was performed to evaluate the significance of data across all the parameters. Analysis of variance and determination of significance among the mean values were conducted with Minitab using one-way and two-way ANOVA. Tukey's Honestly Significant Difference (HSD) test was applied at $p < 0.05$, indicating statistical significance.

3. Results and discussion

3.1. Proximate analysis of instant functional drink powders

The proximate analysis of three formulations of instant drink powders revealed significant variations in their nutritional composition. The moisture content across all formulations ranged from 4.2 ± 0.04% to 5.1 ± 0.02% These moisture levels are considered optimal for instant drink powders, as they fall well below the critical 8% threshold identified by Potosí-Calvache et al. (2017) for preventing microbial growth. The results align with SF et al. (2017), who reported similar moisture contents (4.8-5.2%) in instant sorrel drink powder. Similarly, according to Farzana et al. (2017), vegetable soup powders contain an ash content of 6.2–6.8%. In our study, ash content varied significantly across formulations, ranging from 2.4 ± 0.02% in Sample A (pure mango) to 3.8 ± 0.02% in Sample B (mango with peel). The higher ash content in Sample B is likely due to the mineral-rich pumpkin peel, consistent with findings by Aziah and Komathi (2009), who reported ash levels of 3.4–4.1% in pumpkin peel powder. Sample C had a mid-range ash content value of 3.1 ± 0.03%, comparable to the values of ash content ranging from 2.00 to 3.50% in instant functional beverage powder prepared by Jabeen et al. (2024). The most significant variation was in protein content; Sample C had a very high concentration of 8.4 ± 0.03%, compared to Sample A at 2.1 ± 0.02% and Sample B at 3.2 ± 0.02%. The amount of fat varied significantly. Nyam (2013),The concentration of carbohydrates within samples was inversely proportional to respective concentrations of proteins and fats. For example, Sample C contained 71.8 ± 0.20% carbohydrates, and Sample A consisted of 89.9 ± 0.15%. The present study findings were in line with those of Badsha et al. (2021), who reported that instant mango drink powders contained approximately 90% carbohydrates. Energy values also became dependent on the samples. Sample C recorded the highest energy content ± Samples A and B had nearly the same energy level, ± ± Sample C is more homogeneous in terms of protein and fat content and will meet consumer demand for such a product, as they seek to replace their customary food with nutritionally more beneficial alternatives. In contrast, Sample B contains more fiber but fewer calories. These results are of interest when considered in the context of commercial offerings and the contemporary understanding of functional beverage formulation (**Tables 2 and 3**).

Table 2. Proximate analysis of instant functional drink powders

Parameter	Sample A (Pure Mango)¹	Sample C (Mango+Seed)²	Sample B (Mango+Peel)³
Moisture (%)	4.8 ± 0.03^{A}	4.2 ± 0.04^{AB}	5.1 ± 0.02^{C}
Ash (%)	2.4 ± 0.02^{A}	3.1 ± 0.03^{B}	3.8 ± 0.02^{BC}
Protein (%)	2.1 ± 0.02^{A}	8.4 ± 0.03^{B}	3.2 ± 0.02^{AC}
Fat (%)	0.8 ± 0.03^{A}	12.5 ± 0.04^{B}	1.2 ± 0.01^{AC}
Carbohydrate (%)	89.9 ± 0.15^{A}	71.8 ± 0.20^{B}	86.7 ± 0.18^{AC}
Energy (Kcal/100 g)	375.2 ± 0.25^{AC}	435.3 ± 0.30^{B}	371.4 ± 0.28^{C}

Values are presented as mean ± S.D (n=3). Values in same column within each parameter with different letters were significantly different from each other (p ≤ 0. 05). using a TWO-WAY analysis of variance (ANOVA) and Tukey's HSD test.

