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Athira Mundassery, Jancirani Ramaswamy, Meera Balachandran, Sivasabari Kathirvel, Prema Nedungadi & Kiruba Krishnaswamy

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Processing-induced modulation of nutraceutical and anti-nutritional profiles of beetroot variants targeting enhanced iron retention

Athira Mundassery[a], Jancirani Ramaswamy[b*], Meera Balachandran[cd], Sivasabari Kathirvel[e], Prema Nedungadi[f], Kiruba Krishnaswamy[gh*]

[ab*] *Department of Food Science and Nutrition, Amrita School of Physical Sciences, Coimbatore, Amrita Vishwa Vidyapeetham, Tamil Nadu, India*

[c] *Department of Chemical Engineering and Materials Science, Amrita School of Engineering, Coimbatore, Amrita Vishwa Vidyapeetham, Tamil Nadu, India*

[d] *Centre of Excellence in Advanced Materials and Green Technologies (CoE-AMGT), Amrita School of Engineering, Coimbatore, Amrita Vishwa Vidyapeetham, Tamil Nadu, India*

[e] *Department of Soil Science and Agricultural Chemistry, Amrita School of Agricultural Sciences, Coimbatore, Amrita Vishwa Vidyapeetham, Tamil Nadu, India*

[f] *Amrita Create, Amrita School of Computing, Amritapuri, Amrita Vishwa Vidyapeetham, Kerala, India*

[g] *Department of Chemical and Biomedical Engineering, University of Missouri, Columbia, MO, USA*

[h] *Division of Food, Nutrition and Exercise Science, University of Missouri, Columbia, MO, USA*

[b*] *b_janci@cb.amrita.edu, 0000-0003-0393-8902*

[gh*] *krishnaswamyk@umsystem.edu, 0000-0001-7916-0311*

Abstract

Iron plays a crucial role in the body, despite being a micronutrient. Prolonged iron deficiency can lead to serious health problems, including anemia. This study aimed to optimize different processing methods and beetroot variants to enhance iron retention and promote factors that facilitate iron absorption. The beetroot was subjected to various processing methods, including pressure cooking, steaming, and open pan processing, and divided into three variants: juice, residue, and juice with residue. Results revealed that folate (31.33±0.57-38.33±0.57 µg/100 g DW), β-carotene (12.33±0.57-21.00±1.00 µg/100 g DW), and saponin (12.66±0.57-17.33±0.57 mg/100 g DW) were retained in juice with residue as compared to other variants. Whereas, iron was retained more in the residue variant, followed by juice with the residue variant after thermal processing. The iron absorption inhibitors, like oxalates, tannins, and calcium, were reduced significantly ($p<0.05$) after pressure cooking. Reductions were observed in bioactive compounds, including nitrates, betanin, and vulgaxanthin, by approximately 25%, 91%, and 32%, respectively, in pressure-cooked juice with residue. Despite reductions of these compounds, the overall nutritional improvements make it a favorable option with an iron bioavailability of 4.37±0.05%.

Keywords: beetroot, processing, iron, nutraceuticals, antinutritional

1 Introduction

Iron plays a crucial role in the body by acting as an oxygen carrier and for myoglobin synthesis, red blood cell formation, and brain development [1]. Despite being a micronutrient, iron deficiency is one of the crucial concerns in our society. Food-based approaches like supplementation and fortification are safer, sustainable, effective, and acceptable to the community to prevent deficiency-related problems, including anemia [2]. Iron is available from animal and plant sources in the form of heme and non-heme iron. The body absorbed and utilized heme iron more than non-heme iron due to the presence of certain dietary components in the plant sources. Compounds like citric, ascorbic, tartaric, and malic acids in plants enhance iron absorption, while oxalates, calcium, phytates, and certain polyphenols reduce it. Iron inhibitors bind the ferric form of iron and make it unavailable for the conversion to ferrous form, and thereby prevent its absorption. Whereas, iron enhancers counteract this binding process and accelerate the absorption in the body [3]. The enhancement of the nutrient absorption in the food by another nutrient is termed synergy [4]. While selecting an iron-rich food source, consideration is given to the synergistic approach as it maximizes nutrient bioavailability.

Beetroot (*Beta vulgaris* L.), a plant-based sources has gained attention for its rich nutritional profile, including iron, ascorbic acid, folic acid, nitrates, saponins, triterpenes, carotenoids, alkaloids, polyphenols, and flavonoids [5,6]. Studies have shown that beetroot supplementation can improve iron deficiency anemia, thereby enhancing the oxygen-carrying ability of erythrocytes, increasing folate levels, controlling birth defects, and relaxing smooth muscles. Research on the administration of 200 mL of beetroot juice to the female soccer players for 6 weeks enhanced the hematological parameters such as Hb from 12.77 to 14.21 g/dL, Hct from 39.06-42.22%, iron (79.50-86.10 µg/dL), ferritin level (36.20-37.09 ng/mL), and RBC from 4.31×10^6-5.11×10^6/µL [1]. Similar results were found in female volunteers who consumed 8 g of beetroot for 20 days [7]. In addition, it exhibits anti-oxidant activity, anti-inflammatory properties, action toward carcinogens, and protective effects on the liver and kidneys [5]. Due to these nutraceutical benefits, beetroot has been incorporated into versatile products, including cereal-based products, milk and milk products, meat and meat products, and films and coatings. It was used in various forms/variants such as juice, powder, pomace or pomace powder, extract or extract powder, residue, and peel, and processed using different technologies while maintaining the sensory quality of products [8]. Processing not only enhances sensory characteristics but also boosts certain nutritional compounds while reducing antinutritional factors [9,10].

Limited research has focused on the effect of processing on iron retention, and iron absorption inhibiting and enhancing compounds. Food processing methods such as steaming, pressure-cooking, pressure-steaming, etc. had been found to preserve and influence nutrients such as ascorbic acid and antioxidant value [11]. In addition, food forms/variants influence nutritional compounds due to their solubility and availability. Beetroot juice is widely used for supplementation and dietary interventions, mainly for iron deficiency anemia, in its raw variant. There is a lack of data on which variant and processing methods offer better iron support and nutritional stability. Hence, it is necessary to evaluate and compare processed variants. Considering this, the present study focuses on various processing methods like steaming, pressure-cooking, and open pan-cooking, and converting them to different variants such as Juice, Residue, and Juice with Residue. Optimization of processing methods and beetroot variants for maximal retention of bioactive and nutritional compounds, while reducing antinutrients that interfere with iron absorption, is studied. “The research findings in this paper are protected under a patent (Indian Patent No. 202441067829, 2024) [12].”

2 Materials and Methods

2.1 Selection and preparation of raw material

The dark purplish-red color of beetroot (Ooty-1 variety) was procured from the local market at Coimbatore, India. After primary cleaning, the skin was peeled, sliced into 4 mm-thick uniform slices, and separated into four equal beetroot sample portions.

Raw: One portion of the sample was taken as raw and ground with water (1:0.5 beetroot: water ratio). It was then divided into three variants: Raw Juice (RJ), Raw Residue (RR), and Raw Juice with Residue (RJ+R).

Pressure-Cooked: Another portion was pressure-cooked for 6±2 minutes at 120±5 °C (1:0.5 beetroot: water ratio). The pressure-cooked samples were processed using a domestic juice extractor, and separated into Pressure-cooked Juice (PJ), Pressure-cooked Residue (PR), and Pressure-cooked Juice with Residue (PJ+R) after grinding with water used for pressure cooking.

Steamed: The third portion of the beetroot samples was steamed for 20±5 minutes at 100±5 °C (1:10 beetroot: water ratio) using a stainless-steel steamer. Steamed beetroot was then ground with 50 mL of water used for steaming and then separated into Steamed Juice (SJ), Steamed Residue (SR), and Steamed Juice with Residue (SJ+R).

Open pan: The final portion of the beetroot samples was subjected to open pan-cooking for 45±5 minutes at 100±5 °C (1:4 beetroot: water ratio). After grinding with the cooked water, the samples were separated into Open pan-cooked Juice (OJ), Open pan-cooked Residue (OR), and Open pan-cooked Juice with Residue (OJ+R).

The juice and residue variants were separated using a muslin cloth of grade 90ʹ 1 square yard. Double-Distilled Water (DDW) was used for every processing, including cleaning and cooking. After each thermal treatment, the samples were dried in the freeze-drier to constant weight, and all analyses were performed in the dried powder.

2.2 Determination of antioxidant activity

The antioxidant activity was determined by 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and hydrogen peroxide (H_2O_2) methods. It was expressed as IC_{50} (µg/mL), representing the concentration required to inhibit 50% of the free radicals. A 1 mg/mL stock solution of the sample extract was prepared in a methanolic solution. Different concentrations of working standards (10, 20, 40, 60, 80, and 100 µg/mL) were prepared for antioxidant activity determination. In the DPPH assay, 3 mL of 0.1 mM DPPH (Sisco Research Laboratories Pvt. Ltd.), prepared in methanol, was added to 2 mL of sample extract from different concentrations. The absorbance was recorded at 517 nm in the double beam UV-Visible spectrophotometer (LABMAN-LMSP-UV1900) after 30 minutes of incubation in the dark [13].

The determination of H_2O_2 radical scavenging activity, a 2 mM H_2O_2 (Spectrum Reagents and Chemicals Pvt. Ltd.) solution was prepared by adding an equal volume of 0.2 M phosphate buffer at 7.4 pH. The buffer was made with 50 mL of 0.2 M potassium dihydrogen phosphate and 39.1 mL of sodium hydroxide solution (0.2 M). H_2O_2 (600 µL) was added to 100 µL of each sample concentration. The absorbance was measured at 230 nm after 30 min of incubation in the dark at room temperature [13]. The ABTS assay was determined by the method suggested by Lim et al [14] with minor modifications. In the ABTS assay (Sisco Research Laboratories Pvt. Ltd.), 7 mM ABTS was prepared by adding 192 mg to 50 mL of DDW. It was mixed with an equal volume of 2.45 µM potassium persulphate and kept in the dark for 12-16 h to produce the radical cation. The absorbance value of the $ABTS^{\cdot +}$ solution was modified to 0.700±0.02 at 734 nm by diluting with DDW. ABTS solution (3 mL) was added to 100 µL of different sample concentrations. The absorbance was recorded at 734 nm after 15 min using a UV-Visible spectrophotometer [14].

2.3 Determination of Total Phenolic Content (TPC)

Total phenolic content was estimated by the Folin-Ciocalteu (F-C) method. For the determination, 1 mL of sample was taken from the 1 mg/mL stock solution, diluted with 10 mL of DDW, and 1.5 mL of F-C reagent (Sisco Research Laboratories Pvt. Ltd.). Around 4 mL of 20% Na_2CO_3 was added after 5 minutes of incubation at room temperature. The solution was made up to 25 mL, and the absorbance was read at 765 nm after 30 min incubation in the dark. A calibration curve was prepared using a standard solution of gallic acid (R^2= 0.99). The result was expressed as mg of Gallic Acid Equivalent/100 g of dry weight (mg GAE/100 g DW) [15].

2.4 Determination of Total Flavonoid Content (TFC)

In TFC, 0.5 mL of methanolic extract from the stock solution (1 mg/mL) was treated with 1.5 mL of 95% methanol. Approximately 0.1 mL of 10% aluminum trichloride, 1 M potassium acetate, and 2.8 mL of DDW were added. It was mixed well and incubated in the dark for 30 minutes. The absorbance was read at 415 nm by a double-beam UV-Visible spectrophotometer (LABMAN-LMSP-UV1900). A calibration curve was prepared using a standard quercetin solution (R^2= 0.99). The result was expressed as mg of Quercetin Equivalent/100 g of dry weight (mg QE/100 g DW) [15].

