



Research Article

Development of Apple and Beetroot Blended Ready-to-Serve Drink with Enhanced Nutritional Properties

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Abstract | With the advancement in scientific research, the development of functional beverages with improved nutritional profile is gaining popularity. For this purpose, different vegetables are being blended with fruits to increase the functionality of finished products. This research also aims to develop a beetroot and apple juice blended nutritious ready-to-serve (RTS) drink. For this purpose, five treatments with these formulations were prepared: APBR₀ containing 10% beetroot juice (BJ) and 0% apple juice (AJ), APBR₁ (8% BJ and 2% AJ), APBR₂ (6% BJ and 4% AJ), APBR₃ (4% BJ and 6% AJ), and APBR₄ (2% BJ and 8% AJ). While all treatments contain 15% sugar, 0.05% citric acid, 0.1% sodium benzoate as a preservative and the rest of the portion was water. The blended RTS samples were prepared, packed, and stored at 20±1°C for 60 days. The samples were analyzed for physicochemical and consumer acceptability (3 panels of judges where each panel had 5 judges) after every 15 days. The experiments were conducted in triplicate and analyzed using one-way ANOVA at P≤0.05. The means were separated using LSD. The results After physicochemical analyses, blend of BJ and AJ (6:4) was found most balanced formulation with total soluble solids (16.86±0.39%), pH (4.5±0.19), titratable acidity (0.30±0.06%), reducing sugars (1.48±0.34%), non-reducing sugar (14.52±0.69%), mineral content (0.70 mg/100 mL), ascorbic acid (0.125 mg/100 mL), *L**, *a**, *b** and *C** values of 54.09, 17.07, 9.89, and 18.73 respectively. The treatment also obtained the highest consumer acceptability score of 8.41 on a 9-point hedonic scale throughout the storage period. Thus, after complete analysis APBR₂ emerged as the best formulation with an improved nutritional profile and consumers acceptance. Furthermore, commercial validation should involve shelf-life studies under varied storage conditions and packaging, large-scale production trials, and consumer testing with diverse demographics to confirm scalability and market acceptance.

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Keywords | RTS drink, Beetroot, Apple, Functional drink, Improved minerals, Consumers acceptability.



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Introduction

A notable increase has been observed in the demand for RTS drink as a result of awareness among consumers about the importance of health and nutrition (Giri *et al.*, 2023). RTS drinks are non-fermented and non-carbonated beverage generally prepared by mixing fruit juice, sugar, water, and other additives such as preservatives and stabilizers (Rathinasamy *et al.*, 2022). Previously, the market was dominated by synthetic and carbonated beverages laden with sugar, synthetic flavors and preserving agents, with very low nutritional profile (Tuttle, 2025; Abu-Reidah, 2020). Being the excellent source of essential nutrients such as vitamins and minerals, inclusion of fruits and vegetables in RTS is gaining popularity in modern research (Pillai *et al.*, 2024; Sonker *et al.*, 2018). Inclusion of fruit and vegetables in RTS preparation led to the development of unique taste with enhanced nutrition and functionality (Hemalatha *et al.*, 2018). These fruit and vegetable based RTS are free from synthetic additives, rich in vitamins and minerals making them best choice for human consumption (Alam *et al.*, 2025; Kumar *et al.*, 2020).

Fruit-based drinks are known for their natural sweetness, richness of micro-nutrients, and refreshing taste (Anilakumar *et al.*, 2017). However, combining vegetables with fruit in the preparation of drinks improve their nutritional and functional properties (Bhardwaj and Pandey, 2011). Among different fruit and vegetable, apple (*Malus domestica*) and beetroot (*Beta vulgaris*) juices can be best option for blending with enhanced sensory and nutritional characteristics (Muntean *et al.*, 2022). Apple, mostly grown fruit of temperate region, is well-known for its natural sweetness, pleasant aroma, and high minerals content (Verma *et al.*, 2023; Melke and Fetene, 2014). It is a good source of sugars like glucose and fructose, mineral like iron, magnesium and calcium (Akyereko *et al.*, 2023). Apart from this apple also contain different bio-active compounds like polyphenols and phenolics in addition to various essential vitamins. These diverse group of health promoting compounds impart apple its anti-inflammatory, antioxidant, antimicrobial, anti-depressive, and anti-carcinogenic effects (Guiné *et al.*, 2021).