Table 3. Mineral and Vitamin C analysis of instant functional drink powders (mg/100g)

Mineral Contents	Sample A (Pure Mango)¹	Sample C (Mango+Seed)²	Sample B (Mango+Peel)³
Na	185.4 ± 0.04^{c}	195.6 ± 0.03^{b}	210.3 ± 0.04^{a}
K	3.8 ± 0.03^{c}	8.5 ± 0.02^{a}	5.2 ± 0.03^{b}
Ca	5.5 ± 0.04^{c}	9.8 ± 0.03^{a}	7.4 ± 0.02^{b}
Mg	1.7 ± 0.02^{c}	4.2 ± 0.03^{a}	2.8 ± 0.04^{b}
Cl	56.8 ± 0.03^{c}	58.2 ± 0.04^{a}	57.5 ± 0.03^{b}
P	5.3 ± 0.02^{c}	12.4 ± 0.03^{a}	6.8 ± 0.02^{b}
Fe	0.130 ± 0.002^{c}	0.285 ± 0.003^{a}	0.198 ± 0.002^{b}
Vit-C (mg)	580.5 ± 0.5^{a}	495.4 ± 0.4^{c}	545.6 ± 0.3^{b}

Values are presented as mean ± S.D (n=3). Values in same column within each parameter with different letters were significantly different from each other (p ≤ 0. 05). using a TWO-WAY analysis of variance (ANOVA) and Tukey's HSD test.

3.2. Mineral analysis of functional instant drink powders

The mineral content analysis of three instant drink powder formulations revealed distinctive variations in their compositions. Sample A (pure mango) exhibited a baseline mineral content typical of commercial mango powders, with sodium at 185.4 ± 0.04 mg/100g, potassium at 3.8 ± 0.03 mg/100g, and the highest vitamin C content (580.5 ± 0.5 mg/100g). Similarly, sample C (mango + pumpkin seed) demonstrated significant mineral enhancement, particularly in essential nutrients. Likewise, Sample B (mango + pumpkin peel) showed moderate mineral enhancement. These results are inconsistent with (Kulczyński and Gramza-Michałowska 2019) research on pumpkin seed enrichment. However, vitamin C content decreased to 495.4 ± 0.4 mg/g due to the dilution effect from seed powder addition. The results indicate effective nutrient enrichment through the incorporation of pumpkin by-products, with Sample C achieving the highest mineral content and Sample B offering moderate mineral enhancement alongside improved vitamin C retention.

3.3. Bioactive compounds of functional drink powders

The antioxidant capacity of the formulations was quantitatively assessed, revealing that Sample A (pure mango) exhibited an antioxidant capacity of 21.8 ± 0.03 mg TE/100 g, which is comparable to that of commercially available mango powders. In comparison, Sample C (mango + pumpkin seed) demonstrated a significant enhancement in bioactive compound concentrations, with TAC increasing to 1.15 ± 0.02 mg/100 g, TFC reaching 42.8 ± 0.03 mg QE/100 g, and TPC rising to 45.6 ± 0.02 mg GAE/100 g, contributing to the highest antioxidant capacity of 32.4 ± 0.02 mg TE/100 g among all formulations. Similarly, Sample B (mango + pumpkin peel) exhibited notable improvements in bioactive compounds, with TAC at 0.92 ± 0.01 mg/100 g, TFC at 35.2 ± 0.02 mg QE/100 g, and TPC at 38.4 ± 0.03 mg GAE/100 g. The antioxidant capacity of Sample B was measured at 28.5 ± 0.02 mg TE/100 g, demonstrating a substantial increase compared to the control (Sample A). The result is supported by the findings of (Zdunic *et al.* 2016) regarding the strong antioxidant potential of pumpkin seed powder. Moreover (Altemimi *et al.* 2015) findings also exhibit the strong phytochemistry of pumpkin which favors its utilization for functional product development. These results highlight the successful incorporation of peel bioactives, which may confer superior functional properties in the final product. The progressive increase in bioactive compounds obtained from all samples reveals the potential for both pumpkin seeds and their peel to significantly enhance the functionality of mango-based instant drinks, with the seed showing the most favorable results in improving antioxidant levels. These findings have great importance in furthering functional beverages that enhance health benefits (**Table 4**).

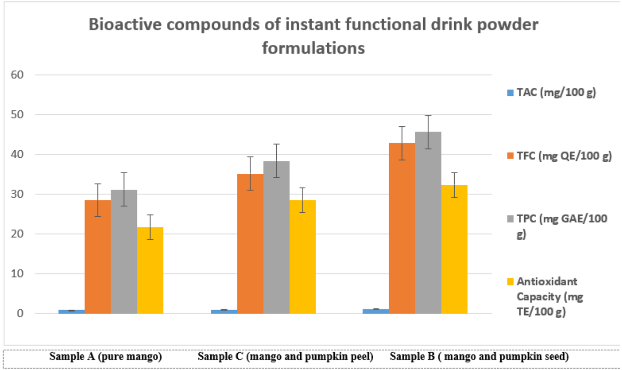


Figure 1. Bioactive compounds of instant functional drink powders formulations.