2.5 Determination of saponin, nitrate, betanin, and vulgaxanthin

The determination of saponin was performed using the method described by Akbari et al [16] using diosgenin as a standard solution. The sample (0.2 mL) was mixed with 0.80 mL of methanol and 0.35 mL of 8% vanillin in ethanol. Sulfuric acid, around 1.2 mL, was added and kept at 60 °C for 10 minutes in a water bath (Labtech, LTWBD-1D). Finally, it was cooled and measured at 544 nm in a double-beam UV-Visible spectrophotometer by keeping methanol as a blank. The nitrate determination was done by adding 10 mL of DDW to 100 mg of the sample, and incubated at 45 °C for an hour. It was then centrifuged at 5,000 rpm for 15 minutes, and 0.2 mL supernatant was mixed with 0.8 mL of 5% salicylic acid, which was prepared in concentrated sulfuric acid. The solution was kept for 20 minutes, and 19 mL of 2N NaOH was added slowly to raise the pH above 12. The absorbance was measured at 410 nm [17]. The saponin and nitrate content were expressed as mg/100 g dry weight (mg/100 g DW).

The determination of betanins and vulgaxanthin was done by adding 0.7 g of sample to 50 mL of phosphate buffer (1.79 g of sodium hydrogen phosphate was mixed with 1.36 g of potassium dihydrogen phosphate and 7.02 g of sodium chloride in DDW, and made up to 1L), mixed for 20 min at 2000 rpm in a vortex shaker. The absorbance of supernatant was read at 476, 538, and 600 nm by keeping the buffer as a blank [18]. The absorbance value for red dye (B) at 538 nm and mg of betanin per 100 of dry weight (mg/100 g DW) was determined from Equation 1 and Equation 2.

$$E_B = 1.095 \times (E_{538} - E_{600}) \qquad \text{Equation 1}$$

$$B = (c \times E_B)/(1120 \times a \times b) \qquad \text{Equation 2}$$

Whereas, E_{538} is the absorbance at 538 nm, E_{600} is the absorbance at 600 nm, and 1.095 is an absorption coefficient at 538 nm resulting from impurities present. c is the mass of the sample with buffer in mg, 1120 value resulting from the absorbance of a 1% betanin solution measured at 538 nm, a is the sample weight (g), and b is the dry matter content (g dry matter/g sample).

The absorbance value for yellow dyes (W) at 476 nm and mg vulgaxanthin /100 g of dry weight (mg/100 g DW) was determined by Equation 3 and Equation 4.

$$E_W = (E_{476} - E_{538}) + (0.667 \times E_B) \qquad \text{Equation 3}$$

$$W = (c \times E_W)/(750 \times a \times b) \qquad \text{Equation 4}$$

Whereas, E_{476} absorbance at 476 nm, E_{538} absorbance at 538 nm, and E_B is the absorbance value for red pigments. Whereas 750 value resulting from the absorbance of a 1% betanin solution measured at 476 nm.

2.6 Determination of anti-nutrients

Phytate was determined according to S. Sadasivam [17]. Weighed a sample having 5-30 mg of phytate in the Erlenmeyer flask. The sample was extracted in 50 mL of 3% TCA for 30 minutes by mechanical shaking, and then for 45 minutes by hand. It was centrifuged for 10 minutes at 10,000 rpm in a centrifuge (Remi, R-24), and 10 mL of an aliquot was treated with 4 mL of ferric chloride. The samples were heated for 45 minutes in a water bath and centrifuged for 10-15 minutes. Decanted the clear supernatant and washed the precipitate twice by dispersing it with 20-25 mL of 3% TCA. It was heated for 5-10 minutes, centrifuged, filtered, and the precipitate was dispersed in 10 mL of DDW. It was treated with 1.5 N NaOH and made up to 30 mL, and heated in boiling water for 30 minutes. The precipitate after filtration was washed with hot DDW. Dissolved the precipitate in 40 mL of 3.2 N hot nitric acid in a 100 mL volumetric flask and washed the precipitate with several portions of DDW. Made up to 100 mL with DDW and transferred 5 mL of the aliquot to another 100 mL of a volumetric flask. Diluted to 70 mL and added 20 mL of potassium thiocyanate (Sisco Research Laboratories Pvt. Ltd.). Read the value at 480 nm within 1 minute and expressed as mg/100 g dry weight (mg/100 g DW).

Tannin was estimated by the vanillin-hydrochloride method suggested by Katoch [19]. One gram of the sample was mixed with 50 mL of methanol and kept for 20-28 h. Around I mL of supernatant after centrifugation was mixed with 5 mL of vanillin-hydrochloride reagent, which was made by mixing an equal volume of 8% HCl and 4% vanillin in methanol. The absorbance was read at 500 nm after 20 minutes. A calibration curve was prepared using a standard tannic acid solution ($R^2 = 0.98$). The result was expressed as mg of Tannic Acid Equivalent/100 g of dry weight (mg/100 g DW). The oxalates were determined according to the method of Stoleru et al [20]. Two grams of powdered sample were mixed with 190 mL of DDW and 10 mL of 6N HCl, and the mixture was digested for an hour at 100 °C. The sample was cooled and diluted to 250 mL with DDW and filtered. To 50 mL of filtrate, 10 mL of 6N HCl was added and placed in a water bath to evaporate till it reached half of its volume. Again, filtered and washed the precipitate using DDW, and made the volume up to 125 mL. It was then treated with 3-4 drops of methyl indicator and ammonium hydroxide concentrates till it turned pale yellow. It was heated to 90 °C, cooled, and filtered. The filtrate was treated with 10 mL of 5% calcium chloride while boiling. It was kept in the dark for twelve hours and filtered. The precipitate was washed with hot DDW, dissolved in 30 mL of water, and concentrated sulfuric acid mixture (3:1). It was warmed at 80 °C for 10 minutes, cooled it and titrated with 0.5% $KMnO_4$ until the pink color persisted for 1 min. 1 mL $KMnO_4$ is equivalent to 2.24 mg oxalate. The oxalate content was expressed as mg/100 g of dry weight (mg/100 g DW).

2.7 Determination of ascorbic acid and citric acid

Ascorbic acid was determined by the spectrophotometric method S. Sadasivam [17]. About 0.5-5 g of sample was treated with 25-50 mL of 4% oxalic acid solution, filtered, and a 10 mL aliquot was transferred to a volumetric flask. It was dehydrogenated using bromine water, and a few drops of 10% thiourea were added to remove excess bromine. The brominated sample was diluted to a known volume with 25-50 mL of 4% oxalic acid solution. A 1 mL sample was made up to 3 mL using DDW and treated with 1 mL of 2,4-Dinitrophenylhydrazine (Sisco Research Laboratories Pvt. Ltd.). The mixture was incubated at 37 °C for 3 h, and the orange-red osazone crystal formed was dissolved in 7 mL of 80% sulfuric acid. Absorbance was read at 540 nm using a double-beam UV-visible spectrophotometer (LABMAN-LMSP-UV1900). The absorbance was calculated from the calibration curve (R^2=0.99) using ascorbic acid as a standard, and the value was expressed as mg/100 g of dry weight (mg/100 g DW). Citric acid estimation was carried out according to the method of Abbas [21] with modifications. Approximately 0.2 g of powdered samples was diluted to 500 mL, and then 10 mL of the diluted sample was titrated against 0.05 N NaOH using phenolphthalein as an indicator till a pink color persisted. The citric acid content was expressed as mg/100 g of dry weight (mg/100 g DW).

2.8 Determination of calcium, iron, folate, and β-carotene

Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES), Shimadzu ICPE-9800 series, was used for the iron estimation. The samples were weighed at approximately 0.5-1.0 g and dried at 110 °C. It was ignited in a muffle furnace at 550 °C for 3 h till all carbon is oxidized. The ash was made up to 100 mL with dilute nitric acid (2%). A standard calibration curve was prepared using 0.1-10 mg/L of standard in nitric acid (R^2=0.99) [22]. Atomic Absorption Spectrophotometry (AAS), Pinnacle 500 series, was used to determine calcium after dry digestion in a muffle furnace. The sample was ignited in a muffle furnace at 450 °C for 5 h and heated for 30 minutes with a watch glass by adding 40 mL of diluted HCl (1:1), and 30 minutes without a watch glass. Cooled and added 10 mL diluted HCl again, filtered into a 100 mL standard flask, and made up the volume. A blank was prepared without the sample. A working standard of calcium of 5 to 25 µg/mL was prepared for the AAS method [23,24]. Calcium and iron were expressed as mg/100 g of dry weight (mg/100 g DW).

The folate was determined by transferring the powdered sample into a 100 mL volumetric flask, exactly equivalent to a laboratory mixture containing 25 mg of meclizine hydrochloride and 2.5 mg of folic acid. Around 60 mL of methanol was added and sonicated for 15 minutes. The solution was filtered through Whatman No.1 filter paper. Washed the residue with methanol, and the absorbance was noted at 540.0 nm against a blank. A calibration curve was prepared with standard folic acid solution (100 µg/mL), and the value was expressed as mg/100 g of dry weight (mg/100 g DW) [25]. The sample was taken in a 150 mL glass stoppered Erlenmeyer flask for the beta-carotene estimation and was treated with water-saturated n-butanol and kept overnight for complete carotene extraction. Filtered the contents through the Whatman No. 1 filter paper. The optical density of the clear filtrate was measured at 440 nm using a spectrophotometer against water-saturated n-butanol as a blank. A calibration curve was made using the carotenoid standard (5 µg/mL), and the value was expressed as mg/100 g of dry weight (mg/100 g DW) [26].

2.9 Determination of color

The color of the samples was analyzed by a portable color meter NH310 (Shenzen Three NH Technology Co Ltd) using CIE lab coordinates, and it was expressed as L*, a*, and b* values. The L^* represents the lightness, a^* is the red/green coordinates, and finally, b^* indicates the yellow/blue coordinates. A higher L^* represents more light in color (L=100 indicates light and L=0 indicates dark). In a^*, positive values move toward the red coordinates and negative values toward the green coordinates. In the case of b^*, positive values move toward the yellow coordinates and negative values toward the blue coordinates.

2.10 Evaluation of sensory parameters

Sensory evaluation of all samples (RJ+R, PJ+R, SJ+R, OJ+R, RJ, PJ, SJ, OJ, RR, PR, SR, and OR) was conducted. Around 50 semi-trained panel members from the food science and nutrition were selected for the sensory analysis. They were instructed about the test, the specific product, the purposes, and the discriminating differences in specific quality characteristics. The triangle test was used to screen the panel members to identify individuals' ability to distinguish specific stimuli. The triangle test is a discriminative sensory test used to determine product differences. The panelist was provided with three coded samples (two identical and one odd), and they had to identify the odd one. Around 40 volunteers were selected from the 50 panel members, and 30 were consistent for the analysis. The experimental protocol was reviewed and approved by the institutional ethics committee, Amrita Vishwa Vidyapeetham University, under approval number AMRITA/SOE/ADMN/DOA/12/2025/001. A volunteer consent was collected from all participants before participation. A 9-point Hedonic Scale (1-Dislike extremely to 9- like extremely) was used to evaluate appearance, flavor, texture, and overall acceptability. Each panelist was presented with a maximum of four samples a day, and samples were randomly assigned along with code numbers. Drinking water was provided for palate cleansing between samples [27].

2.11 Iron bioavailability determination

Iron bioavailability of the optimized sample was determined by the INFOGEST static *in vitro* gastrointestinal food digestion model proposed by Brodkorb et al [28]. The food was exposed to oral, gastric, and intestinal phases for digestion. In the oral phase, 5 g of food was mixed with simulated salivary fluids at a ratio of 1:1 (w/w) with salivary amylase at 37 °C, pH 7 for 2 minutes. The oral bolus was treated with simulated gastric fluids (1:1) and enzymes, pepsin, and lipase at pH 3 for 2 h. The gastric chyme was incubated for 2 h at pH 7 by mixing with simulated intestinal fluids, bile salts, and pancreatic enzymes. The sample after the intestinal digestion was subjected to the Human colon carcinoma (Caco-2) cells, which were obtained from the National Centre for Cell Sciences. It was suspended in 1 mL of culture media containing the filtered sample and incubated in an atmosphere of 5% CO_2 and 95% air at 37 °C for 24 h. The cells were removed from the surface of the plate using trypsin and centrifuged at 2000 rpm for 2 minutes at 4 °C. It was rinsed with 1 mL of ice-cold phosphate-buffered saline (pH 7.2) after eliminating the supernatant. Again, centrifuged at 1000 rpm for 1 minute at 4 °C, and the supernatant was removed. The cells were treated with 1 mL of cytosol buffer (pH 7.5), and the solution was sonicated in a probe sonicator and centrifuged at 14000 rpm for 25 minutes at 4 °C. The supernatant from the centrifugation was used to determine iron content by Inductively Coupled Plasma Mass Spectrometry (ICPMS). The sample (2 mL) was treated with conc. HNO_3, HCl, and a few drops of H_2O_2. It was digested for 1 h at 180 °C in a microwave digestion system, and then diluted and subjected to ICPMS [29–31].