Beetroot, considered as one of the most potent vegetable due to the presence of certain bio-active compounds such as betalains, flavonoids, catechins, saponins, phenolics, and alkaloids (Pandita *et al.*,

2020). Beetroot is primarily composed of minerals like sodium, potassium, calcium, iron, magnesium, zinc and copper in addition to carbohydrates and other vitamins (Mirmiran *et al.*, 2020). These critical components of beetroot have medicinal properties such as antioxidant, antimicrobial, antihypertension, antiviral, analgesic, antihyperglycemic, hepatoprotective, anti-progestogenic, antimigraine, anti-inflammatory, antiallergic, antithrombotic and anti-tumorigenic properties (Thiruvengadam *et al.*, 2024; Pandita *et al.*, 2020). Despite its rich nutritional profile, the earthy taste of beetroot juice limit direct consumption (Kardas *et al.*, 2024). However, combining beetroot juice with fruit not only mask its earth taste but also improve minerals content, antioxidant potential, and functional properties (Paudel, 2022).

Different studies have reported the importance of fruit and vegetable juice blends in enhancing functional properties and nutritional diversity of beverages. Previous researches on RTS drink were primarily focused on conventional fruit juices or single fruit formulations and to the best of our knowledge, limited studies have explored optimized apple and beetroot juice blends for RTS beverages. Though, both apple and beetroot are regarded for their nutritional and organoleptic attributes, however, their combined effect on storage stability, physicochemical characteristics and consumers acceptability remain unexplored. Furthermore, optimum blend ratio that multiply nutritional status and organoleptic characteristics has not been evaluated systematically. Knowledge these features is critical for the development of nutritionally enriched and commercially viable RTS drink that maintain long term shelflife in addition to meeting consumer preferences. Therefore, this study aims to evaluate and develop RTS drink from apple and beetroot juice, specifically focusing on its composition, minerals content, storage stability, and consumers acceptability. The key objectives of the study are: to develop shelf-stable, and standardized RTS drink with optimum blend ratio of apple and beetroot juices, to evaluate the physico-chemical characteristics of the RTS drink during the storage period, and to analyze consumer acceptability of the blended RTS in terms of color, aroma and taste during the 60 days storage duration.

Materials and Methods

Raw materials collection

Firstly, uniform-sized and disease-free beetroots and

apples were collected from a farm at Harichand village of district Charsadda and fruit market of Peshawar. The collected beetroots and apples were transferred to the Food Science & Technology laboratory, Agriculture Research Institute (ARI) Tarnab, Khyber Pakhtunkhwa. The raw materials were thoroughly washed using tap water and left to dry at $25\pm1^{\circ}\text{C}$. After drying the raw materials, peel was removed from each item, cut into slices, and blended in a juicer to get pulp. The pulp was further pressed and filtered through muslin cloth to get clear juice.

RTS preparation

The blended RTS drinks were prepared using different concentrations of beetroot and apple as follows; APBR_0 = only Beetroot juice (BJ) 10% was used as control, APBR_1 = Beetroot juice (BJ) 08% + Apple juice (AJ) 02%, APBR_2 = Beetroot juice (BJ) 06% + Apple juice (AJ) 04%, APBR_3 = Beetroot juice (BJ) 04% + Apple juice (AJ) 06%, and APBR_4 = Beetroot juice (BJ) 02% + Apple juice (AJ) 08%. Other ingredients such as sugar (sucrose), citric acid as stabilizer, and sodium benzoate (preservative) were added in fixed quantity as 15%, 0.05%, and 0.1% respectively. The rest portion of all treatments was water. A total of 18.75 liters (L) of RTS for the whole research with 3 replications per treatment was prepared. After preparation the RTS was pasteurized at 85°C for 15 seconds and poured into 250 mL pre-sterilized plastic bottles and stored at $20\pm1^{\circ}\text{C}$ and analyzed for physico-chemical properties and consumers acceptability at 0, 15-, 30-, 45-, and 60-days intervals.