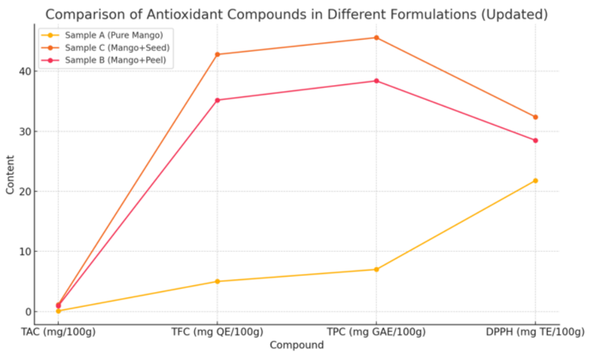


Figure 1a. Comparison of antioxidant compounds (TAC, TFC, TPC, DPPH) in different instant functional drink formulations. Sample C (Mango + Seed) exhibited the highest antioxidant activity, followed by Sample B (Mango + Peel), while Sample A (Pure Mango) showed the lowest values. Placeholder values were used for missing antioxidant parameters in Sample A to maintain comparative trend visualization.

3.4. Microbial assessment of instant functional drink powders

Table 4. Microbial assessment of instant functional drink powders

Sample	TVC (CFU/ml)				TCC (CFU/ml)	E. coli (CFU/ml)	Mold and Yeast (CFU/ml)
	0 days	30 days	60 days	90 days			
Sample A (Pure Mango)[1]	1.5×10^2	1.2×10^3	1.8×10^3	1.0×10^4	ND	ND	ND
Sample C (Mango+Seed)[2]	1.8×10^2	1.4×10^3	2.1×10^3	1.4×10^4	ND	ND	ND
Sample B (Mango+Peel)[3]	2.0×10^2	1.5×10^3	2.3×10^3	1.5×10^4	ND	ND	ND

ND= not detectable, TVC= total viable count and TCC= total coliform count

The control assessment of microbiological quality of three formulations of instant drink powder showed, over the 90 days of storage, differing counts of Total Viable Count. Sample A (pure mango) presented the lowest initial count of TVC at 1.5×10^2; an increase to 1.0×10^4 by day 90 is in line with (Abdul Rahman et al. 2024a), who reported that the kombucha powder exhibited a slight increase in microbial load while maintaining the safe consumption limits. Sample C (mango+pumpkin seed) and Sample B (mango+pumpkin peel) showed similar trends. Importantly, all three formulations proved microbiological safety throughout the entire storage period, with no detectable counts of Total Coliform Count (TCC), *E. coli*, or yeast and mold. The slightly higher Total Viable Count (TVC) in the enriched formulae samples C and B can be attributed to the additives; however, the counts were well within the acceptable limits for dry powder drinks. These findings are in accordance with (Jannah *et al.* 2022) and (Tan *et al.* 2021) who assessed the TVC increase in instant powder product but no pathogenic microorganisms were spotted, ensuring microbial safety. Moreover, (Habiba 2024) observed comparable outcomes for date seed powder-based instant drink. In general, the increase in TVC was at a similar rate across all samples, indicating consistent stability during storage. The absence of pathogenic organisms demonstrates their suitability for commercialization and reinforces consumer confidence in the safety and integrity of functional beverage powders.

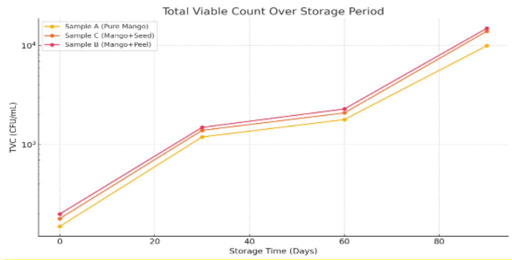


Figure 2. Total viable count (TVC) of instant functional drink powders during 90-day storage. All samples remained within safe microbiological limits. A gradual increase in microbial load was observed over time, with Sample C (Mango + Seed) and Sample B (Mango + Peel) showing slightly higher counts than Sample A (Pure Mango), likely due to the addition of pumpkin by-products. No pathogenic organisms were detected in any formulation.