2.12 Statistical analysis

A factorial design with all possible combinations of factors (juice, residue, and type of cooking) and factor levels was used for the experimental study. A total of 36 formulations, identified by the factorial combinations, were prepared for analysis. The factors and factor levels employed in the experimental design are given in Table 1. Regression models for various responses with cooking method, presence or absence of juice, and residue as factors, obtained from factorial analysis, were evaluated using ANOVA, coefficient of determination (R^2), adjusted R^2, predicted R^2, and lack-of-fit tests. The optimized results were validated by evaluating predicted fit with experimental results, and by statistical evaluation by analysing the standard error fit, 95% of confidence interval, and 95% of predicted interval. All experiments were performed in triplicate, and the results were expressed as mean values±standard deviation (SD). Statistical analysis was also performed using Minitab Version 17. One-way analysis of variance (ANOVA) was carried out to identify the significant effect of treatments. When a significant difference was observed, Tukey's post-hoc multiple pairwise comparison test was applied to compare treatment means. A significance level of $p<0.05$ was considered statistically significant. Error bars in figures represent SD of triplicate measurements. Optimization to identify the formulation with the best nutraceutical and nutritional properties, including iron, was performed using response optimizer functionality in Minitab.

3 Results and discussion

3.1 Effect of thermal processing methods on the nutraceutical properties of various beetroot variants

This study evaluated the impact of different thermal processing methods, such as pressure cooking, steaming, and open pan cooking, on the antioxidant activities, total phenols, flavonoids, nitrates, saponins, betanin, and vulgaxanthin of beetroot variants (Juice, residue, juice with residue) due to their nutraceutical benefits. **Table 2** illustrates the impact of various processing methods on antioxidant activity, measured using DPPH, ABTS, and H_2O_2 assays. They were expressed as IC_{50} values (µg/mL), where lower IC_{50} values represent stronger radical scavenging activity. Among the juice with residue variants, PJ+R and SJ+R exhibited significantly lower IC_{50} values for DPPH and ABTS radical, indicating stronger free radical scavenging activity. Meanwhile, RJ+R and SJ+R showed better scavenging activity towards H_2O_2 radicals. For the juice variant (**Table 2**), pressure and steam-cooking showed significant ($p<0.05$) activity towards DPPH with an IC_{50} value of 39.34±0.55 and 38.48±0.35 µg/mL of sample extract. OJ showed higher activity towards ABTS with an IC_{50} value of 23.29±0.26 µg/mL of sample extract. Moreover, RJ and PJ exhibited significant ($p<0.05$) activity towards H_2O_2 radicals. In contrast, the OR variant revealed higher activity towards DPPH, ABTS, and H_2O_2 free radicals among the residue variants. The antioxidant activity towards a free radical varies depending on the potential compounds present in the plant foods, mainly phenols and flavonoids [32]. TPC, TFC, nitrates, and ascorbic acid contribute substantially to the antioxidant activity of beetroot [5]. The enhanced antioxidant potential of the sample after thermal processing might be partially due to the release of these compounds from the cell matrix and enhanced extractability [33].

The results revealed that steam-processed beetroot juice (**Table 2**) has a high TPC content of 702.81±6.95 mg GAE/100 g DW compared to other processing methods. Among juices with residue, the pressure-cooked one showed greater phenolic contents, around 666.66±6.95 mg GAE/100 g DW. In contrast, a reduction in TPC was observed in the processed beetroot residue variant. This indicated that the thermal processing enhanced the phenolic extractability in the juice fraction but not in the residue. In the case of TFC, it was retained more in steam processed beetroot variants and significantly ($p<0.05$) increased after thermal processing except in the OJ+R. The food matrix, temperature, processing time, and types are the major factors influencing the phenolics and flavonoids [33–35]. In plant foods, they exist either in a free state or bound to the cell membrane. Thermal processing breaks down the cellular compounds in fruits and vegetables and releases the bound phenolics and flavonoids. It degrades the enzymes peroxidases and polyphenol oxidases responsible for the oxidation of phenolics. Thermal processing has both positive and negative impacts on the phenolic and flavonoid compounds [33,35,36]. Thermal processing either causes a transformation of certain phenolic compounds or degradation due to hydrolysis, dimerization, epimerization, oxidation, and polymerization. In addition, thermal treatment weakened the ester linkage of certain phenolics like p-coumaric acid with lignin and hemicellulose and made them available in esterified forms [33]. The thermal stability of flavonoids varies depending on the physical state, food matrix, processing times, and temperatures. TFC of mulberry juice decreased after 25–45 ℃ and it was increased after 45–100 °C of thermal treatment. TFC of teff increased after 7.5 minutes of roasting and decreased after 10 minutes [35]. In this study, the changes in TPC and TFC among different thermal processing and variants might be partially due to the food matrix, transformation, processing duration, and temperature, and also might be due to the degradation of the product that reacts with colorimetric assays.

Similar to TPC and TFC, nitrates can scavenge reactive oxygen species. Dietary nitrates are the exogenous source for endogenous nitric oxide synthesis, which has potential for lowering blood pressure, cardiovascular diseases, and oxidative stress. Research studies revealed that beetroots are a remarkable source of dietary nitrates, and the presence of nitrates is one contributing factor to the health benefits of beetroot [37,38]. This study observed higher amounts of nitrates in RJ+R, as shown in **Table 1** Factors and levels of factors for optimization

Factors	Number of levels	Levels
Juice	2	No, yes
Residue	2	No, yes
Cooking method	4	Raw, pressure cooking, steaming, and open pan cooking

Table 2. The thermal processing reduced the nitrate content in beetroot juice with residue and the residue variant. Whereas, it was enhanced in the juice variant from 50.00±0.10 to 174.12±0.17 mg/100 g DW after processing due to its solubility. Similar to TPC and TFC, thermal processing might leach out the nitrates and enhance their solubility in the cooking medium. This might be the reason for the change in nitrate level in the juice variants. Saponins are another bioactive compound present in plants as a part of their primary protection from pathogens. These compounds possess hypolipidemic activity, antifungal activity, virucidal activity, antimicrobial activity, and hypoglycemic activity. They are considered phytochemical compounds due to their pharmacological activities [5,39]. Beetroot is a notable source of saponin, and the thermal processing of beetroot was found to have significant ($p<0.05$) changes in saponin content. It was enhanced after processing among different beetroot variants as compared to raw beetroot, and retained more in the juice with the residue variant (**Table 1** Factors and levels of factors for optimization **Table 2**). Several studies reported that saponin content was reduced after processing, especially moist heating such as soaking, cooking, blanching, and autoclaving. Whereas dry heating enhanced the saponin content. This is mainly due to the leaching of saponin into the cooked water since it contains water-soluble glycosides [39,40]. The study on saponin content in bitter guard showed a reduction after autoclaving at 121 °C for 20 minutes. Whereas certain saponins were stable at 30 °C, 60 °C, and 100 °C for 20 minutes of boiling [41]. This study observed an increment of saponin from 12.66-17.33 mg/100 g DW in juice with residue, 2-15 mg/100 g DW in juice, and 4-15.33 mg/100 g DW in residue variant. This might be

Factors	Number of levels	Levels
Juice	2	No, yes
Residue	2	No, yes
Cooking method	4	Raw, pressure cooking, steaming, and open pan cooking

due to the inclusion of cooked water for the grinding process. These results are aligned with the observation of Mohsen et al [42], the phytoconstituents were found to be preserved and retained after thermal treatment, like steaming.

The characteristic dark red color of beetroot is due to the presence of compound betalains, a nitrogen-containing water-soluble pigment. The red-violet color is attributed to betacyanin, while the yellow-orange is attributed to betaxanthin. Betalains possess remarkable therapeutic potential, anti-tumor properties, anti-carcinogenic properties, hepato-protective, and regulate vascular homeostasis [5,37]. Betalains are less stable when exposed to thermal treatment and processing time, and the presence of oxygen and enzymes degrades the compound. This might be due to the decarboxylation, isomerization, or epimerization reactions [39]. This study observed a similar result in betanin and vulgaxanthin levels, which were decreased after thermal processing in pressure cooking and steaming. In contrast, a notable increment was observed in open pan processing. A higher concentration of betanin and vulgaxanthin was found in the OR, with values of approximately 43.11±0.04 and 54.37±0.15 mg/100 g DW, respectively. These pigments were better retained in the residue compared to the juice with the residue and juice variants. This may be attributed to reduced pigment leaching, enhanced evaporation rate, and matrix characteristics. In addition, it needs to be noted that the degradation of the product may respond to the colorimetric assays, which may partially reflect in the results.

3.2 Effect of thermal processing methods on the nutritional and antinutritional properties of various beetroot variants

Various processing methods play a vital role in determining the nutritional and antinutritional properties by altering their compositions. Processing, like soaking, cooking, boiling, roasting, steaming, and pressure cooking, has a significant influence on the nutritional characteristics [11,34]. This study focused on the nutritional compounds iron, calcium, ascorbic acid, citric acid, folate, and β-carotene, which were illustrated in **a-l means** values with the same letters are not significantly different according to the Tukey test at $p < 0.05$. Data represent the mean value±standard deviation of three replicates (n=3). IC_{50} values were expressed as µg/mL, representing the concentration of sample extract required to achieve 50% radical scavenging activity. TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; QE: Quercetin equivalent; RJ+R: Raw juice with residue; PJ+R: Pressure-cooked juice with residue; SJ+R: Steamed juice with residue; OJ+R: Open pan-cooked juice with residue; RJ: Raw juice; PJ: Pressure-cooked juice; SJ: Steamed juice; OJ:

Open pan-cooked juice; RR: Raw residue; PR: Pressure-cooked residue; SR: Steamed residue; OR: Open pan-cooked residue.

Table 3. Iron is an essential micronutrient that acts as an oxygen carrier and for myoglobin synthesis, red blood cell formation, and brain development, and its deficiency is a leading factor for anemia [1]. This study observed a reduction in iron after beetroot processing. Iron was retained more in open pan processing as compared to other thermal methods, especially in the residue variant (1.81±0.02 mg/100 g DW). The elongated time for cooking might have helped to release more iron from its complex forms. A higher level of iron was observed in RJ+R, around 2.29±0.04 mg/100 g DW. The processes of pressure cooking, steaming, and open pan processing reduced the iron level by approximately 40%, 34%, and 30%, respectively, compared to RJ+R. Beetroot is widely used and supplemented for anemia in the form of juice. Whereas, there was a reduction in the iron content in RJ by around 68.99% and 15.72% in RR as compared to RJ+R. In the juice and the residue variants, processing reduced 21-63% and 6.2-45% of iron. A similar loss of iron was observed in blanched vegetables (14.1-45.4%), and pressure-cooked and open pan-cooked beans [43,44]. Similar to mineral iron, calcium showed a similar trend; it was decreased after thermal treatment across various beetroot variants. The calcium content was decreased in juice with residue from 4.72±0.14 to 2.02±0.12 mg/100 g DW, in juice from 4.52±0.21 to 1.52±0.05 mg/100 g DW, and in residue from 4.72±0.06 to 2.34±0.19 mg/100 g DW, respectively. A higher amount of calcium was retained in the residue variant after processing. The retention of minerals in the residue might be due to the food matrix.