Physico-chemical analyses

pH, Titratable acidity, Reducing and Non-reducing sugars, Total soluble solids

The pH of the blended RTS formulations was determined using a digital meter (PHS-3C, Pakistan) at 20°C as specified by [AOAC, 2012](#). Titratable acidity was determined through potentiometric titration with 0.1N NaOH solution and reported as % ([AOAC, 2012](#)). Reducing sugars and non-reducing sugars were determined as specified earlier ([AOAC, 2012](#)) and reported as %. The TSS of each sample was determined by recording refractive index at 20°C using a refractometer (ERB-32, ERMA, Japan) as reported earlier ([AOAC, 2012](#)).

Minerals content of blended RTS

The iron, magnesium, calcium, copper, and zinc content of the blended RTS samples were analyzed

using ICP-OES (Avio 220 Max, PerkinElmer, Massachusetts, USA) and UV Spectrophotometer (Specord Plus, double beam, Analytik Jena, Germany) for quantification using the method specified earlier ([Musa and Parmeshwal, 2018](#)). The samples were digested in the presence of nitric acid and diluted to known volume before analysis and final concentration of each mineral was calculated as follows:

$$\text{Mineral content (mg/100mL)} = \frac{IR_s - IR_b \times F_v}{V_s \times 1000} \times 100$$

Where,

IR_s = Inst. Reading of sample (ng/mL)

IR_b = Inst. Reading of blank (ng/mL)

F_v = Final volume (mL)

V_s = Volume of sample (mL)

Ascorbic acid content of blended RTS

The ascorbic acid content of blended RTS samples was measured using titration method with 2,6-dichlorophenol indophenols dye as a decolorizing agent and the results were expressed as mg/100 mL ([AOAC, 2012](#)).

Color of blended RTS

The color changes in RTS samples were measured in terms of color attributes such as L^* (Lightness), a^* (Redness), b^* (Yellowness), and C^* (Chroma) using a colorimeter (PSE-CSM 2, PCE Instruments UK Ltd., United Kingdom) colorimeter ([Jahan et al., 2021](#)).

Consumers acceptability of blended RTS

Consumers acceptability in terms of color, taste and aroma was determined using the method specified by [Juarez-Enriquez et al. \(2015\)](#) with slight modification. Instead of 10-point hedonic scale, 9-point hedonic scale was used (9 = extremely liked, 5 = neutral, and 1 = extremely disliked. Instead of 4 panelists, 3 panels with 5 members in each panel (all members were familiar with beetroot, mixed gender falling within age range of 24-35 years) analyzed the samples for consumers acceptability.

Statistical analysis

The obtained results were analyzed using completely randomized design (CRD) and the means were separated using least significant difference (LSD) at probability level of 5% ($P \leq 0.05$).

Results and Discussion

Changes in pH and TA of blended RTS

The pH in given treatments tend to decrease and titratable acidity tend to increase with increasing concentration of apple juice (Table 1). During the storage period, slight increase was observed in the pH and decrease in TA of all samples, however, the change was not statistically significant ($p \leq 0.05$). The lowest pH and highest TA were observed in APBR₄ (3.5 ± 0.26 and $0.47 \pm 0.03\%$ respectively) and highest pH value and lowest TA value was observed in APBR₀ (4.8 ± 0.27 and $0.24 \pm 0.02\%$ respectively). The decrease in pH and increase in TA by increasing apple juice and decreasing beetroot juice concentration might be due to the innate characteristic of each juice. Previously, different studies have reported pH of beetroot in the range of 6.0 to 6.3 and TA in the range of 0.014 to 0.11% (Mwaijibe and Vicent, 2025; Kale *et al.*, 2018; Koss-Mikołajczyk *et al.*, 2015). Similarly, pH and TA of apple juice has been reported in the ranges of 3.46 to 3.69 and 0.45 to 0.69% respectively (Lončarić *et al.*, 2023; Wilczyński *et al.*, 2019; Włodarska *et al.*, 2018; Moon *et al.*, 2006). Moreover, increase in pH can be attributed to decrease in TA due to oxidation of weak organic acids by ambient temperature and transparent packaging leading to light induced oxidation (Mwaijibe and Vicent, 2025; Tobolková *et al.*, 2020; Kathiravan *et al.*, 2014).

