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Effect of storage conditions on quality, microbial stability, and shelf-life kinetics of beetroot juice concentrate

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Beetroot juice (*Beta vulgaris* L.) with an initial 5.0 °Brix was concentrated to approximately 60 °Brix using forward osmosis (FO). The concentrate was then stored under ambient (25 °C ± 2 °C and 60–70% RH) and accelerated (37 °C ± 2 °C and 90% RH) conditions for 12 weeks. Throughout this period, physical, chemical, and microbiological properties were evaluated at two-week intervals. Results indicated that the quality of the beetroot juice concentrate (BRJC) was influenced by the storage environments over time, with minimal changes in pH, titratable acidity, and total soluble solids. However, betalain content and antioxidant activity were more sensitive to storage time and temperature. The degradation of betalains in ambient and accelerated conditions was 28.53% and 43.57%, respectively, at the end of 12 weeks of storage. The HMF and browning index (BI) levels of BRJC increased more significantly at 37 °C than at 25 °C. Betalain degradation followed first-order kinetics. Overall, the findings suggest that FO is an effective non-thermal method for concentrating beetroot juice as it preserves quality and extends shelf life. Additionally, a moderate to strong correlation was found between betalain content and total color difference in the BRJC stored under ambient and accelerated storage conditions.

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Sustainability spotlight

Beetroot betalains represent a sustainable, multifunctional pigment source aligned with current trends toward natural, safe, and circular product development. While storage and stability issues remain hurdles, ongoing innovations in encapsulation and processing are unlocking their broader potential. In this study, the derived beetroot juice concentrate retained a high amount of betalain content, which is a violet-red pigment and can be used in various food industries, particularly in confectionery, meat, dairy, and beverages. As industries continue to prioritize environmental responsibility, betalains are poised to play a vital role in the next generation of eco-conscious colorants and bioactive compounds.

1 Introduction

Color is one of the very vital factors of fresh and processed foods that affects consumer acceptance. This is a natural indicator of the quality of the food and may be the only factor that a consumer evaluates before selecting or purchasing foods. Hence, it is very important for the producer to consider the visual appearance of the produce. Beetroot (*Beta vulgaris* L.) juice has a natural red food colorant that can be used in jam, jellies, dry mixes, soups, ice cream, sweets, *etc.* The health benefits of beetroot juice include antioxidative, anticancer, antidiabetic, cardioprotective, anti-inflammatory, anti-obesity, and antimicrobial effects.[1,2] The color of beetroot juice originates from its purplish-red and yellow pigments (betacyanin and betaxanthin, respectively), collectively known as betalains. The colorant betalains in beetroot juice also show antioxidant activity.[3,4] Among the numerous natural sources such as amaranth, prickly pear, and dragon fruit, beetroot is the only food containing majorly this class of compounds, betalains .[5,6]

While processed foods are typically colored with synthetic, inorganic, and nature-identical food dye/colorants, there is a growing customer demand for all-natural and clean-label products. This trend has led to an increased interest in beetroot juice betalains as a potential natural alternative to artificial colorants in processed foods. However, like other plant pigments (*e.g.*, anthocyanins), betalains are prone to color degradation during processing and storage. Their low stability and tinctorial strength limit their development, processing, and broader applications.[7–12] Color is a critical sensory attribute of food, making the color reduction of the pigment through processing and storage a crucial factor for maintaining quality. Betalains, in particular, offer a vibrant red hue at acidic pH levels, making the beetroot juice concentrate an excellent choice for coloring fruit juices, nectars, soft drinks, candies, desserts, dairy products, meat products, conserves, jellies and confectionery.[13–15]

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Storage stability is a crucial factor that impacts both the quality and shelf life of the final concentrated products, as well as the overall economic worth of the process. The visual appearance plays a significant role as it is a primary concern for consumer's appeal. The degradation of beetroot juice betalains during food storage significantly impacts the color, quality, and nutritional properties.[16] Therefore, color serves as a crucial indicator of pigment degradation. Minimizing pigment damage during processing and storing is a vital quality parameter, important to both industry standards and consumer preferences. The incorporation of storage studies on forward osmosis-concentrated betalain-rich beetroot juice can cultivate sustainable solutions in food processing involving optimizing storage conditions to enhance product quality and safety. The concentration of beetroot juice, storage conditions of concentrates, and packaging significantly affect its color stability, nutrient retention, and microbial safety. Understanding shelf-life kinetics under various conditions helps in designing eco-friendly preservation methods while minimizing waste. This approach ensures consumer health and product consistency in further applications of beetroot juice concentrates as a food colorant while also supporting broader sustainability goals in the food industry.

Bibliometric analysis offers valuable insights for foretelling future trends within disciplines. It is a quantitative research method that focuses on analyzing the external characteristics of scientific literature. It is commonly used to assess the current research landscape, identify emerging areas, and track the development trajectories of specific fields. Several studies have been conducted on the storage stability of fruit juices, concentrates, processing parameters, concentration methods, and storage conditions. However, to the best of our knowledge, no research has been carried out on the storage stability of beetroot juice concentrates (BRJC). Therefore, this study evaluates the impact of storage duration and conditions on the physical, chemical, organoleptic, and microbiological properties of beetroot juice concentrated using forward osmosis, a non-thermal process.

2 Materials and methods

2.1 Materials

Beetroot (*Beta Vulgaris* L.) was procured from the Devraja local market of Mysore, Karnataka, India. NaOH, citric acid, Trolox, ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)), and DPPH (2,2-diphenyl-1-picrylhydrazyl) were obtained from Sigma-India (Bengaluru, Karnataka, India). All the additional solvents and chemicals used were obtained from Merck (Darmstadt, Germany) and were of HPLC grade or analytical grade.

2.2 Bibliometric analysis

The bibliometric analysis focuses on publications related to the storage of beetroot juice colorant betalains from 2000 to 2025. Data was retrieved from the Scopus database on February 27, 2025, using the following keyword search: TITLE-ABS-KEY (beetroot) and TITLE-ABS-KEY (betalains) and TITLE-ABS-KEY (storage). A total of 67 publications on beetroot juice colorant betalains were identified. The different elements in a collection of publications on beetroot juice storage was examined for similarity visualization (VoS) using VOSviewer software (version 1.6.20) to generate co-occurrences of key terms from the published works.

2.3 Plant materials, juice extraction, clarification, concentration, and storage

2.3.1 Plant material and juice extraction. The mature beetroots were washed with clean water, peeled, and manually cut into 1 cm cubes. The beetroot cubes were added with an equal amount of water (1 : 1, wt/wt) to a juicer. The juice was extracted using a household juicer manufactured by Preethi Appliances, Chennai, India, and then stored under refrigerated conditions before clarification. Each batch of processed juice was set aside separately to minimize seasonal variations. The juice was cleared using centrifugation at 10 000 rpm for 15 minutes using a tabletop centrifuge (M s^{-1} Thermofisher, Waltham, Massachusetts, USA), and the supernatant was collected for future experiments. The clear juice was stored at −20 °C for later usage, concentration, and analysis.

2.3.2 Beetroot juice concentration. The beetroot juice concentrate was prepared using a membrane concentration process, forward osmosis. The juice was concentrated to an ultimate ∼60-degree Brix (°Brix) from an initial °Brix of 5.0 by forward osmosis (FO) process.