In addition to iron and calcium, folate and β-carotene are important dietary nutrients that help to combat anemia and possess synergistic action [45]. Food processing significantly ($p<0.05$) increased the level of folate and β-carotene in different beetroot variants. The concentration of both nutrients was higher in juice with the residue variant, around 37-38.33 µg/100 g DW and 19-21 µg β-carotene/100 g DW, respectively, after processing due to release from the bound form, and making it more extractable. Cooking methods can significantly ($p<0.05$) impact the folate and β-carotene levels in plant foods; some methods can enhance them, and some can lead to their loss. The steaming was found to preserve these nutrients as compared to boiling and microwaving. The thermal degradation of folate and β-carotene is attributed to the leaching into cooking water, which was observed more in boiling and microwaving [46,47]. In this study, the inclusion of cooking water during grinding may be responsible for the observed increase in folate and β-carotene levels.

Ascorbic and citric acids are the organic acids that have synergistic effects on iron absorption in the body. However, ascorbic acid is sensitive to temperature and oxygen, leading to its degradation during heating. The study on prolonged pressure cooking of green leafy vegetables and cowpea pods showed a greater degradation of ascorbic acid levels [48,49]. A substantial loss of this vitamin was observed while processing [11]. A similar observation was found in this study, the ascorbic acid was reduced after various thermal processing, and it was retained more in pressure-cooked variants. Ascorbic acid was degraded by around 29% in juice with residue, 26% in juice, and 49% in residue variant, respectively. A higher level of reduction was observed in the residue variant, and it was higher in the juice variant. The PJ retained a high level of ascorbic acid, by around 7.76±0.02 mg/100 g DW. Heat, exposure to air, and leaching into water are the major causes of ascorbic acid loss. The inclusion of cooked water for grinding and less exposure to air might be the reasons for better ascorbic acid retention in pressure-cooked variants. A similar trend was observed in citric acid (**Table 3**), which was also reduced after thermal processing, except in pressure-cooking. PJ+R was found to preserve citric acid around 0.83±0.11% DW as compared to other processing methods. A significant ($p<0.05$) loss of citric acid was observed in open pan processing. From these observations, it was evident that the food processing and various variants have a significant ($p<0.05$) influence on organic acids.

Similar to the nutritional compounds, antinutrients are naturally present in plant foods and can interfere with the absorption of essential nutrients in the body. The presence of antinutrients, oxalates, tannins, and phytates exhibited an inhibitory effect on minerals, including iron [50]. In this study, the phytate concentration in beetroot was observed below the non-detectable limits. Whereas, the presence of tannin and oxalates was observed and represented in **Table 3**. Tannins are polyphenolic compounds found in plant foods and act as antinutrients [50]. In this study, a significant difference ($p<0.05$) was observed in tannin levels among different processing methods and beetroot variants. It was increased after steaming and the open pan method. In PJ+R and PJ, tannin level was reduced by around 32% and 48%, respectively. A higher tannin content was observed in the juice variant, particularly in SJ, around 528 mg TAE/100 g DW. Similar to the TPC, an increase in tannin might also be due to the leaching of phenolic compounds from the cellular matrix while processing [36]. Similar to tannin, oxalates are available in plant foods and are abundant in green leafy vegetables. They form insoluble complexes with calcium and magnesium, and soluble complexes with sodium and potassium [50]. Boiling, steaming, soaking, and pairing with high-calcium foods were effective strategies to lower oxalate content [51]. Beetroot is also a good source of oxalates; hence, it is required to reduce them. Thermal processing is an effective strategy to reduce the oxalate content in food. This study showed a decrease in oxalate level after processing and a non-significant ($p>0.05$) change in oxalate content among different beetroot variants. It was higher in the RR variant, around 186.66±12.93 mg/100 g DW. There was around a 39% reduction of oxalate level in juice with residue, a 50% in juice variant, and a 56% in residue variant, respectively.

3.3 Effect of thermal processing methods on the color of various beetroot variants

Color analysis from Table 4 indicates that there were significant differences ($p<0.05$) among the processing methods and beetroot variants. The higher L^* in OJ+R and SR indicates a light-colored powder obtained after this treatment. Whereas, a low L^* value was observed in RJ and SJ, and their combination with the residue. The redness (a^*) parameter was reduced in the order of OJ>OR>PJ>PJ+R=PR>RR>SR>OJ+R>RJ+R=SJ+R=RJ=SJ. The reduction in the redness value might be the degradation of pigments during various thermal processing. The b^* value was higher in OJ (4.30±0.01), indicating enhanced yellow pigment. These results indicate that there was an enhancement of color value after thermal processing. The pressure-cooking and open-pan methods showed better results of dark color with red coordinates. Among them, OJ, OR, PJ, PJ+R, and PR resulted in dark, strong red color along with a slight yellow color.

3.4 Effect of thermal processing methods on the sensory parameters of various beetroot variants

The sensory scores attained from different beetroot processing and variants ranged between 3±2.45-7.6±0.55 on the 9-point hedonic scale, indicating a wide range of panel responses from extremely dislike to like very much. Variations in mean sensory scores were observed among the samples, as shown in Figure 1. The ANOVA results indicate that these differences were not statistically significant ($p>0.05$) among the different processing methods and variants. Even though sensory parameters have a significant role in determining consumer acceptance. The mean value (Figure 1) indicates that panel members accepted the raw juice variant when compared to other variants. Among the juice variants, they accepted the RJ and PJ, and mostly beetroot is supplemented in the form of raw juice. The observed variations, which were not statistically significant ($p>0.05$), represent the changes in sensory perceptions of panel members. The variants that received high sensory scores (RJ and PJ) showed a lower iron content (0.71±0.04 and 0.35±0.04 mg/100 g DW, respectively). These finding highlights the need for developing beetroot based value-added products with enhanced sensory acceptability and nutritional quality.

Figure 1 Effect of processing on sensory parameters of different beetroot variants

3.5 Optimization of thermal processing methods and beetroot variants based on iron absorption retention and inhibiting factors

Iron absorption is crucial for maintaining hemoglobin levels, oxygen carrying capacity of the blood, brain development, and to prevent serious health-related complications such as fatigue, dizziness, shortness of breath, and anemia [1]. A sufficient amount of iron in food does not mean that a good amount of iron is bioavailable in the body. There are dietary factors that influence iron absorption. Phytates, tannins, Oxalates, calcium, ascorbic acid, and citric acid are major dietary factors that interfere with iron absorption in the body. Phytate binds with iron and makes it unavailable for absorption. It is found that a low level of phytate, around 500 mg, can substantially reduce iron bioavailability [3]. In this study, phytate levels were below non-detectable limits. Certain polyphenols and flavonoids also exhibit an iron-inhibitory nature. For instance, 50 and 200 mg of bean polyphenols lowered iron bioavailability by 18 and 45%, respectively [3]. Tannin, another type of polyphenol are strong inhibitor of iron absorption. A meal containing 5 mg of tannin reduced iron absorption by 20%, while 100 mg reduced it by 88% [50]. The ratio of iron and tannic acid between 1:0.25-1:1 diminished the bioavailability from 1.4 to 1.3% [51]. These studies emphasize the crucial role of tannin in iron absorption.

The iron-binding properties of oxalates and calcium are influenced by their chemical forms and concentration. The addition of oxalate in the form of calcium oxalate with a ratio of 1:0.25 to 1:1 reduced iron bioavailability by around 1.3-1.2%. Whereas, the addition of oxalate alone decreased iron bioavailability from 1.4-0.3%. This indicated that oxalates play a pivotal role in inhibiting iron absorption in food [51]. Calcium inhibits both non-heme and heme iron, depending on its molar ratio with iron [50]. When calcium is added as calcium carbonate in the ratio of 1:0.25 to 1:1, iron bioavailability was reduced from 2.0-0.95% [51]. And, 1g of calcium oxalate in cabbage reduced iron absorption from 0.32 to 0.19 [52]. The detrimental effect of antinutrients can be counteracted by the presence of organic acids, including ascorbic acid and citric acid, which form soluble complexes with iron or reduce ferric form to ferrous form [50]. Studies have shown that 15 mg of ascorbic acid and 1 g of citric acid enhanced iron absorption from 0.03 to 0.08 and 0.02-0.08, respectively [52]. Ascorbic acid and citric acid combined with iron in $FeSO_4$ solution in ratios of 1.25:1-6.25:1 and 1.25:1-3.75:1 enhanced iron bioavailability from 18.7-88.6% and 27.7-93.5% [51].

This study focused on those factors apart from the iron content to optimize the thermal processing methods and beetroot variants. The general factorial regression model revealed that the three factors and two ways of interactions between the factors (juice*type of cooking and residue*type of cooking) were found to be significant at $p < 0.05$, except in TPC, TFC, and Oxalates (Table 5). From Table 5, a higher R^2 explains the better fitness of the model, and the predicted R^2 is in reasonable agreement with the adjusted R^2. Hence, the results were optimized using a response optimizer. To optimize the results, the antinutrients oxalates and tannins, and the micronutrient calcium were set at a minimum target level (Figure 2) with higher importance as shown in Table 6. Even though TPC and TFC have nutraceutical benefits, they were also set at minimum levels since certain phenolics and flavonoids can inhibit iron absorption. The iron absorption promoters ascorbic acid and citric acid were set as maximum targets with higher importance. Nutrients such as iron, folic acid, β-carotene, betanin, vulgaxanthin, nitrates, and saponin were also set at the maximum goal level. The antioxidant activity was set as a minimum target due to the IC_{50} assay (Figure 2). The standard error of fit was small for most of the responses, as shown in Table 6. It means a precise prediction. The optimum formulations obtained from the response optimizer are represented in Table 7. The actual measured values and predicted values from the Minitab were very close, and there was no significant difference ($p>0.05$) observed.

The optimization study revealed that the PJ+R variant of beetroot was the most suitable formulation within the experimental design, with an overall desirability value of 0.63 (Table 7). Followed by SJ+R and PR with desirability values of 0.51 and 0.46, respectively. showed better optimized results. Compared to the RJ+R variant, levels of oxalates, tannin, and Ca decreased in PJ+R by 26%, 33%, and 43%, respectively. The TPC content was increased by 22%, and TFC exhibited a substantial rise of 152%. However, reductions were observed in iron, nitrates, betanin, and vulgaxanthin by approximately 41%, 25%, 91%, and 32%, respectively. Despite this decrease, there was a notable improvement in other nutrients, such as saponin, which increased by 25%, citric acid by 95%, folic acid by 18%, and β-carotene by 54%. These desirability scores represent how well the responses meet the defined optimization goals for the selected responses. It does not directly indicate enhanced iron absorption or clinical efficacy. The biological relevance of the optimized formulation was interpreted in *in vitro* bioavailability results.

Figure 2 Optimization of thermal processing methods and beetroot variants based on iron absorption

3.6 Iron bioavailability of the optimized sample

The iron bioavailability of a sample has a significant role in the body as it defines how much iron is absorbed and reaches the targeted tissue to accomplish its bioactivity. The bio accessibility measures the amount of iron released from the food matrix for absorption [53]. This study measured iron bioavailability through the INFOGEST static *in vitro* gastrointestinal food digestion model in the optimized sample, PJ+R. It showed approximately 4.37±0.05% bioavailability in Caco-2 cells. Previous studies on spray-dried beetroot powder and beetroot-based sweet and sour sauce showed lower iron bioavailability, which was improved by the addition of ascorbic acid. This highlights the role of enhancers in improving iron absorption [54,55]. Similar study on Mexican foods such as spinach, chard, watercress, purslane, black beans, and lentils, which contain higher amounts of iron by around 67.49, 31.76, 34.06, 32.04, 11.25, and 9.18 mg/100 g, showed a bioavailability of 4.45, 2.38, 1.98, 3.32, 2.45, and 3.53 mg/100 g after cooking. This poor bioavailability was observed due to the presence of anti-nutritional factors in the staple foods [56]. These results highlight that total iron content in the food does not imply the absorption efficacy. The food matrix, processing conditions, and presence of enhancers and inhibitors play a crucial role in the iron bioavailability. In addition, these results can be strengthened by the validation of iron chemical speciation and iron bioavailability of other variants.