Changes in TSS, reducing and non-reducing sugars of blended RTS

The TSS, reducing and non-reducing sugars for different blended RTS is given in table 4.1. The TSS content and reducing sugars increased with decreasing beetroot juice concentration and increasing apple juice, however, non-reducing sugars showed opposite pattern and decreased with increasing apple juice concentration. During storage period, a significant

($P < 0.05$) change in all three parameters were observed. Apple contain higher concentration of reducing sugars and lower non-reducing sugar than beetroot juice, which might be the reason for differences among different RTS blends (Table 1). These findings are aligned with the studies where the TSS of beetroot juice has been reported in the range of 7.96 to 12.5% (Mwaijibe and Vicent, 2025; Dusabumuremyi *et al.*, 2021; Kale *et al.*, 2018). Compared to this TSS range of apple juice has been reported in the range of 11.9 to 13.6% (Mehdi *et al.*, 2020; Wilczyński *et al.*, 2019; Włodarska *et al.*, 2018). Moreover, apple juice contains higher concentration of reducing sugars as compared to beetroot juice that contain more non-reducing sugar (Lončarić *et al.*, 2023; Kuchi *et al.*, 2017; Wosiacki *et al.*, 2007). Moreover, during storage decrease in TSS was observed, and previously certain studies have observed similar changes in TSS of beetroot and apple juice during storage (Mwaijibe and Vicent, 2025; Juarez-Enriquez *et al.*, 2015). This loss of TSS content might be due to oxidation of certain organic acids by heat and light (Tobolková *et al.*, 2020; Kathiravan *et al.*, 2014).

Minerals content of blended RTS

The minerals content of blended RTS formulations such as iron, magnesium, calcium, copper, and zinc are presented in Table 2. The iron content decreased from 0.085 to 0.0749 mg/100mL with increasing apple juice concentration from 0 to 8%. Similar increasing trend was observed in Magnesium, calcium, copper, and zinc. However, no significant changes were observed in mineral composition during storage. Beetroot contains 0.8 mg/100mL iron, 23 mg/100mL magnesium, 16 mg/100mL, 0.36 mg/100mL zinc, and 0.075 mg/100mL copper (Ceclu and Nistor, 2020; Wruss *et al.*, 2015). Similarly, apple juice contains 0.34 mg/100mL iron, 7.0 mg/100mL magnesium, 4.6 mg/100mL calcium, 0.02 mg/100mL zinc and

Table 1: Physico-chemical properties of apple and beetroot blended ready-to-serve drink after 60 days of storage at $20 \pm 1^\circ\text{C}$

Treatments	pH	Titratable acidity (%)	Reducing sugars (%)	Non-reducing sugar (%)	Total soluble solids (%)
APBR ₀	4.8 ± 0.27 a	0.24 ± 0.02 e	1.34 ± 0.51 e	14.67 ± 0.85 a	16.56 ± 0.30 e
APBR ₁	4.5 ± 0.19 b	0.30 ± 0.06 d	1.48 ± 0.34 d	14.52 ± 0.69 b	16.86 ± 0.39 d
APBR ₂	4.2 ± 0.31 c	0.36 ± 0.01 c	1.56 ± 0.11 c	14.46 ± 0.76 c	17.00 ± 0.42 c
APBR ₃	3.9 ± 0.15 d	0.42 ± 0.03 b	1.68 ± 0.17 b	14.39 ± 1.01 d	17.21 ± 0.31 b
APBR ₄	3.5 ± 0.26 e	0.47 ± 0.03 a	1.87 ± 0.21 a	14.22 ± 0.98 e	17.46 ± 0.38 a

The values are means $\pm$ standard deviation of three replicates. Statistical significance was determined using one-way ANOVA ($P < 0.05$), with least significance difference (LSD) post hoc test. Different letters within the columns indicate significance difference.