3.5. Sensory analysis of instant functional drinks

Assessing instantaneous functional beverage powders through sensory evaluation The rating was conducted using a 9-point hedonic scale, where 9 represented "like extremely" and 1 meant "dislike extremely." The results show that pumpkin by-products had been successfully incorporated without compromising sensory acceptability. Sample A(mango), served as the benchmark for acceptance, receiving equal grading for all criteria tested under color (7.5 ± 0.82), flavor (7.8 ± 0.76), taste (7.6 ± 0.65), and overall acceptability (7.5 ± 0.74). Sample C (mango + pumpkin seed) was the most preferred formulation with the highest scores for almost all sensory attributes. Sample B (mango + pumpkin peel) demonstrated acceptable but lower scores across parameters While these scores were slightly lower than the other formulations, they remained within the acceptable range, consistent with (Patel *et al.* 2020) findings on pumpkin-based functional products. Similarly, (Hedegaard and Skibsted, 2024) and (AlJahani and Cheikhousman, 2017) described similar trends. The slight reduction in sensory scores might be attributed to the visible and taste changes introduced by peel powder, though the product maintained satisfactory consumer acceptance levels. Both pumpkin seed and peel-based formulations scored above the midpoint of the 9-point hedonic scale and thus demonstrated general acceptability among consumers (**Table 5**).

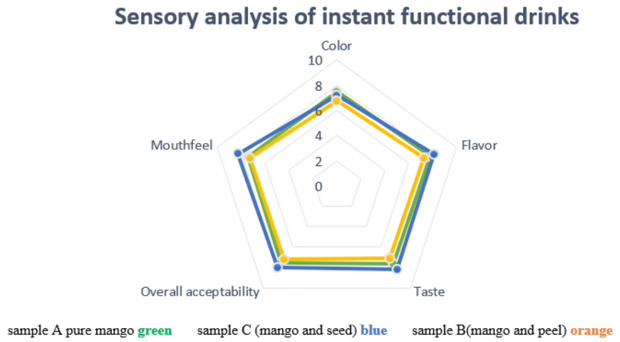


Figure 3. Sensory analysis of instant functional drinks

Figure 3. Radar plot of sensory analysis for instant functional drink powders. Sample C (Mango + Seed, blue) received the highest ratings across flavor, taste, overall acceptability, and mouthfeel, indicating enhanced consumer preference. Sample A (Pure Mango, green) showed consistent acceptability, while Sample B (Mango + Peel, orange) demonstrated slightly lower but still acceptable scores, particularly in taste and mouthfeel.

3.5.1. pH and Acidity Changes During Storage

Across the 90-day storage period, all samples exhibited a steady decline in pH values, with Sample A (Pure Mango) showing the most pronounced drop, from 4.2 to 3.9. Similarly, sample C (mango + seed) showed a decrease in pH from 4.5 to 4.1 over 90 days, and for sample B (mango + peel), the pH decreased from 4.3 to 4.0 during the same period. This steady acidification is consistent with expected biochemical processes, such as enzymatic action and latent fermentation, which are also well-documented in stored, fruit-based powdered drinks. These findings are consistent with the findings of (Abdul Rahman *et al.* 2024), who observed a steady decline in pH accompanied by an increase in acidity in spray-dried kombucha powder. The authors explained that these changes were due to the continuous accumulation of organic acids over time. Further, (Ahmed *et al.* 2023) also recorded similar trends, thereby further supporting this trend of acidification recorded in this study. These results agree with earlier studies described by (Younis *et al.* 2024) (Younis *et al.* 2024) who reported a corresponding decrease in pH with an increase in acidity in orange juice through the addition of freeze-dried cassia javanica extracts' coacervates.