4 Conclusion

The process of steaming was found to be more advantageous in retaining nutraceutical compounds, antioxidant activity, total phenols, flavonoids, and nitrates. Pressure-cooking is found to reduce anti-nutrients and retain other nutritional compounds like folic acid, β-carotene, ascorbic acid, and citric acid. The juice with the residue variant of beetroot provided a better retention of nutrients. Whereas iron was retained more in the residue variant. The above results highlight the significance of evidence-based scientific data for the best processing methods and variants. The optimization model focused on iron inhibitory and absorption factors, and the desirability approach identified that the PJ+R variant was a favorable option due to reduced anti-nutritional factors along with relatively improved nutritional and bioactive profiles. It reduced key inhibitory compounds, oxalates, calcium, and tannin by approximately 42-26%, while enriching nutritional compounds like saponin, citric acid, folic acid, and β-carotene, with an average increase of 67%. However, several bioactive compounds, such as nitrates, betanin, and vulgaxanthin, ascorbic acid, and iron itself, showed a reduction after processing. Therefore, PJ+R cannot be suggested as an effective option for iron retention, whereas it balances the inhibitors and promoters for iron absorption. The iron bioavailability in PJ+R was shown to be around 4.37±0.05% in the Caco-2 cell line model. Followed by PJ+R, SJ+R, and PR were found to be better alternative processes and variants based on the optimization model. Whereas the *in vitro* study was conducted only in the PJ+R variant, further validation through an *in vivo* model and iron speciation analysis is necessary. Moreover, sensory evaluation showed that people preferred the raw and pressure-cooked juice variants, which were low in iron content. The sensory preference and nutritional optimization highlight that there is a need for beetroot based product development strategies that improve acceptability while maintaining nutritional quality.

Statements and Declarations

Author contributions

AM: Conceptualization, formal analysis, Investigation, Writing-original draft, Visualization;

JR: Conceptualization, Supervision, Funding acquisition, Writing-review& editing, Validation;

MB: Formal analysis, Writing- review & editing, validation;

SK: Investigation;

PN: Review & editing;

KK: Supervision, Writing-review& editing, Validation.

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Declaration of Competing Interest

Dr Jancirani Ramaswamy reports financial support was provided by Amrita Vishwa Vidyapeetham University [ASG2022201]. Other 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.

Ethics approval:

The sensory evaluation involving human participants was conducted in accordance with relevant institutional guidelines and regulations for research involving human subjects. The experimental protocol was reviewed and approved by the institutional ethics committee, Amrita

Vishwa Vidyapeetham University, under approval number AMRITA/SOE/ADMN/DOA/12/2025/001. A volunteer consent was collected from all participants before participation.

Availability of data and material

All data generated or analyzed during this study are included in the published article.

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References

1. Lotfi, M., Azizi, M., Tahmasbi, W. & Bashiri, P. The effects of consuming 6 weeks of beetroot juice (*Beta vulgaris* L.) on hematological parameters in female soccer players. *J. Kermanshah Univ. Med. Sci.* **22**, 88-93 (2018).
2. Kaur, N., Agarwal, A. & Sabharwal, M. Food fortification strategies to deliver nutrients for the management of iron deficiency anaemia. *Curr. Res. Food Sci.* **5**, 2094–2107 (2022).
3. Piskin, E., Cianciosi, D., Gulec, S., Tomas, M. & Capanoglu, E. Iron absorption: factors, limitations, and improvement methods. *ACS Omega* **7**, 20441–20456 (2022).
4. Haridas, S., Ramaswamy, J., Natarajan, T. & Nedungadi, P. Micronutrient interventions among vulnerable population over a decade: A systematic review on Indian perspective. *Heal. Promot. Perspect.* **12**, 151–162 (2022).
5. Chhikara, N., Kushwaha, K., Sharma, P., Gat, Y. & Panghal, A. Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chem.* **272**, 192–200 (2019).
6. Liliana, C. & Oana-Viorela, N. Red beetroot: composition and health effects - a review. *J. Nutr. Med. Diet Care* **5**, (2020).

7. Nora, M. A. Effect of red beetroot (*Beta vulgaris* L.) intake on the level of some hematological tests in a group of female volunteers. *ISABB J. Food Agric. Sci.* **8**, 10–17 (2018).

8. Punia Bangar, S., Singh, A., Chaudhary, V., Sharma, N. & Lorenzo, J. M. Beetroot as a novel ingredient for its versatile food applications. *Crit. Rev. Food Sci. Nutr.* **63**, 8403–8427 (2023).

9. Kumar, M. *et al.* Jamun (*Syzygium cumini* (L.) Skeels) seed bioactives and its biological activities: A review. *Food Biosci.* **50**, 102109 (2022).

10. Kumar, M. *et al.* Apple (*Malus domestica Borkh.*) seed: A review on health promoting bioactivities and its application as functional food ingredient. *Food Biosci.* **50**, 102155 (2022).

11. Zor, M., Sengul, M., Karakütük, İ. A. & Odunkıran, A. Changes caused by different cooking methods in some physicochemical properties, antioxidant activity, and mineral composition of various vegetables. *J. Food Process. Preserv.* **46**, (2022).

12. Mundassery, A. R. J. Instant fortified soup formulation and method of preparation thereof. India Pat, 202441067829, filed September 9, 2024.

13. Hussen, E. M. & Endalew, S. A. *In vitro* antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. *BMC Complement. Med. Ther.* **23**, 146 (2023).

14. Lim, J. G., Park, H. & Yoon, K. S. Analysis of saponin composition and comparison of the antioxidant activity of various parts of the quinoa plant (*Chenopodium quinoa* Willd.). *Food Sci. Nutr.* **8**, 694–702 (2020).

15. Madaan R, Bansal G, Kumar S, S. A. Estimation of total phenols and flavonoids in extracts of actaea spicata roots and antioxidant activity studies. *Indian J. Pharm. Sci.* **73**, 666 (2011).

16. Akbari, S., Abdurahman, N. H. & Yunus, R. M. Optimization of saponins, phenolics, and antioxidants extracted from fenugreek seeds using microwave-assisted extraction and response surface methodology as an optimizing tool. *Comptes Rendus Chim.* **22**, 714–727 (2019).

17. S. Sadasivam, A. M. *Biochemical Methods*. (New Age International Publishers, New Delhi, 2007).

18. Nowacka, M. *et al.* The impact of pulsed electric field on the extraction of bioactive compounds from beetroot. *Foods* **8**, 244 (2019).

19. Katoch, R. Methods for nutritional quality evaluation of food materials. *Analytical Techniques in Biochemistry and Molecular Biology* 251–322 (Springer New York, New York, NY, 2011).

20. Stoleru, V. *et al.* Nutritional and antinutritional compounds in leaves of quinoa. *Food Biosci.* **45**, 101494 (2022).

21. Abbas, A. M. & Brima, E. I. Determination of citric acid in soft drinks, juice drinks and energy drinks using titration. *Int. J. Chem. Stud.* **1**, 30–34 (2014).

22. George W. Latimer J. *Official Methods Of Analysis Of AOAC International* (19th edition). (AOAC International, Virginia.USA, 2012).

23. Bazzi, A., Kreuz, B. & Fischer, J. Determination of calcium in cereal with flame atomic absoption spectroscopy. *J. Chem. Educ.* **81**, 1042–1044 (2004).

24. Siang, T. E., Swan Chao, K. & Mizura Shahid, S. Determination of iron in foods by the atomic absorption spectrophotometric and colorimetric methods. *Pertanika* **12**, 313-322 (1989).

25. Chiranjeevi, K., Channabasavaraj, K. P., Srinivas Reddy, P. & Nagaraju, P. T. Development and validation of spectrophotometric method for quantitative estimation of ritonavir in bulk and pharmaceutical dosage forms. *Int. J. ChemTech Res.* **3**, 58–62 (2011).

26. Cuniff Patricia. *Official Methods of Analysis of AOAC International (16th edition).* (AOAC International, Virginia.USA, 1995).

27. Farhan, M. *et al.* Assessment of beetroot powder as nutritional, antioxidant, and sensory evaluation in candies. *J. Agric. Food Res.* **15**, 101023 (2024).

28. Brodkorb, A. *et al.* INFOGEST static *in vitro* simulation of gastrointestinal food digestion. *Nat. Protoc.* **14**, 991–1014 (2019).

29. Boim, A. G. F., Wragg, J., Canniatti-Brazaca, S. G. & Alleoni, L. R. F. Human intestinal Caco-2 cell line *in vitro* assay to evaluate the absorption of Cd, Cu, Mn and Zn from urban environmental matrices. *Environ. Geochem. Health* **42**, 601–615 (2020).

30. Mohanan Deepu, Puthiyedath Rammanohar, G Nandakumar, M G Minsha, N. S. S. & K. S. Evaluation of toxic heavy metal content in marketed Ayurvedic decoctions using closed vessel microwave digestion in ICPMS. *Indian J. Tradit. Knowl.* (2024) doi:10.56042/ijtk.v23i8.13114.

31. Oliveira, A. S. *et al.* Iron-peptide complexes from spent yeast: evaluation of iron absorption using a Caco-2 monolayer. *Food Biosci.* **56**, 103106 (2023).

32. Lalhminghlui, K. & Jagetia, G. C. Evaluation of the free-radical scavenging and antioxidant activities of Chilauni, *Schima wallichii* Korth *in vitro*. *Futur. Sci. OA* **4**, (2018).

33. Mundassery, A., Ramaswamy, J., Natarajan, T., Haridas, S. & Nedungadi, P. Modern and conventional processing technologies and their impact on the quality of different millets. *Food Sci. Biotechnol.* **33**, 2441–2460 (2024).

34. Gao, Y. *et al.* Impact of thermal processing on dietary flavonoids. *Curr. Opin. Food Sci.* **48**, 100915 (2022).

35. Prakash, O., Baskaran, R., Chauhan, A. S. & Kudachikar, V. B. Effect of heat processing on phenolics and their possible transformation in low-sugar high-moisture (LSHM) fruit products from Kainth (*Pyrus pashia* Buch.-ham ex D. Don) fruit. *Food Chem.* **370**, 130988 (2022).

36. Kaur, S., Kaur, N., Aggarwal, P. & Grover, K. Bioactive compounds, antioxidant activity, and color retention of beetroot (*Beta vulgaris* L.) powder: effect of steam blanching with refrigeration and storage. *J. Food Process. Preserv.* **45**, 1–10 (2021).

37. Baião, D. dos S., da Silva, D. V. T. & Paschoalin, V. M. F. Beetroot, a remarkable vegetable: Its nitrate and phytochemical contents can be adjusted in novel formulations to benefit health and support cardiovascular disease therapies. *Antioxidants* **9**, 1–36 (2020).

38. Keller, R. M., Beaver, L., Prater, M. C. & Hord, N. G. Dietary nitrate and nitrite concentrations in food patterns and dietary supplements. *Nutr. Today* **55**, 218–226 (2020).

39. Sharma, S., Kataria, A. & Singh, B. Effect of thermal processing on the bioactive compounds, antioxidative, antinutritional and functional characteristics of quinoa (*Chenopodium quinoa*). *LWT* **160**, 113256 (2022).

40. Sharma, K. *et al.* Saponins: a concise review on food related aspects, applications and health implications. *Food Chem. Adv.* **2**, 100191 (2023).

41. Liu, Y.-J., Lai, Y.-J., Wang, R., Lo, Y.-C. & Chiu, C.-H. The effect of thermal processing on the saponin profiles of *Momordica charantia* L. *J. Food Qual.* **2020**, 1–7 (2020).