2.3.2.1 Forward osmosis. The FO experimental setup comprises an acrylic plate module supported by an SS plate with an active membrane area of 0.0135 m^2, as shown in Fig. 2. The acrylic plate module parts have been mentioned in the previous report by Trishitman (2025) and Trishitman *et al.* (2023).[17,18] The feed solution (beetroot juice) and 6 M NaCl draw solution were circulated on each side of the membrane in a co-current mode by means of a peristaltic pump (Ener Tech Pvt Ltd, India). The active layer of the forward osmosis membrane (cellulose triacetate asymmetric hydrophilic membrane (thickness 0.05 μm)) (developed by Osmotek Inc., Corvallis, OR USA) was placed towards the feed solution during the concentration process. The volume of the feed to the draw solution ratio was kept at 1 : 10, and the draw solution concentration was kept constant throughout the concentration process. All experiments were conducted at a temperature of 25 °C ± 2 °C, with the flow rate of both the feed and osmotic solutions maintained at 150 mL min^{-1}. The betalain-rich beetroot juice was concentrated for 12 h until the ultimate total soluble solids (TSS) reached approximately 60 °Brix.

2.3.3 Storage conditions. The FO-concentrated beetroot juice was transferred into separate glass bottles (25 mL), flushed with nitrogen gas before and after being filled, and sealed securely. The sealed bottles were then piled under ambient conditions of temperature (25 °C ± 2 °C) and 60–70% relative humidity (RH) and accelerated conditions of temperature (37 °C ± 2 °C) and 90% relative humidity (RH). The physicochemical properties and microbial analysis of juice concentrates were conducted at two-week intervals.

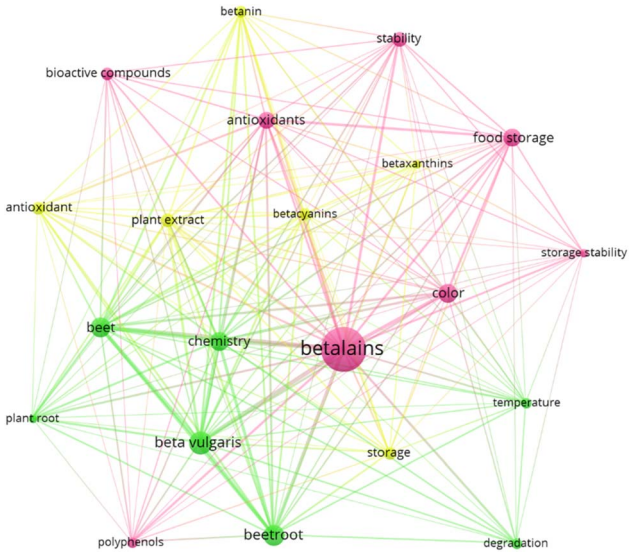

Fig. 1 Network visualization of the bibliometric analysis on the storage of beetroot juice betalains.

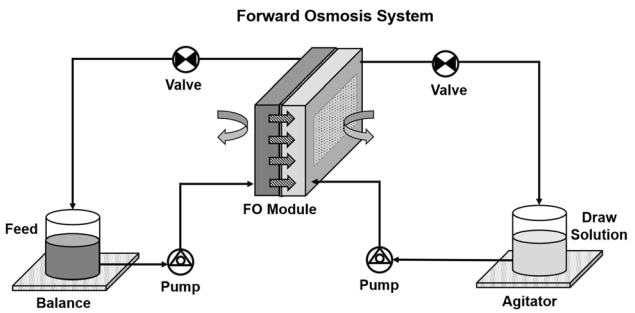

Fig. 2 Schematic of the beetroot juice concentration process using forward osmosis (FO).

2.4 Measurement of pH, titratable acidity and total soluble solids

The pH, titratable acidity (TA), and total soluble solids (TSS) were measured at 25 °C ± 2 °C. The concentrates were used directly for pH measurement using a hand-held pH meter. An Eutech pH 510 (Eutech Instruments, Singapore) was employed, calibrated with pH buffers 4, 7, and 10 for the measurement throughout the studies.

The titratable acidity (TA) was measured following the 965.30 AOAC method, where the juice sample was diluted 10 times and titrated with standardized 0.1 N sodium hydroxide (NaOH) to a pH value of 8.1. The TA was expressed as g L^{-1} of citric acid equivalent (CAE).[19]

TSS was determined using a digital refractometer (Hanna Instruments, Rhode Island, United States) and expressed in ° Brix.

All experiments were performed in triplicate, and the average values are reported with standard deviations.

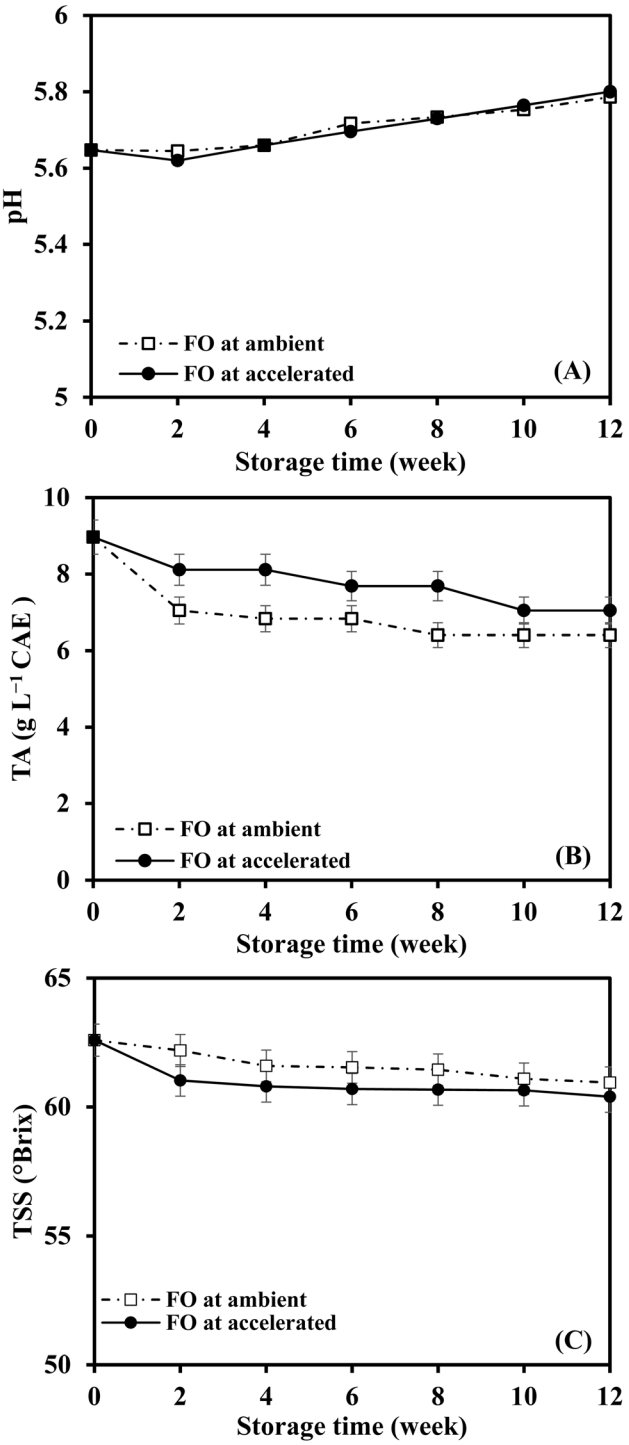

Fig. 3 (A) pH, (B) titratable acidity (TA), and (C) total soluble solids (TSSs) of forward osmosis (FO) concentrated beetroot juice stored under ambient and accelerated conditions for 12 weeks.