Table 5. physiochemical analysis of instant functional drinks

Parameter	Sample A(Pure Mango)^{1} (30 days)	Sample A (Pure Mango)^{1} (60 days)	Sample A (Pure Mango)^{1} (90 days)	Means	Sample C (Mango+Seed)^{2} (30 days)	Sample C (Mango+Seed)^{2} (60 days)	Sample C (Mango+Seed)^{2} (90 days)	Means	Sample B (Mango+Peel)^{3} (30 days)	Sample B (Mango+Peel)^{3} (60 days)	Sample B (Mango+Peel)^{3} (90 days)	Means
Ph	4.2 ± 0.03	4.1 ± 0.02	3.9 ± 0.01	4.067^{A}	4.5 ± 0.02	4.3 ± 0.03	4.1 ± 0.02	4.3^{B}	4.3 ± 0.02	4.2 ± 0.01	4.0 ± 0.02	4.167^{C}
Acidity (%)	0.65 ± 0.01	0.72 ± 0.02	0.81 ± 0.01	0.726^{C}	0.58 ± 0.02	0.64 ± 0.01	0.73 ± 0.02	0.65^{A}	0.61 ± 0.01	0.68 ± 0.02	0.77 ± 0.01	0.68^{B}
TSS (Brix)	18.2 ± 0.3	17.5 ± 0.2	16.8 ± 0.1	17.50^{B}	19.5 ± 0.1	18.9 ± 0.2	18.2 ± 0.1	18.867^{C}	18.8 ± 0.2	18.1 ± 0.1	17.4 ± 0.1	18.1^{A}
Sweetness	7.8 ± 0.2	7.5 ± 0.1	7.2 ± 0.1	7.50A	8.1 ± 0.1	7.9 ± 0.2	7.6 ± 0.1	7.87^{B}	7.9 ± 0.1	7.7 ± 0.1	7.4 ± 0.1	7.67^{C}
Sourness	3.1 ± 0.1	3.4 ± 0.1	3.8 ± 0.1	3.43^{C}	2.9 ± 0.1	3.1 ± 0.1	3.5 ± 0.1	3.167^{A}	3.0 ± 0.1	3.3 ± 0.1	3.6 ± 0.1	3.3^{B}

Values are presented as mean ± S.D (n=3). Values in same column within each parameter with different letters were significantly different from each other (p ≤ 0. 05). using a TWO-WAY analysis of variance (ANOVA) and Tukey's HSD test.

3.5.2. Total Soluble Solids (TSS) stability

Total soluble solids (TSS, Brix), a critical indicator of sugar concentration, exhibited a gradual decline across the storage period, reflecting the natural degradation of sugars and the hygroscopic nature of powdered products, which tend to absorb moisture over time. Specifically, Sample A (Pure Mango), and Sample B (Mango + Peel) demonstrated a decline in TSS Sample C (Mango + Seed) initially exhibited the highest TSS content at 19.5 Brix (30 days), which subsequently reduced to 18.2 Brix (90 days). However, the reductions in TSS observed are in line with the natural breakdown of sugars and the hygroscopic behavior of powdered products, which can cause water absorption over time. This decrease in TSS aligns with the results of Mittal and Bajwa (2014), which show a steady decline in sugar solubility due to crystallization and water absorption. Additionally, noted similar decreases in papaya fruit powder to support the correctness of these outcomes.

4. Sweetness and Sourness

The sweetness in all samples showed a consistent decline over the storage period, with Sample A demonstrating the greatest drop.The observation is consistent with previous research, where a progressive reduction in sweetness was measured in dried sour cherry powder by (Abbasi and Azizpour, 2016) due to sugar degradation and potential Maillard reactions, affecting flavor stability. The results are similar with (Naik *et al.* 2013) who reported the storage study of coconut protein powder. Conversely, sourness levels indicated a rise in all preparations, in line with the progressive acidification characteristic of powdered foods (Abdul Rahman *et al.* 2024b). Surprisingly, Sample A showed the greatest increase in sourness. The change in sensory perception is due to acid hydrolysis reactions and organic acid formation during storage (Bressani *et al.* 2020). The observations are consistent with previous research on alterations in acidity in fruit-based foods, where increased storage resulted in greater acid content due to oxidative and enzymatic reactions(Toy *et al.* 2022).

The enhanced antioxidant capacity of pumpkin by-product-containing foods, particularly Sample C, is primarily due to bioactives like flavonoids and phenolic acids. These bioactives work by scavenging free radicals and metal chelation, and pumpkin seeds contribute phenolics like ferulic and p-coumaric acids, while peels contribute catechins and gallic acid (Gavril *et al.* 2024). Small reductions in pH, TSS, and sweetness after 90-day storage indicate partial degradation of these bioactives, possibly due to oxidation and acidification (Imran *et al.* 2021). Encapsulation methods, freeze-drying, pH correction, and synergistic ingredient mixtures (e.g., with ascorbic acid-containing fruits) are suggested to improve stability and sensory acceptability (Ozdal *et al.* 2020; Bochnak-Niedźwiecka and Świeca 2020).