42. Mohsen, E. *et al.* Impact of thermal processing on phytochemical profile and cardiovascular protection of *Beta vulgaris* L. in hyperlipidemic rats. *Sci. Rep.* **14**, 27539 (2024).

43. Feitosa, S. *et al.* Effect of traditional household processes on iron, zinc and copper bioaccessibility in black bean (*Phaseolus vulgaris* L.). *Foods* **7**, 123 (2018).

44. Latunde-Dada, G. O. Effect of processing on iron levels in and availability from some nigerian vegetables. *J. Sci. Food Agric.* **53**, 355–361 (1990).

45. World Health Organization. *World Health Organization* https://www.who.int/news-room/fact-sheets/detail/anaemia (2024).

46. Shin, J.-A., Heo, Y., Seo, M., Choi, Y. & Lee, K.-T. Effects of cooking methods on the β-carotene levels of selected plant food materials. *Food Sci. Biotechnol.* **25**, 955–963 (2016).

47. Czarnowska-Kujawska, M., Draszanowska, A. & Starowicz, M. Effect of different cooking methods on the folate content, organoleptic and functional properties of broccoli and spinach. *LWT* **167**, 113825 (2022).

48. Deol, J. K. & Bains, K. Effect of household cooking methods on nutritional and anti nutritional factors in green cowpea (*Vigna unguiculata*) pods. *J. Food Sci. Technol.* **47**, 579–581 (2010).

49. Hailemariam, G. A. & Wudineh, T. A. Effect of cooking methods on ascorbic acid destruction of green leafy vegetables. *J. Food Qual.* **2020**, 1–5 (2020).

50. Milman, N. T. A review of nutrients and compounds, which promote or inhibit intestinal iron absorption: making a platform for dietary measures that can reduce iron uptake in patients with genetic haemochromatosis. *J. Nutr. Metab.* **2020**, 1–15 (2020).

51. Jyothi Lakshmi, A., Gupta, S. & Prakash, J. Comparative analysis of influence of promoters and inhibitors on *in vitro* available iron using two methods. *Int. J. Food Sci. Nutr.* **57**, 559–569 (2006).

52. Gillooly, M. *et al.* The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. *Br. J. Nutr.* **49**, 331–342 (1983).

53. Rodrigues, D. B. *et al.* Trust your gut: bioavailability and bioaccessibility of dietary compounds. *Curr. Res. Food Sci.* **5**, 228–233 (2022).

54. Igual, M. *et al.* The *in vitro* simulated gastrointestinal digestion affects the bioaccessibility and bioactivity of *Beta vulgaris* constituents. *Foods* **12**, 338 (2023).

55. Sheir, M. A., Ramadan, M. M., El-Messery, T. M. & Mohamed, E. N. Enhancing iron bioavailability and bioactive stability in sweet-sour beetroot sauce through liposomal vitamin C. *Food Chem. Adv.* **7**, 101020 (2025).

56. Sotelo, A., González-Osnaya, L., Sánchez-Chinchillas, A. & Trejo, A. Role of oxate, phytate, tannins and cooking on iron bioavailability from foods commonly consumed in Mexico. *Int. J. Food Sci. Nutr.* **61**, 29–39 (2010).

Figure legends

Figure 1 represent the sensory parameters of different processing methods of beetroot variants. RJ+R, PJ+R, SJ+R, OJ+R, RJ, PJ, SJ, OJ, RR, PR, SR, and OR represent raw juice with residue, pressure-cooked juice with residue, steam-cooked juice with residue, open pan-cooked juice with residue, raw juice, pressure-cooked juice, steam-cooked juice, open pan-cooked juice, raw residue, pressure-cooked residue, steam-cooked residue, and open pan-cooked residue. Error bars in figures represent standard deviation of triplicate measurements.

Figure 2 represents the optimization of beetroot processing methods and variants. Minimum and Maximum represent the minimized and maximized variables for the optimization process.

Table 1 Factors and levels of factors for optimization

Factors	Number of levels	Levels
Juice	2	No, yes
Residue	2	No, yes
Cooking method	4	Raw, pressure cooking, steaming, and open pan cooking

Table 2 Effect of thermal processing methods on the nutraceutical properties of various beetroot variants

Different processing methods and beetroot variants	DPPH (IC_{50} μg/mL)	ABTS (IC_{50} μg/mL)	H_2O_2 (IC_{50} μg/mL)	TPC (mg GAE/100 g DW)	TFC (mg QE/100 g DW)	Saponin (mg/100 g DW)	Nitrates (mg/100 g DW)	Betanins (mg/100 g DW)	Vulgaxanthin (mg/100 g DW)
RJ+R	316.86±0.09^{b}	216.08±0.02^{b}	49.52±0.31^{i}	546.18±6.95^{h}	109.28±4.70^{h}	12.66±0.57^{de}	200.10±0.10^{a}	21.43±0.03^{e}	29.90±0.10^{d}

PJ+R	58.34 ± 0.50^{g}	83.54 ± 0.32^{g}	141.31 ± 0.33^{c}	666.66 ± 6.95^{cd}	275.95 ± 4.70^{c}	15.66 ± 0.57^{ab}	157.37 ± 0.17^{c}	17.28 ± 0.02^{f}	23.19 ± 0.03^{g}
SJ+R	54.70 ± 0.07^{h}	129.15 ± 0.11^{e}	41.69 ± 0.01^{j}	646.58 ± 6.95^{de}	349.72 ± 4.70^{a}	17.33 ± 0.57^{a}	124.62 ± 0.17^{e}	12.41 ± 0.03^{j}	20.76 ± 0.07^{i}
OJ+R	184.62 ± 0.08^{e}	186.68 ± 0.29^{d}	214.61 ± 0.31^{b}	618.47 ± 6.95^{f}	81.96 ± 8.10^{i}	15.66 ± 1.15^{ab}	111.87 ± 0.17^{g}	39.84 ± 0.04^{b}	47.60 ± 0.24^{b}
RJ	58.57 ± 0.39^{g}	199.66 ± 0.38^{c}	57.25 ± 0.31^{h}	650.60 ± 6.95^{cd}	76.50 ± 4.70^{i}	2.00 ± 0.01^{g}	50.00 ± 0.10^{i}	15.49 ± 0.02^{h}	23.50 ± 0.04^{fg}
PJ	39.34 ± 0.55^{i}	53.68 ± 0.06^{k}	68.41 ± 0.39^{g}	698.79 ± 0.01^{ab}	139.34 ± 8.10^{g}	13.33 ± 0.57^{cd}	171.12 ± 0.17^{b}	13.17 ± 0.09^{i}	18.97 ± 0.11^{j}
SJ	38.48 ± 0.35^{i}	58.33 ± 0.49^{j}	129.03 ± 0.01^{d}	702.81 ± 6.95^{a}	366.12 ± 4.70^{a}	10.66 ± 0.57^{ef}	174.12 ± 0.17^{b}	3.60 ± 0.10^{l}	11.80 ± 0.08^{k}
OJ	87.37 ± 0.23^{f}	23.29 ± 0.26^{l}	121.08 ± 0.07^{f}	590.36 ± 12.04^{g}	221.31 ± 8.10^{e}	15.00 ± 1.00^{bc}	118.50 ± 0.25^{f}	11.59 ± 0.06^{k}	21.80 ± 0.14^{h}
RR	282.28 ± 0.22^{c}	101.65 ± 0.23^{f}	331.87 ± 0.02^{a}	674.69 ± 6.95^{bc}	139.34 ± 8.10^{g}	4.00 ± 0.01^{g}	125.60 ± 0.49^{e}	34.79 ± 0.03^{c}	43.03 ± 0.38^{c}
PR	334.68 ± 0.18^{a}	78.43 ± 0.47^{h}	126.56 ± 0.11^{e}	622.48 ± 18.40^{ef}	245.90 ± 4.70^{d}	9.00 ± 1.00^{f}	137.12 ± 0.17^{d}	22.47 ± 0.04^{d}	28.64 ± 0.05^{e}
SR	263.35 ± 1.94^{d}	217.59 ± 0.26^{a}	214.52 ± 0.46^{b}	586.34 ± 6.95^{g}	330.60 ± 4.70^{b}	11.66 ± 0.57^{de}	107.87 ± 0.17^{g}	16.11 ± 0.10^{g}	23.83 ± 0.14^{f}

OR	58.30 ± 0.12^{g}	72.52 ± 0.19^{i}	26.74 ± 0.23^{k}	610.44 ± 6.95^{fg}	163.93 ± 8.10^{f}	15.33 ± 0.57^{abc}	64.50 ± 0.35^{h}	43.11 ± 0.04^{a}	54.37 ± 0.15^{a}

$^{a\text{-}l}$ means values with the same letters are not significantly different according to the Tukey test at $p < 0.05$. Data represent the mean value±standard deviation of three replicates (n=3). IC_{50} values were expressed as µg/mL, representing the concentration of sample extract required to achieve 50% radical scavenging activity. TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; QE: Quercetin equivalent; RJ+R: Raw juice with residue; PJ+R: Pressure-cooked juice with residue; SJ+R: Steamed juice with residue; OJ+R: Open pan-cooked juice with residue; RJ: Raw juice; PJ: Pressure-cooked juice; SJ: Steamed juice; OJ: Open pan-cooked juice; RR: Raw residue; PR: Pressure-cooked residue; SR: Steamed residue; OR: Open pan-cooked residue.

Table 3 Effect of thermal processing methods on the nutritional and antinutritional properties of various beetroot variants

Different processing methods and beetroot variants	Ascorbic acid (mg/100 g DW)	Citric acid (%)	Iron (mg/100 g DW)	Folate (µg/100 g DW)	β-carotene (µg/100 g DW)	Tannin (mg TAE/100 g DW)	Oxalates (mg/100 g DW)	Calcium (mg/100 DW)
RJ+R	7.01 ± 0.02^{c}	0.43 ± 0.05^{cd}	2.29 ± 0.04^{a}	31.33 ± 0.57^{d}	12.33 ± 0.57^{f}	172.83 ± 2.88^{h}	171.46 ± 13.39^{a}	4.72±0.
PJ+R	6.90 ± 0.05^{c}	0.83 ± 0.11^{a}	1.36 ± 0.02^{f}	37.00 ± 1.00^{ab}	19.00 ± 1.00^{ab}	116.16 ± 2.88^{j}	126.93 ± 12.93^{bc}	2.67±0.
SJ+R	4.97 ± 0.02^{g}	0.42 ± 0.02^{cd}	1.50 ± 0.03^{e}	38.33 ± 0.57^{a}	21.00 ± 1.00^{a}	400.5 ± 0.50^{e}	126.93 ± 12.93^{bc}	2.02±0.
OJ+R	5.38 ± 0.02^{f}	0.05 ± 0.02^{f}	1.60 ± 0.02^{d}	38.33 ± 0.57^{a}	20.33 ± 0.57^{ab}	455 ± 0.50^{c}	104.53 ± 12.93^{cde}	2.66±0.
RJ	8.18 ± 0.02^{a}	0.51 ± 0.09^{bc}	0.71 ± 0.04^{h}	30.00 ± 1.00^{d}	12.00 ± 1.00^{f}	322.83 ± 2.88^{f}	149.33 ± 12.93^{ab}	4.52±0.2
PJ	7.76 ± 0.02^{b}	0.27 ± 0.04^{de}	0.35 ± 0.04^{j}	34.00 ± 1.00^{c}	15.33 ± 0.57^{de}	167.83 ± 2.88^{h}	126.93 ± 12.93^{bc}	2.71±0.