2.5 Measurement of total betalain content

The betalain content was determined using a UV-1800-spectrophotometer (M s^{-1}, Shimadzu Corporation, Kyoto, Kyoto, Japan) as described by Nilsson (1970) and Trishitman *et al.* (2021) using the following equation[20,21]

Table 1 Color profile of beetroot juice concentrates stored at ambient (25 °C) and accelerated (37 °C) conditions for 12 weeks[a]

Storage conditions	Storage time (weeks)	L^*	a^*	b^*	ΔE
Ambient (25 °C)	0	27.57 ± 0.09^{a}	54.23 ± 0.09^{g}	43.63 ± 0.20^{a}	0.00 ± 0.00^{a}
	2	34.82 ± 0.05^{d}	49.55 ± 0.07^{f}	52.88 ± 0.09^{d}	12.65 ± 0.08^{b}
	4	33.17 ± 0.01^{b}	45.68 ± 0.05^{e}	51.71 ± 0.09^{c}	13.03 ± 0.05^{c}
	6	33.23 ± 0.01^{b}	45.32 ± 0.05^{d}	52.68 ± 0.19^{d}	13.90 ± 0.15^{d}
	8	36.10 ± 0.03^{e}	44.07 ± 0.06^{c}	52.91 ± 0.12^{d}	16.19 ± 0.11^{f}
	10	34.87 ± 0.04^{d}	41.74 ± 0.07^{b}	53.55 ± 0.21^{e}	17.54 ± 0.14^{g}
	12	33.34 ± 0.02^{c}	41.47 ± 0.03^{a}	51.10 ± 0.13^{b}	15.87 ± 0.07^{e}
Accelerated (37 °C)	0	27.57 ± 0.09^{A}	54.23 ± 0.09^{G}	43.63 ± 0.20^{A}	0.00 ± 0.00^{A}
	2	40.83 ± 0.05^{D}	38.97 ± 0.06^{F}	58.10 ± 0.15^{G}	24.86 ± 0.10^{B}
	4	40.27 ± 0.03^{C}	33.77 ± 0.05^{E}	55.63 ± 0.10^{D}	26.91 ± 0.05^{C}
	6	39.68 ± 0.03^{B}	33.17 ± 0.06^{D}	58.03 ± 0.17^{F}	28.24 ± 0.04^{D}
	8	41.46 ± 0.03^{E}	29.77 ± 0.03^{C}	54.91 ± 0.19^{C}	30.31 ± 0.08^{E}
	10	44.31 ± 0.01^{F}	26.76 ± 0.03^{B}	57.44 ± 0.08^{E}	35.01 ± 0.03^{F}
	12	52.66 ± 0.02^{G}	24.98 ± 0.05^{A}	52.35 ± 0.06^{B}	39.51 ± 0.05^{G}

[a] Letters (lowercase and uppercase separately) that differ within the same column denote statistical significance at $p < 0.05$.

$$\text{Total betalains}\left(\frac{\text{mg}}{100\ \text{mL}}\right) = 1.33 \times A_{480} + 0.893 \times A_{540} \quad (1)$$

Here, A_{480} and A_{540} represent the maximum absorption at 480 nm and 540 nm, respectively.

The beetroot juice samples were centrifuged at 5000 rpm for 15 minutes, and the supernatant was analysed directly in a UV spectrophotometer. All the concentrated samples were diluted with distilled water before measurements, and the results are reported after multiplying with the appropriate dilution factor. All experiments were performed in triplicate, and the average values are reported with standard deviations.

2.6 Color measurement

The color of beetroot juice concentrates were measured by a CM-5 Minolta colorimeter (M s^{-1}. Konica Minolta Sensing Inc., Osaka, Japan), which assessed the juice based on three

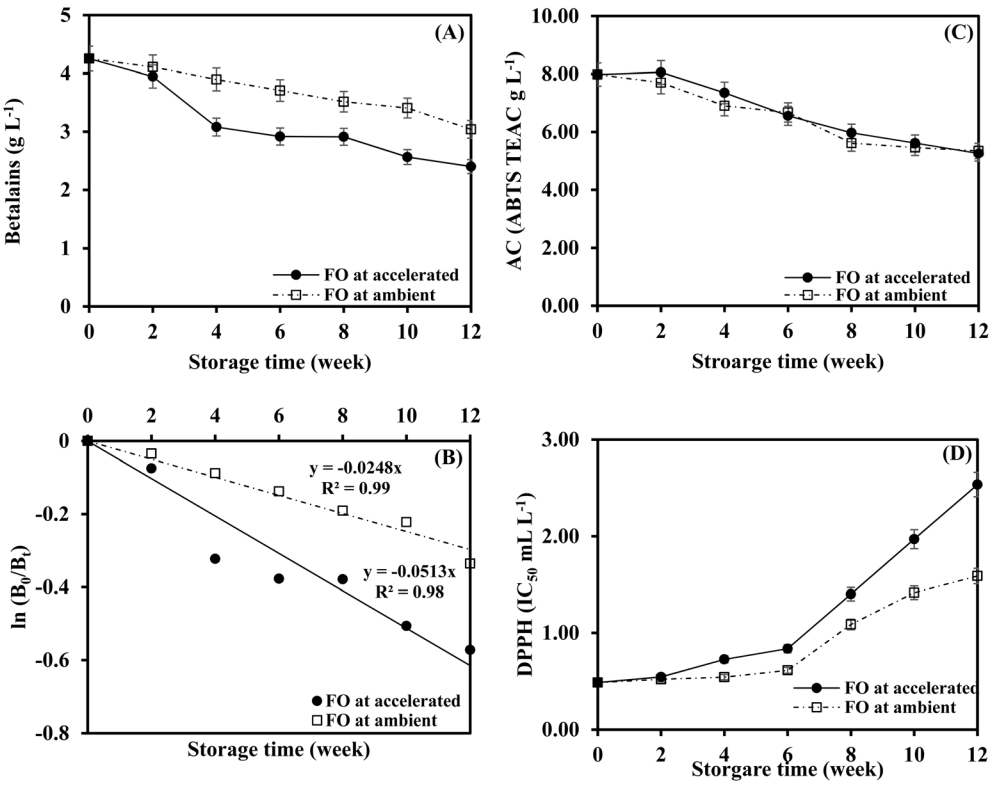

Fig. 4 Effect of storage on (A) betalain content, (B) betalain degradation kinetics, antioxidant activity measured by (C) ABTS and (D) DPPH of forward osmosis (FO) concentrated beetroot juice under ambient and accelerated conditions for 12 weeks.