0.007 mg/100mL copper (Francini *et al.*, 2021; Henríquez *et al.*, 2011). In this way, ABPR2 provides approximately 1% of the adult RDA for iron per serving. The higher concentration of each mineral in beetroot can be the perfect reason for higher mineral content in those samples containing higher beetroot juice (Ceclu and Nistor, 2020; Henríquez *et al.*, 2011).

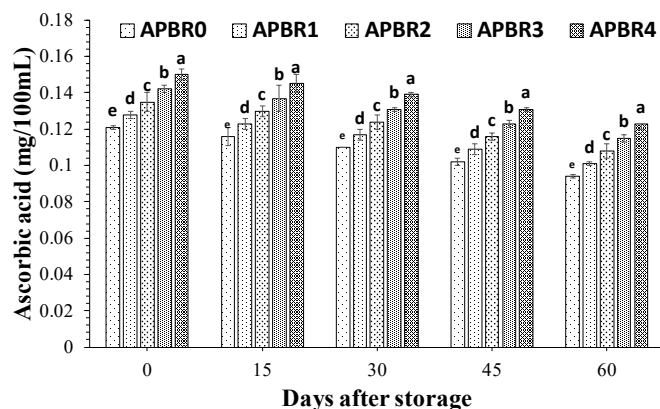


Figure 1: Ascorbic acid (mg/100 mL) in apple and beetroot blended ready-to-serve drink stored for 60 days at $20\pm1^{\circ}\text{C}$. The values are means $\pm$ standard deviation of three replicates. Statistical significance was determined using one-way ANOVA ($P<0.05$), with least significance difference (LSD) post hoc test. Different letters above columns indicate significance difference and the bars represent SD.

Ascorbic acid content of blended RTS

The ascorbic acid content of beetroot and apple juice blended RTS showed increasing trend with increasing apple juice concentration (Figure 1). Moreover, a significant decrease was also observed in ascorbic

acid of all treatments during storage ($p<0.05$). The ascorbic acid in given treatments (APBR₀ to APBR₄) decreased to 0.094, 0.101, 0.108, 0.115, and 0.123 mg/100mL on day 60. Apple juice generally contains more ascorbic acid as compared to beetroot juice. Apple contains more ascorbic acid than beetroot, that might be the possible reason for high ascorbic acid in treatments having higher concentration of apple juice. Previously, in cultivated apples, ascorbic acid has been reported in the range of 1.05 to 27.85 mg/100g (Li *et al.*, 2021; Fang *et al.*, 2017). Similarly, different studies have reported ascorbic acid content of beetroot juice in the range of 4.20 to 10.01 mg/100mL (Theba *et al.*, 2024; Kale *et al.*, 2018). Dambalkar *et al.* (2015) observed similar decreasing trend in ascorbic acid when beetroot concentration was reduced in RTS made from beetroot, ginger and orange juice. Decrease in ascorbic acid during storage can be attributed to its susceptibility to light, heat, oxygen, enzymes and non-enzymes driven reactions (El-Dakak *et al.*, 2017). During storage ascorbic acid is degraded into dehydroascorbic acid ultimately converting into biologically inactive compound (Yin *et al.*, 2022). The oxidation process further intensified by elevated temperature and presence of iron and copper ions (Murekhina *et al.*, 2022; Shen *et al.*, 2021). Furthermore, increase in pH can also speed up ascorbic acid degradation as it is more stable in acidic conditions (Yin *et al.*, 2022).

Color changes of blended RTS

Color changes in blended RTS in terms of L^* , a^* , b^* , and C^* are given in Figure 2. The L^* and b^* values