SJ	6.01±0.02^{e}	0.42±0.02^{cd}	0.26±0.01^{k}	29.66±0.57^{d}	12.66±0.57^{f}	528±0.50^{a}	119.46±12.93^{bcd}	1.52±0.
OJ	6.55±0.05^{d}	0.04±0.02^{f}	0.56±0.01^{i}	34.66±0.57^{c}	16.66±0.57^{cd}	515±0.50^{b}	74.66±12.93^{e}	2.23±0.
RR	3.93±0.05^{h}	0.25±0.04^{e}	1.93±0.03^{b}	25.66±0.57^{e}	5.00±0.01^{g}	92.83±2.88^{k}	186.66±12.93^{a}	4.72±0.
PR	3.43±0.05^{i}	0.60±0.08^{b}	1.06±0.03^{g}	30.33±0.57^{d}	13.33±0.57^{ef}	137.83±2.88^{i}	104.53±12.93^{cde}	3.78±0.
SR	3.33±0.05^{i}	0.17±0.02^{e}	1.75±0.04^{c}	34.33±0.57^{c}	15.33±0.57^{de}	446.5±0.50^{d}	104.53±12.93^{cde}	2.34±0.
OR	2.01±0.02^{j}	0.04±0.02^{f}	1.81±0.02^{c}	36.00±1.00^{bc}	18.66±0.57^{bc}	315±0.50^{g}	82.13±12.93^{de}	4.34±0.

$^{a\text{-}l}$ means values with the same letters are not significantly different according to the Tukey test at $p < 0.05$. Data represents the mean value±standard deviation of three replicates (n=3). TAE: Tannic acid equivalent; RJ+R: Raw juice with residue; PJ+R: Pressure-cooked juice with residue; SJ+R: Steamed juice with residue; OJ+R: Open pan-cooked juice with residue; RJ: Raw juice; PJ: Pressure-cooked juice; SJ: Steamed juice; OJ: Open pan-cooked juice; RR: Raw residue; PR: Pressure-cooked residue; SR: Steamed residue; OR: Open pan- cooked residue.

Table 4 Effect of thermal processing methods on the color of various beetroot variants

Different processing methods and beetroot variants	L*	a*	b*
RJ+R	0.01±0.0^{h}	0.01±0.0^{h}	0.01±0.0^{g}
PJ+R	12.26±0.01^{d}	10.35±0.03^{d}	0.40±0.01^{f}
SJ+R	0.01±0.0^{h}	0.01±0.0^{h}	0.01±0.0^{g}
OJ+R	15.90±0.01^{a}	8.46±0.01^{g}	1.21±0.01^{d}
RJ	0.01±0.0^{h}	0.01±0.0^{h}	0.01±0.0^{g}
PJ	11.83±0.04^{e}	10.55±0.02^{c}	0.46±0.01^{e}

SJ	0.01±0.0^{h}	0.01±0.0^{h}	0.01±0.0^{g}
OJ	6.88±0.01^{g}	17.24±0.01^{a}	4.30±0.01^{a}
RR	12.54±0.02^{c}	10.03±0.02^{e}	0.43±0.01^{f}
PR	12.26±0.02^{d}	10.35±0.01^{d}	0.42±0.02^{f}
SR	15.58±0.02^{b}	9.46±0.01^{f}	2.25±0.01^{c}
OR	9.15±0.02^{f}	14.73±0.01^{b}	2.85±0.02^{b}

$^{a\text{-}h}$ means values with the same letters are not significantly different according to the Tukey test at $p < 0.05$. Data represents the mean value±standard deviation of three replicates (n=3). RJ+R: Raw juice with residue; PJ+R: Pressure-cooked juice with residue; SJ+R: Steamed juice with residue; OJ+R: Open pan-cooked juice with residue; RJ: Raw juice; PJ: Pressure-cooked juice; SJ: Steamed juice; OJ: Open pan-cooked juice; RR: Raw residue; PR: Pressure-cooked residue; SR: Steamed residue; OR: Open pan- cooked residue.

Table 5 General factorial regression model summary

DPPH versus Juice, Residue, Type								
Source	DF	Adj SS	Adj MS	F-Value	P-Value	R^2	R^2 (adj)	R^2 (pred)
Model	11	497320	45210.90	112716.71	0.00	0.10	0.10	0.10

Linear	5	257237	51447.30	128264.98	0.00			
Juice	1	45427	45427.10	113255.66	0.00			
Residue	1	57257	57257.10	142749.41	0.00			
Type	3	52324	17441.40	43483.72	0.00			
2-Way Interactions	6	227014	37835.60	94329.21	0.00			
Juice*Type	3	181918	60639.4	151182.03	0.00			
Residue*Type	3	57932	19310.8	48144.45	0.00			
ABTS versus Juice, Residue, Type								
Model	11	158396	14399.70	163546.15	0.00	0.10	0.10	0.10
Linear	5	76734	15346.90	174304.33	0.00			
Juice	1	7910	7910	89840.94	0.00			

Residue	1	29496	29496.20	335007.29	0.00			
Type	3	47226	15741.90	178790.63	0.00			
2-Way Interactions	6	75336	12555.90	142605.69	0.00			
Juice*Type	3	43047	14348.90	162969.86	0.00			
Residue*Type	3	19806	6601.80	74981.34	0.00			
H_2O_2 versus Juice, Residue, Type								
Model	11	267546	24322.40	339801.31	0.00	0.10	0.10	0.10
Linear	5	103677	20735.30	289687.27	0.00			
Juice	1	23920	23919.60	334174.09	0.00			
Residue	1	1909	1909.40	26676.34	0.00			

Type	3	60227	20075.60	280471.10	0.00			
2-Way Interactions	6	218412	36402.00	508562.46	0.00			
Juice*Type	3	193736	64578.60	902210.47	0.00			
Residue*Type	3	30718	10239.40	143051.51	0.00			
TPC versus Juice, Residue, Type								
Model	11	75139.9	6830.90	94.12	0.00	0.97	0.96	0.94
Linear	5	28580.2	5716.00	78.76	0.00			
Juice	1	96.8	96.80	1.33	0.26			
Residue	1	10669.2	10669.20	147.00	0.00			
Type	3	15580.4	5193.50	71.56	0.00			

2-Way Interactions	6	46096.0	7682.70	105.85	0.00			
Juice*Type	3	33144.6	11048.20	152.22	0.00			
Residue*Type	3	14443.3	4814.40	66.33	0.00			
TFC versus Juice, Residue, Type								
Model	11	361320	32847.30	800.02	0.00	0.99	0.99	0.99
Linear	5	183610	36721.90	894.39	0.00			
Juice	1	1355	1354.90	33.00	0.00			
Residue	1	70	70.00	1.70	0.20			
Type	3	181299	60433.10	1471.89	0.00			
2-Way Interactions	6	65458	10909.70	265.71	0.00			

Juice*Type	3	12239	4079.70	99.36	0.00			
Residue*Type	3	59065	19688.40	479.52	0.00			
Saponin versus Juice, Residue, Type								
Model	11	756.97	68.816	145.73	0.00	0.98	0.97	0.96
Linear	5	687.51	137.50	291.18	0.00			
Juice	1	170.67	170.66	361.41	0.00			
Residue	1	155.04	155.04	328.32	0.00			
Type	3	470.13	156.70	331.85	0.00			
2-Way Interactions	6	122.39	20.39	43.20	0.00			
Juice*Type	3	57.00	19.00	40.24	0.00			
Residue*Type	3	91.13	30.37	64.32	0.00			
Nitrates versus Juice, Residue, Type								
Model	11	634.833	57.71	2579.39	0.00	0.99	0.99	0.99

Linear	5	302.102	60.42	2700.43	0.00			
Juice	1	95.940	95.94	4287.95	0.00			
Residue	1	24.675	24.67	1102.81	0.00			
Type	3	206.155	68.71	3071.31	0.00			
2-Way Interactions	6	387.297	64.55	2884.99	0.00			
Juice*Type	3	33.246	11.08	495.30	0.00			
Residue*Type	3	357.710	119.23	5329.17	0.00			
Betanins versus Juice, Residue, Type								
Model	11	4892.80	444.80	131684.08	0.00	0.10	0.10	0.10
Linear	5	3165.34	633.07	187421.40	0.00			
Juice	1	244.29	244.29	72322.76	0.00			
Residue	1	831.90	831.90	246287.29	0.00			

Type	3	1129.34	376.44	111447.97	0.00			
2-Way Interactions	6	730.29	121.71	36033.85	0.00			
Juice*Type	3	100.44	33.47	9911.47	0.00			
Residue*Type	3	559.48	186.49	55211.50	0.00			
Vulgaxanthin versus Juice, Residue, Type								
Model	11	5381.02	489.18	18750.63	0.00	0.99	0.99	0.99
Linear	5	3580.15	716.03	27445.80	0.00			
Juice	1	302.74	302.74	11604.33	0.00			
Residue	1	771.69	771.69	29579.14	0.00			
Type	3	1503.12	501.04	19205.06	0.00			
2-Way Interactions	6	664.00	110.66	4241.91	0.00			
Juice*Type	3	83.18	27.73	1062.81	0.00			

Residue*Type	3	434.61	144.87	5553.00	0.00			
Ascorbic acid versus Juice, Residue, Type								
Model	11	125.39	11.39	5862.66	0.00	0.99	0.99	0.99
Linear	5	112.30	22.46	11551.03	0.00			
Juice	1	50.02	50.02	25727.63	0.00			
Residue	1	6.77	6.77	3483.48	0.00			
Type	3	12.02	4.00	2061.50	0.00			
2-Way Interactions	6	4.41	0.73	378.70	0.00			
Juice*Type	3	3.26	1.08	559.63	0.00			
Residue*Type	3	0.09	0.03	15.48	0.00			
Citric acid versus Juice, Residue, Type								
Model	11	1.97	0.18	65.40	0.00	0.96	0.95	0.92
Linear	5	0.72	0.14	52.76	0.00			
Juice	1	0.17	0.17	62.02	0.00			

Residue	1	0.09	0.09	32.42	0.00			
Type	3	0.54	0.18	65.89	0.00			
2-Way Interactions	6	0.52	0.08	31.78	0.00			
Juice*Type	3	0.05	0.02	6.69	0.00			
Residue*Type	3	0.39	0.13	47.55	0.00			
Iron versus Juice, Residue, Type								
Model	11	44.65	4.06	295.01	0.00	0.99	0.98	0.98
Linear	5	31.37	6.27	455.98	0.00			
Juice	1	0.46	0.46	33.18	0.00			
Residue	1	0.25	0.25	18.04	0.00			
Type	3	30.88	10.29	748.07	0.00			
2-Way Interactions	6	1.83	0.31	22.21	0.00			
Juice*Type	3	0.68	0.23	16.48	0.00			
Residue*Type	3	1.54	0.51	37.32	0.00			

Folate versus Juice, Residue, Type								
Model	11	510.31	46.39	83.50	0.00	0.97	0.96	0.94
Linear	5	327.89	65.57	118.04	0.00			
Juice	1	130.67	130.66	235.20	0.00			
Residue	1	104.17	104.16	187.50	0.00			
Type	3	170.33	56.77	102.20	0.00			
2-Way Interactions	6	95.56	15.92	28.67	0.00			
Juice*Type	3	16.33	5.44	9.80	0.00			
Residue*Type	3	44.83	14.94	26.90	0.00			
β-carotene versus Juice, Residue, Type								
Model	11	662.97	60.27	127.63	0.00	0.98	0.97	0.96
Linear	5	431.51	86.30	182.76	0.00			
Juice	1	155.04	155.04	328.32	0.00			
Residue	1	96.00	96.00	203.29	0.00			
Type	3	259.46	86.48	183.15	0.00			

2-Way Interactions	6	109.28	18.21	38.57	0.00			
Juice*Type	3	26.13	8.70	18.44	0.00			
Residue*Type	3	48.67	16.22	34.35	0.00			
Oxalates versus Juice, Residue, Type								
Model	11	36551.00	3322.80	19.75	0.00	0.90	0.85	0.77
Linear	5	26223.00	5244.60	31.17	0.00			
Juice	1	1014.00	1014.00	6.03	0.02			
Residue	1	1326.10	1326.10	7.88	0.01			
Type	3	24649.00	8216.30	48.83	0.00			
2-Way Interactions	6	3774.30	629.00	3.74	0.00			

Juice*Type	3	1590.50	530.20	3.15	0.04			
Residue*Type	3	830.40	276.80	1.64	0.20			
Tannin versus Juice, Residue, Type								
Model	11	872790	79345	18488.06	0.00	0.99	0.99	0.99
Linear	5	581614	116323	27104.36	0.00			
Juice	1	8759	8759	2040.99	0.00			
Residue	1	56648	56648	13199.57	0.00			
Type	3	464704	154901	36093.51	0.00			
2-Way Interactions	6	57223	9537	2222.27	0.00			
Juice*Type	3	34329	11443	2666.35	0.00			
Residue*Type	3	10801	3600	838.89	0.00			
Calcium versus Juice, Residue, Type								

Model	11	44.14	4.01	249.87	0.00	0.99	0.98	0.98
Linear	5	28.85	5.77	359.24	0.00			
Juice	1	3.61	3.61	225.30	0.00			
Residue	1	0.44	0.44	27.74	0.00			
Type	3	21.74	7.24	451.14	0.00			
2-Way Interactions	6	3.84	0.64	39.94	0.00			
Juice*Type	3	2.61	0.87	54.34	0.00			
Residue*Type	3	0.27	0.09	5.75	0.00			

TPC: Total phenolic content; TFC: Total flavonoid content.