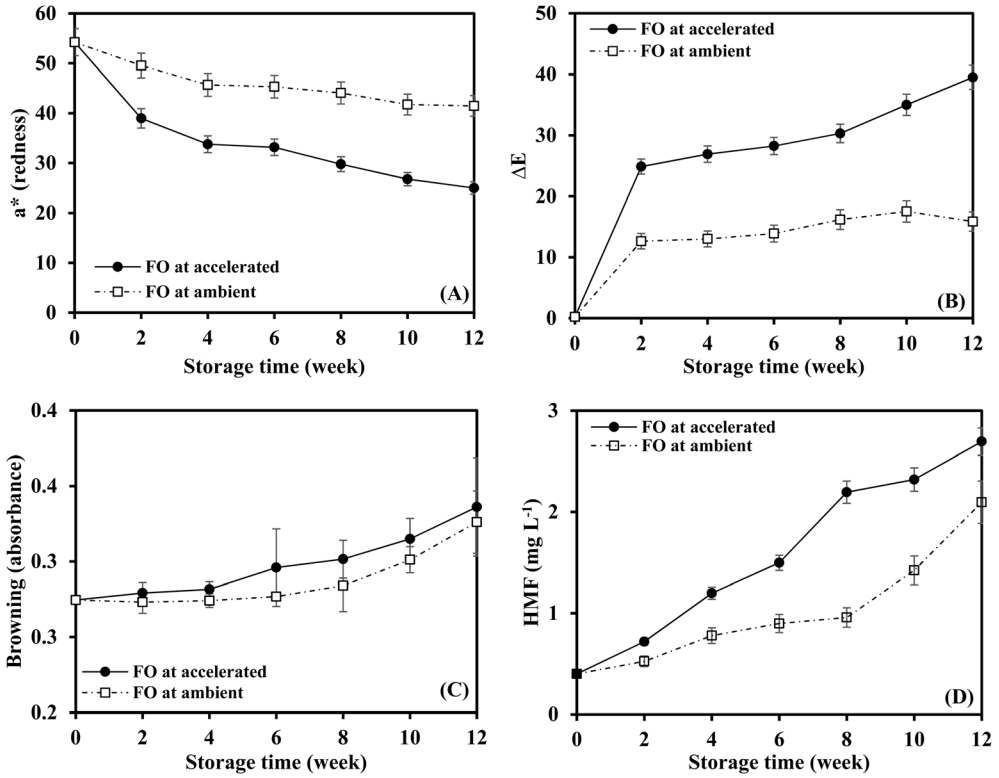

Fig. 5 Changes in (A) a^* (redness), (B) ΔE (total color difference), (C) browning index (BI), and (D) hydroxymethyl furfural (HMF) of forward osmosis (FO) concentrated beetroot juice stored under ambient and accelerated conditions for 12 weeks.

color coordinates: L^* (lightness/brightness), a^* (red/green), and b^* (blue/yellow). The data were recorded in terms of these three coordinates referring to the CIE Lab color system.

The total color difference (TCD) (ΔE) was calculated with respect to the original beetroot juice as reference:

$$\Delta E = \sqrt{\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2} \quad (2)$$

2.7 Measurement of antioxidant activity (DPPH and ABTS methods)

To determine the antioxidant activity, each sample of the beetroot juice concentrate (BRJC) was diluted using deionized water. The radical scavenging activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams *et al.* (1995), with modifications to the reaction time.[22] The antioxidant concentration by DPPH was measured by the 50% inhibition of lipid peroxidation (IC_{50}), which was calculated in triplicate at five different antioxidant concentrations.

Additionally, the ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation ($ABTS^{\bullet+}$) method was also used, as described by Re *et al.* (1999).[23]

Calibration curves in the range of 0.001–0.01 mg mL^{-1} were used to quantify the antioxidant activity from both methods. The investigation was conducted in quintuplet ($n = 5$), and the

Table 2 Degradation kinetic parameters (zero, first and second order) of betalain content and ΔE (total color difference) of beetroot juice concentrates at ambient and accelerated storage conditions for 12 weeks

Storage conditions	Betalains				ΔE (total color difference)			
	Ambient (25 °C)		Accelerated (37 °C)		Ambient (25 °C)		Accelerated (37 °C)	
Kinetics order	k (weeks)	R^2	k (weeks)	R^2	k (weeks)	R^2	k (weeks)	R^2
Zero	−0.0969	0.98	−0.1517	0.90	−0.3426	0.88	−0.9164	0.95
First	−0.0265	0.97	−0.0492	0.97	0.0237	0.90	0.0320	0.95
Second	0.0073	0.96	0.0149	0.95	−0.0017	0.91	−0.0011	0.06

Table 3 Degradation kinetic parameters of beetroot juice concentrate stored under ambient and accelerated conditions for 12 weeks

Storage condition	Process	B_0 (g L^{-1})	k (weeks)	$t_{1/2}$ (weeks)	D Value (weeks)	R^2
Ambient (25 °C)	Forward osmosis	4.25	−0.0265	26.16	86.9	0.97
Accelerated (37 °C)	Forward osmosis	4.25	−0.0492	14.09	46.8	0.97

Table 4 Correlation between betalain content and total color difference during storage of beetroot juice concentrate under ambient and accelerated conditions for 12 weeks

	Forward osmosis	
Storage condition	Pearson correlation, r	p-Value
Ambient (25 °C)	−0.746	0.054
Accelerated (37 °C)	−0.886	0.008

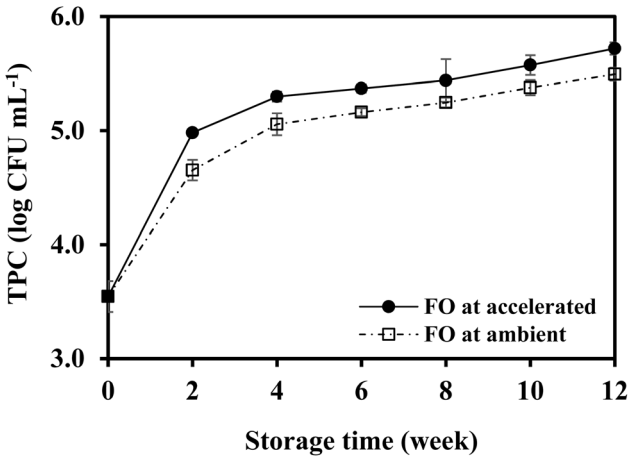

Fig. 6 Effect of storage on the total plate count of forward osmosis (FO) concentrated beetroot juice during storage at ambient and accelerated conditions for 12 weeks.

data were stated as mean ± standard error, with units in mg Trolox per Litre (mg Trolox L^{-1}).

2.8 Browning measurement by UV-spectroscopy

Beetroot juice concentrate (BRJC) samples were centrifuged at 5000 rpm for 15 min, and the supernatant was diluted (600 times) with deionized water. The browning of the diluted juice was assessed by measuring the absorbance of the supernatant at 420 nm using a Multiplate reader in triplicate ($n = 3$) as defined by Baxter (1995) and Selen Burdurlu and Karadeniz (2003).[24,25]

2.9 Measurement of HMF

HMF (5-hydroxymethylfurfural) measurement is used to assess the quality of juice concentrates. It is typically absent in fresh juices; however, its concentration increases during processing and storage. Carrez I solution and Carrez II solution were used to measure the HMF content of the juice concentrates throughout the storage as described by White Jr (1979).[26] The absorbance of the sample mixed solutions was measured at wavelengths of 284 and 336 nm. The HMF concentration was quantified using the following equation:

$$\text{HMF (mg L}^{-1}) = (A_{284} - A_{336}) \times 149.7/\text{sample} \quad (3)$$

$$\text{Factor } (F) = \frac{126}{16830} \times \frac{1000}{10} \times \frac{1000}{5} = 149.7$$

Here, 126 = mol. wt of HMF; 16 830 = molar absorptivity of HMF at 284 nm; 1000 = mg g^{-1}; 10 = centimetre per L; 1000 = mL juice reported; 5 = nominal sample weight.

All experiments were performed in triplicate, and the average values are reported with standard deviations.

2.10 Measurement of total plate count

One mL of a series of decimally diluted juice concentrate samples was inoculated onto plate count agar medium and incubated at 37 °C for 24 h to analyse the total plate count (TPC), as described in FSSAI.[27]

2.11 Data analysis

2.11.1 Shelf-life kinetics of beetroot juice concentrate for betalain content and total color difference. The degradation kinetics of betalains content of the concentrate was studied using zero and first-order reactions, which are given as follows:

$$\text{Zero-order: } B_t = -B_0 + k \times t,\ t_{1/2} = B_0/2k_0 \quad (4)$$

$$\text{First-order: } \ln(B_t/B_0) = -k_1 \times t,\ t_{1/2} = -\ln 0.5/k \quad (5)$$

$$\text{Second-order: } 1/B_t = -k_2 \times t + 1/B_0,\ t_{1/2} = 1/(k \times B_0) \quad (6)$$

B_t and B_0 are the betalain contents at time t and t_0, respectively, k_0, k_1 and k_2 are the zero, first and second order kinetic constants, respectively, and t is the storage time (week).