5. Conclusion

This study highlights the significant potential of utilizing pumpkin by-products, particularly seeds and peels, in the formulation of instant functional drink powders. The incorporation of these nutrient-dense by-products not only enhances the nutritional profile of the beverages but also contributes to sustainability by minimizing agricultural waste. Among the formulations tested, the pumpkin seed-enriched variant demonstrated superior protein, fat, and antioxidant content, making it an excellent candidate for the development of functional beverages. In contrast, the pumpkin peel formulation demonstrated enhanced fiber and mineral content, offering additional health benefits. The physicochemical assessments confirmed the stability of the formulations over a 90-day period, with all samples maintaining safe microbiological limits. Sensory analysis revealed that the pumpkin seed-enriched formulation was the most preferred among panelists due to its superior taste and overall acceptability. These findings suggest that pumpkin by-products can be successfully integrated into instant drink powders without compromising consumer appeal. From a commercial perspective, this study provides a strong foundation for the development of innovative functional beverages that cater to modern consumer demands for nutritionally enhanced, convenient, and sustainable food products. Conversion of Cucurbita maxima by-products into functional ingredients aligns with current sustainable food innovation trends. Emerging digital technologies facilitate to enhance processing efficiency and product development (Wen *et al.* 2025), while digital trade platforms facilitate expanded market access to value-added products (Liu *et al.* 2025). Addition of pumpkin powder to instant drinks supports the circular economy and promote sustainable utilization of resources (Wang *et al.* 2025; Ma *et al.* 2025). In addition, green innovation in the food industry relies on aggregate energy and financial industry trends, which highlights the need for combined product design and marketing strategies (Wu *et al.* 2025; Tong, 2025).

6. Future Research Directions

In future studies, the mechanism of the contribution of specific bioactive components (e.g., flavonoids, polyphenols, etc.) in pumpkin by-products to the antioxidant activity of functional beverages, as well as the change of the stability of these components during storage, can be further analyzed in depth. In addition, combining with the existing literature, we will explore how to further improve the antioxidant capacity and sensory quality of the products by optimizing the formulation or processing. To fully utilize pumpkin by-products in functional food systems, future research needs to focus on their integration into diversified food matrices, like fermented dairy replacers, nutraceutical candies, or fiber-fortified baked foods, to evaluate cross-category functional compatibility and consumer acceptability. In addition, deployment of biotechnological innovation, such as enzymatic bioconversion, probiotic fermentation, or nanoencapsulation, can potentially increase the bioefficacy, stability, and sensory neutrality of such bio-residues significantly. At the same time, cross-disciplinary research combining behavioral economics and sensory science must be conducted to finally demystify the complexity of consumer perception, market segmentation, and value-based pricing strategies. Such integrative approaches will make the transformation of waste-derived components into marketable, health-promoting, and sustainable food innovations feasible.

Author Contributions

Ifrah Usman and Farhan Saeed: provided the conceptualization; Ali Imran and Muhammad Umair Arshad: finalized the methodology; Ifrah Usman, Muhammad Umair Arshad and Ali Imran: validation, visualization, and software; Ifrah Usman and Farhan Saeed: wrote the original draft and performed the analysis; Ifrah Usman, and Ali Imran; review and editing; Ali Imran: supervision and project administration.

Declarations of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be provided on a request basis.

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Ethics and Consent Statement

The sensory evaluation study was conducted in Pakistan, and ethical approval from an institutional ethics committee was not required for this study, as per national research ethics regulations. According to the applicable ethical guidelines, ethics committee approval is only required when research involves: human embryos, body cells, or tissues, socially vulnerable groups, clinical or interventional methods, animal testing, tracking or observing individuals without their knowledge, sensitive topics that may cause psychological distress and collection of confidential personal data that could impact participants' privacy or reputation. None of these conditions applied to this study. Participants voluntarily took part in the sensory evaluation, fully aware of the study's purpose, the product composition, and any potential allergens. They were provided with clear information about their role in the research and could withdraw at any time without facing any consequences. The product samples tested in this study were made from safe, commercially available ingredients. While chemical and microbiological safety of pumpkin by-products such as seeds and peels were confirmed through laboratory testing. Neither the product formulations nor the sensory evaluation process posed any risk to the physical or mental well-being of the participants.

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