Table 6 Response optimization results

Response	Goal	Lower	Target	Upper	Weight	Importance	SE fit
Oxalates	Minimum	--	67.20	201.60	1	2	7.49
Calcium	Minimum	--	1.48	4.88	1	2	0.07
Tannin	Minimum	--	89.50	528.50	1	2	1.20
TPC	Minimum	--	542.16	710.84	1	1	4.92
TFC	Minimum	--	73.77	368.85	1	1	3.70

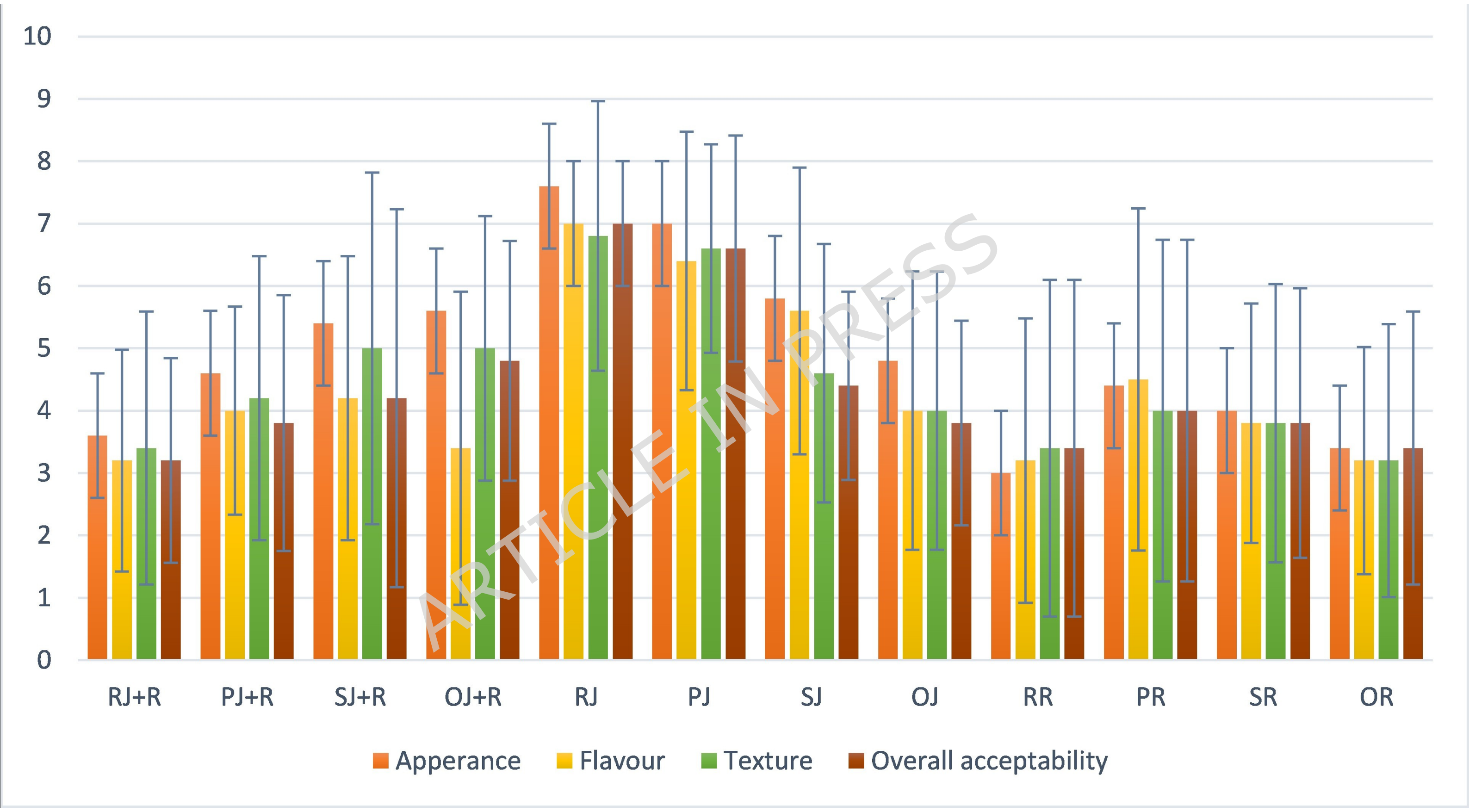
Ascorbic acid	Maximum	2	8.2	--	1	2	0.02
Citric acid	Maximum	0.03	0.96	--	1	2	0.03
Iron	Maximum	0.25	2.32	--	1	4	0.16
Folate	Maximum	25	39	--	1	2	0.43
β-carotene	Maximum	5.0	22.0	--	1	2	0.39
Nitrates	Maximum	4.90	20.20	--	1	1	0.08
Saponin	Maximum	2.00	18.00	--	1	1	0.39
Betanin	Maximum	3.50	43.14	--	1	1	0.03
Vulgaxanthin	Maximum	11.71	54.52	--	1	1	0.09
DPPH	Minimum	--	38.10	358.96	1	1	0.36
ABTS	Minimum	--	23.10	217.79	1	1	0.17
H_2O_2	Minimum	--	26.53	331.91	1	1	0.15

TPC: Total phenolic content; TFC: Total flavonoid content.

Table 7 Optimized solutions from Minitab with predicted value and desirability

	Solution 1	Solution 2	Solution 3
Juice	Yes	Yes	No
Residue	Yes	Yes	Yes
Type of cooking	Pressure cooking	Steaming	Pressure cooking
Oxalates fit	126.93	126.93	104.53

Calcium fit	2.67	2.02	3.78
Tannin fit	116.16	400.50	137.83
Total phenol content fit	666.66	646.58	622.49
Total flavonoid fit	275.95	349.72	243.16
Ascorbic acid fit	4.96	7.76	3.43
Citric acid fit	0.83	0.42	0.59
Iron fit	1.36	1.50	1.06
Folate fit	37.00	38.00	30.33
β-carotene fit	19.00	21.00	13.33
Nitrates fit	15.75	12.46	13.70
Saponin fit	15.66	17.33	9.00
Betanin fit	17.28	12.41	22.46
Vulgaxanthin fit	23.19	20.76	28.63
DPPH fit	58.33	54.69	263.34
ABTS fit	83.54	129.15	78.43
H_2O_2 fit	141.31	41.69	126.56
Composite desirability	0.63	0.51	0.46


10
9
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RJ+R
PJ+R
SJ+R
OJ+R
RJ
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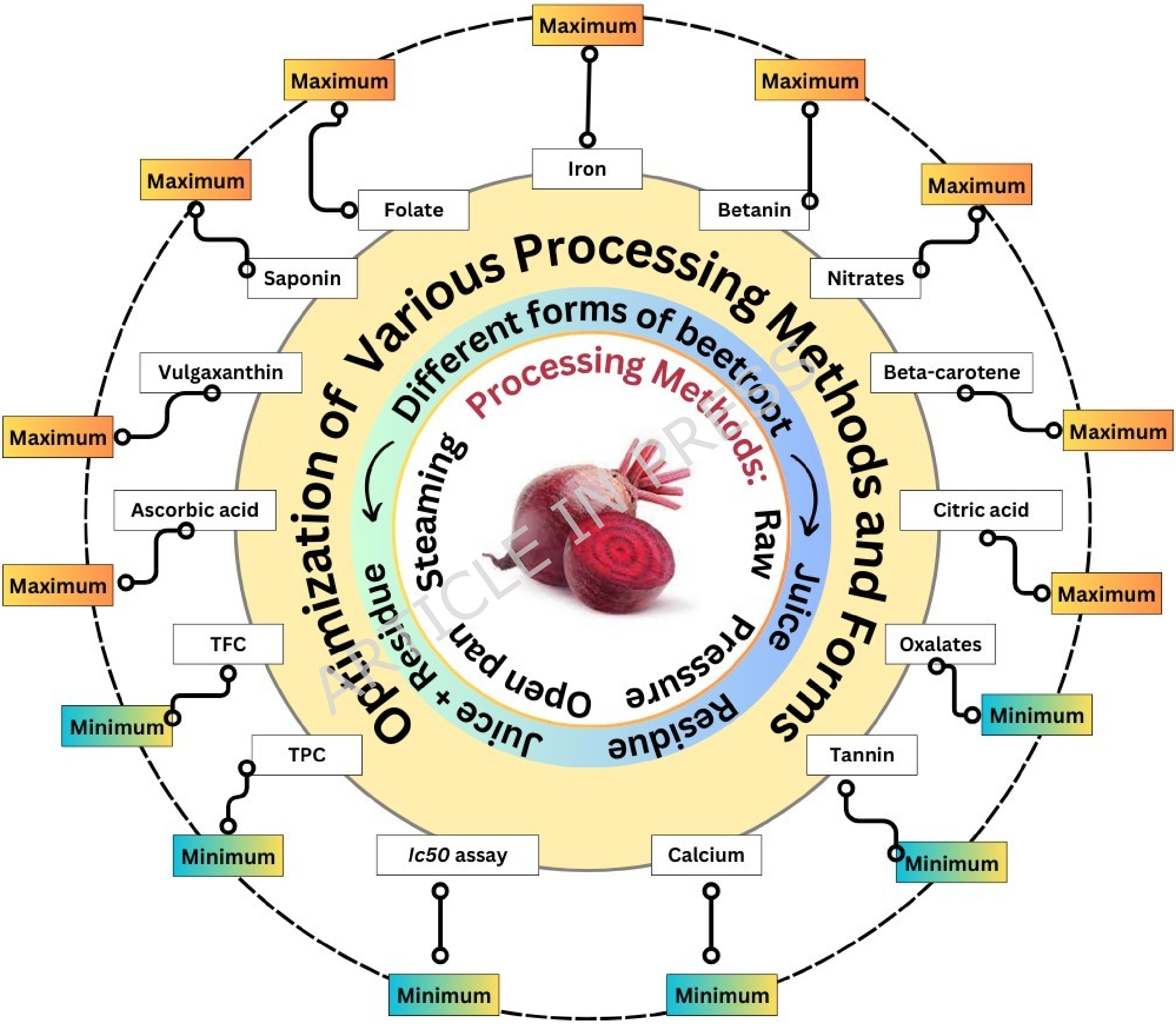
SJ
OJ
RR
PR
SR
OR
Apperance
Flavour
Texture
Overall acceptability


Optimization of Various Processing Methods and Forms
Different forms of beetroot
Juice
Residue
Juice + Residue
Processing Methods:
Raw
Pressure
Open pan
Steaming
Iron
Maximum
Folate
Maximum
Betanin
Maximum
Saponin
Maximum
Nitrates
Maximum
Vulgaxanthin
Maximum
Beta-carotene
Maximum
Ascorbic acid
Maximum
Citric acid
Maximum
TFC
Minimum
Oxalates
Minimum
TPC
Minimum
Tannin
Minimum
Ic50 assay
Minimum
Calcium
Minimum