Further, the half-life value $t_{1/2}$ of the total betalain content was calculated; D-value (the time required for 90% degradation of betalains was also calculated as $D = \ln 10/k$, as described by Alighourchi and Barzegar (2009) and Cisse *et al.* (2012).[28,29]

2.11.2 Statistical analysis. All experiments were conducted in triplicate, and the data are presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 12.0 (SPSS, Inc., Chicago, IL, USA). Group comparisons were made using Duncan's multiple range test, along with one-way analysis of variance (ANOVA). Differences were considered statistically significant at a p-value of <0.05.

3 Results and discussion

3.1 Bibliometric analysis of the storage of beetroot juice colorant betalains

The bibliometric analysis, carried out through a co-occurrence analysis of the storage of beetroot juice colorant betalains using the Scopus database and VOSviewer software, is illustrated in Fig. 1. The figure presents three clusters: betalains (red-violet), beetroot (green), and storage (yellow), categorized by their significance. The correlation between the terms related to beetroot juice storage reflects the co-occurrence of keywords from a dataset of 67 articles published between 2000 and 2025. The analysis reveals the prominence of research in betalains (red-violet), beetroot (green), and storage (yellow). However, there is a noticeable gap in research focused on the storage of beetroot juice colorant betalains under different storage conditions, highlighting a substantial opportunity for further investigation and development in this field.

3.2 Changes in pH, titratable acidity and total soluble solids

The pH of the FO samples were varied between 5.69–5.80 during 12 weeks of storage under ambient (25 °C) and accelerated (37 °C) conditions (Fig. 3A). Bull *et al.* (2004) and Rodrigo *et al.* (2003) did not detect any substantial changes in the pH values of dissimilar juices during their storage for 12 weeks.[30,31]

The initial TA values of BRJC were 8.97 g L^{-1} for the FO concentration process. The TA values for the FO BRJC samples were slightly decreased and varied between 6.40 to 8.97 and 7.04 to 8.97 g L^{-1} during storage under ambient (25 °C) and accelerated (37 °C) conditions, respectively (Fig. 3B). The substantial decrease in TA throughout the storage was possibly related with the degradation of oxalic acid, which is majorly present in beetroot juice.[32]

The primary total soluble solids (TSS) of the concentrated beetroot juice obtained through forward osmosis (FO) were 62.3 °Brix. The TSS values of FO concentrated BRJCs were slightly decreased under both ambient (25 °C) and accelerated (37 °C) storage conditions, and varied between 60.7–62.3 °Brix during 12 weeks of storage (Fig. 3C). These results are consistent with those previously stated by Ayhan *et al.* (2001) and Bull *et al.* (2004) for pasteurized juices.[30,33]

3.3 Changes in color profile during storage

Changes in the color profile of the BRJC samples by FO throughout the storage are presented in Table 1. A significant increase in L^* values was observed in both ambient (25 °C) and accelerated (37 °C) storage conditions for the BRJCs during 12 weeks. The lightness (L^*) of FO BRJC degraded from 27.57 to 33.34 at ambient storage conditions (25 °C) after 12 weeks. The lightness (L^*) of the FO BRJCs changed in the range of 27.57 to 52.66 at accelerated storage conditions (37 °C).

Differences in a^* (redness) and b^* (yellowness) were observed between the storage under ambient (25 °C) and accelerated (37 °C) conditions in FO BRJCs. The loss of a^* values of FO BRJCs changes from 54.23 to 41.47 and 24.98 at 25 °C and 37 °C storage temperature throughout the 12 weeks of storage (Fig. 5A). The b^* values of the FO beetroot juice concentrates (BRJCs) increased from 43.63 to 51.10 and 52.35 after 12 weeks of storage at ambient conditions (25 °C ± 2 °C and 60–70% RH) and accelerated conditions (37°C ± 2 °C and 90% RH), respectively.

The total color difference (ΔE) was visible in the BRJC samples kept in ambient (25 °C) and accelerated (37 °C) storage conditions for 12 weeks. The changes in ΔE values for FO samples at ambient (25 °C) and accelerated (37 °C) conditions were 15.87 and 39.51, respectively (Fig. 5B). Similar effects were observed by Herbach *et al.* (2007, 2006b, 2006a) for L^* and ΔE, which increased slowly in the purple pitaya juice kept under light and dark at 20 °C for 6 months.[34–36]

The bright violet-red color of BRJCs becomes tawny as indicated by the decrease in a^* values, suggesting that the color loss of the BRJCs could be attributed to betalain degradation during storage. Many researchers have reported that the stability of betalains depends on pH, temperature, light, water activity, presence of oxygen and metal ions, in particular.[10,15,21] The decrease in L^* and a^* values can be attributed to the degradation of betalains at a high temperature and fading of the typical red color of beetroot juice or puree.[8,11] The heat destroyed betalain pigments, which are unstable in the presence of oxygen, and high water activity explained the decrease in L^* and a^* values during the different storage conditions.[10,15] The increase in b^* values indicates deep red to red to brown to yellowing of red pigment betalains.[11,13,14] The increase in b^* values resulted in the formation of isobetanin due to the isomerization of betanin (red in color), and decarboxylation leads to an increase in vulgaxanthin, which is yellow in color.[35] It also indicates a rise in yellowness (betaxanthins) or a reduction in redness (betacyanins), resulting from the decarboxylation of betanin into vulgaxanthin-I and the formation of other betaxanthin derivatives.[15,34]

The decline in ΔE values was observed in both FO BRJC samples during the 12-weeks storage, and this may be attributed to enzymatic or non-enzymatic browning of the juice concentrates and fading of color from vivid to duller that could reflect the decolorization of betalains.[9,14] The increasing trend of total color difference (ΔE) values in the BRJC samples indicates a more noticeable visual color change during storage.[37]

3.4 Changes in betalain contents of beetroot juice concentrates (BRJCs)

Betalains are the major constituents of BRJC and are a crucial quality factor that influences the acceptance of the juice and its use as a colorant. The bright violet-red color of the BRJCs becomes tawny as indicated by betalain degradation throughout the storage. The loss of betalains in BRJCs by FO samples was visible, as shown in Fig. 4A. The initial betalain content of FO BRJCs was 4.25 g L^{-1}. The betalain degradation of the FO sample in ambient (25 °C) and accelerated (37 °C) conditions was 28.53% and 43.57%, respectively, at the end of 12 weeks of storage. These results indicate that betalain content was well preserved when stored at 25 °C compared to 37 °C during storage for 12 weeks. Similar results were described by

Siow and Wong (2017), who found 93% and 67% betacyanin retention in red-fleshed dragon fruit at 4 and 25 °C after 8 weeks of storage.[38] Herbach *et al.* (2007) demonstrated a 60% reduction in betacyanins in purple pitaya juice kept at 20 °C in the dark without ascorbic acid for 6 months.[34] The higher retention of betalains in the BRJCs may probably indicate betalain regeneration as previously reported by numerous authors, who found higher color retention in betanin solution, red beet and red-fleshed dragon fruit juice at low storage temperatures.[9,38,39]