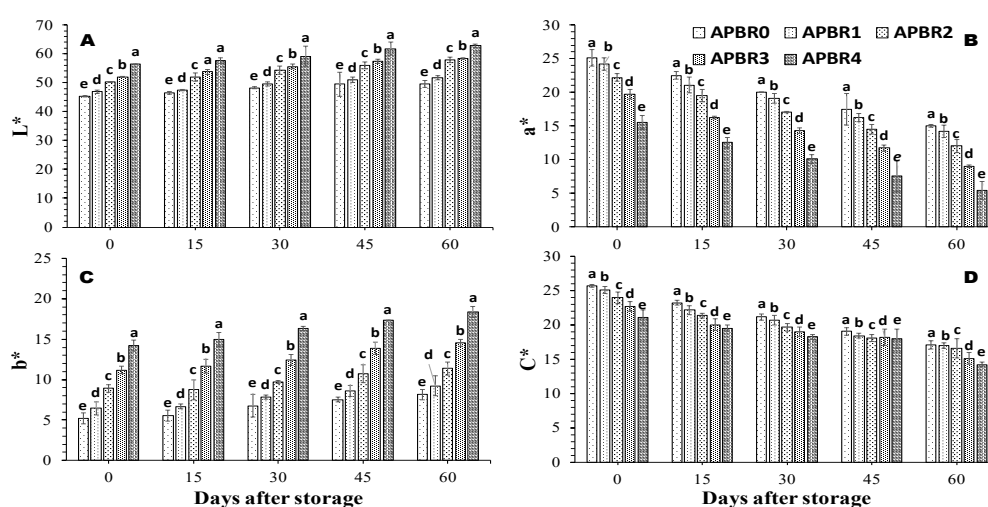


Figure 2: Color analysis of apple and beetroot blended ready-to-serve drink in terms of (L^* , a^* , b^* , and C^* values) during 60 days storage at $20\pm1^{\circ}\text{C}$. The values are means $\pm$ standard deviation of three replicates. Statistical significance was determined using one-way ANOVA ($P<0.05$), with least significance difference (LSD) post hoc test. Different letters above columns indicate significance difference and the bars represent SD.

increased with increasing apple juice concentration among the treatments, however, a^* and C^* values decreased with increasing apple juice and decreasing beetroot concentration. The L^* and b^* values for all treatments significantly increased ($P < 0.05$), while, the a^* and C^* values for all treatments significantly decreased during the storage period of 60 days. Furthermore, APBR₂ stable a^* (redness) likely contributed to its higher acceptability. Similar changes in color parameters were observed in several studies (Tobolková *et al.*, 2020; Prieto-Santiago *et al.*, 2020). Color is one of the most focused parameters for analyzing product quality as it significantly affects consumer preferences. The increase in L^* values might be due to browning precipitation of unstable and suspended particles during storage (Kayın *et al.*, 2019; Kathiravan *et al.*, 2015; Casati *et al.*, 2012). The decrease in a^* and increase in b^* values can be attributed to degradation betalains in beetroot and anthocyanins in apple juice to other compounds imparting yellow color (Tobolková *et al.*, 2020; Knebel *et al.*, 2018). C^* value is dependent on a^* and b^* values and drops during storage indicating non-enzymatic browning and less saturation in the color of the drink (Fikry *et al.*, 2023; Tobolková *et al.*, 2020).

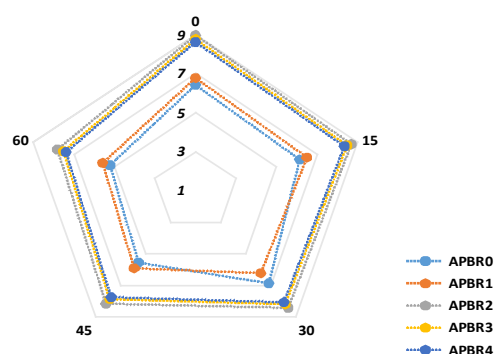


Figure 3: Consumers acceptability of apple and beetroot blended ready-to-serve drink in terms of organoleptic characteristics such as taste and color during 60 days storage at $20 \pm 1^\circ\text{C}$. The values are means of three replicates.

Statistical significance was determined using one-way ANOVA ($P < 0.05$), with least significance difference (LSD) post hoc test.