3.5 Kinetic change of betalain content during storage of beetroot juice concentrates

Fig. 4B shows the betalain content in beetroot juice concentrate (BRJC) following a first-order kinetic reaction model as a function of storage time. In this study, the first-order kinetic model was preferred over the zero-order and second-order models, as it resulted in a higher R^2 value, indicating the best fit for the data (Table 2). The reaction rate constant (k), initial betalain content (B_0), half-life ($t_{1/2}$), D value, and coefficient of determination (R^2) were determined and are presented in Table 3. The negative values of the reaction rate constant indicate a reduction in betalain content over time. The BRJCs stored at the accelerated temperature of 37 °C (−0.0492) exhibited a higher degradation of betalains compared to those stored at ambient conditions of 25 °C (−0.0265), indicating a greater loss of betalains at the higher temperature. The half-life of FO BRJC was 26.16 weeks at ambient storage conditions (25 °C), whereas the half-life of BRJC samples at accelerated conditions (37 °C) was 14.09 weeks. The D-values of the FO BRJC samples based on betalain content was 86.9 and 46.8 weeks at ambient (25 °C) and accelerated (37 °C) storage conditions, respectively. These results are in accordance with the k values of red-fleshed dragon fruit juice reported by Siow and Wong (2017), which were 0.0102, 0.04 and 0.0828 weeks for a storage temperature of 25, 37 °C and 45 °C.[38] Similar results were reported by Derossi *et al.* (2010), where the k value at 25 °C was 0.198 days and 0.130 days for a storage temperature of 25 °C by Odriozola-Serrano *et al.* (2009).[40,41] Kırca *et al.* (2007) reported the k values for 64 °B black carrot juice anthocyanins were 0.00276 and 0.02489 at a storage temperature of 20 °C and 37 °C of and the $t_{1/2}$ values were 35.9 and 4 weeks at 20 and 37 °C.[42,43]

3.6 Correlation between betalain content and total color difference

The degradation kinetics parameters for the total color difference of FO-concentrated BRJCs stored at different conditions (ambient (25 °C ± 2 °C and 60–70% RH) and accelerated (37 °C ± 2 °C and 90% RH) conditions) are shown in Table 2. First-order reaction kinetics provided the best fit compared to zero-order and second-order models. For the BRJC samples stored in ambient conditions, the degradation was less compared to the samples stored at accelerated conditions, consequently resulting in less color degradation and greater retention. The decreasing trend in betalain content in FO BRJCs stored under both conditions is correlated with the total color difference (TCD) of the concentrate over time. The correlation between betalain content and TCD of BRJC of FO samples is shown in Table 4. The Pearson correlation of BRJC kept at ambient conditions of 25 °C was −0.746, which showed a high to moderate negative correlation between the two variables. A strong correlation of −0.886 was observed between betalain content and TCD in stored BRJCs under accelerated conditions at 37 °C. Various studies have reported different degradation patterns of betalains at varying temperatures.[8,10,15,44]

3.7 Changes in antioxidant capacity

The changes in antioxidant capacity of beetroot juice concentrates by FO, during storage, were evaluated by ABTS and DPPH. The antioxidant capacity of BRJCs was measured using ABTS radical scavenging methods and expressed in terms of Trolox equivalent antioxidant capacity (TEAC) per litre of juice concentrates (Fig. 4C). The initial antioxidant capacity of BRJCs evaluated by ABTS radical scavenging methods was 7.98 g trolox equivalent per litre for FO BRJC. The TEAC of FO BRJCs was reduced to 5.34 and 5.26 g Trolox equivalent per litre at the end of 12 weeks at ambient (25 °C) and accelerated (37 °C) storage conditions, respectively. The loss in antioxidant capacity of BRJCs by FO was 33.03% and 34.11% at the end of 12 weeks of storage at ambient (25 °C) and accelerated (37 °C), conditions, respectively. The decrease in antioxidant activity, measured with the ABTS+ radical cation method in rosehip nectars during 8 months of storage at 25 °C, 35 °C and 45 °C, of glass-bottled samples was 11.2%, 16.6%, and 27.8% respectively.[45]

The antioxidant activities of BRJCs were measured using the DPPH method and expressed as IC_{50} values, representing the mL of juice required to inhibit 50% of DPPH per litre. The DPPH assay also showed a similar variation trend of ABTS radical scavenging methods and a pronounced drop of antioxidant capacity was visible (Fig. 4D). The antioxidant activity of BRJCs by FO was reduced from 0.49 to 1.59 and 2.54 mL L^{-1} at ambient (25 °C) and accelerated (37 °C) storage conditions at the end of 12 weeks. The reduction in antioxidant activity of the BRJCs was similar to the previous study by Klimczak *et al.* (2007), where the antioxidant activity of orange juice measured by DPPH assays declined by 18%, 45% and 84% after 6 months of storage at 18 °C, 28 °C and 38 °C, respectively.[46]

The different results from TEAC and DPPH assays could be attributed to different electron-transfer mechanisms. The degradation of antioxidant capacity of BRJCs may be associated with the loss of vitamin C during storage.[11,47] The decrease in the antioxidant capacity of orange juices and carrots was related to the decrease in polyphenols and vitamin C during storage. Kalt *et al.* (1999) reported a linear relationship between phenolic compounds and antioxidant activity upon storage of small berries, which could be reflected by the decrease in antioxidant capacity of beetroot juice concentrate during 12 weeks of storage.[48–50]

3.8 Changes in browning index (BI)

Browning is one of the significant reactions that occurs during the processing and storage of juices and juice concentrates. Enzymatic and non-enzymatic browning can both affect the

quality of concentrates, depending on the type of foods and degrade the visual appearance of foods.[51] Enzymatic browning is related to the oxidation of phenolic compounds, whereas non-enzymatic browning is referred to as the Maillard reaction, where reducing sugars degrade in reaction with amino acids, peptides or proteins. The initial absorbance value of the BI of FO BRJC was 0.265. The browning index of all the BRJCs predominantly increased after 6 weeks of storage at ambient (25 °C) and accelerated (37 °C) storage conditions (Fig. 5C). FO BRJCs BI the BI absorbance values of FO BRJCs changed to 0.326 and 0.336 after 12 weeks of storage at 25 °C and 37 °C, respectively. The higher changes in the FO juice concentrate samples were visible at a higher storage temperature of accelerated (37 °C) compared to ambient (25 °C) storage conditions due to exposure at a higher temperature for a longer time.

Lyu *et al.* (2018) demonstrated a significant impact of storage time of over 40 days and temperature on the browning degree of peach juice, with browning degree values increasing by 0.382 at 25 °C and 0.533 at 37 °C.[52] The reaction between dehydroascorbic acid and proteins, amino groups of amino acids and other amines forms aldehydes, carbon dioxide and α-amino carbonyl compounds, leading to the formation of pigments (melanoidins).[53–55] Chandran *et al.* (2014) and Güneşer (2016) reported that the color of the beetroot puree transformed from a deep violet-red to a yellowish-brown during heating, and this change occurs due to the degradation of betalains, which may lead to an increase in the BI of BRJCs.[8,10]