Consumers acceptability of blended RTS

The consumers acceptability of apple and beetroot blended RTS is presented in Figure 3. On day 0, APBR₂ get higher score of 8.96, followed by APBR₃ (8.76) APBR₄ (8.61), APBR₁ (6.77), and APBR₀ (6.41). Throughout the storage study, these formulations showed similar decreasing consumer acceptance trend. On day 60 the score for mentioned treatments reduced to 7.82, 7.53, 7.39, 5.58, and 5.22 respectively. A significant difference was observed in overall acceptability score for all treatments. The APBR₂ treatment consistently maintained higher consumer acceptability during the entire study period. Consumer acceptance of a product depends upon sensory characteristics and mostly people are used to of using apple products (Drkenda *et al.*, 2021; Endrizzi *et al.*, 2025). Beetroot beverage on the other hand is new to the consumers and the earthy flavor associated with beetroot negatively affects consumers preferences (Casciano *et al.*, 2022). The result clearly expressed that 10% beetroot was not as appealing to consumers as in the 6:4 (beetroot and apple juice) concentration. Moreover, the difference in consumers acceptance may be due to varying physicochemical properties. As beetroot contains more minerals and sugar content (Mwaijibe and Vicent, 2025; Dusabumuremyi *et al.*, 2021) while apple juice contains more ascorbic acid and reducing sugars (Loncaric *et al.*, 2023; Li *et al.*, 2021).

Conclusions and Recommendations

The beetroot and apple blended RTS were analyzed for physicochemical and consumers acceptability for 60 days. Overall, treatments containing higher concentration of beetroot juice showed higher pH,

Table 2: Minerals content of apple and beetroot blended ready-to-serve drink after 60 days of storage at $20 \pm 1^\circ\text{C}$

Minerals content (mg/100mL)					
Treatments	Iron	Magnesium	Calcium	Copper	Zinc
APBR ₀	0.085±0.0002 a	1.970±0.001 a	1.710±0.003 a	0.0050±0.00001 a	0.0199±0.002 a
APBR ₁	0.082±0.0001 b	1.756±0.003 b	1.633±0.007 b	0.00493±0.00003 b	0.0197±0.007 b
APBR ₂	0.079±0.0003 c	1.541±0.004 c	1.555±0.002 c	0.00485±0.00004 c	0.0193±0.005 c
APBR ₃	0.077±0.0001 d	1.3269±0.006 d	1.477±0.008 d	0.00477±0.00001 d	0.0191±0.003 d
APBR ₄	0.075±0.0001 e	1.1125±0.002 e	1.399±0.004 e	0.00469±0.00005 e	0.0190±0.003 e

The values are means $\pm$ standard deviation of three replicates. Statistical significance was determined using one-way ANOVA ($P < 0.05$), with least significance difference (LSD) post hoc test. Different letters within the columns indicate significance difference.

total soluble solids, non-reducing sugar, and minerals content. Treatments containing higher concentration of apple juice showed higher titratable acidity, reducing sugars, and ascorbic acid content. The consumer acceptability scores, however, showed different pattern with APBR₂ (6:4 Beetroot and apple juice) emerging as the most accepted treatment throughout the storage period compared to all other treatments. As clear from the physicochemical analyses results, this treatment also contains balance amount of each parameter, with low pH, non-reducing sugars, and high TA, reducing sugars and ascorbic acid. Thus, it can be concluded that beetroot and apple juice in 6:4 concentration should be used for the preparation of blended RTS. Considering the scope of this study, commercial validation should include large-scale production trials, shelf-life testing in opaque packaging to minimize ascorbic acid degradation and consumer testing with diverse demographics to confirm stability and market acceptance.

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Novelty Statement

The study represents a novel approach to develop a functional RTS beverage by blending beetroot and apple juices in different ratios to improve both nutritional value and consumers acceptability.

Authors contributions

Ibrar Alam: Designed the study, conducted the laboratory work, analyzed the data, and wrote the manuscript.

Majid S. Hashmi: Contributed to the study design, supervised the research, and assisted in the finalization of the manuscript.

Mukaram Shah: Supervised the laboratory work and contributed to manuscript writing.

Muhammad Ayub: Supervised, reviewed and assisted in the finalization of the final draft.

Sahib Alam: Proofread the manuscript and contributed to its improvement.

Ayaz Ahmad: Assisted with data analysis and

manuscript finalization.

Zahid Alam: Supported laboratory work and data compilation.

Generative AI or AI assisted technology statement

The authors declare that no generative AI or AI-assisted technology was used in the writing or editing of this manuscript.

Conflict of interests:

The authors declare that there are no conflicts of interest related to this work.

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