3.9 Changes in HMF

The formation of HMF was visible (Fig. 5D) in all the samples kept at ambient (25 °C ± 2 °C and 60–70% RH) and accelerated (37 °C ± 2 °C and 90% RH) conditions during the 12-weeks storage. The initial values of HMF for FO BRJCs were 0.40 mg L^{-1}. The accumulation of HMF for samples was significant at accelerated storage conditions (37 °C) and by the end of 12 weeks, it increased to 2.69. Meanwhile, the formation of HMF in ambient storage conditions (25 °C) for BRJCs by FO was comparatively low and the values increased readily after 8 weeks of storage; at the end of 12 weeks, it increased to 2.10 mg L^{-1}. The development of HMF in fruit juices in storage primarily depends on the juice type, pH, concentration, and storage temperature.[56] The HMF content of fresh peach juice was 1.75 mg L^{-1}, while the values increased to 6.63 and 8.06 mg L^{-1} at the end of 40 days of ambient (25 °C) and accelerated (37 °C) storage conditions, respectively.[52]

HMF development is more common in fruit juice concentrates than in fresh juices, as the reaction rate is influenced by the TSS levels and storage conditions.[57,58] The HMF can be formed by the enolization and dehydration of glucose or fructose, under acidic conditions.[59] The formation of HMF depends on the storage conditions. In previous studies, it was found that the formation of HMF at lower temperatures was comparatively lower than at higher storage temperatures. The International Federation of Fruit Juice Processors (IFFJP) has established a maximum limit of 5–10 mg L^{-1} for fruit juices and 25 mg kg^{-1} for concentrates, while the European Union has set a recommended limit of 20 mg kg^{-1} for 5-HMF in juices intended for children.[57] The whole FO BRJCs have lower HMF initially and a slight increase in HMF were observed during storage at both the conditions; however, for all the samples the HMF content was under the permissible limits.

3.10 Changes in microbial growth

The changes in the growth of microorganisms at ambient (25 °C ± 2 °C and 60–70% RH) and accelerated (37 °C ± 2 °C and 90% RH) storage conditions for FO BRJCs are shown in Fig. 6. The initial total plate count found for BRJCs was 3.55 log CFU mL^{-1}, respectively. The maximum increase in the total plate count (TPC) was visible after the 2nd week of storage for all the BRJCs at ambient (25 °C) and accelerated (37 °C) conditions. The FO samples showed an increase in TPC and were measured at 5.49 and 5.72 log CFU mL^{-1}, respectively, at ambient (25 °C) and accelerated (37 °C) storage conditions after 12 weeks. The increase in TPC during ambient (25 °C) and accelerated (37 °C) storage conditions was impacted due to the initial load of 3.55 log CFU mL^{-1} of the juice concentrates. The increase in the TPC was around 2 log CFU mL^{-1} during both the ambient (25 °C) and accelerated (37 °C) storage conditions.

Beetroot is a subterranean crop that is exposed to microorganisms present in the soil, irrigation water, and during handling after harvest, due to which beetroot juice may contain a significant initial microbial load. Also, the juice may be prone to cross-contamination during processing and concentration. This can be avoided by initial pasteurization of the juices before concentration or after concentration before storage. The presence of lactic acid-forming anaerobic microorganisms, such as *Leuconostoc* spp and *Lactobacillus* spp, may be the reason for the increase in TPC, which more frequently occurs in unpasteurized juices during storage.[60,61] According to good manufacturing practices, the maximum acceptable limit for total yeast count during the storage of food products is set at 6 log CFU mL^{-1} for pasteurized juices.[62] Therefore, the FO BRJCs stored at ambient (25 °C) and accelerated (37 °C) conditions for 12 weeks were considerably safe.

4 Conclusions

Beetroot juice concentrate obtained through forward osmosis was stored at ambient conditions (25 °C ± 2 °C and 60–70% RH) and accelerated conditions (37 °C ± 2 °C and 90% RH) for 12 weeks, which showed significant changes in betalain content, total color, antioxidant capacity, browning index, HMF and microbial count. The betalain content and antioxidant capacity in BRJCs by the FO process generally decreased along with the storage time at both storage conditions; however, at ambient conditions, the reduction was less compared to that at accelerated conditions. The beetroot juice concentrate is significantly impacted by storage conditions (temperature and relative humidity), affecting its color stability, nutrient retention, and microbial load. Despite the substantial rise in microbial count, the FO BRJCs were still acceptable as the total plate counts were considered within the permissible limit. The study on the FO

BRJCs focused on the physical, chemical, and microbiological properties, which can further help in improving the storage conditions to enhance product quality and safety. The betalain content and TCD in BRJCs by FO decreased over the storage duration under both conditions, with the degradation of betalains following a first-order reaction model. The degradation was more pronounced under accelerated storage conditions. A moderately strong correlation was observed between the betalain content and TCD in BRJCs stored under ambient conditions, while a strong correlation was found for those stored under accelerated conditions. These insights and control of spoilage mechanisms will also reduce product loss due to quality and microbial degradation. To minimize the betalains and other physicochemical degradation, it is recommended that beetroot juice concentrates be stored at refrigerated conditions as soon as they are produced.

This study on shelf-life stability will help to ensure optimal product quality throughout the supply chain, enabling wider distribution, including export to distant markets, without the need for synthetic preservatives. Furthermore, the red betalain colorant-rich beetroot juice concentrate can be used as a supplement or as a colorant in different foods. Additionally, it can help manufacturers optimize natural preservation techniques to meet consumer demand for minimally processed, additive-free beverages, supporting more sustainable food systems and offering both economic and environmental benefits.

Author contributions

Das Trishitman: conceptualization, methodology, formal analysis, investigation, data curation, software, and writing – original manuscript.

Conflicts of interest

The author declares that there is no conflict of interest.

Data availability

The data supporting the findings of this study are available within the article and its cited references. Additional information or SI can be provided by the authors upon reasonable request

SI Table 1 changes in total soluble solids, pH and titratable acidity of beetroot juice concentrate stored at ambient and accelerated conditions for 12 weeks. See DOI: **https://doi.org/10.1039/d5fb00331h**.

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References

1 R. Domínguez, J. L. Maté-Muñoz, E. Cuenca, P. García-Fernández, F. Mata-Ordoñez, M. C. Lozano-Estevan, P. Veiga-Herreros, S. F. da Silva and M. V. Garnacho-Castaño, *J. Int. Soc. Sports Nutr.*, 2018, **15**, 2.
2 M. Ormsbee, J. Lox and P. Arciero, *Nutr. Diet. Suppl.*, 2013, **5**, 27.
3 J. Escribano, M. A. Pedreño, F. García-Carmona and R. Muñoz, *Phytochem. Anal.*, 1998, **9**, 124–127.
4 J. Kanner, S. Harel and R. Granit, *J. Agric. Food Chem.*, 2001, **49**, 5178–5185.
5 R. L. Jackman and J. L. Smith, in *Natural Food Colorants*, ed. G. A. F. Hendry and J. D. Houghton, Springer US, Boston, MA, 1996, pp. 244–309.
6 S. J. Schwartz and J. H. Von Elbe, *J. Agric. Food Chem.*, 1980, **28**, 540–543.
7 A. Baublis, A. Spomer and M. D. Berber-Jiménez, *J. Food Sci.*, 1994, **59**, 1219–1221.
8 J. Chandran, P. Nisha, R. S. Singhal and A. B. Pandit, *J. Food Sci. Technol.*, 2014, **51**, 2678–2684.
9 J. H. V. Elbe, S. J. Schwartz and B. E. Hildenbrand, *J. Food Sci.*, 1981, **46**, 1713–1715.
10 O. Güneşer, *Food Chem.*, 2016, **196**, 220–227.
11 K. Ravichandran, N. M. M. T. Saw, A. A. A. Mohdaly, A. M. M. Gabr, A. Kastell, H. Riedel, Z. Cai, D. Knorr and I. Smetanska, *Food Res. Int.*, 2013, **50**, 670–675.
12 J. H. Von Elbe, J. T. Klement, C. H. Amundson, R. G. Cassens and R. C. Lindsay, Evaluation of betalain pigments as sausage colorants, *J. Food Sci.*, 1974, **39**(1), 128–132.
13 H. M. C. Azeredo, *Int. J. Food Sci. Technol.*, 2009, **44**, 2365–2376.
14 K. m. Herbach, F. c. Stintzing and R. Carle, *J. Food Sci.*, 2004, **69**, C491–C498.
15 K. M. Herbach, F. C. Stintzing and R. Carle, *J. Food Sci.*, 2006, **71**, R41–R50.
16 S. M. Duyar, F. Sari and H. A. Karaoglan, Production of red beetroot juice by different methods: Kinetics of microbial growth, sugar consumption, and acid production, *Heliyon*, 2024, **10**(9), e30448.
17 D. Trishitman, P. S. Negi and N. K. Rastogi, *Food Chem.*, 2023, **399**, 133972.
18 D. Trishitman, *J. Food Eng.*, 2025, **399**, 112622.
19 W. Horwitz and G. W. Latimer, *Association of Official Analytical Chemists International, Official Methods of Analysis of AOAC International*, AOAC International, Gaithersburg, Maryland, 2006.
20 T. Nilsson, *Lantbrukshogskolans Annaler*, 1970, **36**, 179–219.
21 D. Trishitman, P. S. Negi and N. K. Rastogi, *LWT–Food Sci. Technol.*, 2021, **145**, 111522.

22 W. Brand-Williams, M. E. Cuvelier and C. Berset, *LWT–Food Sci. Technol.*, 1995, **28**, 25–30.
23 R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, *Free Radical Biol. Med.*, 1999, **26**, 1231–1237.
24 J. H. Baxter, *J. Food Sci.*, 1995, **60**, 405–408.
25 H. Selen Burdurlu and F. Karadeniz, *Food Chem.*, 2003, **80**, 91–97.
26 J. W. White Jr, *J.-Assoc. Off. Anal. Chem.*, 1979, **62**, 509–514.
27 FSSAI, 2020, **https://www.fssai.gov.in/cms/food-safety-and-standards-regulations.php**, accessed 11.25.20.
28 H. Alighourchi and M. Barzegar, *J. Food Eng.*, 2009, **90**, 179–185.
29 M. Cisse, F. Vaillant, A. Kane, O. Ndiaye and M. Dornier, *J. Sci. Food Agric.*, 2012, **92**, 1214–1221.
30 M. K. Bull, K. Zerdin, E. Howe, D. Goicoechea, P. Paramanandhan, R. Stockman, J. Sellahewa, E. A. Szabo, R. L. Johnson and C. M. Stewart, *Innovative Food Sci. Emerging Technol.*, 2004, **5**, 135–149.
31 D. Rodrigo, J. I. Arranz, S. Koch, A. Frígola, M. C. Rodrigo, M. J. Esteve, C. Calvo and M. Rodrigo, *J. Food Sci.*, 2003, **68**, 2111–2116.
32 J. Wruss, G. Waldenberger, S. Huemer, P. Uygun, P. Lanzerstorfer, U. Müller, O. Höglinger and J. Weghuber, *J. Food Compos. Anal.*, 2015, **42**, 46–55.
33 Z. Ayhan, H. W. Yeom, Q. H. Zhang and D. B. Min, *J. Agric. Food Chem.*, 2001, **49**, 669–674.
34 K. M. Herbach, C. Maier, F. C. Stintzing and R. Carle, *Eur. Food Res. Technol.*, 2007, **224**, 649–658.
35 K. M. Herbach, F. C. Stintzing and R. Carle, *J. Agric. Food Chem.*, 2006, **54**, 390–398.
36 K. M. Herbach, M. Rohe, F. C. Stintzing and R. Carle, *Food Res. Int.*, 2006, **39**, 667–677.
37 V. Sanchez, R. Baeza and J. Chirife, *J. Berry Res.*, 2015, **5**, 243–251.
38 L.-F. Siow and Y.-M. Wong, *Int. J. Food Prop.*, 2017, **20**, 623–632.
39 A. S. Huang and J. H. V. Elbe, *J. Food Sci.*, 1987, **52**, 1689–1693.
40 A. Derossi, T. De Pilli and A. G. Fiore, *LWT–Food Sci. Technol.*, 2010, **43**, 590–595.
41 I. Odriozola-Serrano, R. Soliva-Fortuny and O. Martín-Belloso, *J. Food Sci.*, 2009, **74**, C184–C191.
42 A. Kırca, M. Özkan and B. Cemeroğlu, *Food Chem.*, 2007, **101**, 212–218.
43 A. Kırca, M. Özkan and B. Cemeroğlu, *Food Chem.*, 2007, **101**, 212–218.
44 D. Trishitman, *Ultrasonics*, 2025, **153**, 107676.
45 N. Duru, F. Karadeniz and H. S. Erge, *Food Bioprocess Technol.*, 2012, **5**, 2899–2907.
46 I. Klimczak, M. Małecka, M. Szlachta and A. Gliszczyńska-Świgło, *J. Food Compos. Anal.*, 2007, **20**, 313–322.
47 C. Vijaya Kumar Reddy, D. Sreeramulu and M. Raghunath, *Food Res. Int.*, 2010, **43**, 285–288.
48 N. Koca and F. Karadeniz, *Int. J. Food Sci. Technol.*, 2008, **43**, 2019–2025.
49 A. Del Caro, A. Piga, V. Vacca and M. Agabbio, *Food Chem.*, 2004, **84**, 99–105.
50 W. Kalt, C. F. Forney, A. Martin and R. L. Prior, *J. Agric. Food Chem.*, 1999, **47**, 4638–4644.
51 M. Villamiel, M. Castillo and N. Corzo, in *Food Biochemistry and Food Processing*, 2007, 2nd edn, pp. 71–100.
52 J. Lyu, X. Liu, J. Bi, X. Wu, L. Zhou, W. Ruan, M. Zhou and Y. Jiao, *J. Food Sci. Technol.*, 2018, **55**, 1003–1009.
53 M. Eskin, C. Ho and F. Shahidi, *J. Food Biochem.*, 2013, 245–289.
54 B. M. Tolbert and J. B. Ward, Dehydroascorbic Acid, in *Ascorbic Acid: Chemistry, Metabolism, and Uses, Advances in Chemistry*, American Chemical Society, 1982, pp. 101–123, DOI: **10.1021/ba-1982-0200.ch005**.
55 M. Wong and D. W. Stanton, *J. Food Sci.*, 1989, **54**, 669–673.
56 S. Wibowo, T. Grauwet, G. B. Gedefa, M. Hendrickx and A. Van Loey, *Food Res. Int.*, 2015, **78**, 410–423.
57 J. S. Thakur, *Curr. Res. Nutr. Food Sci.*, 2018, **6**, 227–233.
58 D. Trishitman, PhD thesis, Central Food Technological Research Institute, 2022.
59 H. S. Lee and S. Nagy, *J. Food Process. Preserv.*, 1990, **14**, 171–178.
60 K. R. Aneja, R. Dhiman, N. K. Aggarwal, V. Kumar and M. Kaur, Microbes Associated with Freshly Prepared Juices of Citrus and Carrots, *Int. J. Food Sci.*, 2014, **1**, 408085.
61 S. E. Keller and A. J. Miller, in *Microbiology of Fruits and Vegetables*, 2005, pp. 211–230.
62 C. Stannard, Development and Use of Microbiological Criteria for Foods, *Food Sci. Technol. Today*, 1997, **11**(3), 137–177.